International Journal of Multidisciplinary Research and Growth Evaluation.

Illegal trade of canines: Identifying suspected samples of Tigers (*Panthera tigris*) and Bears (*Melursus ursinus*)

Abinaya Nadarajan [1*], Prithiviraj S [2], Vasantha Kumari [3], Kanchana Rangasamy [4], Debasis Jana [5]
[1-5] Centre for Wildlife Forensic Sciences, Advanced Institute for Wildlife Conservation, Tamil Nadu Forest Department, Chennai, Tamil Nadu, India

* Corresponding Author: **Abinaya Nadarajan**

Article Info

ISSN (online): 2582-7138
Volume: 03
Issue: 01
January-February 2022
Received: 23-01-2022;
Accepted: 12-02-2022
Page No: 531-540
DOI:
https://doi.org/10.54660/anfo.2022.3.1.25

Abstract
Poaching is one of the major reasons for declining heterotrophic wild populations attributed to illegitimate economic gain. It causes serious threats not only to the specific population but also generates a negative impact on the entire world population of wildlife. In recent years prohibited trade for canines is going through an upward trendline, adversely impacting a number of significant species of mammals. Increasing reports of canine seizures demand forensic inspection and thorough investigation of evidence for species identification. Though examination of canine samples through morphometric technique is acceptable, it falls short when the complete sample is not recovered for analysis. Thus, molecular analysis provides error-free and reliable proof of evidence in identifying species. This report investigates two separate canine seizures using combined approaches of morphometry and DNA analysis. Seizure 1 comprised a 10 years old broken canine sample and seizure 2 contained 4 canine samples (n=4). Morphometrically, physical examination, X-ray analysis and mensuration were undertaken. For molecular analysis, the mitochondrial regions of Cytochrome b (Cytb), 12S rRNA and 16S rRNA were targeted. BLASTn search and comparison with the genetic repository at Advanced Institute for Wildlife Conservation (AIWC) clearly indicated that seizure 1 belongs to Tiger *(Panthera tigris)* and seizure 2 belongs to Sloth bear *(Melursus ursinus).* To examine wildlife forensic case samples, both morphometric and molecular databases must be strengthened to increase the conviction rate while prosecuting under the Wildlife (Protection) Act, 1972 of India.

Keywords: Canine, Illegal trade, Morphometry, DNA analysis, Wildlife forensics, India

1. Introduction
Tigers are one of the flagship and umbrella species given prime importance globally with utmost protection status as per Wildlife (Protection) Act (WPA), 1972 of India and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), to contain the commercial international trade (Ramesh, 1999; Musing, 2020) [1, 3]. Sloth bears are also given the same protection status (Willcox, 2016) [6]. Both tigers and bears are targeted for illegal trade of canine teeth, skin, bones, and other body parts (Nijman & Shepherd, 2015) [2], though stringent laws and various survey methods are in use to deduce the verifiable and reliable population numbers. Several factors are contributing to the decline in population, including habitat loss and fragmentation of forest covers, but the illegal trade and trafficking of animals and their parts pose the primary threat to their existence (Goodrich et al., 2015) [4]. Almost all body parts and products of tigers and bears are given high trade value leading to huge monetary gain. Teeth, claw, and bones are used for ornamental purposes and clothing, and the meat for consumption (Nowell, 2000) [5] and medicinal purposes.

Canines are long and pointed, with sharp tips, and sometimes edges that make them effective tools for piercing and penetrating prey (Anderson, 2018) [7]. They are the four elongated teeth situated in the front of the jaws, and at first glance all canine teeth look alike (Van Valkenbourgh, 1996) [10]. The canine teeth of bears (Ursidae) and cats (Felidae) are prized for use as ornaments and fetishes because of their intrinsic shape and symbolism. They are also faked (carved from bone or ivory) or substituted (as bear for tiger, or vice versa). Preliminary identification is possible, at least to the level of Family (Yates, 1996) [12] through morphometric analysis. Such limitations are circumvented through advanced molecular techniques to accurately identify the sample till species level. Mitochondrial loci are mostly chosen for challenging forensic samples owing to their unique features such as high copy number and less shear stress. The commonly used mitochondrial regions are cytochrome b (Cytb), 12S rRNA and 16S rRNA. In this paper, the authors describe the forensic techniques used in examining two different seizures of canine samples to find the authenticity and species identification of the presented samples.

2. Materials & Methods

2.1 Case history

The Tamil Nadu Forest department officials confiscated canines from two separate seizures, seizure 1 (Fig 1) with one broken canine, which was a 10 years old sample and seizure 2 (Fig 2) with four canine samples. The investigating team collected the samples from crime sites and forwarded to the Centre for Wildlife Forensic Sciences, Advanced Institute for Wildlife Conservation Institute (AIWC), Vandalur, Chennai. The cases were registered to perform morphometric and molecular analysis for species identification to provide fool-proof evidence in the Court-of-law.

2.2 Morphometric analysis: Laboratory procedures

2.2.1. Physical Examination

The teeth were examined physically (Yates, 1996) [12] to identify the species. Examination was done in stereomicroscope (Lynx) and observations were noted down.

2.2.2. X-ray analysis

X-ray images of each tooth were obtained to visualize the pulp cavity to verify the seized articles were original or fake. Radiography has played a central role in forensics almost immediately from the discovery of the X-ray image. Radiography is an integral part in forensic odontology, mainly to establish identification (Breecher et al., 1969) [11].

2.2.3. Morphometric measurements

Canine root of seizure 1 sample measurements were taken with slight modifications from (Smuts et al,. 1978) [9] by measuring the root height along with maximum mesio-distal width of root. This method was followed only for the seizure where the tooth crown was completely absent.

For seizure 2, Canine measurements were taken as follows: crown height in straight line from the center of the alveolar margin to the apex, and anteroposterior (AP) and lateromedial (LM) diameters measured at seven intervals along the crown: at the alveolar margin, and at 15, 30, 45, 60, 75, and 90% of crown height, respectively, from the alveolar margin (Christiansen, 2007) [8]. A digital Vernier Caliper (Mitutoyo) with a measurement accuracy of ±0.02 mm was used to measure the dimensions of the teeth (Fig. 1 & 2).

Fig 1: Seizure 1, Canine root with the measurement of maximum-mesio distal width of root

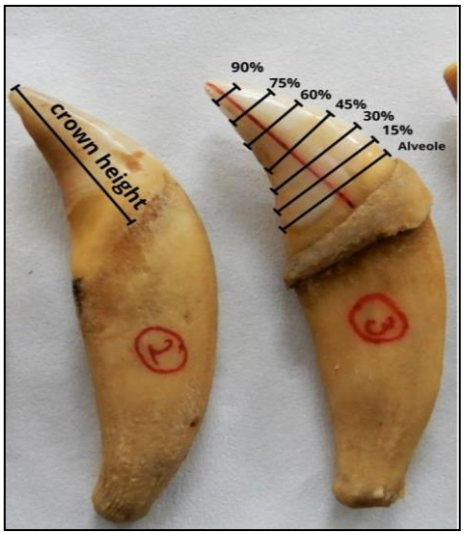

Fig 2: Seizure 2 dislaying crown height and anteposterior diameter of canine being measured

2.3 DNA analysis: Laboratory procedures

2.3.1. Washing, DNA extraction, PCR amplification and Sequencing

Twenty mg of each canine sample was drilled with the help of BOSCH kit from Seizures 1 and 2 and was taken in separate 2 ml microcentrifuge tubes and subjected to wash using 0.5 M EDTA with 24 hours of incubation at room temperature. On completing the wash, the supernatant was discarded by centrifuging at 12000 rpm for 5 minutes. DNA was extracted following the commercially available Qiagen DNeasy Blood & Tissue kit procedure (Qiagen, Germany). The samples after wash were kept under extraction digestion

With digestion buffer and proteinase K, for the period of 48 hours totally, of which 24 hours was at room temperature and 24 hours in circulating water-bath at 56 ℃. The DNA extracted from canine samples was subjected to amplification of mitochondrial regions of 16S rRNA (Guha & Kashyap, 2006) [15] and 12S rRNA (Kitano et al., 2007) [16] from Seizure 1 yielding 600bp and 240bp respectively. Cytochrome b (Kocher et al., 1998) [14] and 12S rRNA (Kitano et al., 2007) [16] were chosen for seizure 2 yielding 370bp and 240bp respectively. The PCR amplification was carried out in Eppendorf Nexus GSX1 Mastercycler. 10 µL PCR reactions were prepared comprising 1X Taq Buffer (KAPA Biosystems, SIGMA), 0.25 mM dNTPs, 0.4 µM of both forward and reverse primer, 2.5 mM $MgCl_2$, 0.25 U Taq DNA Polymerase (KAPA Biosystems, SIGMA) and 1 µL of 10- 50 ng/µL template DNA. Cycling conditions consisted of 5 min. of initial denaturation at 95 ˚C, followed by 35 cycles of 30 seconds of denaturation at 95 ˚C, annealing at 55 ˚C (for Cytochrome b primer) and 57˚C (for 12S and 16S primer), 45 seconds of extension at 72 ˚C and final extension at 72˚C for 10 min. The 12S PCR was set up with primer concentration of 0.5 µM of both forward and reverse primer. The PCR reactions were set with positive and non-template controls. The bands were visualized in 2% agarose gel with novel juice stain and documented using BioRad XR+ gel doc system. The PCR samples were purified using QIAquick gel extraction kit (Qiagen, Germany) and sequences were obtained through Sanger sequencing performed in ABI 3730 Genetic Analyzer (Applied Biosystems, USA).

2.3.2 Data Analysis

The samples with forward and reverse sequences were matched against the NCBI database using nucleotide BLAST and they were processed using MEGA X software (Kumar S et al., 2018) [18]. The similar hits of homologous sequences with a percentage match of greater than 99% were extracted from the repository and subjected to multiple sequence alignment with the query sequence using CLUSTALW. Phylogenetic tree was constructed by the Neighbour Joining method using the Kimura-2-Parameter model with 500 bootstrap replications and pairwise distances were computed using MEGA X (Tamura K et al, 2004) [17]. The seizure 1 query sequences of each mitochondrial regions were analyzed with similar and dissimilar mammal species such as Tiger *(Panthera tigris)*, Asiatic lion *(Panthera leo)*, Leopard *(Panthera pardus)*, Snow leopard *(Panthera uncia)*, and seizure 2 query sequences were compared to Sloth bear *(Melursus ursinus)*, Himalayan brown bear *(Ursus arctos isabellinus)*, Brown bear *(Ursus arctos)* for determining the species identification.

3. Results & discussion

3.1 Morphological analysis

3.1.1. Physical examination

The tooth of seizure 1 was devoid of tooth crown and it had started to crack longitudinally like in all canines when dried (Yates, 1996) [12]. The proximal end was tapering. The four canines/ teeth of seizure 2 showed no presence of grooves and had fine brown rings in the enamel which is characteristic for Ursidae (Fig. 3) (Yates, 1996) [12]. The teeth started to crack longitudinally like in all canines when dried (Yates, 1996) [12].

Fig.3. Enamel showing the presence of fine brown rings in the canine

3.1.2. X-ray analysis

The x-ray images from both seizures showed the presence of pulp cavity, which was used as a sign to find the originality of teeth (Fig. 4 & 5).

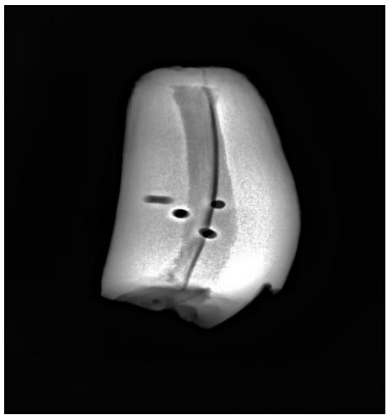

Fig 4: Seizure 1, X-ray image of the canine root showing the presence of pulp cavity

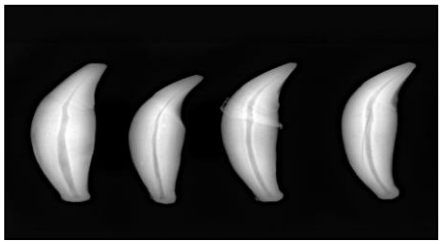

Fig 5: Seizure 2, X-ray image of the canine teeth showing the presence of pulp cavity.

3.1.3. Morphometric measurements

The seized canine root was measured and compared with the available canine roots of Tiger (*Panthera tigris*) since the seized canine root was larger than most canine roots (Table. 1).

Table 1: Canine root measurements compared with data of Tiger (*Panthera tigris*)

Sample name	S.No	Maximum mesiodistal width of root (mm)	Root height (mm)
Canine Root	1	32.23	69.41
Tiger Root	n = 4	26.03	62.85

The morphometric measurements of seizure 2 with four canines were compared with the known standards of Sloth

Bear (*Melursus ursinus*) due to the wide distribution of the species in the Indian subcontinent (Table. 2, 3 & 4).

Table 2: Crown height of four teeth compared with the reference data (Christiansen, 2007) [8].

S.NO	Crown Height (mm)
1	22.48
2	24.34
3	25.56
4	25.72
Sloth Bear	37.21 ± 3.34

Table 3: Anteroposterior diameter of four teeth compared with the reference data of Sloth Bear (Christiansen, 2007) [8].

Anteroposterior Diameter (mm)							
S. No	**Alveole**	**15%**	**30%**	**45%**	**60%**	**75%**	**90%**
1	16.44	12.65	10.5	8.55	6.78	5.54	3.88
2	18.17	16.55	14.5	12.56	10.64	8.57	5.95
3	20.64	16.08	14	11.22	9.1	7.24	4.91
4	19.34	16.46	14.46	12.22	10.27	8.45	7.05
Sloth Bear	20.4±2.24	20.06±2.31	16.90±2.14	13.2±1.73	10.47±1.25	8.09±1.02	5.66±0.88

Table 4: Lateromedial diameter of four teeth compared with the reference data for Sloth Bear (Christiansen, 2007) [8].

Lateromedial Diameter (mm)							
S.NO	**Alveole**	**15%**	**30%**	**45%**	**60%**	**75%**	**90%**
1	11.67	9.68	7.85	8.03	6.46	5.59	5.06
2	11.65	16.03	12.65	12.38	10.39	8.43	5.51
3	12.06	11.21	10.17	8.85	7.71	6.66	5.73
4	12.82	11.13	10.29	9.19	7.97	6.51	5.39
Sloth Bear	14.60±1.12	14.47±1.10	13.08±1.41	10.55±1.09	8.78±0.88	7.16±0.89	5.26±0.7

DNA analysis

The genomic DNA concentration of the isolated samples resulted in the range of (10 - 50) ng/µL. The mitochondrial genes of 16S rRNA and 12S rRNA from seizure 1 were successfully amplified and sequenced, as cytochrome b failed to amplify. Whereas, Cytochrome b and 12S rRNA were chosen for seizure 2 samples. The lengths yielded were 600bp in 16S rRNA, 240bp in 12S rRNA and 370bp in Cytb region respectively. The query sequences of seizure 1 and 2 were submitted to the NCBI database (Table 5).

Table 5: Query samples' submissions to NCBI database and accession numbers

Seizure No.	**Species Name**	**Accession number**	
		16S rRNA	12S rRNA
Seizure 1 - Canine	*Panthera tigris*	OL960591	OL989143
		Cytochrome b	12S rRNA
Seizure 2 - Canine 1	*Melursus ursinus*	OL978589	OL989146
Seizure 2 - Canine 2	*Melursus ursinus*	OL978590	OL989147
Seizure 2 - Canine 3	*Melursus ursinus*	OL978591	OL989148
Seizure 2 - Canine 4	*Melursus ursinus*	OL978592	OL989149

The query sequences of seizure 1 were 99.66% similar to the Tiger *(Panthera tigris)* using 16S ribosomal RNA sequence, whereas 100% similarity was obtained through 12S ribosomal RNA sequence (Fig. 6 & 7). Similarly, the query samples of seizure 2 were 99.07 % of Cytb region and 100% of 12S rRNA region to the Sloth bear *(Melursus ursinus)* (Fig. 8 & 9).

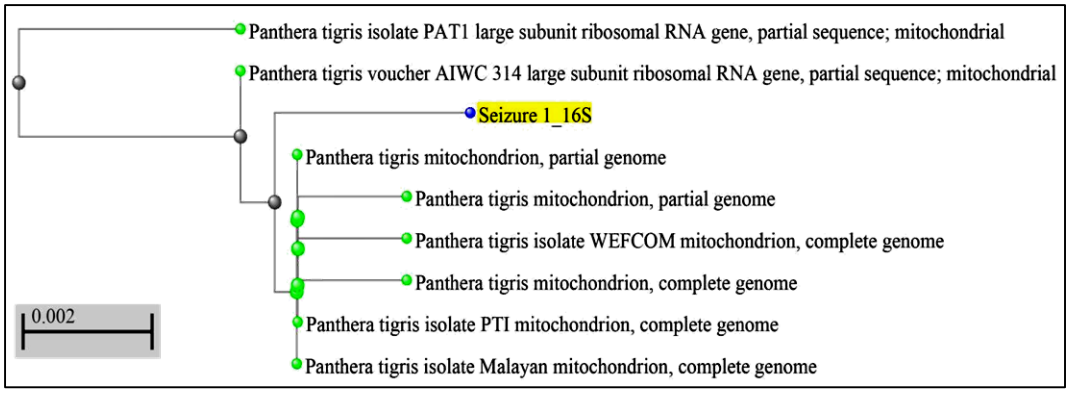

Fig 6: Seizure 1 BLAST tree view generated upon pairwise alignments using 16S rRNA on the NCBI database

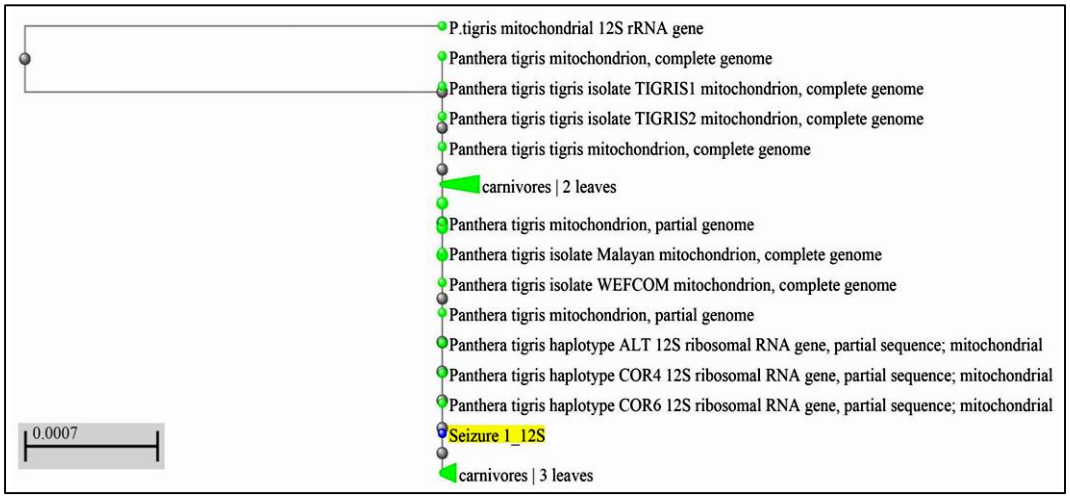

Fig 7: Seizure 1 BLAST tree view generated upon pairwise alignments using 12S rRNA on the NCBI database

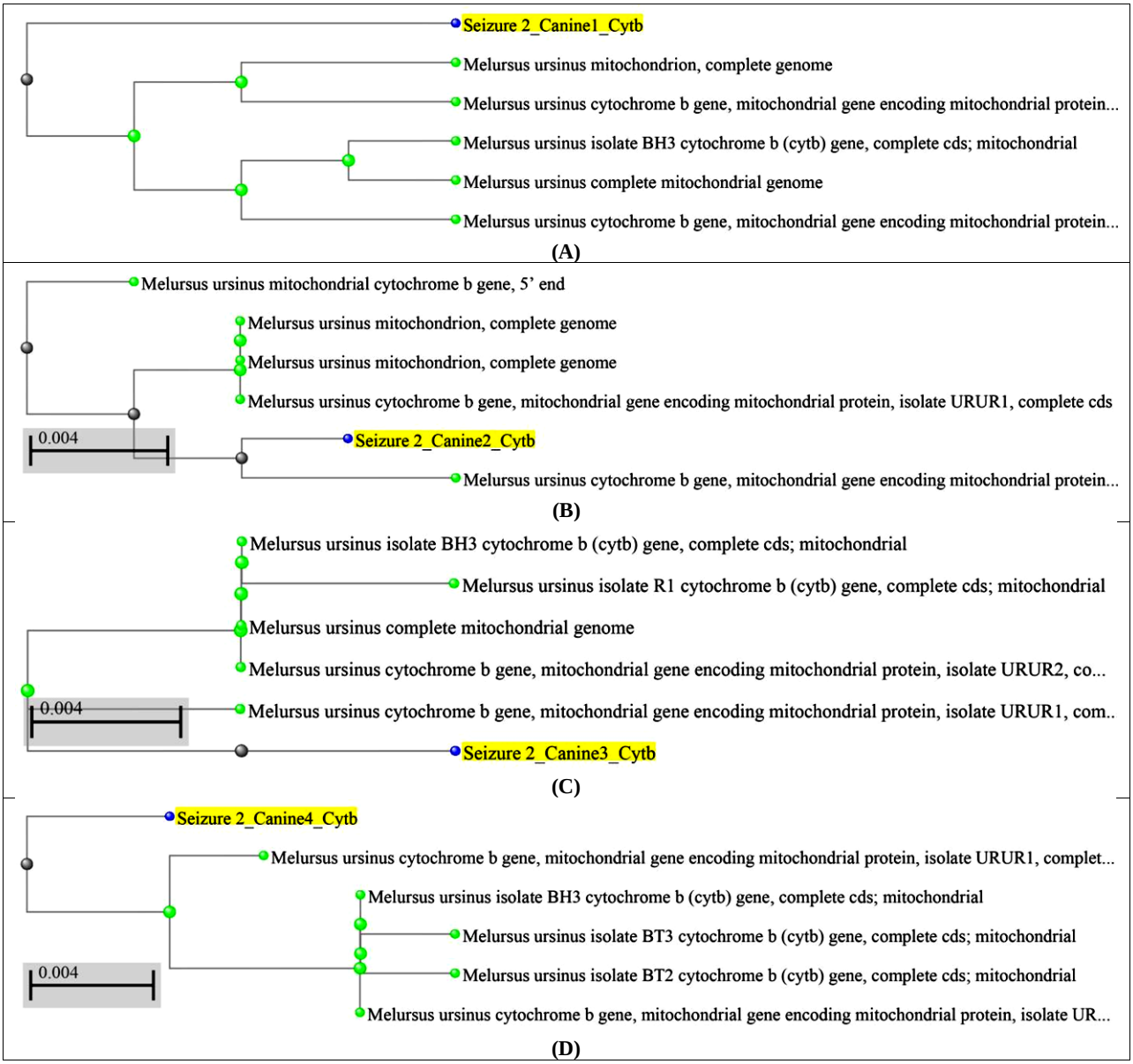

Fig 8: Seizure 2 BLAST tree view generated upon pairwise alignments using Cytochrome b of canine 1(A), 2(B), 3(C) and 4(D) on the NCBI database

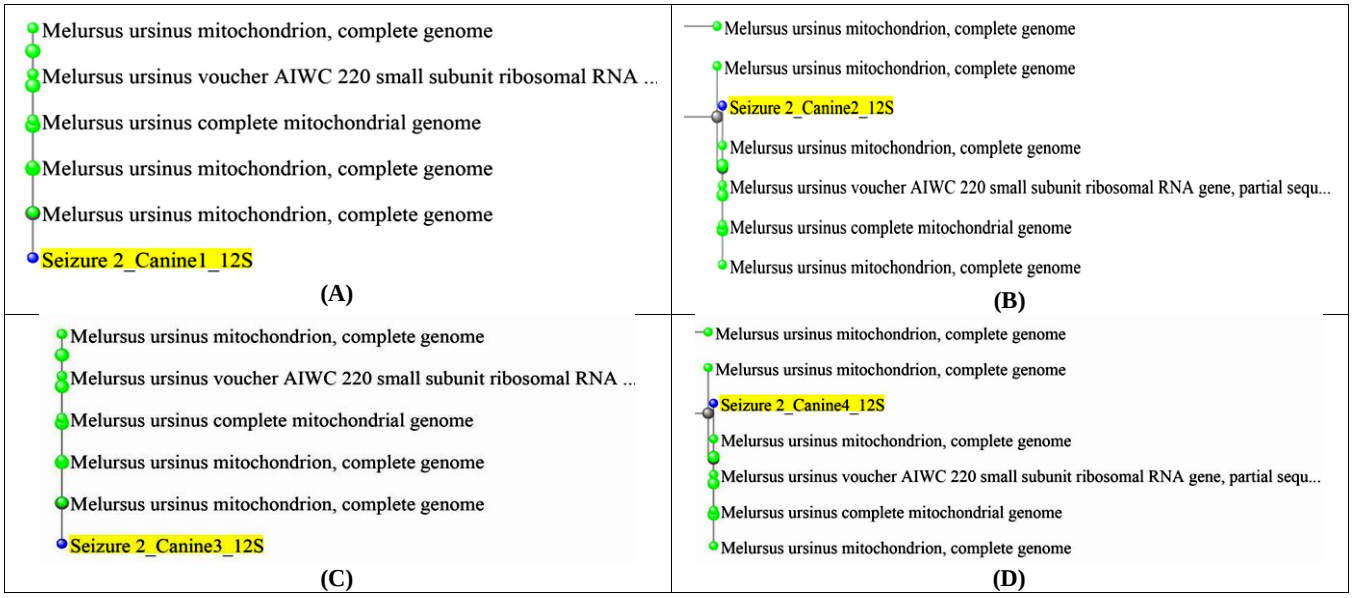

Fig.9. Seizure 1 BLAST tree view generated upon pairwise alignments using 12S rRNA of canine 1(A), 2(B), 3(C) and 4(D) on the NCBI database

To validate the BLAST search results, evolutionary divergence matrix and neighbour-joining (NJ) tree was constructed for each gene region (Tamura K et al. 2004) [17]. In evolutionary distance matrix construction, the number of base substitutions per site between sequences are displayed (Kumar S. 2018) [18]. Analyses were conducted using the Maximum Composite Likelihood model between related species. The distance computed between the 16S query sequences and the best hit species from the NCBI database ranged from 0.0000 to 0.0330. The least divergence from the query sequences was observed in *Panthera uncia* with (0.0241) and most divergence was found with the species *Panthera leo* (0.0330). Likewise, the distance computed between the 12S query sequences and the best hit species from the NCBI database ranged from 0.0000 to 0.0089. The divergence from the query sequences was observed equal (0.0089) in *Panthera leo* and *Panthera leo* species. (Table 6).

Table 6: Estimates of evolutionary divergence between related species of 16S rRNA and 12S rRNA sequences of seizure 1. The standard error estimations are shown above the diagonal within the table. The analysis was carried out using Kimura two-parameter model.

Species	16S rRNA			
	1	**2**	**3**	**4**
Seizure_1_Canine		0	0.0080	0.0068
KR132595.1_Panthera_tigris	0.0000		0.0080	0.0068
MW257216.1_Panthera_leo	0.0330	0.0330		0.0049
LC147065.2_Panthera_uncia	0.0241	0.0241	0.0137	
Species	12S rRNA			
	1	2	3	4
Seizure_1_Canine		0.0000	0.0064	0.0063
KR132595.1_Panthera_tigris	0.0000		0.0064	0.0063
MW257216.1_Panthera_leo	0.0089	0.0089		0.0061
KJ866876.1_Panthera_pardus	0.0089	0.0089	0.0089	

The distances between Cytochrome b sequences of query samples (seizure 2) and NCBI database species ranges from 0.0000 to 0.0549 (Table. 7). The least divergence was observed with *Ursus arctos* (0.0347) and highest divergence was observed with *Ursus arctos isabellinus* (0.0549). And, the distances between 12S rRNA sequences of query samples (seizure 2) and NCBI database species ranges from 0.0000 to 0.0271. The least divergence was observed with *Ursus arctos* (0.0139) and highest divergence was observed with *Ursus arctos* from Canada (0.0271).

Table 7: Estimates of evolutionary divergence between related species of Cytochrome b and 12S rRNA sequences of seizure 2. The standard error estimations are shown above the diagonal within the table. The analysis was carried out using Kimura two-parameter model.

Species	Cytochrome b						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Seizure_2_Canine_1_Cytb		0.0000	0.0000	0.0000	0.002	0.0232	0.0252
Seizure_2_Canine_2_Cytb	0.0000		0.0000	0.0000	0.0000	0.0192	0.0205
Seizure_2_Canine_3_Cytb	0.0000	0.0000		0.0000	0.002	0.028	0.0298
Seizure_2_Canine_4_Cytb	0.0000	0.0000	0.0000		0.0022	0.0283	0.0304
MG366863.1_Melursus_ursinus	0.0014	0.0000	0.0014	0.0015		0.0248	0.0269
EU567094.1_Ursus_arctos	0.0423	0.0347	0.0506	0.0513	0.0439		0.0081

MG066705.1_Ursus_arctos_isabellinus	0.0456	0.0364	0.0540	0.0549	0.0472	0.0095	
Species	**12S rRNA**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Seizure_2_Canine_1_12S		0.0000	0.0000	0.0000	0.0000	0.0109	0.0125
Seizure_2_Canine_2_12S	0.0000		0.0000	0.0000	0.0000	0.0148	0.0163
Seizure_2_Canine_3_12S	0.0000	0.0000		0.0000	0.0000	0.0148	0.0163
Seizure_2_Canine_4_12S	0.0000	0.0000	0.0000		0.0000	0.0148	0.0163
MZ427323.1_Melursus_ursinus	0.0000	0.0000	0.0000	0.0000		0.0114	0.0130
AP012579.1_Ursus_arctos	0.0139	0.0242	0.0242	0.0242	0.0159		0.0047
OK512954.1_Ursus_arctos_from_Canada	0.0168	0.0271	0.0271	0.0271	0.0187	0.0025	

The NJ tree analysis for seizure 1 was carried out based on 16S ribosomal RNA and 12S ribosomal RNA regions of mitochondrial genes which resulted in concrete discrimination from related species with distinctive branching of the tree. Similarly, the phylogenetic analysis of seizure 2 sequences was carried out based on Cytb and 12S ribosomal RNA regions of mitochondrial gene resulted in with accurate discrimination of closely related species.

The 16S query sample of seizure 1 clustered with the *Panthera tigris* with 100% bootstrap value, and other related species such as *Panthera leo*, *Panthera uncia*, were grouped separately. Likewise, the 12S query sample displayed clustering with *Panthera tigris* 100% bootstrap value with 81% bootstrap value with other closely related species (Fig. 10).

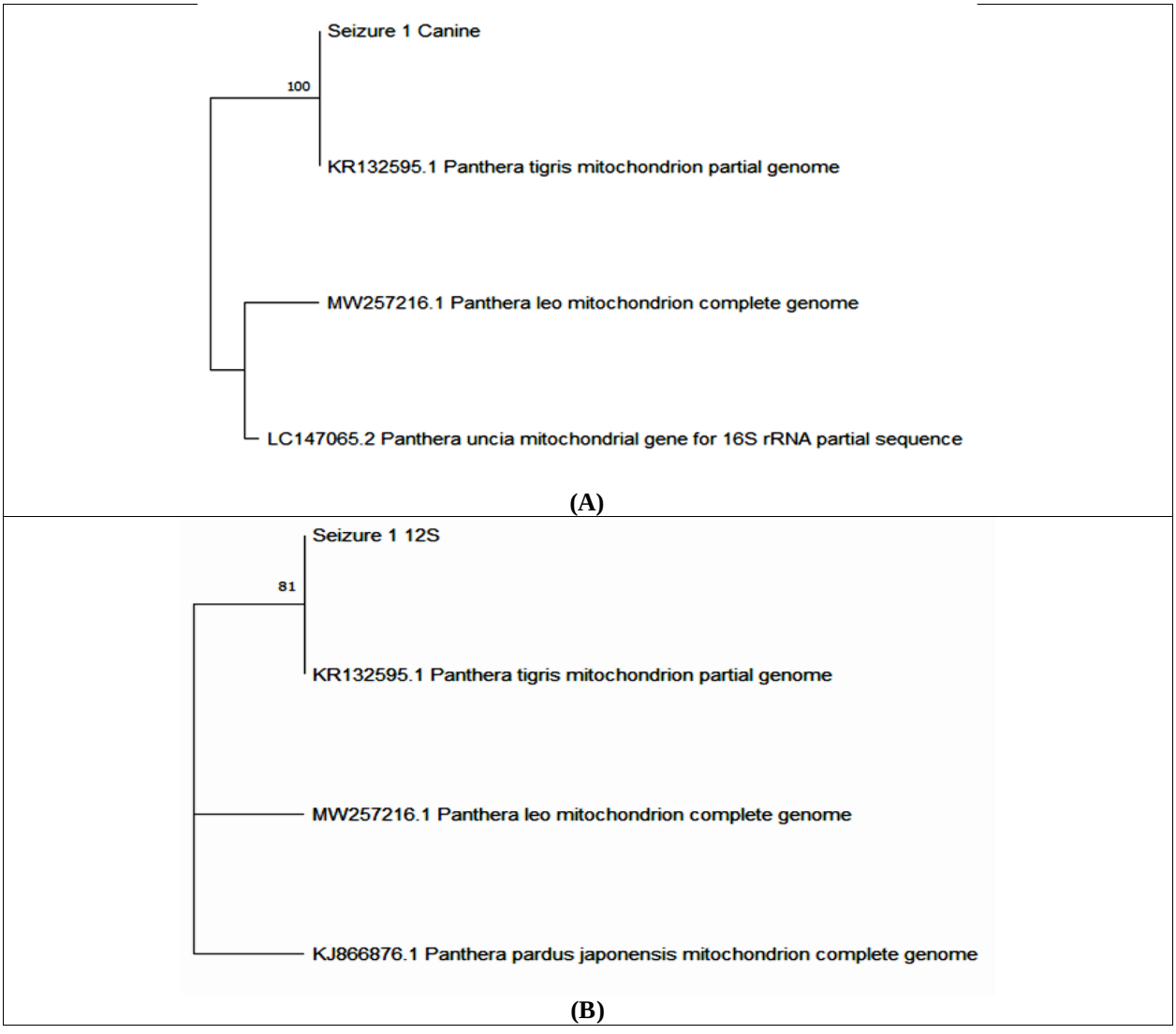

Fig 10: 16S rRNA (A) and 12S rRNA (B) gene regions-based tree topology displaying the query sequences as Seizure 1 with the most similar species obtained from the NCBI database through BLAST search.

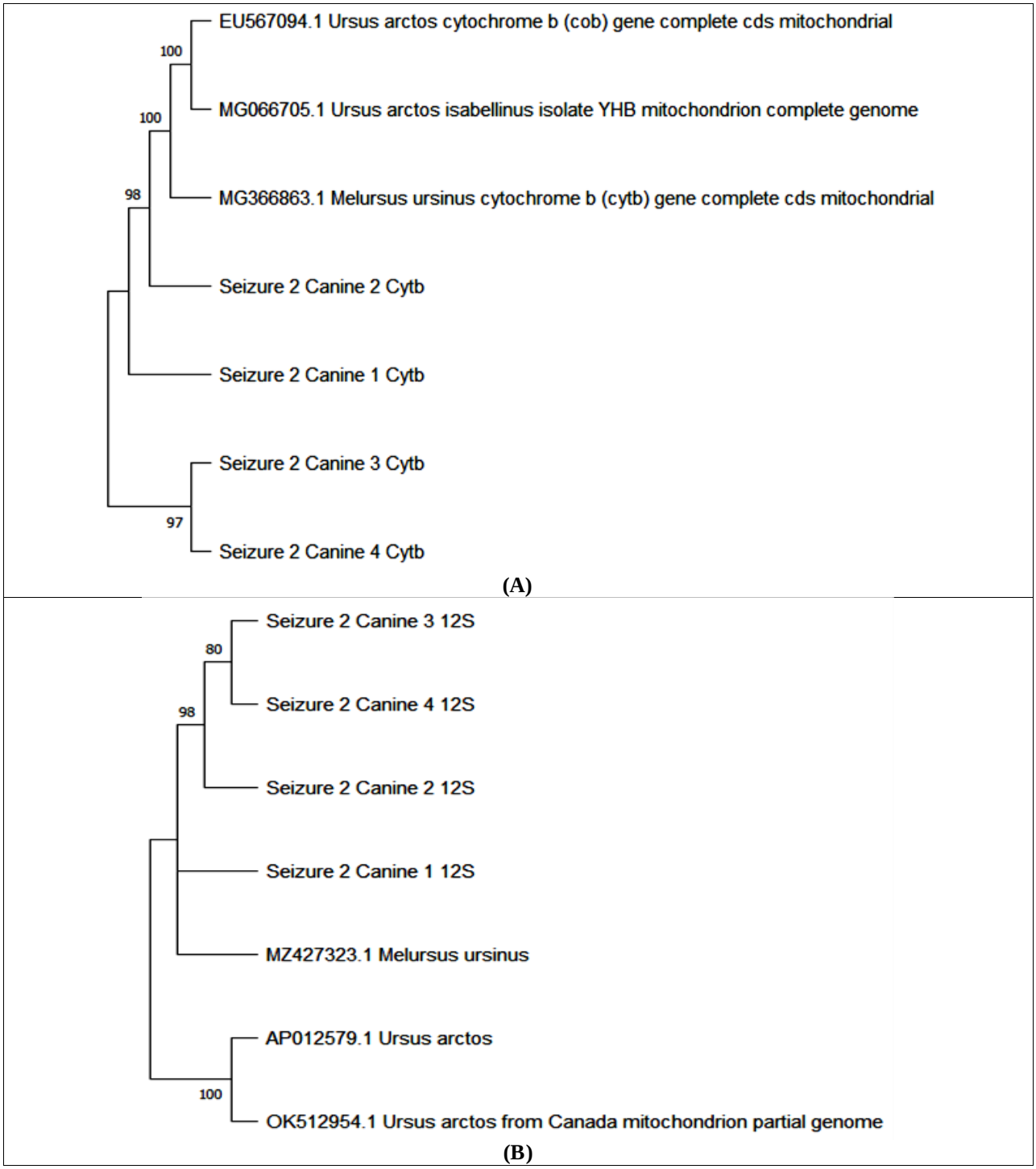

Fig 11: Cytb (A) and 12S rRNA (B) gene regions-based tree topology displaying the query sequences as Seizure 2 with the most similar species obtained from the NCBI database through BLAST search.

The NJ tree analysis for seizure 2 was carried out based on Cytochrome b and 12S ribosomal RNA regions of mitochondrial gene distinguished the related species with separate branching within the tree. The Cytb query sample of seizure 2 clustered with *Melursus ursinus* with 100% bootstrap value, and other related species such as *Ursus arctos*, *Ursus arctos isabellinus*, were clustered together. Likewise, the 12S query sample displayed clustering with *Panthera tigris* with 98% bootstrap value and other closely related species grouped together (Fig. 11).

Seven percent of the totally seized Tiger (*Panthera tigris*) seizures are teeth (World wildlife crime report 2020). Hence it has become important to identify the species from seized canines. The originality of the canines was checked from the presence of crack that commonly develops in the centre of dried canines (Yates 1996) [12] and x-ray images also showed the presence of pulp cavity which is a sign to confirm the seized teeth as original teeth and not made out of fake articles. From X-ray image, the canine without a crown was confirmed to be the original tooth. Morphometric measurements of seized canine root were compared with the available canine root of tiger (*Panthera tigris*) since the root was wide and large in size. The seized canine root was slightly wider than the referred data. This could be because of drying, cracking and expansion of the canine root. There was a lack of data on the metrics of canine root. Hence, we

were not able to confirm the species through morphological analysis.

Similarly, the canines from the second seizure were confirmed to be the original canine based on X-ray imaging. Bears have distinctive brown rings that encircle the teeth (Laurel, 2009) [13]. Therefore, with physical analysis we were able to confirm that the seized canine teeth belonged to Ursidae. Morphometric dimensions of the teeth which included crown height, anteroposterior diameter and lateromedial diameter were measured and compared with the existing data of Sloth Bear (Christiansen, 2008) [8]. The seized canines were smaller in size compared to the referred data. It could be due to multiple reasons like age, sex, worn out crown, etc. Identification of the teeth up to Family level was possible through morphological analysis in the second seizure and it was not possible to identify the species using morphological analysis.

The sample type such as tooth/ canine poses a difficulty in molecular examination as well; though it is well advanced, it clearly depends on the availability of number of cells in the sample, enabling successful DNA isolation with intact target regions. In this study of seizure 1 and 2 comprising 5 canine samples totally, the examination through both morphometry and molecular approaches are obligatory to explore and study the nuances present in each sample, with which wildlife forensics are also strengthened with enough data (Sharma et al., 2016) [20].

4. Conclusion

Identification of the species from tough samples such as tooth/canine often requires precision and accuracy in sample processing for investigation. Morphometric analysis necessitates close observation of details and molecular analysis is crucial to implement wildlife protection laws to improve conviction rate. This study describes the techniques used for identification of canine seizures using combined approaches of morphometry and DNA. Even though broad identification of canines through morphometric analysis is feasible, DNA-based analysis is required as a confirmatory method for identifying the species of wildlife articles.

Declaration of Competing Interest

The authors report no declarations of interest.

Funding

The work was funded by the allocation of core funds of Advanced Institute for Wildlife Conservation, State Forest Department of Tamil Nadu.

Ethics approval and consent to participate

Not applicable

Consent for Publication

Not applicable

Acknowledgements

The authors are grateful to the Forest officials of Tamil Nadu Forest Department, for their support.

References

1. Ramesh MK. The Wildlife Protection Act, 1972 of India: An Agenda for Reform. Asia Pac. J Envtl. L. 1999; 4:271.
2. Nijman V, Shepherd CR. Trade in tigers and other wild cats in Mong La and Tachilek, Myanmar-A tale of two border towns. Biological Conservation. 2015; 182:1-7, https://doi.org/10.1016/j.biocon.2014.10.031
3. Musing L. Falling through the system: The role of the European Union captive tiger population in the trade in tigers. A TRAFFIC and WWF report. Cambridge, UK, 2020.
4. Goodrich J, Lynam A, Miquelle D, Wibisono H, Kawanishi K, Pattanavibool A, *et al*. Panthera tigris. The IUCN Red List of Threatened Species. 2015:e. T15955A50659951, http://dx.doi.org/10.2305/IUCN.UK.2015- 2. RLTS.T15955A50659951.en
5. Nowell, K. (2000). Far from a cure: the tiger trade revisited.
6. Willcox D, Nguyen MDT, Gomez L. An assessment of trade in bear bile and gall bladder in Viet Nam. TRAFFIC. Petaling Jaya, Selangor, Malaysia, 2016.
7. Anderson PSL. Making a point: shared mechanics underlying the diversity of biological puncture. Journal of Experimental Biology 221.J Exp Biol. 2018; 221:22. https://doi.org/10.1242/jeb.187294
8. Christiansen P. Evolutionary implications of bite mechanics and feeding ecology in bears. Journal of Zoology. 2007; 272:423-443, https://doi.org/10.1111/j.1469-7998.2006.00286.x
9. L Smuts, JL Anderson, JC Austin. Age determination of African Lion (Panthera Leo). Journal of Zoology, London. 1978; 185:115-146, https://doi.org/10.1111/j.1469-7998.1978.tb03317.x
10. Van Valkenburgh B. Feeding behavior in free-ranging, large African carnivores. Journal of Mammalogy. 1996; 77(1):240-254, https://doi.org/10.2307/1382725
11. Breecher R, Breecher E. The rays: a history of radiology in the United States and Canada Baltimore: Williams and Wilkins, 1969.
12. Yates BC. Types of mammalian teeth. IDentification Note M-96-2. IDentification Notes and other Lab information, 1996. Available at: http:/www.lab.fws.gov/for-lab.htm
13. Laurel A Neme. Animal Investigators: How the World's First Wildlife Forensics Lab is solving crimes and saving Endangered Species. University Press of Florida, 2009, 166-169.
14. Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, *et al*. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. PNAS. 1989; 86(16):6196-200, https://doi.org/10.1073/pnas.86.16.6196
15. Guha S, Kashyap VK. Molecular identification of lizard by RAPD & FINS of mitochondrial 16S rRNA gene. Leg. Med. 2006; 8(1):5-10, https://doi.org/10.1016/j.legalmed.2005.07.01
16. Kitano T, Umetsu K, Tian W, Osawa M. Two universal primer sets for species identification among vertebrates. International journal of legal medicine. 2007; 121(5):423-427, https://doi.org/10.1007/s00414-006-0113-y
17. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA). 2004; 101:11030-11035, https://doi.org/10.1073/pnas.0404206101
18. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA

X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution. 2018; 35:1547-1549, Doi: 10.1093/molbev/msy096
19. UNODC. World Wildlife Crime Report. Trafficking in Protected Species. United Nations Publications, 2020, 79-81
20. Sharma V, Sharma CP, Kumar VP, Goyal SP. Pioneer identification of fake tiger claws using morphometric and DNA-based analysis in wildlife forensics in India. Forensic science international. 2016; 266:226-233. https://doi.org/10.1016/j.forsciint.2016.05.024