CHARACTERIZATION OF WILDLIFE ARTICLES SEIZED AND RECENT TRENDS IN ILLEGAL WILDLIFE TRADE IN INDIA: IMPLICATION FOR WILDLIFE FORENSICS

Thesis submitted for the award of the degree of

Doctor of Philosophy in

WILDLIFE SCIENCE

by

CHANDRA PRAKASH SHARMA

to

SAURASHTRA UNIVERSITY

Rajkot-360005 (Gujarat)

Under the supervision of

Dr. Gopal Singh Rawat

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

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DECLARATION

I hereby declare that the work conducted under this thesis titled "**Characterization of wildlife articles seized and recent trends in illegal wildlife trade in India: Implication for wildlife forensics**" is a record of original and independent research work done by me and subsequently submitted for the award of the degree of **Doctor of Philosophy in Wildlife Science** to the **Saurashtra University, Rajkot (Gujarat).** This research work has been carried out under the guidance and supervision of Dr. Gopal Singh Rawat, Director (Retd.) of Wildlife Institute of India, Dehradun. The work has not formed the basis for the award of any other degree, diploma or any other qualification. I also declare that the thesis embodies my own work, analysis, observation, understanding and the particulars given in it are true to the best of my knowledge.

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CERTIFICATE

This is to certify that the thesis by **Sh. Chandra Prakash Sharma** titled "**Characterization of wildlife articles seized and recent trends in illegal wildlife trade in India: Implication for wildlife forensics**" is an original and independent research work submitted to the **Saurashtra University, Rajkot (Gujarat)**, for the award of the degree of **Doctor of Philosophy in Wildlife Science.**

Mr. Chandra Prakash Sharma has put more than six semesters of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

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I certify that the research work was appreciated by all who were present and the comments made by the faculty and researchers have been appropriately included in this thesis.

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This Ph. D thesis is a reflection of my long association as a practitioner of wildlife forensics with an aim to be voice of voiceless wild animals, which could not be possible without continuous support and encouragement from all the noble people around me, and thus provides me an opportunity to acknowledge the several debts I have accumulated over the years in my professional and personnel journey.

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Chandra Prakash Sharma

Contents

List of publications

Sharma, C. P., Sharma, S., Sharma, V., and Singh, R. (2019). Rapid and non-destructive identification of claws using ATR-FTIR spectroscopy–A novel approach in wildlife forensics. Science & Justice. doi:10.1016/j.scijus.2019.08.002.

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Sharma, C. P., Rawat, G.S. (2021) "Microscopic analysis of opportunistic collected hair from inside the completely shaved or singed musk deer pods- implications in wildlife forensics" ((Virtual), International E-Conference on Forensic Biology, Government Institute of Forensic Science, Nagpur, Maharashtra, 28-29 January 2021

Sharma, C. P., Rawat, G.S. (2022) "Comparative morphological characterization of mandible fragments of three sympatric carnivores, the tiger, leopard and hyaena, seized in illegal wildlife offense: implications for wildlife forensics" (Virtual), 1st International conference on Forensic Science, Department of Forensic Science, JAIN (Deemed to be University}, Bengaluru, 10th -12th February, 2022

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Executive Summary

Illegal trade in wildlife parts and products has emerged as an organized trans-national crime, threatening the existence of many wild species across the globe. Man is dependent on natural resources including various forms of wildlife for subsistence since its arrival on this planet. Recently, thousands of wildlife including plant, animal and other organisms are subjected to unlawful exploitation for subsistence or illegal wildlife trade for commercial gains. While typical uses of wildlife products were limited to subsistence, sorcery and traditional medicine, more recently, animals or their derivatives have found demand for brazen displays, such as shahtoosh shawls, Ivory products, tiger bone, rhino horn, and mammal/reptilian pelt products (Graham-Rowe 2011; Pragatheesh et al. 2022). Unrestrained and unsustainable exploitation of fauna and flora for illegal commercial gain has resulted in environmental crisis resulting in hasty loss of biodiversity and ecosystem services (Clifton & Rastogi, 2016), and are major contributing factors for pushing few species to the extermination, especially the charismatic species and megafauna (Miller 2005; Challender et al. 2015). The lesser known species i.e. Varanas spp., Golden Jackal etc., (Sharma et al. 2019, 2022) and other groups are also in heavy demand and are threatened from unabated illegal wildlife trade. United Nations office on Drug and Crime (UNODC) has listed offenses against wild animals as one of the major Green crimes (UNODC 2020).

In any wildlife forensic investigation, analysis of biological evidences has perpetual significance in getting conviction of accused or proving innocence. Appropriate analysis of such evidence, not only help in identification and proving possession of a contraband by the accused but also help in establishing link between the suspect, the wildlife, and the crime scene in poaching scenarios. Forensic

identification of species from parts and products recovered in various seizures of wildlife articles by various enforcement agencies is the core for implementation of Wild Life (Protection) Act, 1972. The illegal wildlife trade is very dynamic, and new challenges in identification of wildlife articles or its derivatives crop up sooner, placing scientific characterization protocols of paramount importance. Trade and seizure of fake or imitation wildlife articles, wrongly identified by enforcement agencies, have opened up new frontiers for law enforcement agencies and practicing forensic experts to identify whether the submitted evidence is genuine or made of some other animal part or a synthetic article (Rajpoot et al. 2018; Sharma et al. 2022b). Therefore, development of fast and efficient scientific tools for characterization of wildlife parts and products for better detection, seizure and identification in wildlife crime investigation is of paramount importance.

Since, the data on wildlife crimes is scarcely available and is scattered unevenly with different enforcement agencies, data majorly available with Wildlife Forensic and Conservation Genetics cell (WFCGC) of Wildlife Institute of India (WII) in combination with data scooped from open sources was used to understand the emerging trends in crime against wildlife. Record of 6809 entries for wildlife offenses from 2011 to 2020 was reviewed in the current study and after thorough scrutiny and authentication by data cleaning and removal of entries having any ambiguity, duplicity or missing fields, 5817 wildlife offence empirical entries were taken up for the study in detail. This study provides growing understanding of wildlife crime related research that has identified and explained major trends and patterns of crime against wildlife in India during the last decade.

While dealing with the results we have considered the fact that the reported offense data is skewed towards few states as there is very less offenses/reporting from few others. The study shows that during the reported period, eight states in alphabetical order, viz. Chhattisgarh, Gujarat, Madhya Pradesh, Odisha, Punjab, Tamil Nadu, Uttarakhand and Uttar Pradesh constitute more than 60% reported wildlife offences.

A peek through the long term data of submitted wildlife offenses indicate no major shift in number of seizures per year among the states. There are species which are more in demand for minting quick money while others are preferred for subsistence only or for social or cultural rituals. Similarly, there are identified hot spots for poaching while others serve as collection or trading centers. Mammals were the most prevalent group in all reported wildlife offenses with 76.6% share followed by reptiles (10%) and birds (8.6%). Wild pig and leopard are the top two represented animals in the wildlife crime database.

Large quantity of seizures of marine animals especially sea horses and sea cucumbers needs special attention from marine enforcement agencies to avoid any threat to large scale extermination of these species. Specific pattern and equipment's used to poach a wild animal was observed, as indicated by use of firearms, mostly country made for large herbivores (Elephant, Rhino, Nilgai) and water birds, snare/jaw traps for big and lesser cats, wild pig, pheasants/birds, hunting dogs for cervids, pangolins, monitor lizards, potash bomb for wild pig and other herbivores, plant poison (Aconitum spp.) is found in elephant poaching case, and tractor tubes with net were used for illegal fishing in protected areas. This study not only has highlighted varied numbers of charismatic species involved but also targeted demand for lesser

known species like the golden jackals is brought to fore which is heavily poached all over India for "Jackal's Horn"

Although, around 50% of wildlife offenses have one suspect, but those cases are overwhelmingly trade related where carrier was apprehended. For committing poaching, it is almost always more than one person is involved. Due to magnitude of seizure data, all India analysis shows the trends and pattern are less to cater the international illicit trade and more likely a reflection of socio-cultural demands for certain products and opportunistic crimes including illicit trafficking of wildlife parts.

Morphological techniques in combination with statistical and other tools employed in this research are hugely expedient in wildlife forensics. In the present research work for developing forensic morphological protocols, wildlife articles available in the repository of Wildlife Institute of India were subjected to a variety of macroscopic, microscopic comparison techniques and morphometric measurements were taken and results were obtained employing statistical analysis to develop identification protocols to the family, genus, or species level. Selection of species and targeted parts studied for this study was done based on the review of available data at Wildlife Forensic & Conservation Genetics cell (WFCGC) of WII and published information accessed through Google Scholar search engine using key words relevant to illegal wildlife trade and wildlife crime in India. It also aimed at filling up the gaps in WII's ongoing research and development program (1995 to present) for development of protocols for identification of wildlife parts and derivatives encountered in wildlife offenses. The availability of sufficient samples for a particular species is also one of the key factor for undertaking this study. Identification protocols for broken mandibles, C1 bone, canines of four sympatric species, i.e. tiger, leopard, hyaena and sloth bear

along with opportunistic identification of musk deer pod by microscopic hair analysis and "jackals horn" were developed during this study.

Under this study the applicability of attenuated total Reflectance-Fourier transforms infrared (ATR FT-IR) spectroscopy for development of forensic protocols for uses in wildlife forensics was undertaken. The analysis of wildlife evidences has had an overwhelming impact on the species/individual identification by using DNA profiling. However, a limitation of DNA typing is that it cannot provide success when the sample size is limited and DNA extracted is not sufficient for analysis as in highly processed/finished wildlife articles (Gupta et al. 2022). Also, in wildlife poaching cases, the crime scene usually gets compromised before picking up of crucial evidences, due to many obvious reasons including, the crime is detected late as it usually happens in remote/secluded areas, movement of scavengers and other animals etc. and therefore contaminations are quite recurrent. Identification of species based on non- destructive FTIR, technique is vehemently used in characterization of biological evidences in human forensics (Sharma & Kumar 2018; Sharma et al. 2021) and recently claws and hair of wildlife were successfully characterized based on this technique (Sharma et al. 2019b, Sharma et al. 2022a).

Work was undertaken to characterize and differentiate the selected wildlife articles commonly found in wildlife offense seizures, sent for species identification to WII using ATR FT-IR spectroscopy. Sufficient known biological samples of each of the selected wildlife article (blood, Musk pod grains, claws, shahtoosh) were obtained from repository of WII. ATR FT-IR spectroscopy showed its usefulness in the field of wildlife investigation for the characterization of musk sample, both complete and in powder form. The method is rapid, does not need time-consuming sample preparation,

sensitive, reliable, and environment friendly. It gives rapid information about the characterization of musk sample by providing information about proteins as well as minor constituents. ATR FT-IR spectroscopy is able to differentiate fake, leopard and tiger claws sample from each other by unique, dominant peaks being present or absent in the spectra. For differentiation of shahtoosh and pashmina wool hair, through only visual inspection it is quite difficult to differentiate the spectra of shahtoosh and pashmina wool, as visual discrimination is not legible and subjective in nature, therefore further advanced chemometric tools were employed to extract the information-rich signals to get the results in an objective manner without biasness. ATR FT-IR spectroscopy together with advanced statistical tools shows excellent potential for the rapid and non-destructive discrimination of blood collected from Tiger, Elephant, Leopard, and Domestic pig. By using this method, obtained spectra from selected species were substantially discriminated with 100% accuracy without any overlapping

The study of the judgements from trial courts of four diverse states for which sufficient data was available helps in better understanding of the reasoning behind the judgements and provide remedial actions, if any. The purpose was to find out the trial procedures followed in trial courts cases under study and reasons behind the judgment, either of conviction, or, of acquittal. This exercise will provide a holistic idea of the overall pattern of the crime, conviction rate, challenges and basis of rulings of the trial courts. For undertaking this study records covering a time-span of one decade, from 2011 to 2020 were studied to understand comprehensively about the procedures adopted, lacunas, if any and basis of rulings of the trial courts. It has provided an understanding about the pattern of crime and the level of veracity in implementation of the provisions of the Wild Life (Protection) Act, 1972.

The pattern of wildlife offenses has shades of poaching for subsistence, conflict of interest or for making quick money by dealing in prohibited articles in illegal wildlife trade in all the four states taken up for detailed study. As expected, Delhi emerged, as a major collection Centre for illegal cash rich wildlife articles like shahtoosh, elephant ivory, large cat's skins and wildlife souvenir i.e. marine shells and sea fans. Madhya Pradesh reported more of conflict offenses like illegal entry, destruction of habitat, illegal cattle grazing, cutting of trees, ignition of fire and collection of river bed material (RBM) from protected areas than the poaching or illegal trade of wildlife. In Rajasthan, more judgements are for illegal entry, destruction of habitat by collection of RBM and for dealing in illegal wildlife parts. The pattern of wildlife crime in Uttarakhand usually has poaching and illegal trade activity, but the pattern is more inclined towards the cases involving illegal trade of targeted large cat parts and turtles.

The data on arrested suspected persons in wildlife offense cases across all four states indicate crime against wildlife is a gang like activity and that more than one person is involved to poach an animal and extract the valuable part of the animal. From the study of availble case documents it is found out that a single person arrest while committing a wildlife crime, indicate usually a poacher for lesser animal or bird, a carrier or a trader. A combined study of four states, indicates that in 51% incidents, there is only one suspect. Delhi with 78% cases and Uttarakhand with around 60% case having single accused while Madhya Pradesh and Rajasthan reported single person's arrest in 49.8% and 45.5% cases, respectively. Arrest of one accused in cases from Delhi and Uttarakhand can be explained as in all these cases the offense booked was under section 39 (Govt. Property) or 49B (illegal trade) of WPA. Rajasthan and MP also shows a bit higher arrest of single accused per case which is due to the cases booked for illegal entry (Section 27 of WPA), destruction of habitat (Under section 29

of WPA) for grazing of cattle or extraction of RBM using tractor and the driver is usually booked for such cases.

The conviction rate is much above (>84%) for Uttarakhand, which is quite good in comparison to other studies states of Delhi, Madhya Pradesh and Rajasthan. One of the main reason came into light, is that in Uttarakhand almost all the wildlife offense cases were referred to scientific Institutions for species identification of biological evidences recovered from accused.

The present study is the first detailed study encompassing the whole gamut of enforcement of wildlife protection laws in the country involving understanding the dynamics of the wildlife crime, issues related to enforcement of protection laws and legal procedures and challenges before the judiciary. While discussing the dynamics of wildlife offenses, pros and cons in enforcement of wildlife protection laws based on empirical data, this study is in no way an expression of the total wildlife crime rate of any state or region as the data is only based on the reported incidents and does not account for actual crime against the wildlife. Working in the field of wildlife forensics and interacting with enforcement agencies on dynamics of illegal trade in wildlife since more than two and half decades, the work presented here is not just a scientific offering but an outpouring of a practitioner.

Chapter 1:

GENERAL INTRODUCTION

1.1 Background

Use of natural resources including various forms of wildlife for subsistence is inherent to human evolution since its arrival on this planet. Blitzkrieg effect, caused by tremendous increase in predation efficiency for subsistence was attributed as one of the reason for waves of decimation of mega faunal species of the Pleistocene era by human settlers in fresh land masses (Wroe *et al*. 2004). Global wildlife trade generates approximately $23 billion a year and has links with other organised crimes like human and drug trafficking, and illegal arms trade making it as a "global threat" (FATF 2020*)*. While typical uses of wildlife products were limited to subsistence, sorcery and traditional medicine, more recently, animals or their derivatives have found demand for brazen displays, such as *shahtoosh* shawls, Ivory products, tiger bone, rhino horn, and mammal/reptilian pelt products (Graham-Rowe 2011; Pragatheesh *et al*. 2022). Unrestrained and unsustainable exploitation of fauna and flora for illegal commercial gain has resulted in environmental crisis resulting in hasty loss of biodiversity and ecosystem services (Clifton & Rastogi, 2016), and are major contributing factors for pushing few species to the extermination, especially the charismatic species and megafauna (Miller 2005; Challender *et al*. 2015). Not only the more charismatic mammalian species like Tiger, Rhino and Elephants face severe threat from targeted poaching for bone, horn and ivory but the unabated illegal wildlife trade is also threatening the lesser known species in other groups.

A wildlife offence refers specifically to crime against wild flora and fauna and involves the illicit taking, transport, trade, or possession of animal or their derivatives thereof in contravention of existing domestic and international laws and treaties (Sosnowski *et al*. 2022). Illegal trade in wildlife and their parts is also reported to be

responsible for the spreading of invasive species along with some veterinary and zoonotic diseases (Gomez & Aguirre 2008; Palvin *et al*. 2009).

Poaching and illegal trade in wild animals and their parts or derivatives form is a lucrative trade of enormous size, generating huge monetary gain annually (Nellemann *et al*. 2014; APG & UNODC 2017). Wildlife seizures range in size and numbers, from a single small animal part i.e. hemi-penis of *Varanus spp*., a *shahtoosh* shawl to multi-ton packages with thousands of individual animals/parts, i.e. sea horses and sea cucumbers, live turtles, birds etc. (Wyler & Sheikh 2013).

Several factors contribute to the recent increase in illegal wildlife trade including easy access to wildlife markets, better connectivity to wildlife habitats, rising affluence, unemployment, poor law enforcement, lack of awareness of regulations and conservation concerns (Vira *et al*. 2014; World Bank 2018; Singh et al. 2019; Haines *et al.* 2021; Sosnowski et al. 2022). Easy access to internet at affordable price and easy transfer of faceless digital money in far flung but biodiversity rich regions, often aggravate crime against wildlife by providing a key platform for convenient communication between the poachers and traders (Basnet 2003; Lavorgna 2014; Baker *et al*. 2013; Sharma *et al*. 2019a; Pragatheesh *et al.* 2022). More recently, use of social media in closely knitted groups has emerged as a tool of choice for advertising, sourcing, and the online illegal trade of wildlife (Hinsley *et al.* 2015; IFAW 2014). The e-commerce websites and social media platforms has made online shopping of illegal traded wildlife increasingly popular, creating new demands in both charismatics to less alluring species of wild and endangered fauna and flora, along with fake or imitating wildlife articles (Sharma *et al* 2019a; Pragatheesh *et al.* 2021). This requires an urgent challenge for enforcement agencies to prepare and synchronize themselves in cyber space through OSINT (open source intelligence) in combating illegal wildlife trade of

wildlife and wildlife products on priority basis. (Xiao & Wang, 2015; Xiao *et al*. 2017; TRAFFIC 2019; Pragatheesh *et al*. 2022).

The extensive use of traditional medicines in developing countries, and the rapidly growing acceptance of alternative therapies in developed countries, have resulted in the field of zoo therapy, which is the use of medicines obtained from animals or ultimately derived from them (Labadie 1986; Friant *et al.* 2022). Body parts of wild animals both raw and processed (e.g., gall bladders, fat, antlers, paws, skins, bones, feathers, horns/hooves, tusks) form important ingredients in the preparation of curative, protective and preventive medicine (Adeola 1992; Anageletti *et al*. 1992; Griffiths *et al.* 2010; Yujin & Yongde 2021) in many traditional pharmacopeias. The World Health Organization estimated that 80% of the world's developing countries rely on animal and plant-based medicines as only choice for easy access and affordable treatment, but recently trends indicate that traditional medicines are rapidly gaining acceptance in developed countries as well where allopathic medicines are readily available and can be afforded. Apart from use of wildlife in traditional pharmacopeia and for subsistence, hostility towards wildlife in man-animal negative interaction is another factor responsible for wildlife crimes.

Among all the factors discussed, the illegal wildlife trade in one of the major factor threatening the survival of many species. It is estimated that there in an exponential margin of monetary profit on wildlife products as we go up the supply chain involving poacher, carriers and ultimately to major traders at various stages, thus making these offenses very lucrative to everybody all along the supply chain (Damania *et al*. 2003; WWF 2012; Ayling, 2013). The prospect of considerably high profitable, low investment and relatively low risk of detection as compared to other illicit trades makes this trade much attractive (Brisman & South 2018).

Apart from conservation related issues, wildlife crime has significant impacts beyond the environmental or ecological realm of conservation, as the proceeds earned from illicit trafficking are even linked to funding source for many terror networks (Warchol 2004; South & Wyatt 2011; Ayling 2013: Wyler & Sheikh, 2013; Douglas & Alie, 2014; Nellemann *et al*. 2014; Maguire & Haenlein 2015). Studies have shown that non-state armed groups in Africa and Asia, which happen to be located in biologically diverse areas, are financing terrorist and politically destabilizing activities using natural resources (Margot 2013; Sharon *et al.* 2008; Kasper & Jonathan 2013). It has been assumed that trans-national criminal networks, already involved in the other illicit transnational trades, through their established contacts and routes exploit 'opportunistic trafficking' of wildlife, whenever they get a chance (Elliot 2012, 2016; Haas & Ferreira, 2015). Thus, negative impact of illegal wildlife trade extends beyond biodiversity loss, as criminals involved in illegal wildlife trade may destabilize governments and economies (Felbab-Brown 2017) and damage livelihoods and security for those living in wildlife rich areas.

1.2 Need and emergence of Wildlife Forensic Science

Crime related to wildlife is very dynamic in nature. It is influenced by geographical and global effects, maneuvering clandestinely with changing demands, place, innovating *modus operandii* for, species specific poaching, smuggling and finding newer markets, purely for the making quick money. The illegal wildlife trade involves both national and trans-national criminal syndicates. The ease of communication and movement of goods and money in the era of free trade, also facilitate the illegal activities involving protected wildlife (UNODC 2020). Another enticing factor for the national and trans-national crime syndicates is the perception of low risk and high profit in illegal wildlife trade (Nellemann *et al.* 2014). To overcome

above challenges the successful enforcement of wildlife protection law goes beyond the seizure of illegal contraband and arrest of offenders. The law and its application in totality plays a crucial role, with severe penalties, which may serve as a strong deterrent. Along with an effective enforcement and robust judicial system, scientific intervention is a must to deal with wildlife offenses considering the scope, and dynamics and transnational nature of illegal wildlife trade.

Due to inherent reasons, different regions of the world respond differently in implementation of wildlife protection laws. Whereas countries in Africa, Asia and South America are more vigorously enforcing wildlife conservation laws, the countries in the global North are lacking any such urgency (Sollund & Runhovde 2020). Under "Convention on International Trade of Endangered Species of wild fauna and flora" (CITES), it is the responsibility of host country to ensure that the import and export of protected species comply with the provisions of the convention. Unfortunately, the detection of wildlife is not a priority with many countries and most of the illegal wildlife seizures reported were accidental. But, the common thing in both the areas is that, even if offenses are booked for wildlife trafficking, they usually lead to meagre penalties with fines or with suspended imprisonment (Sollund & Runhovde 2020). Even the large seizures involving illegal live animal trade, animal trophies and ivory products may incur considerable low penalties, due to indifferent attitude of enforcement agencies towards environmental crimes or lack of forensic tools to detect and identify wildlife derivatives that can withstand the scrutiny of legal system.

India, one of the mega biodiversity countries, not only harbors a rich floral and faunal diversity but also is home to a number of highly threatened, rare and endangered species (Pironon *et al.* 2020). The challenges for our country can be gauged from the confessions of transnational illegal traders that most of the tiger and leopard skins

available for sale in China as home decor, clothing, and prestigious gifts or non-financial bribes have been sourced from India and Nepal (EIA 2016, 2018). India, acts as a source (Bear bile, musk pod, felids bones), transit (African Rhino horn) and consumer country (*shahtoosh*) with the illegal wildlife trade syndicates, forming close knit web of networks around the world to fulfill the demands for traditional medicine, fashion and pet animal trade. Not only the easy access to wildlife, porous border and difficult terrain towards the major transit and consumer countries of East Asia makes wildlife law enforcement difficult, lack of scientific identification protocols of plethora of involved species and their derivatives makes detection and conviction even more challenging. Successful implementation of provisions of Wild Life (Protection) Act, 1972 and other regulations in India in wildlife offenses is somehow erratic, with few states doing sufficiently good employing scientific approach during investigation and getting backing of wildlife forensic facilities available in the country while others are performing poorly, more dependent on the confessions/statements of accused and witnesses which fail miserably in the court of law without any scientific tools of investigation (internal communication).

Under the Wild Life (Protection) Act, 1972, it is mandatory to identify the species from the wildlife article as different species are kept in various schedules depending on the level of protection they enjoy and thus the amount of penalties they incur for any crime against them. Therefore, having well established and specialized wildlife forensic facilities will result in shot in the arm for enforcement agencies by providing scientific reports with expert witness testimony that corroborates investigators story "beyond doubt" in the court of law, allowing for successful prosecution.

Failure in detection and correct scientific identification of illegally harvested wildlife parts and products can be a big challenge for the law enforcement agencies and the events of prosecution often led to miserably low conviction in the trial courts. This requires a systematic and comprehensive approach with robust database and scientific protocols to maximize scientific evidences for getting successful prosecution. In order to tackle the tremendous challenges posed by complex wildlife offenses, well established wildlife forensic facility and knowledge base is a must. Indirect measures of illegal wildlife trade (IWT), such as seizure data, provide some indication of trade hot spots and its quantity (Rosen & Smith, 2010), but they contain detection and reporting biases (Underwood *et al.* 2013), and largely gave status of relative trends happening in traded species, leaving aside other wildlife offenses like for subsistence and other cultural and ritual demands. Seizure data of IWT tends to be biased towards charismatic species (e.g., Tiger parts, ivory) and may constitute less than 10% of all illegal trade (van Uhm, 2018). While trends in the transnational and *legal* wildlife trade are relatively well documented (Harfoot *et al*., 2018), little has been done to analyze holistically the scope of wildlife crime in relation to IWT in India (TRAFFIC 2008, 2019; Pragatheesh *et al.* 2021).Therefore, information linked to underlying factors and trends shaping wildlife offenses for commercial gain, subsistence, sports and other factors are very important, although difficult to obtain since are lying scattered, in developing a Pragmatic approach towards identifying and mitigating the factors responsible for wildlife crime in India.

Thus it is amply evident from above discussion that knowledge about illegally traded wildlife species and derivatives is lacking and their detection and identification is a challenge for enforcement agencies to establish an offender’s guilt 'beyond a reasonable doubt' and constitute main reasons for miserably low conviction in the court

of law. Therefore, wildlife forensics' needs to be developed based on a multi-pronged, systematic and comprehensive approach with empirical evidences and scientific protocols to maximize evidentiary outputs for framing up an appropriate policy, field responses and getting prosecutions to tackle the complex wildlife offenses

Wildlife Forensic & Conservation Genetics Cell (WFGCG) at Wildlife Institute of India (WII) was established with aim to develop Wildlife Forensics capacity in India in mid-nineties under collaboration with United States Fish and Wildlife Service (USFWS) for sharing scientific acumen available in the field of wildlife forensics and strengthening infrastructure for efficiently developing facility for CITES scientific authority in India. This facility has received around 5000 wildlife offense/opinion cases and is providing scientific/ forensic analysis report to all enforcement agencies in India tasked for conservation of wildlife, implementation of regulations prohibiting wildlife offenses and maritime security. Apart from it, this facility is also being utilized by forensic labs of neighboring countries, i.e. Nepal, Bangladesh and Sri Lanka and other countries (Singapore and Switzerland) for forensic analysis or second opinion in wildlife offense related cases. The wildlife offense data and reference specimens from repository of WFCGC was freely used for under taking study of illegal WL trade dynamics and morphological analysis.

For undertaking development of morphological protocols under this study, all analysis was done at WFCGC with reference material procured from WII's repository. For Infra-Red Spectroscopic studies, powdered/scraped samples from WII were taken with due approval from competent authority and analysis was performed at Instrument facility of Department of Forensic Science, Punjabi University, Patiala, Punjab.

1.3 Status of Wildlife Forensics: A Review

Thousands of wildlife including plant, animal and other species are subjected to unlawful exploitation for subsistence or illegal wildlife trade for commercial gains. United Nations office on Drug and Crime (UNODC) has listed offenses against wild animals as one of the major Green crimes (UNODC 2020). Those who are mute and deserve to be protected the most, are generally excluded from debates on criminology as the victim wildlife or its near and dear ones are not capable of expressing their loss in our language (Wyatt 2012; Spapens 2014; Lynch & Stretesky 2014). Also, due to its covert nature, complexity, remoteness of crime scene and involvement of thousands of species and their processed parts, the absolute dimension and magnitude of wildlife offenses is challenging to assess. wildlife crime, one of the more severe green crimes against natural treasure for any region is invaluable for humankind and no monetary value can be assigned to it but studies have placed it among the top five illegal transnational trades, along with human trafficking, arms and drugs (van Uhm, 2016; UNODC, 2016).

Wildlife offenses, especially IWT directly threatens a wide range of targeted taxonomic groups, leading to severe declines across species, from African elephants (Wittemyer *et al*. 2014) to Southeast Asian slipper orchids (Hinsley & Roberts 2018). IWT also threatens biodiversity indirectly, through introduction of invasive species (Carrete &Tella, 2008) and disease (Palvin *et al*. 2009; Gomez & Bouhuys 2018), habitat destruction, and bycatch of non-target species (Riskas *et al.* 2018).

Detection of wildlife contraband is the first step in initiating any prosecution against illegal wildlife offenders, but failure in this is one of the most serious issue, owing to many reasons including, the exorbitant number of traded species of mammals,

reptiles, birds, marine animals, invertebrates, their processed parts and derivatives, making it a herculean task for one agency or one country to plug this crime. There are specific species which are sought for subsistence, others for sorcery and many others only for commercial gain. There exists no centralized facility to have wildlife crime database to understand this problem, its dynamics, magnitude, the crime syndicates their *modus operandii* etc. and chalk out strategies to combat it, although CITES maintains database for legally traded and seizures of wildlife at customs areas. But this database does not hold any references to seizures happening outside the customs area and also the database provided in not sufficient.

Wildlife Forensics' help enforcement agencies in determining cause and manner of death and identifying the species from parts or products recovered as evidence in an offense so as to link the suspect with the crime beyond any doubt. Wildlife forensics is comparatively a new science when compared to Human Forensics'. The first dedicated laboratory "The National Fish and Wildlife Forensics Laboratory, devoted to wildlife laws was established at Ashland, Oregon, United States, in 1988 under United States Fish and Wildlife Service. Later, other Wildlife forensic labs were established in other parts of the world including the "Wildlife Forensic & Conservation Genetics Cell" at Wildlife Institute of India, Dehradun, India in 1995.

For overall development of wildlife forensics, understanding of the crime, i.e. illegal extraction of wildlife from their ranges, hotspots for illegal trade and gaps in scientific interventions needed to be fulfilled along with judicial scrutiny is a must and can be studied as multi-pronged strategies, including

i) Trends in wildlife crime involving dynamics and scope of illegal wildlife trade

ii) gaps in the identification protocols that need to be fulfilled and

iii) review of judicial scrutiny from trial court judgements.

Unlike lot of studies available providing an insight to the magnitude and scope of illegal wildlife trade from outside India, very scarce information is available for wildlife offense scenario in the country. Also, most of the work is based on the analysis of seizure data at the customs point of entry or exit (Niraj *et al.*, 2011; IFAW, 2014; Nijman *et al*. 2016; TRAFFIC 2019; UNODC 2020) and therefore only discuss about the charismatics species or megafauna or specific targeted species. Thus, all these studies show the tip of the problem, as these does not consider the offenses occurring against wildlife at the source, i.e. for subsistence, traditional medicines, social practices and local trade. Hundreds of seizures of wildlife articles are reported every year by various enforcement agencies and this information is lying scattered with various enforcement agencies There is no specific study available on these aspects of illegal wildlife trade in India, taking into account the overall dynamics of crime against wildlife from source to destination.

Apart from deficient knowledge about the dynamics and scope of wildlife offenses, another aspect causing impediment in proper implementation of Wild Life (Protection) Act, 1972 is the detection and accurate scientific identification of the animal parts or their derivative seized in offenses against wildlife. The illegal wildlife traffickers use diverse *modus operandi* to conceal the identity by miss-declarations of wildlife article, masking the originality or by selling imitations (Vipin *et al*. 2016; Sharma *et al.* 2019a). Hence, law enforcement becomes ineffective in detecting such undercover wildlife poaching and trading in illegal business (Bennett, 2011; van Uhm 2016) and enforcement agencies are increasingly seeking forensic support to answer

investigative questions in suspected wildlife crime's (Kumar et al. 2016; Vipin *et al*. 2016; Sharma *et al*. 2022b).

Since wildlife forensics is still an evolving science, various techniques from related fields were employed by wildlife forensic labs world over for identification of species from wildlife parts seized including morphology based comparative analysis, molecular or spectroscopic techniques. Studies presenting relation between morphology and wildlife conservation are abound in the technical wildlife literature, but mostly, this relationship is unidirectional, taking into account the role of morphology, performance, and physiology in management and conservation. Biologists in the field of wildlife conservation are using morphology along with standard morphometrical measurements to identify age (Karels et al. 2004; Marti & Ryser-Degiorgis 2018; Roy et al. 2022), sex (Sheuer 2002; Walker 2008; Priya *et al.* 2016), and species including remains from archeological sites. (Christensen & Sylvester 2008) in a great diversity of taxonomic groups. As most of the studies are done keeping zoo archeology or functional morphology in vision, these studies, although useful, remain short and lack understanding of the specific requirements of enforcement agencies or forensic morphologist, dealing in detection and identification of species in wildlife forensic investigation. Wildlife articles or their derivatives reported to wildlife forensic labs are often found broken /incomplete lacking vital morphological characteristics or are highly processed. Recently, lot of fake or imitation biological articles are also reported which need to be distinguished from genuine wildlife article. The routine morphological analysis described for identification of species using complete specimens, therefore fell short and wanting in wildlife forensics. Therefore, there is a need to fill the gap in identification of species by characterization of wildlife articles where morphological identification tools are not available.

The review of aforementioned studies makes it amply clear that there is a dire necessity to understand the dynamics and scope of wildlife offenses in India by studying the wildlife crime data available leading to assessment for required development in morphological and spectroscopic characterization of species from seized wildlife parts. This will not only help in early detection by enforcement agencies but also provide fast and effective tool for wildlife forensic scientists. Such a multifaceted study is now a pre-requisite to effectively combat the menace of illegal activities against wildlife in India. In the following sections, the specific objectives of the study are enlisted and overview of data based analysis and laboratory methods are discussed, that we intend to use.

1.4 Present Study

Seizures of wildlife articles in exponential numbers are reported every year by various enforcement agencies like State Police, Central Bureau of Investigation (CBI) and Forest departments, Wildlife Crime Control Bureau (WCCB), Indian Customs, Foreign post, Indian Coast Guard (ICG), Indian Navy and other agencies. For forensic identification of species, theses wildlife evidences are sent to Wildlife Institute of India (WII), Dehradun and other scientific institutions. Trade and seizure of fake or imitation wildlife articles, wrongly identified by enforcement agencies, have opened up new frontiers for law enforcement agencies and practicing forensic experts to identify whether the submitted evidence is genuine or made of some other animal part or a synthetic article. Often fake biological articles are seized or wildlife articles wrongly identified due to lack of morphological protocols at the spot of seizure resulting in the negative impact during prosecution of the case. The challenge posed is to identify not only real or fake but also the species involved to make the imitation wildlife article has also to be identified, like whether it belong to another common or less protected wild

species or a domestic animal, or is a non-biological synthetic product. Fraudulence in illegal wildlife trade has been reported related to meat (domestic vs wild origin), elephant bone as tusk, fake rhino horn, skins, claws, canines, hemi-penis of *varanus spp*., "Jackal's Horn" etc. (Rajpoot *et al.* 2018; Sharma *et al*. 2022b). Therefore, there is a need to develop scientific tool for fast and efficient characterization of wildlife parts and products for better detection, seizure and identification in wildlife crime investigation.

Biological evidences sent for analysis to WII are sent in accompaniment of documentary evidences relating to case history and seizures. These documents are a treasure trove to understand the dynamics, scale, scope, modus operandi, emerging trends of the wildlife offenses. There is no specific study available on these aspects of wildlife offenses as a whole including illegal wildlife trade in India and the information is lying scattered with various enforcement agencies. This research work was conducted mostly on database available at Wildlife Forensic & Conservation Genetics Cell of WII and other open source data from various organizations working in the field of wildlife law enforcement and digital media.

For having a holistic idea of the culmination of various seizures in wildlife offenses during court trial, verdicts under Wild Life (Protection) Act, 1972 covering a time-span of one decade, from 2011 to 2020 were studied to understand comprehensively about the procedures adopted, lacunas, if any and basis of rulings of the trial courts. It will not only allow us to understand the level of veracity in implementation of the provisions of the Wild Life (Protection) Act, 1972 but also allows us to document the evidentiary value of scientific investigation tools.

The illegal wildlife trade is very dynamic, and new challenges in identification of wildlife articles or its derivatives crop up sooner, placing scientific characterization protocols with enforcement agencies and forensic scientists is of paramount importance. Practicing wildlife forensic morphologists are constantly striving to deliver the very best, by providing protocols for detection and identification of wildlife parts to enforcement agencies. As the illegal wildlife trade keeps on changing with time and space, to further augment the efforts of the scientific community continuous and broad study on the nature and scope of wildlife offenses across the region, noticeable gaps in scientific species identification protocols remains to be fulfilled, so as to strengthen hands of judiciary in getting successful conviction.

Due to often unsystematic approach lacking basic wildlife crime data and knowledge about the dynamics of wildlife offenses, enforcement of wildlife protection laws for curbing the wildlife offenses at all stages, i.e. poaching at source, processing and local transfer, and smuggling to the demand site is usually very casual. To effectively confront this ever-growing challenge, it requires exploration and application field and forensic laboratory tools. Therefore, in this study efforts are made to holistically analyze data showing overall crime scenario against wildlife, so as to enable enforcement agencies, forensic labs, law officers and conservation managers to devise strategies for better enforcement of wildlife protection laws. This thesis has findings of a detailed study based on a combination of an intensive field and laboratory based assessment of wildlife offenses in India during last decade (2011-2020) and help in formulating scientific protocols for wildlife evidences identification for corroborating investigating agencies story beyond doubt to effectively counter wildlife offenses.

The study of the judgements from trial courts of four diverse states for which sufficient data was available helps in better understanding of the reasoning behind the

judgements and provide remedial actions, if any. The purpose was to find out the trial procedures followed in trial courts cases under study and reasons behind the judgment, either of conviction, or, of acquittal. This exercise will provide a holistic idea of the overall pattern of the crime, conviction rate, minimum time needed for culmination of a booked wildlife offense, challenges and basis of rulings of the trial courts.

While discussing the dynamics of wildlife offenses, pros and cons in enforcement of wildlife protection laws based on empirical data, but it is in no way an expression of the total wildlife crime rate of any state or region as the data is only based on the reported incidents and does not account for actual crime against the wildlife. Working in the field of wildlife forensics and interacting with enforcement agencies on dynamics of illegal trade in wildlife since more than two and half decades, the work presented here is not just a scientific offering but an outpouring of a practitioner.

1.5 Study objectives

Objectives of the present study are as follows:

1. To develop morphological protocols for identification of species from available wildlife articles seized in illegal wildlife trade.
2. To document and analyze the recent trends in the illegal wildlife trade in India based on forensic evidences.

Specific components of the study included

i. Documentation of the type and extent of wildlife trade reported across various states/regions of India
ii. Analyze recent trends in illegal wildlife trade in the country based on seizure data
iii. Developing protocols morphological characterization of parts and products of illegally traded species
iv. Document the prosecution scenario based on trial court judgements from four selected states

Chapter 2:

METHODOLOGY: FORENSIC TOOLS AND EVIDENCES USED IN THE STUDY

2.1 Introduction

In absence of a robust database of illegally traded wildlife parts and scientific protocols for detection and identification of huge magnitude of confiscated wildlife parts and derivatives, involving local criminals to trans-national criminal gangs, it is quite challenging for the law enforcement agencies to enforce wildlife protection laws. Therefore, to identify the gaps and develop the required scientific protocols for filling up these identified gaps, is the foremost concern for combating wildlife crimes. A systematic and comprehensive approach in developing empirical database from enforcement agencies and scientific protocols are needed to maximize chances of prosecutions in wildlife offense cases.

Indirect measures of illegal wildlife trade (IWT), such as seizure data provide some indication of trade hotspots and trends (Rosen & Smith, 2010), but they contain detection and reporting biases (Underwood *et al.* 2013), and largely gave status of relative trends happening in traded species for commercial gains only, while other wildlife offenses including for subsistence and other cultural or ritual demands are left neglected. Seizure data of IWT tends to be biased towards charismatic species (e.g., Tiger parts, elephant ivory) and may constitute less than 10% of all illegal trade (van Uhm 2016). While trends in the transnational and *legal* wildlife trade are relatively well documented (Harfoot *et al.* 2018) little has been done to analyze holistically the scope of wildlife crime in relation to illegal wildlife trade (IWT) in India. Therefore, information linked to underlying drivers and trends shaping wildlife offenses for commercial gain, subsistence, sports and other factors are very important, although difficult to obtain, in developing a Pragmatic approach towards identifying and mitigating the factors responsible for wildlife crime in India.

Wildlife forensics always follows crime against wildlife and has emerged to assist the need that is driven to create a deterrent against wildlife crimes in society by not only getting prosecution of criminals that specifically target wildlife for personnel gains or commercial purposes but as a conservation strategy for threatened species in the region. Currently, wildlife crimes including illegal trade in wildlife and their derivatives is one of the single biggest global challenges, the cost of which is estimated at US $10–20 billion per year, roughly 5% of the international drug trade (Wilson-Wilde 2010). Although crimes against wildlife for commercial gain is a global challenge, Southeast Asia is considered one of the main global hubs as producer/suppliers, transit points and consumers for this illegal trade (Rosen and Smith, 2010).

To help in investigation and substantiate prosecution, the wildlife forensic scientists are required to essentially undertake identification of species of wild animals and their derivatives confiscated, along with ascertaining the cause and manner employed for poaching, *modus operandii,* time elapsed since poaching took place and possible number of accused involved.

This chapter provides a summary of forensic tools and biological evidences found in wildlife offense cases. These tools and techniques were tested during the course of present study

2.2 Basic approaches

2.2.1 Necropsy and physical examination

While dealing wildlife offense cases involving delayed detection of carcasses of wild animals in deep forests, lying in open sky under natural conditions poses serious challenges for veterinary pathologists in determining cause and manner of death. While

performing necropsy, a trained veterinary pathologist can deduce and report on cause and manner of death through visual observations, wounds/injuries pattern, radiography, and microscopy (Cooper and Cooper 2008). Further in cases of suspected poisoning, disease or when conclusive opinion can't be made, visceral samples collected and preserved meticulously were sent to analysis for toxicology and histopathology in a forensic laboratory.

2.2.2 Molecular tools

DNA based analysis is very useful tool not only in identification of species in highly processed, powdered, cooked, pickled tissues (Chang *et al.* 2014; Ciavaglia *et al.* 2014; Davitkov *et al*. 2017; Gupta 2018; Ewart *et al.* 2020), but also for individualization (genotyping) of an individual in paternity identification, in man--animal negative interactions by identifying the problem animal (Yadav *et al.* 2020; Jabin *et al*. 2020) and zoo-geographical origin of wildlife derivative (Wasser *et al.* 2007, 2015). This technique is at present widely used in wildlife forensics in combination with other tools in reaching conclusive results.

2.2.3. Chemical analysis

Chemical analysis is performed for identification of

i) Specific wildlife derivatives (e.g. bear gall bladders) (Hagey *et al*. 1993, Dubois *et al*. 2022), teeth, antler derivatives (Singh *et al*. 2006; Singh *et al.* 2022), bone fragments (Coals *et al.* 2021), civet and American beaver anal gland secretions through chemical analysis.

ii) Suspected death due to poisoning by the analysis of viscera samples for any poison and its validation and verification through extensive chemical reference libraries available (Otieno *et al.* 2010).

iii) Stable isotopes, often referred to as the "dietary" isotopes, used to address wildlife forensic questions, for ascertaining if the specimen in question is legally acquired through inheritance or before the enactment of wildlife protection laws or got illegally in recent times. (Hobson 1999; Hillson S, 2005). This tool is rather used rarely.

2.3 Tools used in present study

2.3.1 Morphological analysis

Morphological identification of species from biological evidences submitted, an essential step in wildlife crime investigations, uses the characterization of specific functional traits evolved during interface of an organisms with its environment in evolutionary process (Trail 2021). These specific morphological characters in a species have an intimate association with vital survival functions i.e. feeding (dentition) and locomotion (gait pattern/running), for that species, which influences its ecological interactions and natural selection for resource use, important for its existence and propagation (Bock & von Wahlert 1965; Arnold 1983; Christensen 1996).

In a wildlife forensic context, a forensic morphologist uses physical comparison of submitted evidence to identify wildlife parts and products, typically to the family, genus, or species source. Depending on the nature of the evidence, a variety of macroscopic and microscopic comparison techniques may be employed and results are compared with repository on known specimen for conclusive identification. The analyses performed by a forensic wildlife morphologist are mostly based on class characters (family/genus/species level), and not the individual characters. Individualization based on morphological characters is rarely attempted in wildlife cases, except in cases of identification of problem animals (large carnivore) from

excreta/scat analysis or matching an individual Tiger skin with available camera trap images. Thus morphological comparison technique is not only useful to taxonomists, anatomists, paleontologists, and archaeologists but also have vital importance for wildlife morphologists.

Review of literature clearly shows majority of work on identification of bony remains was mostly on humans and domestic animals, from archeological sites based on fossilized dentition available that makes the base for species identification by following standard protocols (von den Driesch 1976). Enough work has also been reported from study of specific features of bones resulting from basic survival skills leading to definite adaptation and functional morphology, mostly of skull, in a species (Christiansen & Sylvestor 2008; Sims 2012). But these studies are limited to few charismatic groups or species like large cats (Tiger and leopard) and mega herbivores (Elephants) and others, with limited dataset for complete specimens.

Forensic morphologists use all available scientific data and literature while conducting analysis of wildlife remains but considering the gigantic number of species involved in wildlife offenses and type of evidence recovered is dependent on specific demand, *modus operandii* while poaching or processing before smuggling, resulting in injuries or deformations, therefore wildlife morphologists need specific protocols for characterization of species from available wild animal remains.

But more efforts are required with available data to fill the gaps for wildlife articles derived from rare or lesser known animals and other biological parts for which sufficient scientific research is not available. Since wildlife offenses at local level are region specific depending on availability of particular species and social and financial status of people, it requires separate set of tools for identification than reported in

international seizures. e.g. tiger parts in TCM was highest world over but in India it is bones, skin and claws that are more seized than traditional medicine having tiger parts as ingredient (UNODC 2020). Another recent aspect observed is the illegal trade in exotic wildlife parts (Lavorgna 2014; Pragatheesh 2021).

The morphological methods used in wildlife forensics are utilized primarily for identification of wildlife article seized in wildlife offenses. These methods are based on anatomical identification of animal's body parts and require a considerable understanding of comparative functional anatomy at the macroscopic and microscopic scale.

2.3.2 Macroscopic and microscopic analysis

By applying gross osteology, identification of a single bony element including teeth by the unique diagnostic morphological attributes or morphological characters can be ascertained. In wildlife forensics, animal identifications are far more challenging because of numerous possibilities and inter- and intra-species variants. To overcome this, by invoking the exclusion method first the human and other distant groups are ruled out and then by unique bony character's specific species or genus (taxon) is zeroed and identified through detailed analysis. Depending on the mode of poaching of wild animal, especially large cats (Tiger and Leopard) by trapping using steel jaw traps and striking it by hitting hard on the skull to kill it instantly and also bones are usually buried by poachers to avoid detection while transportation without cleaning and whenever recovered after long elapsed time, few bony elements lose their original structure and found separated from joints/sutures/sockets. Still, by the type of bony joint, overall size and shape, ligamentous insertion sites and morphometric measurements provide diagnostic characteristic features of a particular species (Christensen 1996; Mazák

2010; Sims 2012; Otárola-Castillo 2018). Estimation of minimum number of individual of same or multiple species present becomes important when a large consignment of bones is seized. Thus by applying this approach, by identifying a single skeletal element like no. of skulls, C1/atlas bone or scapula pair present, minimum numbers of individuals can be estimated. When dentition is present, the chance of identification increases immensely, since dentition is unique to most species (Evans & Sanson, 2003; Smith 2015).

Microscopic analysis at low magnification can be performed where fragmentary remains (teeth, elephant molars to distinguish African and Asian elephant (Espinosa and Mann 1993) are recovered and higher magnification analysis where minute evidences are recovered (*shahtoosh*, hair/wool, hemipenis of *varanus spp*. (Brunner and Comman 1974; Moore *et al*. 1974; Yates et al. 2010; Sharma *et al.* 2019a; Gupta *et al*. 2022).

2.3.3 Spectroscopic analysis: FT-IR

In any Wildlife forensic investigation, analysis of biological evidences has perpetual significance in getting conviction of accused or proving innocence. Appropriate analysis of such evidence, not only help in identification and proving possession of a contraband by the accused but also help in establishing link between the suspect, the wildlife, and the crime scene in poaching scenarios. The analysis of wildlife evidences has had an overwhelming impact on the species/individual identification by using DNA profiling. DNA profiling is considered a cornerstone in the field of forensic science and is a sensitive and the most reliable means of identification of a species or an individual. However, a limitation of DNA typing is that it cannot provide success when the sample size is limited and DNA extracted is not

sufficient for analysis as in highly processed/finished wildlife articles (Gupta *et al*. 2022). Also, in wildlife poaching cases, the crime scene usually spoiled before picking up of crucial evidences, due to many obvious reasons including, the crime is detected late as it usually happens in remote/secluded areas, movement of scavengers and other animals etc. and therefore contaminations are quite recurrent. As identification of species is core to wildlife forensics, for the investigative leads, accurate identification methods for challenging evidences would be helpful to avoid the performance of costly and redundant analysis of deficient wildlife evidences or simulated/fake wildlife articles of non- biological origin. Identification of species based on FTIR, can also be a pre-cursor or screening tool for other available robust conventional methods definitively utilized for species discrimination, but the destructive nature of these tests is the most significant concern that requires to be consigned along with the cost involved for such analysis of large consignments of wildlife contrabands being declared as legal entities. The ATR-FTIR technique is vehemently used in characterization of biological evidences in human forensics (Sharma & Kumar 2018; Sharma *et al*. 2021) and recently claws and hair of wildlife were successfully characterized based on this technique (Sharma *et al.* 2019b, Sharma *et al.* 2022a).

Biological parts encountered in illegal wildlife trade are often found as fragments, dressed or as derivatives lacking few or all morphological identification characteristics thereby posing a great challenge to identify confidently during detection for initiating any legal action.

2.4 Animal parts selected for the study

Crime against wildlife is very dynamic and involves a large number of species from various taxonomic orders. Availability of scientific protocols for detection and

identification of processed or incomplete wildlife parts remained a challenge for enforcement agencies and therefore frequently encountered biological parts were taken for morphological identification in current study. Care has been taken to select only those animal parts for which sufficient references were available in WFCGC.

2.4.1 Cranial bones and C1 bone of cranio-vertebral joints

One of the most serious threats presently to large carnivores specially to tigers and other large felids survival is the use of their bones in traditional medicine (Mills & Jackson 1994; Nijman *et al.* 2012; Chen *et al.* 2015; Yujin & Yongde 2021) and pet trade (Coals *et al.* 2021). With the dwindling population of tigers resulting in scarcity of supply, it has been noticeable increase in poaching of other sympatric large carnivores for their bones and illegal traders trying to mix these in large consignment of bones and pass them as original tiger bones.

Although closely related species have similar basic bone features and design but structural variations occur to cater the specific performance requirements for feeding and survival (Arnold 1983; Wainwright, 1994). The predatory performance in carnivores is directly related to the mode and size of prey it captures and reflected in the cranial-mandibular morphology (Christiansen 2007; Slater *et al*. 2009), and these morphological characteristics can be for scientifically determining the closely related species in wildlife forensic investigation (Christiansen & Adams 2008; Sims 2012).

The skull is specialized for providing support and function of top predators (Christiansen & Adams 2008). Along with the skull the cranio-vertebral joint of first cervical work as a coherent mechanical unit optimized for extra stability and enhanced mobility (Goel *et al.* 2011). In wildlife bone seizures, that are devoid of the skull and other major bones identification of species for booking an offense is challenging for

law border enforcement agencies. Thus, in seizures lacking major bones or mixed bones from many species, the identification of species from often neglected lesser bones, especially the C1 of atlas bone may be of immense use in wildlife forensics.

Suspected bones of tiger, leopard, hyaena and sloth bear are regularly refereed to WFCGC under wildlife offense cases for species identification and make up 6.5% (N= 4046) of the total case submitted. In this study the C1 vertebrae of tiger, leopard, hyaena, and sloth bear is compared to characterize species in Wildlife forensic investigation.

2.4.2 Canines teeth

Canines are teeth posterior to incisors and are generally elongated, pointed and single rooted and with a conical sharp monocuspid crown. These teeth are ideal for piercing, tearing off flesh, and offense and defense (Feldhamer *et al.* 2015; Bhamrah & Juneja 1990). This spear-like tooth is more prominent in carnivores with most of them having a total of four canines. In herbivores, canines have usually been lost or greatly reduced (Savage & Long 1986), but in some animals is present but their number is less than four. Molar teeth of carnivores and large herbivores like elephant and rhinos are also encountered in illegal wildlife trade

Canines are usually recovered in wildlife offenses attached to skull but loose canines or studded as pendants are also common (Nadarajan *et al*. 2022). Canines not only have to be identified for specific species but also as genuine or fake as lot of canines made of synthetic material or from some other animals, including from domestic animals are also submitted during wildlife offense investigation. Through morphology and morphometric measurements, comparative study of canine tooth of Tiger, Leopard and Fake (synthetic material and Horse) is done to identify and

differentiate them. Musk deer canines are also frequently seized in wildlife offenses and are characterized in this study for use in forensic identification of species.

2.4.3 Musk Pod

Musk deer have been extensively hunted for harvesting "musk", an odiferous secretion from the preputial follicle of sexually mature male musk deer, due to its fixative and scent properties in expensive perfumes and within indigenous systems of medicine in many parts of the world. This secretion is collected in a "sac" called as musk pod formed by the in folding of the skin on the abdomen (Seth *et al.* 1975; Green 1985, 1986, 1987). The use of musk ("Kasturi" in Hindi) is quite known to ancient Indian practicener's of indigenous system of Ayurveda medicine as described in Sushruta Samhita, a compendium in Sanskrit language, on medicine and surgery (Ray *et al.* 1980; Meulenbeld 1999).

Continued poaching of deer in substantial numbers to cater the burgeoning demand of musk, the musk deer are now critically endangered, or in some cases, extinct from their habitat as a result of poaching and illegal trade (Homes, 1999). The Himalayan musk deer (Moschus spp.), is included in the schedule I of the Wildlife (Protection) Act, 1972 and Appendix I of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The International Union for the Conservation of Nature and Natural Resources (IUCN) lists it as a near-threatened species. Strong enforcement of law in the area of origin but constant high demand for illegal Wildlife articles result in illicit traders dealing in substitute species or spurious items to make quick money (UNODC 2020).

More often the pods seized in illegal wildlife trade are found with other skin removed and the contents are found only in the muscular pouch. Efforts were made to

identify the species from the musk deer pouch by opportunistic collection of guard hair from inside the musk gland and performing analysis based on microscopy. Musk contents received as powder or as constituent of traditional medicine were characterized based on IR spectroscopic analysis (ATR-FTIR).

2.4.4 Claws

Work on morphological identification and differentiation has already been done (Vipin *et al.* 2016), but in case of cut or processed claws studded in pendants, identification of species was difficult as the studded claws loses major identifying characteristics. Therefore, for small fragments of claws, IR based spectroscopic analysis (ATR-FTIR) was employed for differentiating Tiger, Leopard and fake claws made of animal keratin material (Sharma *et al.* 2019b).

2.4.5 Shahtoosh

Shahtoosh, is derived from the underfur of Tibetan antelope or Chiru (*Pantholops hodgsonii*), endemic to the Tibetan Plateau which is listed as a critically endangered species by the International Union for Conservation of Nature (IUCN) and has been protected under Schedule 1 of The Wild Life (Protection) Act-1972. Owing to its fineness and rarity, Tibetan antelope is heavily exploited for *shahtoosh* to make beautiful luxury products and these *shahtoosh* fabrics, are among the most sought-after articles in the illegal wildlife trade (Maron 2019). Since, for the manufacturing of a single *shahtoosh* shawl, wool from skins of 3-5 adult animals are required, the huge demand of *shahtoosh* in the illegal wildlife trade has led to the large-scale poaching of the Tibetan antelope (IFWA 2001). Deliberations attended by eleven source, transit and consumer countries, CITES secretariat and Interpol in 2016, raised the lack of sufficient database to understand scope and magnitude of this illegal trade and stressed for a need

to have better coordination among concerned countries (FDHA 2016**).** In CITES secretariat report on Tibetan Antelope (CITES, 2016), consistently high number of seizures of "*shahtoosh* shawls" or "Shawls of Shame" were reported, especially from Switzerland with origins from India and Italy.

The illegal *shahtoosh* is often blended with Pashmina wool or *Cashmere*, collected after shearing wool from domesticated Pashmina goat (*Capra aegagrus hircus*) to reduce the cost, increase profit (Gupta 2011), and mis-declared as Pashmina product to avoid detection by enforcement agencies. Conventionally, physical and morphological characteristics of the guard hairs are examined for the identification of *shahtoosh* and its differentiation from Pashmina wool using light (Donn and Yates 2002; Sahajpal *et al.* 2009) and scanning electron microscopy for wool hair (Phan *et al*.2000; Bahuguna & Mukherjee 2000; Choudhary *et al.* 2014). The use of modern technology and machines for de-hairing of coarser guard hair and adulteration of pashmina wool has therefore, made identification of *shahtoosh,* challenging for enforcement and scientific agencies. Therefore, having reliable scientific protocols for identification and discrimination of *shahtoosh* from other species especially from Pashmina is of utmost importance in wildlife forensics.

Currently, DNA based species identification is the most commonly used and widely accepted reliable method for the identification of species using species-specific genetic markers from a variety of biological materials. Though DNA is unique to the species and can be used to identify and differentiate species, the cognizance of the fact that in many cases hair strands do not exist with enough root tissue attached with follicular tag, posing challenge for DNA typing (Gupta *et al*. 2022). Another technique is FT-IR spectroscopy that seems to be promising owing to its non – destructive, reliable, and rapid nature. The resolving power of FT-IR spectroscopy has been applied

as an indispensable tool in diverse field of forensics to identify trace evidence such as fibers (Lang *et at*. 1986; Howell & Davis 1991), hairs (Manheim *et al*. 2016; Boll *et al*. 2017) and biological fluids (Elkins 2011; Sharma *et al*. 2019c, 2021). Recently, another keratin material from wildlife, i.e. claws of Indian Tiger (*Panthera tigris tigris*) and common leopard (*Panthera pardus fusca*) were successfully differentiated using ATR FT-IR spectroscopy supplemented with chemometrics tools (Sharma *et al*. 2019b).

In this study 1771 suspected woolen shawls submitted in Wildlife Institute of India (WII) for species identification under 12 wildlife offense cases were studied

2.4.6 Blood

Offences against wildlife involve illegal exploitation of a wide range of species including insects, reptiles, amphibians, fish and mammals for commercial gain. This illegal exploitation of wildlife has become substantial in its scope and dimensions in recent years globally, largely for subsistence, illegal wildlife trade, societal/religious rituals luxury items, and use in traditional medicines (Nijman *et al.* 2012; UNODC 2016). In recent times the illegal trade in wildlife has become increasingly sophisticated with new tools and weapons for poaching of wildlife, not only for high value species, such as tigers, elephants and rhinoceros, but also for lesser animals like monitor lizard and pangolin parts, birds and turtles and availability of distant but secure digital platforms for secure supply (Burn *et al.* 2011, Sharma *et al.* 2019a, Pragatheesh *et al*. 2021). For implementation of wildlife protection laws, identification of scene of crime and species from varied biological evidences recovered from crime scene is of foremost importance.

The identification of blood as evidence in various forms and on various substrates, i.e. weapon, instrument, soil, dried litter, carrying bags etc., is central to any

poaching incident. Blood as evidence makes 8.2% of total biological evidences (N=4408) submitted in last 20 years (2001-2020) to WFCGC of Wildlife Institute of India (WII), Dehradun

Not only analysis of blood provide clue about the species involved but also its evaluation is crucial to substantiate the *Corpus delicti,* i.e. establishing procedure to kill animal, primary site of poaching, single or multiple species involved, number of individuals, Road-traffic accidents and involvement of suspect (s) if blood is found on personnel belongings and space like cloths/vehicle/house thus proving guilt beyond doubt.

In recent years, analytical chemistry has become one of the expanding areas in trace evidence analysis. The identification of species from body fluids analysis is a significant aspect in criminal investigations. Lately, plethora of research has been conducted for the purpose of species identification and differentiation from blood traces using various techniques including spectroscopic techniques with varying success. Raman spectroscopy and ATR FT-IR spectroscopy standalone was successfully used to discriminate the spectra of blood from human and animal origin (De weal *et al.* 2008; Virkler & Lednev 2009), followed by discrimination of species using a combination of ATR FT-IR and advance chemometric tools (dog, rabbit, boar, ram, and bull) Apart from blood, other body fluids and their stains were also differentiated using FT-IR technique (Zhang *et al*. 2014; Mistek & Lednev 2015, Wei *et al*. 2021).

In present study ATR FT-IR spectroscopy is used for the first time to analyze the dry blood samples of wild animal species including Elephant, Tiger, Leopard, and Domestic Pig for paramount application in wildlife species identification. The present work expanded upon the work of species identification using a non-destructive and

rapid approach that is ATR FT-IR spectroscopy with chemometric tools. Based on the success of the previously reported studies for the purposes of species discrimination, same approach was envisaged for the current study also. The foremost rationale of the current research is to demonstrate the principle that blood samples could be spectroscopically discriminated according to the species of origin which may be an important tool in discriminating species while analyzing blood evidence in wildlife offense cases.

2.4.7 Suspected Jackal's horn

Globally, illegal wildlife trade usually involves highly charismatic species, but quite often, derivatives of lesser-known species are also encountered and receive less attention. Considering the huge demand, profit and short supply, many fake articles derived from either domestic or wild animals are frequently encountered in the wildlife trade. Trafficking and unsustainable trade of internationally high-value or charismatic species are widely reported and addressed. Illegal trade in lesser-known species is widely exploited. It receives less attention, thereby jeopardizing the whole concept of conservation, leading to the decline or local extinction of such species (Bennett 2011). Body parts of some of such species like monitor lizards (*Varanus spp*.), pangolin (*Manis spp.*) and jackal (*Canis aureus*) are frequently encountered in illegal wildlife offenses (Douglas and Alie 2014; Aiyadurai 2011; Mendiratta *et al* 2017; Sharma *et al.* 2019a). Moreover, due to short supply and high demand, many types of fake wildlife items, such as claws, canines, skin, elephant tusk, musk deer pod, rhino horn etc. are found in the illegal wildlife trade markets (Martin and Stiles 2000; Chawla *et al.* 2020). These fake articles are either made of non-biological material or biological material obtained from domestic or other less charismatic wild species. Jackal (*Canis aureus*) horn (locally known as "Siyar or Gidar singhi") is one such fake item that is widely

used in sorcery and other occult practices available through offline and online trading platforms. The major threat to jackal's survival is poaching for "Jackal Horn" (locally called Siyar or Gidar singhi), claimed to be a rare horn-like protrusion or deformity behind Jackal's sagittal crest. Jackal horns were well marketed by astrologers, sorcery practitioners, and religious ritual traders and strengthened through social media (Sharma *et al.* 2022b).

Although jackal horns were considered counterfeit items, but there exists no proper documentary evidence to know what kind of non-biological or biological materials were being used to prepare them. Therefore, in this study, morphological and molecular approaches were applied to

i) identify whether jackal horn samples sold in wildlife markets were genuine or fake and if fake,

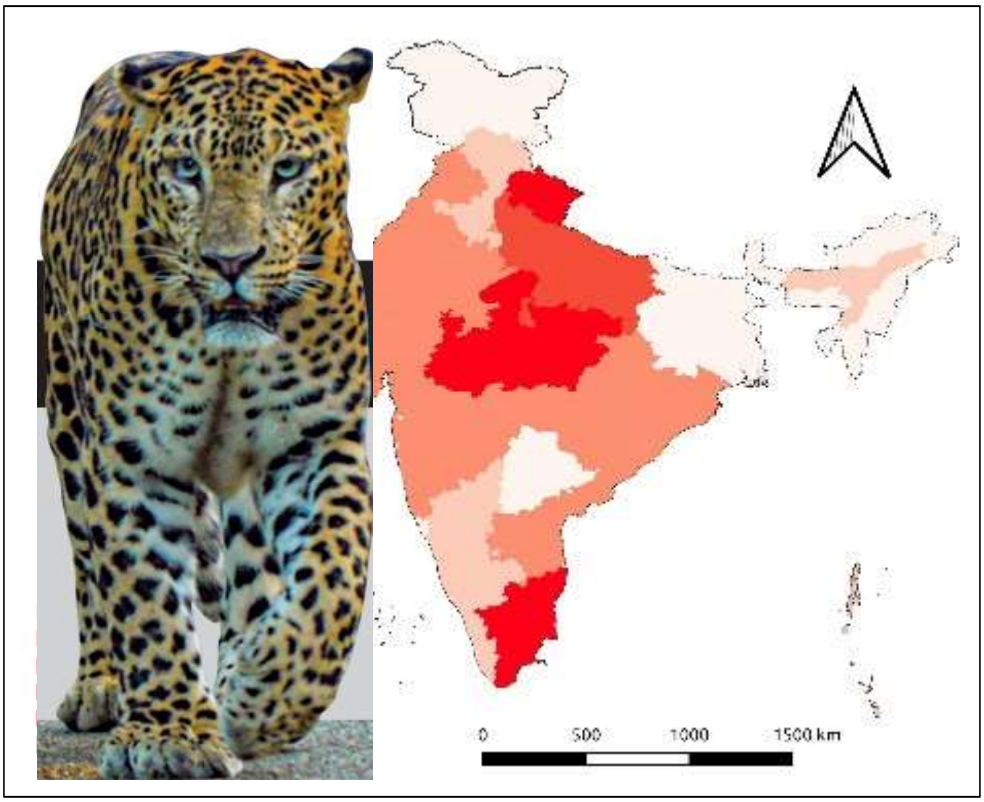
ii) what kind of non-biological or biological materials are used and

iii) if the derivatives of domestic or wild species for making such items.

The results so obtained will provide an opportunity to understand the true origin of the jackal horns upon which law enforcement agencies can rely and decide enforce the existing wildlife laws, which may help in the conservation and management of species.

Chapter 3:

RECENT TRENDS IN WILDLIFE OFFENSES ACROSS INDIAN STATES



3.1 Introduction

One of the many reasons for extermination of many species is the illegal trade in wildlife parts due to its highly lucrative nature and now counted as one of the "global threat" in international discussions (FATF 2020). In this chapter the complex dynamics of the wildlife offenses from India are specifically examined. While there is enough database for illegal trade in charismatic species and articles, it does not explain the overall offenses of much greater spans involving a range of species for subsistence, occult practices, traditional medicines, jewelry/ornaments, clothing, lucky charms or souvenirs and for pets. Trends on wildlife offenses are based on the data available on illegal wildlife trade from CITES, Customs points during transit or from carriers and is typically focused on more charismatic species. However, much is left to understand about the crime against other lesser known species, backward and forward linkages, the supply chains and understanding the *modus operandii*, offender profiles and motivations (Challender *et al.,* 2015, Sharma *et al.* 2022). Janssen and Shepherd (2018) have also shown that although there is plenty of research available to support the argument that wildlife seizure data provide a good baseline understanding of the impact of illegal wildlife trade, but still, such studies remain inundated by discrepancies.

The vast area and varying geographical conditions in India led to occurrence of animals in different taxonomic orders and also posing unique challenges in implementation of wildlife protection laws. Wildlife Forensic & Conservation Genetics cell is receiving biological evidences for species identification from all over India, as mandated by the law in wildlife offense cases. A peek through the long term data of submitted wildlife offenses indicate no major shift in number of seizures per year among the states. There are species which are found to be more is demand for minting quick money while others are preferred for subsistence only, while some others are for

social or cultural uses. Similarly, there are identified hot spots for poaching and others serve as collection or trading centre's.

In this chapter, trends gathered from study of long term (2011-2020) empirical data of reported wildlife offenses will be studies along with specifically dealing with the trends in illegal trade in Tibetan antelope wool or *shahtoosh*.

3.2 Data Sources

Data on wildlife crimes is scarcely available and is spread sporadically with different enforcement agencies. Wildlife offense cases referred to Wildlife Forensic and Conservation Genetics cell (WFCGC) of Wildlife Institute of India (WII), as mandated under wildlife protection laws for species identification from various enforcement agencies was used in this study. Wildlife crime related data was also scooped from multiple sources including data available at open source, social sites and media. Empirical data of 6809 entries for wildlife offenses from 2011 to 2020 were reviewed in the current study. The entries were thoroughly scrutinized and authenticated during data cleaning and entries having any ambiguity, duplicity or missing fields were removed, resulting in 5817 wildlife offences taken up for the study in detail. To overcome the imbalance of uneven distribution of available reported data from different states, only those states were considered for detailed study from where sufficient data on wildlife offenses was reported and available, assuming the Pareto's principle of factor sparsity, that at least 80% of results will come from 20% of the input entries (Arnold B C. 1983).

To quantify the trends and dynamics of wildlife offenses during the period 2011-2020, 5817 offenses were studied in detail. While dealing with the results the fact was kept in consideration that the reported offense data is skewed towards few states as there is very less offenses/reporting from few others. The study shows that during the reported period, eight states in alphabetical order, viz. Chhattisgarh, Gujarat, Madhya Pradesh, Odisha, Punjab, Tamil Nadu, Uttarakhand and Uttar Pradesh constitute more than 60% reported wildlife offences. (**Fig. 3.1**)

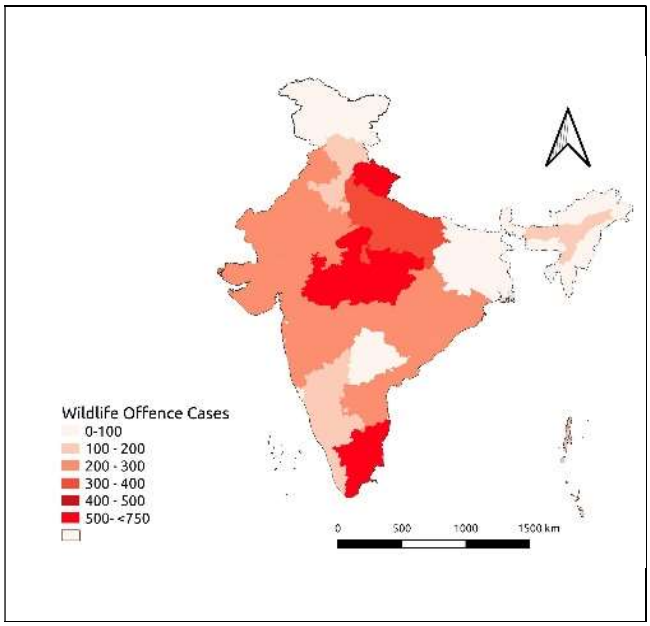


Fig 3.1: Distribution of reported wildlife offense cases for period from 2011 to 2020

3.3 Broad Trends

3.3.1 Year wise reporting of the wildlife offense cases

There is a slight increase in reported incidents during the initial period of the last decade with maximum reported wildlife offense incidents reaching 774 in 2013 and thereafter the reported incidents remain almost constant with steep decrease in year 2019 and 2020, due to reasons not identified. (Fig. 3.2)

3.2.2 State wise reporting of wildlife offense cases

There is a trend observed in the reporting of wildlife offense incidents from states, is that number of states reporting >5% incidents increased to eight from 5 in the initial years. Whereas only five states were contributing major share in reported

incidents during the initial years, later on, although number of reported incident saw a dip but the incidents were shared by other states also, showing spread of better enforcement/reporting from other states.

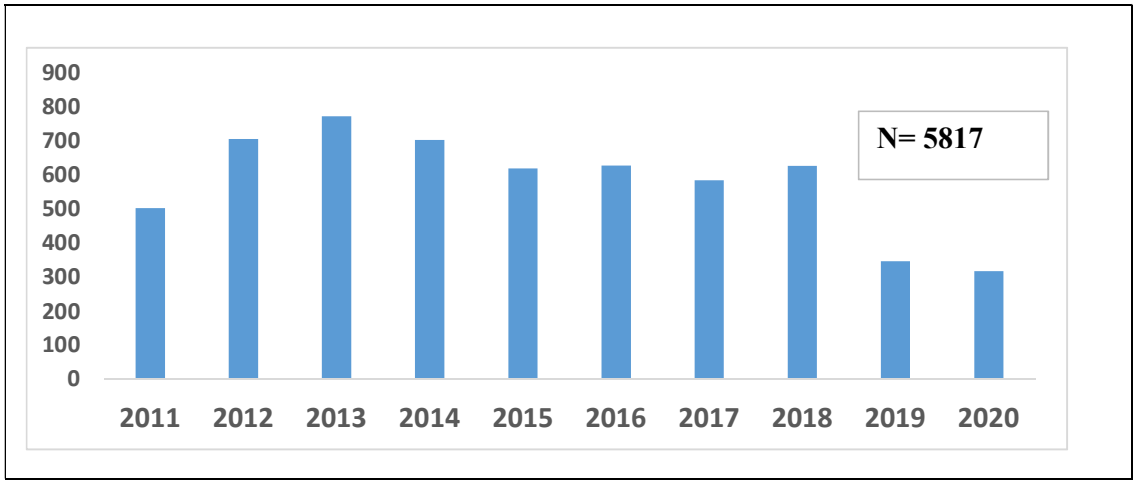


Fig. 3.2: Year wise distribution of reported wildlife offense incidents

The reporting of wildlife incidents from various districts/regions is not symmetrical with twenty-one districts/regions were identified as hot-spots, reporting >1% or 37% of total reported incidents (N= 2152) of seizure of wildlife articles led by Delhi and followed by Erode, Coimbatore, Dehradun and Tirunelveli respectively as top five districts/region.

Table 3.1: Reported incidents from states over the decade 2011-2020

Year	Top Five states reporting maximum Wildlife offenses					Reported WL offenses
2011	Madhya Pradesh (21.2%)	Uttarakhand (12.5%)	Tamil Nadu (8.1%)	Uttar Pradesh (7.5%)	Punjab (29%)	504 (5 states > 5%)
2012	Tamil Nadu (26.3%)	Madhya Pradesh (17.4%)	Andhra Pradesh (7.1%)	Uttarakhand (7.1%)	Gujarat (4.2%)	707 (5 states > 5%)
2013	Madhya Pradesh (23.%)	Tamil Nadu (21.3%)	Uttarakhand (9.9%)	Uttar Pradesh (5.7%)	Rajasthan (4.9%)	774 (5 states > 5%)
2014	Tamil Nadu (26.7%)	Madhya Pradesh (13.6%)	Uttarakhand (5.7%)	Punjab (5.4%)	Andhra Pradesh (5.1%)	704 (5 states > 5%)
2015	Madhya Pradesh (12.3%)	Tamil Nadu (9.7%)	Uttar Pradesh (9.2%)	Andhra Pradesh (7.4%)	Chhattisgarh (6.5%)	620 (8 states > 5%)
2016	Uttarakhand (11.4%)	Chhattisgarh (9.8%)	Gujarat (8.9%)	Madhya Pradesh (8.3%)	Maharashtra (7.2 %)	629 (8 states > 5%)
2017	Uttarakhand (16.4%)	Uttar Pradesh (9.2%)	Chhattisgarh (7.9%)	Karnataka (7.4%)	Gujarat (7.2%)	585(8 states > 5%)
2018	Uttar Pradesh (12.8%)	Uttarakhand (11%)	Rajasthan (7.8%)	Punjab (7.1%)	Chhattisgarh (7%)	628 (8 states > 5%)
2019	Uttar Pradesh (19.3%)	Uttarakhand (11.2%)	Maharashtra (8.6%)	Rajasthan (8.1%)	Odisha (6.9%)	347
2020	Uttarakhand (20.3%)	Rajasthan (9.1%)	Odisha (8.5%)	A & N Islands (6.3%)	Uttar Pradesh (6.3%)	319 (7 states > 5%)

3.3.3 Department wise reporting of wildlife offense cases

As expected, the state forest department's reported maximum wildlife offense incidents (Fig. 3.3). Indian customs and Marine enforcement/security agencies were specifically active at airport (IGI Delhi) and at high seas (Andaman & Nicobar Islands). The cases intercepted by border para-military forces like, Sashatra Seema Bal (SSB), Assam Rifles etc. were taken as police cases The maritime security agencies, the Indian Navy and Indian Coast Guard intercepted the wildlife offense in high seas and referred the investigation/prosecution to local forest department. The Wildlife Crime Control Bureau (WCCB), after 2018 did not booked any case but referred all cases to Indian Customs for prosecution.

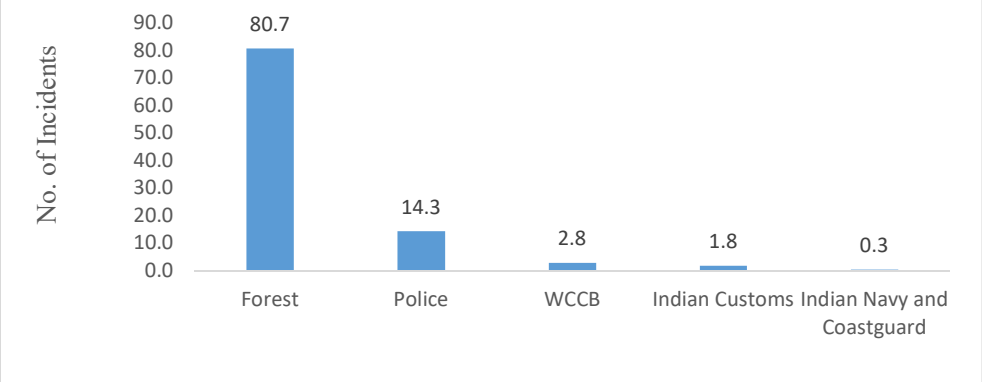


Fig. 3.3: Wildlife Offenses reported by various enforcement agencies

3.3.4 Season wise reporting of wildlife offense cases

Seasonal capture of trends of reported wildlife offences indicates a lull during the monsoon (August-September) as expected due to inaccessibility during monsoon but an unexplained dip was also observed during the month of November in overall data for the decade.

3.3.5 Major species reported in wildlife offense cases

The most sought after taxon in wildlife offences was found to be Mammals (76.6 %) followed by Reptile (10 %), Birds (8.6 %), Fish (2.8 %), Invertebrates (1.9 %), and amphibians (0.04 %). Even top ten most sought species were of mammals except one, Indian Peafowl.

A total of 66 mammalian species were reported in wildlife offenses during the last decade led by the wild pig (18.2%)and Leopard (14.7%) are top two species and represent most sought after species in herbivores and carnivores, respectively. Among all mammal's, four species, i.e. wild pig, leopard, chital and sambar constitute >50% incidents (N=3711). (Table 3.2)

Table 3.2: Representation of mammalian species in wildlife offense incidents

	Reported biological parts (% age representation among all mammalian species) (N=3711)										
Species/ Percent	Wild Pig 18.2	Leopard 14.7	Chital 11.6	Sambar 9.8	Nilgai 5.5	Tiger 4.8	Elephant 3.9	Hares3.8	Bear 2.5	Pangolin 2.4	Muntjak 2.3

3.3.6 Major mammalian groups represented in wildlife offense cases

Among the groups in mammalian species, deer are most common in wildlife offenses followed by large cats, pigs (including Andaman wild pig), antelopes and elephants as top five. Figure 3.4 shows mammalian group for which >1% offense incidents were reported

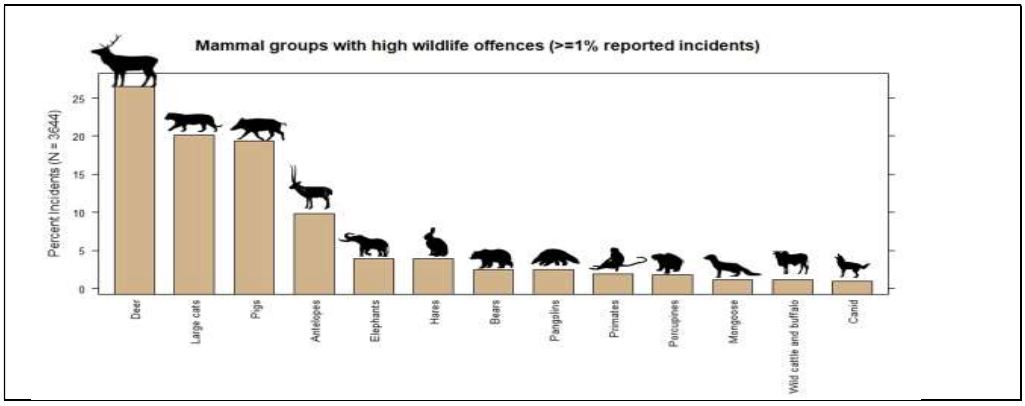


Fig. 3.4: Mammalian groups reporting >1% offense incidents

The data shows that Madhya Pradesh, Uttarakhand, Tamil Nadu, Uttar Pradesh, Chhattisgarh and Andhra Pradesh each reported> 5% of wildlife offenses against mammals in descending order during the reported decade. Offenses against tigers, one of the highly protected species has wide reporting with eight states reporting >5% incidents of the total reported incidents (N=178).

Leopards are the most sought after big cat species among four big cats in India and two Himalayan states of Uttarakhand and Himachal Pradesh were leading in reporting incidents against leopard along with Chhattisgarh and Madhya Pradesh which also reported > 5% of incidents against leopard. Wild pig (*Sus scrofa scrofa*) and Andaman wild pig (*Sus andamansis*) constitute the pigs group lead the reported incidents against herbivores. Pigs are mostly targeted for bush meat and for their canines for use in occult or ornamental purposes from almost all states having its population. Madhya Pradesh and Tamil Nadu were way ahead in reporting crime against this species than other states. Chital (*Axis axis*), Sambar (*Rucervus unicolor*), Nilgai (*Boselaphus tragocamelus*) and Asian Elephant (*Elephus maximus*) follows pigs in targeted killing of these species. Among herbivores, in bovid group, booked offenses shows Nilgai (*Boselaphus tragocamelus*) as most frequently reported whereas offenses

related to Black buck (*Antelope cervicapra*), Tibetan antelope (*Pantholops hodgsonii*), Chinkara (*Gazella bennettii*) and Four-horned antelope (*Tetracerus quadricornis*) are also common. Chital and Sambar are major contributors in incidents against deer group which also includes Musk and Mouse deer, although Mouse deer is reported in only few incidents.

3.3.7 Indian, exotics species and other wildlife offenses

The data analysis indicates that exotic species parts too are encountered in wildlife offense. Since there are reports of illegal trade of "*shahtoosh* wool" from Tibetan Autonomous Region (TAR) and no confirmed reported poaching of Tibetan antelope in India, it is grouped with exotic species parts with African Elephant ivory, Black Rhino horn, skin of Arctic fox, Reindeer, European hare, trophies of wart hog, chamois, teeth (Incisor and canines) of Hippopotamus and live exotic birds and turtles for pet trade. Nine hundred forty-eight suspected wildlife offenses from this study were categorized as "others" for reasons as mentioned in table 3.3.

Table 3.3: Exotic species in reported wildlife offense incidents

Wildlife Species	Total species reported	Total Incidents (5817)	Species
Indian	163	4801	Species as mentioned in schedule I-IV of WPA, 1972
Exotic	12	68	Tibetan antelope (56 incidents), African Elephant, African Black Rhino, Arctic fox, Chamois, European Hare, Hippopotamus, Reindeer, Scottish Red deer, Warthog, exotic birds and turtles
Not Applicable	Others	948	Attempt to poach-321, Domestic animals- 122, Fake article-66, No Opinion- 421 and college specimen's-18)- Cat, dog, Buffalo, Goat, Sheep, Pashmina, Cattle, Pig, Yak etc.

3.3.8 Representation of reptiles

Turtles are most commonly encountered sub-group among reptiles for pet trade and for meat. *Varanus spp.* are the other common sub-group among reptiles, majorly poaching for its hemi-penis also known as "Hattha Jodi" much sought in occult practices. Tamil Nadu, Uttarakhand, Uttar Pradesh, Gujarat and Maharashtra states were top five states in reporting incidents against reptiles.

3.3.9 Representation of Birds

Among birds, pheasants were most sought after group in wildlife offenses especially, Indian peafowl (*Pavo cristatus*) which constitutes >99 % of the reported wildlife offenses among pheasants. Other major sub groups of birds along with Indian peafowl, involves parrots and water birds (wading birds, ducks and rails) together constitutes >80% of total reported incidents from Gujarat, Madhya Pradesh, Rajasthan, Tamil Nadu and Uttar Pradesh.

3.3.10 Representation of aquatic species

Sea-cucumbers, sea-horses, sea- fans and other marine shells represent the major sub-groups in marine products seizures from coastal states like Andaman & Nicobar Islands, Tamil Nadu, Gujarat and Karnataka, except for Delhi which is a major hub for illegal trade in sea shells including finished products (e.g. *Trochus spp*.) and sea-fans, as ornamental/display article. Six states viz. Punjab, Rajasthan, Uttar Pradesh, Tamil Nadu, Madhya Pradesh and Telangana reported >5% of offenses booked for seizures against protected species of fishes and/or fishing inside protected areas and mostly involve fresh water fishes.

3.3.11 Wildlife offense cases without recovery of any wildlife part

Reported wildlife offenses not only involved seizures or illegal trade of wildlife articles but also involve enforcement of other provisions enshrined in Wild Life

(Protection) Act, 1972, fake articles seized or no opinion report was given due to contamination or wrong preservation of samples. Such cases make 15.72 % of the total wildlife offenses recorded for this study (N=915). It involves attempt to poach (5.5%), seizure of fake articles (1.1%), domestic animals (2.0%) and where no opinion was given (7.1%) due to contamination of sample, insufficient sample, wrong preservation or technique not available mainly for body fat.

3.4 Trends in the illegal *Shahtoosh* trade

The underfur obtained from highly endangered Tibetan Antelope (*Pantholops hodgsonii* Abel, 1826) also known as *shahtoosh*, is a highly valued commodity in illegal wildlife trade due to its rarity, softness and warmth. The illegal trade in *shahtoosh* is highly detrimental to the survival of this highly endemic species of Tibetan plateau may lead to extinction of this species (Schaller 1998, 2012; Fei *et al*., 2014). The Tibetan Antelope is highly protected and any trade is prohibited under Wild Life (Protection) Act 1972, and other international laws and treaties. Though the trade in *shahtoosh* is globally forbidden, but due to the high margin of profit and international demand the illegal trade in it continued worldwide. India, act as consumer for *shahtoosh* wool and as an exporter for finished '*Shahtoosh* products" (FDHA 2016). In order to avoid detection, the *shahtoosh* wool and its products were traded by traffickers by miss-declaring the consignments as *Pashmina*, *Cashmere* and *Shahmina* or by concealment in large consignments of lookalike woolen products. In the present study the wildlife offense incidents for two decades, i.e. from 2001 to 2010 and from 2011 to 2020 were compared for trends in *shahtoosh* trade, changing patterns of products and challenges encountered during analysis at Wildlife Forensic Conservation Genetics cell (WFCGC) of Wildlife Institute of India (WII).

Seizure data from wildlife offense referred to WFCGC for species confirmation along with details from Wildlife Crime Control Bureau (WCCB) and Indian Customs was studied for this purpose, as a two-pronged strategy involving lab and enforcement agencies both. A total of 120 incidents involving 1771 suspected *shahtoosh* products were studied in India from which were reported to WII for species identification. The decade from 2001 to 2010 involved 553 suspected *shahtoosh* products in 54 incidents, while 1218 suspected *shahtoosh* products were referred to WII from 2011 to 2020 in 66 wildlife offense incidents against Tibetan antelope.

Increased global demand for *shahtoosh* products was not only evident from the increased number of seizures but also in increased number of new demand destinations. Wildlife Institute of India is receiving suspected wildlife articles seized under wildlife protection laws from various enforcement agencies. Most of the *shahtoosh* products were detected at the entry or exit customs points. During the decade 2001 to 2010, 553 suspected products were seized in 54 incidents, whereas the incidents rose to 66 with 1218 suspected *shahtoosh* products seizures during 2011 -2020. During the two decades of data analysis, 1771 suspected *shahtoosh* products were referred to WII for analysis. Delhi remained the major reporting station for wildlife offenses in both the decades with Bengaluru (Karnataka), Chennai (Tamil Nadu), Dharamshala, Manali (Himachal Pradesh), Jaipur (Rajasthan) and Siddhartha Nagar (UP) also reporting suspected *shahtoosh* products seizures. During the period 2011-2020, a major shift is observed in seizures from Delhi that all the seizures happened at the customs exit or entry points by Indian customs or WCCB whereas in previous decade customs reported nil *shahtoosh* seizure incident. Amritsar in Punjab, emerged as another major transit point along with Delhi, for smuggling of suspected *shahtoosh* products through Land Customs, Integrated Check Post (ICP) Attari, Punjab bordering Pakistan during last years of the

decade 2011-2020, mainly because of closure of all international air routes due to Covid-19. Interestingly, seventeen shawls made of *shahtoosh* wool were seized from Thekkady in Kerala, while in four instances *shahtoosh* products were recovered at arrival of Delhi Airport in cargo/personnel baggage's while coming back from Almaty (Kazakhstan) - 5 shawls, Dubai (UAE)- 15 shawls, Riyad (Saudi Arabia)- 32 shawls and Washington (USA)- 25 shawls, while returning back to India as unsold part of *shahtoosh* products after selling/ participating in trade fairs, clearly pointing the *modus operandii* of smuggling of *shahtoosh* products in parcels or in personnel baggage's .

While during the decade 2001-2010 CBI, Delhi Forest Department and WCCB made all the suspected *shahtoosh* seizures, during the next decade, it was mainly by Indian Customs and WCCB. During the period 2001-2010, majority of suspected seizures were held in Indi or were meant for Europe, Middle east Asia or North America, but the quantity of consignment was very less and usually in personnel baggage's, but during the period of 2011-2020, new markets emerged with Mauritius in Africa, Kazakhstan in Central Asia, Hong Kong, Beijing and Tokyo in East Asia, Muscat, Dubai, Doha, Kuwait in Middle east Asia and Lahore in South Asia. The data from 2011-2020 clearly shows extension of demand for *shahtoosh* product in Mauritius in Africa and Asian countries.

There is an observed shift in demand for type of *shahtoosh* product also. While during the period of 2001-2010, the highest number of *shahtoosh* products detected were 74.68% shawls, followed by *shemagh* 15.01%, scarfs 6.69% and others 3.62%. On the other hand, square or *Shemagh* came into more demand during 2011-2020 with 52.8% share in all seizures followed by shawls 29.1% and scarf 17.1% (Table 3.4). Comparison shift between trends in demand among *shahtoosh* fabric in last two decades, which clearly shows a shift in demand for square/*shemagh*, and scarfs.

Table 3.4: Type of *shahtoosh* product in demand

Decade/Type of *shahtoosh* product	Square/*Shemagh*	Shawl	Scarf	Others
<2010	15.01%	74.68%	6.69%	3.62%
>2010	52.8%	29.1%	17.1%	0.3%

These is a specific shift in color preference in later decade (2011-2020) under study as beige color losing its sheen to Ivory, dark colors, and multi-color patterns. Embroidery also saw a big shift with plain shawls with no embroidery giving way to heavy embroidered *shahtoosh* products, mainly E1, E2 pattern for Square/*Shemagh* fabrics. Use of heavy threads of gold and silver was also observed along with prints, deviating from original Kashmiri embroidery pattern of buta/petals. The weaving is also finding new experiments with Cheveron, and towel live weaving along with traditional Bulbul eye or mosaic and plain weaving pattern (Table 3.5)

Table 3.5: Comparison of pattern of color, embroidery and weaving in *shahtoosh* products

Color	Brown	Ivory	Red	Blue	Green	Black	Multi	Yellow	Double	
<2010	71.0	10.5	6.4	5.5	2.4	2.2	1.7	0.4	0.0	
>2010	34.9	17.2	11.2	17.6	8	4.4	4.5	1.4	0.7	
Embroidery	E0	E1	E2	E3	E4	E5	E6	E6	E6+	Print
<2010	53.3	14.3	10.8	9.9	3.3	5.5	2.0	0.2	0.6	0.0
>2010	23.6	22.8	24.2	18.6	2.6	2.5	2.9	0.0	2.2	0.7
Weaving pattern	Chevron	Cloth	Mosaic	Plain	Turkey Towel					
<2010	0	0	2.5	97.5	0					
>2010	0.2	0.1	5.9	93.4	0.3					

In the present study the data of 2001-2010 and 2011-2020 was analyzed to identify the major destination of *shahtoosh* products (Fig. 3.6). It was observed that

>90% seizures happened in Delhi (85.7%) and Dubai (5.4%) during 2011-2020 while in next decade 90% seizures were reported from nine destinations including Muscat (23.15%), Lahore (22.82%) and Dubai (20.85%) making above 20% seizures (Table 3.7).

Table 3.6: Demand destination for *shahtoosh* products

	Region	Africa	Central -Asia	East Asia	Europe	Middle east	North America	South Asia
<2010	Incidents	0	0	0	4	1	1	0
Destination		NA	NA	NA	Bern, Madrid, Paris Rome	Dubai	Washington	NA
>2010	Incidents	1	1	3	5	4	1	1
Destination		Port Louis	Almaty	Beijing, Hong Kong, Tokyo	Bern, London Frankfurt, Madrid, Milan	Doha, Dubai, Kuwait, Muscat	Washington	Lahore

Table 3.7: Comparative increased markets for *shahtoosh* products in last two decades

<2010	Delhi	Dubai	-	-	-	-	-	-	-
	85.7	5.4	-	-	-	-	-	-	-
>2010	Muscat	Lahore	Dubai	Beijing	Hong Kong	Doha	Delhi	Bern	Washington
	23.15	22.82	20.85	5.91	5.83	4.52	3.61	2.87	2.3

Based on the analysis of the data submitted along with the evidences in lab, specific demand for different types of *shahtoosh* products namely Square or *Shimagh*, Shawls, Scarfs/stoles, and other fabrics were specific to destination. It is evident from Table 3.4 that there is a definitive surge in the demand of Square or *shemagh* (52.79%) made of *shahtoosh* during the later decade 2011-2020 in comparison to previous decade 2001-2010 (15%) of total seizure, in direct relation to opening of new destinations in East Asian and Middle-East Asian countries. *Shemagh* or square

shahtoosh product is most common (Ca 84%) in Middle- east countries (Oman, Qatar, Saudi Arabia and UAE), which can be linked to the traditional head gear sported by men in these regions (*Amjad, & Sameer, 2019)*. In East Asian countries demand for *shahtoosh* emerged only during 2011-2020 with *shemagh* or square type product (53%) and stole/scarf (39%) in reported seizures. Stole/scarfs are much in demand in East Asia and in Europe. It was observed that among the varied *shahtoosh* products seized the *shemagh* scarf or square shawls were usually bound for Western Asia (Oman, Qatar, Saudi Arabia and UAE).

Therefore, we can see that the demand for square/*shemagh* and stole/scarf is increased during the period 2011-2020 in comparison to previous decade with main market in Middle-east Asia, East Asia and Europe. The analysis of data from various cities of India indicated demand in tourist destinations or in big cities. Also, the high percentage of seizures of *shahtoosh* products during 2001-2010 outside the customs area in Delhi can be explained by most of the seizures were made by CBI or Delhi Forest Departments and very few by customs/WCCB at the airport. Analysis of data from south Asia which involved only Lahore in Pakistan was not analyzed due to lack of concrete information about its final destination whether Lahore is the final destination or some other destinations in Middle east or Europe during COVID-2019 as international passenger and cargo flights were at standstill.

Also noticeable trend observed in the analysis data was perceivable decrease in purity of *shahtoosh* shawls measured by presence/absence of guard hair of Tibetan antelope found weaved in the suspected *shahtoosh* products. The percentage of non-availability of Tibetan guard hair in suspected *shahtoosh* products (NSh) shot up from 17.2% in 2001-2010 period to 29.6% during the later period while the pure *shahtoosh* products reduced from 73.8% to 47.3%. This trend either shows better technology to

remove guard hair from the wool before weaving or due to large scale mixing with other wool mainly of *pashmina* goat. Usually small amount of *pashmina* is mixed with *shahtoosh* to give strength to the product, but during 2011-2020 large scale mixing was observed in with 25% purity for 3.1% products, 50% for 8.5% and 75% purity for 11.5%.

In recent years the dynamics and nature of the illegal wildlife trade has transformed significantly. The burgeoning demand for *shahtoosh* products worldwide is seriously impacting the very survival of the existing populations of the Tibetan Antelope, in their distribution range. The analysis presented in this study has highlighted the extent of seizures in *shahtoosh* products in India from 2009 to 2020. The present study not only provides the first empirical detailed countrywide assessment of illegal wildlife trafficking of "*shahtoosh*" products, destinations, but also deduced the major trends available regarding probable destination for specific type of *shahtoosh* fabric, weaving and embroidery pattern and provided an assessment of guard hair present in the submitted product to get a reasonable idea of the purity of the *shahtoosh* product. The part findings of this study were published (Pragatheesh *et al.* 2022) as "Operation Soft Gold – Integration of cyber intelligence in curbing illegal *shahtoosh* trade in India".

Sufficient guard hair for the analysis of microscopic hair characteristics were found in 82.8% (N=458) suspected *shahtoosh* products during 2001-2010 while only 70.4% % (N=858) are with different level of contamination/mixing but having sufficient guard hair as required for microscopic hair analysis protocol. Since the technique for identification of species is by microscopic analysis of guard hair present in the shawls, the suspected *shahtoosh* products which are found devoid of any guard hair remained a challenge for identification. As the wool of Tibetan antelope in being

mixed with other wool also, like of pashmina and also while weaving raw wool is combed to remove coarser guard hair, washed and treated with chemical/soap many times to make it soft before weaving, resulting in destruction of hair, molecular technique for identification of species through wool hair is found with limitations for forensic studies Part of the findings were already published (Gupta *et al.* 2022) in peer reviewed journal. Therefore, efforts were also taken up to identify the species by analysis of wool hair based on Infra-red technique and success was achieved in that, as discussed in other chapter.

3.5 Conclusions

This study contributes to the growing understanding of wildlife crime related research that has identified and explained major trends and patterns of crime against wildlife in India during the last decade. Major conclusions are as follows:

i) While overall the offense cases reported for the decade usually remain stable, there is slight variation in reported cases from each state during the years of the decade, requiring enforcement agencies to be a persistent in implementation of wildlife preservation regulations. Sixteen states reported >5% of total reported wildlife offenses in India during 2011-2020, with Uttarakhand reporting >5% wildlife offenses (9 times), Uttar Pradesh (7 times), Madhya Pradesh (6 times) and Tamil Nadu (4 times). The reports need not necessarily relate with the proportion of illegal trade in wildlife products. Top five cities/districts reporting maximum wildlife offenses during the decade includes Delhi, Erode, Coimbatore, Tirunelveli and Dehradun. The above areas not necessarily represent the poaching areas but also collection, or social and cultural centers.

ii) The State Forest Department's reported maximum wildlife offenses, followed by police including Central Bureau of Investigation (CBI). Indian Customs

reported majority of cases from Indira Gandhi International Airport, Delhi. Indian Navy and Indian Coast Guards intercepted sea cucumbers and sea horse consignments in high seas and reported through forest department of Andaman & Nicobar Islands, during mid of the decade but there is a lull in recent years. The seasonal trend observed indicate less reporting during monsoon season which can be explained due to inaccessible forest roads and other reasons but an overall slight dip of seizures in the month of November has to be explored further.

iii) Mammals were the most prevalent group in all reported wildlife offenses with 76.6% share followed by reptiles (10%) and birds (8.6%). Wild pig and leopard are the top two represented animals in the wildlife crime database. This study on wildlife offenses found that illegal trade in wildlife is very dynamic and certain species disproportionately coming in only specific forms (i.e. TA-shahtoosh products, Mongoose - painting brush), are at variable risk status, in various taxonomic groups, from particular export regions and offenses show a seasonal pattern also.

iv) Large quantity of seizures of marine animals especially sea horses and sea cucumbers needs special attention from marine enforcement agencies to avoid any threat to large scale extermination of these species. With the expansion of new International routes, airports and seaports tend to facilitate greater illegal wildlife imports (exotic pet trade) and exports (*shahtoosh*, star tortoise).

v) A peek into the invoices of *shahtoosh* seizures meant for exports, indicate that many consignments although seized at IGI, New Delhi, were booked from Jaipur, Sawai Madhopur and Jaiselmer towns in Rajasthan, which is very unexpected.

vi) Specific pattern and equipment's used to poach a wild animal was observed, using firearms for large herbivores (Elephant, Rhino, Nilgai) and water birds, snare/jaw traps for big and lesser cats, wild pig, pheasants/birds, hunting dogs for cervids, pangolins, monitor lizards, potash bomb for wild pig, plant poison (*Aconitum spp*.) for elephant, tractor tubes with net for illegal fishing in protected areas.

vii) Although, around 50% of wildlife offenses have one suspect, but those cases are overwhelmingly trade related where carrier was apprehended. For committing poaching, it is almost always more than one person is involved. Due to magnitude of seizure data, all India analysis shows the trends and pattern are less to cater the international illicit trade and more likely a reflection of socio-cultural demands for certain products and opportunistic crimes including illicit trafficking of wildlife parts.

viii) This study not only has highlighted varied numbers of charismatic species involved but also targeted demand for lesser known species like the golden jackals is brought to fore which is heavily poached all over India for *"Jackal's Horn*"

Chapter 4:

MORPHOLOGICAL ANALYSIS OF ANIMAL PARTS FOUND IN ILLEGAL WILDLIFE TRADE

4.1 Introduction

Charles Darwin, described morphology as the "*study of form and it is soul of natural history*" (Gans 1985). Based on random evolutionary processes, new structural modifications happen in overall morphology of an organism, that are useful to remain adaptive in changing ecological situations. These specific structural or morphological characters in a species have an intimate association with vital survival functions i.e. feeding (dentition) and locomotion (gait pattern/running), for that species, which in turn influence its ecological interactions and natural selection for resource use, important for its existence and propagation (Bock & von Wahlert 1965; Arnold 1983; Christensen 1996). Morphology has evolved from detailed anatomical descriptions of organismal structures, to descriptive morphology for use in natural history descriptions, phylogenetic systematics and understanding evolution of specific traits in species. Morphological identification of species from biological evidences submitted, an essential step in wildlife crime investigations, uses the characterization of specific functional traits evolved during interface of an organisms with its environment in evolutionary process (Trail 2021).

By comparing the physical characteristics of submitted biological evidences with the repository samples, a forensic morphologist identifies wildlife parts and products and assign the family, genus, or species to which it belongs. Most analyses performed by a wildlife morphologist are based on class characters (family/genus/species level), and not individual characters. Individualization, in contrast, requires the recognition of characters uniquely identifying a particular individual.

4.2 Selection of animal parts and statistical methods

Crime against wildlife is very dynamic and involves a large number of species from various taxonomic orders. Also, the illegal trade in wildlife involves targeted poaching of wildlife in live, their parts and processed or derivative forms, making their detection and identification difficult for enforcement agencies.

4.2.1 Selection of species and wildlife parts

Selection of species and targeted parts studied for this study was done based on the review of available data at Wildlife Forensic & Conservation Genetics cell (WFCGC) of Wildlife Institute of India (WII) and published information accessed through Google Scholar search engine using key words relevant to illegal wildlife trade and wildlife crime in India. The selection of wildlife parts is also aimed at filling up the gaps in WII's ongoing research and development program (1995 to present) for development of protocols for identification of wildlife parts and derivatives encountered in wildlife offenses. Information obtained through informal interviews and discussions with Forest Department as well as other enforcement agencies officials were also synthesized, leading to selection of parts and species. The availability of sufficient samples for a particular species is also one of the key factor for undertaking study on those parts.

4.2.1 (a) Mandible of tiger, leopard and hyaena

Targeted poaching or large carnivores, mostly felids for their bones to fulfill the demand in traditional medicine pharmacopeia, has emerged as one of the most serious threats to already fragile status of these species world over (Mills & Jackson, 1994). Bones of Tigers (*Panthera tigris*), highly demanded for use in traditional medicines or for health tonics, were often received at WFCGC of Wildlife Institute of India (WII) for species identification. Recently, due to the heightened vigilance by enforcement agencies and scarce number of remaining wild tigers, the

demand has widened and seizures (n=11) have been reported to WII where tiger bones are found with cranium missing and mixed with bones of Leopard (*Panthera pardus*) (n=8) or Hyaena (*Hyaena hyaena*) (n=3). In 13 cases, bones including skull of Tiger and leopard were seized together. Also, the increasing use of steel jaw traps for trapping of large carnivores, and killing the animal by striking its skull with a wooden log, is resulting in broken skull and mandible (n=34) making identification a challenging task for forensic scientists. From a heap of bones, morphological analysis of complete mandible based on dentition has long been used as a fast and reliable method for species identification but it becomes wanting when mandible is found in fragments, with teeth missing due to long period of burial, ante or post-mortem injuries or canines removed to be sold separately to get more profit.

Complete known mandibles (n=5 each) of adult Tiger (*Panthera tigris*) and Leopard (*Panthera pardus*), and four mandibles of hyaena (*Hyaena hyaena*) were taken from repository of WII to characterize mandibular bone of above species in the present study.

4.2.1 (b) Cervical 1 (C1) bone of cranio-vertebral joints of tiger, leopard, hyaena and sloth bear

Bones or skeletal remains have been used to identify species, sex, probable age or manner of death since long in paleontology, Forensics, and in Anatomical studies. However, most of the work was done on cranial or skull and long bones but the importance of vertebrae has been usually neglected (Boonsri *et al.*, 2021). Although closely related species have similar basic bone features and design but structural variations occur to cater the specific performance requirements for feeding and survival (Arnold 1983; Wainwright 1994). The predatory performance in felids is directly related to the mode and size of prey capture and reflected in the cranial-mandibular morphology with larger felids having relatively larger gape and increased bite force for restraining the struggling prey than small felids (Christiansen & Adams 2008; Slater *et al.* 2009; Christiansen

& Harris 2012). A rapid and non-destructive method, ATR-FTIR spectroscopy combined with chemo-metrics (Sharma *et al.* 2019) was successfully applied to identify the species from wildlife parts, but like molecular and other techniques it also requires lab based analysis and interpretation of data. The simple and easily accessible morphological identification of complete bones through physical characteristics, morphometric measurements and comparison with reference material is one of the quick, reliable and crucial aspects for scientifically determining the species in wildlife forensic investigation (Sims 2012). Closely related species need a combination of morphological diagnostic features for comparative analysis (Christiansen 2006). One of the most serious threats presently to large carnivores especially to tiger and other felids survival is the use of their bones in traditional Asian medicine. With the dwindling population of tigers resulting in scarcity of supply, it has been noticed that the poachers are killing other large carnivores/omnivores and trying to pass their bones as original tiger bones. Therefore, morphometrical characterization of skeletal remains through identification of first cervical vertebrae, C1 was undertaken as a tool for identification of species in wildlife forensics.

The suspected bones of tiger, leopard, hyaena and sloth bear seized in illegal wildlife trade and submitted for forensic identification at Wildlife Institute of India (WII) make up 6.5% (N= 4046) of the total case submitted. The skull is specialized for providing support and function of top predators (Christiansen & Adams 2008). Along with the skull the cranio-vertebral joint of first cervical (C1 bone), work as a coherent mechanical unit augmented well for extra stability and enhanced mobility (Goel *et al.* 2011). From a heap of bones, therefore species and number of animals involved can easily be identified based on the denture and profile of skulls present in the seizure (Pocock, 1939). But often the seizures are devoid of the skull and other major bones making it difficult for field enforcement officers and forensic morphologists to identify the species. The usefulness of C1 or atlas bone is often left neglected in identification of species in wildlife forensics

especially when seizures lack major bones and bones of different species were mixed. Despite tiger and leopard being top predators, hyaena being scavenger and sloth bear an omnivore, the basic pattern of structure and joint remains similar in all four species, but to accommodate the specific functional requirements of the animal the structural variations in the **C1** in relation with cranio-vertebral joint were compared. Here, we describe the general morphology of C1 vertebrae and compare it between Tiger, Leopard, Hyaena, and Sloth to characterize species for use in Wildlife forensic investigation.

4.2.1 (c) Canines of Tiger, leopard and fake canines

Teeth are present in all mammals except few specialized myrmecophagus such as aardvarks and pangolins. Depending on specific function they perform, the teeth can be classified into four types, an incisor, canine, premolar, or molar which collectively involves structure, kind, number, and arrangement of teeth to form dentition of an animal. Collectively the main function of the dentition is to hold the food or prey and prevent its escape from the mouth (Feldhamer *et al.* 2015). Morphologically a tooth can be divided into two main zones: a portion above gum line called *crown* and a section below the gum line is known as the root. In between crown and root, lies the *neck*, the portion that remains embedded in gum. Crown is covered by the *enamel,* the hardest mineralized tissue found in the body, and the root is covered by the *cementum*, which is attached to the periodontal ligament. In a tooth, the dentine makes up the bulk of the structure, surrounding a central cavity consisting of pulp, which is the living tissue that contains nerves, blood vessels, and odontoblasts (Feldhamer *et al. 2015*) for growth and development of tooth.

Canine teeth, four in numbers in carnivores are spear-like projections, posterior to incisors and are usually elongated, pointed, single rooted and with a conical sharp mono-cuspid crown. Root of canines may either found closed or open. The open root in a canine indicate either it is a milk or deciduous teeth or a permanent tooth that is still growing. In a permanent canine tooth, the

root end is found closed restricting further growth. The jaw bones which bears the teeth, surround and fix the root of canine tooth firmly by a layer of cement and a vascular peridontal ligament, giving the root a rough surface. Canines in carnivores are ideal for grasping, piercing, tearing off flesh with great pressure, and for defense (Bhamrah & Juneja 1990; Valkenburgh & Ruff 1987). In all carnivores there two sets of canines based on their position, the maxillary or upper dentition and the mandibular or lower dentition. In herbivores, canines have usually been lost, but in some animals these are present i.e. equids, few cervids, but greatly reduced in size and number. Rodents and Lagomorphs do not have canines, but have long incisor's. (Feldhamer *et al. 2015*).

The characterization of canine is an important aspect to identify the particular species in trade from a heap of biological evidence lying in front of a wildlife morphologist. By characterization of dentition, considering clues it provides regarding the diet of the animal, not only the group can be classified in certain animals (Bhamrah & Juneja, 1990), but also species can be deduced by morphological and morphometric techniques for use in zoo archeology, wildlife forensics and conservation biology studies. The basic purpose of this study is to provide a basic scientific protocol to both, the enforcement agencies for detection and to a forensic morphologist for characterization of species of a canine referred as a forensic evidence.

Big cats (Sub-family Pantherinae) by virtue of their inherent charisma but delicate status globally, finds special attention for them in illegal wildlife trade. There is particularly huge demand for Tigers and their parts have in trade since historical times for many reasons. All big cats are placed in Schedule-I of the Wild Life (Protection) Act, 1972, prohibiting trade of their body parts. Also, all big cats, except the African lion, are listed in CITES Appendix I, meaning international commercial trade in these species is prohibited. Canines of tiger and leopard, are commonly found in local trade as separated, pendants, magical amulets for good luck charm, witch-craft and other occult practices, fixed with the skull as trophies, ornaments, necklace, souvenirs (Mukherjee 1996;

Martin 1992, 1997; Martin & Phipps 1996; UNODC 2020). It has been observed that in many instances, fake canines made of non-biological material or of some domestic animals, also found their way in grey markets and traded as tiger canines.

Although fake canines, made up of non-biological material can be differentiated by careful morphological analysis, like synthetic material used, show less or no wear and tear, no demarcation between root and crown, under low magnification or x-ray radiograph, unnatural bubbles or craters can be sighted, but once these have been seized as suspected wild animal's canine, forensic morphologist has to prove it beyond doubt as a pre-requisite of justice.

4.2.1 (d) Musk pod

Musk pod, as it is known in illegal wildlife trade is found as a dried round, oval to semi-circular pouch/sac in wildlife offense seizures, is the preputial gland of the sexually mature male musk deer (Moschus spp. Moschidae) (Green, 1986, Sokolov *et al.,* 1987, Sathyakumar *et al.,* 2015). In India, Musk deer is distributed in Jammu & Kashmir, Himachal Pradesh and Uttarakhand in Western Himalayas and Sikkim in Eastern Himalayan ranges which together belongs to one of the four biodiversity hotspots in India and are treasure trove for a range of biota (Venkataraman, & Sivaperuman, 2018). The musk, an odiferous secretion from the preputial follicle of sexually mature male musk deer remains one of the most valued and expensive sought after natural products in the world since long for its distinct fixative and scent properties (Seth et al., 1975, Sanjeev et al., 2015), leading to substantial poaching of this species.

The extensive poaching of all musk deer populations to illegally exploit musk to cater the escalating demand musk in expensive perfumes and in Traditional Chinese medicine (TCM's) pharmacopoeia and other indigenous systems of medicine in many parts of the world (Bensky *et al.,* 1993; Mills & Jackson 1998; Homes, 1999), along with habitat fragmentation and degradation

due to anthropogenic pressure has led to decimation of all populations or local extinction (Green 1987). There is an enormous demand for musk in traditional Chinese and Korean medicine with around 300 pharmaceutical preparations have musk as a major ingredient (Mills & Jackson. 1998). The ethno zoological practice using different animal parts and their products for siddha/folk medicines by tribal populace in few states in North-East and South India is well documented. The use of musk ("Kasturi" in Hindi) is quite known to ancient Indian practitioners of indigenous system of Ayurveda medicine and surgery (Ray et al., 1980; Meulenbeld, 1999).

The Himalayan musk deer (*Moschus spp*.), is included in the schedule I of the Wildlife (Protection) Act, 1972 and all musk deer species *Moschus* spp. have been included in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1979, *(CITES 2016).* The International Union for the Conservation of Nature and Natural Resources (IUCN) lists it as a near-threatened species.

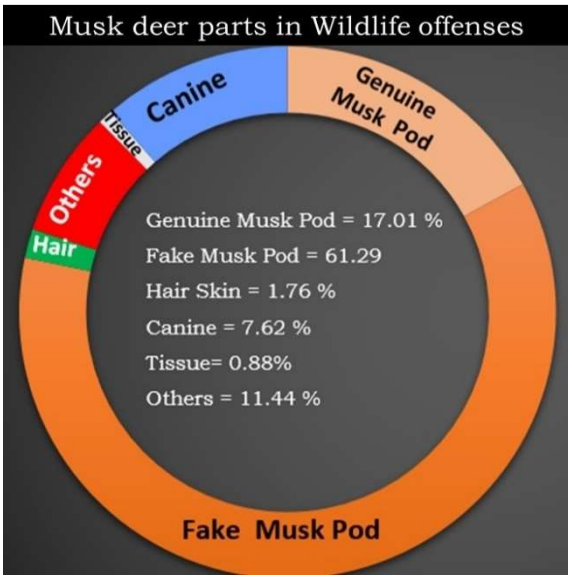


Fig 4.1: Musk deer parts found in wildlife offense cases

The high demand and value but limited supply of musk for ethno-zoological practices and use in traditional medicines has led to widespread adulteration and fake products by illicit traders like any other illegally harvested wildlife part (*UNODC 2020, Homes 1999).* Musk deer are usually poached for their musk gland but their meat and canines are also frequently encountered in seizures. Wildlife Institute of India (WII) is regularly receiving suspected musk deer glands for species identification from all over India. The genuineness of musk gland for forensic identification requires knowledge of the morphological,

molecular or chemical protocols and their informative significance in the court of law. For developing forensic identification protocols of musk gland, baseline information on seizures forms and types is used from the database of WII and scientific protocol based on microscopic hair identification is proposed.

Wildlife Institute of India (WII) has received 63 wildlife offense cases involving suspected musk deer parts (N=341) for species confirmation, involving 45 wildlife offense cases of suspected musk deer pods (N=267) seizures. A detailed study of physical characteristics of all genuine (N=58) musk pods indicated that most of the pods were lacking sufficient hair required for analysis by microscopic tools, as the poachers or traders usually shave or singe the surface hair of the pod to avoid detection. Thus, developing protocol for identification of such case property by easily accessible tools were required for enforcement and scientific agencies.

The suspected musk pods lacking surface hair were cut open from one side, to spread it open, and the hidden hair on the inverted edge of constricted orifice and loose hair embedded in the musk contents were collected for microscopic analysis (Brunner and Coman, 1974*)*. The microscopic structure was viewed and microphotographs were taken at 200-400X magnification under 'Optika" compound microscope (Optika B-383 PLi) and images grabbed with "Proview" digital camera software. The microscopic structure of hair was than compared with reference hair of musk deer skin (n=2) and hair from musk pods (n=4) provided to WII repository by Uttarakhand Forest department, were taken.

4.2.1 (e) Suspected "Jackals horn" or "siyar singhi"

International illegal trade of parts of few charismatic species such as tiger, rhinoceros, snow leopard, and elephants are widely reported, but, illegal trade in body parts of lesser-known species receives little attention leading to the decline or local extermination of such species (Douglas &

Alie 2014). Illegal trade of parts of monitor lizard's (*Varanus spp.*), pangolin 's (*Manis* spp.), and golden jackals (*Canis aureus*), are widely encountered in illegal wildlife offenses in the South-east Asia *(*Aiyadurai A 2011; Mendiratta *et al.* 2017; Sharma *et al.* 2019; Chawla *et al.* 2020). Along with genuine wildlife parts, imitated wildlife parts such as fake claws, canines, skin, elephant tusk, musk pod, and rhino horns are also found in plenty to mint huge profit from high demand in comparison to short supply (Martin and Stiles 2000; Sharma *et al.* 2016). These fake articles are either made up of non-biological material or biological material obtained from domestic or other less charismatic wild species. Rampant poaching of the golden jackal is widely reported in India for meat, and other body parts (Chawla *et al.* 2020). Jackal is listed under the "Least Concern" category in IUCN Red List due to its stable population and wide distribution. Nevertheless, in India, it is protected under Schedule II of Wild Life (Protection) Act, 1972 (WPA), and Appendix III of CITES (Convention of International Trade in Endangered Species of Wild Fauna and Flora). The major threat includes poaching for "Jackal Horn" (locally called Siyar or Gidar singhi), a horn-like protrusion or deformity behind the sagittal crest of this species. Jackal horns have been traded surreptitiously by the quacks, astrologers, and sorcery practitioners for a long time and more recently through social media (Chawla *et al.* 2020).

A total of 342 seized jackal horn samples belonging to eleven wildlife offense cases were sent to the Wildlife Forensic and Conservation Genetics (WFCG) Cell of Wildlife Institute of India (WII) by law enforcement agencies for species identification. External characteristics and hair morphology examination Morphological examinations of the "horns" were noted based on physical characteristics, including the shape, weight, hair color, and hair bands on hair follicles. Subsequently, the "horns" were cut open, and information on the type of material used as filling along with attachment types was also recorded. The jackal horn samples were further pooled based on the abovementioned morphological characteristics, and 30 representative samples were taken

for the microscopic hair analysis. Cuticle and medullary patterns of all the 30 hair samples were observed using methodology as detailed in the earlier study (Singh *at el*. 2020). The cuticle and medullary patterns were compared with the hair patterns of wild and domestic animals available in the repository of WFCG Cell, WII (Bahuguna & Mukherjee 2010).

4.2.2 Morphological and Statistical approaches

Since wildlife crime involves a large number of species from different taxonomic orders and in various forms, a single of few technique is not sufficient for detection and identification of wildlife species involved. Mandibles of selected species were differentiated morphologically by comparative anatomical studies, while cervical 1 bone and canines were differentiated among closely related species involving morphological and morphometric approaches. Shaved or singed musk pods were identified by employing physical characteristics and microscopic characterization of opportunistic collected guard hair from inside the musk pod while the skin of species involved in making pods of suspected "jackal's horn" were characterized by physical and microscopic tools while the wildlife parts in the pod contents were further confirmed by molecular technique.

The variables for statistical analysis were selected based on peer reviewed scientific work (von den Driesch, 1976, Christiansen & Harris, 2012), and positively correlated variables were selected for obtaining classification tree (Breiman *et. al.* 1984). The classification tree approaches are computationally intensive but comparatively recent in wildlife forensics for differentiating species from wildlife parts, especially bony remains in seizures. Nevertheless, classification tree is found very useful in differentiating the bony remains of closely related species.

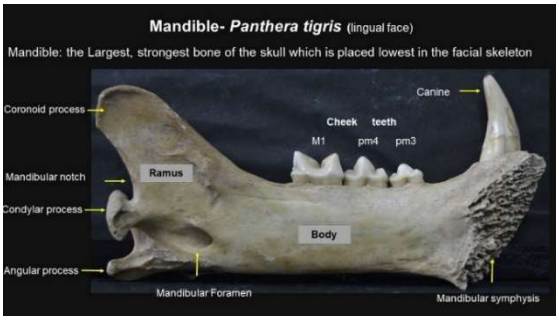
The study and reference material for anatomical comparisons were obtained from repository of WFCGC.

4.3 Result and Discussion

4.3.1 Morphological characterization of mandible of tiger, leopard and hyaena:

Morphological characterization of mandibular bone of above three sympatric carnivore species will not only provide a cheap, fast and reliable forensic tool for differentiation of species while dealing bones in wildlife forensic studies but also will allow early detection of species during seizures, to book the offense under stipulated schedule of Wild Life (Protection) Act, 1972.

Fig 4.2: Mandibular fragments from multiple species seized in wildlife offense case



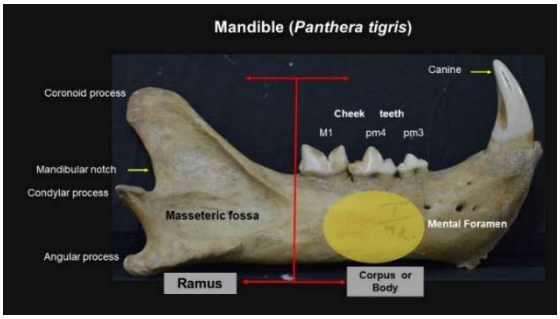


Fig. 4.3: Mandible bone parts

Table 4.1: Comparative morphological study to differentiate mandible of tiger, leopard and hyaena

If only anterior part of mandible is available: Position of mental foramen

Species	Mandible Pic	Description
Tiger		More than two mental foramina present, if a line is drawn from posterior most foramen to alveolar border the line always meet at the anterior part of the premolar
Leopard		More than two mental foramina present, if a line is drawn from posterior most foramen to alveolar border the line always meet at the anterior part of the premolar.
Hyena		There is only one large mental foramina present, if a line is drawn from this foramen to alveolar border the line always meet at the centre or posterior of 1st premolar.

Comparative morphology- Mandible Ramus

Species	Photograph	Character	Observations
Tiger		Shape of Coronoid process	Sharp, makes a "V"
		Angle of Mandibular notch	Massive, below the lower margin of condylar process
		Mandibular Foramen	
Leopard		Shape of Coronoid process	Makes almost 90^0 but lower margin vanishes abruptly over condylar process
		Angle of Mandibular notch	Massive, below the lower margin of condylar process
		Mandibular Foramen	
Hyena		Shape of Coronoid process	Broad, makes a "C"
		Angle of Mandibular notch	Reduced, pointing above the upper margin of condylar process
		Mandibular Foramen	

Comparative mandible morphology- Complete half

Species	Photograph	Character (Ventral border)
Tiger		Ventral border is concave, so it touches the ground only at its extremities.
Leopard		Ventral border slightly convex, so it touches the ground at the middle, and also rest on the proximal end.
Hyena		Ventral border is convex, touches the ground at the middle and the proximal end is raised.

Temporomandibular Joint

If the mandible is broken and only anterior part is available: Position of mental foramen on rami

Species	Condyle Pic	Description
Tiger		Condylar process has smooth articulating part and rough non articulating part laterally. (arrow in the photo)
Leopard		Condylar process has only smooth articulating part
Hyena		A sharp oblique ridge is present which actually demarcation between smooth articulating and rough non articulating part.(arrow in photo). Thick at the centre (Black arrow)

4.3.2 Characterization of C1 or atlas bone among various species

The analysis of variation among living organisms fundamentally involves the concept of size and shape. The morphology of the organism is more reliably predicted by the shape of the bone than the size of it (Jolicoeur & Mosimann 1960) as biometrical variation in size of most organisms is more affected by external environment/ecology than their shape (McCoy *et al.* 2006). Therefore, there remain ambiguity morphological dataset while differentiating size with shape in multivariate analysis (Claude 2008). To overcome this, we addressed the issue by z transforming all the measurements for standardization or auto-scaling of data with mean = 0 and standard deviation =1 to make z-Scores comparable.

Summary statistics of the variables were calculated. Bivariate correlations between the variables across all species pooled and as well as separately for each species were examined. Exploratory Factor analysis was conducted to examine the composite structure of the measured variables (dimension reduction) and to see if they help in segregation of species. All variables were Z-transformed (zero mean and 1 S.D) before conducting the Factor analysis. Three Factors were extracted after Varimax rotation, these explained 83% of the variation. Factor scores were calculated through the regression method and saved for further graphical analysis. A decision tree (Breiman *et al.* 1984, De'ath & Fabricus 2000) was constructed using tree partitioning algorithms.

All analysis was conducted in R statistical software environment v 3.6.3 (R Core Team, 2020). Graphical examination of correlations was done using the library "corrplot" **(**Wei & Simko 2021). Factor analysis was conducted using the native routine included in R. Tree partitioning and classification was conducted using the "rpart"

library **(**Therneau & Atkinson 2001). Plotting of the decision tree was done using the library "rpart.plot" (Milborrow 2021).

1st Cervical vertebrae- Measurements (Tiger)

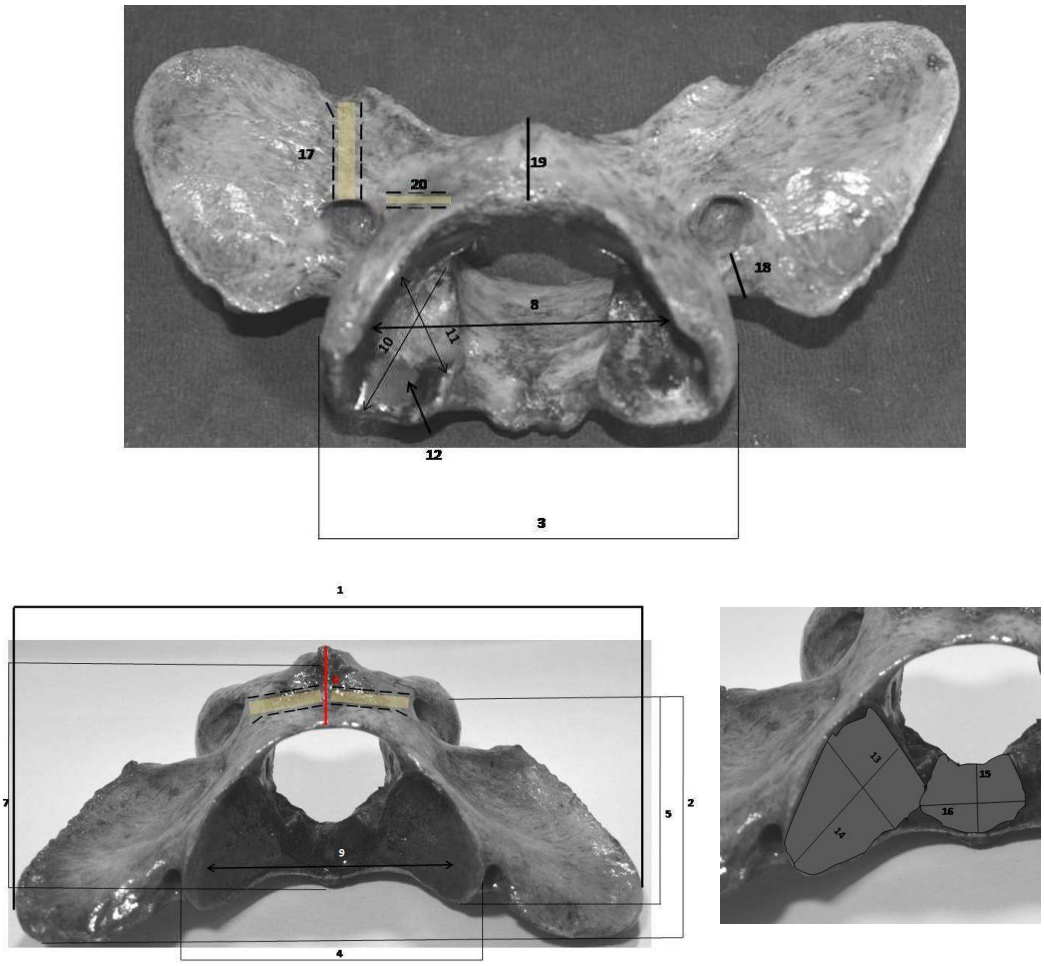


Figure 4.4 a, b and c: C1 bone measurements- 1- Greatest breadth over the wings, **2**-Greatest Length over the wings, **3**- Greatest breadth of the cranial articulation surface, **4**- Greatest breadth of the caudal articulation surface, **5** –Max. width between cranial & caudal articulation surface, **6**- Length of dorsal arch, **7**- Height of Atlas, **8**- Inner breadth of cranial articulation surface, **9**- Inner breadth of caudal articulation surface**, 10**- max. length of condyle articulation facet, **11**- max. breadth of condyle articulation facet, **12**- max. depth of condyle articulation facet, **13-** - max. length of articulation facet of axis, **14**- max. breadth of articulation facet of axis, **15**- max. length of *fovea dentis,* **16**- max. breadth of *fovea dentis,* **17**- Length of transverse canal, **18**- distance from alar incisures to alar foraman, **19**- Thickness of Posterior arch, **20**- length of short canal

Table 4.2: Morphometric measurements in C1 bone

S.no.	Species → / Feature ↓	Variable code	Tiger (mm)	Leopard (mm)	Hyaena (mm)	Sloth Bear (mm)
1	Greatest breadth over the wings	WngMxB	101.45-166.07	59.62-105.93	123.55-139-78	114.64-138.99
2	Greatest Length over the wings	WngMxL	57.32-93-61	30.23-60.8	66.34-75.68	56.72-71.06
3	Greatest breadth of the cranial articulation surface	CraMxB	50.07-77.09	40.8-51.58	48.57-51.68	63.03-66.52
4	Greatest breadth of the caudal articulation surface	CauMxB	54.85-107.37	35.26-50.11	41.15-46.58	54.5-56.14
5	Max. width between cranial & caudal articulation surface	CraCauMxB	48.33-75.47	29.71-50.12	45.47-51.16	44.21-51.58
6	Length of dorsal arch	DorarchL	22.31-39.63	14.38-27.43	26.71-28.42	25.27-27.1
7	Height of Atlas	AtlasHt	32.81-53.13	23-35.12		
8	Inner breadth of cranial articulation surface	CrainrB	28.94-67.26	37.05-47.05	43.2-46.63	57.06-59.13
9	Inner breadth of caudal articulation surface	CauinrB	42.31-69.9	33.11-48.08	38.15-44	51.32-54.05
10	Max. length of condyle articulation facet	CondMxL	24.27-48.08	16.85-28.82	27.49-29.35	27.44-30.33
11	Max. breadth of condyle articulation facet	CondMxB	15.15-25.8	6.83-17.19	16.28-21.13	21.59-26.93
12	Max. depth of condyle articulation facet	CondMxD	5.06-18.12	2.12-10.42	8.79-9.88	6.78-9.9
13	Max. length of articulation facet of axis	AxsMxL	18.55-33.4	12.64-21.53	17.42-19.54	18.68-21.16
14	Max. breadth of articulation facet of axis	AxsMxB	21.14-31.65	14.64-21.53	8.33-19.39	20.02-25.06
15	Max. length of *fovea dentis*	FovdenMxL	11.32-21.57	10.4-15.01	14.46-17.46	14.21-19.18
16	Max. breadth of *fovea dentis*	FovdenMXB	11.34-22.45	12.06-15.20	15.64-18.78	17.18-21.14
17	Length of transverse canal	TrncnlL	31.06-50.7	23.72-37.29	28.37-36.15	34.88-41.67
18	distance from alar incisures to alar foraman	AlrincAlrforDst	5.74-12.05	5.14-9.78	6.53-8.99	5.36-6.75
19	Thickness of Posterior arch	PostarchThk	7.14-11.7	3.51-7.37	5.49-7.13	5.56-8.19
20	length of short canal	ShrtcnlL	8.91-19.94	6.8-15.3	8.22-8.97	15.24-22.1
21	Weight of C1 (in gms)	C1wght	33-122	5.0-36	34-50	41-61

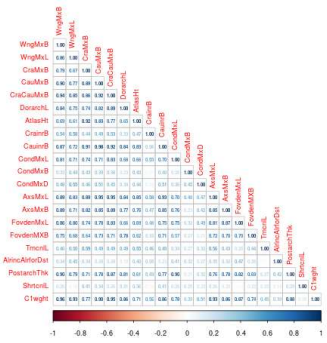
Table 4.3**:** Morphological variations in C1 bone

Species	**Tiger**	**Leopard**	**Hyaena**	**Sloth Bear**
Feature				
Anterior arch	Convex anterior surface with deep notch dorsally on cranial aspect	Convex anterior surface with shallow notch dorsally on cranial aspect	Convex anterior surface with shallow notch dorsally on cranial aspect	Shallow convex anterior surface, **notch absent,** almost continuous edge
Anterior articular facet	Large, oval, **cup shaped**, approach each other dorsally in front before bending downwards. Ventral edge curved unevenly and meet ventral arch forming a deep broad notch.	Large, oval, **cup shaped**, approach each other dorsally in front before bending downwards. Ventral edge curved unevenly and meet ventral arch forming a deep broad notch.	Large, oval, **cup shaped**, approach each other in front and bending slightly downwards. Ventral edge curved unevenly and meet ventral arch forming a deep broad notch.	Large, oval, concave-**saucer shaped,** approach each other in front and merging with dorsal anterior arch edge medially. Both dorsal and ventral edges are almost on same plane
Dorsal tubercle	Very prominent, rough surface	Flaccid, rough surface	Prominent, rough surface	Rudimentary
Alar incisure	Large deep Notch	Broad Notch	Almost flat	Very reduced, Narrow
Ventral arch	Thick, convex, “C” like on the cranio- ventral aspect	Thick, convex, “C” like on the cranio-ventral aspect	Thick, curved on the cranio ventral aspect with a **sharp short notch** medially	Thick, **almost straight**, curving and merging with base of anterior articular foveae on the cranio-ventral aspect, thick, rudimentary posterior tubercle
Ventral articular facets	Oval shape on posterior- medial face of wings in line with edge	Oval shape on posterior- medial face of wings separated by	Oval shape **pointing upwards** dorsally on posterior- medial face of	Oval shape on posterior-medial face of wings separated by thick, almost

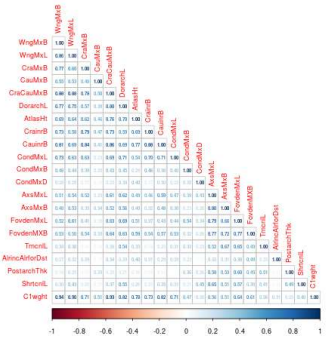
	of wings, separated by concave fovea dentis on ventral arch	concave fovea dentis on ventral arch	wings, lower end open merging with flat fovea dentis	flat fovea dentis on posterior arch
fovea dentis	Large, concave, slanting upward caudal-cranially	Concave, slanting upward caudal-cranially	Large, shallow, slanting upward caudal-cranially	Very large, shallow, slanting upward caudal-cranially
Transverse process (Dorsal Aspect- Caudal region)	Massive, broad, very rough conical bony plate of bone that bent downwards and backwards. Very thick caudal edge, Large fossa	Short, rough, almost straight lateral edge, large fossa	Thin, broad and oval bony plate with smooth edge, dorsally touch the surface at caudal tip, very large fossa, only	Narrow cylindrical plate of bone, very thick caudally, irregular edges, fossa is narrow canal like
Transverse foramen (caudal)	Large, positioned at the base of wings laterally	Small, positioned at the base of wings laterally	Large, positioned at the base of wings laterally	Small, positioned at the base of wings laterally
Lateral vertebral Foramen (pair)	Large, cranially positioned on dorsal surface preceded by a large fossa	Small,, cranially positioned on dorsal surface preceded by a large fossa	Large, cranially positioned on dorsal surface preceded by a large fossa	small, cranially positioned on dorsal surface, fossa absent instead a narrow channel moves behind the raised surface of Anterior articulating surface
Transverse-vertebral canal	Small, moves diagonal caudally	Very small, moves diagonal caudally	Large, moves diagonal caudally	Very small, moves diagonal caudally, further reduced in vertebral opening
Alar canal	Absent	Absent	**Present**	Absent

Correlation among variables: All variables are positively correlated in Tiger and Leopard samples, some variables show negative correlations in Hyaena and Sloth bear samples. Thus suggesting structural differences at least at a Family level. When all species are pooled together, all variables are positively correlated with each other, mainly because of influence of much larger sample sizes of tiger and leopard which mask the patterns of other species.

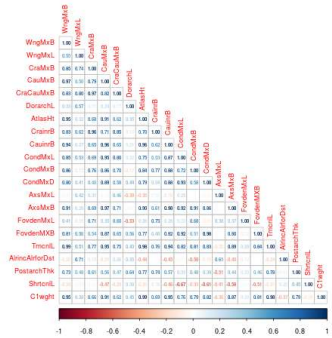
Figure 4.5: Correlation among variables in C1 bone



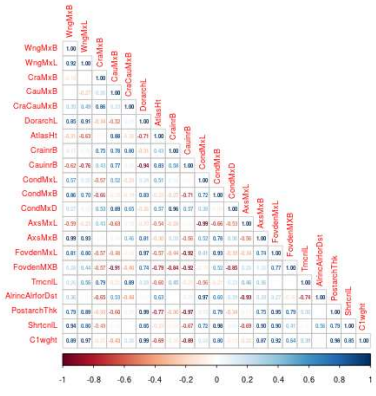
Correlation plot- Tiger (N=22)



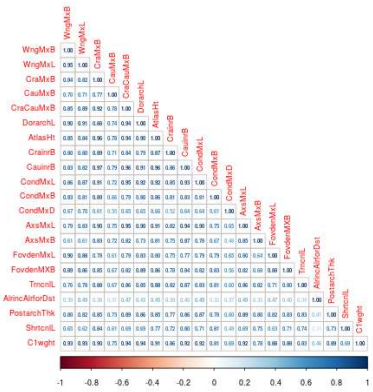
Correlation plot- Leopard (N=31)



Correlation plot- Hyaena (N=05)



Correlation plot- Sloth bear (N=04)

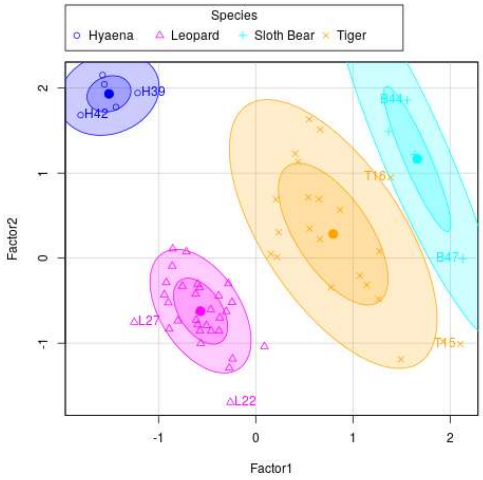


Correlation plot- All species pooled (Tiger, Leopard, Hyaena and Sloth bear) (N=62)

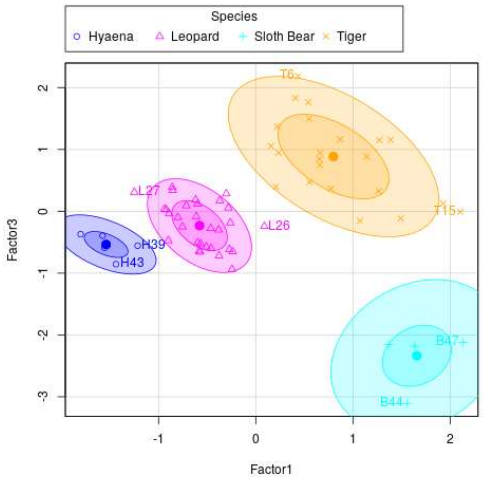
Factor analysis: Three factors were extracted which explained a cumulative 83.5% of the total variance. Scatter plots between Factors with 90% confidence ellipses showed good segregation among species (Figure 4.6 a & b)

Factors 1 & 3 showed the most separation of species in the factor space. Tiger samples had high positive loadings on both Factor 1 & 3, Sloth bear had high positive loadings on Factor 1 and high negative loadings on Factor 3. Leopard occupied almost the middle space between Factor 1 & 3, while Hyaena had high negative loadings on Factor 1 and low negative loadings on Factor 3. However, Hyaena and Leopard were more distinctly segregated on Factor 1 & 2, with Hyaena highly positively loaded on Factor 2 and negatively loaded on Factor 1. Leopard was negatively loaded on Factor 2 and had an intermediate position on Factor 1.

Fig. 4.6 a & b: Plot between Factors in C1 bone



Plot between Factor 1 & 2. Shaded ellipses depict 0.5 and 0.9 confidence levels



Plot between Factor 1 & 3. Shaded ellipses depict 0.5 and 0.9 confidence levels

Table 4.4 : Variance explained by Factor analysis in C1 bone

	Factor 1	Factor 2	Factor 3
SS loadings (Eigenvalues)	6.892	5.944	4.692
Percentage variance explained	32.8	28.3	22.3
Cumulative variance explained	32.8	61.1	83.5

Table 4.5: Factor loadings- C1 bone (correlation of variables) values > 0.5 are in bold

S no	Variable	Factor 1	Factor 2	Factor 3
1	WngMxB	0.41	**0.81**	0.36
2	WngMxL	0.35	**0.77**	0.48
3	CraMxB	**0.79**	0.45	0.40
4	CauMxB	**0.54**	0.37	0.46
5	CraCauMxB	**0.55**	0.46	**0.68**
6	DorarchL	0.48	**0.60**	**0.58**
7	AtlasHt	**0.70**	0.47	0.48
8	CrainrB	**0.66**	0.49	0.36
9	CauinrB	**0.72**	0.41	**0.54**
10	CondMxL	**0.57**	**0.50**	**0.59**
11	CondMxB	**0.69**	**0.60**	0.18
12	CondMxD	0.29	**0.51**	0.38
13	AxsMxL	**0.57**	0.40	**0.67**
14	AxsMxB	**0.71**	0.16	**0.54**
15	FovdenMxL	0.38	**0.75**	0.35
16	FovdenMXB	**0.52**	**0.69**	0.33
17	TrncnlL	**0.65**	0.42	0.45
18	AlrincAlrforDst	0.09	0.28	0.43
19	PostarchThk	**0.53**	0.49	**0.54**
20	ShrtcnlL	**0.80**	0.35	0.13
21	C1wght	0.49	**0.64**	**0.55**

Classification Tree:

The classification tree used only 4 variables, CondMxB, CraCauMxB, CraMxB, WngMxB in the tree construction. The complexity parameters and cross-validated errors were smallest for a 4 splits tree, corresponding to the four species (Figure 4.6 a & b).

The primary split (CondMxB < 17) segregated all leopard samples from the remaining three species (with only one Hyaena sample requiring further split). The tiger samples split neatly at the second level with CondMxB > 17, and CraCauMxB < 53. (Fig. 11).

These rules can be simplified into the following statements

if CondMxB < 17, and if WngMxB >=116, Hyaena, else All Leopard

if CondMxB > 17, and if CraCauMxB < 53, All Tiger

if CondMxB > 17, and if CraCauMxB > 53 and if CraMxB < 57, Hyaena, else all Sloth bear.

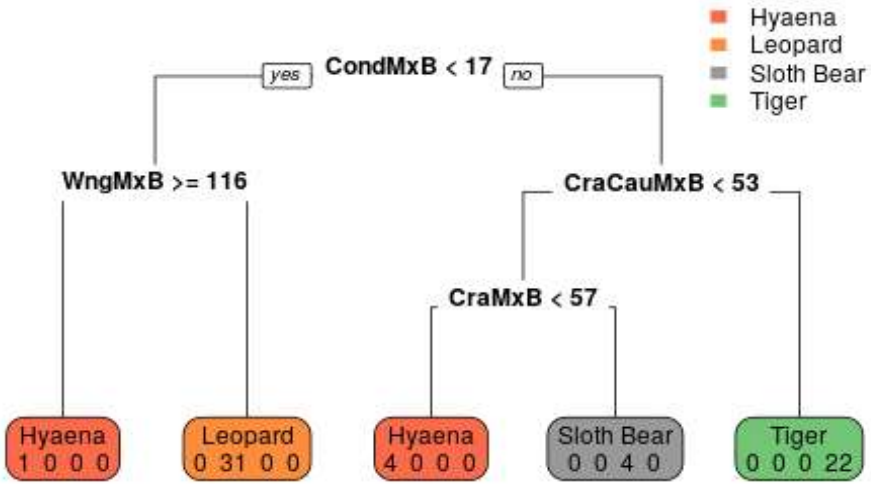


Fig. 4.7: Classification Tree showing differentiation in C1 bone among sympatric carnivore species

Conclusions: Despite similarities in basic morphological features and joint alignments, the structural variations crept in C1 vertebrae for performing specific functional requirements for survival in each species allows differentiation based on the morphological feature of large felids (tiger and leopard) with that of other sympatric carnivores/ species like hyaena and sloth bear. The quantitative analysis of morphometric data further allows differentiation between tiger and leopard. Based on only four morphometric measurements, species identification is possible in seizures of multiple species bones where skull and other major bones are not available.

4.3.3 Characterization and differentiation of Tiger, leopard and fake canines:

Canines of tiger, leopard, sloth bear, hyaena, musk deer, barking deer, wild pig and fake claws are usually found in seizures sent for species identification at WFCGC. Some of the teeth/canines of few species are easy to distinguish morphologically, such as wild pig, musk deer, and sloth bear canines. By presence or absence of groove felid canines can be distinguished from those canid and other groups, with exception when grooves are found missing in felid canine also, mainly in young animals. Lot of fake canines of non-biological material or other animals (Horses) are also regularly referred to WFCGC which are seized as suspected tiger or leopard canines. Therefore, it is very pertinent to study and devise protocols for characterization of genuine canines of commonly found species in wildlife offenses.

Real canines have two layer, enamel and dentine, but fake canine has only one layers lacking any pulp cavity, which can be detected by X-ray radiograph. Also, distinct feature of genuine canines like ridges, grooves, rings, outgrowths and distinct root ad crown separated by neck of tooth is missing in fake canines. For genuine canines, along

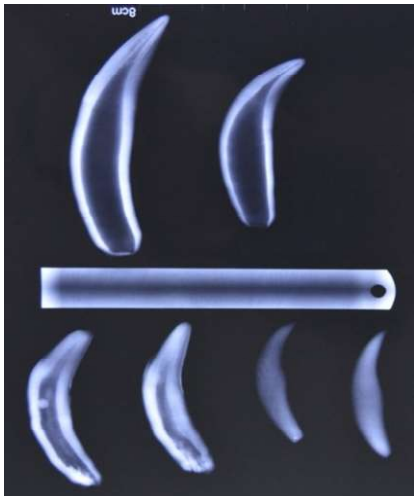
with morphological characters, morphometric measurements may be used to differentiate genuine Tiger and leopard canine from other canines of domestic animals. All the suspected canines were ruled out by morphological analysis in combination with radiograph analysis.

Canines of felids, are well developed to suit their feeding habit and prey base. These are high pointed crowns, usually with one distal ridge, and bulging in the cervical region and have a single long and stout root. Longitudinal grooves are present on the labial and lingual side of the canines in felids. (Nigam *et al*. 2016). These grooves, if present, are parallel with the mesial ridge of the tooth, but there is some variation in length. The maxillary teeth have on the labial and on the lingual side, two, one or no grooves. The mandibular teeth have two, one or no grooves on the labial side and never grooves on the lingual side. Many families including the felids and viverridae have distinct grooves on the crown of canines (Lamerichs, 1985, Folinsbee *et al*. 2007).

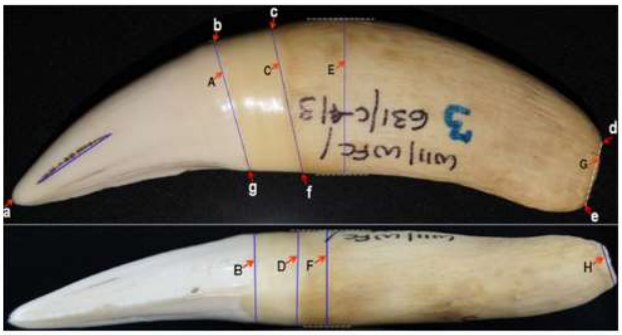
The upper canine in Tiger and leopards is more elongated, slightly laterally compressed and develops a slight backward curve to pair with mandible for a tight closing to the mouth. The mandibular canine is smaller than maxillary canine but more curved for fitting tightly into the incisive bone of maxilla to allow for making a firm grip. The anterior and more particularly the posterior border acquire a serrated edge, to give a good tearing edge (Savage & Long, 1986). The distinct features present on the crown of tiger and leopard canine include the presence of 0-4 grooves, and prominent ridge is present on the dorsal side. The Leopard has the same structure of canines, but are much smaller than a tiger canine. All the canines in the study were having very smooth root.

X-Ray Radiographs: to distinguish genuine and fake canine

A portable X-ray machine was used to examine some of the specimen and as they were in trophy form and tied by the help of wires and nails thus, the quality of radiograph is low. X-ray radiographs of available specimen were observed. The size of pulp cavity decreases with age from deposition of secondary dentine and therefore can be taken for estimation of age of animal (Fisher, 1941, Kuehn & Berg, 1981). Canines with closed root and narrow pulp cavity are considered as adult (Kuhen & Berg, 1981) and can be used as a tool along with root opening to differentiate adult leopard canine from sub-adult Tiger canine of same size.



(a)



(b)

Fig. 4.8 a : X-ray radiograph of canines**:** Smooth surface of root and pulp cavity in tiger & leopard in comparison with horse with rough root and absent pulp cavity in fake synthetic canine
Fig. 4.8 b: Linear measurements of Tiger canine

Further, morphometric measurements of canines of tiger, leopard and fake (Horse canines) were taken and differentiated using statistical tool. Canines for reference repository of WFCGC were taken for developing protocols for differentiation of species. The details of the canines taken for the current study is provided in table

below. The morphological characteristics of canines were noted and comparative study was done between tiger, leopard and fake canines which are traded together.

Table 4.6: Canine sample size

Species	Tiger	Leopard	Fake (Horse)	Fake (Non biological)
No. of samples	20	20	20	5

After noting the morphological characteristics morphometric measurements were taken as detailed below and comparative study was done. Linear measurements were recorded by digital Vernier Calipers, (MITUTAYO, Japan), while curve measurements were taken with the help of a fine thread and scale, and weight (gm) was taken by weighing balance (ACZEL CG 602). The following measurements were taken as per fig. 4.8b and table 4.7.

Table 4.7: Linear measurements of canines

Length (in mm)			
CN1	**ad -** Length from crown tip to root tip (labial)	**CN2**	**ae -** Length from crown tip to root tip (lingual)
CN3	**ab -** Crown length (labial)	**CN4**	**ag -** Crown length (lingual)
CN5	**bc -** Gum line length (labial)	**CN6**	**gf -** Gum line length tip (lingual)
CN7	**cd -** Root length ((labial)	**CN8**	**fe -** Root length (lingual)
Width (in mm)			
CN9	**A -** Crown width (labio- lingual)	**CN10**	**B -** Crown width (laterally)
CN11	**C -** Gum line width (labio- lingual)	**CN12**	**D -** Gum line width (laterally)
CN13	**E -** Maximum width (labio-lingual)	**CN14**	**F -** Maximum width (laterally)
CN15	**G – (de)**Root tip width (labio-lingual)	**CN16**	**H -**Root tip width (laterally)
Miscellaneous			
CN17	Number of grooves		
CN18	Weight (in gms)		

Classification Tree:

The classification tree used only 2 variables, CN1_1 and CN3 in the tree construction. The complexity parameters and cross-validated errors were smallest for a 3 splits tree, corresponding to the three species (Figure 14 and 15).

Tiger, Leopard and fake (Horse) canines:

The primary split (CN1_1>=81) segregated all leopard canine samples from the remaining two species. The tiger canine samples split neatly at the second level from horse with CN3<39. Fig. 4.9

These rules can be simplified into the following statements

if CN1_1 >= 81, either Tiger or Horse, all leopard <= 8, are thus separated,

if CN1_1>=81 and CN3< 39, All Horse

if CN1_1>=81 and CN3> 39, All Tiger

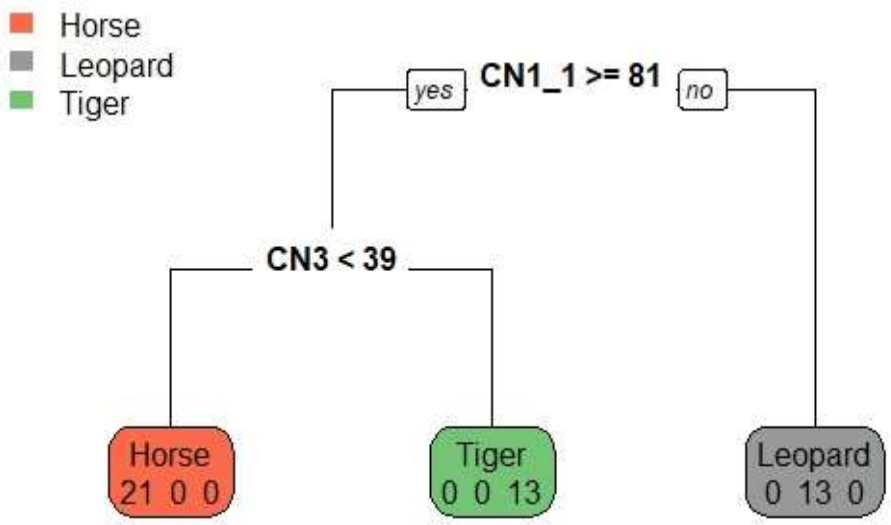


Fig. 4.9: Decision tree differentiation three species based on two parameters

4.3.4 Characterization of Musk Deer Pod by opportunistic microscopic analysis of hair

The musk deer pod is a ventrally flat preputial gland with a muscular constriction orifice, covered on the ventral side with skin having thick, white wrinkled hair. The orifice of musk deer pod is well surrounded by a thick lining of hair aligned in the direction of the sac that holds back leaking of musk contents when the pod is freshly harvested, thus the poacher usually ties a knot with some thread at the orifice end (N=12). The dorsal surface, which remains inside the skin is just a muscular pouch containing musk contents. To avoid detection immediately after harvesting the pod, in a bid to hide the animal origin of musk pods, the poachers, either peel off the outer skin or shave or singe the ventral hair. The fresh pod after removing the ventral skin becomes bare and soft and can be easily molded into any shape. The weight of musk pods from wild caught/seized pods (N= 58) range from 3.50 – 39.00 gms, depending on size, season of collection and degree of drying when seized while weight of musk pods from captive animals (N= 04) range from 9.89-17.24 gms. Most of the pods seized under wildlife offense cases are round to oval in shape (N=55). The musk deer pods seizures include (N=57) pods with shaved/trimmed or singed hair

The orifice of musk pod is lined with thick hair aligned in the direction of the sac that hold back leaking of musk and usually the neck of orifice is found to be knotted with some thread. The musk pods seized were usually spherical with thick ventral skin with trimmed hair (N= 5), spherical with very fine layer of muscular pouch (N= 51) and Semi-circular/ bucket shape pod with trimmed white hair (N= 1) are also referred under seizures. All the shaved/trimmed/singed pods initially thought to be unsuitable for microscopic hair analysis, were cut from centre and opened to find tuft of hair present on the in-folds of the skin of orifice, these few hairs found folded inside and not

visible from outside and hence survive the shaving or burning. Some loose hair detached from roots are found embedded in the brownish black grains of musk content, which can be collected carefully. These hairs were than washed with alcohol to remove any dirt or grease and analyzed under the microscope following standard protocols for species identification.

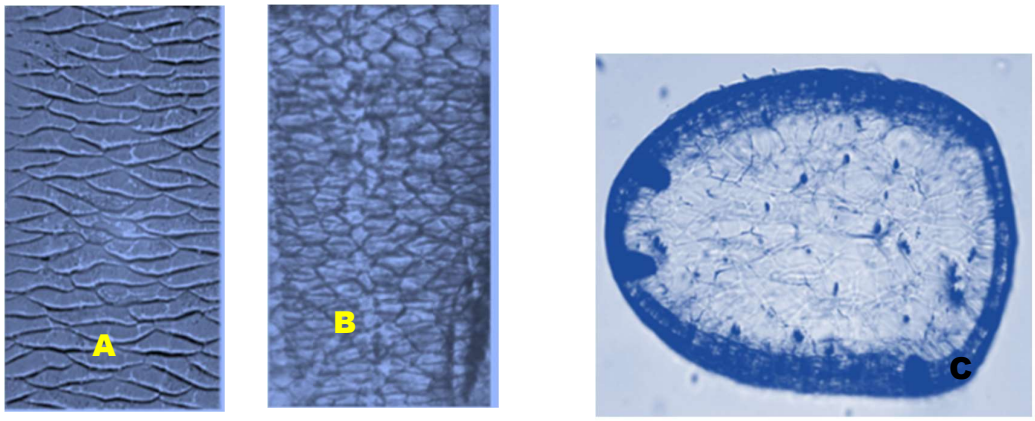
The fake musk deer pods are round to oval in shape with a skin pouch having white or brown hair. The skin is folded into a pouch and stitched at one end which is visible to naked eye, usually filled with black charcoal or incense powder. The weight of fake pods (N=209) ranges from 12-60 gms.

Table 4.8: Summary of musk pod characteristics referred to WII for species identification

Musk Deer Characteristics	Shape of suspected musk pods			Wt. (gms)	Orifice inverted or everted	ventral skin present Absent	Hair present on pod orifice	Colour and texture of hair, if present
	Round /oval	Flat/ pressed	Semi-circular/ bucket					
Real Musk Pods (N=58)	55	2	01	3.5-39	Inverted=46 Everted =12	P= 6 A= 51	Hair/stub on orifice= 58	Thick, white with dark tip, wrinkled
Fake Musk Pods (N=209)	209	00	00	12-60	Inverted = 00 Everted = 209	P= 209 A= 00	No orifice as such, instead a pouch of skin is folded and stitched at one end	Fine, white/soft= Goat skin and Brown/coarse= Horse skin

Fake musk pods and adulteration: Less sophisticated fake pods, usually spherical in shape is a pouch of goat or horse skin having incense powder, charcoal with perfume, ball of wood powder and added lead pieces to increase the weight. In one case from Chhattisgarh in central India, white round testicles of goat were seized as musk pod. Adulterations like Musk content mixed with tobacco (n=1) and indigenous medicines claimed to be having musk are also found in illegal trade.

Microscopic examination of opportunistic recovered hair from musk deer pod:



A. Cuticle, B. Medulla and C. cross- Section of Musk deer guard hair

Figure 4.10: Microphotograph of Musk deer hair

Table 4.9: Microscopic characteristics of hair found on musk pod

Microscopic Hair Characteristics	Reference Musk deer hair	Hair collected from inside the shaved or singed musk pod
Cuticle		
Margin of scale	Smooth	Smooth
Distance between scales	Wide	Wide
Scale Pattern	Regular wave forming ……	Regular wave forming ……
Medulla		
Medulla shape	Wide medulla lattice	Wide medulla lattice
Medulla %age	**90%**	
Cross- section		
Cross-section shape	Round to oval (crinkled hair)	Round to oval (crinkled hair)
Medulla shape	Wide medulla lattice forming reticulum	Wide medulla lattice forming reticulum

4.3.5 Fraudulence in illegal WL trade- Jackal's horn

A total of 342 seized jackal horn samples belonging to eleven wildlife offense cases were sent to the Wildlife Forensic and Conservation Genetics (WFCG) Cell of Wildlife Institute of India (WII) by law enforcement agencies for species identification.

Morphological and microscopic analysis:

The jackal pods' outer physical characteristics, including the shape, weight, hair color, and hair bands on hair follicles, were noted. Subsequently, the pods were cut open, and information on the type of material used as filling along with attachment types was also recorded (Fig.4.11). The jackal horn samples were further pooled based on the above-mentioned morphological characteristics, and nearly 30 representative samples were taken for the microscopic hair analysis. Cuticle and medullary pattern of all the 30 hair samples were observed following methodology as detailed in the earlier study (Singh *et al.* 2020). Examination of seized jackal horn pods (n=342) revealed variations in the composition of outer physical characteristics and internal filling materials (Fig. 4.11). The shape of the pods was round (n=231) followed by whorl (n=130) and conical (n=11), whereas the weights ranged between 0.68 and 15.51 gms, with the majority of samples between 6-8 gms (n=104). The color of hair follicles was brownish (n=157) followed by white (n=65) and black (n=40) with ventral hair in major proportion (n=223) observed on pods. The internal filling material was mostly dry clay or flour balls (n=235) followed by charcoal powder (n=63). The other materials used were wooden dust, sponge, the fold of skin, and paw with pads. Mainly, talon (n=204) was used as an attachment followed by tooth (n=61) with few samples observed without any attachment (n=48) (Fig. 4.12). Moreover, cuticle and medullary patterns of examined hair samples when compared with repository samples of WFCGC were matched with multiple domestic and wild species (Fig 4.13).

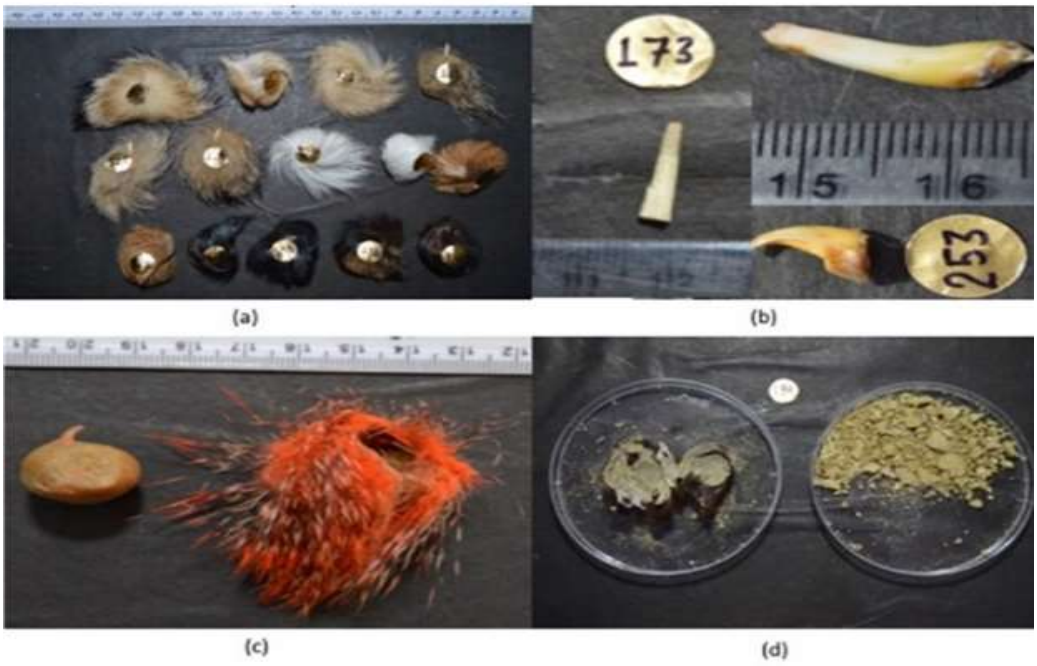


Fig. 4.11. Confiscated Jackal's horn pods (a) showing various attachments (b) and filling material (c and d) used to make fake "jackal's horn" or "siyar singhi"

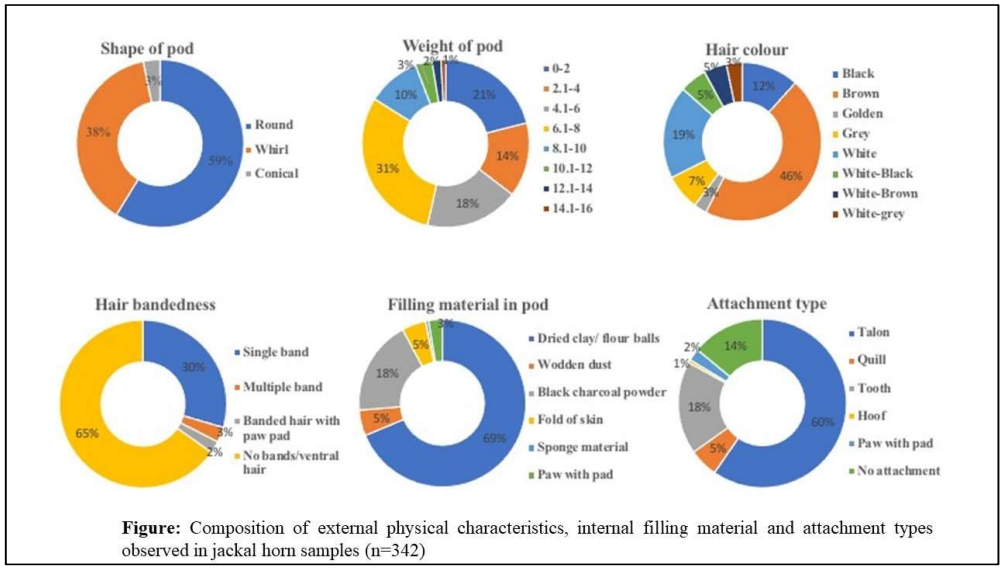


Figure: Composition of external physical characteristics, internal filling material and attachment types observed in jackal horn samples (n=342)

Fig. 4.12. Variations in jackal's horn constitutents

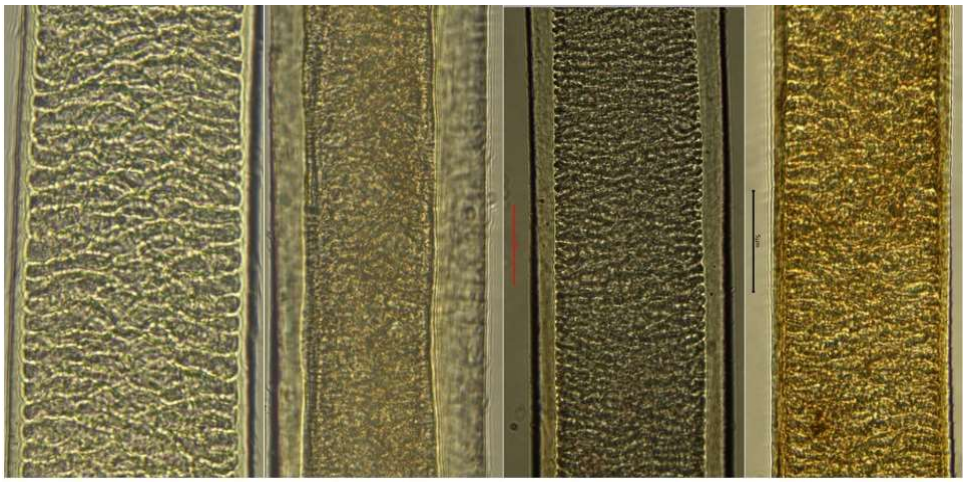


Fig. 4.13 Variation in medulla pattern observed in the hair of suspected Siyar singhi, "Jackal's horn" referred to WII for species identification, A. Goat B. Canid, C. Mongoose, D. Sheep

For further confirmation of the species involved in attachments, the samples were subjected to molecular analysis and species identification was done. The results shows that the claimed "jackal horn" is a fake article and made of parts of multiple domestic and wild species.

4.4 Discussion

Identification of species from seized wildlife parts is vital for the enforcement agencies to implement protection laws effectively (Broad & Roe 2003; Mendiratta *et al.* 2017). But, considering many different species and fake items being encountered in the illegal wildlife trade markets, identification becomes even more challenging. The seized articles are often made from materials derived from either domestic animals or lesser charismatic species. Jackal horn is one such item that is considered fake and artificially made by using biological and non-biological materials. Enforcement agencies often seize jackal horn samples, but they receive less attention due to a lack of information on the biological origin of such samples. Therefore, for the first time, we attempted to identify the trustworthy source of jackal horns and the materials, including the non-biological and biological, which were used in making it.

Morphological examination of outer physical characteristics and internal filling materials revealed variations in shape, weight, hair color, presence and absence of bands on the hair strand, attachment types, and the non-biological material used. The attachments were initially covered with dried clay, flour balls, and charcoal powder and then stitched with a skin sample giving either a spherical or whorl shape with weights ranging between small pad to large spherical ball structure. The hair samples were without any bands indicating the ventral hairs or whiskers used in making these pods. The attachments that gave the feel of the horns-like structure were made of either bird talons, an inferior umbilical portion of the calamus, or animal's hoof. Microphotographs of cuticle and medullary patterns from hairs collected on skin showed that multiple species were used to make the jackal's horns pods. Molecular analysis identified several wild species e.g., jackal, red fox, Indian grey mongoose, Indian peafowl, and wild pig, were used either for their skin for outer covering or other body parts for a horn-like attachment. Several other domestic species, such as goat, cat, sheep, fowl, and pigeons, were also used to make fake jackal horn pods. Largely red fox, domestic cat and goats were used in both the skin and attachments among the confiscated samples. The findings of this study were published (Sharma *et al.* 2022b), 'Unraveling the mystery of confiscated "jackal horns" in India using wildlife forensic tools', in International Journal of Legal Medicine.

4.5 Conclusions

The wildlife crime usually involves modified extracted parts of target wildlife species. Llack of prompt and proper identification of the wildlife part lying before an enforcement agency is a major hindrance to initiate any confiscation. Most of the seizures are chance findings or during routine checking, therefore these agencies need to be equipped with scientific protocols for confident seizures. The conjoint approach

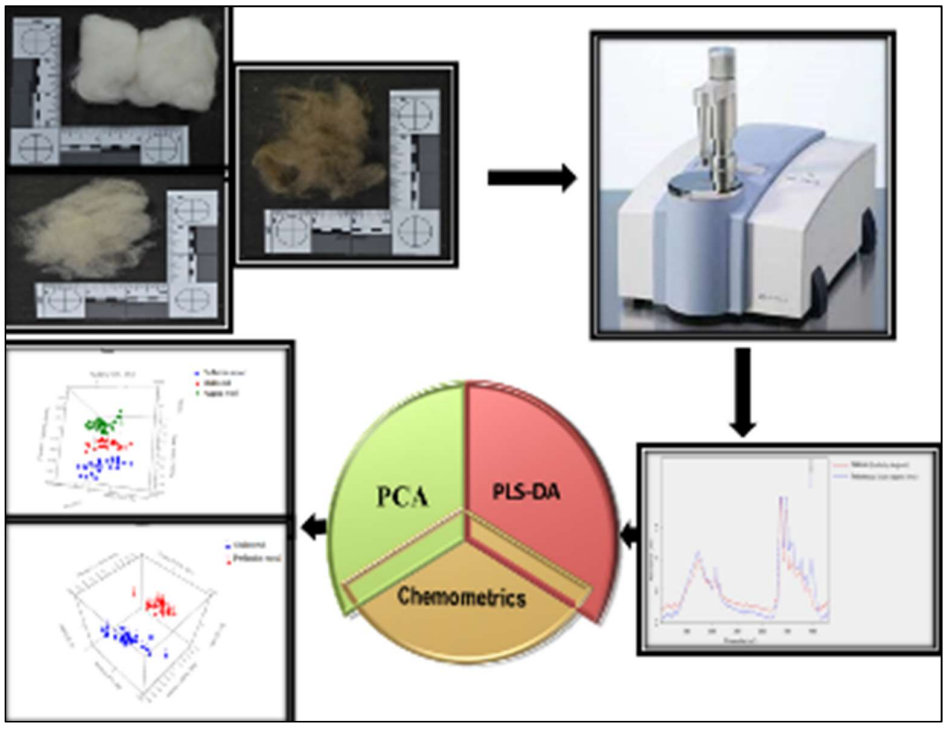
of understanding of illegal wildlife trade and scientific identification protocols of parts involved will enable enforcement agencies in checking the crime against venerable wildlife and chalking out mitigating measures against wildlife crime.

Morphological techniques in combination with statistical and other tools employed in this chapter are hugely expedient in wildlife forensics. Since, detection and identification of a wide variety of wildlife parts/ derivatives pose a serious challenge before the enforcement agencies and forensic scientists, the techniques discussed above provides conclusive, and reliable results in a non-destructive, and eco-friendly manner and possesses disparate advantages over other methods which depend on destruction of vital forensic evidence's. Morphological analysis has almost unlimited shelf life as evidence remains intact of repeat analysis whenever required and the results obtained are rapid and reliable. Also, a combination of morphological and statistical approaches, could not only be successfully used to ascertain the species involved but also to differentiate genuine or fake wildlife article, or if multiple individuals are involved.

In view of the above discussion, it is clear that morphology in combination with statistical tools is a game changer for enforcement agencies for effective implementation of Wild Life (Protection) Act, 1972 and other relevant acts but requires extensive repository of reference material from different age groups and representing various zoogeographical regions and comprehensive validation studies especially for sympatric and closely related species should be performed. The present work is the base work in wildlife forensics and hope it will definitely undergo further investigations on the applicability of morphology for differentiating species from wildlife parts in wildlife forensics.

Chapter 5:

SPECTROSCOPIC TOOLS (FT-IR) FOR IDENTIFICATION OF WILD ANIMAL SAMPLES FOUND IN ILLEGAL WILDLIFE TRADE



5.1 Introduction

The present work deals with characterization and differentiation of the selected wildlife articles, using ATR FT-IR spectroscopy commonly found in wildlife offense seizures. While performing the analysis, effect of contamination with other biological material including other body fluids, environmental conditions, false-positive test, and chemical treatments, if any were considered simulating with the actual case conditions.

5.2 Sample collection

Sufficient known biological samples of each of the selected wildlife article (blood, Musk pod grains, claws, *shahtoosh*/hair) were obtained from repository of Wildlife Institute of India (WII).

5.2.1 Musk pod constituents

Grains from musk pods (n=11) were taken from the repository of Wildlife Forensic and Conservation Genetics Cell (WFCGC) of Wildlife Institute of India and two samples from fake pods were taken. One sample drug ("Hew" capsule) which claimed to have "muscone" as ingredient was also taken as blank sample.

5.2.2 Claws

Samples used in the present study were obtained from the repository of Wildlife Institute of India (WII), Dehradun. Details of the samples are provided in table 5.1. As this technique does not require any sample preparation, so the samples were analyzed without any pre-treatment. Claws were scrapped with the help of sterile blades and the scraping is directly placed on the crystal surface for analysis.

Table 5.1**:** Details of claw samples for FT-IR analysis

S .No.	**Claw samples**	**Sample number(n)**
1-10,151,152,153, 154,155,156	Claw scrapes of tiger	16
11-20, 86,87,88,89,90	Claw scrapes of Indian leopard	15
61-70	Claw scrapes of fake claws	10
Unknown/blind samples		
163,164, 53,54,81,82,41		7

5.2.3 *Shahtoosh*

The reference underfur hair samples of Shahtoosh (Tibetan antelope), Pashmina and Angora rabbit were collected from the repository of WFCGC. Details of all collected samples are enumerated in table 5.2.

Table 5.2**:** Detailed description of wool samples for FT-IR analysis

Common Name /Wool Type	Scientific names of species involved	Number of individuals	Number of hair strands per individual (*n*=)
Shahtoosh	*Pantholops hodgsonii*	05	25
Pashmina	*Capra aegagrus hircus*	05	25
Angora wool	*Oryctolagus cuniculus domesticus*	08	40

5.2.4 Animal Blood

In the present study, wild animal blood specimens collected for research purposes and deposited with due permission were taken from the refrigerated repository of wildlife institute of India, Dehradun and brought to Punjabi University, Patiala in cold chain. The

blood samples were acquired from four species, namely Leopard, Tiger, Elephant, and Domestic pig. Details of collected samples are enumerated in Table 5.3.

Table 5.3**:** Details of collected blood samples for FT-IR analysis

Common name	**Scientific name**	**Number of blood samples (*n*=)**
Elephant	*Elephus maximus*	6
Domestic pig	*Sus scrofa domesticus*	8
Leopard	*Panthera pardus*	7
Tiger	*Panthera tigris*	14

The human blood sample collected from superficial vein was employed to test the validation method. The human blood was collected from fifteen consented volunteer donors by trained phlebotomist. An aliquot of 2 mL blood was withdrawn from each donor in dipotassium EDTA anticoagulant at a concentration of approximately 1.8 mg K2 EDTA per 2 mL of blood and were kept at 2-4°C.

5.3 Sample Analysis

Samples were analyzed at Forensic Science Department of Punjabi University, Patiala, Punjab using Bruker Alpha, eco ATR FT-IR spectrometer (Fig XX) with a Smart Orbit, ZnSe crystal accessory and OPUS (v 7.2) software equipped with an air-cooled DLATGS detector. All samples were scanned for 24 times at 4 cm-1 resolutions within the MIR range of 4000-600 cm-1 infrared region. ATR crystal was cleaned with a pre-wetted ATR cleaning tissues (part No. 1008033), containing deionized water and isopropyl alcohol prior to analysis of any new sample. Positive and negative controls were analyzed, and a homogenous contact was ensured between the crystal and sample surface with ATR anvil. All operating parameters and specifications were described as follows in Table 5.4

5.3.1 Model Validation

The parameters included for the validation study were false-positive rate, false-negative rate, sensitivity, specificity, precision, and accuracy. These parameters can be calculated using the following formulas (Gregório et al., 2017; Morillas et al., 2018).

Sensitivity = True positives/ (True positives + False negatives) × 100

Specificity = True negative/ (True Negatives +False Positives) × 100

Accuracy = True positive+ True negative/(True positive+ True negative+ False positive+ False negative)× 100

Precision = True positive (True positive+ False positive) × 100

False positive rate= False positive/ (True positive + False negative) × 100

False negative rate = False negative/ (True negative+ false positive) × 100

Model validation was carried out in reciprocation of only two output that is ("selected wildlife article" or "not")

5.3.2 Operating parameters for the analysis of biological material using ATR FT-IR spectroscopy

Table 5.4. Operating parameters for the analysis of biological material using ATR FT-IR spectroscopy

S.no.	Parameters	Operating Parameters
1.	Scans	24
2.	Resolution	4 cm-1
3.	Detector	DLATGS
4.	ATR crystal	ZnSe
5.	Spectral processing software	OPUS
6.	Software version	v 7.2

5.3.3 Multivariate data analysis and data Pre-processing

All chemometric tools were performed using unscrambler X software (Version 10.5.1 (64 bit), CAMO AS, Norway). To perform the chemometrics, data were saved as a .dpt file and imported in unscrambler X software. PCA, PLSR, and LDA were applied on the collected spectral data. Before applying multivariate data analysis, different pre-processing techniques were applied to evade any unwanted effect of noise and to normalize the generated difference in the spectra due to the amount of deposited samples. The data was pre-processed using- baseline offset and linear baseline correction, smoothing with Savitzky- Golay algorithm with 2 polynomial orders in a symmetric kernel, and normalization by range. Subsequently, NIPALS algorithm was used with cross-validation method (Gautam *et al.,* 2015; Lee et al., 2017).

5.4 Results and Discussion

ATR FT-IR spectroscopy is an analytical technique that can be utilized to identify the physical and chemical characteristics of the molecule (Petibois *et al*. 2001). In the field of biomedical science, this technique has generally been used to identify bio-molecular structures and components. Every molecule has unique fingerprint spectra, which makes ATR FT-IR spectroscopy an extremely specific technique for the molecular identification and to obtain both qualitative and quantitative information (Bunaciu & Aboul-Enein, 2016; Willard *et al*. 1989).

Biological evidences referred for species identification are composed of specific bio-molecules with varying concentration, which can be helpful to effortlessly distinguish them on the basis of their unique bio-molecular structure. To obtain the vibrational spectra of each selected wildlife biological article, the samples were analyzed untreated form in the mid-infrared region (4000-600 cm-1) of the electromagnetic spectrum. The spectrum of the wildlife biological article so obtained, is primarily divided into three significant

macromolecules groups; lipids (3000-2800 cm^{-1}), proteins (1700-1600 cm^{-1}), and nucleic acid (1250-1000 cm^{-1}). In this study, for the analysis of body fluids, dried sampling approach was adopted.

5.4.1 Characterization of Musk contents

A total of 11 samples were collected from 11 individual musk deer glands. The measurement scans were recorded in the mid-infrared region (MIR) that is 4000-600 cm^{-1}. In the obtained spectrum, total 16 characteristic peaks were observed as enumerated in table 4. The obtained peaks were assigned to various biomolecules including carbohydrates, lipids, and proteins. However, proteins are the major contributor for the peaks in spectral region. Table 1 detailed about the peak assignment with specific component identification and vibrational group. The majority of characteristic peaks in the spectrum of musk were observed in the range of 1600 to 600 cm^{-1} i.e. fingerprint region. This region contains the major amide bands. The typical musk spectrum consists of some dominant peaks with some smaller peaks. The peak positioned at approximately 3271 cm^{-1} was the medium intensity peak and peaks at 1620 cm^{-1} and 1538 cm^{-1} were categorized as strongest, intense, and dominant peaks. Rests of the peaks are weak due to their low peak intensity. The medium peak at approximately 3271 cm^{-1} corresponds to Amide A and the vibrational group is H bonded O-H stretching and N-H symmetric stretching. The strong peak positioned at approximately 1620 cm^{-1} and 1538 cm^{-1} corresponds to Amide I and Amide II respectively (Elkins KM 2011; Narayanan *et al.* 2011; Orphanou 2015; Quinn & Elkins 2017).

Table 5.5: ATR FT-IR peak component for the characterization of musk

Peak number	Wave number (cm^{-1})	Vibrational group	Component identification	Reference
1	3271	(Amino acid) Amide A	H bonded OH stretching, NH stretching	(Garidel & Schott 2006; Orphanou 2015)
2	3062	Amide B	N-H stretching	-
3	2918	-	Asymmetric CH2 stretching	(Movasaghi *et al.* 2008)
4	2852	Methylene stretches of lipids	Asymmetric and symmetric CH2 stretching	(Orphanou 2015)
5	1620	Amide I (ß sheet)	C=O stretching	(Garidel & Schott 2006; Movasaghi *et al.* 2008; Barth 2007)
6	1538	Amide II (ß sheet)	N-H bending and C-H stretching	(Garidel & Schott 2006; Barth 2007; Movasaghi *et al*. 2008)
7	1439	Methylene bending of amino acid, lipids, and proteins	Asymmetric CH3 bending	(Movasaghi *et al.* 2008; Narayanan *et al*. 20114)
8	1389	Fibrinogen/ amino acid side groups	Symmetric CH3 bending	
9	1320-1286 (1306)	Amide III	N-H bend in plane and C-N stretching	(Movasaghi *et al.* 2008; Narayanan *et al*. 20114)
10	1233			
11,12	1170-1000 cm^{-1} (1111, 1053)	Sugar moieties Carbohydrates	CH2OH groups, CO stretching and bending COH groups, symmetric PO2 - stretching	(Narayanan *et al.* 20114)
13	880	-	Out of plane	
14	810		bending vibrations ; C-0- C stretching in ethers	
15	687 (625-770)	Amide IV	O=C-N deformation	(Garidel & Schott 2006)
16	620	-	CH out-of-plane bending vibrations	Movasaghi *et al.* 2008

In IR spectroscopy, total of eight characteristic peaks with polypeptide repeat units within proteins and amino acids were recorded (Table 5.6). These amide peaks are termed as amide A, B, I-VI in order of decreasing frequency (Olsztynska-Janus et al. 2012). To investigate the secondary structure of protein only amide I-III has been reported as useful (Table 5.7). Peaks of Amide I and Amide II are the two most prominent vibrational bands, which confers the protein backbone (Kong and Yu 2007).

Table 5.6: Amide peaks (Barth 2007; Dong, Huang, and Caughey 1990; Narayanan et al. 2011; Pelton and McLean 2000)

Designation	**Approximate frequency (cm^{-1})**	**Description**
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Amide I	1600-1690	C=O stretching
Amide II	1480-1575	CN stretching, NH bending
Amide III	1229-1301	CN stretching, NH plane bending
Amide IV	625-767	O=C-N deformation
Amide V	640-800	Out-of-plane NH bending
Amide VI	537-606	Out-of-plane C=O bending

Table 5.7: Vibrational groups of amides (Pelton and McLean 2000)

Amide peaks	**Vibrational group**
Amide I	80% C=O stretch
Amide II	60% N-H bend and 40% C-N stretch
Amide III	40% C-N stretch, 30% N-H bend

Amide peaks in the musk spectrum represents the secondary structure of proteins, which can be identified at the significant peak position corresponds to amides A, I, II, III, and IV (Barth & Harris 2009; Kong & Yu 2007). The amide A peak positioned in the range of 3310-3270 cm^{-1}, corresponds to N-H symmetric stretching vibrations (Olsztynska-Janus et al. 2012). The amide A peak cannot confer the protein structure due to the N-H group which is basically insensitive to protein backbone conformation (Barth 2007). In the infrared spectra of an organic molecule, the amide I

peak is the most sensitive and intense peak observed and it provides the majority of information regarding the protein secondary structure (Pelton & McLean 2000). It positioned at approximately 1620 cm^{-1} in the region of 1700-1600 cm-1 originates 80% from C=O stretches corresponding to peptide–linked backbone of proteins. The rest 20% arises from N-H bending and C-N stretching vibrations (Bandekar 1992; Barth 2007; Garidel & Schott 2006; Olsztynska-Janus *et al.* 2012). Due to the unique hydrogen-bonding pattern, molecular geometry, and couplings among transition dipoles, the secondary structure of proteins provides somewhat distinct stretching frequency in the amide I region. Due to the different structure of secondary amides, the peaks produce at a different vibrational frequency and thus lead to the elucidation of structural identifications as given in Table 6 (Barth 2007; Barth and Haris 2009; Garidel & Schott 2006). The second most intense peak in the musk spectrum was Amide II, observed in the range of 1557-1507 cm^{-1}. The peak corresponds to the vibrational mode of in-plane N-H bending (40-60%), C-N stretching (18-40%), in plane C=O bending and C-C stretching. In the obtained spectrum of musk sample, the amide I peak is positioned at an average of 1620 cm^{-1}, that can be due to the Beta (β) sheets structures. Amide II peak positioned at 1538 cm^{-1} that is also due to the Beta (β) sheets secondary structures (Table 5.8).

Table 5.8: Secondary structure of amide bands (Barth 2007; Elkins 2011; Garidel & Schott 2006)

Secondary Amide Structure	**Amide I wavenumber (cm^{-1})**	**Amide II wave number (cm^{-1})**
Beta (β) sheets	1620-1640 1670-1695	1530-1550* 1510-1530†
Unordered or non- ordered structures	1640-1650	-
α – helix (alpha helices)	1650-1658	-
β-turns	1662-1686	-

*parallel, †anti-parallel

The peak positioned in the range of 1170-1000 cm-1 at approximately 1116 cm^{-1} corresponds to C-H stretching vibrations (Garidel & Schott 2006; Mohan 2004; Olsztynska-Janus *et al.* 2012; Pelton and McLean 2000). Amide III is the weakest peak in amides, positioned within the range of 1320-1286 cm^{-1} (Movasaghi et al. 2008). The dominant vibrational mode responsible for the amide III peak attributed due to C-N stretches in combination with in-plane N-H bending vibrations and on the other hand weaker contributions for amide III peaks arise due to in-plane C=O bending and C-C stretching vibrations. Peak positioned at 687 cm-1 in the range of 625-770 cm^{-1} attributed to Amide IV with O=C-N deformation. Another peak at 1439 cm^{-1} corresponds to methylene bending of amino acid, lipids, and proteins with asymmetric CH3 bending (Movasaghi *et al.* 2008; Narayanan *et al.* 2011). Peak at 2852 cm^{-1} is due to the methylene stretches of lipids with asymmetric and symmetric CH2 stretching (Orphanou 2015). Another peak at 1389 cm^{-1} is due to the fibrinogen/ amino acid side groups with symmetric CH3 bending.

Comparison of real and fake musk pods

Two samples from fake pods were collected and comparative overlaid spectra are shown in Figure 2 and Figure 3. As shown in figure 2, number of additional bands (1635, 1348, 1356, 1147, 1076, 996, 847, 796, 692) was observed in the spectrum of fake musk sample. A sharp and intense peak was positioned at 996 cm^{-1}. In case of fake pod sample 2, the number of additional bands were observed.

ATR FT-IR spectroscopy showed its usefulness in the field of wild-life investigation for the characterization of musk sample, both complete and in powder form, obtained from musk deer. The method is rapid, does not need time-consuming sample preparation, sensitive, reliable, and environment friendly. It gives rapid

information about the characterization of musk sample by providing information about proteins as well as minor constituents. To construct spectral libraries, including different species, may be helpful for the identification and classification by using chemometric analysis such as PCA, PLS-DA, and LDA. This approach deserves attention for conducting further studies, including more number of musk samples from different musk deer sub-species to check the reliability, specificity, and sensitivity of the method.

5.4.2 Characterization of Tiger, Leopard and fake claws

ATR FT-IR spectroscopy is able to differentiate fake, leopard and tiger claws sample from each other by unique, dominant peaks being present or absent in the spectra. Since by visual inspection of the spectra the discrimination of samples was not possible, PLS DA method was applied to discriminate the spectra, collected in the mid infra-red range.

The spectra were measured from 4000-600 cm^{-1} with a resolution of 4 $cm^{-1.}$ The signal was averaged from 23 scans. ATR FT-IR spectroscopy is able to differentiate selected wildlife fake, leopard and tiger claws samples from each other by unique, dominant peaks being present or absent in the spectra below the range of 4000-600 cm^{-1} region. Thus, the results obtained within this study demonstrated that the technique ATR FT-IR spectroscopy combined with multivariate data analysis can successfully yield spectra that obtain characteristic peaks attributable to components independently. Each of these samples have demonstrated different spectral patterns, despite some of the peaks which originating from common macromolecules.

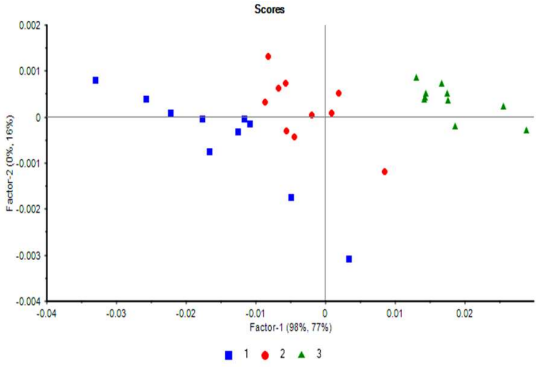


Fig. 5.1a: PLS DA score plot to differentiate fake claws from genuine animal claws

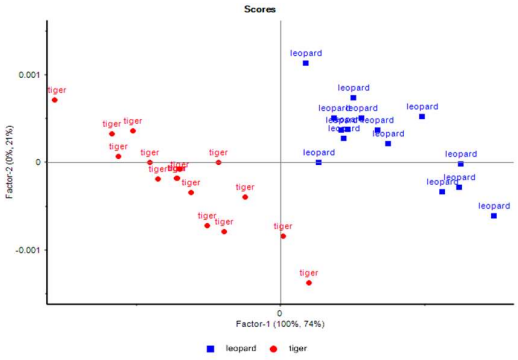


Fig. 5.1b: PLS DA model to differentiate between tiger and leopard claws

In conclusion, coupling of ATR-FTIR spectroscopy with PLS-DA and LDA has a potential to present an accurate, rapid, non-destructive, reliable and an eco-friendly alternative to the existing methods for the accurate identification and differentiation among Royal Bengal Tiger and Indian Leopard claws. The present method for analysis of unknown claws could accelerate the investigation process and corroborate with the conclusions made through other examination methods.

5.4.3 Characterization of "*Shahtoosh*" wool

Spectral analysis of training dataset Fig. 5.2 represents the composite hair spectra of Shahtoosh and Pashmina wool. The recorded spectra were measured in the midinfrared range that is 4000–600 cm− 1 with average scan of 24. Chemically hairs are composed of 65–95 % of proteins; therefore, the peaks of hairs are dominated by amide peaks associated with keratins. Thorough examination of these spectra allows us to confirm the presence of frequencies characteristics of keratin as assigned in Table XX. The spectra were measured by visual inspection followed by PCA and PLS-DA methods. Reproducibility was checked by analyzing three replicates of each collected hair strands and homogeneity test was

performed on all the hair strands by analyzing the same strand from three different points (left, central and right point). On the basis of overlaid spectra, no significant difference on direct ocular inspection was observed.

The obtained results demonstrated excellent reproducibility and repeatability. As shown in Fig. 5.2, ATR-FTIR spectra of *Shahtoosh* and Pashmina wool are almost similar and exhibit nearly identical values of wavenumbers however, only minor difference exist in the region of 1200–1000 cm− 1. This particular region is associated with the vibrations of sulphur oxgen groups of keratin **[34].** Sample of Pashmina wool did not exhibit the prominent band at the position of 1036 cm− 1 peak. It can be concluded that the intense band at 1036 cm− 1 observed due to the cysteine oxides and of cysteic acid.

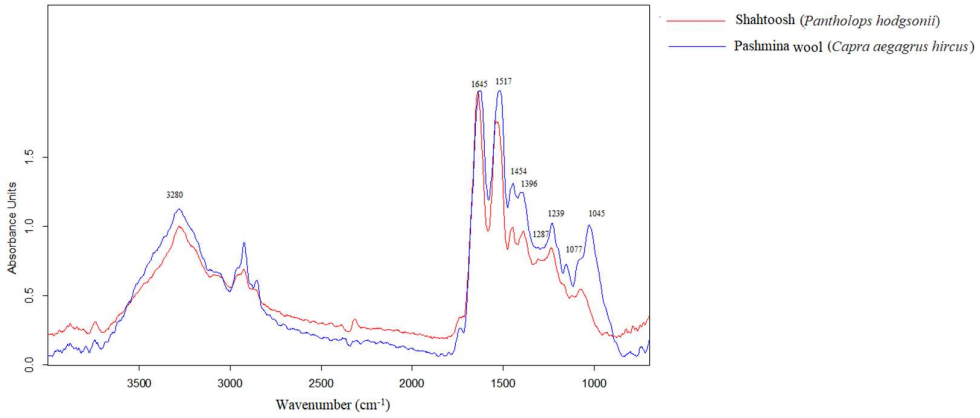


Fig. 5.2: Overlaid spectra of species of Pashmina and Chiru species in MIR range

However, it is quite difficult to differentiate the spectra of *shahtoosh* and *pashmina* wool only on the basis of their visual inspection. The visual discrimination is not legible and moreover subjective in nature, therefore further advanced chemometric tools were needed to extract the information-rich signals to get the results in an objective manner without biasness in the obtained results. The primary objective of this study is to differentiate the *shatoosh* and Pashmina wool through ATR FT-IR spectroscopy using PCA, and PLS-DA model. A PLS-DA model was constructed using training data set containing 50 spectra (25

each) of *Shahtoosh* and Pashmina wool. To check the performance of classification model, external validation and blind testing were also done. Collected hair samples of *Shahtoosh* and Pashmina wool for blind testing were not the part of training data set. For external validation test, samples were also collected separately from different species. In this study, an attempt has been made for the differentiation purposes when obtained spectra are somewhat inherently visually similar but differ chemically.

Construction of a binary PCA model

The PC-1 and PC-2 (2-D) score plot for the classification of hairs of Shahtoosh and Pashmina wool was performed. Here, PC1 explained 65% of variation and PC2 accounted for 21% of variation in the dataset. In total, cumulative variation of 86% was achieved using the PCA model. From the score plot, it was concluded that scattered distribution was although obtained, there is not a clear separation between class 1 and class 2.

Table 5.9: Assignment of prominent peaks of hair**: (**Hopkins *et al.* 1991; Akhtar *et al.* 1997; Espinoza *et al.* 2008; Gao 2013; Manheim *et al.* 2016)

Peak observed	**Group frequency**	**Designation**
3280	Symmetric N–H stretching. in 1◦amide	Amide A
1645	C = O stretching H-O–H Bending	Amide I (α-Helix)
1517	N–H deformations in 2◦amide	Amide II
1451	Asymmetric CH_3 bending	
1374	symmetric CH_3 bending	
1239	N–H bending	Amide III
1161	Stretching modes of the C-OH groups of serine, threonine, and tyrosine residues of cellular proteins	
1090, 1036	Cysteic acid	Cystine oxides

Construction of a PLS-DA model for the discrimination between *Shahtoosh* and *Pashmina* wool

To get the better classification rate, PLS-DA model was established for achieving the accurate discrimination between hair spectra of *shahtoosh* and Pashmina wool. During the process of establishing PLS-DA model, total 50 hair spectra (25 each) of *shahtoosh* and *pashmina* wool were imported.

Based on the PLS-DA model, the spectra of two wool *shahtoosh* (class 1) and *pashmina* (class 2) were successfully discriminated without any misclassification, hence achieved 100% accuracy for the classification of spectra of two wools. Highly significant R-square value for PLS-DA model was achieved that is 0.995; hence, it indicates greater predictive accuracy with minimum error rate. An important step after the establishment of PLS-DA model (discrimination of *shahtoosh* and *pashmina* wool) is to perform the validation study (blind and external validation tests) to verify the predictive capacity of the PLS-DA model. The loading plots showing the similar profile with the original dataset and highlights the important regions which convey important pieces of information. In loading plot, the region of Amide I (α- Helix) and amide II are the most important variables for the discrimination of *shahtoosh* and *pashmina* wool.

5.4.4 Characterization of species from Blood: Results and discussions

Figure 5.3 displays the ATR FT-IR spectrum of neat blood deposited on a clean glass slide and dried in situ for 24 hours and scraped out for analysis. Table 5.10 describes the identified peaks of blood with their corresponding wavenumbers (cm^{-1}). The results obtained for the characterization of blood peaks in the current work correlated with various reported literature of body fluid analysis using ATR FT-IR spectroscopy (Lang *et al*. 1986; Dong *et al*. 1990; Fearn 2000; Faridel & Schott 2006;

De Weal *et al*. 2008; Mou & Rabalais 2009; Manheim *et al.* 2016; Boll et al. 2017). In the present work, the position and frequencies of all the noteworthy peaks of blood are very similar with the peaks of blood reported in the published literature.

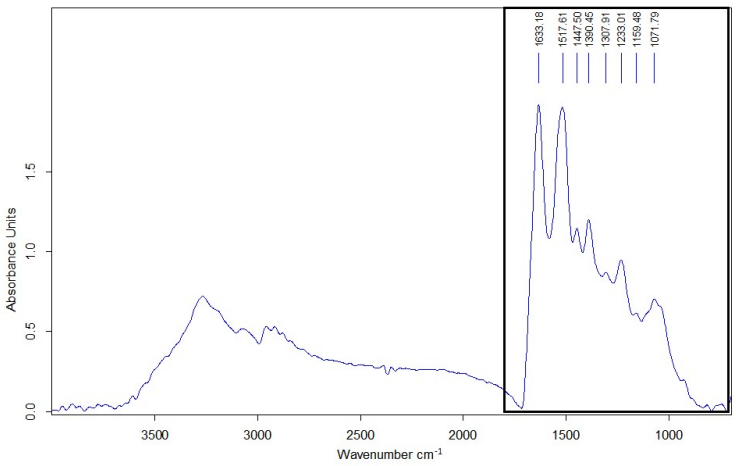


Fig. 5.3. Representative spectra of blood

Typical neat blood spectrum (% Absorbance vs. Wavenumber) consists of some broader and narrower peaks. The two peak positioned at approximately 1633, and 1517 cm^{-1} were categorized as intense, strong, and dominant peaks corresponds to amide I and amide II, respectively. Rest of the peaks was weak/small due to their low peak intensity.

Two weak peaks at approximately 1447 and 1390 cm^{-1} are due to the methyl bending of amino acids, proteins, lipids with asymmetric CH_3 bending and fibrinogen, amino acid side groups with symmetric CH_3 bending, respectively. Amide III was the weakest peak in amides and showed distinct "M'' shaped peak, positioned at 1307 and 1233 cm^{-1} as shown in fig. 5.3. The dominant vibrational modes responsible for the amide III peak are attributed due to C-N stretches in combination with in-plane N-H bending vibrations and on the other hand weaker contributions for amide III peaks arise due to in-plane C=O bending and C-C stretching vibrations. The lower apex region of

the spectrum that is 1250-925 cm^{-1} attributed to the carbohydrates (sugar moieties) with vibrational groups of C-O symmetric stretching. Other peaks positioned at approximately 1071 cm^{-1} corresponds to haptoglobin, fibrinogen, IgA, IgG, and IgM with C=O stretching. Table 5.10 showcases the characteristic band assignments of blood.

Table 5.10: ATR FT-IR peak components for the identification of blood

Wave number (cm^{-1})	Component identification	Vibrational mode	Peak intensity
3277	Amino acid (Amide A)	H bonded O-H stretching, N-H Symmetric Stretching (Water and hydroxyl group)	Medium peak
2948	Methyl stretches of lipids in plasma	C-H stretching	Weak
1643	Amide I (α-helix) (HSA and Hb is the major contributor)	C=O symmetric stretching	Strong and most intense peak
1527	Amide II(HSA and Hb is the major contributor)	N-H in plane bending vibration strongly coupled to C-N stretching vibration of protein	Strong and most intense peak
1445	Methyl bending of amino acids, proteins, and lipids	Asymmetric C-H scissoring of $-CH_3$ bending	Weak
1389	Fibrinogen and amino acid side groups	Symmetric CH_3 bending	Weak
1298 and 1240 (1229-1301)	Amide III (plasma proteins, transferring, and α1 – acid glycoprotein) { HSA and Hb is the major contributor }	C-N stretching	Weak(M shape peak in absorbance mode)
1162	Carbohydrates (glucose)	C-O symmetric stretching	Weak
1093	Fibrinogen, haptoglobin, IgA, IgG, and IgM	C=O stretching	Weak

Visual discrimination between Leopard, Tiger, Elephant, and Domestic pig

Comparative ATR-FTIR spectra of Leopard, Tiger, Elephant, and Domestic pig showed similar bands at similar wavenumbers as illustrated in Fig. 5.11. It is evident from the given figure that to the eye no visual spectral differences were observed between the Leopard, Tiger, Elephant, and Domestic pig. However, differentiation between spectra was impossible through visual inspection, therefore, advanced chemometric tools are needed for further discrimination.

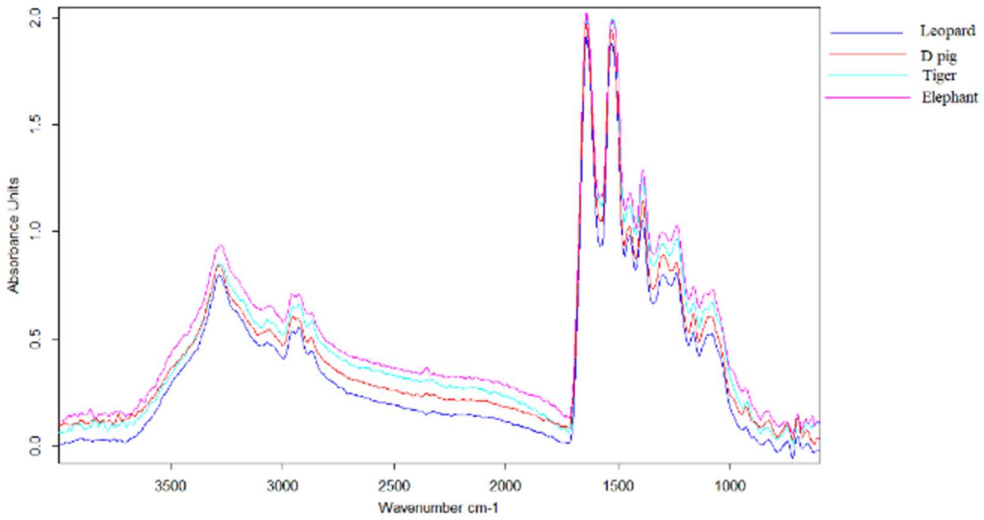


Figure. 5.4: Comparative ATR-FTIR spectra of Leopard, Tiger, Elephant, and domestic pig

Multivariate discrimination between the Leopard, Tiger, Elephant, and domestic pig

Discrimination using PCA

The principal component score plot of 3 PCS (principal components); PC1, PC2, and PC3 are exemplified in Figure 3. PC1 contributed 73 %, and PC2 accounted 15%, and PC3 added 8% of the total variance in the given dataset. The collective percentage (%) of total variance of PC1, PC2, and PC3 was 96%. The PCA could not

be discriminative enough through score plot, for blood spectra collected from different animal species. The spectra were dispersed or scattered on the plot and showed overlapping between spectra of different species; and assembled in a larger cluster; however, not clustered appropriately in their respective groups. Since the differentiation was not subtle, the scores for twenty principal components extracted from animal blood spectral datasets were further analyzed using PLS-DA predictive tools to characterize them in a more conclusive manner.

Discrimination using PLS-DA model: Tiger, Leopard, Domestic pig and Elephant blood differentiation was carried out by constructing the PLS-DA model. The prediction results of PLS-DA model are given in Figure 4. The PLS-DA plot illustrated complete discrimination or separation among ATR FT-IR blood spectra acquired from different species and no misclassification was observed in any case.

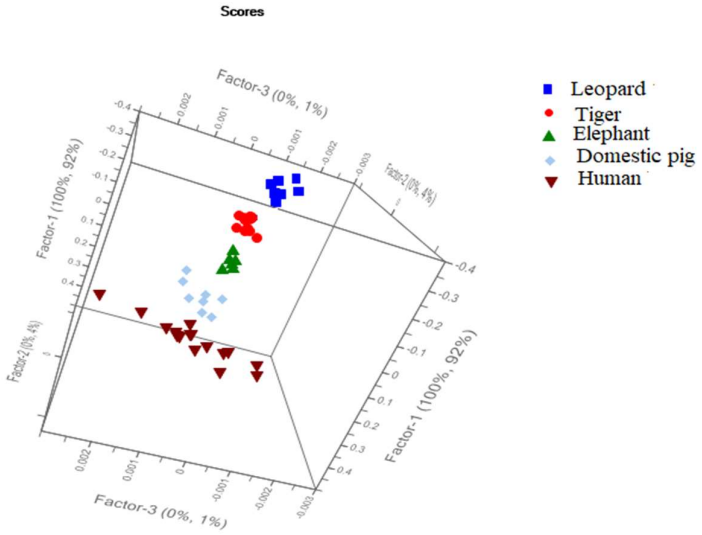


Fig. 5.5 Discrimination of Human blood from animal (Indian leopard, Bengal Tiger, Asian Elephant and domestic pig) blood

The obtained R-square value for calibration data set was 0.99 and for validation data set 0.98. The RMSE value for calibration data set was 0.29 and for validation dataset was 0.11. The slope value was nearer to 1 (0.99) for both calibration

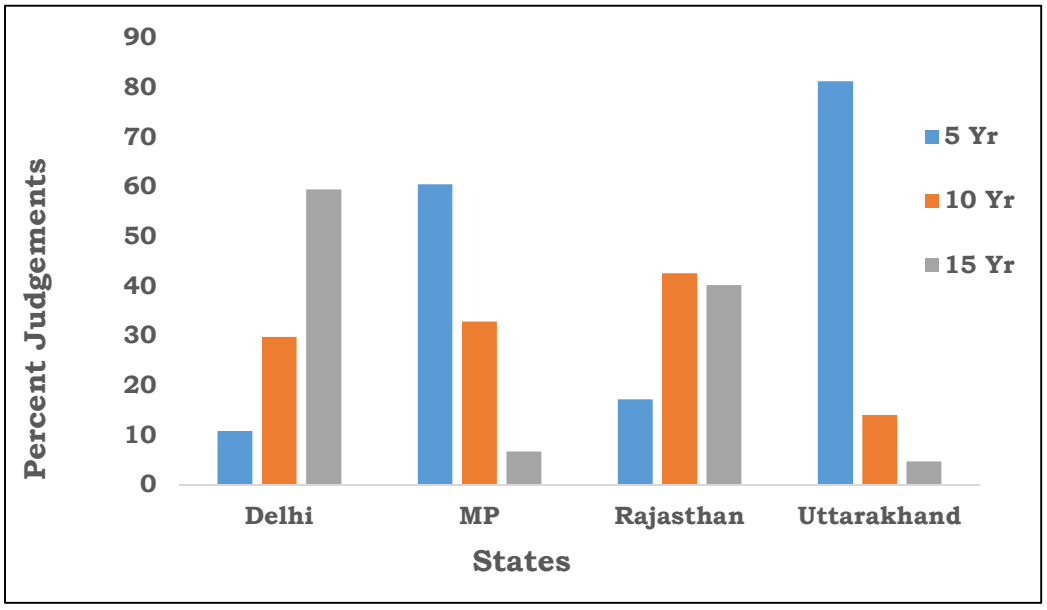
and validation dataset. To carry out the external validation test, 15 samples of human blood were collected and analyzed under the similar set of experimental conditions. The spectral data of human blood samples were incorporated in the PLS-DA model for the purpose of predictions. The obtained results of PLS-DA model are illustrated in Figure 5. The obtained R-Square value for PLS-DA model for the discrimination of human blood from animal blood is 0.972, which is highly significant.

Conclusions

Discriminating or identifying individual species from an unknown sample is highly significant for a variety of forensic applications. ATR FT-IR spectroscopy in tandem with advanced statistical tools shows excellent potential for the rapid and non-destructive discrimination of blood collected from Tiger, Elephant, Leopard, and Domestic pig. By using this method, obtained spectra from selected species were substantially discriminated with 100% accuracy without any overlapping. Human blood samples were used to construct the validation model and resulted no misclassification of human blood from animal blood. Due to the distinctive performance of the current method with rapid and non-destructive nature, this technique is highly promising for the identification of species in wildlife offences. However, before applying this technique to practical forensic applications, future work is needed to address the related impediments associated with the scene of crime. ATR FT-IR spectroscopy is quick, robust, eco-friendly and nondestructive technique that can potentially be executed in identification of species in future work after having a sufficient data base of species and comprehensive classification and discriminant model.

Chapter 6:

POLICY, LEGAL AND ENFORCEMENT FRAMEWORK IN WILDLIFE OFFENSES: WITH SPECIAL REFERENCE TO UTTARAKHAND



6.1 Introduction

Enough studies are available about the illegal wildlife trade, but as discussed earlier also, information is lying scattered with various enforcement agencies in India regarding the actual wildlife offenses booked and their fate during legal scrutiny. Any analyses on wildlife crime data or seizures made, are mainly dependent on regular and systematic reporting by state and central government agencies, media outlets which can further be corroborated from wildlife forensic laboratories. It has been observed from the seizure data that reporting often vary from state to state and region to region due to differences in reporting protocols, level of awareness among staff responsible for data reporting and varying level of social and print media and public interest and awareness. For instance, seizures of wildlife and wildlife products from scheduled and charismatic species (like tigers, elephants, *shahtoosh* fiber) and often well-documented and exciting stories related to negative interactions of wildlife with humans receive more public attention and media coverage, and are therefore more frequently found in the wildlife offense database. Due to this reporting bias a particular species overshadows other venerable lesser known species and also few areas show higher wildlife crime than other areas.

To verify the unsubstantiated claims of low prosecution and conviction rates in India, related to wildlife offenses, a sample study on booked wildlife offences and judgements delivered during the last decades from four states was undertaken. The four states include i) Uttarakhand, having rich biodiversity and mostly hilly terrain with porous border with Tibet & Nepal, ii) Delhi with scarce wildlife available but claimed to be illegal wildlife trade hotspot and Central Indian states of iii) Madhya Pradesh with rich biodiversity and a large tribal population and Western state of iv) Rajasthan with

totally different terrain and biodiversity. In all the above four states the lower court uses either Hindi or English for day today work and for writing judgements. Due to paucity of available online/offline court data from other states and also due to available judgements from lower courts in vernacular, this study was limited to four states only, but together they contribute more than 50% of wildlife offenses booked during the last decade

The objective of studying the "Policy, legal enforcement framework" was to understand comprehensively about the procedures adopted, lacunas, if any and rulings of the trial courts under Wild Life (Protection) Act, 1972 covering a sufficient time-span of one decade, from 2011 to 2020. Along with the wildlife offense data, the empirical data on fate of complaints filed before the trial courts by enforcement agencies was collected. It will allow us to understand the level of veracity in implementation of the provisions of the Wild Life (Protection) Act, 1972. A good rate of convictions under this act is a good indicator that the objectives of the act have been achieved and depends on not only employing winnable legal tactics, but also on the efforts made to collect and present the evidences before the court by more scientific interpretation. A peek into the judgments will also help in understanding in detail, to which extent the objectives of the act have been fulfilled and what are the reasons if any, for failure in implementation of the said act. This study will deal with legal scrutiny of investigation of wildlife offenses, its relevance in our contemporary times is very important and will allow us in formulating future strategies in dealing such crimes.

6.2 Material and Methods

For legal scrutiny of the wildlife offenses, empirical data was collected for analysis from e-court website being maintained by Department of Justice, Ministry of

Law and Justice, Government of India for the District Courts across the country and through collection of certified copies for each judgement. The key words for search involved State, District, Court name, Wild Life (Protection) Act, 1972FoWildlife. Four States from which sufficient judgements were available in Hindi or English were downloaded for study purpose. From Uttarakhand, certified copies were also sought from district courts, in addition to judgements downloaded from e-courts website. Along with the availability of sufficient judgments in Hindi or English language, the trends studied from wildlife offense data also led to selection of above four major states from north and central India, having 30.5% of reported offenses in national data of wildlife offenses (N=5817), variety of wildlife offense cases as representative sample from majority of states and involving various sections of the Wild Life (Protection) Act, 1972.

6.3 Limitation of the study

The wildlife offense dataset for Uttarakhand, underlying this analysis spans from 1st January 2011 to 31st December 2020, and focuses on the database available with Wildlife Forensic and Conservation Genetics Cell (WFCGC) of WII and Information sourced from open sources such as social and news media outlets, NGO's and state governments. The judgement study in wildlife offense cases studied here is not necessarily of those booked during the studied period from 2011-2020, as it sometimes takes longer than a decade to bring culmination of the prosecution. Sincere efforts were made to extract sufficient data from e-courts website and through collection of physical copy of judgements. Data was collated and scrutinized for double entry. In few cases reverse entry of data was also done, where detail and used in datasets of seizure were not available but from copy of judgement all details of the offenses were extracted. Judgements which were available in Hindi or English were only taken

and this was one of the main limitation of this study which prohibited its pan India scope. Many states were left out due to functioning of district courts in vernacular language or sufficient data was not available. The data not considered reliable or could not be deciphered properly is also left out in this study.

Acquisition of above mentioned data was highly opportunistic and by no means exhaustive, but is included in this analysis as it offers an empirical, wide-ranging overview of the scale of wildlife offenses, the provisions of the act, legal interpretation and enforcement lacunas, if any. As, the review is based on judgements delivered in trial courts only and the final results arrived upon should be seen considering deliberations and appeal in appellate and superior courts as data may change after appeal.

Using wildlife articles in seizures as a measure of impact on targeted species, is complicated because of different attributes of the seizures, i.e. a seizure of two elephant tusks or a bear gall bladder reported from Uttarakhand is inherently different from a seizure of 2000 mongoose hair brushes, 100 kgs of pangolin scales or 500 *shahtoosh shawls*. Therefore, using variables such as quantity alone, makes it difficult to get a true picture of the scope of the issue. However, these data provide some level of quantitative understanding of wildlife offenses reported from India. Also, the reported seizure data is dependent on the data source and only captures reported seizures and does not show entire crime against wildlife that were unsuccessful to confiscate or may have not been reported in the dataset. Thus, the study presented here only takes into account the data of crime against wildlife which was reported by various enforcement agencies indicating operative enforcement rather than entire account of wildlife offenses happening in India. Considering the dynamic nature and clandestine nature of these offenses, continued research related to these knowledge gaps are therefore needed.

6.4 Results and Discussions

For this study a total of 720 judgements delivered by trial courts during a period from 2011-2020 from four states, viz. Delhi, Madhya Pradesh, Rajasthan and Uttarakhand were collected as per details below (Table 6.1).

Table. 6.1: Details of judgement under study from each state

State	Total Judgements extracted	Judgements found sufficient for this study
Delhi	41	41
Madhya Pradesh	481	377
Rajasthan	123	123
Uttarakhand	75	75
Total	**720**	**616**

There is a huge diversity in type of cases booked for wildlife offenses in four states under study due to diverse geographical areas, biological diversity and status as primary supplier or consumer or trade centres, and thus varied problems in enforcement of the provisions of Wild Life (Protection) Act, 1972.

A peek through the dataset of wildlife offenses reported from Delhi, Madhya Pradesh, Rajasthan and Uttarakhand project a diverse field ov view. Delhi, by virtue of negligible protected area or forest cover or presence of wildlife with seizures of wildlife articles in every wildlife offese recorded, indicating basically a trade centre, whereas in Madhya Pradesh (45.9%) and Rajasthan (31.7%) reported wildlife offenses without any recovery of wildlife article also, i.e. offenses booked for illegal entry inside prohibited area, destruction of habitat or wildlife including collection of wood, River bed material (RBM), grazing of bufflao's etc. Although, Uttarakhand has many protected area and enough wildlife, there is no reporting of wildlife offenses where wildlife article is not recovered, thus despite having numerous protected areas, the data shows a trend similar

to Delhi in wildlife offense seizures. The prosecution data is similar for Delhi and Uttarakhand with success rate of much above at 82.4% and 84.1% respectively, in comparison to Madhya Pradesh and Rajasthan having conviction rate of 44.6% and 31.1% respectively. One disturbing trend noted from the dataset for Madhya Pradesh was that, in cases where wildlife articles were recovered, were having less conviction, i.e. more conviction for illegal entry or illegal grazing or destruction of habitat than for poaching or illegal trade in wildlife articles.

A deep look into the acquittal's from Madhya Pradesh and Rajasthan, shows a grim reality in that the enforcement agencies are incapable of understanding the intrinsic nature of wildlife crimes, evidences to be looked upon, scientific analysis and miserably failing in legal procedure. Since, the penal section 51 of the WPA, stipulates the punishment of an imprisonment for a term which may extend to three years or with fine which may extend to 25000/- or with both, with no minimum punishment, in cases of crimes including recovery of wildlife derivatives from non-scheduled animals, the scrutiny in courts about collected evidences is much low and enforcment agencies easily get minimum punishment.

But in cases involving recovery of wildlife articles from schedule I or part –II of schedule-II and crimes cimmitteed inside a sanctuary and Tiger reserve where minimum punishment is prescribed, the court will not allow any lacuna in investigation or in legal procedure and therefore both Rajasthan and Madhya Pradesh failing in getting conviction in those cases (27.4% and 34.9% respectively (fig. 6.1).

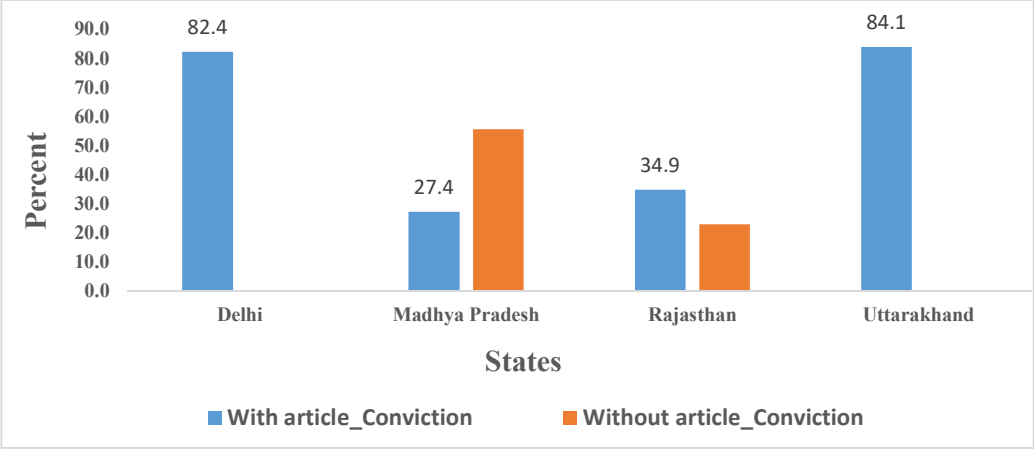


Figure 6.1: Conviction status for wildlife article recovery and non-recovery cases

Since, crime against wildlife is a gang like activity, requiring more than one person to maim the animal, the same translate in the arrest of suspected persons in wildlife offense cases. A combined study of four states, indicates that in 51% incidents, the suspect is one person, followed by 17.6%, 14.2%, 8.2% and 3.5% for 2,3,4 and >5 persons arrested in each wildlife offense cases. A further statewise breakup of this data shows that in Delhi single suspect was involved in 78% cases, indicating specifically carrier or trade activity whereas in Madhya Pradesh, Rajasthan and Uttarakhand, single person was found in 49.8%, 45.5% and 57.3 persons in the same order, the rest cases involve more that one person in crime against wildlife, more specific poaching and collection of illegal wildlife for susbsistence, occult practices or for local trade (Fig. 6.3 and Table 6.2).

Table 6.2: No. of accused persons apprehended in each case

State (N)	1	2	3	4	5	6	7	8	9	10
Delhi (41)	78.0	14.6	2.4	2.4	2.4	0.0	0.0	0.0	0.0	0.0
Madhya Pradesh (474)	49.8	15.2	14.1	10.3	3.8	**3.0**	**1.9**	**0.4**	**0.6**	**0.8**
Rajasthan (123)	45.5	26.0	15.4	6.5	2.4	**0.8**	**1.6**	**1.6**	0.0	0.0
Uttarakhand (75)	57.3	17.3	17.3	2.7	4.0	0.0	1.3	0	0	0

The judgement data from four states under study shows that it took even more than twenty years for a wildlie offenses case to be decided in Madhya Pradesh (0.2%) and Rajasthan (2.5%). In Delhi, although the conviction rate is above 80%, but it took more than 10 years to get judgement in 62.1% cases, whereas only 10.8% cases were decided within 5 years. None of the case was decided under 1 year in Delhi.

In Madhya Pradesh 62.8% cases were decided in five years and 96.7 % within 10 years, whereas for Rajasthan it is 17.3% and 59.4% respectively. In Uttarakhand trial courts delivered judgements in 81.5% wildlife offenses within 5 years and 96.3% within 10 years. In Delhi and Rajasthan require >10 years to reach a judgement in 62.1% and 40.5% wildlife offense cases repectively which is 3.2 and 4.6% for Madhya Pradesh and Uttarakhand

Table 6.3: Time taken in wildlife offense cases to reach the culmination

Duration/ State	**1 day**	**<1 Year**	**1-5 Years**	**5-10 Years**	**11-15 Years**	**15-20 Years**	**20+ Years**
Delhi	0.0	0.0	10.8	27.0	29.7	32.4	0.0
MP	0.4	17.9	44.5	33.9	1.9	1.1	0.2
Rajasthan	0.8	3.3	13.2	42.1	32.2	5.8	2.5
Uttarakhand	1.5	23.1	56.9	13.8	3.1	1.5	0.0

So, from above disscussion it is evident that in Delhi and Rajasthan, usually it took >10 years to get judgement in wildlife offense cases. Efforts were made to deduce this pattern from state of Delhi. While appearing as expert scientific witness in wildlife offense cases in special Act court at Tis Hazari Courts in Delhi, it was observed that in cases of Shahtoosh seizures, which make 75.67% of total judgements in this study for Delhi, the accused are usually well off traders from Jammu & Kashmir, having good connections and hire reputed lawyers from Delgi High Court or even from Superior court. Also, since the accused were usually from Jammu & Kashmir, application for allowing absence of accused due to many reasons was upheld by trail court regularly.

Also, many a time the witnesses, who have to join prosecution from other departments and far off places, also request for absence. All these reasons delayed the process of prosecution in court of law and this is evident from our data from Delhi, where none of the shahtoosh case was adjudicated within 5 years, 30% within 10 years while rest of 70% cases took more than 15 years for adjudication (Fig6.2).

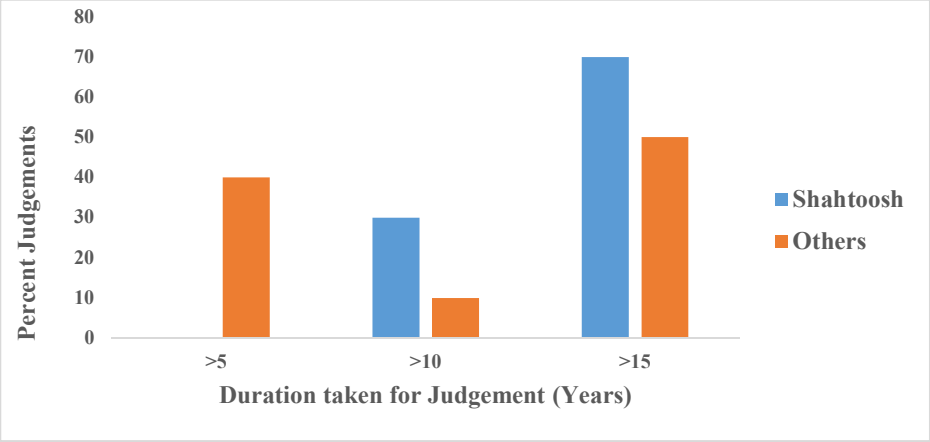


Fig. 6.2: Duration for getting adjuducation in Shahtoosh cases in Delhi Court's

6.5 Enforcement in Uttarakhand

Uttarakhand lies on the southern slope of the Himalaya range, and the climate and vegetation vary greatly with elevation, from glaciers at the highest elevations to tropical forests at the lower elevations. The highest elevations are covered by ice covered bare rock mountains in Chamoli, Bageshwar and Pithoragarh districts with the highest altitude of 7,816 metres (25,643 ft) (Nanda Devi) above sea level, whereas Sharda Sagar Reservoir in Udham Singh Nagar, is the lowest land point of Uttarakhand with the altitude of 187 metres (614 ft). Uttrakhand shares its north-west border with Himachal Pradesh, while in south lies Uttar Pradesh and has International border with Tibet in north and Nepal in the east. Uttarakhand State comprises of thirteen districts of which 07 are in Garhwal Region and remaining 06 in Kumaon Region. Uttarkashi,

Chamoli and Pithoragarh districts of Uttarakhand share International boundary in the north-west with Tibet and in the east, the districts of Pithoragarh, Champawat and Udham Singh Nagar share International boundary but with Nepal. Uttarkashi and Dehradun share inter-state boundaries with Himachal Pradesh in the north-west, while Dehradun, Haridwar, part of Nainital and Udham Singh Nagar touches the southern boundary with Uttar Pradesh.

Uttarakhand falls under Central Himalaya which is rich in biodiversity as this area exhibits large number of plants and animals. The 86% of total geographical area of Uttarakhand is mountainous and 65% is covered by forest. This variation in elevation and climatic conditions resulted in rich diversity of flora and fauna in Uttarakhand. There are about 7000 species of plants and 500 species of fauna in Uttarakhand state. The faunal diversity of Uttarakhand has 35 faunal endemic species including 11 vertebrates and 24 invertebrates. Many plant and animal species are highly threatened therefore major initiatives in the form of creating many protective areas for wildlife were taken for sustainable ecological balance (Sundriyal & Sharma B (2016). It is clear from the forgone discussion that by virtue of rich biodiversity and International open border with Nepal, Uttarakhand is very important as a consumer, transit and exporter for wildlife offenses especially for illegal wildlife trade.

For the sake of our study the wildlife offense data from all thirteen districts was studied with fields such as animal sub-groups, species, part of animal recovered in wildlife offense and conviction rate. A total of 26 wildlife sub-groups were identified in the dataset distributed across the thirteen districts. Large cats were represented in all thirteen districts with reaching top reported group in ten and except in Haridwar, Nainitaal and Udham Singh Nagar where these are in second position pushed below by deer species and turtles (both live and meat) species (Table 6.4).

Table 6.4: Distribution of wildlife sub-groups in all thirteen districts

S.no	Sub-Group	AL	BA	CL	CP	DD	HA	NA	PG	PI	RP	TG	US	UT	Grand Total
1	Antelopes	0	0	0	0	0	2	0	0	0	0	0	1	0	3
2	Badgers	0	0	0	0	0	1	0	0	0	0	0	0	0	1
3	Bears	1	4	5	0	9	1	4	1	4	2	3	0	3	37
4	Canid	0	1	0	0	0	1	0	0	0	0	0	0	0	2
5	Civets	1	0	1	0	1	0	0	0	0	0	2	0	0	5
6	Corals	0	0	0	0	0	1	0	0	0	0	0	0	0	1
7	Deer	1	1	10	1	27	10	21	4	1	3	3	12	7	101
8	Elephants	1	0	0	0	3	2	4	3	1		3	3	0	20
9	Fish (FW)	1	0	0	0	0	0	0	0	0	0	0	0	0	1
10	Hares	0	0	0	0	0	2	0	0	0	0	0	0	0	2
11	Large cats	**9**	**14**	**34**	**17**	**53**	**11**	**17**	**17**	**39**	**9**	**10**	**11**	**13**	254
12	Lizards	0	0	0	0	5	6	0	0	0	0	0	0	0	11
13	Mongoose	0	0	0	0	0	0	1	0	0	0	0	0	0	1
14	Fake/NA	1	1	3	0	16	8	11	6	4	1	1	2	1	55
15	Otter	1	0	0	0	0	0	0	0	0	0	0	0	0	1
16	Pangolins	0	0	0	0	13	1	0	1	0	0	0	1	0	16
17	Pheasants	0	1	0	0	0	1	0	0	0	0	0	0	0	2
18	Pigs	0	1	0	0	2	0	3	1	0	0	0	3	0	10
19	Porcupines	0	0	0	1	4	2	0	1	0	0	0	0	0	8
20	Owls	0	0	0	0	0	1	0	0	1	0	0	0	0	2
21	Shells-marine	0	0	0	0	1	0	0	0	0	0	0	0	0	1
22	Small cats	0	0	0	0	2	1	0	0	1	0	0	0	1	5
23	Snakes	0	0	0	0	1	9	0	0	0	0	0	0	0	10
24	Turtles	0	0	0	0	8	18	1	1	0	0	1	10	0	39
25	Water birds	0	0	0	0	0	0	0	0	0	0	0	2	0	2
26	Wild Sheep/ Goat	0	1	3	2	1	0	0	0	3	0	1	0	0	11
	Grand Total	**16**	**24**	**56**	**21**	**146**	**78**	**62**	**35**	**54**	**15**	**24**	**45**	**25**	**601**

AL- Almora, BA- Bageshwar, CL- Chamoli, CP- Champawat, DD- Dehradun, HA- Haridwar, NA- Nainital, PG- Pauri Garhwal, PI- Pithoragarh, RP- Rudraprayag, TG- Tehri Garhwal, US- Udham Singh Nagar, UT- Uttarkashi (FW- Fresh Water, NA- not applicable)

A detailed study for fate of large cats which included Tiger, Leopard and Snow leopard found in Uttarakhand, common leopard (*Panthera pardus*) was targeted in all districts (>3%) with abundantly with maximum seizures from Dehradun, Pithoragarh and Chamoli all three reporting >15% seizures. For parts of Tiger (*Panthera tigris*), Nainital (43.5%), Dehradun (21.7%) and Champawat (13%) districts are hot spots, whereas 6 districts didn't report any tiger parts seizure. Snow leopard parts have three seizures, one from Dehradun and two from Pithoragarh (Fig 6.3).

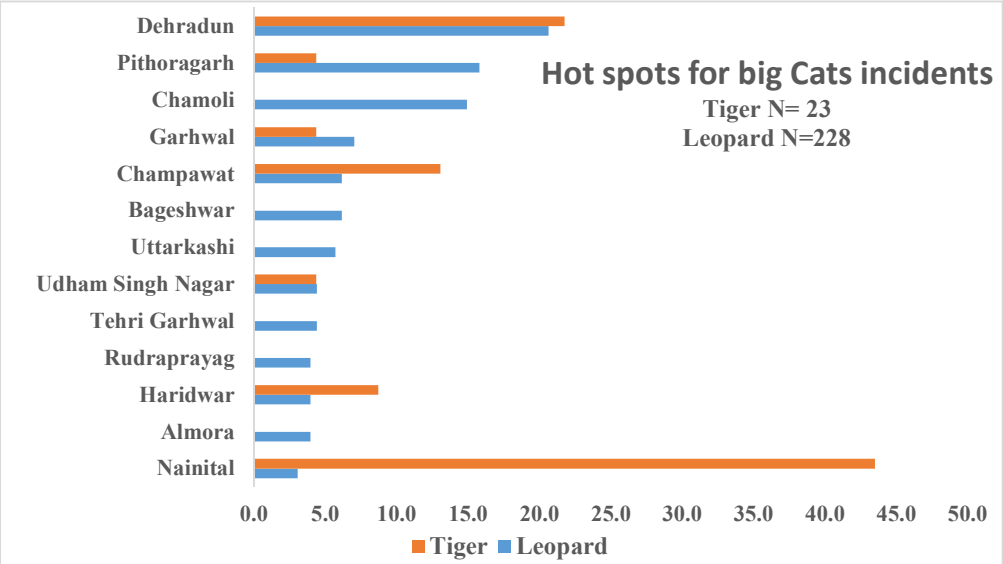


Fig.6.3: Distribution of Leopard (*Panthera pardus*) and Tiger (*Panthera tigris*) parts in districts of Uttarakhand

Apart from large cat's seizures, the other more frequent targeted sub-group of wildlife are deer and turtles. Sambar deer is most targeted species among deer family including musk deer in Uttarakhand represented in 47.5% (N=101) incidents. Sambar deer is reported from all districts of Uttarakhand except for Bageshwar and Pithoragarh. Dehradun (27.1%), Haridwar (16.7%) and Nainital (14.6%) shared the top place in incidents against sambar in Uttarakhand. Chital represented 26.7% of deer species in wildlife offense cases with Nainital (33.3%), Uttarkashi (29.6%) and Tehri Garhwal

(25.9%) represented >25% incidents. Bageshwar (7.45) and Dehradun (3.7%) incidents also reported cases against chital. Rest of the districts didn't report any incidents against chital.

Musk deer parts were represented in 16 (15.8%) incidents with Chamoli district reporting maximum 43.8% incidents, whereas Dehradun and Uttarkashi both contributed 12.5% incidents. Other districts reporting musk deer parts in offenses includes Pithoragarh, Garhwal, Nainital and Bageshwar, each representing 6.3% incidents. Maninly the reported seizures include musk deer pods, canines and skin. Hog deer was reported in 2% incidents from Nainital and Udham Singh Nagar while Swamp deer antlers were reported from Dehradun in 1% reported incidents.

Table 6.5: Number of incidents involving deer species in each thirteen districts of Uttarakhand

Districts →	**AL**	**BA**	**CL**	**CP**	**DD**	**HA**	**NA**	**PG**	**PI**	**RP**	**TG**	**US**	**UT**	**% incidents**
Species ↓	**Number of Incidents**													
Sambar	1	0	3	1	13	8	8	3	0	2	2	3	4	47.5
Chital	0	0	0	0	7	2	9	0	0	0	1	8	0	26.7
Musk Deer	0	1	7	0	2	0	1	1	1	1	0	0	2	15.8
Indian Muntjak	0	0	0	0	4	0	2	0	0	0	0	0	1	6.9
Hog Deer	0	0	0	0	0	0	1	0	0	0	0	1	0	2
Swamp Deer	0	0	0	0	1	0	0	0	0	0	0	0	0	1

AL- Almora, **BA**- Bageshwar, **CL**- Chamoli, **CP**- Champawat, **DD**- Dehradun, **HA**- Haridwar, **NA**- Nainital, **PG**- Pauri Garhwal, **PI**- Pithoragarh, **RP**- Rudraprayag, **TG**- Tehri Garhwal, **US**- Udham Singh Nagar, **UT**- Uttarkashi

Dehradun (26.7%, Nainital 19.8%, US Nagar 10.9%, Haridwar and Chamoli (9.9) and Uttarkashi 6.9% represented >80% of offenses against deer family. Haridwar reported more offenses (46.2%) against turtles than any other district in Uttarakhand followed by Udham Singh Nagar (25.6%) and Dehradun (20.5%). Most of the incidents involve Indian Soft Shell Turtle (Lissemys punctata), found live and or in meat form. along with. Other species involed includes Indian Tent Turtle (Pangshura *tentoria*),

Brown roofed turtle (*Pangshura smithii*), Indian *eyed turtle* (Morenia petersi), *black pond turtle* (*Geoclemys hamiltonii*) etc. mostly for pet trade

6.5.1 Conviction in Uttarakhand

The legal framework in Uttarakhand, with respect to other states compared during this study is found to be quite satisfactory with >80% convictions in sample dataset. The salient features in district wise study of the conviction rate is worth mentioning here.

i) Bageshwar reported 19% of total wildlife offenses in Uttarakhand, it got maximum conviction, in 81.8% of total cases filed in Bageshwar and Garud courts and the contribution of it in state percent of conviction is 18.8% of total 58 wildlife offense cases.

ii) Chamoli district has reported 40% of successful conviction in 8.6% of state reporting and contribute 4.2 in state conviction rate. Gopeshwar court has zero conviction in three cases that had completed the trial.

iii) Champawat reported 22.4% cases with success rate of 69.2% which contributed to 18.8% to the successful convictions in Uttarakhand.

iv) Nainital district reported 22.4% cases from Haldwani court, with 100% conviction and contributing to 4.2% to State conviction rate.

v) Similarly, Haridwar reported 1.7% to the total reported wildlife offenses with a contribution of 2.1% to the state conviction rate.

vi) The most sensitive border district but successful district also, was Pithoragarh which reported 44.8% of wildlife offenses in Uttarakhand with a successful conviction rate of 96.2% from Pithoragarh and Didihat courts, including one case in Juvenile Justice Board, Didihat. This district contributes 52.1% of successful conviction rate in Uttarakhand (Table 6.6).

Table 6.6: Conviction rate in six major wildlife offense prone Uttarakhand districts

Conviction rate		
District	State % age of reporting (N=58)	% conviction in state (N=48)
Bageshwar	19	18.8
Chamoli	8.6	4.2
Champawat	22.4	18.8
Nainital	3.4	4.2
Haridwar	1.7	2.1
Pithoragarh	44.8	52.1

6.6 Conclusion

From the foregone discussion, it is concluded that there is a pattern of crime for target killing of wildlife for subsistence or for illegal trade for minting quick money and even the target species are specific to each crime. The police, legal and enforcement framework can be better understood as:

6.6.1 Pattern of crime

For all four states, the pattern of wildlife offenses has shades of poaching for subsistence, conflict of interest or for making quick money by dealing in prohibited articles in illegal wildlife trade. Delhi is emerging, as expected, as a major hub for collection and illegal trade of cash rich wildlife articles like *shahtoosh*, elephant ivory, large cat's skins and marine shells including sea fans as display items. In Madhya Pradesh, the reporting and judgements are more for conflict resulting in registration of more offenses in illegal entry, destruction of habitat including illegal cattle grazing, cutting of trees, ignition of fire and collection of River bed material (RBM) from protected areas. In Rajasthan, more judgements are for illegal entry, destruction of habitat by collection of RBM and for dealing in illegal wildlife parts. The pattern of wildlife crime in Uttarakhand usually has poaching and illegal trade activity, but the pattern is more inclined towards the cases involving illegal trade of targeted large cat

parts and turtles. Number of accused per case apprehended in 57 % cases is one and >90% cases having 3 or less indicating that in Uttarakhand, the wildlife crime usually involves targeted species for illegal trade.

Since, crime against wildlife is a gang like activity and it is assumed that more than one person to poach an animal and extract the valuable part of the animal. This assumption is verified by the data on arrested suspected persons in wildlife offense cases across all four states. A combined study of four states, indicates that in 51% incidents, there is only one suspect. Delhi with 78% cases and Uttarakhand with around 60% case having single accused, indicate specifically carrier or trade activity whereas in Madhya Pradesh and Rajasthan single person was found in 49.8% and 45.5% cases. Arrest of one accused in cases from Delhi and Uttarakhand can be explained as in all these cases the offense booked was under section 39 (Govt. Property) or 49B (illegal trade) of WPA. Rajasthan and MP also shows a bit higher arrest of single accused per case which is due to the cases booked for illegal entry (Section 27 of WPA), destruction of habitat (Under section 29 of WPA) by grazing of buffalo or extraction of RBM using Tractor and the driver is usually booked for such cases.

The rest cases involve more than one person in crime against wildlife, more specific poaching and collection of illegal wildlife for susbsistence, occult practices or for local trade.

6.6.2 Conviction Rate

The conviction rate is much above (>84%) for Uttarakhand, which is quite good in comparison to other studies states of Delhi, Madhya Pradesh and Rajasthan. One of the main reason came into light, is that in Uttarakhand almost all the wildlife offense cases were referred to scientific Institutions for species identification of biological evidences recovered from accused. Since, in Wild Life (Protection) Act, 1972 (WPA),

the prohibited species are placed in schedules of the act, identification of species is a must to proceed under penal section 51 of the act. In has been very prominently found out that the prosecution in almost all the cases, didn't tried hard to get evidences to prove the motive of illegal trade or link perpetrator with the crime and primary crime scene of poaching, which is well translated into judgements from hon'ble trial courts while giving the judgements for conviction, always release the accused in section 9 and 49B of the WPA. Uttarakhand gets higher conviction rate of >84% cases mainly under section 39 read with section 51 of Wild Life (Protection) Act, 1972, proving illegal possession of the contraband. Also, noted that, it is well documented fact that in Traditional Chinese Medicine (TCM) pharmacopeia use of tiger and leopard bone powder is much recommended and these two large cats are usually poached for that only, but the seizure data shows that the seizures of skins is not matching with the miniscule seizures of bone consignments. There is a need to change the attitude of our enforcement agencies mainly in cases involving leopards and tigers to always investigate the backward and forward linkages, while investigating tiger and leopard parts seizures.

6.6.3 Challenges before the court: Lenient view by Judiciary

While dealing offenses against mute wild animals, the judiciary comes with its own set of challenges, as the presiding officers often tend to take a lenient view in case of wildlife crime. Various factors like old age, poor health, poverty, tribal villager make the accused as the aggrieved party, rather than the wildlife that is poached, resulting in probation, less punishment, only fine etc. even for scheduled wildlife, against the actual punishment as stipulated in section 51 of WPA. Also, too much emphasis was also given to prosecution independent eyewitnesses, and whenever independent eyewitnesses are not present or become hostile as usually they belong to same locality

or due to stretching of trial too long, the benefit often goes to the accused rather than to the prosecution.

Another aspect detrimental to success in getting prosecution is the lack of intent shown by the government appointed public prosecutors in trial courts. Most of them are overburdened with the sheer load of legal proceedings in different acts and hence wildlife-related cases are least on their priorities. Also, by virtue of WPA being a special act, less shared in academic curriculum, the majority of them lack interest in the provisions of this Act, leading to failure to invoke the specific provisions of the Act, which are quite robust. For example, in Bageshwar, Uttarakhand while the Police failed to prevent acquittal to the accused even in leopard skin seizures, whereas in same court, during the same period the Forest Department won all the wildlife offense cases.

A deep look into the acquittal's from Madhya Pradesh and Rajasthan, shows a grim reality in that the enforcement agencies are incapable of understanding the intrinsic nature of wildlife crimes, evidences to be looked upon, scientific analysis and miserably failing in legal procedure. Since, the penal section 51 of the WPA, stipulates the punishment of an imprisonment for a term which may extend to three years or with fine which may extend to 25000/- or with both, with no minimus punishment, in cases of crimes including recovery of wildlif pderivatives from non-scheduled animals, the scrutiny in courts is much low and enforcment agencies easily get minimum punishment. But in cases involving wrecovery of wildlife articles from schedule I or part –II of schedule-II and crimes cimmitteed inside a sanctuary and Tiger reserve where minimum punishment is prescribed, the court will not allow any lacuna in investigation or in legal procedure and therefore both Rajasthan and Madhya Pradesh failing in getting conviction in those cases.

Reasons for acquittal in cases where wildlife derivative is recovered includes huge contradiction in the statements of key witnesses in relation to time and space,

hostile independent witnesses, lenient view of judiciary "since there is no boundary wall, buffalos came inside for grazing of their own" or "villagers are living inside the forest area, and therefore claim that they entered the protected are illegally is not tenable"

In case of wildlife derivative recovery, even for scheduled wildlife, the main reasons of acquittal involves no scientific analysis for species identification of wildlife derivative, half cooked species identification report given by veterinary officer from meat or bony fragments, firearms were not sent for analysis in for ballistic analysis, if they are in working condition or not, wrong preservation or contamination of tissue sample collected from crime scene led to "No opinion" reports from forensic laboratories, conscious possession of prohibited item with accused not proved beyond doubt, absconding accused (s) were not conclusively identified due to darkness or long distance, ownership of the land on which crime was found remained doubtful, and reasons mentioned above regarding discrepancies in documentation and statements of witnesses, among others.

Role of independent witnesses, who usually belong to the same locality as of the accused, needs to be discussed when we talk about the dismal performance of conviction rate in wildlife offense cases. Due to closeness, societal pressure and over-stretching of legal battle, in majority of cases the independent eyewitness are becoming hostile resulting into detrimental effects on judgements. Therefore, the public prosecutors need to educate the presiding officers of the court about the intrinsic nature of wildlife crime and secluded crime scenes where independent witnesses are very rare and the departmental witnesses are one whom the court has to believe after proper scrutiny.

The Wild Life (Protection) Act, 1972 as amended time to time, is one of the most comprehensively drafted acts, which lays a lot of stress on protection of wildlife

and prevention of crimes leading to overall conservation of nature. Despite this to get successful prosecution in the courts, it requires relevant evidences and witnesses, both independent and departmental. Usually the site of occurrence of the crime is also a critical factor in deciding the prosecution outcome of a crime. Since most of the crime against wildlife happens in secluded places, wildlife seizures happen in remote locations, inside protected areas where no common people are allowed to enter or during late hours rendering difficult to get independent witnesses. Therefore, scientific meticulous collection of evidences, their analysis and interpretation is foremost in getting conviction in wildlife offense cases.

The study of the judgements from four diverse states for which sufficient data was available helps in better understanding of the reasoning behind the judgements and provide remedial actions, if any. The purpose was to find out the trial procedures followed in trial courts cases under study and reasons behind the judgment, either of conviction, or, of acquittal. If conviction, whether it needs further augmentation and if acquittal, the lacunas to be addressed. Through empirical data collected this study amply documents the most likely reasons of low conviction rate in wildlife offense cases and identifies the deficiencies during the investigation of in trial procedure. It brings forth to light the problem faced by the prosecution during investigation to get independent witnesses for general public is usually not willingly ready to be involved as a witness or witnesses are from local village who become hostile due to family pressure during the course of trial in court. The trial court in many instances pointed out the shoddy investigation of the Investigation agency as they fail to produce the relevant incriminating evidence against the accused to buttress their case.

Chapter 7:

PROSECUTION OF WILDLIFE OFFENSE CASES: A SYNTHESIS

7.1 Introduction

Crime against wild animals, majorly its illegal trade for subsistence, traditional pharmacopeia, fashion, artefacts and fraudulences had adversely affected the enforcement of laws for protection of the wild faunal diversity. Sustainable use of natural resources including all forms of wildlife for subsistence is inherent to mankind development since its arrival on this planet. This sustainable use, involves very balanced, intricate but fragile interactions among living beings and their surrounding environment. A slight deviation due to unrestrained and unsustainable exploitation, can result in havoc for flora and faunal species at different levels, locally or over a large area, which becomes noticeable in short term or over a long period (Clifton & Rastogi, 2016). This over exploitation of biodiversity as a whole is one of the important contributing factors for pushing few species to the extermination, especially the charismatic species and megafauna (Challender et al. 2015). World over different societies and cultures have different prominences on sustainable use of wildlife for subsistence and have varied mode of collection and economic rationales (Bhattacharya 2016). The ethics and ethos of conservation of nature is inherent in Indian culture and many animals and plants are considered sacred, and accorded protection due to social, religious, traditional and other reasons.

In general terms a “wildlife offense” refers specifically to crime against wild flora and fauna and involves the illicit taking, transport, trade, or possession of animal or their derivatives thereof in contravention of existing domestic and international laws and treaties (Sosnowski & Moreto, 2021). Crime related to wildlife is very dynamic in nature, influenced by geographical and global effects maneuvering clandestinely with changing demands, place, innovating suitable techniques for poaching for species specific poaching, purely for the minting quick money.

India, by virtue of its strategic location, is considered one of the key country in the illicit trade in wildlife because of its role as a source, transit and destination country [Organization for Economic Co-operation and Development (OECD), 2016]. In relentless efforts for better conservation of biodiversity, wildlife protection regulations and laws in India, are framed and amended time to time, and enforcement agencies specifically employ, Wild Life (Protection) Act, 1972 (WPA) along with other wildlife protection laws and rules for achieving it. The provisions of WPA, not only prohibits trade in any wildlife or part of it, but also stipulates certain prohibitions for management of protected areas in the country, and contravention of any provision will amount to an offense against wildlife and the offender is liable for prosecution under this act.

Implementation of existing wildlife protection laws, needs a cohesive effort from park managers, other enforcement agencies, scientific Institutions and legal fraternity considering the increased recognition of the potential consequences of depleting wildlife due to un sustained exploitation of wildlife for commercial gains. The efficacy of WPA comes under challenge bearing in mind that despite multi-agencies efforts to curb illegal wildlife trade, poaching of scheduled species and illicit trafficking of their parts remains unabated. Apart from commonly cited hindrances in responding to this persistent illicit activity including legal gaps and lack of coordination among enforcement agencies and with forensic labs, lack of capacity for early detection of wildlife crime is one of the major issue. As discussed earlier, the illegal wildlife trade is very dynamic, and new challenges in identification of illegal wildlife article or its derivative crop up sooner, placing fast and robust morphological characterization protocols with enforcement agencies and forensic scientists is of paramount importance.

Practicing wildlife forensic morphologists are constantly striving to keep pace for characterization of wildlife derivatives, but considering the vast numbers of species and their derivatives involved along and lack of broad study on the nature and scope of wildlife offenses across India, noticeable gaps in having robust and reliable species identification protocols remains to be fulfilled. Examining the wildlife offense data for a decade (2011-2020) gives a peek into the dynamic nature and broad spectrum of one of the most noticeable Green crime. Using amalgamation of basic tenets of wildlife sciences and forensics resulted in developing morphological protocols for identification of wildlife derivatives. All efforts were made to provide scientific morphological protocols which can be easily understood by officers implementing law and used by scientist dealing case property in Wildlife Forensic Laboratory.

This pioneer study combines an intensive field fed dataset and laboratory based assessment of wildlife offenses in India in recent times and allows us to understand the gaps and provide remedies for early detection by enforcement agencies and characterization of species from wildlife parts submitted to forensic laboratories. It is hoped that this work will be a valuable resource for wildlife forensic scientists, forest, police, customs and other enforcement agencies who are tasked for the protection of our National treasure of wild fauna. The following submissions, which emanated from this research will seek to address emerging issues, if any and help in formulating national and regional multifaceted policy initiatives by focusing on interdisciplinary enforcement, technical assistance, and capacity building, to effectively counter wildlife offenses.

Enforcement of wildlife protection laws for curbing the wildlife offenses at all stages—poaching at source, processing and intermediate transfer, and smuggling to the demand site—the approach is often casual, and unsystematic due to absence of basic

data and knowledge about the dynamics of wildlife offenses. To effectively confront this ever-growing challenge, it requires exploration and application of both, field and forensic laboratory tools. Therefore, in this study efforts are made to holistically analyze data showing overall crime scenario against wildlife, so as to enable enforcement agencies, forensic labs and conservation managers to devise strategies for better enforcement of wildlife protection laws.

Efforts were also made to highlight vulnerabilities of enforcement and legal system while dealing wildlife offenses as a sample study, therefore making it multi-faceted.

7.2 Trends in Wildlife Offenses

Quantification of wildlife offense data to get the trends and dynamics of crimes happening against wild animals during the period 2011-2020, 5817 offenses were studied in detail. It has been observed that the format for submission of offense data is not uniform in all states. The fields were casually filled up in many cases with common English name was not mentioned or vernacular name was provided, common name is not matching with the scientific name and many times scientific name of a species which is exotic is written against some Indian Species. While dealing with the data it was clearly visible that reported offense data is highly skewed towards few states while some other states reported very few cases. As reported, during the reported period, eight states in alphabetical order, viz. Chhattisgarh, Gujarat, Madhya Pradesh, Odisha, Punjab, Tamil Nadu, Uttarakhand and Uttar Pradesh constitute more than 60% of reported wildlife offences. It doesn't imply that in other states the wildlife offenses are less, may be the offenses are not reported.

The detailed study of the data presented, contributes to the growing understanding of wildlife crime research that has identified and explained major trends and patterns of crime against wildlife in India during the last decade. Since, the data, apart from seizures during illegal trade of wildlife, also takes into account the other crimes as stipulated in the Wild Life (Protection) Act, 1972, provides a holistic understanding of the wildlife crime and to chalk out remedial measures. The salient features can be summarized as follows:

The wildlife offense cases reported for the decade usually remain stable for the country, with slight variation in reported cases among states during different years, requiring enforcement agencies to be a persistent in implementation of wildlife preservation regulations. The offense data shows slight downward trend during the last two years, more understandably owing to less data entry during 2020 for the year 2019 and COVID during 2020-2021.

i) The enforcement of wildlife protection laws is not uniform among states of India as evident from reported data, with few states doing really good job in enforcement of WPA and reporting too, while others need to augment their enforcement and reporting mechanism.

ii) There are few hot-spots identified in this study involving the collection and illegal trade centres. Few of them were identified as collection/trade centres for wildlife derivatives specifically meant for international trade, i.e. *shahtoosh*, large cat skin while others are more notorious for applying special *modus operandii* for poaching of targeted species, local trade for subsistence, Occult practices and local beliefs. various wildlife crime hotspots in India: for different targeted animals there are different crime hotspots like in Uttarakhand, Chamoli

district is notorious for Musk deer and Asiatic Black Bear parts whereas in Bageshwar and Pithoragarh districts it is leopard derivatives. Also, with open border with Nepal, Pithoragarh is the main transit point to Nepal for Leopard, Musk deer and Asiatic Black bear parts. Udham Singh Nagar and Haridwar districts reported majority of soft-shelled turtle seizures, for subsistence, along with few other species for pet trade in Haridwar. Documents submitted with wildlife offenses, also reveals about the major role being played by National capital region of Delhi, being a major collection and transit point for cross country and trans-national illegal trade in wildlife (*shahtoosh*, Tiger & Asian Elephant products) routes. Similarly, Special Task Force and CB-CID, Odisha has identified Siliguri in West Bengal as a major collection and transit centre in North-east India for pangolin scales along with other wildlife articles for trans-national illegal trade to Myanmar via Champai in Mizoram. Thus, the enforcement agencies have to be very meticulous in designing strategies for each specific area considering special enforcement measure and one tactics will not work for whole nation.

iii) There are few taxonomic groups/species identified that need special attention for protection, as they are specifically targeted for lucrative transnational illegal trade. As the dynamics and scope of illegal wildlife trade is much dependent on the international demand, and also with the advent of social media, demand for species and their derivatives, which were not traditionally in the illegal wildlife trade has emerged, i.e. monitor lizard, pangolin, sand boa, sea fans, *trochus spp*. etc.

iv) Although the more charismatic and traditional species viz. tiger, leopard, snow leopard, elephant, Rhinoceros take the limelight in reported wildlife offenses or

international trade Share of traded vs subsistence species (large cats, Elephant, Rhino vs others)

v) Almost all the enforcement agencies directly or indirectly were involved in enforcing the wildlife protection laws in India. The majority of offenses, as expected, were reported from State Forest departments, along with Police department including Central Bureau of Investigation (CBI) and National Investigation Agency (NIA), Central Armed Police Force's (CAPF) like Sashastra Seema Bal (SSB), Assam Rifles, Indo Tibetan Border Police (ITBP) through Forest department, Indian Customs (Air Intelligence unit, Cargo and Land customs points) including Directorate of Revenue Intelligence (DRI), Wildlife Crime Control Bureau (WCCB). Indian Navy (IN) and Indian Coast Guards (ICG) also made several seizures in the high seas of Andaman and Nicobar Islands transferred to Forest department for filing the case in court.

vi) This study on wildlife offenses found that illegal trade in wildlife is very dynamic and has wide scope with certain species are disproportionately represented in seizures and found in only specific forms (i.e. Tibetan antelope for "*shahtoosh*" products, Mongoose - painting brush, Varanus *spp.* for hemipenis, pangolin for scales, sea horses/cucumbers- in dried forms and are at variable risk status in wildlife conservation laws, represent various taxonomic groups, from specific collection or trade regions (Star tortoises- southern India and Fresh water turtles- train/road route to west Bengal via Uttar Pradesh)

vii) Large quantity of seizures of marine animals especially sea horses, sea cucumbers, sea fans and Trochus *spp.* needs special attention from marine

enforcement agencies to avoid any threat to large scale extermination of these species.

viii) Seasonal variation observed on the expected lines, with monsoon season reporting less seizures, owing to inhospitable access routes for patrolling of staff or for dense vegetation not suitable for poachers to venture inside,

ix) With the expansion of new International routes, airports and seaports tend to facilitate not only the illegal wildlife export (shahtoosh, star tortoise) but also greater illegal wildlife imports (exotic pet mammals, birds and reptiles trade). Therefore, the enforcement agencies have not only to look for export of our wildlife treasure, but also arrest the import of exotic animals usually for pet trade and zoo displays. The exotic species, both live and derivative forms includes live turtles, primates, birds, Rhino horn (Black Rhino), Chamois and Wart hog head mounts, Hippopotamus canines and others. These species may not directly come under wildlife protection laws in India, but enforcement agencies should not lower their guard in stopping these, as under CITES their trade is restricted and also in live form that may be a danger to endemic populations or may carry zoonotic pathogens.

x) Specific pattern and equipment's used to poach a wild animal was observed, i.e. large caliber firearms for large herbivores (Elephant, Rhino, Nilgai) and shotgun for water birds, snare/jaw traps for big and lesser cats, wild pig, pheasants/birds, hunting dogs for lagomorphs, pangolins, monitor lizards, pigs, cervids, catapult for peafowls, potash bomb for wild pig, plant poison (root paste of *Aconitum spp*.) for elephant, tractor tubes with net for illegal fishing in protected areas are common tools/equipment's of poaching.

xi) Although, 51% of wildlife offenses have one suspect, but those cases are overwhelmingly illegal trade related where carrier was apprehended. Other offenses have more than one person involved while committing a wildlife crime, clearly indication that wildlife crime is more sort of a gang like activity where 49% cases have multiple suspects. For committing poaching, it is almost certain except in poaching of few birds related crimes or in retaliatory killing of problematic animals by poisoning or electrocution cases, that more than one person is involved.

xii) While analyzing seizure data of such a large magnitude, our results shows an equilibrium towards charismatic and lesser known species both in contrast to other trends analysis studies by Indian or International scientists, as those were based on the illegal or CITES trade data, which mainly involves targeted species of commercial interest. Our results show that the overall National trends and pattern of wildlife offenses in India are inclined more towards the subsistence or local trade, a likely reflection of socio-cultural demands for certain products and opportunistic crimes including illicit trafficking of wildlife parts and proportionately less to cater the international illicit trade.

xiii) Noticeable, is the fact that apart from the recently added lesser known species like varanus *spp*., pangolins, other species which does not make headlines but are much frequent in seizures involve Black bear, Musk deer, Sambar, Lagomorphs (Black napped hare) and Jackals derivatives (siyar singhi or "Jackals Horn") which are widespread all over India in their distribution ranges.

xiv) The study has not only highlighted varied numbers of species involved and large scale inflow of imitating wildlife but also the growing role of online

marketplaces and mobile and social media-platforms to facilitate faceless display, sale and transfer of money warranting a coordinated response from enforcement agencies (Specifically illegal "*shahtoosh*" trade analysis).

xv) A peek into the invoices of *shahtoosh* seizures meant for exports, not only indicate the increased numbers of destinations in new regions but also indicate that many consignments although seized at Indira Gandhi International (IGI) Airport, New Delhi, were actually booked from Jaipur, Sawai Madhopur and Jaiselmer towns in Rajasthan, to avoid any detection. Also, as a common feature, all the seizures were miss-declared as pashmina products and also are mixed with pashmina products consignment. During the decade 2011-2020 the woolen products seized, suspected to be made of "*shahtoosh*" were found with low percentage of guard hair available for analysis in comparison to period from 2001-2010. This has resulted due to more awareness among enforcement agencies by continuous sensitization towards "*shahtoosh*" resulting in choking of supply routes for illegal trade of raw wool of Tibetan antelope inside India, thus mixing of large amount of pashmina goat wool with "*shahtoosh*" and also employing more sophisticated tools to comb out guard hair or "dehair" Tibetan antelope raw wool. The weaving and embroidery pattern also shows variation with machine weaving, now a new normal instead of hand looms and in embroidery it was observed that heavy weaving is more in supply now, making the shawls very heavy, some of them using gold/silver threads for weaving or having stories depicted scenes completely filling the shawl surface with embroidered threads. The size also shows a trend in variation with *shemagh* (square shawls) and stoles are more in demand from middle-east Asia and East-Asia which are new destinations during the later decade. One more important

aspect observed during analysis of data identified a new frontier for smuggling of *shahtoosh* products in the form of land route, the Land Customs, Integrated Check Post (ICP) Attari, Punjab bordering Pakistan, which was not known earlier. This was mainly during severe COVID -19 lockdown period when all the international air transportation was halted. These above findings along with cyber intelligence, were of immense use for enforcement agencies in developing concrete information about the suspected consignments and are published in peer reviewed journal.

7.3 Morphology: as a tool in wildlife forensics

One of the major issues in enforcement of wildlife (Protection) Act, 1972 in India is lack of knowledge and thus intent, with other enforcement agencies except for forest department, about prohibited wildlife parts and products found in raw and processed form.

Regarding identifying the gaps in protocols available for morphological characterization of species, enough work was already done for identification of large cat species mainly Tiger and leopard, the two sympatric species from major bones. But all the work done was on the complete skulls, whereas in wildlife forensics around 40% seizures reported broken skulls, loosing many identifying features. Therefore, a comparative study was undertaken and found that only three features which are usually found, even in broken bones can be used to differentiate tiger bones from leopard bones. Also, the first cervical or Atlas bone is also very commonly available (% percent) with the seizures of bones and can be a good evidence to identify the species from a heap of bones, without any skull. The atlas bone of four sympatric species, tiger, leopard, sloth

bear and hyaena was characterized cent percent based on only three parameters by using morphometric analysis.

Similarly, canines are also found regularly in the seizures and main species involved includes Tiger, leopard, Musk deer and Wild pig. Fake canines made of synthetic material were also recovered regularly in wildlife offense cases. Morphological characterization with statistical analysis protocols were successfully developed, to differentiate Tiger, leopard and horse canines, which will be a boon for wildlife forensic laboratories. The above protocol to differentiate animal parts among sympatric and similar size species whose derivatives are regularly found mixed in wildlife offense seizures, based on morphometric analysis by decision tree, has opened up new avenues for use in wildlife forensics for fast and conclusive results.

7.4 Fourier Transform Infrared Spectroscopy

As discussed above, the illegal trade in wildlife keeps pace with new demands and always strive to avoid detection by enforcement agencies by transforming the identifiable morphological features in derivatives. Some of these derivatives are processed so much that even extraction of DNA is much difficult rendering the analysis impossible. For such cases, characterization of species from wildlife articles based on Infra-red based spectroscopy comes as a rescuer. Owing to much smaller size of instrument, with sufficient training, it can even be used in a small lab at the air or sea port or any other forensic laboratory. Since this technique requires almost nil preparation and no destruction of samples, is fast and accurate, it is the future in wildlife forensics along with existing techniques. During this study the wool hair of *shahtoosh* and pashmina, which otherwise are difficult to differentiate conclusively by molecular or microscopy techniques were successfully differentiated. Also, claws of tiger, leopard and fake (made of animal keratin- hoofs and horns) were also differentiated from a

small scrape, which are usually found carved and embedded in pendants, losing major morphological features. Efforts were also made to characterize and differentiate species from blood by FTIR technique. The findings of the above research on claws, *shahtoosh* and blood were published in international peer reviewed journals.

Musk deer is a highly protected species in India, and is poached mainly for illegal trade for scent gland (Musk pod) found only in the males of this species. Since, the secretion from this scent gland were stored in a muscular pouch in the ventral region (belly) of the males, the pouch is covered with skin having long, wrinkled white hair. But, it has been observed in seizures of the scent gland that, poachers to avoid detection, shave or singe the hair of the skin thus leaving useless for identification of species by microscopic analysis of hair. Also, in many cases fake musk deer pods with pouch made of some domestic animal skin and containing incense powder/ charcoal with perfume and medicines claimed to contain musk powder were also recovered. To overcome this problem of identification of hair less genuine musk pod and musk contents, the grains of genuine musk deer scent gland and two fake glands were successfully characterized by ATR- FTIR technique. Based on the same line ATR-FTIR technique was found successful in characterizing the blood of Tiger, Leopard, Asian Elephant, wild pig and human as control sample, and differentiate to species level for forensic studies. The above four species make up 41.57% of all the reported wildlife offenses among mammals.

7.5 Enforcement & Legal ramifications

This study takes into account all types of wildlife offenses into considerations for holistically evaluate the dynamics, scope and legal implications of wildlife offenses and vary from other similar studies in that, in other studies data only for illegal trade (seizures) and CITES trade data is usually taken to draw conclusions. This has allowed to understand the type of wildlife targeted, procedures adopted during investigation, in

filing of the case, lacunas, if any and rulings of the trial courts under Wild Life (Protection) Act, 1972 covering a sufficient time-span of one decade, from 2011 to 2020. This ultimately results in verification of the unsubstantiated claims of low prosecution rates in India in wildlife offenses. The four central Indian selected for this study has varied concerns in implementation of wildlife protection laws and provide a comprehensive vision representative to other regions of India. These states include Uttarakhand, having rich biodiversity and mostly hilly terrain with porous border with Tibet & Nepal, Delhi with scarce wildlife available but claimed to be illegal wildlife trade hotspot and Central Indian states of Madhya Pradesh with rich biodiversity and a large tribal population and Western state of Rajasthan with totally different terrain and biodiversity. Despite, hindrances in getting certified copies and language issues being judgements in vernacular language, since these four states together have contributed more than 50% of wildlife offenses booked during the last decade, a comprehensive study was possible which provided a feel of state of affairs in legal scrutiny of wildlife offense cases.

The duration for reachning the culmination in wildlife cases in courts, was calculated from the delivered judgements, and was found to be a long process, in sink with offenses as mentioned in other acts including Criminal Procedure Code (CrPC). From one day, for those cases where court was not convinced about the varacity of the case to few milder offenses where the offender has admitted guulty and was fined or impriosnment till the court raise for the day, the duration has reached >20 years for wildlife offenses, i.e. in Madhya Pradesh (0.2%) and Rajasthan (2.5%). In Delhi, although the conviction rate is above 80%, but it took more than 10 years to get judgement in 62.1% cases, whereas only 10.8% cases were decided within 5 years. None of the case was decided under 1 year in Delhi.Therefore, thorough documentation is not only necessary for getting the conviction in the court of law but also along with

it continuous monitoring of the legal process by responsible officials is a must. Any relaxation in approach may result in detrimental consequences for the prosecution.

The statistical data of the judgements studied shows that approach of prosecuting authorities and judicial officers, both is not always in consonant with pro-wildlife as can be visualized by low conviction rate in few states and sometimes lesser punishment than stipulated in the law after convicting the accused.

The reporting and thus the trial in courts of the wildlife offenses in highly skewed, higher towards few states, while others showing very low wildlife offenses, contrary to common beliefs. But, still at micro level a deeper look at the districts/regional level, few hot spots for wildlife offenses were identified and considering their status as trade, transit or collection/poaching centres, special remedial measures may be adopted for enforcement and legal aid is provided to staff in getting sufficient punishment against offenders so as to set a benchmark deterrent in the community.

The lucid reasons identified for failure in prosecution of wildlife offense cases in trial courts can be explained as:

Witness credibility: Most witnesses happen to be department witnesses which at times hurts the credibility of such witness. As usually the independent and eye witnesses are from same locality, during long pendency of case, these turn hostile or do not cooperate.

Lack of awareness of the provisions and the intent behind the wildlife laws: Too much reliability of prosecution on witnesses and lack of acquaintance and training of department witnesses, to the provisions of Wild Life (Protection) Act, 1972, and insecure mindset while deposing for examination in chief or to tackle the art of cross-examination deployed by defense is one of the main reason identified for acquittals in

wildlife offense cases. The results reflect somewhat grim picture of the state of affairs in dealing wildlife offenses legally. Although, convictions are pronounced in many cases, but despite the stringent Wild Life (Protection) Act, 1972 due to lack of understanding of the provisions and intent behind the act and procedures to be followed, many cases involving recovery of Schedule-I species derivatives, were lost in court. In such cases, the court pronounced the acquittal by holding that conscious possession of the said article could not be proved beyond reasonable doubt.

Lack of Motivation in Public Prosecutor: The wildlife offenses are dealt by the public prosecutors on behalf of enforcement agencies, unless department hire special public prosecutors for dealing only such cases. While appearing for court cases as expert scientific witness, it has been observed and data also indicate that the public prosecutors, while dealing all sorts of cases in trail courts, are laid back and lacks motivation to win as they do not take it as serious a crime as against a human or their incentives are not subject to their winning or losing the case (Garud & Bageshwar courts in Uttarakhand). Further, in few cases mostly in big or capital cities, where the accused can pay hefty fees and can hire very senior superior court defense counsel's, further add demotivation of public prosecutors as in Shahtoosh cases in Delhi, Allahabad in Uttar Pradesh.

Since, wildlife offenses are against the mute animals, instances involving misuse of the "law of plea bargaining" wherein most offenders, imploring their lower social status, poverty, old age, ignorance and inadvertent or accidental death of wildlife (electrocution of schedule-I species in agriculture fields by electric fencing), bargain the punishment they deserve with the much lesser one and in many cases succeed in getting it than what is stipulated in the WPA without any resistance from the public prosecutors.

Lengthy Trial: All wildlife-related cases are criminal cases, and take a long time to get disposed-off. This delay generally goes in favor of the accused, with witnesses turning hostile and evidence losing credibility. Also, during such long period, the investigating officers were transferred to many places and to other responsibilities also, the public prosecutors changed and even the Magistrate's get transferred many times in between the trial. With time, the memory and interest of the witnesses, investigating officers, public prosecutors get clouded, resulting a favorable ground for the accused's.

Insufficient efforts: Efforts rendered for search and recovery of evidences to link the recovery with the scene of crime-backward or forward linkage to prove *mens rea* and *actus reus*, mostly weapon/instrument in poaching cases, possession of wildlife or presence of accused at the scene of recovery during transit of illegal wildlife or money trail to prove illegal trade.

Loss of Physical evidence: Lack of understanding of the importance of scientific collection and interpretation of physical evidences to link the perpetrator with the crime scene and thus failed to prove guilt of the accused's.

Wrong identification of species and nomenclature used for the wildlife crime report. The collected information shows that in several cases, species scientific name is missing, wrongly written or only vernacular name of species was written. Even in few cases species which are not reported within the geographic distribution of the country or region were found in the official records.

Missing details about the form, dimensions and other identification features of seized articles sent for identification of species to forensic laboratory: The details of form and dimensions of wildlife article, i.e. skin was dry or fresh when seized and its size, any puncture/cut mark on it, claws were present on skin and if yes, their numbers, bones seized were dug out from the pit were clean or with meat, were fresh

or old, with skull or not, if yes all teeth including canine present or absent etc. These all have serious repercussion during framing of charges and identification of seized property.

***Lacking fool-proof "Chain of Custody"*:** Maintaining the sanctity of the case property, as it changes hands in one of the most important issues in wildlife offense cases. The concept of importance of 'Sample seal" was found lacking, mostly the staff from the forest department were unaware about the proper sealing procedure of the evidence property and few case properties were received, sealed with molten lac having impression of "Ashoka emblem" of currency coins. Few other case properties were received by "Courier" service, a private entity, which is challengeable during trial. It is to be noted that "Chain of Custody" has to be maintained in any circumstances and any accidental or intentional break of official seal or unauthorized handling of the case property is breach of "Chain of custody" and definitely the benefit of doubt will be for the accused leading to acquittal. It is highly advisable to educate the enforcement staff about the importance of 'Chain of Custody" in wildlife offense cases, as the two most important aspects in getting convictions, i.e. proving possession and the species identification of biological evidence by forensic laboratories will become useless if accidental or intentional breach is proved.

Lack of scientific analysis report: Lack of or no scientific analysis for species identification of suspected wildlife derivatives under investigation. In few cases, the veterinary officer gave the species identification report randomly of wildlife parts, when no identifying feature were available

Wrong preservation of Biological evidences: Cases booked under Wild Life (Protection) Act, 1972 (WPA) mandatorily requires species identification of wildlife as the punishment varies with the species status in different schedules of the WPA. It is commonly observed the during necropsy the veterinary officers and forest officials also

usually put the biological samples in formalin as preservative for analysis by molecular technique. This is flawed as formalin render the tissue sample useless for analysis by not allowing DNA extraction for analysis. So, 'No Opinion" reports are issued in such cases, which ultimately hamper the prosecution case.

Analysis of the statistical data from four states for which sufficient data was available, it is clear that provisions of wildlife (Protection) Act, 1972 are not succeeding strictly and uniformly pan India. In Delhi and Uttarakhand the conviction rate is 84.1% and 82.4% respectively, whereas for Madhya Pradesh (44.6%) and Rajasthan (31.1%) it is much below, during period 2011-2020. Unfortunately, in many cases prosecution gets conviction for illegal entry and destruction of habitat whereas in cases where actual wildlife articles were seized, the prosecution failed miserably. So, When the law is same, agencies responsible for enforcing the wildlife protection laws are equipped with similar powers, to prohibit wildlife offenses, this sort of disparity needs better understanding of the dynamics of wildlife crime, scientific collection of evidences, handy scientific tools and protocols for detection and identification of the wildlife articles, better interaction with forensic laboratories, and thorough knowledge of wildlife protection laws.

Wildlife offenses, a major "Green Crime" is very dynamic and has very vast scope considering number of species involved, their derivatives, imitations available in market and illegal trade in National and transnational grey market. As illegal wildlife traders usually have better knowledge of the trade routes, local and transnational cooperation, therefore operate several steps ahead of enforcement agencies, this present a serious challenge to all enforcement agencies including legal and scientific communities to have better intelligence sharing, identification and concrete prosecution in court of law to set a deterrent in society. We have to understand that, wildlife forensics, cannot work in isolation as it is the Interface between various enforcement,

scientific and legal entities, which teaches wildlife to them and incorporates policing and science in it. The above laid down statistical facts, figures and study of judgements helps in better understanding of the overall scenario, identified the gaps in detection and identification of wildlife derivatives and lacunas during legal scrutiny and provide remedial actions and reasoning leading to help in chalk out strategies for providing justice to wild life. Although there is no dichotomy between the written act and procedure for its implementation on field but the capacity and sometimes will also, for its implementation is lacking among various enforcement agencies. These all have to be augmented, considering the challenges faced in the present scenario, for proper & effective implementation of wildlife protection regulations especially provisions enshrined in Wild Life (Protection) Act, 1972, by continuous scientific intervention in documentation of events, identification of targeted species, their derivatives, collection and preservation of evidences, protocols for species identification from raw and processed wildlife parts along with educating field officials in court craft is highly desirable. This will go a long way in formulating wildlife conservation policies and implementing wildlife protection laws in the country, ultimately leading to positive impact on International illegal trade in wildlife.

Efforts in this study were sincerely made to identify and highlight vulnerabilities of enforcement and legal system while dealing with wildlife offenses therefore making it multi-faceted in providing a holistic assessment of overall wildlife offense enforcement, policy and legal framework. Since this study discusses the dynamics of wildlife offenses, pros and cons in enforcement of wildlife protection laws based on empirical data, it is in no way an expression of the total wildlife crime rate of any state or region as the data is only based on the reported incidents and does not account for actual crime against the wildlife.

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Rapid and non-destructive identification of claws using ATR-FTIR spectroscopy–A novel approach in wildlife forensics

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ABSTRACT

Differentiation and identification of Royal Bengal Tiger (*Panthera tigris tigris*) and Indian Leopard (*Panthera pardus fusca*) claws is a challenging task in wildlife forensics, due to similarity in their morphology, anatomy and chemical compositions as both the species are closely related to each other genetically. ATR-FTIR spectroscopy, which offers a non-destructive and safe alternative technique to other conventional methods, has been employed in the present work to differentiate claws of Royal Bengal Tiger and Indian Leopard. An attempt has been made to differentiate 31 reference claw samples from 16 different Royal Bengal Tigers, 15 different Indian Leopards, and 10 fake claws using ATR-FTIR spectroscopy supplemented with PCA, PLS-DA, and LDA. PCA could not distinguish the samples of two closely related species among themselves as well as from the fake claws. On the other hand, PLS-DA and LDA models both yielded highly significant classification rate for differentiation among the samples of Royal Bengal Tiger, Indian Leopard, and their fake counterparts. Further, seven blind claw samples that were pretended to be unknown to the analyst of both the species are also examined and identified correctly to their respective groups. The R-Square value obtained for PLS-DA model to differentiate Royal Bengal Tiger, Indian Leopard, and fake claws is 0.99, which is highly significant for predictive accuracy. This study shows that ATR-FTIR spectroscopy with PLS-DA/LDA has a potential to present a rapid, non-destructive, reliable, and eco-friendly approach for the accurate identification and differentiation of Royal Bengal Tiger and Indian Leopard claws.

1. Introduction

Wildlife crime is posing a serious threat to biodiversity and environment. A huge amount of money, approximately $ 32 billion involved in the illegal trade of wild animals, their bodily parts and products thereof is raising serious issues with internal security of many nations across the globe as well [1–3].

Every year, wild animals in large numbers are poached to fulfill crazy and money-grubbing desires of human being. The incident of poaching of Royal Bengal Tiger (*Panthera tigris tigris*) and Indian Leopard (*Panthera pardus fusca*) among all other wild cats are more frequent due to the heavy demand of their body parts and products in this trade [4,5]. In India, both species are declared critically endangered and are protected under schedule 1 of the Wildlife (Protection) Act-1972. At the international level, both are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which prohibits international commercial trade of these species, their body parts or other derivatives between all CITES Parties. India has been a signatory to CITES since 1976 [6].

Along with skin and bones, claws are the most sought-after articles in the market and constitute to 3.8% of total articles ($n = 2899$) related to wildlife crime received at Wildlife Institute of India, Dehradun for forensic examination [7]. Claws are used to make pendants, sculptures, items of religious worship and Traditional Chinese Medicines (TCMs) etc. High demand for Royal Bengal Tiger and Indian Leopard claws has caused the heavy influx of fake claw articles in this trade as well [7]. Due to the extensive or wide range of similarity in appearance and morphology of Royal Bengal Tiger and Indian Leopard claws and their fake counterparts, it is of paramount importance to differentiate and identify them for the successful implementation of laws related to wildlife protection and conservation.

For the analysis of claws various techniques such as of X-ray analysis (Radiography), Burn test, morphological and DNA-based techniques have been used in past [7]. X-ray analysis and morphological

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examination, which require thorough knowledge of claw morphology and anatomy is rapid and non-destructive in nature however cannot be applied to the samples, which are fragmented/mutilated/modified. The Burn test (a keratin-specific pungent smell is produced, when a small piece of claw is burnt in a flame, due to the formation of sulfur compounds) is both destructive and subjective. DNA based species identification is the most commonly employed and widely accepted method to identify claws. However, DNA analysis from keratinized materials is a laborious and costly process, which involves usage of harmful chemicals and destructive as well [8].

Recently, spectroscopic techniques have emerged as a tool for species identification from a variety of biological materials. Human, cat and dog blood was successfully differentiated using Raman and ATR-FTIR spectroscopy coupled with principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) respectively [9,10]. Human and animal blood was successfully differentiated using Raman spectroscopy [11–14], Diffuse reflectance spectroscopy [15,16], Near-infrared diffuse transmittance spectroscopy combined with PLS-DA method [17,18] and ATR-FTIR spectroscopy [19]. Pickering et al. [20] reported the discrimination of maggot's species and its life cycle stages simultaneously by using ATR-FTIR spectroscopy in combination of principal component- discriminant function analysis (PC-DFA), and support vector machine (SVM). In addition, human, cats, dog hair [8], human and non-human bone [21] were also successfully differentiated by using ATR- FTIR spectroscopy. The application of FTIR for non-destructive differentiation of hard tissues of animals especially in members of closely linked families is still in its infancy stages. However, the following works can be considered as a stepping stone to move forward. Espinoza et al. [22], Guo et al. [23], Walker, [24], and Nagaraju [25] have worked on the identification of Elephants and Giraffe hair, straight guard hair of Golden cat and Indian Leopard cat, various animal hard tissues, and skin and appendages of wild animals respectively. Therefore, this study is a step further in this direction.

In this pilot study, ATR-FTIR spectroscopy supplemented with chemometrics is used to differentiate and identify claws of Royal Bengal Tigers, and Indian Leopards with external and blind validation testing. As per the authors' best of knowledge, no study has been reported for the purpose of species identification from claws using ATR-FTIR spectroscopy till date. From a forensic perspective, this technique could be an ideal tool because of its sensitivity, rapid analysis, eco-friendly, and non-destructive nature with a high degree of confidence and minimal to no sample preparation requirements. The main purpose of utilizing the chemometric methods for such kind of problems is that the spectroscopic analytical methods (such as FTIR in the present study) generate a huge amount of dataset creating difficulties in the interpretation of the results from these dataset. This kind of problems could potentially be overcome through the use of chemometric techniques. The data/spectrum obtained through ATR-FTIR analytical method varies from sample to sample and these chemometric methods extract different useful information for the purpose of classification and prediction. The individualization and classification in a particular class of samples is known as 'chemical pattern recognition' [26]. These pattern recognition methods are having two types namely supervised and unsupervised pattern recognition. PLS-DA and LDA which come under supervised pattern recognition have been utilized for classification purpose here.

2. Materials and methods

2.1. Sample collection details

A total 31 Claw samples from 16 different Royal Bengal Tigers (*Panthera tigris tigris*) and 15 different Indian Leopards (*Panthera pardus fusca*) as training data set were obtained from the repository of Wildlife Institute of India (WII), Dehradun. Additionally, a test dataset of 10 claw samples, proven fake (with the help of X-ray, morphometric measurements, and burn test) was prepared for external validation test to assess the performance of classification model. The fake samples were made up from keratinized materials most likely hooves and horn of other species. A set of seven samples (41, 53, 54, 81, 82, 163, and 164) was also prepared for the blind test. These samples were pretended to be unknown for the analyst and their actual identity was not revealed until predictions were completed. These samples were not the part of training data set.

2.2. Sample preparation

Fine scrapings across the claws samples were obtained by using surgical blades (Pulse™). The scrapings were placed on the crystal surface and analyzed directly without giving any pre-treatment to the samples.

2.3. Sample analysis

A Bruker Alpha Fourier Transform Infrared (FT-IR) spectrometer with a Smart Orbit; ZnSe crystal attenuated total reflectance (ATR) accessory and OPUS (V 7.2) software equipped with an air-cooled DTGS detector was used to collect the spectra of all samples. Acetone was used throughout the experiments to clean the ATR stage supplied by Loba Chemie Company to avoid any cross contamination. The spectra were measured from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}. The signal was averaged from 23 scans. The small amount of sample was put directly on a ZnSe crystal and pressed carefully with an ATR pressure anvil. Background was analyzed each time before the analysis. Principal component analysis (PCA) and Partial least square- discriminant analysis (PLS-DA) were applied using the trial version of Unscrambler X (CAMO Software AS, Oslo, Norway) software on the infrared spectroscopic data. Data were imported in opus format in the whole mid infrared spectral range (4000–600 cm^{-1}) in Unscrambler X software. The LDA analysis was performed with SPSS software (20.0 IBM). The result analysis and the graphical plots were performed using the Origin Pro 8 (Origin Lab Corporation, Massachusetts, USA) and SPSS software (20.0 IBM).

2.4. Chemometric methods

Chemometric is the application of mathematical and statistical methods to design, extract, and interpret the information from the large dataset obtained from various analytical techniques [27].

2.4.1. Principal component analysis

PCA is an unsupervised dimensionality-reduction technique which reduces large data set to few significant variables or co-ordinates called principal components for easy pattern recognition and relationships in data. It is used to reduce multicollinearity and explains the variance in a data set without any loss of information. PCA results are commonly displayed in the form of score plots that represent the similarities and differences between the data sets that helps in easy interpretation of the original data. In score plots, sample with similar score forms a group and samples with different score are placed distant from one another [27–29].

2.4.2. Partial least square-discriminant analysis (PLS-DA)

Partial least square-discriminant analysis (PLS-DA) has evolved from PLSR algorithm which combines features of both PLS (determination of suitable variables) and discriminant analysis (classification of samples based on the extracted variables). PLS-DA is a supervised linear classification chemometric technique exhibiting the properties of PLSR with the discrimination power of predictive and classification ability with the ability to handle correlated and multicollinear variables in the data [30,31].

Firstly, the generated classification model is calibrated, and then unknown samples are predicted in the pre-defined classes. PLS-DA

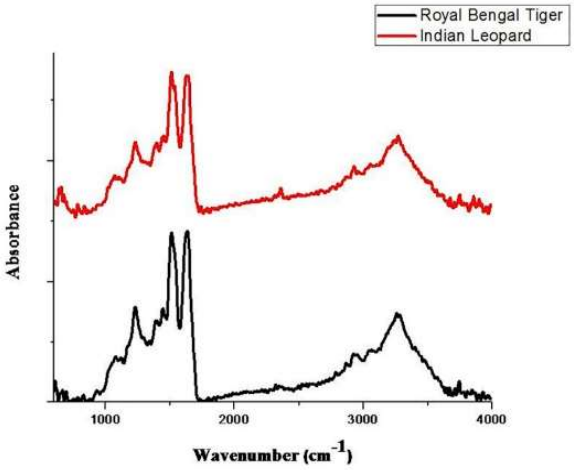

Fig. 1. Overlaid ATR-FTIR spectrum of Royal Bengal Tiger, and Indian Leopard in 4000–600 cm^{-1} wavenumber range.

searches for the latent variables with highest covariance with the Y-variables. It uses dummy values for the Y matrix as in if the value of predicted class is less than or more the value of threshold indicates that the sample belongs to other class [21,32].

The raw datasets obtained from ATR-FTIR spectroscopy were processed using PLS-DA analysis to identify the significant differences and similarities within score plots. The plot can be used to interpret differences and similarities among samples and samples within clusters are similar to each other. The pre-treatment applied for PLS-DA model was baseline offset and linear baseline correction, de-resolve transform and orthogonal signal correction. The algorithm used in the PLS-DA model was non- linear iterative partial least squares (NIPALS) with random cross validation method. This algorithm handles missing values and tends to be faster than Kernal-based algorithms.

2.4.3. Linear discriminant analysis (LDA)

LDA is the method of constructing linear combinations of canonical variables with minimum to no loss in the original information present in the datasets. This is done in order to maximize the separation among the already existing classes. LDA helps to build a model of prediction that allows the grouping of the unknown sample to the known classes based on the similarities and dissimilarities amongst them [28].

3. Results and discussion

Before analyzing any sample, the important experimental parameters were appropriately optimized. It is well understood that when the number of scans are increased, it will give a spectrum with good SNR (signal to noise ratio) and therefore improvement in the spectral quality. The resolution of spectrometer measures the ability of the instrument that how well it will separate two or more closer peaks. The spectra were measured from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}.The signal was averaged from 23 scans. The obtained spectra are interpreted by peak comparison/visual inspection, PLS-DA and LDA chemometric methods.

3.1. ATR-FTIR spectrum of Royal Bengal Tiger and Indian Leopard claws

Claws are composed of keratin proteins, the significant peaks were observed in the fingerprint region ranges from 1600 to 600 cm^{-1} as shown in Fig. 1.

This region contains the major amide bands. The major peaks are located at approximately 3271 cm^{-1} [Amide A]; 1645 cm^{-1} [C═O stretching (Amide I)], 1517 cm^{-1} [CN stretching, NH bending (Amide II)], 1116 cm^{-1} (C—H stretching), 1454 cm^{-1} (asymmetric CH_3 bending vibration), 1396 cm^{-1} (symmetric CH_3 bending, aliphatic side groups of amino acid residues), 1233 cm^{-1} [CN stretching and NH bending vibrations (Amide III)], 702 cm^{-1} [out of plane N—H bending vibrations (Amide IV)], and 1080 cm^{-1} [cysteine oxidase (S—O) and cysteic acid] [25,33–39].

As shown in Fig. 1, ATR-FTIR spectra of Royal Bengal Tiger and Indian Leopard claws are almost similar and exhibit nearly identical values of absorbance and wavenumbers. It is difficult to differentiate the claws of Royal Bengal Tiger and Indian Leopard on the basis of their visual inspection.

3.2. Discrimination using PCA model

As shown in Figs. 2 and 3, using PCA the samples could not be differentiated into separate clusters of Royal Bengal Tiger and Indian Leopard class (Fig. 2), and class of Royal Bengal Tiger, Indian Leopard and fake claws (Fig. 3) and show scattered distribution which results in overlapping of Royal Bengal Tiger and Indian Leopard claw spectra. PC1 accounted for 80% of total variation, PC2 accounted for 8% of the variation, and PC3 summarized 4% of the remaining variation. Total 92% of the variation was observed with PC1, PC2, and PC3. Three-dimensional PCA scatter plot was generated which is shown in Fig. 2.

For the discrimination for Genuine Royal Bengal Tiger and Indian Leopard claws from their fake counterparts, PC1 accounted for 63%, PC2 accounted for 16%, and PC3 showed 4% of total variation. Cumulative variance of PC1, PC2, and PC3 was obtained to be of 83%. It was also observed that only 70% of fake claws (7 out of 10 samples) were accurately classified as a distinct cluster and henceforth, could be differentiated from their real counterparts (Fig. 3).

3.3. Discrimination between Royal Bengal Tiger and Indian Leopard claws using PLS-DA model

In an attempt to get better classification rate PLS-DA is applied. Two dimensional PLS-DA model is constructed using training dataset of 31 Infrared spectra of Royal Bengal Tiger and Indian Leopard claws with two specified latent variables or factors. The primary aim of the study is to differentiate Royal Bengal Tiger and Indian Leopard claws using FT-IR spectroscopy and chemometric methods. Based on PLS-DA score plot, Royal Bengal Tiger and Indian Leopard claws entirely separated from each other along with factor 1, which explains most of the variability as shown in Fig. 4. All the samples of Royal Bengal Tiger and Indian Leopard claws are categorized in their respective classes. No false negative and positive assignments were observed in this study and hence achieved 100% accuracy in terms of positive classification. The R-Square value obtained for this model is 0.999, which is highly significant. High R-square value denotes low error rate and high predictive accuracy. Thus the generated model is a good fit for the samples of the present study.

3.3.1. External validation test

For the external validation test, 10 fake Royal Bengal Tiger and Indian Leopard claws (not included in original training data set) were acquired. The samples were analyzed under similar set of experimental conditions. The samples were loaded in the PLS-DA model for the predictions. The results obtained with PLS-DA score plot are shown in Fig. 5.

From the score plot (Fig. 5), it is evident that complete differentiation of selected fake and genuine claws is achieved. The results demonstrate that, it would be very difficult for fake claws to be misidentified as a genuine Royal Bengal Tiger and Indian Leopard claws. This remarkable performance of the classification ability of PLS-DA model and the non-destructive approach of ATR-FTIR spectroscopy make this technique well suited for the forensic species discrimination.

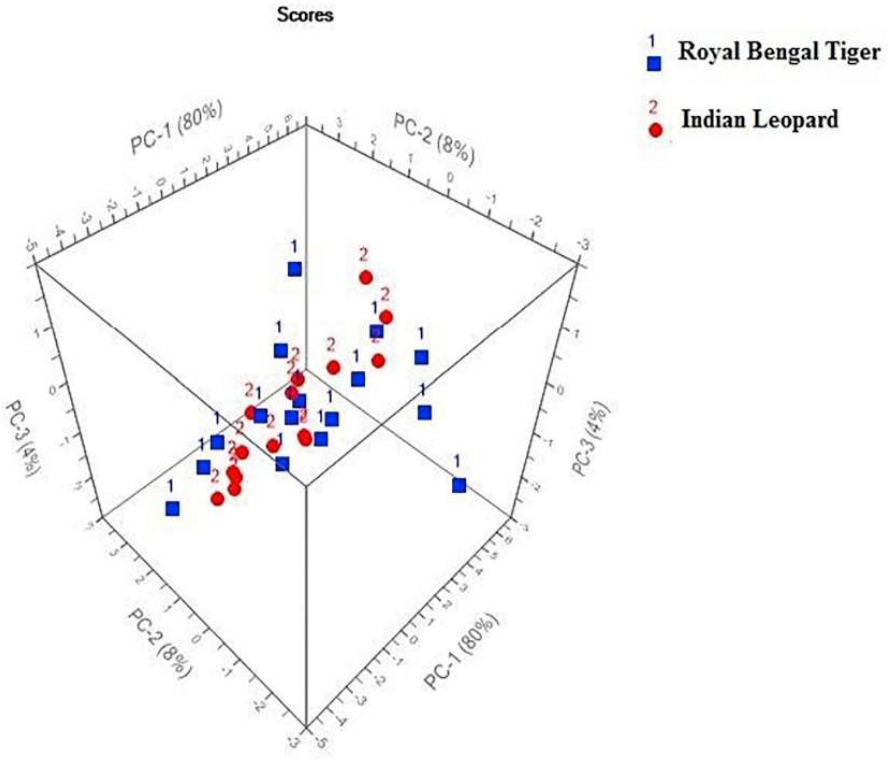

Fig. 2. PCA score plot to discriminate samples of Royal Bengal Tiger and Indian Leopard claws.

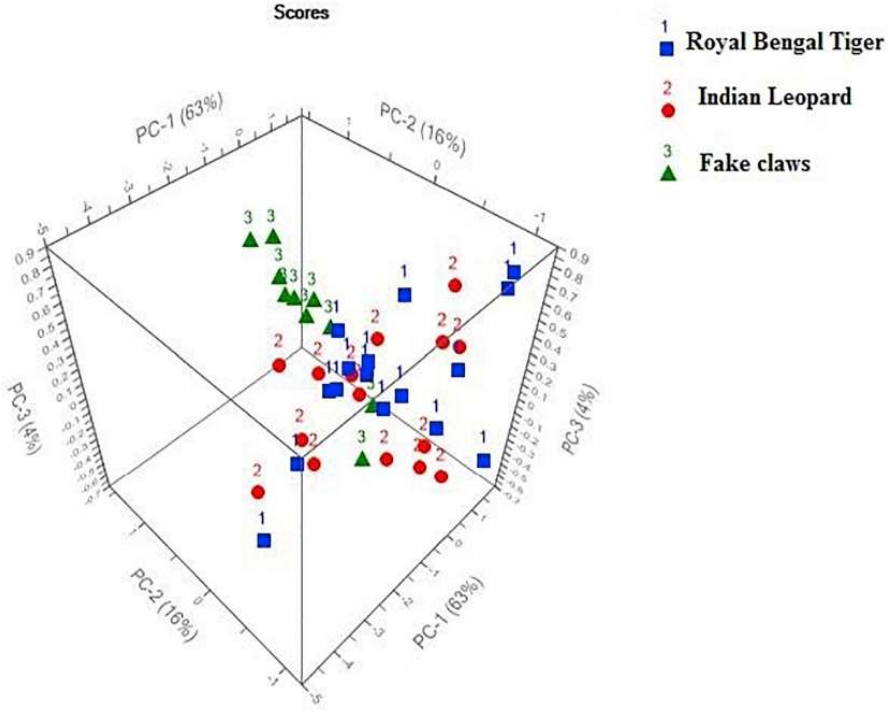

Fig. 3. PCA score plot to discriminate samples of Royal Bengal Tiger, Indian Leopard, and Fake claws.

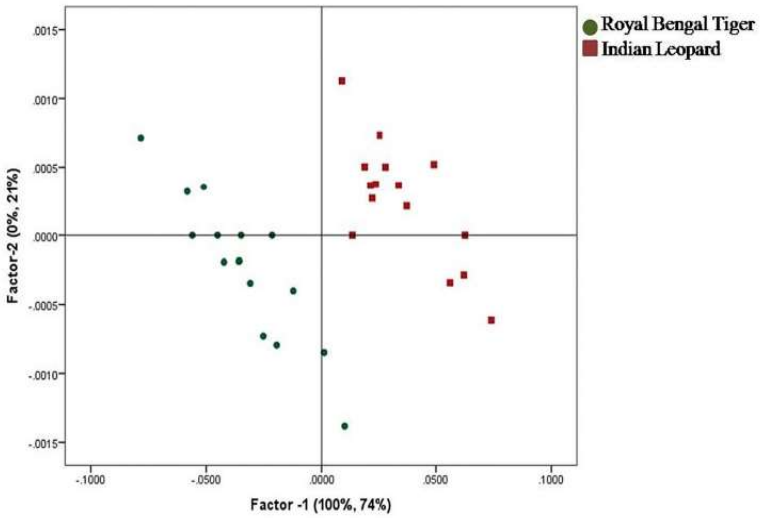

Fig. 4. Scatter plot to differentiate between Royal Bengal Tiger and Indian Leopard claws.

The results suggested that the complete differentiation is possible between fake and genuine claws with PLS-DAscore plot. The R-Square value obtained for this model is 0.998.

3.3.2. Blind test

To confirm the integrity of model that is the classification ability of this model, a blind validation test is performed. Seven unknown Royal Bengal Tiger and Indian Leopard claw samples were obtained (not included in training dataset). These samples were pretended to be unknown to the analyst and their actual identity was not revealed until predictions were completed.

The spectra of these samples are loaded into the PLS-DA model. Unknown samples can be identified by its nearest position of known group (Royal Bengal Tiger-1, Indian Leopard-2), which were made on the basis of training data set. In prediction columns, it is observed that all the unknown samples were positioned correctly in their respective class. The prediction results for these samples are displayed in Figs. 6, 7 and Table 1. In Fig. 6, marks with blue color are the predicted class for unknown samples. In Fig. 7, predicted Y represents groups of known class (1- Royal Bengal Tiger, 2- Indian Leopard, 3and 4-Unassigned) and X-axis represents samples of unknown claws. Sample number 163, 164, 53, and 54 lie in group 1 that is Royal Bengal Tiger class and sample number 41, 81, and 82 lie in group 2 that is Indian Leopard class. (see Table 2.)

These results demonstrate the model's ability to assign all the unknown samples to their respective class correctly that is Royal Bengal Tiger (Class1) and Indian Leopard (Class 2). The model resulted in 100% accuracy for the unknown claws sample's prediction, as none of samples is misclassified.

3.4. Linear discriminant analysis (LDA)

Although the PLS-DAmodel showed very good results in order to achieve the objective of the study. However one limitation in PLS-DA model is that the statistical software is needed to be run every time for predicting the unknown samples to establish whether they belong to

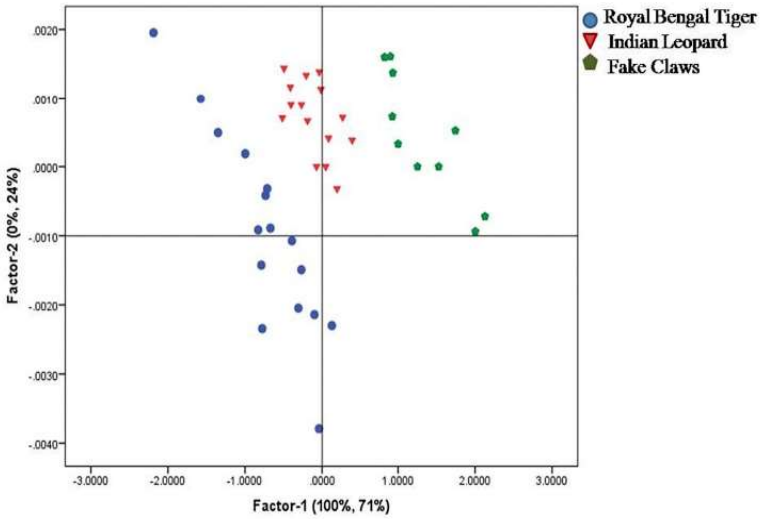

Fig. 5. PLS-DA score plot to differentiate fake claws from genuine claws of Royal Bengal Tiger and Indian Leopard class.

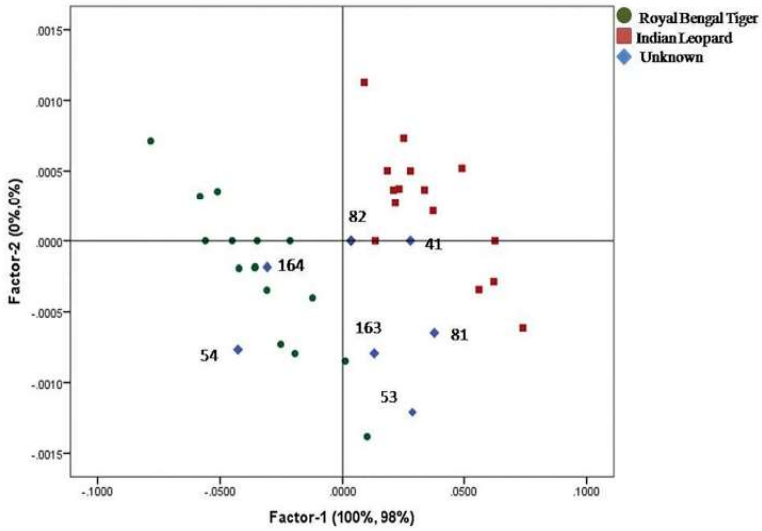

Fig. 6. Predicted Score plot to identify the blind test samples.

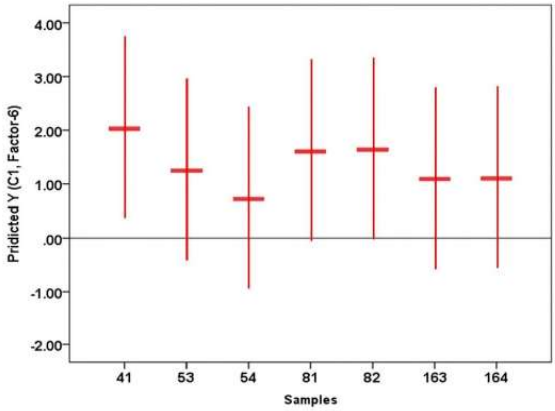

Fig. 7. Predicted Y (Claws factor) represents groups of known class (1- Royal Bengal Tiger, 2- Indian Leopard, 3 and 4- Unassigned) and X-axis represents samples of unknown claws.

Table 1
Summary of PLS-DA model predictions from blind validation test spectra.

Unknown/blind samples	Predicted class	Deviation	Actual identity
Unknown 163	1.09	0.13	1
Unknown 164	1.10	0.07	1
Unknown 41	2.03	0.07	2
Unknown 53	1.25	0.10	1
Unknown 54	0.72	0.11	1
Unknown 81	1.60	0.11	2
Unknown 82	1.64	0.09	2

Sensitivity, specificity and accuracy of this model are calculated to measure the discrimination of samples using PLS-DA.
Sensitivity = True positives/True positives + False negatives × 100.
Specificity = True positives/True negatives + False positives × 100.
Accuracy = True positive + True negative/(True positive + True negative + False positive + False negative) × 100.
The model performed 100%, sensitivity, specificity and accuracy with blind and external validation test.

Table 2
Sensitivity, specificity and accuracy values.

Claws	Sensitivity (%)	Specificity (%)	Accuracy (%)
Royal Bengal Tiger claws	100	100	100
Indian Leopard claws	100	100	100
Fake claws	100	100	100

Royal Bengal Tiger or Indian Leopard class or in general to their respective class. However for forensic scientist, there is a need to develop a method where the data from IR spectra directly be fed into an equation to predict one particular class. Such statistical models will have high forensic application in linking the claw with the endangered species as it minimizes the chances of false positive/negative results. Linear Discriminant function Analysis (LDA) is utilized for this type of classifications. The discussion and development of the model is as follow;

The absorbance values at a particular wavenumbers (cm^{-1}) that is 3266, 1634, 1540, 1509, 1469, 1452, 1418, 1393, 1338, 1232, 1112, 1071, 1052, 1024, 977, 948, 936, 893, 834, 787, 771, 746, 728, 716, 699, 665, 642, 626 and 612 are elected manually as the variables which have to be entered in the software for discriminant analysis because most of the claw samples show peaks at these wavenumbers only. The canonical discriminant function analysis has been used for classifying the claw samples into three groups that is Group I (Royal Bengal Tiger Claws), Group II (Indian Leopard Claws) and Group III (Fake Claws). The absorbance values at aforementioned wavenumber are subjected for LDA analysis. The achieved canonical correlations and eigen values are very much significant and hence, are able to classify the claw samples into their corresponding groups.

3.4.1. Analysis of variance (ANOVA)

This test allows us to select the best predictor variables for group membership. Among all the variables entered in the classification software, only the absorbance values at 1634, 1418 cm^{-1}, 1393, 1232, 1071 and 612 cm^{-1} shows significant p-values and therefore entered in the final model. It means that these absorbance values are the most appropriate for classification purposes. The developed model shows the lowest Wilks's Lambda i.e. 0.003 and 0.074 for two discriminant functions respectively with a significant p-value = 0.00. Moreover, the Box's M statistics also show significant results.

Table 3
Discriminant function's group centroids values for all three groups.

Groups	Function	
	1	2
Royal Bengal Tiger claw	− 6.156	− 1.830
Indian Leopard claw	0.694	4.460
Fake claw	5.461	− 2.630

3.4.2. The canonical discriminant function coefficient

The discriminant function equations (DF) have to be developed by using unstandardized coefficients. A prerequisites conditions of eigen value > 1 and canonical correlation > 0.35 should be followed to develop a good model. In this study, as mentioned earlier, the model provides high eigen values that is 28.42 and 12.57 which is much greater than unity and the value of canonical correlation are 0.983 and 0.962 approaching towards the unity which are higher than 0.35 for both the discriminant functions respectively. Therefore, the developed equations will elucidate a good grouping model for unknown claw samples. The DF equations for predicting the respective claw's groups are as follow;

$$DF1 = -123.488 + (67.525 \times \text{Abs. at } 1634\ cm^{-1}) + (-55.251 \times \text{Abs. at } 1418\ cm^{-1}) + (52.885 \times \text{Abs. at } 1393\ cm^{-1}) + (-24.485 \times \text{Abs. at } 1232\ cm^{-1}) + (25.641 \times \text{Abs. at } 1071\ cm^{-1}) + (18.333 \times \text{Abs. at } 612\ cm^{-1}) \quad (1)$$

$$DF2 = -58.249 + (36.202 \times \text{Abs. at } 1634\ cm^{-1}) + (-53.220 \times \text{Abs. at } 1418\ cm^{-1}) + (5.980 \times \text{Abs. at } 1393\ cm^{-1}) + (21.70 \times \text{Abs. at } 1232\ cm^{-1}) + (15.744 \times \text{Abs. at } 1071\ cm^{-1}) + (15.321 \times \text{Abs. at } 612\ cm^{-1}) \quad (2)$$

Further, a centroid value is calculated which helps in revealing the group membership of unknown claw samples. It's basically a boundary, which uses the discriminant function scores for the classification of claw samples to their respective groups as shown in Table 3. It should be noted that the outcome of the discriminant function equation has to be examined cautiously. For example, if the values of discriminant functions come out near to −6.156 and −1.830, the sample will be grouped into Group I (Royal Bengal Tiger claw) and similarly for other two groups. The calculated value from discriminant equations for the unknown sample will closely be matched with respective groups' centroid values.

3.4.3. Classification results

It is concluded from the 'goodness of fit' model that the original classification shows 100% correct classification of all the samples in their predefined groups. Leave one out cross validation was employed to authenticate these outputs and the outcomes of cross-validation also reveals 100% accurate classification of claw samples. The calculated results represent the excellent grouping equation for unknown claws classification. All three types of samples are correctly clustered in their respective groups.

4. Conclusions

The main objective of this study was to develop a rapid and non-destructive method to differentiate Royal Bengal Tiger and Indian Leopard claws. In this study, ATR-FTIR spectroscopy combined with chemometrics is successfully used to achieve the desired objective.

The PLS-DA and LDA models both demonstrated complete separation between Royal Bengal Tiger, Indian Leopard, and fake claws whilst PCA could not discriminate between these two closely related species and fake claws as well. The external and blind validation test confirmed the classification ability of model with 100% accuracy. External validation test resulted in zero percent false positive and negative assignments for the Royal Bengal Tiger and Indian Leopard class. All samples from the blind test are assigned to their proper classes in both the models. The R-Square value obtained for each PLS-DA model for differentiating Royal Bengal Tiger, Indian Leopard and fake claws (in external validation test) is 0.99, which is highly significant for predictive accuracy.

Among both these models, LDA is having advantages over PLS-DA, because in PLS-DA, the statistical software is needed to be run every time for predicting the unknown samples to establish whether they belong to Royal Bengal Tiger or Indian Leopard class. On the other hand, the expert has two choices to select any model i.e. either PLS-DA or LDA as per the availability at his/her end for these kinds of classification problems.

In conclusion, coupling of ATR-FTIR spectroscopy with PLS-DA and LDA has a potential to present an accurate, rapid, non-destructive, reliable and an eco-friendly alternative to the existing methods for the accurate identification and differentiation among Royal Bengal Tiger and Indian Leopard claws. The present method for analysis of unknown claws could accelerate the investigation process and corroborate with the conclusions made through other examination methods.

This study offers preliminary approach of claws discrimination with limited sample size (only two species), yet provides a potential tool for such type of discrimination. This method should not be taken as a substitute to DNA based species identification, which is a well-established method for this purpose and should be treated as complementary to it.

More extensive studies covering more species with large sample size are required to explore and test full potential of ATR-FTIR spectroscopy. Nevertheless, in principle if claws of two closely related species like Royal Bengal Tiger (*Panthera tigris tigris*) and Indian Leopard (*Panthera pardus fusca*) can be differentiated then claws of distant species can also be differentiated and identified using this approach. Further, this method can also be performed on the spot (as portable ATR-FTIR instruments are commercially available), which offers quick screening of genuine/fake claw samples for the successful implementation of wildlife laws and to prevent false accusations.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Unraveling the mystery of confiscated "jackal horns" in India using wildlife forensic tools

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Abstract

Internationally, illegal wildlife trade involves highly prized and charismatic species and their derivatives. At the same time, common or less known species and their parts are also encountered but receive less attention than charismatic species. Given the increasing demand for wildlife products in many parts of the world, profit, and short supply, many fake articles derived from domestic or wild animals are frequently encountered in the wildlife trade. Jackal horn (locally known as "Siyar or Gidar singhi") is one such fake item widely used in sorcery and other occult practices available through offline and online trading platforms within India. We used a combination of morphological, microscopic hair, and molecular approaches (Cyt *b* and 16 s rRNA genes) to reveal the true identity of confiscated "jackal horns" ($n = 342$). Detailed morphological study of the jackal horns showed that it varied in size, shape, color of hair, attachment material, and filling material. The microscopic hair and molecular approaches revealed that all the items sold as jackal horns were fake and made up of protected wild species and domestic animals. Our results confirm the use of the biological samples from few wild species protected under the Wild Life (Protection) Act, 1972, of India. Therefore, the law enforcement agencies are cautioned to get forensic opinions while dealing with such counterfeit items.

Keywords Illegal wildlife trade · Jackal horn · Morphology · Mitochondrial DNA · Wildlife forensics

Introduction

Illegal trade in wildlife parts, excessive poaching for subsistence, and habitat destruction are the major drivers of biodiversity loss and species extinction [1]. Like the illegal trafficking of drugs and arms, the illicit wildlife trade is considered the most complex and multi-billion-dollar industry [1, 2]. The illegal wildlife trade is ever-evolving and poses a severe threat to wild species of flora and fauna [3]. Humans have been hunting wild animals for ages for food, rituals, witchcraft, and fulfilling superstitious beliefs worldwide [2, 4, 5]. However, consumption of wildlife parts and products has increased in many parts largely out of superstitious beliefs, crazy desires, and greed leading to over-exploitation and decimation of wild populations, driving them to the verge of extinction [5, 6].

Trafficking and illegal international trade of high-value parts of charismatic species such as tiger, rhinoceros, snow leopard, and elephants are widely reported and addressed by various countries [7]. However, illegal trade of lesser-known species receives hardly any attention leading to the decline or local extinction of such species [7]. Body parts of some of such species, such as monitor lizard (*Varanus benghalensis*), pangolin (*Manis* spp.), and golden jackals (*Canis aureus*), are frequently encountered in illegal wildlife offenses in the Indian sub-continent [8–11]. Moreover, the grey market on wildlife parts is also notorious for adulterated items such as fake claws, canines, skin, elephant tusk, musk pod, and rhino horns to meet the short supply and high demand [12, 13]. These fake articles are either made up of non-biological material or biological material obtained from domestic or other less charismatic wild species.

Rampant poaching of the golden jackal is widely reported in India for meat, body parts (skin, tail, claws, skull and

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teeth) for religious practices, cultural beliefs, and traditional medicine [11]. Jackal is listed under the "Least Concern" category in IUCN Red List due to its stable population and wide distribution. Nevertheless, in India, it is protected under Schedule II of Wild Life (Protection) Act, 1972 (WPA), and Appendix III of CITES (Convention of International Trade in Endangered Species of Wild Fauna and Flora). The major threat includes poaching for "Jackal Horn" (locally called Siyar or Gidar singhi), a horn-like protrusion or deformity behind the sagittal crest of this species. Jackal horns have been traded surreptitiously by the quacks, astrologers, and sorcery practitioners for a long time and more recently through social media [11].

Identification of genuine and fake wildlife products poses a big challenge in wildlife forensics as species identification requires a multi-prong approach. Since "jackal horns" are considered counterfeit items, there is no evidence to show what kind of non-biological or biological material is used to fabricate this item [11]. To unravel this, we used morphological and molecular approaches to verify whether "jackal horns" illegally traded in the illegal wildlife market are real or fake. If fake, what kind of material is used to make such items and whether this item is derived from domestic or wild species. The findings of this study would help in understanding the true origin of the "jackal horns" traded illegally and for law enforcement.

Materials and methods

Case report

A total of 342 seized jackal horn samples belonging to eleven wildlife offense cases were sent to the Wildlife Forensic and Conservation Genetics (WFCG) Cell of Wildlife Institute of India (WII) by law enforcement agencies for species identification.

External characteristics and hair morphology examination

Morphological examinations of the "horns" were noted based on physical characteristics, including the shape, weight, hair color, and hair bands on hair follicles. Subsequently, the "horns" were cut open, and information on the type of material used as filling along with attachment types was also recorded (Fig. 1). The jackal horn samples were further pooled based on the abovementioned morphological characteristics, and 30 representative samples were taken for the microscopic hair analysis. Cuticle and medullary patterns of all the 30 hair samples were observed using methodology as detailed in the earlier study [14]. The cuticle and medullary patterns were compared with the hair patterns of wild and domestic animals available in the repository of WFCG Cell, WII [15].

Fig. 1 Different forms of confiscated jackal horn pods (**a**) along with attachments (**b**) and filling materials (**c** and **d**) used for making fake jackal horns

Genetic identification

Owing to budgetary constraints, we randomly selected 129 samples out of 342 for species identification using molecular tools. The selected samples were morphologically characterized, and 129 skin and 116 attachments (total 245 samples) were used for molecular analysis. Genomic DNA was isolated using Qiagen DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufacturer's instructions. Species identification was carried out by amplifying partial fragments of conserved mitochondrial cytochrome *b* (Cyt *b*) and 16S rRNA genes [16, 17]. PCR amplification and sequencing were carried out according to earlier described methodologies [14]. Isolated DNA samples and PCR amplified products were visualized in UV light on 0.8% and 2% agarose gels respectively stained with ethidium bromide. Species confirmation was carried out by validating the generated sequences using GenBank BLAST (http://blast.ncbi.nlm.nih.gov/Blast/). Conserved Cyt *b* and 16S rRNA failed to differentiate between *Canis* spp., i.e., jackal, wolves, and dog [18]; therefore, we amplified partial fragments of Canid-specific mitochondrial Cyt *b*: Canid L1 & H15149 [16, 19] and aligned them with other available *Canis* species sequences for confirming the species. The Cyt *b* and 16S rRNA gene sequences generated in this study were aligned with the reference sequences (obtained from the WFCG Cell repository) using CLUSTAL W multiple alignment algorithms implemented in BioEdit v7.2 [20] and submitted to GenBank (Accession No.- 16srRNA: MW862805-MW862829; Cyt *b*: MW862830-MW862975).

Results

Morphological analysis and hair characteristics

Examination of seized jackal horns ($n=342$) revealed variations in the composition of outer physical characteristics and internal filling materials (Fig. 2). The shape of the pods was round ($n=201$) followed by whorl ($n=130$) and conical ($n=11$), whereas the weights ranged between 0.68 and 15.51 g, with the majority of samples between 6 and 8 g ($n=104$). The color of hair follicles was mostly brownish ($n=157$) followed by white ($n=65$) and black ($n=40$) with ventral hair in major proportion ($n=223$) observed on "horns." The internal filling material was mostly dry clay or flour balls ($n=235$) followed by charcoal powder ($n=63$). The other materials used were wooden dust, sponge, the fold of skin, and paw with pads. Mainly, talon ($n=204$) was used as an attachment followed by teeth of unknown animals ($n=61$) with few samples observed without any attachment ($n=48$). Moreover, cuticle and medullary patterns of examined hair samples when compared with repository samples of WFCG Cell were matched with multiple domestic and wild species.

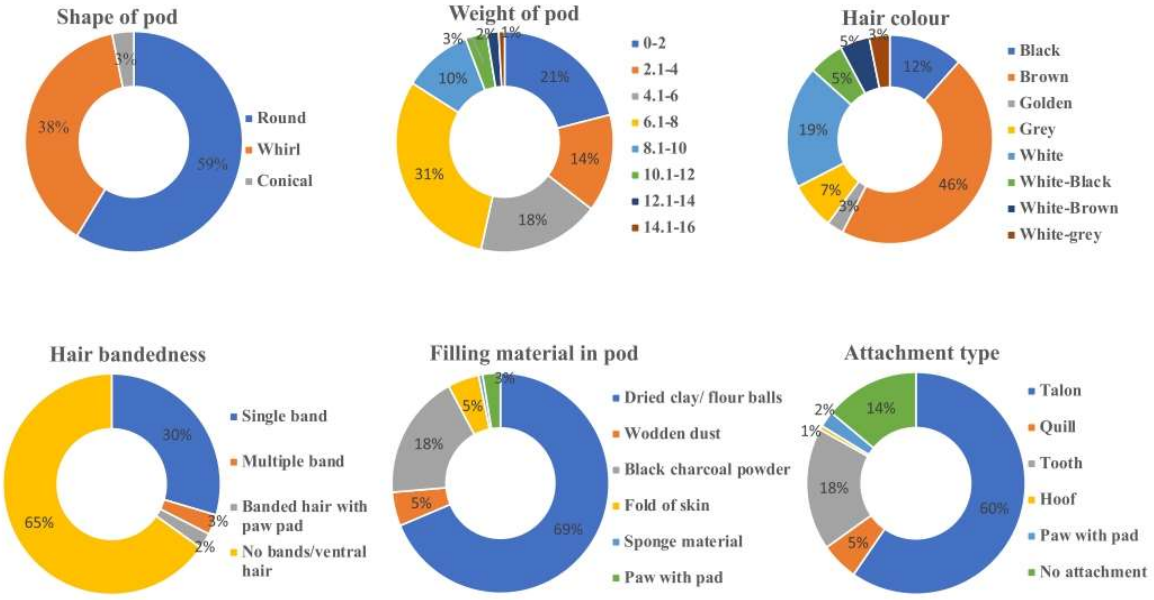

Fig. 2 Composition of external physical characteristics, internal filling material, and attachment types observed in jackal horn samples ($n=342$)

Molecular analysis

Of the 245 samples, DNA was successfully extracted from 180 (96 skin samples and 84 attachments). Species identification was achieved by amplifying partial fragments of Cyt *b* and 16S rRNA genes. The GenBank BLAST search analysis using Cyt *b* and 16S rRNA gene fragments showed similarity with multiple domestic and wild species. Skin samples were made of biological materials originated from wild species (Supplementary Table 1), viz., red fox (*Vulpes vulpes*, $n=22$), jackal ($n=7$), and Indian grey mongoose (*Herpestes edwardsi*, $n=9$), along with domestic species, viz., goat (*Capra hircus*, $n=45$) and domestic cat (*Felis catus* $n=13$). Similarly, the molecular analysis also identified wild species such as Indian peafowl (*Pavo cristatus*, $n=19$), wild pig (*Suc scrofa*, $n=2$), and red fox ($n=1$) along with red jungle fowl (*Gallus gallus*, $n=24$), cat ($n=9$), goat ($n=3$), blue rock pigeon (*Columba livia*, $n=6$), and domestic sheep (*Ovis aries*, $n=20$) from the attachments. Interestingly, none of the attachment samples matched with the DNA of the jackal, whereas red fox is found in both the skin and attachment samples.

Discussion

Identification of species from seized wildlife parts is vital for the enforcement agencies to implement wildlife protection laws effectively [1, 3, 21–23]. Considering different species and fake items used for adulteration of illegally traded products, identifying such items becomes even more challenging. The seized articles are often made from materials derived from either domestic animals or lesser-known wild animals. "Jackal horn" turned out to be one of the widely traded items but invariably fabricated using biological and non-biological material. Enforcement agencies in India often seize jackal horn samples, but they receive less attention due to a lack of information on the biological origin of such samples. Therefore, we have established the nature and composition of this product for the first time; constituents of which are largely spurious, including biological and non-biological material. It was found that horns or so-called pods varied in shape, weight, hair color, presence and absence of bands on the hair strand, attachment types, and the non-biological material used. The attachments were initially covered with dry clay, flour balls, and charcoal powder and then stitched with a skin sample giving either a spherical or whorl shape with weights ranging between small pad to large spherical ball structure. The hair samples were without any bands indicating the ventral hairs or whiskers used in making these pods. The attachments that gave the feel of the hornlike structure were made of either bird talons, an inferior umbilical portion of the calamus, or animal hooves. Microphotographs of cuticle and medullary patterns from hairs collected on skin showed that multiple species were used to make the fake jackal horns.

Sample quality for species identification using molecular tools is considered as the major challenge in forensic studies. Selection of mitochondrial molecular markers with enough variations within and between the species for accurate species identification is another key factor. Conserved molecular markers such as 12S rRNA, 16S rRNA, CO*I*, and Cyt *b* genes are commonly used and found suitable in forensic research [23]. But in this study, we successfully isolated DNA from 180 out of 245 samples with nearly 74% success rate. The low success rate could be attributed to low quantity, degradation, and PCR inhibitors carried along with case work samples used in this study [23, 24]. Though 16S rRNA and Cyt *b* gene fragments were found useful in accurate identification of samples to species level, we observed more amplification success with Cyt *b* in comparison to 16S rRNA gene. Molecular analysis identified several wild species, e.g., jackal, red fox, Indian grey mongoose, Indian peafowl, and wild pig, used either from their skin for outer covering or other body parts for a horn-like attachment. Most of the identified wild species are also given special protection under WPA, 1972, in India for their long-term conservation. Several other domestic species, such as goat, cat, sheep, fowl, and pigeons, were also used to make fake jackal horns. Largely species like red fox, domestic cat, and goats were used in both the skin and attachments among the confiscated samples.

Conclusion

Using a combination of morphological and molecular approaches, we have successfully ascertained that commonly traded wildlife product illegally sold in the market in the name of "jackal horn" is primarily fake, comprising parts of domestic or lesser known wildlife. Even though domestic species were used in most of the skin and attachments, few wild species were also observed, which are listed as schedule species under WPA and CITES appendices. We therefore emphasize that enforcement agencies must obtain forensic opinion for species identification and take action accordingly rather than discarding these samples as fake wildlife products as they might contain some parts or derivatives of other ecologically important protected wild species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00414-022-02773-6.

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Declarations

Ethics approval This study did not use experiment/capture of live animal (all the used samples were confiscated by enforcement agency); hence, Institutional Animal Ethics Committee approval was not required.

Consent to participate This study did not use research involving human participant; hence, informed consent was not required.

Research involving human participants, their data, or biological material This study did not use research involving human participant and their data.

Conflict interest The authors declare no competing interests.

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Research Paper

Rapid and non-destructive differentiation of Shahtoosh from Pashmina/ Cashmere wool using ATR FT-IR spectroscopy

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ABSTRACT

Shahtoosh, the most expensive and sought-after wool in the illegal wildlife trade is obtained from the underfur of a critically endangered species-the Tibetan Antelope (*Pantholops hodgsonii*). It is often adulterated or mixed with the wool of Pashmina goat (*Capra aegagrus hircus*) for making shawls, scarves and other woolen articles to maximize the profit. The comparable fineness, color and texture, makes it a challenging task in wildlife forensics to differentiate them. In this study, an attempt has been made to differentiate 50 reference unprocessed underfur hairs from five individuals of each species using ATR FT-IR spectroscopy in combination with chemometric tools such as PCA, and PLS-DA. Results of PCA model demonstrated slight overlap and thus failed to distinguish hairs of these two species. Subsequently, PLS-DA model was employed, and also validation tests (external and blind testing) were carried out to ensure the predictive ability of the model, which resulted in 100% accuracy. The results of PLS-DA model exhibited complete differentiation between Shahtoosh, Pashmina and Angora (*Oryctolagus cuniculus domesticus*) wool used for external validation study with highly significant predictive ability (R-square value 0.99). This proof-of-concept study illustrates the potential of ATR FT-IR spectroscopy to complement current forensic microscopic and DNA based technique to analyze hair evidence in wildlife investigations owing to its rapid and non-destructive nature with high degree of confidence, and its ease-of-use with minimal to no sample preparation.

1. Introduction

Shahtoosh (Persian meaning "king of wools") is derived from the underfur of Tibetan antelope locally known as Chiru (*Pantholops hodgsonii*), a rare and critically endangered high altitude species endemic to the Tibetan Plateau. It is the warmest, finest and lightest natural fiber known to the mankind, exploited to make luxury shawls, scarves and other woolen garments. A Shahtoosh shawl because of its beauty and rarity was once regarded as a valued dowry in India, is now among the most sought-after and costliest trade items/articles in the illegal wildlife trade and can cost around $20,000 [1]. For the manufacturing of a single Shahtoosh shawl (2 × 1 m for women and 3 × 1.5 m for men), skins of 3–5 adult animals are required as a single adult produces only 100–120 gms of hairs [2]. The huge demand of Shahtoosh in the illegal wildlife tradehas led to the large-scale poaching of the Tibetan antelopes. It has been reported that demand surged in the late 1980 s and over 1500 kg of Shahtoosh has been seized in January 2013 in Nepal, which translates into the poaching of around 10,000 Tibetan antelopes [3]. The poaching of this scale has wiped outaround 90 percent of the Tibetan antelope population and is posing the gravest threat to its survival [1].

The Tibetan antelope has been identified and listed as a critically endangered species by the International Union for Conservation of Nature (IUCN). In India, the Tibetan antelope has been protected under Schedule 1 of The Wildlife (Protection) Act-1972. Hunting or trade in any of its body part or product amounts to an offence which is punishable with imprisonment of up to 7 years and fine, apart from mandatory confiscation of the product [4,5]. At the international level, it is also listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and hence, since 1979 any international trade in Shahtoosh and other Tibetan antelope

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body parts or products thereof is banned between all CITES Parties [6]. India has been a signatory to CITES since 1976 [7].

Interestingly, high altitude areas in Ladakh and other parts on Trans-Himalaya produce a similar wool called as Cashmere or Pashmina. This wool is obtained from the Pashmina goat (*Capra aegagrus hircus*) which is of comparable fineness and warmth and often passed on as Shahtoosh. Sometimes, along with Pashmina, wool of Angora rabbit (*Oryctolagus cuniculus domesticus*) is also blended with Shahtoosh to reduce the cost and increase profit. The trade in these domestic species is sustainable and legal, but if a shawl contains even1% of Shahtoosh it is still an illegal product [8].

In this aspect, the identification and discrimination of Shahtoosh from the hairs/fur of other species especially from Pashmina is a crucial facet of wildlife forensics to combat wildlife crime and for the successful implementation of laws related to wildlife protection, and conservation across the globe.

Conventionally, the physical appearance and morphological characteristics of the guard hairs are examined for the identification and differentiation of Shahtoosh from Pashmina wool using light [9–11] and scanning electron microscopy [8]. Species can be identified by using various guard hair characteristics such as hair color, length, diameter/thickness, cuticle scale pattern, medulla pattern, scale count index and medullary index in combination. But, recently due to mechanized dehairing techniques employed to removes the coarser guard hair from the raw wool to get finer quality Shahtoosh, presence of guard hair in shawls is hugely reduced. Also, stringent enforcement of India's Wildlife (Protection) Act, 1972, led to reduced supply of Shahtoosh wool forcing shawl weavers using cost effective but comparable and legal wool or "pashm", derived from Pashmina goat as an adulterant [12]. These woolen fabrics are passed off as legal Pashmina shawls posing a serious challenge for enforcement agencies to identify the species from wool hair only. Wildlife Institute of India (WII), Dehradun has received 1755 suspected Shahtoosh shawls/wool under 119 wildlife offense cases during the period from 2001 to 2020. Sufficient guard hair for the analysis of microscopic hair characteristics were found in 63.36% (N = 1112) whereas rest 643 shawls/wool were having insufficient or devoidof any guard hair.

Currently, DNA based species identification is the most commonly used and widely accepted reliable method for the identification of species using species-specific genetic markers from a variety of biological materials. Though DNA is unique to the species and can be used to identify and differentiate species, however, the cognizance of the fact that in many cases hair strands do not exist with enough root tissue attached with follicular tag, which could be troublesome for DNA typing. A DNA profiling method for Shahtoosh which utilized nested PCR and sequencing of a *cytochrome b* gene fragment have been reported to identify and differentiate Shahtoosh, Cashmere, and Sheep wool fibers [13]. Recently, a specially designed rapid and simple TaqMan real-time PCR-based method has been reported for identification and differentiation of Shahtoosh, Cashmere, and Sheep wool fibers [6].

The characteristics of ATR FT-IR spectroscopy are extremely attractive and are rapidly gaining popularity in the analytical and biomedicine fields owing to its non – destructive, reliable, and rapid nature. Few articles exemplify the use of FTIR spectroscopy supported with chemometric tools for the differentiation of infrared spectra from various types of hairs. In 1998, Rintoul and the group amalgamates FTIR microscopy and chemometric tools such as PCA and SIMCA for the differentiation of Asian and Caucasian hairs [14]. In 2004, Panayiotou [15] analyzed cat fur, parrot feather, sheep wool, human, cow, horse, and dog hair using FTIR micro-spectroscopy in transflection mode coupled with chemometrics. Further, Espinoza et al. (2008) emphasized on the identification of tail hair of elephant /giraffe origin, commonly used to manufacture indigenous artifacts such as earrings, fingerings, bracelets etc. using horizontal-attenuated total-reflection Fourier transform infrared (HATR FTIR) spectroscopy [48]. Guo et al. [16], have worked on straight guard hair of Golden cat (*Catopuma temminckii*) and Indian leopard cat (*Prionailurus bengalensis*) using FTIR spectroscopy. Of late, Manheim et al. in 2016 used ATR FT-IR spectroscopy for the differentiation of human, cat, and dog hairs augmented with PLS-DA model. Notwithstanding successful discrimination between hairs of human, cat and dog origin, unknown hairs were also predicted accurately in their respective class which ascertains the accuracy of the model. Recently, Sharma et al. [17] successfully differentiated claws of Indian Leopard and Royal Bengal Tiger using ATR FT-IR spectroscopy.

However, the available conventional methods definitively utilized for species discrimination, the destructive nature of these tests is the most significant concern that requires to be consigned. Moreover, DNA based methods are the feasible biological approaches towards the species identification and discrimination. Although backed by definite advantages of superior specificity and sensitivity, the DNA based approaches suffer from the relentless limitations of complex methodology which are time consuming in nature and uses of various chemicals in which some are carcinogenic and expensive in nature [18]. On the other hand, Raman spectroscopy has been successfully used for the species identification and discrimination. Certainly, the obtained results from Raman spectroscopy are satisfactory, but to analyze stains on fabrics is bothersome due to strong Raman signals generated by the fabrics and its dye [19,20]. Besides, the cost of Raman spectroscopy is exceedingly high in contrast to the FTIR spectroscopy [21]. Quite the reverse, FTIR spectroscopy undoubtedly has colossal potential to analyze stains on fabrics and other simulated surfaces due to its less penetration depth and inherent surface sensitivity [21–23].

All-inclusive from a forensic perspective, ATR FT-IR spectroscopy is highly amenable and would be complementary to microscopic examination, DNA examination and other available methods. Herein, the present study aimed to discriminate the hair spectra of Shahtoosh and Pashmina wool (unprocessed) via ATR FT-IR spectroscopy with appropriate chemometric tools that are PCA, and PLS-DA. The foregoing method is apt, robust, simple in operation, non-destructive (no harm to the object in question), eco-friendly, and rapid for identification and differentiation of hairs and can gain further insights in the results using chemometric tools. Moreover, the results are objective in nature and do not create any cynicism in the interpretation of results.

The main aim of using multivariate data analysis or chemometric tools with ATR FTIR spectroscopic methods is to reduce large dataset to make the easy interpretation of results as in this case the data points generated is large which otherwise creates difficulty in the interpretation of results [24].

2. Materials and methods

2.1. Hair sample collection

The reference underfur hair samples of Shahtoosh, Pashmina and Angora wool (unprocessed) were collected from the repository of WII, Dehradun, Uttarakhand in India. Details of all collected samples are enumerated in Table 1. The images of collected Shahtoosh, Pashmina, and Angora wool is shown in Figure SI, SII, and SIII of supplementary file, respectively.

Table 1
Detailed description of Samples.

Wool Type	Scientific names of species involved	Number of individuals	Number of hair strands per species (*n* =)
Shahtoosh	*Pantholops hodgsonii*	05	25
Pashmina	*Capra aegagrus hircus*	05	25
Angora	*Oryctolagus cuniculus domesticus*	08	40

2.2. Sample preparation and spectral collection

Samples were analyzed using Attenuated total reflectance (ATR) technique accessory contained ZnSe crystal plate attached with Fourier transform- infrared spectroscopy (Bruker alpha eco-ATR; Billerica, MA). Hair strands were not pre-treated and no cleaning, decontamination or altering preparation was given to samples. A single hair strand was taken out and placed directly on the crystal cell of ATR FT-IR instrument. Spectra were recorded by pressing carefully with an ATR pressure anvil/ arm at the midpoint of each hair strand to facilitate good contact between hair sample and the crystal surface. All the samples were analyzed within the mid infrared range (MIR) i.e. 4000–600 cm^{-1} [absorbance (%) vs wavenumber (cm^{-1})] with 4 cm^{-1} resolution, averaging 24 scans per spectrum. ATR FT-IR spectral data were detected using Opus software (version 7.2). A background spectrum of the empty ATR cell was collected before analyzing any new hair sample under similar set of conditions. The crystal face of ATR was properly cleaned with ATR wet tissue (part number 1008033), containing isopropyl alcohol and deionized water before the analysis of a new hair sample. Reproducibility was checked by analyzing three replicates of each collected hair strands. Homogeneity test was performed on all the hair strands by analyzing the same strand from three different regions (proximal, medial and distal) to study homogenous distribution of components within samples.

2.3. Applied chemometric methods

The principal component analysis (PCA), and partial least square discriminant analysis (PLS-DA) model was created to discriminate total 50 spectra (25 wool hairs from each species) obtained from Shahtoosh and Pashmina wool in the mid infrared range i.e. 4000–600 cm^{-1}. PCA and PLS-DA were applied by using the Unscrambler X 10.4 (CAMO Software AS, Oslo, Norway) software on the infrared spectroscopic data.

2.3.1. Principal components analysis (PCA)

PCA is a well-known unsupervised dimensionality-reduction or bilinear modeling tool which helps to present the significant information into simpler plots. The algorithm used in PCA is to figure out the relationship among the highly correlated variables hence, these correlated and orthogonal variables are known as principal components [25]. It employs eigen space method (also known as Karhumen- Loeve transform) and reflected as an imperative tool to exhibit the significant relationships in the selected samples. The results of PCA are most commonly displayed in the form of score plots. It represents the significant differences and similarities in the datasets and thus elucidates easy interpretation of the results [24,26–28].

Herein, in the present study, PCA was used for the discrimination of total 50 spectra (25 wool hairs from each species) obtained from Shahtoosh and Pashmina wool. To perform the PCA, the numbers of principal components were selected by using the Kaiser Criterion test. On the basis of Kaiser Criterion test, 3 PCs were selected which could describe a total possible variance in the dataset. The singular value decomposition (SVD) algorithm with random cross validation method was used to construct the PCA model. The PCA was performed in the range of 1700–600 cm^{-1}.

2.3.2. Partial least square- discriminant analysis (PLS-DA)

PLS-DA is a well-known linear classification technique, combines the characteristic features of PLSR (partial least square regression) and the discrimination power of a classification method. PLS-DA is a versatile method based on the PLSR algorithm for calibration which finds the latent variables with a maximum covariance with the Y- variables. It can be used for the descriptive modeling, predictive modeling and the same time for the discriminative variable selection and thus produces multiple outcomes. PLS-DA model considered as a favored modeling tool when dealing with high dimensional/ hyper spectral dataset to extract the valuable information [29,30]. When the obtained predicted value is greater than the threshold value, the sample belongs to the current class and if the predicted value is less than the threshold value, it belongs to the other class. It plays an important role in exploratory data analysis and thus provides good insights for the purpose of discrimination using loadings and weights [31]. Eventually, various studies suggest that PLS-DA has proved a superior classification approach than PCA and PCA-LDA [29,32]. The algorithm used in the PLS-DA model is non– linear iterative partial least squares (NIPALS) with random cross validation method. This algorithm handles missing values and tends to be faster than Kernal-based algorithms.

2.4. Pre-processing of raw data matrix

Pre-processing of data is a mathematical manipulation prior to the primary analysis of data to remove and diminish any noise and variation due to the irrelevant sources. ATR correction was applied on all the obtained spectra before any pre-processing methods. To perform pre-processing method, and for ATR correction, The Unscrambler X 10.4 (CAMO Software AS, Oslo, Norway) software was used.

Pre-processing methods applied for PCA:

1. Baseline offset and linear baseline correction: Baseline offset (the value of the lowest point in the spectrum is subtracted from all the variables) and linear baseline correction (transform a sloped baseline into a horizontal baseline) used to correct the baseline of the samples.
2. Smoothing with Savitzky- Golay algorithm: Smoothing is a row-oriented transformation and basically helps to minimize the noise in the data without diminishing the variables numbers. Savitzky-Golay is an averaging algorithm which finds a data value by making a polynomial to fit the data by utilizing a number of data points on each side. Therefore, from the polynomial equation, the value to be averaged is predicted.14 smoothing points and 2 polynomial orders in a symmetric kernel were selected.
3. Normalization by range: Normalization is used to "scale" samples in order to get all data on approximately the same scale. In range normalization each row is divided by its range, i.e."max value – min value".
4. Standard normal variate (SNV): SNV is a row-oriented transformation which removes scatter effects by scaling and centering from each individual spectra.

Pre-processing methods used for PLS-DA.

1. Baseline offset and linear baseline correction
2. Deresolve transform: This function could be used for changing the apparent resolution of the instrument. It changes the high resolution of spectrum to a low resolution. However, it may also be utilized for some noise reduction.
3. Orthogonal signal correction (OSC): It could be utilized as a method of transformation for constructing the PLS regression models from the infrared spectral data. It helps to remove the extraneous variance from the × data that are not related to some responses, and building the model more accurate, reliable, parsimonious, and easier to interpret. OSC with Kernel based PLS algorithm was used to construct the PLS-DA model.

2.5. Validation test

Reliability of model was checked by conducting blind and external validation test. For external validation test, 40 samples from 8 different individuals of Angora rabbit (5 samples per individual) were collected and analyzed using ATR FT-IR spectroscopy. To check the blind testing, 10 hairs which were not the part of training data set (belong to the Shahtoosh and Pashmina wool) were provided to the investigator. The

real identity of all these samples was disclosed to the analyst only after the test was completed.

False-positive, false-negative, sensitivity, precision, specificity, and accuracy rate were calculated to validate the generated model with the following formulas [22,33]:

$$FP = FP/(TP + FN) \times 100$$

$$FN = FN/(TN + FP) \times 100$$

$$Precision = TP(TP + FP) \times 100$$

$$Sensitivity = TP/(TP + FN) \times 100$$

$$Specificity = TN/(TN + FP) \times 100$$

$$Accuracy = TP + TN/(TP + TN + FP + FN) \times 100.$$

TP is the number of true positives; TN is the number of true negatives; FN is the number of false negatives; and FP is the number of false positives.

3. Results and discussion

3.1. Spectral analysis of training dataset

Fig. 1. represents the composite hair spectra of Shahtoosh and Pashmina wool. The recorded spectra were measured in the mid-infrared range that is 4000–600 cm^{-1} with average scan of 24. Chemically hairs are composed of 65–95 % of proteins; therefore, the peaks of hairs are dominated by amide peaks associated with keratins. Thorough examination of these spectra allows us to confirm the presence of frequencies characteristics of keratin as assigned in Table 2. The spectra were measured by visual inspection followed by PCA and PLS-DA methods.

Reproducibility was checked by analyzing three replicates of each collected hair strands and homogeneity test was performed on all the hair strands by analyzing the same strand from three different points (left, central and right point). On the basis of overlaid spectra, no significant difference on direct ocular inspection was observed as shown in figure SIV and SV (Supplementary file). The obtained results demonstrated excellent reproducibility and repeatability.

As shown in Fig. 1, ATR-FTIR spectra of Shahtoosh and Pashmina wool are almost similar and exhibit nearly identical values of wavenumbers however, only minor difference exist in the region of 1200–1000 cm^{-1}. This particular region is associated with the vibrations of sulphur oxgen groups of keratin [34]. Sample of Pashmina wool did not exhibit the prominent band at the position of 1036 cm^{-1} peak. It can be concluded that the intense band at 1036 cm^{-1} observed due to the cysteine oxides and of cysteic acid.

Table 2
Assignment of prominent peaks of hairs [34–38].

Peak observed	Group frequency	Designation
3280	Symmetric N–H stretching. in 1°amide	Amide A
1645	C = O stretching H-O–H Bending	Amide I (α-Helix)
1517	N–H deformations in 2°amide	Amide II
1451	Asymmetric CH_3bending	
1374	symmetric CH_3bending	
1239	N–H bending	Amide III
1161	Stretching modes of the C-OH groups of serine, threonine, and tyrosine residues of cellular proteins	
1090, 1036	Cysteic acid	Cystine oxides

However, it is quite difficult to differentiate the spectra of Shahtoosh and Pashmina wool only on the basis of their visual inspection. The visual discrimination is not legible and moreover subjective in nature, therefore further advanced chemometric tools were needed to extract the information-rich signals to get the results in an objective manner without biasness in the obtained results.

The primary objective of this study is to differentiate the Shatoosh and Pashmina wool through ATR FT-IR spectroscopy using PCA, and PLS-DA model. A PLS-DA model was constructed using training data set containing 50 spectra (25 each) of Shahtoosh and Pashmina wool. To check the performance of classification model, external validation and blind testing were also done. Collected hair samples of Shahtoosh and Pashmina wool for blind testing were not the part of training data set. For external validation test, samples were also collected separately from different species. In this study, an attempt has been made for the differentiation purposes when obtained spectra are somewhat inherently visually similar but differ chemically.

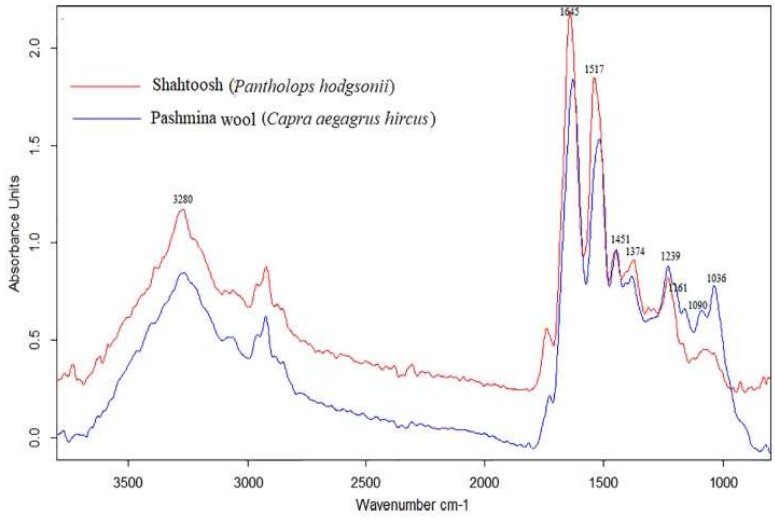

Fig. 1. Overlaid spectra of Shahtoosh and Pashmina wool in MIR range.

3.2. Construction of a binary PCA model

The PC-1 and PC-2 (2-D) score plot for the classification of hairs of Shahtoosh and Pashmina wool are presented in Fig. 2. Here, PC1 explained 65% of variation and PC2 accounted for 21% of variation in the dataset. In total, cumulative variation of 86% was achieved using the PCA model. From the score plot, it was concluded that there is not clear separation between class 1 and class 2; however, scattered distribution was obtained as shown in Fig. 2. Therefore, the result is that it appears not possible to differentiate the Shahtoosh and Pashmina wool with 100% accuracy using PCA model.

Fig. 3 shows the loading plot of PC1 in the range of 1700–600 cm^{-1}. The loading plot was basically used to identify the major contributing spectral region for the differentiation of samples. It is apparent from the loading plot that major peaks positioned at 1645 (Amide I), 1517 (Amide II), and 1036 (Cysteic acid) cm^{-1} contributing for the discrimination of shahtoosh and Pashmina wool.

3.3. Construction of a PLS-DA model for the discrimination between Shahtoosh and Pashmina wool

To get the better classification rate, PLS-DA model was established for achieving the accurate discrimination between hair spectra of Shahtoosh and Pashmina wool. During the process of establishing PLS-DA model, total 50 hair spectra (25 each) of Shahtoosh and Pashmina wool were imported. Based on the PLS-DA model, as enumerated in Fig. 4, the spectra of two wool Shahtoosh (class 1) and Pashmina (class 2) were successfully discriminated without any misclassification, hence achieved 100% accuracy for the classification of spectra of two wools. Highly significant R-square value for PLS-DA model was achieved that is 0.995; hence, it indicates greater predictive accuracy with minimum error rate. An important step after the establishment of PLS-DA model (discrimination of Shahtoosh and Pashmina wool) is to perform the validation study (blind and external validation tests) to verify the predictive capacity of the PLS-DA model.

Fig. 5 shows the factor 1 loading plot. The loading plots showing the similar profile with the original dataset and highlights the important regions which convey important pieces of information. In loading plot, the region of Amide I (α- Helix) and amide II are the most important variables for the discrimination of Shahtoosh and Pashmina wool.

3.4. Blind test

To figure out the performance of the PLS-DA model, which is used for the classification, a set of 10 unknown hair samples of Shahtoosh and Pashmina wools were collected which was not the part of training dataset. The samples were assigned as unknown samples and designated with UNK1-UNK10. The obtained spectral data were loaded into the PLS-DA model. All unknown samples (UNK1-UNK10) could be acknowledged by its adjacent position of known group (class 1- Pashmina wool and class 2- Shahtoosh), which were constructed based on the training data set. In prediction column, it was observed that all unknown samples were accurately placed in their corresponding class. The results of prediction for these blind samples are displayed in Fig. 6 and Table 3. In Fig. 6, Y-axis represents the groups of known class (Class 1-Pashmina wool, Class 2-Shahtoosh, and Class 3-Unassigned) and X-axis represents the samples of unknown class (UNK1-UNK10). Unknown sample number UNK1, UNK2, and UNK3 belong to the class 1 that is Pashmina wool and UNK4-UNK10 belong to the class 2 that is Shahtoosh (Fig. 6 and Table 3). PLS-DA model, resulted 100% accuracy for the unknown class prediction, as none of the unknown sample was misclassified.

3.5. External validation test

To evaluate the performance of PLS-DA model, predictions were carried out by using samples of external dataset. 40 wool hair samples from 8 individuals of Angora rabbit (5 from each individual) were collected to evaluate the performance of prediction model. The representative ATR FT-IR spectrum of Angora wool is given in Figure SVI (supplementary file). It is used for external validation test to investigate how the model classifies hair samples of Angora rabbit outside the dataset of Shahtoosh and Pashmina wool. The results of prediction are displayed in Fig. 7. Blue square represents the class of Pashmina wool and red circles represent the class of Shahtoosh and a green triangle designates the class of Angora wool. To construct the external validation model, the samples of Angora wool were projected onto the original model. It was observed that the deposit spectra of Angora wool were classified separately from Shahtoosh and Pashmina wool and thus the model performed 100% accuracy for unknown class predictions. This demonstrates that it would be very unlikely for hairs of Angora wool to be misidentified as Shahtoosh and Pashmina wool. The obtained R-Square value for the current model is 0.99.

The obtained results from this study, confirms the ability of PLS-DA model to discriminate hair of Shahtoosh and Pashmina wool. The non-destructive nature of ATR FT-IR spectroscopy and the classification performance of PLS-DA model make this technique well suited for the forensic species discrimination from a single strand of unknown wool hair sample of aforementioned species. This is highly useful when sample is encountered in trace amount and the destructive approach adopted for species identification could not be afforded. As portable ATR

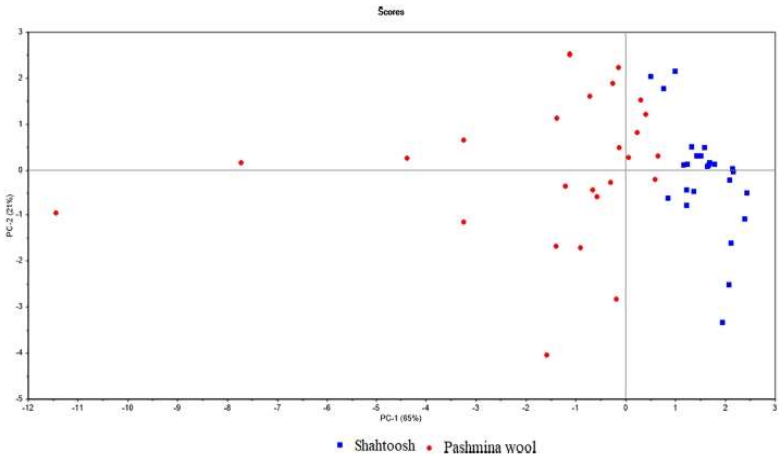

Fig. 2. Discrimination of Shahtoosh and Pashmina wool using PCA model (1700–600 cm^{-1}).

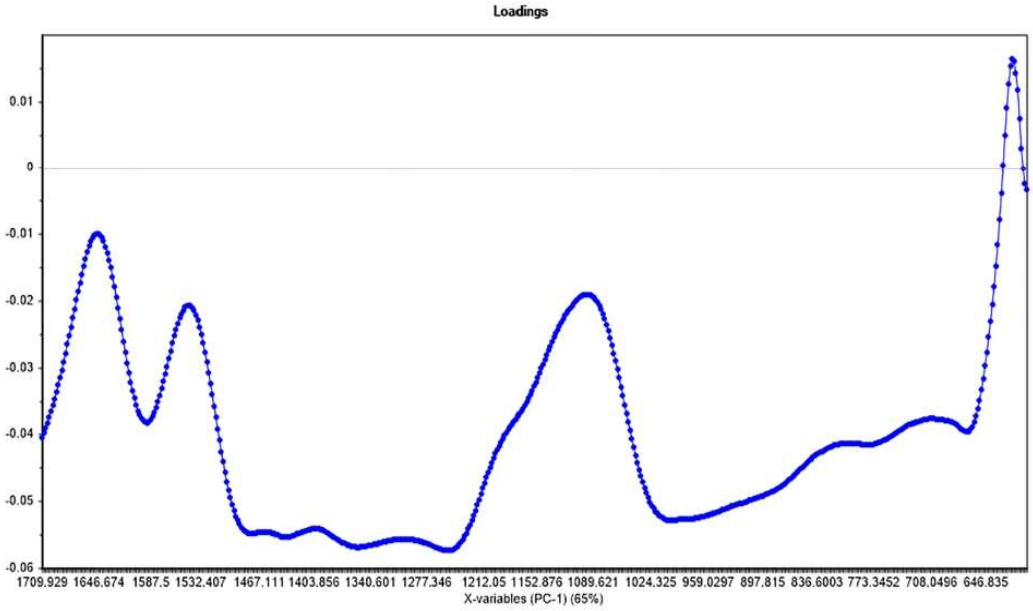

Fig. 3. Factor loading plot of PC1 in the whole MIR range (1700–600 cm^{-1}).

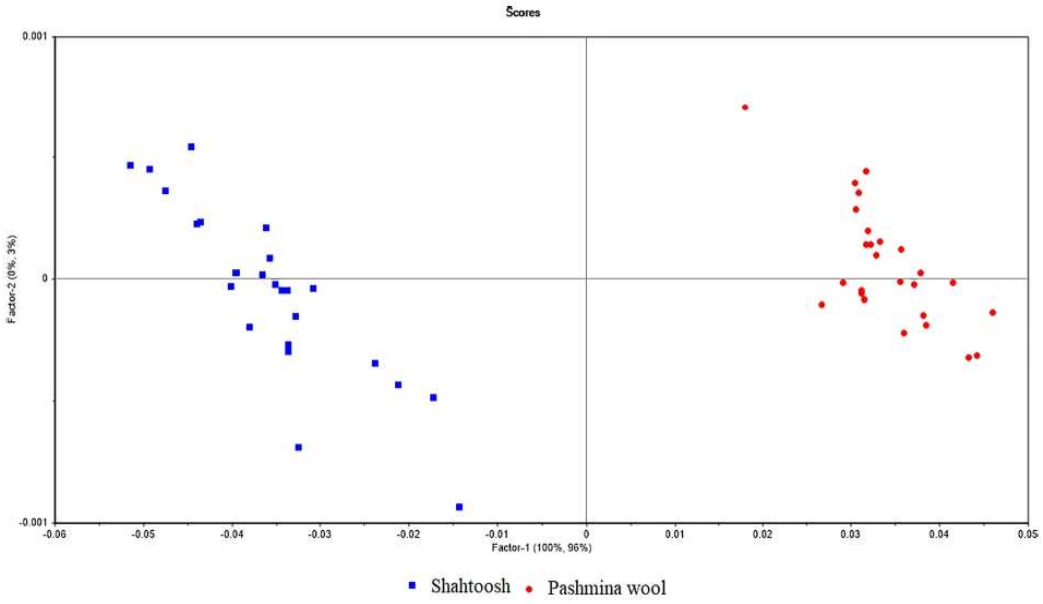

Fig. 4. Discrimination of Shahtoosh and Pashmina wool using PLS-DA model in whole MIR range.

FT-IR instruments are available in various laboratories, therefore rapid species discrimination is also feasible in on– field. This technique would be of great use due to its rapid and non-destructive nature and also successful probability predictions were obtained without analyst bias.

Based on the blind and external validation results, accuracy, precision, false-positive, false-negative, sensitivity, accuracy, and specificity rate of the model are calculated (Table 4). The model performed 100%, sensitivity, specificity and accuracy with 0% rate of false positive and false negative rate.

The obtained results showed that ATR FT-IR spectroscopy is solely good enough for the discrimination of unprocessed Shahtoosh, Pashmina underfur hairs and Angora wool. Since this application of this technique has just entered into the wildlife forensic arena, it needs to be validated across the laboratories with samples from real case scenario like any other promising technique. Therefore, to be on the safer side as this technique is at initial stages, its usage is recommended in combination with microscopic and DNA techniques in the following order:

Firstly, the examination of Shahtoosh wool hairs under microscope for color, length, and thickness (non-destructive). Secondly, ATR FT-IR spectroscopy-based again non-destructive analysis of unprocessed Shahtoosh which is possible from wool hairs in the absence of guard hairs and provides conclusive differentiation from Pashmina and Angora

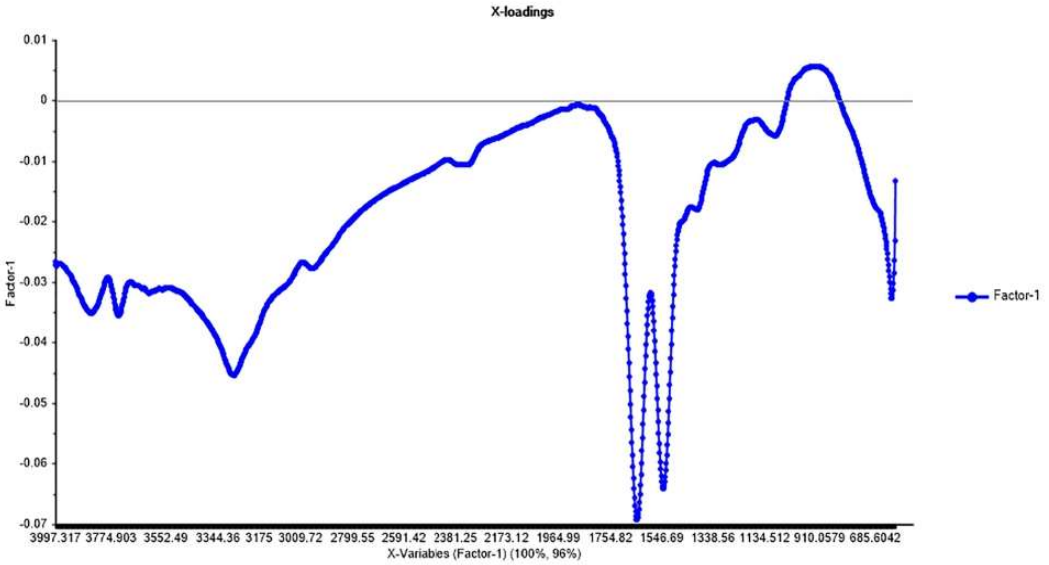

Fig. 5. Factor-1 loading plot of PLS-DA model to discriminate the Shahtoosh and Pashmina wool in the whole MIR range.

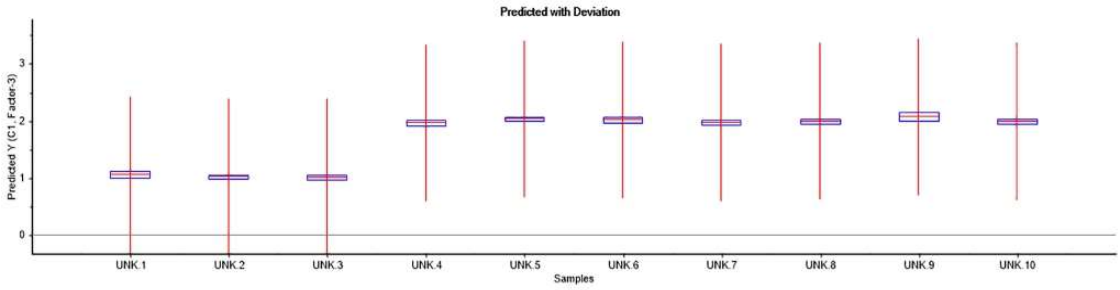

Fig. 6. Predicted Y represents groups of known class (Class 1- Pashmina wool, Class 2- Shahtoosh, and Class 3-Unassigned) and X-axis represents samples of unknown hairs.

Table 3
Summary of PLS-DA model predictions from blind validation test spectra.

Unknown/Blind samples	Predicted class	Deviation	Actual Identity
UNK1	1.05	0.06	1
UNK2	1.01	0.03	1
UNK3	1.00	0.04	1
UNK4	1.96	0.05	2
UNK5	2.02	0.04	2
UNK6	2.01	0.05	2
UNK7	1.96	0.04	2
UNK8	1.99	0.04	2
UNK9	2.06	0.07	2
UNK10	1.98	0.03	2

wool from unprocessed samples. Thirdly, examination of Shahtoosh guard hairs (if available) under microscope for cuticle scale patterns, medulla (pattern and index) and cross-section (shape and pigment distribution) as methodology involved is destructive in nature. Lastly, DNA based analysis which is possible from both guard and wool hairs for confirmed species identification.

4. Conclusions

The rationale that contributes to the overwhelming application of ATR FT-IR spectroscopy in combination with chemometrics is its non-destructive, rapid, facile, and eco-friendly nature. It was possible to differentiate unprocessed underfur Shahtoosh and Pashmina wool hair by applying ATR FT-IR spectroscopy in combination with chemometrics, regardless of the fact that, to the eye, the obtained spectra could not be sufficiently distinguished. Consequently, chemometric tools have been shown exceedingly powerful to extract out the information from the spectral data. First, we used PCA model for the purpose of discrimination between hair spectra of Shahtoosh and Pashmina wool; however, which resulted scattered distribution and thus cannot be considered sufficient for the purpose of classification. Further, the PLS-DA model revealed very good classification of Shahtoosh and Pashmina wool with zero occurrences of false-negative and positive assignments. In external validation test, hair samples of Angora wool were also excluded and classified separately from the training data set.

Overall, a resulting performance shows that ATR FT-IR spectroscopy, concurrently with chemometrics has surged as a powerful and non-destructive tool to distinguishing the unprocessed underfur hairs of Shahtoosh and Pashmina wool often encountered in wildlife investigations. However, since this technique is at initial stages, it is recommended that it must be used as a screening tool preceded by conventional microscopic examination and followed by well-established DNA based species identification. This approach needs further validation studies on processed and finished Shahtoosh articles recovered in

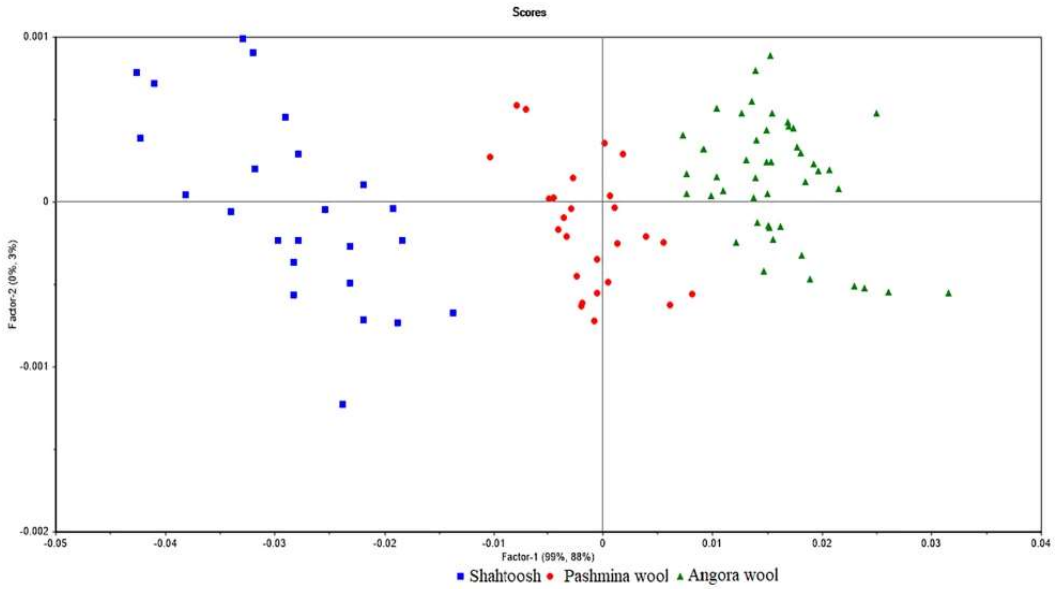

Fig. 7. PLS-DA score plot to differentiate Angora wool from Shahtoosh and Pashmina wool.

Table 4
Sensitivity, specificity and accuracy values.

	FP (%)	FN (%)	Accuracy (%)	Specificity (%)	Sensitivity (%)	Precision (%)
Shahtoosh	0	0	100	100	100	100
Pashmina wool	0	0	100	100	100	100
Angora wool	0	0	100	100	100	100

wildlife investigations. Nevertheless, this proof-of-concept study will open up a new dimension for the rapid screening of confiscated wildlife trade items suspected for the presence of Shahtoosh (as portable ATR FT-IR instruments are commercially available now a days), which will be very helpful to the law enforcement agencies to implement wildlife laws, and prevent false accusations as well.

CRediT authorship contribution statement

Chandra Prakash Sharma: Conceptualization, Data curation, Formal analysis, Supervision, Investigation. **Sweety Sharma:** Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. **Gopal Singh Rawat:** Conceptualization, Writing – review & editing. **Rajinder Singh:** Conceptualization, Data curation, Methodology, Formal analysis, Supervision, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Operation Soft Gold – Integration of cyber intelligence in curbing illegal Shahtoosh trade in India

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ABSTRACT

The fine wool Shahtoosh obtained from the Tibetan Antelope (*Pantholops hodgsonii* Abel, 1826) which is endemic to Tibetan Plateau, is highly valued for its rarity, warmth and lightness. The illegal trade in Shahtoosh may lead to extinction of this species. The Tibetan Antelope is protected from commercial trade throughout its range, both nationally and internationally thereby prohibiting any commercial use or trade of any product in India and in the international market. Though globally banned the illegal trade of Shahtoosh driven by international demand has continued to be actively operational undercover in India. In order to bypass this trade ban, the traffickers misdeclare Shahtoosh consignments as Pashmina, Cashmere or conceal by mixed them in shipments between look-alike woolen products. In the present study the illegal Shahtoosh trade was analysed from 2009 to 2020. The Wildlife Crime Control Bureau has envisaged Operation Soft Gold to curb this. A total of 62 confirmed Shahtoosh cases were detected in India from 2009 to 2020. Most of them were detected at exit points and intended for international smuggling. The Indri Gandhi International airport New Delhi in India was the most preferred airport, while Air Cargo and Air Courier were often used by the traffickers. Our analysis shows that the illegal Shahtoosh trade network is going on between the Western Asian countries, Oman, China, Japan, Pakistan, UK, Spain, Hong Kong and Switzerland are either destination or transit countries for illegal Shahtoosh products.

1. Introduction

Shahtoosh, the "king of wool" is the finest wool obtained from the under fur of the Tibetan Antelope or Chiru (*Pantholops hodgsonii* Abel, 1826), an iconic species endemic to the Tibetan Plateau [1–3]. Shawls and other items made up of Shahtoosh are considered as a sign of nobility and appreciated for their lightness, warmth and softness [2–4]. Every year large number of antelopes are killed in their natural distributional range from time immemorial [2,3,5] and the hair obtained are smuggled to Jammu and Kashmir, India where skilled Kashmiri weavers used to weave them into varied Shahtoosh products [6]. The finished Shahtoosh products used to illegally transported across India and other countries.

The Tibetan antelope has been accorded the highest protection by both national and international laws. Thus the trade in raw wool, finished products as well as any body part of the Tibetan antelope is prohibited. Due to the recovery of some population this the Tibetan Antelope was reclassified as Near Threatened (NT) by International Union for Conservation of Nature (IUCN. 2022), in Appendix - I of the Convention on International Trade in Endangered Species (CITES) and Schedule I of The Wild Life (Protection) Act, 1972 of India. As per estimate,125–130 g of wool is obtained from single individual and for weaving one shawl, wool obtained from four to five individual animal is required [7,8]. More than a million Tibetan Antelope were present at the beginning of the last century but the population was reduced to less than 50% in just 20 years of the 20th century due to habitat fragmentation, increased poaching and smuggling [1,8,9]. According to an estimate, 684.5 kg of Shahtoosh wool seized in 1997 must have been extracted after killing about six thousand individuals [10] and at least 10,000–20, 000 Tibetan Antelopes are killed annually in their distributional range [11,12]. The major constraints in combating illegal Shahtoosh trade are an insufficient database on illegal trade, unclear modus operandi for trafficking, and a lack of awareness for the detection of Shahtoosh by enforcement agencies.

The Wildlife Crime Control Bureau (WCCB), India commissioned a dedicated enforcement operation having code-name "Operation Soft

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Gold" from October 2018 to September 2020 with a focus on bringing special attention of enforcement agencies within India and beyond towards the illegal trade in "Shahtoosh products". The WCCB along with the Wildlife Institute of India (WII), Dehradun, imparted capacity building and hands-on training for identification of Shahtoosh products and wool to approximate more than 450 officials of different enforcement agencies of Indian Customs, State Forest departments and Border Guarding Paramilitary Forces deployed at various border entry and exit points.

In the present study, Open-source intelligence (OSINT) tools were used for collecting intelligence about the suspects whose consignments were seized. Suspected Shahtoosh seizures were referred to the WII for forensic analysis. All such data were used to formulate strategies for combating organized wildlife crime involving the Tibetan Antelope, increased detections and quick convictions which will act as a major deterrent in combating the threat of illegal trade of products made from Tibetan Antelope.

2. Material and methods

A two-pronged strategy was developed for collecting information on Shahtoosh wool smuggling into India, concealment and trafficking of Shahtoosh finished products out of the country. Synchronized digital and human intelligence interface were used to extract information resulting in the confiscation of Shahtoosh products at various main land and exit points of India.

2.1. Intelligence collection through OSINT

For monitoring active illegal dealings in Shahtoosh products online, manual screening of different common social media platforms namely Facebook, Instagram, Twitter, YouTube, Pinterest, Tumblr, Reddit, Flicker, Vimeo, Dailymotion and trade web portals like Etsy, IndiaMART and Alibaba was done using OSINT tools with a fixed set of keys words were selected based on multiple hashtags used by the trader in the social media. The geo-location and contact details of the group/person involved in the illegal wildlife trafficking of the Shahtoosh shawls were extracted using available OSINT tools. We followed the guidelines on ethical decision making and internet research [13].

To understand the criminal network the information pertaining to entities, links and attributes was tabulated as specific information dataset which was used for mapping the connections among the illicit Shahtoosh traders using IBM i2 Analyst's Notebook. The entire study period was divided into two periods namely, Before Operation Soft Gold (BOSG) from January 2009 to September 2018 and Operation Soft Gold (OSG) from October 2018 to September 2020 to facilitate a comparative study to understand the trend in trading of Shahtoosh products, shift in quality of Shahtoosh products and mode of smuggling. The available literature, news reports, peer-reviewed research articles and seizure details of Shahtoosh by enforcement agencies at entry/exit points were analysed to confirm the baseline information on the border smuggling routes as well as trade hot spots.

2.2. Enforcement

On-site physical examination of suspected shipments was carried out at main land and exit points following the standard protocols [12,14]. All the suspected Shahtoosh consignments were referred for scientific opinion, as a mandatory requirement under The Wild Life (Protection) Act, 1972. A detailed scientific examination of the seized Shahtoosh shawls was carried out on physical properties and microscopic hair characteristics following standard forensic protocols [12]. The shipment details from invoices of consignments extracted namely number of items, billing address, description of the material along with the value of the consignment as declared by the trader, number of Shahtoosh product detected, and mode of transport were noted for analysis.

The data was analysed for both the periods (BOSG and OSG) separately to understand the possible shift in the quality of Shahtoosh products, adulteration, mode of concealment and modus operandi of the illegal trade. In the present study each suspected seizure that was confirmed as Shahtoosh is treated as one unit (mentioned as "n"). The judgments/orders passed by various courts during 2009–2020 were also reviewed for assessing judicial convictions. The entire data was analysed using IBM i2 Analyst's Notebook and Circos Table Viewer v0.63–9 © 2008–2021 [15]. As it was hypothesized that there is a significant influence of invoice details and reasons for suspicion of the Shahtoosh products in the consignment the information retrieved from the invoice details of conformed Shahtoosh product seizures from 2009 to 2020, the information were regressed accordingly using statistical software IBM SPSS version 19.

3. Result

3.1. Seizures of confirmed Shahtoosh products

From 2009–2020 a total of 62 seizures made by various enforcement agencies at prominent sites identified on the basis of the intelligence were confirmed for presence of Shahtoosh during the study period which included raw wool to finished Shahtoosh products such as Shawls, Square shawls, Stole/Scarf (Table 1; Fig. 1). Though the trade of Shahtoosh was globally banned from 1979 under CITES to which India is a signatory, BOSG the seizure data from 2009 to 2018 (Table 1 and Fig. 1) indicates that the trade of the Shahtoosh has been actively operational undercover in India in order to cater to the worldwide demand. On the basis of the consistent intelligence inputs regarding continuous undercover activities and active organized syndicates of Shahtoosh traders, during the OSG (from September 2018 to October 2020) period a total of 28 confirmed Shahtoosh seizures were made within two year span in India.

During the analysis of the invoice details, it was observed that to overcome this embargo, the traffickers mis-declared Shahtoosh consignments as Pashmina, Cashmere and Woolen product which is legal and derived from domestic goats and legal to trade. Sometime Shahtoosh products were concealed among other legally traded in order to get goods clearance from customs. We categorized the data as per the declarations in the invoice by the exporter to clearance of shipment at Customs point.

3.2. Illegal trade of Shahtoosh before operation soft gold (BOSG)

It was observed that during the study period BOSG a total of 34 confirmed Shahtoosh cases (n = 292) were detected at various Airports and other likely places (Main land) in India (Table 2). The details of the specific airport along with the Shahtoosh products seized are given in Table 2. Our study signifies that the IGI Airport, New Delhi is found to be the most favored point of exit which accounts for making 91% (N = 29) of total Shahtoosh detections. During this period, it was found that 47% (N = 16) of the illegal Shahtoosh products were booked through courier agencies (cargo and courier mode) which provide door to door pickup and delivery services. Terminal 3, IGI Airport, New Delhi accounted for 20% of cases including (N = 5, n = 58) Shahtoosh products which were of persons returning back to India after selling the Shahtoosh products abroad which were smuggled out of the country earlier. Among them (n = 32) Shahtoosh products (N = 1) were brought back to India from Riyad, Saudi Arabia, followed by n = 15, N = 2 from the United Arab Emirates, n = 06, N = 1 from the United States of America and n = 05, N = 1 from Kazakhstan (Table 1). All the Shahtoosh products were illegally smuggled out of India and the same was sold in respective countries where they were smuggled to, and the unsold products were brought back by the persons in their personal check in luggage along with them. The person was intercepted by Indian Customs official at IGI Airport, New Delhi while attempting to cross green channel along with

Table 1
Details of confirmed Shahtoosh seizures from January 2009 to September 2020.

Period	Total seizures of confirmed Shahtoosh	Shipment value[a]		Type of Shahtoosh product (n)				
		INR	USD	Shawl	Stole/scarf	Square	Wool	Total
BOSG	N = 34	56,772,672.00	1,208,725.00	185	37	69	1	292
OSG	N = 28	50,468,436.55	716,738.60	158[b]	64	308	0	530
Combined BOSG and OSG	N = 62	107,241,108.55	1,925,464.00	343	101	377	1	822

[a] Shipment Values as given on invoices related to consignments containing confirmed Shahtoosh products.
[b] At ICP Attari along with Shahtoosh shawls a Shathoosh fabric was seized.

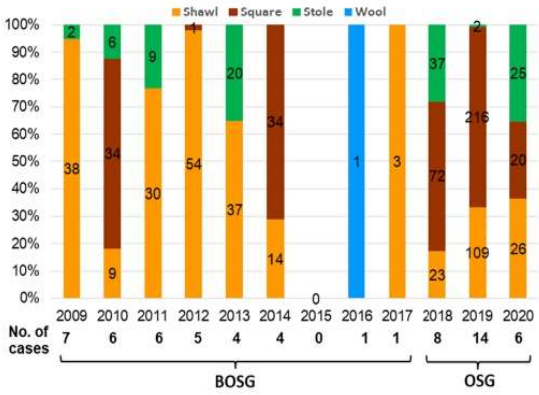

Fig. 1. Shahtoosh products seized by enforcement agencies from January, 2009 to September 2020.

the Shahtoosh products.

Apart from export in large quantities, the Shahtoosh products are also directly sold on retail to affluent buyers by high end fashion emporium mostly in tourist destinations. Shahtoosh products are usually sold to interested buyers after verifying their genuine interest and price negotiations. Based on the intelligence developed by the enforcement agencies, 7% (N = 4) of Shahtoosh cases (n = 20 Shahtoosh product and 1 Shahtoosh wool) were detected in main land in India in which (n = 9) Shahtoosh products (N = 1) were detected at Thekkady, Kerala, (n = 8) Shahtoosh products (N = 1) at Chennai, Tamil Nadu and (n = 3) Shahtoosh products (N = 1) in New Delhi. In all the three locations the Shahtoosh products were illegally kept and sold to the prospective customers. Human carriers trying to smuggle out Shahtoosh products by concealing few shawls, stoles etc. in hand baggage or checked in luggage were also apprehended. Suspected Shahtoosh raw wool detected at Punjab, Puh (Himachal Pradesh), Kheri- Sampurana Nagar, Pilibhit (Uttar Pradesh) and Srinagar (Jammu and Kashmir) out of which the case (N = 1) from Srinagar (Jammu and Kashmir) was confirmed to be Shahtoosh.

3.3. Open source intelligence to trace Shahtoosh trafficking in India

The Illegal trade of Shahtoosh products online is rampant. During the investigation of online trade through social media, 22 keywords were identified which were used for # Hastag purposes to advertise the Tibetan Antelope/Chiru (Shahtoosh) products like shawls, square shawls, stole /scarf etc for sale. The social media platforms were searched using these key words, by using advanced search option and scrutinized as *date posted: Latest, posts from: public, Location: Anywhere*. It was observed that Instagram (95%) was most frequently used for advertising Shahtoosh products followed by Facebook (1.4%) and Pinterest (1%) and then others namely Twitter, YouTube, Tumblr, Reddit, Flicker and Vimeo which in total accounts for 1.8%. The maximum number of hashtag or advertisements of Shahtoosh products were observed between the months of September and February during OSG on Instagram and Facebook.

While investigation of social media, free listings websites and e-commerce, which were observed for active sellers of various Shahtoosh products across the globe, a total of 59 such profiles were identified. From which 32 active profiles were found in the social media and free listing websites and 27 active sellers were identified by screening of e-commerce sites namely IndiaMART (n = 16 https://www.indiamart.com/), Alibaba (n = 6 https://www.alibaba.com/) and Etsy (n = 5 https://www.etsy.com/in-en/). Out of the 59 profiles investigated 27 active profiles are of owners based in India or having direct relation with Indian trader and actively advertising Shahtoosh product on social media for prospect buyer. Using this information, these total 27 active profiles and sellers were further investigated through Open Source Intelligence Tools (OSINT). All the information was converted into entities, link, attributes and mapped by using IBM i2 Analyst's Notebook (Fig. 2), which demonstrates the importance of OSINT tools in the identification of the illegal Shahtoosh traders that are active on social media. Based on intelligence developed through OSINT, OSG was launched from October 2018 to September 2020 in India.

3.4. Status of Shahtoosh products trade during operation soft gold (OSG)

As the OSG is a dedicated two yearlong operation specially launched by the enforcement WCCB to curb the illegal Shahtoosh trade going on in India, in the present study it was concluded that during OSG based on

Table 2
Detention of different Shahtoosh product at various local location based on intelligence.

Local location		No. of case and Shahtoosh products							
		(Shawl)		(Square shawl)		(Stole/Scarf)		(Wool)	
		BOSG	OSG	BOSG	OSG	BOSG	OSG	BOSG	OSG
IGI Airport, New Delhi	Air Cargo Terminal	7 (45)	12 (115)	4 (35)	5 (288)	6 (32)	5 (44)	0	0
	Air Courier Terminal	6 (43)	1 (2)	1 (34)	1 (20)	0	0	0	0
	Passenger Terminal-3	4 (53)	1 (15)	0	0	1 (5)	0	0	0
Netaji Subhash Chandra Bose International Airport (NSCBI), Kolkata		1 (24)	0	0	0	0	0	0	0
Integrated Check Post (ICP) Attari, Amritsar, Punjab		0	2 (26[a])	0	0	0	1 (20)	0	0
Main Land: Kerala, Tamil Nadu, New Delhi and Srinagar		3 (20)	0	0	0	0	0	1	0
Total		**21 (185)**	**16 (158)**	**5 (69)**	**6 (308)**	**7 (37)**	**6 (64)**	**1**	**0**

[a] At ICP Attari along with Shahtoosh shawls a Shahtoosh fabric was seized.

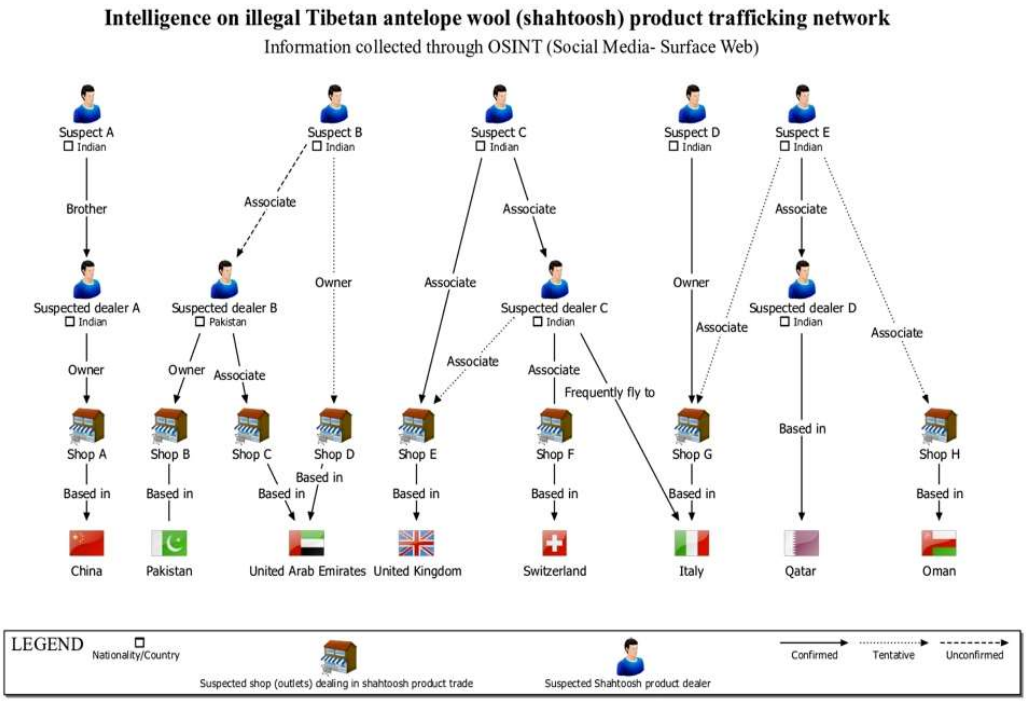

Fig. 2. Representative network chart for illegal Tibetan Antelope wool and Shahtoosh products trafficking developed using IBM i2 Analyst Notebook.

intelligence inputs developed through OSINT and coordinated operations a total 28 confirmed Shahtoosh (n = 530) cases were detected at IGI Airport, New Delhi and Attari, Punjab, (Table 1) among them 84% (N = 22, n = 447) Shahtoosh products were detected at Air Cargo Terminal, IGI Airport New Delhi. Air Courier Terminal, IGI Airport accounts for 4% (N = 2, n = 22) while 3% (N = 1, n = 15) were from passenger Terminal-3, IGI Airport. During the period 9% (N = 3, n = 46) were detected at the Integrated Check Post (ICP) Attari while attempting to smuggle to Pakistan through land route of ICP Attari. The ICP Attari is located at about 30 Kms from the holy city of Amritsar in the State of Punjab on the India-Pakistan Border (Table 2). Among them, 76% (N = 19) of the detections were mis-declared as pashmina followed by 20% (N = 5) as woolen products and 4% (N = 1) as cashmere products respectively. The mode of export of mis-declared goods was mostly from the commercial Air Cargo Terminal from IGI Airport New Delhi which accounts for 79% (N = 22) of total shipments while 7% (N = 2) were through Air Courier Terminal, 4% (N = 1) from Passenger Terminal 3, IGI Airport New Delhi and 11% (N = 3) from ICP Attari, Punjab. It was

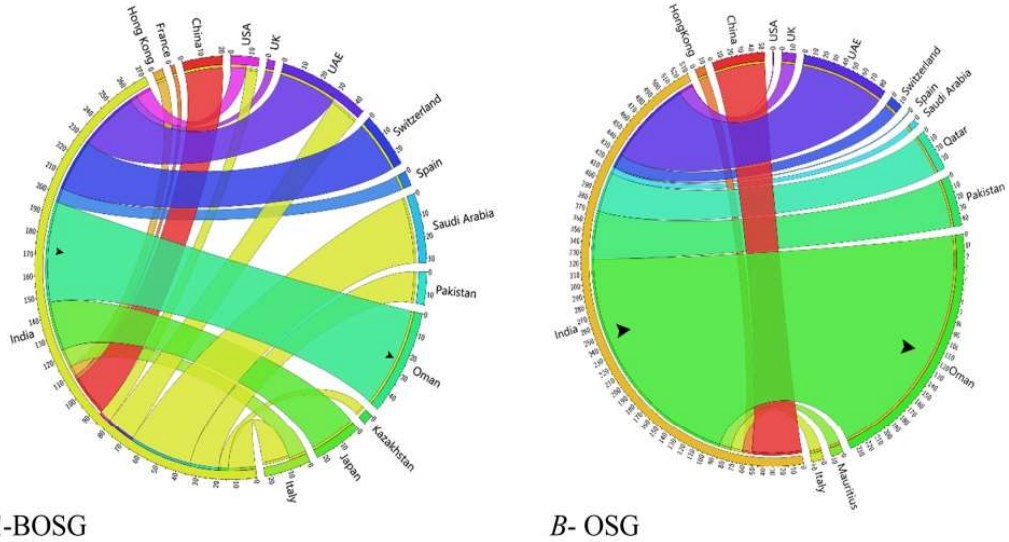

Fig. 3. Trade routes identified for illegal Shahtoosh trafficking (Number of Shahtoosh products denoted through tick marks, trade flow ribbons of different color adjacent to the name of country indicate outflow while trade flow ribbons with a gap shows inflow.

found that illegal Shahtoosh products (64%) were mostly booked through international courier agencies (cargo and courier mode).

3.5. Shahtoosh product trade route

In the present study we analysed the data of BOSG and OSG with the aim to identify the major importers and exporters of Shahtoosh product. It was observed that BOSG with relation to its different types of product (n = 271) (Fig. 3A), 18% of Shahtoosh product (n = 49) were bound for Oman followed by 17% for the United Arab Emirates (n = 45), 12% for Riyad, Saudi Arabia (n = 32) and 10% for Switzerland (n = 26). The details mentioned in invoices reveals that among the ongoing illegal Shahtoosh trade there is a specific demand for different types of Shahtoosh products namely Shahtoosh Shawls, Stoles, Square Shawls, Fabrics and Raw Wool in different countries. It is evident from Table 2 that 63% (n = 185) of Shahtoosh shawls, 24% (n = 69) of Shahtoosh square shawls, 13% (n = 37) of Shahtoosh stoles/scarves and 0.3% (n = 1) of Shahtoosh wool were seized where 13% of the Shahtoosh shawls were bound to Japan (n = 24) followed by Switzerland (11%, n = 20). It was observed that 67% of Shahtoosh square shawls were bound for Oman (n = 46) and UAE (33%, n = 23) respectively while stoles/scarves were bound for China (54% n = 20) and Switzerland (16%, n = 6).

The OSG period witnessed 45% of the Shahtoosh product (n = 238) were bound for Oman followed by 16% (n = 86) for the United Arab Emirates, 10% (n = 52) for China and 09% (n = 46) for Pakistan (Fig. 3*B*) It is evident from Table 2 that 38% of the Shahtoosh shawls seized were bound for UAE (n = 60) and Pakistan (16% n = 25). 77% of Shahtoosh square shawls were bound for Oman (n = 236) followed by Qatar (12%, n = 37). Shahtoosh stoles/scarves (58%) were bound for China (n = 37) and Pakistan (31%, n = 20).

3.6. Trends in the illegal trade of Shahtoosh products from 2009 to 2020

Rapidly evolving air transport systems play a significant role in increasing the wildlife trafficking of highly valued products across the globe [16]. India too now has a well-developed air network connecting important cities with most of the Countries. Analysing the seizure data of confirmed Shahtoosh products cumulatively from 2009 to 2020 it becomes clear that the market trend in the various Shahtoosh products shifted with respect to type of Shahtoosh products, year and destination countries. It was observed that among the varied Shahtoosh products seized the square shawls were usually bound for Western Asia (Oman, Qatar, Saudi Arabia and UAE) which can be linked to the traditional wearing particular head gear known as Shemagh by men in these areas [17]. As the market value of Shahtoosh products is usually very high, it can be inferred that the per capita income of countries and the inhabitants influence procurement of the luxury Shahtoosh products strongly (Write and Kumar, 1997). Another Shahtoosh product which is smaller than a traditional shawl called a stole is lower in weight and usually becomes the prime choice of fashion industry [17,18]. In the present study it was observed that Stoles are preferred in Switzerland, Hongkong, China, UK, Spain and France.

It was observed that the details of the shipment value given in the invoice of the detained consignment on suspicion following the intelligence inputs that were confirmed for Shahtoosh after forensic analysis plays an important role in developing strategies to identify and to sort the consignments which are suspected to contain Shahtoosh. Multiple regression analysis (Table 3) was performed in which shipment details of the consignments proven to contain Shahtoosh were regressed taking confirmed Shahtoosh products as dependent variable and shipment details as predicting variables (Number of items in invoice, Shipment value, Total number of shawls in the shipment, Number of boxes in the shipment) to test the hypothesis H_1 that there is a significant relationship between the shipment details declared by the exporter and the consignment seized on suspicion. The multiple regression analysis (R2 = 0.845) clearly explains 84.5% of the variance in suspected Shahtoosh products due to the shipment details.

Table 3
Multiple regression analysis for identifying the significance of details mentioned in the invoice in laying suspicion of Shahtoosh products in the consignment.

Model summary					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	
1	.920[a]	.846	.845	26.537	
ANOVA[b]					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	3,032,788.199	4	758,197.050	1076.632	.000[a]
Residual	552,820.935	785	704.230		
Total	3,585,609.134	789			
Coefficients[b]					
Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	30.503	1.486	1.572	20.524	.000
No_of_Box	11.130	.617	-1.151	18.039	.000
T_Shawls	-0.075	.004	.392	-17.479	.000
Ship_Val	3.150E-6	.000	-0.310	11.949	.000
No_of _Items	-3.668	.214		-17.099	.000

Note: *p < 0.05; No_of_Box: Number of boxes in the shipment; T_Shawls: Total number of shawls in the shipment; Ship_Val: Shipment Value; No_of _Items: Number of items in invoice.

[a] Predictors: (Constant), Number of items in invoice, Shipment value, Total number of shawls in the shipment, Number of boxes in the shipment.

[b] Dependent Variable: Suspected Shahtoosh products seized for the shipment.

On the basis of analysing the Shahtoosh product seizers and literature available, we developed a network which reflects possible illegal trade of in Tibetan antelope products using IBM i2 Analyst's Notebook (Fig. 4). Trend of varied Shahtoosh products seized from 2009 to 2020 by enforcement agencies at various checkpoints in India are given in Fig. 5.

3.7. Status of legal prosecution in India

In India, the Tibetan antelope is listed in Schedule I of Wild Life (Protection) Act, 1972. Dealing, transporting and possession of the Tibetan antelope and the articles derived are prohibited U/s 39(3)/44/49/48A/49B(1) of the Wild Life(Protection) Act,1972 and shall be punishable with imprisonment for a term which shall not be less than three years but which may extend to seven years and also with a fine which shall not be less than ten thousand rupees U/s 51 of the said Act. Since 2009–2018, 34 cases Shahtoosh products and 01 Shahtoosh wool case were detected; in 6 cases the accused got a conviction for 3 years and fines varied from Rs.10,000/- to 10,00,000/-. 04 cases are in the trial phase in different courts. The rest of the 24 cases are being adjudicated by the Customs officers under the Indian Customs Act 1962 which are pending for trial under the provisions of The Wild Life (Protection) Act 1972 also.

4. Discussion

Increased global demand for Shahtoosh products was evident from the 62 seizures from 2009 to 2020. During the study period, 822 diverse illegal Shahtoosh products were detected which were bound for export to meet the global demands. Out of them 97.44% were seized at the exit points, while a total of 21 Shahtoosh products (2.56% were seizures were made from main land including one seizure of Shahtoosh wool.

During the period of OSG, 90% of the Shahtoosh shawls were attempted to be smuggle to Asia followed by Europe (8%), Africa (2%) and North America (less than 1%). In Asia 70% of Shahtoosh shawls

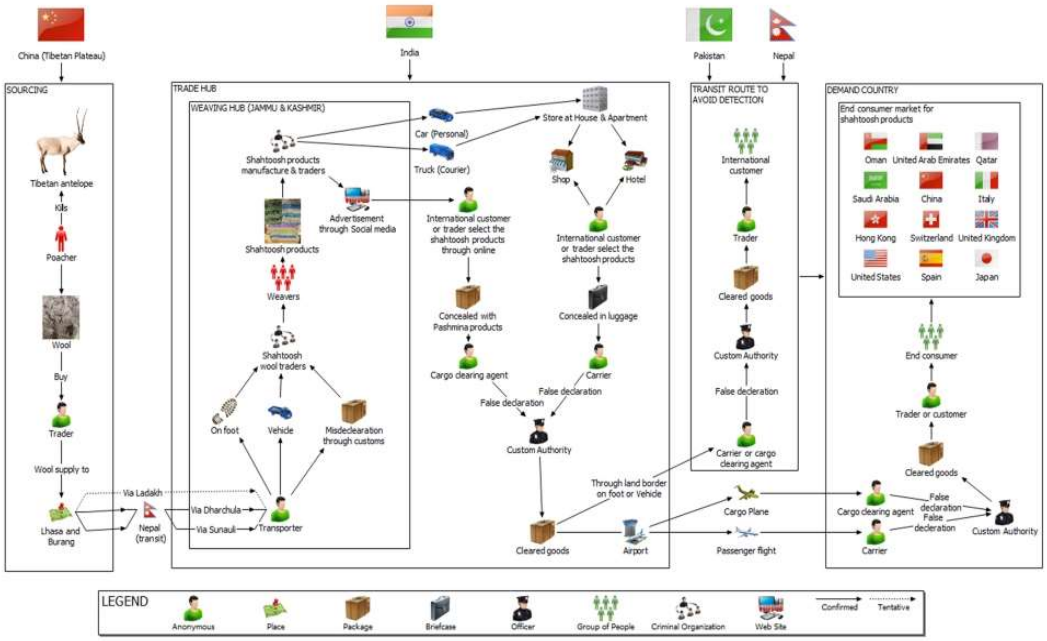

Fig. 4. Illegal Tibetan antelope wool (Shahtoosh) and the product trafficking network.

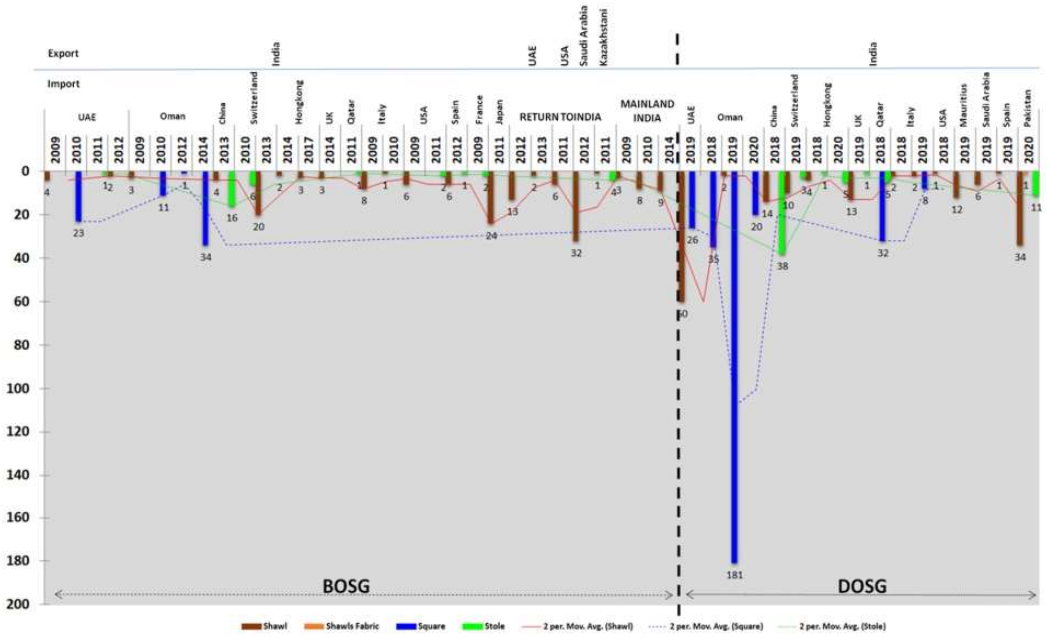

Fig. 5. Graph showing trend of various Shahtoosh products seized, inflow and outflow of the products among different countries.

were attempted to be smuggled to West Asian countries like Oman (45%), UAE (16%), Qatar (7%) and Saudi Arabia (1%), followed by East Asian (12%) countries like China (10%) and Hong Kong (2%). The outcome of the date from the Operation Soft Gold here clearly shows that there is high demand for Shahtoosh product in West Asian countries. According to the report of [11] it was earlier European and American countries which were having high demand of Shahtoosh products.). Our study shows that 9% Shahtoosh shawls were attempted to be smuggled to Pakistan.

Although India is the source country for manufacturing Shahtoosh shawls, during the intelligence collection through OSINT we found a lot of suppliers in Pakistan offering Shahtoosh shawls through social media. From the Social Network Analysis it was observed that the suppliers in Pakistan have a close association with dealers or manufacturers in India. It was also observed that during OSG the key traders were engaged in covert conversation through WhatsApp or Facebook (Messenger) to

confirm their involvement in illegal Shahtoosh products trade. It was also observed that the traders were using international courier services for sending the Shahtoosh products across the world. The most usual payment means used by dealers/traders was Western Union, MoneyGram and Paypal.

The highest number of Shahtoosh products detected during BOSG was 185 Shahtoosh shawls, with 69 square shawls and 37 stole/scarf. During OSG, 158 Shahtoosh shawls, 308 square shawls and 64 stole/scarf were seized. It is illustrated from Table 1 and Fig. 5 that the demand for Shahtoosh square shawls varies with respect to destination country. The majority of Shahtoosh products seized since 2009 were destined for Middle East Asia with an increased demand for square shape shawls in Oman during OSG.

Before OSG, the enforcement officers (Customs or Wildlife Inspectors) checked the shipments randomly based on the declaration provided by the exporter and the destination of the shipments. This study revealed that the Shahtoosh shawls/products are mis-declared as pashmina shawls for easy clearance at Indian custom points. Based on the declaration and destination, enforcement authorities detected 292 Shahtoosh product (3–4 cases and 29 Shahtoosh product per year). But during OSG, the enforcement authorities detected 530 Shahtoosh products (14 cases and 266 Shahtoosh shawls per year). The increased detection is due to the intelligence developed through Open Source Intelligence (OSINT) in identifying the global syndicate of Shahtoosh traders. At exit points, Shahtoosh products are mostly mis-declared as pashmina or concealed in large export consignments of other woolen products to avoid detection. The majority of Shahtoosh products were detected at IGI Airport, New Delhi, as per assumption of the traders that enforcement agencies are not able to detect the concealed Shahtoosh products due to underlying reasons of huge volume of transactions, convenience of location and connectivity with rest of the world.

An increase in Shahtoosh shipments concealed as pashmina and other wool booked through Air Cargo (IGI New Delhi) was observed during OSG. The non-commercial shipments like Air Courier and passenger hand/check-in luggage were also used by the traders to carry and able to make escape from customs examination. In the present scenario where many cargo and courier services are provide door to door pickup services, for consumers as well as suppliers end ensuring fast, safe and guaranteed delivery it was observed that Shahtoosh product traders specifically prefers international courier agencies (cargo and courier mode) which are renowned international courier services (Fig. 4). Though the main land of Jammu and Kashmir is considered as origin of Shahtoosh products [1,2,6], only one confirmed Shahtoosh wool case was detected by the enforcement agency until now. While 3 cases were recorded each in 2009, 2010 and 2014 from other main land areas of India, no Shahtoosh shawl case was detected in mainland after 2014. From our study the trade route as well as the participants countries involved in active illegal Shahtoosh product trade identified for the very first time. The detection at mainland is very low despite of efforts laid by enforcement agencies.

During OSG we saw the opening of a new frontier in the form of land route which was not known to be used for the smuggling of Shahtoosh products until then. This was discovered after seizures were made at Land Customs, Integrated Check Post (ICP) Attari, Punjab bordering Pakistan. After completion of Operation Soft Gold, in March 2021 a Shahtoosh wool case was detected at Land Custom Station (LCS), Sonauli, Maharajganj, in Uttar Pradesh which borders Nepal. Since 2009 very few seizures of wool coming into India have been detected in comparison to seizures of Shahtoosh products intended for export from India. Although 35 Shahtoosh cases have been under judicial trial from 2009 to 2020, only 6 Shahtoosh cases ended in conviction under the Wild Life (Protection) Act 1972.

5. Conclusions

In recent years the scale and nature of the illegal wildlife trade transformed significantly. The ever increasing demand of Shahtoosh products worldwide is imposing additional fatal pressure on the existing populations of the Tibetan Antelope, in their distribution range which is already a starved habitat. The present study has highlighted the extent of illegal trade in Shahtoosh products in India from 2009 to 2020. The study also uncovered/revealed the involvement of various groups involved in trading of Shahtoosh products across the globe, identified the local as well as global hotspots of Shahtoosh trade and the modus operandi used by the syndicates despite having stringent national and international laws which clearly prohibit trade in Tibetan Antelope in any form. The traders involved in the Shahtoosh products are very well aware of all such laws, and devise mechanisms to escape enforcement eyes. There is urgent need to address the dynamics of wildlife crime attempted by the organized criminal groups. The present study provides the first and most detailed countrywide assessment of illegal wildlife trafficking of Shahtoosh products, major trends and patterns of illegal export-import along with the modus operandi adopted in India. From the invoice details the value of seized Shahtoosh products from 2009 to 2020 is estimated to be ~ USD 1925,464 (ranges from 1 USD to INR- at the rate of 48.41–76.38). Western Asia has emerged as the main market for illegal Shahtoosh products from India. There is an urgent need to address the issues at various levels of wool origin, weaving, production, shipment and transportation in a comprehensive way to combat the illegal trafficking of Shahtoosh products.

6. Recommendations and way forward

Airports are playing a bottle neck roles. The Indian Customs and border guarding enforcement agencies keep rotating their personnel from border and custom areas therefore consistent capacity building for identification of Shahtoosh is needed. Due to strict vigilance at IGI Airport the illegal trade of Shahtoosh product may shift to other airport/exit point, therefore awareness and sensitization is required in all exit point in India.

The results of this study regarding information on invoices, modus operandi and exit points as well as countries of destination must be taken into account in order to focus enforcement efforts

The neighboring countries of India need to have strict vigilance for in and outflow of Tibetan antelope wool (Shahtoosh) and products and a deep study is required to understand modus-operandi of the international market for Illegal Shahtoosh with regard to sink and transit points.

Monitoring the illegal Shahtoosh trade through OSINT is much needed.

Since 2014 all the Shahtoosh product seizure have taken place at exit points only. No seizures have taken place in main land. The Enforcement authority within India (Police and Forest department) need to be sensitized about the illegal trade of Tibetan antelope wool (Shahtoosh) and products.

The results of this study need to be taken into account by the countries of destination that have been identified and appropriate measures must be implemented at ports of import as well as within the countries at locations of sale.

There must be a transfer of knowledge and best practices on an international scale in order to implement effective measures.

To develop on spot condition based Shahtoosh identification protocols which help enforcement agencies to detain the consignments more robustly as the Shahtoosh traders are very well aware of the techniques used for scientific identification from Shahtoosh products containing guard and wool hair.

Prosecution on the Tibetan antelope cases need to be speedup in India.

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CRediT authorship contribution statement

A.Pragatheesh: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Visualization, Writing – Original draft preparation, Reviewing and Editing, **Vinita Sharma:** Formal analysis, Data curation, Software, Writing – Original draft, Reviewing and Editing, **C.P. Sharma:** Data curation, Writing – Reviewing & Editing, **H.V. Girisha:** Writing – Reviewing & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Species discrimination from blood traces using ATR FT-IR spectroscopy and chemometrics: Application in wildlife forensics

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ABSTRACT

Efficient tools for the identification and discrimination of species are imperative in wildlife conservation since they can endow with information of species exploitation and also abet in solving problems related to forensic science. Herein, a non-destructive and rapid analytical method (ATR-FTIR Spectroscopy) coupled with PCA and PLS-DA was employed to analyze the dry blood samples for the species discrimination. Asian Elephant (*Elephas maximus*), Indian Leopard (*Panthera pardus fusca*), and Royal Bengal Tiger (*Panthera tigris tigris*) species were used to construct the chemometric models. Additionally, Domestic Pig (*Sus scrofa domesticus*) and Human (*Homo sapiens*) blood were taken for the external validation study. The evaluation results illustrate that the ATR FT-IR Spectroscopy in combination with PLS-DA model showed statistically confident discrimination among selected species from dry blood traces. ATR-FTIR spectroscopy supported with predictive models has been a robust, ideal, and suitable tool for species discrimination from dry blood traces recovered in wildlife investigations.

1. Introduction

Offenses against wildlife involve illegal exploitation of a wide range of species for commercial gain. This illegal exploitation of wildlife has become substantial in its scope and dimensions in recent years globally, largely for subsistence, illegal wildlife trade, societal/religious rituals luxury items, and use in traditional medicines etc. [1–3]. In recent times the illegal trade in wildlife has become increasingly sophisticated with availability of distant but secure digital platforms for secure supply along with new tools and weapons for poaching of wildlife, not only for high value charismatic species, such as Tigers, Elephants and Rhinoceros, but also for lesser animals like Monitor lizard and Pangolin's, birds and turtles [4,5].

Large felids like Tigers and Leopards and mega herbivores like Elephant and Rhinoceros are mostly poached for commercial purposes. Tigers are poached primarily for their bones to be used in "Traditional medicines" but other parts like pelts, fat, claws and canines are also traded and used locally. Recently the affluent consumers are demanding Tiger bone wine and glue to show off their wealth instead of investing in their health by demanding traditional medicines containing Tiger bones. Since 2002, over 22,000 Elephant carcasses have been so categorized, with between 1000 and 2000 observations per year between 2007 and 2018 [6].

For the implementation of wildlife protection laws, identification of species from biological evidences recovered from crime scene is of foremost importance. A major portion of evidences seized while enforcing wildlife protection laws involve seizures of biological evidences from victim wild animal. Blood as evidence is central to poaching incidents during wildlife crime investigations due to struggle and injury to wild animal and often found in various forms on various substrates and makes 8.2 % of total biological evidences (n = 4408) submitted in last 20 years (two decades-2001–2020) to Wildlife Forensic and Conservation Genetics cell of Wildlife Institute of India (WII), Dehradun. The evaluation of blood as evidence in wildlife crimes is crucial to

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substantiate the alleged accusations on suspect (s) apart from *Corpus delicti* in establishing procedure to kill animal, primary site of poaching, single or multiple species involved, number of individuals, Road-traffic accidents and linking suspects if blood is found of personnel belongings and space like cloths/vehicle/house thus proving guilt or innocence of suspects during criminal proceedings. Forensic analysis of minute blood evidence found on the floor of the forest can also help in identifying, locating and preventing injured/bleeding long range animals like Elephants and Tigers, to reach closer to Human settlements in injured state and avoid any conflict with Human thus mitigating the Human-wildlife negative interactions. It will also allow in preventing any retaliatory killings by Humans after personnel or monetary loss by wild animals.

In recent years, analytical chemistry has become one of the expanding areas in trace evidence analysis. The analysis of body fluids is a significant aspect in criminal investigations. However, to establish the species of origin of sample is another crucial facet in the analysis. Lately, plethora of research has been conducted for the purpose of species identification and differentiation from blood traces using various techniques with varying success. A number of approaches have been executed that entail the species differentiation from DNA based methods such as polymerase chain reaction (PCR)-based approach [7], mitochondrial DNA (mtDNA) and ribosomal RNA (rRNA) [8], HyBeacon probe technology [9], Cytochrome c oxidase I [10], high resolution melt (HRM) analysis [11], and next generation sequencing [12]. Besides DNA based methods, other approaches have also been explored for the purpose of species discrimination. Zailer et al. in 2017 mentioned 100 % accurate classification and discrimination of blood species (Human, cats and dogs) using 1 H NMR (nuclear magnetic resonance) spectroscopy in combination with principal component analysis [13]. Inoue et al. [14] used reverse-phase high performance liquid chromatography (HPLC) for species identification from blood and bloodstains. Espinoza and other team members have employed the electrospray-ionizing mass spectrometry to identify and differentiate blood samples of different species [15].

Virkler and Lednev group in 2009 [16] have used Raman spectroscopy supported with chemometric (PCA) to discriminate the spectra of blood from Human and animal (cat and dog) origin with confidence level of 99 %. Another research was conducted on the discrimination of Human and non-Human bloodstains by using portable Raman spectroscopy and chemometric tools that is PCA. Blood species including Cat, Cow, Chicken, Horse, Dog, Mouse, Rabbit, Pig, Sheep, Rat, and Human) were accurately discriminated [17]. Further McLaughlin and group [18] successfully discriminated the animal and Human blood traces via Raman spectroscopy. McLaughlin and co-workers also worked on the discrimination of various species from bone samples using Raman spectroscopy [19].

Further near-Infrared diffuse transmittance spectroscopy [20,21] and visible diffuse reflectance Spectroscopy [22] in amalgamation with PLS-DA model was successfully used for the discrimination of Human and animal blood.

Indeed, Raman spectroscopy provides reasonable results in this aspect, but it is bothersome in case of stains' particularly on fabrics therefore strong interfering signals are produced owing to the fabrics and dyes. In addition, Raman spectroscopy is very expensive than ATR FT-IR spectroscopy. Conversely, FTIR spectroscopy unequivocally has a high potential for the detection of biological fluids on various fabrics and other simulated substrates due to its intrinsic surface sensitivity and shorter depth of penetration (less than 10 μm) [23–25].

De Wael and group was the first who mainly focused on the problem associated with species identification from bloodstains using ATR FT-IR spectroscopy; however, results showed inability to discriminate among blood from Human and animal (Cat and Dog) origin without using any chemometric tool [26]. Further Zhang et al. [20] worked on the identification and discrimination of three species of blood such as Human, Macaque, and Mouse by using near-infrared diffuse transmitted spectra combined with advanced multivariate data analytical approach that is PLS-DA model. Mistek [27] successfully discriminated animal (Cat and Dog) and Human blood via ATR-FTIR spectroscopy supported with PLS-DA model. Of late, Wei et al. [28] used combination of ATR FT-IR and advance chemometric tools for the discrimination of Human and animal (Dog, Rabbit, Boar, Ram, and Bull) semen stains. Sharma et al. [29] successfully differentiated claws of Indian Leopard and Royal Bengal Tiger using ATR FT-IR spectroscopy.

Besides, species identification ATR FT-IR spectroscopy is an exceedingly flexible analytical approach with an array of other applications in forensic science such as for the identification and discrimination of biological fluids [30–33], inks [34], cosmetics [35–38], paints [39–41], explosive particles [42], hairs [43,44], fibers [45,46] etc. ATR FT-IR spectroscopy is advantageous in the field of forensic science in the wake of rapid and non-destructive results with limited sample preparation, and the possibilities of in-field analysis with portable ATR FT-IR instruments. It is a non-destructive, non-invasive, and rapid method exhibiting the fingerprint profile.

Considering the accomplishments of previously reported studies for the purposes of species discrimination from blood using ATR FT-IR spectroscopy, the present study was envisaged and expanded upon some critically endangered, and protected wild species of south Asia i.e., Asian Elephant (*Elephas maximus*), Indian Leopard (*Panthera pardus fusca*), and Royal Bengal Tiger (*Panthera tigris tigris*). In fact, this is first of its kind study to the best of author's knowledge wherein the data set mainly focuses on blood spectra from protected wild species, whereas earlier studies mainly focused on domestic species.

It is highly imperative to expand upon the previous methodology and explore the versatility and applicability of chemometric models by investigating blood samples from new animal species. The foremost rationale of the current research is to demonstrate the principle that blood samples could be spectroscopically discriminated according to the species of origin.

2. Materials and method

2.1. Sample selection

2.1.1. Animal blood

In the present study, animal blood specimens were collected from the repository of wildlife institute of India, Dehradun. The blood samples were acquired from four species, namely Asian Elephant, Indian Leopard, Royal Bengal Tiger, and Domestic pig. Details of collected samples are enumerated in Table 1.

2.1.2. Human blood

The Human blood samples ($n = 14$) collected from superficial vein was employed to test the validation method. The human blood was collected from fourteen consented volunteer donors by a well-trained phlebotomist. An aliquot of 2 mL blood was withdrawn from each donor in dipotassium EDTA anticoagulant at a concentration of approximately 1.8 mg K2 EDTA per 2 mL of blood and were kept at 2–4 °C.

The human blood samples were collected after the due approval of institutional human ethical committee (IEC/03–2017/08) and prior to sample collection written informed consent was obtained from each volunteer donor.

Table 1
Details of collected blood samples.

Common name	Scientific name	Number of blood samples ($n = $)
Asian Elephant	*Elephas maximus*	5
Indian Leopard	*Panthera pardus fusca*	6
Royal Bengal Tiger	*Panthera tigris tigris*	13
Domestic pig	*Sus scrofa domesticus*	8

2.2. Sample preparation

For the analysis, the ZnSe ATR crystal face was properly cleaned with acetone wipes. The background scan was executed without placing any sample on the surface of ATR crystal for the spectral acquisition. The blood samples were prepared by placing an aliquot of 10–20 µl of blood of each selected species on clean and sterile glass slide and each sample was allowed to dry completely for further analysis. Then, using sterile spatula, dried sample was scraped out and homogeneously deposited on the surface of ATR crystal and directly analyzed for spectral acquisition. For each selected animal blood species, three replicate spectra were acquired from separate aliquots to check the reproducibility.

2.3. Instrumentation

The eco- ATR FT-IR spectrometer (Bruker Alpha) enclosed with Smart Orbit, ZnSe crystal face with OPUS (v 8.0) software was utilized for the scanning of blood samples in the MIR spectral range that is 600–4000 cm^{-1}. Samples were scanned with accumulations of 24 scans at 4 cm^{-1} resolution. The cleaning of ATR face was carried out after each scanning by using pre-wetted ATR cleaning tissues containing de-ionized water and isopropyl alcohol.

2.4. Chemometrics

PCA is an unsupervised exploratory or dimension reduction multivariate data analytical tool which reduces large number of the dataset into simple easier form and helps to recognize the patterns and relationships in datasets. PLS-DA is a further extension of the principal component analysis that accounts to maximize the class separations. In this case, the selected classes for the species identification are Royal Bengal Tiger (class 1), Indian Leopard (class 2), and Asian Elephant (class 3).

2.5. Data pre-processing

Multivariate data analysis was executed using Unscrambler X, version 10.5.1, 64 bit software (CAMO AS, Norway). To carry out the chemometric tools, firstly data were saved as opus file and imported in unscrambler X software. Then PLS-DA was applied on the collected spectra in the whole MIR range. Prior conducting multivariate data analysis, various pre-processing methods were applied to circumvent any redundant effect of noise and to normalize the obtained differences in the spectral data owing to the quantity of analyzed samples.

To perform the PCA, pre-processing methods chosen were: baseline offset and linear baseline correction, smoothing with Savitzky–Golay algorithm with 9 smoothing point and 2 polynomial orders in a symmetric kernel, and normalization by range. To construct the PLS-DA model, the spectral data was pre-processed by using the baseline correction (baseline offset and linear baseline correction), deresolve transform and OSC (orthogonal signal correction). The de-resolve transform function is a row-oriented transformation and cannot be used to non-numeric data [47]. To construct the PLS-DA and PCA model, non- linear iterative partial least squares (NIPALS) with random cross validation method was used. This algorithm handles missing values and tends to be faster than kernel-based algorithms.

This function can be utilized to transform the noticeable resolution of an instrument, changing a spectrum of higher resolution to lower resolution, which in turn could be used to reduce the noise. OSC is another pre-processing method which eliminates disparity from the X-data (not related to a few responses). The hypothesis is that the regression model which trained on the transformed spectral data will be further robust, parsimonious, and easier to interpret [48].

2.6. Model validation

Method validation for ATR-FTIR analysis was executed by collecting additional 14 Human blood samples and 8 samples of domestic pig blood. These additional samples were included as an external group in the constructed model to confirm the performance of the model. The experimental conditions for these external samples were the same as training dataset samples.

The parameters included for the validation study were false-positive rate, false-negative rate, sensitivity, specificity, precision, and accuracy. These parameters can be calculated using the following formulas.

Sensitivity = True positives/ (True positives + False negatives) × 100.

Accuracy = True positive+ True negative/(True positive+ True negative+ False positive+ False negative)× 100.

False positive rate= False positive/ (True positive + False negative) × 100.

False negative rate = False negative/ (True negative+ false positive) × 100.

Specificity = True negative/ (True Negatives +False Positives) × 100.

Precision = True positive (True positive+ False positive) × 100.

3. Results and discussions

3.1. Characterization of blood spectra

Fig. 1. displays the ATR FT-IR spectrum of neat blood deposited on a clean glass slide and dried in situ for 24 h and scraped out for analysis. Table 2 describes the identified peaks of blood with their corresponding wavenumbers (cm^{-1}). The results obtained for the characterization of blood peaks in the current work correlated with various reported literature of body fluid analysis using ATR FT-IR spectroscopy [23,33,49–56]. In the present work, the position and frequencies of all the noteworthy peaks of blood are very similar with the peaks of blood reported in the published literature.

Typical neat blood spectrum (% Absorbance vs. Wavenumber) consists of some broader and narrower peaks. The two peaks positioned at approximately 1633, and 1517 cm^{-1} were categorized as intense, strong, and dominant peaks corresponds to amide I and amide II, respectively. Rest of the peaks was weak/small due to their low peak intensity.

Two weak peaks at approximately 1447 and 1390 cm^{-1} are due to the methyl bending of amino acids, proteins, lipids with asymmetric CH_3 bending and fibrinogen, amino acid side groups with symmetric CH_3 bending, respectively. Amide III was the weakest peak in amides and showed distinct "M'' shaped peak, positioned at 1307 and 1233 cm^{-1} as shown in Fig. 1. The dominant vibrational modes responsible for the amide III peak are attributed due to C-N stretches in combination with in-plane N-H bending vibrations and on the other hand weaker contributions for amide III peaks arise due to in-plane C═O bending and C-C stretching vibrations. The lower apex region of the spectrum that is 1250–925 cm^{-1} attributed to the carbohydrates (sugar moieties) with vibrational groups of C-O symmetric stretching. Other peaks positioned at approximately 1071 cm^{-1}corresponds to haptoglobin, fibrinogen, IgA, IgG, and IgM with C═O stretching. Table 2 showcases the characteristic band assignments of blood.

3.2. Visual discrimination between Asian elephant, Indian Leopard, and Royal Bengal Tiger

Comparative ATR-FTIR spectra of Asian Elephant, Indian Leopard, and Royal Bengal Tiger showed similar bands at similar wavenumbers as illustrated in Fig. 2. It is evident from the given figure that to the eye no visual spectral differences were observed between the Indian Leopard, Royal Bengal Tiger, and. However, differentiation between spectra was impossible through visual inspection, therefore, advanced chemometric

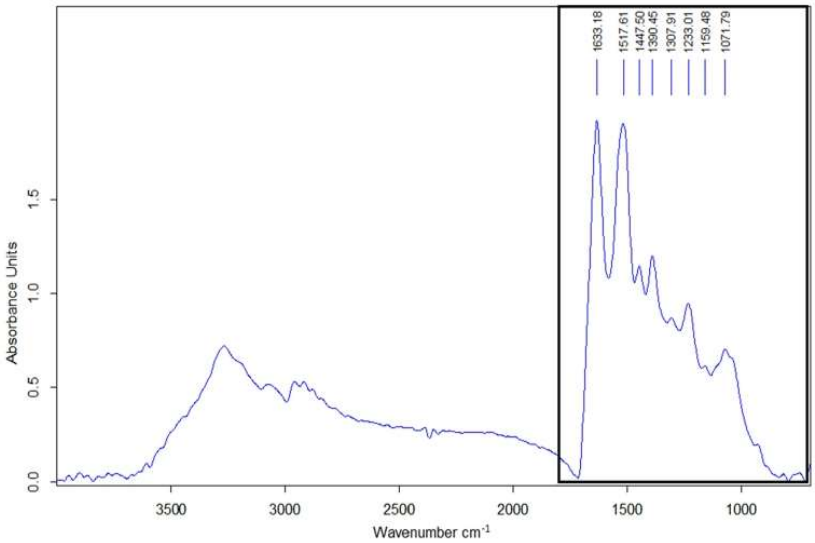

Fig. 1. Representative spectra of blood.

Table 2
ATR FT-IR peak components for the identification of blood.

Wavenumber (cm^{-1})	Component identification	Vibrational mode	Peak intensity
3277	Amino acid (Amide A)	H bonded O-H stretching, N-H symmetric Stretching (Water and hydroxyl group)	Medium peak
2948	Methyl stretches of lipids in plasma	C-H stretching	Weak
1643	Amide I (α-helix) (HSA and Hb is the major contributor)	C═O symmetric stretching	Strong and most intense peak
1527	Amide II(HSA and Hb is the major contributor)	N-H in plane bending vibration strongly coupled to C-N stretching vibration of protein	Strong and most intense peak
1445	Methyl bending of amino acids, proteins, and lipids	Asymmetric C-H scissoring of -CH_3 bending	Weak
1389	Fibrinogen and amino acid side groups	Symmetric CH_3 bending	Weak
1298 and 1240 (1229–1301)	Amide III (plasma proteins, transferring, and α1 – acid glycoprotein) { HSA and Hb is the major contributor}	C-N stretching	Weak (M shap peak in absorbance mode)
1162	Carbohydrates (glucose)	C-O symmetric stretching	Weak
1093	Fibrinogen, haptoglobin, IgA, IgG, and IgM	C═O stretching	Weak

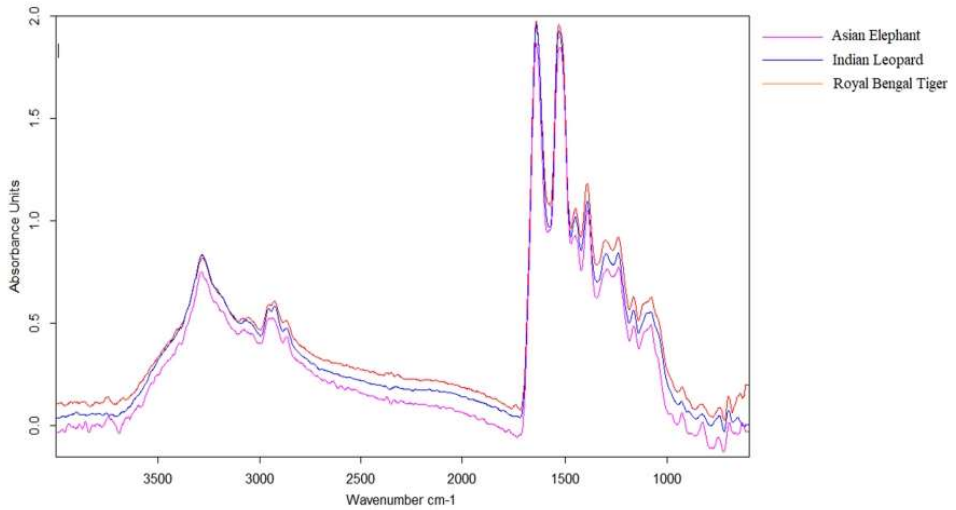

Fig. 2. Comparative ATR-FTIR spectra of Asian Elephant, Indian Leopard, and Royal Bengal Tiger.

tools are needed for further discrimination.

3.3. Multivariate discrimination between the Asian Elephant, Indian Leopard, and Royal Bengal Tiger

3.3.1. Discrimination using PCA

The principal component score plot of 3 PCS (principal components); PC1, PC2, and PC3 are exemplified in Fig. 3. PC1 contributed 82 %, and PC2 accounted 11 %, and PC3 added 3 % of the total variance in the given dataset. The collective percentage (%) of total variance of PC1, PC2, and PC3 was 96 %. It was evident from the score plot as given in Fig. 3 that PCA could not discriminative enough for blood spectra collected from different animal species. The spectra were dispersed or scattered on the plot and showed overlapping between spectra of different species; and assembled in a larger cluster; however, not clustered appropriately in their respective groups. Since the differentiation was not subtle, the scores for twenty principal components extracted from animal blood spectral datasets were further analyzed using PLS-DA predictive tools to characterize them in a more conclusive manner.

3.3.2. Discrimination using PLS-DA model

Using PCA since the discrimination was not very clear therefore to obtain better results further PLS-DA was applied. Three-dimensional (3-D) PLS-DA model was constructed using training dataset containing 5 spectra of Elephant, 6 spectra of Leopard, and 13 spectra of Royal Bengal Tiger blood with three specified latent factors/variables. Factor 1, factor 2, and factor 3 are useful for the study, as these three variables summarize more variation in the dataset.

The prediction results of PLS-DA model are given in Fig. 4. The Asian Elephant class was assigned as group 1; Indian Leopard class was assigned as group 2; and Royal Bengal Tiger class was assigned as group 3. The PLS-DA plot illustrated complete discrimination or separation among ATR FT-IR blood spectra acquired from different species and no misclassification was observed in any case. The PLS-DA model demonstrated accuracy of 100 % for the discrimination between different classes of species by using the spectra of blood. The obtained calibration vs. validation values are enumerated in Table 3. Fig. 5 shows the loading plot of factor 1. The loading plot is showing the profile similar to the original dataset and consequently it conveys most significant part of information.

In loading plot, the region of amide I (1643 cm^{-1}), amide II (1527 cm^{-1}), amide III (1229–1301 cm^{-1}), and fibrinogen, haptoglobin, IgA, IgG, and IgM (1093 cm^{-1}) are the most important variables for the discrimination of all selected species from blood.

3.3.3. External validation test

To carry out the external validation test, 14 samples of Human blood and 8 samples of Domestic Pig were collected and analysed under the similar set of experimental conditions. The spectral data of Human blood and Domestic Pig samples were incorporated in the PLS-DA model for the purpose of predictions. The obtained results of PLS-DA model are illustrated in Fig. 6. The obtained R-Square value for PLS-DA model for the discrimination of blood collected from Human, Domestic Pig, Asian Elephant, Indian Leopard, and Royal Bengal Tiger is 0.972, which is highly significant. The model performed 100% accuracy for the unknown class predictions as Human blood designated with diamond shape and Domestic Pig designated with inverted triangle. The calibration and validation dataset values are given in Table 4.

From the PLS-DA score plot, it is apparent that complete discrimination was accomplished between Human blood and other selected species. The obtained results make obvious that, it might be very difficult for Human blood to be falsely identified as animal blood. None of the spectra of blood associated to Royal Bengal Tiger, Indian Leopard, Asian Elephant, and Domestic pig were falsely classified as Human blood samples. The obtained results from the validation study showed no false positive and negative assignments for these species. It is amenable that this approach has superb ability to discriminate the ATR FT-IR spectra of animal and Human blood.

4. Conclusions

Discriminating or identifying individual species from an unknown sample is highly significant for a variety of forensic applications. In this paper, ATR FT-IR spectroscopy and chemometric tools are utilized for the discrimination of blood of critically endangered and protected wild species from south Asia. ATR FT-IR spectroscopy in tandem with

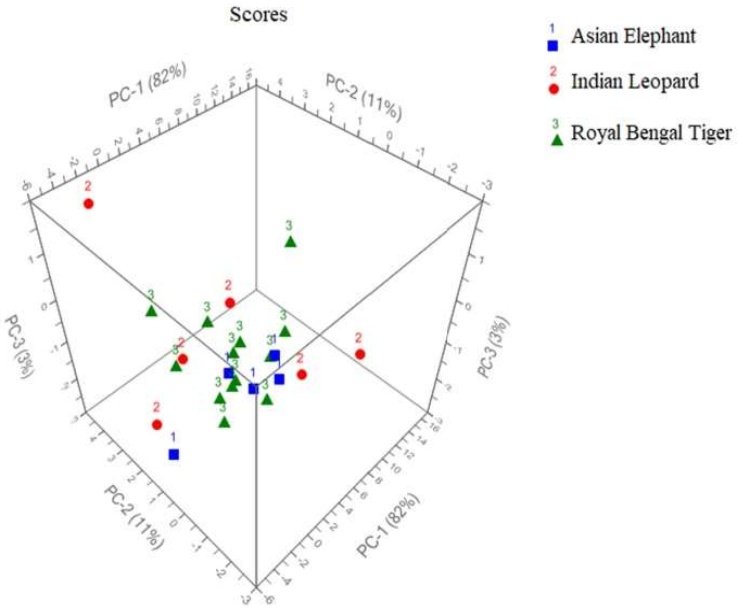

Fig. 3. PCA plot to discriminate the blood spectra of Asian Elephant, Indian Leopard, and Royal Bengal Tiger.

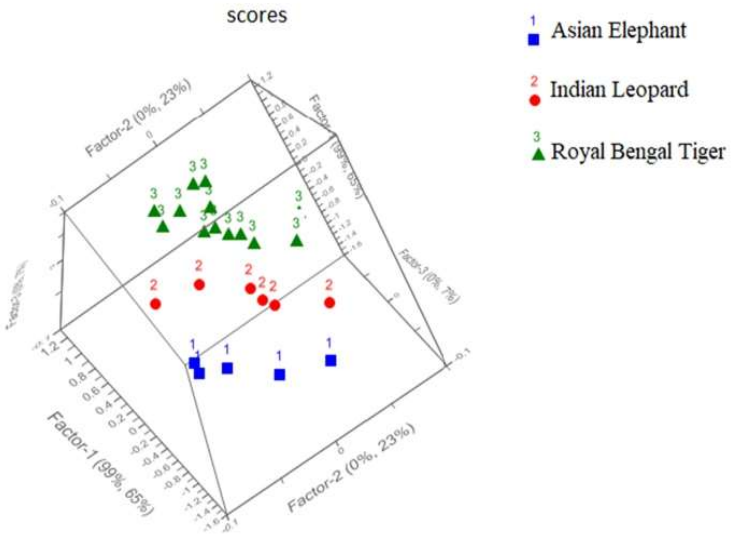

Fig. 4. PLS-DA plot to discriminate the blood spectra of Asian Elephant, Indian Leopard, and Royal Bengal Tiger.

Table 3
Predictive vs. reference values for the differentiation of Asian Elephant, Indian Leopard, and Royal Bengal Tiger from dry blood traces.

	Slope	Offset	RMSE	R-Square
Validation	0.9	0.02	0.08	0.9
Calibration	0.6	0.8	0.5	0.6

advance statistical tools shows excellent potential for the rapid and non-destructive discrimination of blood collected from Asian Elephant, Indian Leopard, and Royal Bengal Tiger. By using this method, obtained spectra from selected species were substantially discriminated with 100 % accuracy without any overlapping. Human and Domestic pig blood samples were additionally used to construct the validation model and resulted no misclassification of Human blood from animal (Domestic Pig, Asian Elephant, Indian Leopard, and Royal Bengal Tiger) blood as well. Due to the distinctive performance of the current method with rapid and non-destructive nature, this technique is highly promising for the application in practical case scenarios. However, before applying this technique to practical forensic applications, future work is needed to address the related impediments associated with the scene of crime. Finally, future work requires to add-on more species and to construct a comprehensive classification and discriminant model. Nevertheless, this proof-of-concept study will open up new horizon for the species discrimination from blood traces recovered in wildlife investigations as well in a quick, robust, eco-friendly, and non-destructive manner.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the Institutional Ethical Committee (IEC), Punjabi university, Patiala 147002 with letter number IEC/03–2017/08. All the participants were informed about the study and their consent were duly recorded.

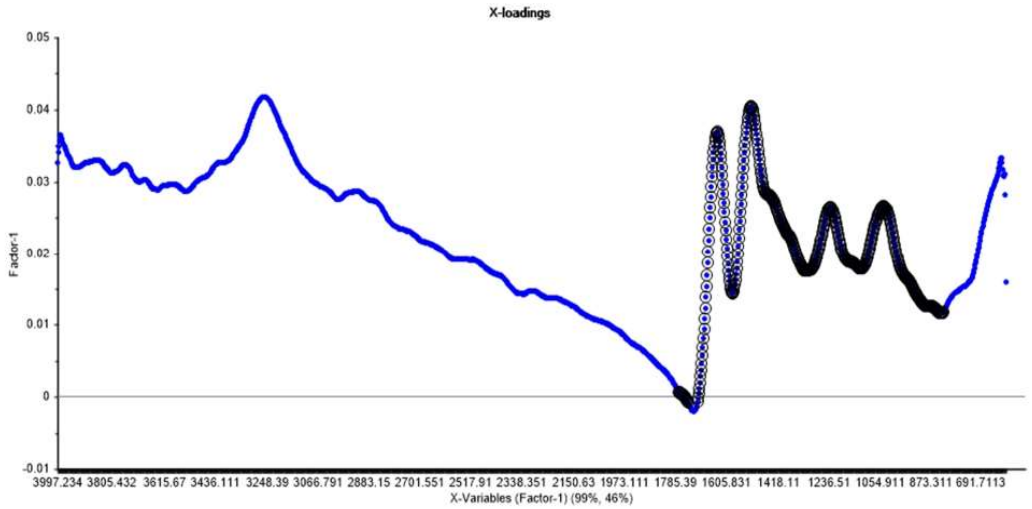

Fig. 5. Factor loading plot.

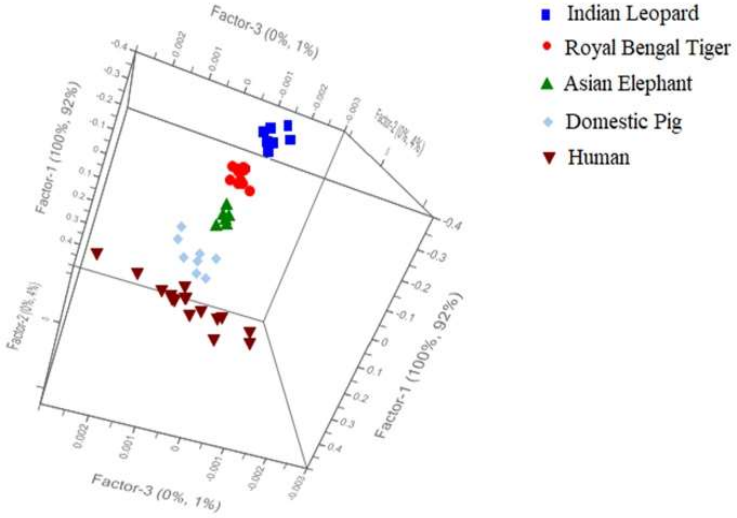

Fig. 6. Discrimination of Human blood from animal (Indian Leopard, Royal Bengal Tiger, Asian Elephant, and Domestic pig) blood.

Table 4
Predictive vs. reference values for the differentiation of dry blood traces collected from Human, Domestic Pig, Asian Elephant, Indian Leopard, and Royal Bengal Tiger.

	Slope	Offset	RMSE	R-Square
Validation	0.9	0.1	0.2	0.9
Calibration	0.9	0.1	0.2	0.9

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Further reading

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ANNEXURE-I

Permits and Ethical clearance

Due permission was obtained from Director/Dean/RDC, Wildlife Institute of India (WII) for generating data in this study using database of offense cases and biological sample from repository of Wildlife Forensic & Conservation Genetics Cell (WFCGC) of WII for studying trends in wildlife offense and developing morphological and FT-IR based protocols.

All procedures performed for developing FT-IR based protocol involving human participants were in accordance with the Institutional Ethical Committee (IEC), Punjabi university, Patiala 147002 with letter number IEC/03–2017/08. All the participants were informed about the study and their consent were duly recorded.

ANNEXURE –II

Conference Certificates

Proud to host KHELO INDIA

Azadi Ka Amrit Mahotsav

JGI JAIN

JAIN (Deemed-to-be University)
School of Sciences
Department of Forensic Science

Certificate of Achievement

This is to certify that

Chandra Prakash Sharma

has participated for Paper Presentation and presented his / her Paper Titled –

Comparative morphological characterization of mandible fragments of three sympatric carnivores, the Tiger, Leopard, and Hyena, seized in illegal wildlife offenses: Implications in wildlife forensics

at the 1st International Conference on Forensic Science, organised by Department of Forensic Science, School of Sciences, JAIN (Deemed-To-Be University), Bengaluru, held on 10th to 12th February, 2022.

Dr Vichar Mishra
Coordinator
DC-Department of Forensic Science

Dr Reena Susan Philip
Head of the Department
Department of Forensic Science

Dr Asha Rajiv
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JAIN (Deemed to be University)

Dr Arun Sharma
Former Director, Forensic Services,
Himachal Pradesh

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Government of India, MHRD NIRF India Ranking- 2020, College (Rank Band- 101-150)

INTERNATIONAL E-CONFERENCE ON FORENSIC BIOLOGY

Certificate

ID- IeCFB20211063

This is to certify that,

Mr. Chandra Prakash Sharma, Wildlife Institute of India, Dehradun has participated in two days **"International e-Conference on Forensic Biology"** organised by Department of Forensic Biology and Internal Quality Assurance Cell (IQAC), Government Institute of Forensic Science, Nagpur held on **28th and 29th January, 2021**.

Dr. Archana Mahakalkar	Dr. Hariprasad Paikrao	Dr. Rajesh Patil	Dr. Virendra Shende	Dr. Sanjay Thakre
Organising Co-ordinator	Organising Co-ordinator	IQAC Co-ordinator	Organising Secretary	Convener/Director