ECOLOGY AND POPULATION GENETICS OF SELECTED CARNIVORES

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Certificate

This is to certify that the dissertation report entitled **"Ecology and Population Genetics of Selected Carnivores"** submitted by **Ms. Udisha Pal** (Roll no. 602304013) in partial fulfilment of the requirement for the award of the degree of **Masters of Technology in Biotechnology**, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, is an authentic record of students work during the period of one year from July 2024 to July 2025. This report has not been submitted for the reward of any other degree or certificate or any other University or Institute.

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ABSTRACT

Carnivores play a vital role in maintaining ecosystem balance by regulating prey populations, yet many face threats from habitat fragmentation, prey depletion, and genetic isolation. This study aimed to investigate the ecology and population genetics of selected carnivores with a focus on *Panthera tigris* in Corbett Tiger Reserve (CTR), Uttarakhand, using non-invasive scat analysis.

A total of 68 field-collected samples and 35 samples from CTR were analyzed, out of which 36 and 27, respectively, yielded DNA suitable for sequencing. Species identification confirmed the presence of Jungle Cat (n=12), Wild Cat (n=1), Rusty-spotted Cat (n=2), Golden Jackal (n=3), and Mongoose (n=4), while all usable samples from CTR were identified as Tiger (*Panthera tigris*), confirming the reliability of DNA-based scat identification.

Population genetic analysis of tiger samples using microsatellite markers indicated low genetic diversity (Ho = 0.248; He = 0.656) with moderate inbreeding. Bayesian clustering (K=3) and Discriminant Analysis of Principal Components revealed mixed populations and three genetic clusters. Dietary analysis of 26 tiger scats revealed a preference for medium-sized ungulates, with Chital (42.31%) and Sambar (26.92%) as primary prey, followed by Nilgai (15.38%), Wild Pig (11.54%), and Barking Deer (7.69%).

The findings demonstrate the effectiveness of non-invasive scat analysis for species monitoring, while highlighting concerns of low genetic diversity in tigers of CTR. Dependence on wild ungulates underscores the importance of prey base management. This study provides valuable baseline data for conservation and management strategies focusing on prey restoration, habitat connectivity, and genetic monitoring to ensure long-term viability of carnivore populations in Corbett Tiger Reserve.

SUMMARY

This study utilized non-invasive scat analysis and molecular techniques to assess carnivore diversity, genetic health and feeding ecology across multiple sites, including the Corbett Tiger Reserve. Field samples revealed the presence of several carnivores, with the Jungle cat being most frequently detected, indicating high adaptability.

In Corbett, tiger presence was confirmed through DNA sequencing and microsatellite analysis showed moderate genetic diversity and population structure. Dietary analysis further revealed a strong preference for prey like Chital and Sambar, highlighting the importance of maintaining a healthy prey base. Overall, the study showcases the effectiveness of combining genetic and ecological approaches for informed conservation and management strategies and prey base planning.

CHAPTER 1: INTRODUCTION

Carnivora is very alluring order of mammals and is the fifth-largest among twenty-nine extant orders. It exists on almost every large landmass, including continent Antarctica, where its marine members (like seals) are present and they occupy every major habitat on Earth, from ice-cold Arctic regions to hot-dry regions of Sahara Dessert. This order comprises a portion of most incredible, marvellous and admirable species including Least Weasel, which is the world's smallest carnivore species, its size is so small that it could be squeeze though a small size bangle and it weighs 10,000 times less than a Polar Bear, which is the largest species of terrestrial region but unfortunately, some of them comes under Endangered in species in Red Data Book.

Fig. 1. Tiger (*Panthera tigris*), AG (7 Feb 2019), Unsplash, URL: https://unsplash.com/photos/brown-and-black-tiger-on-focus-photography-YuQgNYku1M8

Carnivora order is divided into two primary suborders, which diverged approximately around 45 to 50 million years ago, though the actual split may have occurred even earlier due to limited availability of fossil evidences. There is a suborder *Feliformia*, it includes 'cat-like' families such as *Felidae*, *Hyanidae*, *Eupleridae*, *Viverridae* and *Nandiniidae*. On other hand, the suborder *Caniformia*, it includes 'dog-like' families

such as *Canidae*, *Ursidae*, *Procyonidae*, *Ailuridae*, *Mephitidae* and *Mustelidae*, along with three families of *Pinnipeds* (Hunter, 2018).

Mammalian carnivores pose significant challenges for researchers and conservationists due to their ecological and behavioural characteristics. Positioned at the top of the food chain, these species typically exist at low population densities. They are often nocturnal, elusive, and occasionally pose safety risks, making them difficult to observe and study. As a result, many carnivore species remain insufficiently understood. This lack of reliable data hinders conservation and recovery efforts, even for high-profile species such as the tiger (*Panthera tigris*), lion (*Panthera leo*) and wolf (*Canis lupus*). For numerous species, information remains limited to anatomical details and general distribution maps, with critical gaps in our understanding of their diet, social behaviour, ecological roles, population dynamics and genetics. Wild carnivores are charismatic species that attract human interest for various reasons. They have historically competed and conflicted with humans over resources such as food and space across both evolutionary and historical timelines (Inskip & Zimmermann, 2008). Human perceptions of carnivores are influenced by a range of factors, including fear, admiration and traditional beliefs. Additionally, these species are often valued for their utilitarian benefits, including meat, pelts and their potential to support tourism (Karanth and Chellum, 2009).

On a more optimistic note, despite the growing threats they face, many carnivore species exhibit demographic characteristics—such as early reproductive age, large litter sizes and high reproductive rates—that enhance their resilience. For instance, the red fox (*Vulpes vulpes*) has one of the widest geographic distributions among terrestrial mammals. In some cases, certain carnivore species have even become invasive in non-native regions, where they pose ecological challenges by adversely affecting native wildlife (Ibarra et al., 2008). Notably, certain rare and threatened carnivore species are showing signs of recovery in response to recent land-use changes and shifts in cultural attitudes. For example, in parts of North America, the puma (*Puma concolor*) is benefiting from the conversion of farmland back to woodland habitats. Similarly, in Europe, species such as the Eurasian lynx (*Lynx*

lynx), wolf, and brown bear (*Ursus arctos*) have experienced range expansions, supported by growing public opposition to hunting and increased concern for animal welfare.

Carnivores are prime concerns for strategies related to conservation of biodiversity, hence they acquire top positions in the food chain of ecosystems, so their conservation is very important to secure other biodiversity elements also (Gittleman et al. 2001; Ray 2005). For example, there are large carnivores like lions (*Panthera leo*) and brown bears (*Ursus arctos*), they are basically the important predators because they are regulation the population of preys existing there, through ecological cascades, over all they are having intense effect on the construction of the entire community (Nowak 2005; Ripple et al. 2014). This is the reason why carnivores serve as a very important tool for organisation and structurisation of the overall health of the ecosystem (Soulé and Terborgh 1999). This indicates that the protection of the area where carnivores are existing is important for the stability of their populations, and this can lead to the conservation of other biodiversity elements occupying that area, this is kind of so-called “Umbrella effect” (Roberge and Angelstam 2004; Sergio et al. 2008). Other than this, there effectiveness in conservation and functioning of biodiversity and ecosystem respectively, carnivores provide great service to human well-being also by performing scavenging and predatory roles. For example, they are helpful in the increasing the yielding of agriculture by consuming the species which are destroying the crops, through reduction in competition they are also preventing cultural values, other than these they are helpful in the regulation of zoonotic diseases (infections that can be transmitted between animals and humans or vice versa), disposal of waste etc (O'Bryan et al. 2018; Willcox 2020; Sepúlveda and Martín, 2022).

The diversity of carnivores includes more than 230 species, and these exists across all the continents excluding Antarctic region, from dry deserts to rain forests, from Arctic to tropics, and urban areas which are densely populated, almost in all habitats, even from sea level to more than 5000m. Even though, plenty of them also adapted

to habitat which are made by humans, this is contrary to the thought as usually carnivores are correlated with untamed lands which usually exists in remote location from human lives. Many North American cities are colonised by numerous Raccoons (*Procyon lotor)*, sometimes they are lounging in the spaces under the roof but most of the time they found denning inside hollow trees, and some other species like striped skunks *(Mephitis mephitis)* also exist which usually found under houses. In European cities, lives some Stone Martens *(Martes foina)* in dense urban areas, they usually live under the roofs of houses and hoods of cars. As a matter of fact, there are many carnivores which are very strict to their environment type like Black-Footed Ferrets *(Mustela nigripes)* and Giant Pandas *(Ailuropoda melanoleuca),* there are also many more carnivores which are remarkably adaptable to their habitat which are made by humans depending on how they are treated. There are brown and black Bears (*Ursus arctos* and *Ursus americanus* respectively) along with wolves *(Canis lupus)* which use to scavenge on dumps, black Bear makes dens under the houses of people also in winter season. Sometimes, the Polar Bear *(Ursus maritimus)* and Tigers *(Panthera tigris)* use to hunt people in villages and towns. Habitat is not the only thing to affect the diversity of carnivores. These species exhibit remarkable range in body size—spanning over four orders of magnitude—from the small female least weasel (*Mustela rixosa*, formerly *Mustela nivalis*), weighing under 50 grams, to the massive polar bear, which can exceed 600 kilograms. Their population densities also vary significantly, from urban raccoons with densities surpassing 100 individuals per square kilometre, to wide-ranging species such as wolverines (*Gulo gulo*) and large bear species (polar, brown, and black bears), as well as wolves, which occupy home ranges from hundreds to thousands of square kilometres.

While some species, such as the black-footed ferret, are recovering from critically low population numbers, others remain highly endangered. In contrast, some carnivores, like the small Asian mongoose (*Herpestes javanicus*), have become invasive, particularly on the West Indies and Hawaiian Islands.

Although the term “carnivore” traditionally refers to meat-eaters, members of the order Carnivora display a wide range of dietary adaptations. These range from strict carnivory (seen in many felids and mustelids), to scavenging and omnivory (common among canids, ursids, and procyonids), to insectivory (observed in certain mongooses, canids and the aardwolf *Proteles cristatus*). Notably, the giant panda is an obligate herbivore.

Carnivores also exhibit diverse hunting strategies, including ambush, stalking, pursuit, and coordinated group hunting. Many species are highly social and demonstrate complex behavioral patterns. In fact, some—like the grey wolf (*Canis lupus*)—have coevolved with humans, eventually giving rise to domesticated dogs (*Canis familiaris*) (Boitani and Powell, 2012).

Global distributions of carnivores frequently overlap with landscapes extensively utilized by humans (Ripple et al., 2014). These multi-use, heterogeneous areas can serve as important supplementary habitats that support populations of various carnivore species, presenting significant conservation opportunities (Carter NH, Linnell JD, 2016). However, prevailing conservation strategies, particularly in developing countries, remain focused on the establishment and management of protected areas (Joppa and Pfaff, 2009; Jenkins and Joppa, 2009). This approach is limited in scale, as such reserves typically cover only 4–11% of the total land area in many nations (Venter et al. 2014).

Conserving large carnivores in human-use landscapes is further complicated by socio-cultural, financial, and political challenges, making it difficult to design policies that simultaneously promote carnivore conservation and safeguard human lives, livelihoods and property.

The spatial convergence of carnivore ranges with human-dominated areas heightens the potential for human–carnivore interactions, often leading to conflict (Chapron and Lo´pez-Bao, 2014). These situations are especially prevalent in developing regions where communities are heavily dependent on land-based livelihoods such as agriculture and livestock rearing (Woodroffe et al., 2005). Forests are also frequently used by local populations for firewood collection and extraction of non-timber forest products (Barve et al. 2005 and Vaidyanathan et al. 2009), while simultaneously serving as grazing grounds for domestic animals (Davidar et al. 2010).

On the other hand, carnivores are increasingly seen venturing into agricultural fields, rural settlements, and urban environments (Odden et al. 2014), resulting in contested 'shared spaces' between people and predators. Such encounters may lead to livestock loss, personal safety concerns, and economic impacts related to avoidance of carnivore-occupied areas. In retaliation, carnivores often face injury, displacement, or lethal responses. Notably, public attitudes toward carnivores may be shaped more by perceived risks than actual instances of loss (Agarwala et al. 2010 and Athreya et al. 2015).

The Tiger (*Panthera tigris*) is among the most iconic and easily recognized of all wild cats, distinguished by its unique striped coat. Though often regarded as the largest living feline species, this claim is subject to debate. Across its wide geographical range—from the temperate oak forests of the north to the tropical rainforests near the Equator—the tiger displays considerable variation in size, coloration, and markings, shaped by the diversity of habitats it occupies. Understanding this ecological and morphological variation is crucial for effective conservation, yet its full significance remains unclear. Exploring the tiger's two-million-year fossil history offers valuable insights into the evolutionary processes that have shaped the species' present-day diversity. However, conserving tigers poses significant challenges, especially in densely populated regions like Asia, where allocating large, undisturbed areas for wildlife is difficult. Many protected areas are too small to support viable tiger populations over the long term. Therefore, India's conservation

strategy emphasizes preserving these smaller habitat patches as part of larger, interconnected landscapes, managing them within a meta-population framework to ensure the long-term survival of the species. Throughout history, tigers have played a prominent role in human societies, particularly in areas where they continue to share habitats with people. Their impressive size and reliance on large prey have often led to conflicts, including livestock predation and occasional attacks on humans. Despite this, tigers have been admired for their strength, agility and hunting skills. Unfortunately, the demand for tiger skins, as well as body parts used in traditional medicine, persists even today, threatening wild populations despite existing legal protections. Furthermore, the tiger's image is widely exploited in global markets, symbolizing power and luxury in commercial branding. As human activities continue to encroach upon the remaining fragmented tiger habitats, it becomes increasingly important to revisit the fundamental question: what defines a tiger? Efforts to classify tiger populations—whether through identifying subspecies or distinct evolutionary lineages—have so far produced inconsistent results. These uncertainties complicate conservation planning, both in natural habitats and in captivity. A deeper understanding of tiger diversity is essential for designing targeted conservation measures, ensuring the long-term survival of this iconic species and the ecosystems it represents (Hemmer et al. 1974).

SPECIES IDENTIFICATION

In recent years, various DNA-based techniques have emerged for species identification, such as DNA hybridization, restriction enzyme analysis, random PCR amplification, species-specific primers, and DNA sequencing. Each method should be critically assessed for its ability to differentiate species accurately and its consistency across samples. A significant advancement in molecular biology has been the development of reliable tools to detect and analyse variations in DNA sequences swiftly. This report emphasizes the value of mitochondrial DNA (mtDNA) sequencing, in combination with bioinformatics, as a powerful approach for identifying animal species from tissue samples.

Mitochondria are present in nearly all eukaryotic cells, and their genome is a circular, double-stranded DNA molecule approximately 16 kb long, comprising about 1–2% of a mammal's total DNA. The mitochondrial genome encodes 13 proteins essential for oxidative phosphorylation, including seven subunits of Complex I (ND1-6, ND4L), one subunit of Complex III (cytochrome b), three subunits of Complex IV (COI–III), and two subunits of Complex V (ATPase 6 and 8). In addition, it encodes two ribosomal RNAs (12S and 16S rRNA) and 22 transfer RNAs necessary for mitochondrial protein synthesis. Mitochondria also contain their own systems for DNA replication, transcription, and translation.

Animal mtDNA is characterized by a high mutation rate and a compact structure with minimal non-coding regions. This rapid rate of evolution leads to noticeable genetic differences even between closely related species, making mtDNA an effective tool for species identification. Additionally, mtDNA is typically inherited maternally, due to mechanisms like the exclusion or degradation of sperm mtDNA after fertilization. This mode of inheritance simplifies data interpretation in species identification. Furthermore, mtDNA is often recoverable from degraded samples or small tissue quantities—such as hair—because it exists in multiple copies per cell, offering a practical advantage over nuclear DNA-based methods (Yang et al. 2014).

POPULATION GENETICS

The field of population genetics primarily focuses on developing and accessing hypotheses about genetic diversity within groups of organisms. It aims to understand how such variations arise and are maintained. Researchers seek to predict factors like the extent of genetic differences in a population, how these differences change over time and the traces of genetic patterns that biological processes may leave behind as they occur across generations or regions.

To create these predictions, scientists rely on foundational concepts known as first principles — basic assumptions and rules that explain how natural systems operate

at their core level. An example from physics would be the principle of gravity. In population genetics, these foundational ideas include the fundamental mechanisms of Mendelian inheritance and various biological processes such as mutations, mating behaviours, gene flow and natural selection — all of which contribute to changes in genetic makeup over time. By combining these principles, population geneticists strive to form a robust framework that explains genetic patterns in any organism. The ultimate objective is to create a reliable model that can be applied broadly across different species and genetic systems (Hamilton, 2021).

DIET ANALYSIS

Predatory species, particularly mammalian carnivores, are vital for ecosystem health and possess high conservation significance due to their influential ecological roles. Their population sizes are often directly influenced by the abundance and biomass of prey species (Hatton et al. 2015), making precise understanding of their dietary patterns essential for evaluating broader ecosystem dynamics. Moreover, in ecosystems where multiple carnivore species coexist, competition for common prey is thought to be a key factor driving interspecific conflicts (Donadio & Buskirk, 2006; Ritchie & Johnson, 2009). Thus, unravelling the underlying causes of such interactions requires accurate data on carnivore feeding ecology.

From a conservation standpoint, understanding carnivore diets is also important because conflicts with humans—such as livestock predation and competition with hunters—have significantly contributed to the global decline and even local extinction of many carnivore species (Ceballos & Ehrlich, 2002; Treves & Karanth, 2003). Therefore, detailed diet analysis helps estimate crucial ecological parameters like trophic niche width, dietary specialization, and prey preferences, all of which are valuable for conservation planning and ecological research.

Traditionally, carnivore diet has been assessed by examining undigested remains in fecal matter, or scats, a method widely adopted due to its non-invasive nature (Klare

et al. 2011). This approach typically assumes that scats can be accurately assigned to a species based on physical characteristics alone. However, since the 1990s, molecular techniques have revealed that such morphological identification can be unreliable (Hoss et al. 1992; Kohn et al. 1995), potentially introducing errors in ecological conclusions (Martínez-Gutiérrez et al. 2015; Morin et al. 2016; Weiskopf et al. 2016). The use of genetic tools has therefore become increasingly important in ensuring more accurate species-level identification of scats for dietary studies.

CHAPTER 2: REVIEW OF LITERATURE

This section presents a review of existing literature related to the carnivore species included in the present study. Although the primary focus is on species identification—particularly of tiger—the literature compiled here highlights relevant findings from earlier studies, including aspects like hair morphology, to support and contextualize the identification process.

2.2. Tiger the Great

निर्वनो वध्यते व्याघ्रो निर्व्याघ्रं छिद्यते वनम्।
तस्माद्व्याघ्रो वनं रक्षेद्वयं व्याघ्रं च पालयेत् ॥

-महाभारत – उद्योग पर्व : ५.२९.५७

If there is no forest, then the tiger gets killed; if there is no tiger, then the forest gets destroyed. Hence, the tiger protects the forest and the forest guards the tiger!

-Mahabharat (Kumbhaghonam Edition) – Udyoga Parva: 5.29.57

It is the leading species of Asian forests ecosystem. In last two decades it has lost almost 40% of its range. This decline is primarily driven by profit-motivated poaching, habitat degradation and hunting (Dinerstein et al. 2007). As Asia is densely populated, allocating sufficient land for tiger conservation becomes a significant challenge. The situation is further complicated by the fact that the protected areas are often too small to support a sustainable tiger population in the long term (Wikramanayake et al. 1998). Although, as there are high prey densities, some protected areas which support tigers in dense population and if such areas are part of an unbroken stretch of forest, they can contribute to maintain and support the survival of a healthy tiger population within the entire habitat. Hence, the strategy for tiger conservation in India emphasizes safeguarding small forest fragments within

broader connected ecosystems, treating them as components of a metapopulation framework (Qureshi et al. 2014).

Tigers spend a significant portion of their time searching for food, often covering large distances in pursuit of sufficient prey. Due to the challenges of monitoring their movements, precise data on the distances tigers travel during nightly hunts remains limited. Although they may occasionally hunt during daylight hours, tigers are predominantly active at night, with their activity patterns largely aligning with those of their main prey species (Sunquist, 1981; Karanth and Sunquist, 2000). While hunting, tigers do not roam aimlessly. Their movements are often purposeful, as if guided by a mental map of known hunting grounds and familiar pathways within their territory. They typically navigate between productive hunting areas by following established routes such as trails, forest tracks, and gullies, which allow them to move quietly and with less effort. In snowy regions like the Russian Far East, tigers avoid areas where snow exceeds 30 cm in depth, not only because prey are scarce there, but also because deep, unstable snow hampers silent movement and increases energy expenditure. To overcome these challenges, tigers prefer to travel along frozen riverbeds, ungulate trails, valley floors, or other paths where snow is less deep and movement is easier (Heptner and Suidiskii, 1992).

A tiger's hunting success depends on complete physical commitment during the final ambush. Once the moment comes, the tiger must launch an explosive, decisive charge, fully ignoring the risks posed by defensive kicks or antlers from prey. Any hesitation can result in failure or, worse, injury — a potentially fatal outcome, as an injured tiger may starve if it can no longer hunt effectively. Direct observations of tigers making kills are rare in the wild; much of what is known comes from incidents involving livestock predation. Studies from Chitwan National Park have provided valuable insights, revealing the adaptability of tiger hunting strategies. These observations show that tigers adjust their attack methods depending on the prey's escape tactics. The position and technique of the killing bite also evolve with the tiger's age, size, and hunting experience. This behavioural flexibility enables tigers to successfully hunt a wide variety of prey species, ranging in both type and size (Seidensticker and McDougal, 1993). The availability of prey plays a crucial role in determining the population density of carnivores like tigers and significantly affects

various aspects of their ecology. Factors such as the size of individual territories, daily energy requirements, the number of breeding females, and the presence of transient individuals are all influenced by prey abundance. Additionally, prey density directly

impacts reproductive success, including the survival rates of cubs and juvenile tigers (Karanth, 2003; Miquelle et al. 1999; Karanth and Stith, 1999).

However, it is not just the quantity of prey that matters, but also its size. Research consistently shows that tiger populations are more closely linked to the presence of large-bodied ungulates. A healthy population of sizable prey species is essential for sustaining viable tiger populations. Even if smaller prey is plentiful, they alone cannot meet the dietary and energetic adequate numbers often marks the difference between a tiger population merely persisting and one that is truly flourishing (Sunquist and Karanth, 1999).

Conservation status (2022)

Project Tiger, initiated on 1 April 1973, at the Jim Corbett National Park in Uttarakhand (then part of Uttar Pradesh). It was initiated by the Government of India under Prime Minister Indira Gandhi, with the aim of conserving the declining population of tigers (*Panthera tigris*) and preserving their natural habitats through the creation of protected areas known as tiger reserves. It was envisioned to leverage the ecological importance and public appeal of the tiger to mobilize support and resources for the conservation of representative ecosystems. Since its launch, the initiative has grown significantly—from an initial network of nine tiger reserves covering approximately 18,278 km² to 53 reserves spanning 75,796 km², which collectively represent around 2.3% of India's total land area.

Despite this progress, many tiger reserves and protected areas today remain isolated fragments within broader landscapes dominated by ecologically unsustainable land-use practices. In several cases, tiger populations are restricted to these small, protected pockets. While some habitat corridors do facilitate tiger movement between these areas, many of these connecting zones lie outside formal

protection, making them vulnerable to degradation from human exploitation and developmental pressures. As a result, these corridors often fail to support effective wildlife movement, posing challenges to long-term population viability.

Given the wide ecological range occupied by tigers across India, tiger-inhabited regions have been classified into five major landscapes based on biogeographic characteristics and habitat connectivity

1. Shivalik-Gangetic Plains,
2. Central India and Eastern Ghats,
3. Western Ghats,
4. North-Eastern Hills and Brahmaputra Floodplains, and
5. Sundarbans.

Each landscape is treated as a distinct ecological unit, as the environmental factors influencing tiger abundance vary across regions. These landscapes are not only geographically unique but also represent functionally cohesive biological systems—allowing for shared individuals, genetic exchange, and potential dispersal among tiger populations within the same landscape.

Considering the increasing emphasis on landscape-level conservation strategies, and the rarity of tiger movement between landscapes in contemporary times, this classification is ecologically relevant. It supports more context-specific management decisions and effective conservation planning at the landscape scale.

Tiger conservation efforts in India can be broadly categorized into two distinct phases. The first phase, initiated during the 1970s, was marked by the enactment of the Wildlife Protection Act and the creation of protected areas, which played a pivotal role in safeguarding both tiger populations and the broader tropical forest ecosystems they inhabit.

The second phase began around 2005–2006, characterized by a shift toward a landscape-level conservation approach and the implementation of rigorous monitoring protocols. This strategic transition led to a notable rise in the national tiger population—from 1,411 individuals in 2006 to 2,967 by 2018.

Over four successive cycles of tiger monitoring, India has witnessed transformative changes in conservation policy and management. Key initiatives have included:

- Demarcation and notification of critical core and buffer zones within tiger reserves
- Establishment of new tiger reserves
- Recognition of tiger landscapes and ecological corridors
- Integration of conservation with development planning
- Formulation of reintroduction and supplementation strategies for tigers and their prey
- Targeted conservation investments aimed at protecting genetically distinct and vulnerable populations

These efforts have also fostered scientific awareness among forest personnel and promoted the use of technology for transparent data collection and analysis. Collectively, these outcomes underscore the importance of science-based conservation in informing policy and decision-making, ultimately supporting both biodiversity preservation and societal well-being.

India's national tiger monitoring program began in 2006 with the division of the country into uniform sampling units of 100 km^2 grids, which have remained consistent to date. Each grid was assigned a unique code to ensure comparability across survey cycles. While the total surveyed area for Phase I has stayed constant, the distribution of camera-trapped areas versus model-predicted areas for estimating tiger populations has evolved.

The assessment follows a three-phase approach:

- Phase I involves field data collection at the forest beat level by forest department personnel across 10,146 grids.
- Phase III includes intensive camera-trap sampling at 174 sites, covering 32,588 locations, which generated 47,081,881 photographs, including 97,399 tiger images.
- Phase II, conducted by the Wildlife Institute of India, focuses on landscape-level assessments using remote sensing and secondary data.

This extensive exercise required 641,102 man-days and stands as the largest wildlife survey ever conducted globally.

Tiger occupancy increased from 1,758 grids in 2018 to 1,792 grids in 2022. The number of individually identified tigers rose from 2,461 (2018) to 3,080 (2022). The minimum population estimate in 2022 is 3,167. Notable growth was observed in the Shivalik & Gangetic plains, followed by Central India, North Eastern Hills, the Brahmaputra floodplains and Sundarbans, while Western Ghats showed a population decline with certain populations remaining stable.

In the Shivalik Hills and Gangetic Plains landscape, a significant population increase was recorded with 804 unique tigers photographed, surpassing the 2018 estimate of 646 (SE: 567–726). New photographic records in Uttar Pradesh and Himachal Pradesh suggest potential range expansion. To secure long-term conservation success, there is a need for repopulation efforts in Shivalik Forest Division, enhanced protection in Suhelwa, and focused conservation of the genetically distinct Valmiki tiger population.

The western–eastern corridor of Rajaji Tiger Reserve has become functionally extinct for tigers and elephants due to linear infrastructure projects, necessitating the adoption of green infrastructure solutions. Uttarakhand and Uttar Pradesh must also address growing human-wildlife conflicts as tiger and large herbivore populations expand beyond protected areas.

The Central Indian landscape showed an upward trend with 1,161 individual tigers captured, exceeding the 2018 estimate of 1,033 (SE: 885–1,193). Tigers have recolonized areas in Madhya Pradesh and Maharashtra, yet local extinctions have occurred in places like Sri Venkateswara National Park, and reserves such as Kawal, Satkosia and Sahyadri.

Although the expansion of tiger habitats is encouraging, urgent intervention is required in regions experiencing population declines or extinction, especially in Jharkhand, Odisha, Chhattisgarh, Telangana and Andhra Pradesh. Of particular concern is the small, genetically unique population in Simlipal, which demands immediate conservation attention.

Major threats in these regions include:

- Habitat encroachment
- Illegal hunting
- Human-wildlife conflicts
- Unregulated cattle grazing
- Overharvesting of non-timber forest products
- Forest fires
- Mining
- Expanding infrastructure

Mitigation strategies such as eco-sensitive construction, minimizing mining impact, and restoration of degraded sites are essential. The successful implementation of wildlife mitigation structures, like the one on National Highway 44, sets a positive precedent for balancing development with biodiversity conservation.

Western Ghat Landscapes

The protected areas within the Western Ghats represent some of the most biologically rich ecosystems in the country. However, increasing developmental pressures have led to growing human-wildlife interface issues. In 2018, the estimated tiger population in this region was 981 individuals (SE: 871–1093). By 2022, camera trap data recorded 824 unique tigers, reflecting a decline in certain areas, while populations in well-managed tiger reserves remained stable.

The Nilgiri cluster supports the world's largest contiguous tiger population, but overall tiger occupancy across the Western Ghats has reduced, with exceptions such as the Kali (Anshi Dandeli) landscape. While populations within protected areas have either stabilized or increased, a significant decline in occupancy has been observed in landscapes like Wayanad, BRT Hills, and the border regions of Goa and Karnataka. Similarly, the Mookambika–Sharavathi–Sirsi landscape and Bhadra showed marked reductions in occupancy. Outside the Anamalai-Parambikulam complex, tiger presence also declined. Although the Periyar landscape has maintained a stable

population, tiger presence outside its boundaries has decreased. Local extinctions have occurred in Sirsi, Kanyakumari and Srivilliputhur. To maintain ecosystem integrity, management interventions are needed to control invasive species and safeguard native flora and fauna diversity.

North-Eastern Hills and Brahamaputra Plains

This landscape continues to remain relatively secure, with 194 individual tigers photographed in 2022. The estimated population in 2018 was 219 (SE: 194–244). The tigers of the Northeast are genetically distinct but exist in small, isolated populations, necessitating intensive conservation efforts.

This region holds exceptional ecological and cultural importance due to its high biodiversity, endemism, and the presence of indigenous communities. However, it faces serious threats from habitat fragmentation, poaching, and increasing human-wildlife conflict. Despite the presence of several tiger reserves, substantial tiger populations are found only in Kaziranga and Manas. To ensure long-term survival, enhanced anti-poaching efforts, strengthened protected area management, and local community engagement to reduce traditional hunting are essential.

Sundarban Landscapes

The Sundarbans, located near Kolkata, is critical for conserving the unique mangrove ecosystem. It is, however, vulnerable to sea-level rise and climate change, resulting in natural processes of erosion and accretion. Surrounded by forest-dependent villages, the region experiences biotic pressure from fishing, timber and palm extraction and waterway expansion.

Tigers in this region are well-adapted to the mangrove habitat but remain confined due to the limited range. In 2018, the estimated population was 88 (SE: 86–90), while 100 individual tigers were captured through camera traps in 2022, indicating a stable trend with minimal potential for spatial expansion. Ensuring long-term viability will require cross-border collaboration with Bangladesh and knowledge-sharing to manage the landscape sustainably (Qureshi et al. 2023).

2.3. Jungle cat

The jungle cat *(Felis chaus)* typically exhibits a greyish-brown or buff coat with black-tipped fur, giving it a grizzled appearance. Jungle cat (*Felis chaus*) shares a close evolutionary relationship with the domestic cat (*Felis catus*) (Kitchener et al. 2016). Distinctive black stripes can be seen on its forelegs and tail, while the ear tips are adorned with tufts of dark hair. Melanistic individuals have been documented in the Indian subcontinent (Chakraborty et al. 1988). The species has a slender facial structure marked by dark tear lines running along the cheeks. In India, the average adult jungle cat weighs approximately 4 kilograms. It is considered the most commonly found wild cat species in the country.

Fig.2 Jungle Cat *(Felis chaus)*. (Steve DiMatteo, Published on May 30, 2023) https://images.unsplash.com/photo-1685456017747-b300a9f4eef9?q=80&w=1074&auto=format&fit=crop&ixlib=rb-4.1.0&ixid=M3wxMjA3fDB8MHxwaG90by1wYWdlfHx8fGVufDB8fHx8fA%3D%3D

Jungle cats inhabit a variety of ecosystems, including grasslands, scrublands, dry and evergreen forests and the reed-covered banks of marshes (Menon, 2014). Classified as Least Concern (LC) on the IUCN Red List (Gray et al. 2016), their

geographical range extends from the Nile Valley in Africa to Southeast Asia (Abu-Baker et al. 2003). In Nepal, they have been recorded at elevations ranging from 3,000 to 3,300 meters in the Annapurna Conservation Area (Bikram et al. 2020). In Pakistan, jungle cats are mostly observed in protected regions; however, their presence was also confirmed by camera traps in the unprotected Haripur district in 2019 (Anjum et al. 2020). India is home to four recognized subspecies of the jungle cat:

- *Felis chaus affinis* (Gray, 1830) – found in the Himalayas and Northeast India
- *Felis chaus kutas* (Pearson, 1832) – distributed across Peninsular India, up to the north of the Krishna River
- *Felis chaus prateri* (Pocock, 1939) – found in Western India
- *Felis chaus kelaarti* (Pocock, 1939) – present in Southern India

Studies have also highlighted the jungle cat's ability to adapt to various agricultural landscapes, particularly where rodent populations are high (Ogurlu et al. 2010). Rodents form a significant portion of the jungle cat’s diet; however, their prey spectrum also includes hares, deer fawns, primates, reptiles, and birds (Majumder et al. 2011). Jungle cats are primarily nocturnal and use ambush techniques to hunt their prey (Majumder et al. 2011; Mukherjee et al. 2014). The activity patterns of carnivores like the jungle cat are often influenced by the movement of prey species as well as interactions with other carnivores (Lynam et al. 2013).

Geographic Distribution: The jungle cat (*Felis chaus*) exhibits a wide yet discontinuous distribution across its range. In Africa, its presence is confined to Egypt, specifically along the Nile River Valley extending south to Aswan, as well as in the oases of El Faiyum, Farafara, Dakhla and Kharga (Glas, 2013). In Southwest Asia, its populations are mainly concentrated near riparian habitats and regions with reliable water sources. The species occurs across Israel, southern Lebanon, northwestern Jordan, western Syria, Turkey, Iraq and northern Iran—particularly in provinces like Golestan and Mazandan, extending westward along the Caspian Sea coast and into the Hyrcanian forests up to West Azarbaijan Province (Abu-Baker et al. 2003; Sanei et al. 2016).

In Turkey, jungle cats are sparsely distributed, predominantly in the southern regions and wetlands (Gerngross, 2014). Recent records have confirmed their presence in areas such as the Büyük Menderes Delta in Aydin Province, the Akyatan Wildlife Conservation and Development Area in Adana Province, Akyatan Lagoon and near Manavgat in Antalya Province (Avgan, 2009; Gerngross, 2014). However, their overall conservation status in Turkey remains uncertain (Avgan, 2009).

The species is also found in the Caucasus region—up to elevations of 1,000 meters—in countries including Georgia, Armenia, Azerbaijan, and parts of Russia. Its range extends west and south of the Caspian Sea, as well as around the Aral Sea and along associated river systems. In Central Asia, jungle cats inhabit Kazakhstan, Uzbekistan, Turkmenistan, Tajikistan and possibly Kyrgyzstan and Afghanistan.

Within South Asia, the species is widely distributed, ranging from western Pakistan across most parts of India and extending to Sri Lanka, Bangladesh, Bhutan and Nepal. In the Himalayan foothills, they are recorded at elevations up to 2,400 meters. In Southeast Asia, the jungle cat's range includes Myanmar, Thailand, Cambodia, Laos, Vietnam and southern China. However, it is absent from the Malayan Peninsula south of the Isthmus of Kra (Nowell & Jackson, 1996). Its distribution in Indochina remains poorly understood, especially in Myanmar, though historical and recent records from Laos, Cambodia, and Vietnam provide some insight (Duckworth et al. 2005).

2.4. Rusty Spotted Cat

The rusty-spotted cat (*Prionailurus rubiginosus*), described by I. Geoffroy Saint-Hilaire in 1831, is recognized as the world's smallest wild feline species (Prater 1971; Nowell & Jackson 1996; Menon 2014). It is native to India, Nepal, and Sri Lanka (Mukherjee et al. 2016) and, due to its rarity and limited data on population status, it has been listed under Schedule I of India's Wildlife Protection Act (1972). While the species was previously classified as Vulnerable on the IUCN Red List, recent records from various Parts of India and Nepal have contributed to its current Near Threatened status (Khan & Mukherjee 2008; Mukherjee et al. 2016). Despite

this, habitat fragmentation and anthropogenic land-use changes continue to pose significant threats to its distribution (Mukherjee et al. 2016).

Fig. 3. Rusty-Spotted Cat (*Prionailurus rubiginosus*). (Sandaru Muthuwadige; Published on November 28, 2023).https://images.unsplash.com/photo-1701179020800-ce65eb437c67?q=80&w=764&auto=format&fit=crop&ixlib=rb-4.1.0&ixid=M3wxMjA3fDB8MHxwaG90by1wYWdlfHx8fGVufDB8fHx8fA%3D%3D

Ecological niche modeling across the species' range suggests that rusty-spotted cats primarily inhabit dry and moist deciduous forests in three major biogeographic zones: the Western Ghats, the southern Deccan plateau, and the Himalayan foothills (Silva et al. 2014). More recent observations (Aditya & Ganesh 2016; Devkar et al. 2016; Ghaskadbi et al. 2016; Jena et al. 2016; Guptha & Ramanujam 2017; Palei & Debata 2017) indicate that their distribution may also extend into semi-arid landscapes (Nayak et al. 2017). Nonetheless, substantial gaps remain in our understanding of

the species' ecology, particularly regarding local habitat preferences—a crucial component for effective conservation planning.

The species' elusive nature and low densities present considerable challenges for direct observation. As a result, camera trapping has emerged as a key tool for studying cryptic wildlife species in tropical habitats (Karanth et al. 2004). This non-invasive technique enables the collection of data on habitat use, activity patterns, and geographic distribution. Using presence-only data obtained from camera traps, researchers can apply ecological niche models to identify suitable habitats and estimate potential distribution ranges for rare or threatened species (Graham et al. 2004; Thorn et al. 2009; Wilting et al. 2010; Aryal et al. 2016). Furthermore, camera trap detections can help assess whether certain habitats are preferentially used, providing insights into habitat selection (Neu et al. 1974; Manly et al. 2002).

2.5. Mongoose

The popular perception of mongooses as skilled snake-killers has been significantly shaped by Rudyard Kipling's story of *Rikki-tikki-tavi*, in which a mongoose defends a British family in India by eliminating cobras in their garden. Although fictional, Kipling's portrayal reflects real behavioural traits observed in mongooses. These animals are known for their diverse diet, which includes insects, rodents, snakes, and other potentially harmful species. Their role as natural pest controllers, much like domestic cats, has long been acknowledged by humans.

Historically, their utility has been noted across various cultures. The ancient Romans, for example, credited the Egyptian ichneumon (a species of mongoose) with controlling crocodile populations by consuming their eggs—an ecological interaction believed to help maintain navigability of the Nile River (Diodorus Siculus, as cited in Oldfeather, 1933). The symbolic importance of this relationship extended into religion, particularly in Heracleopolis, where the mongoose was deified. This cult is thought to have been in ideological opposition to the worship of the crocodile in nearby Fayyam (Flinders Petrie, 1955).

Fig. 4. Indian Grey Mongoose *(Herpestes edwardsii).*

Glen Carrie., Published on February 19, 2025. https://images.unsplash.com/photo-1739960041256-140d01954328?q=80&w=1239&auto=format&fit=crop&ixlib=rb-4.1.0&ixid=M3wxMjA3fDB8MHxwaG90by1wYWdlfHx8fGVufDB8fHx8fA%3D%3D

Classical texts by Aristotle, Pliny the Elder and Strabo also document the mongoose's antagonistic relationship with snakes. However, it is in India that the mongoose has become most renowned for this trait, particularly in its ability to combat venomous snakes like the cobra. During British colonial rule, mongooses were often kept as domestic pets to help control venomous wildlife such as snakes and scorpions.

Due to their effectiveness as pest controllers, Indian mongooses were introduced to regions such as Fiji, the Caribbean and Hawaii in the late 19th century to mitigate rodent damage in sugarcane plantations. However, these introductions have had unintended ecological consequences. Mongooses have been observed preying on poultry and damaging crops and in some island ecosystems, they have contributed to the decline of native ground-nesting bird populations.

As a result of these ecological impacts, regulations were enacted in certain regions. In Hawaii, the breeding or keeping of mongooses is prohibited except for scientific purposes (Hawaiian Revised Statutes 142.92–93, 1976). Additionally, the species is classified as a biosecurity risk in the continental United States and its importation is strictly banned (U.S. Code, Title 18, Paragraph 42, 1976).

In their native habitats, mongooses are generally viewed more favourably. While they held religious significance in ancient Egypt, in most parts of the Old World, including India, they are more commonly seen as beneficial animals in folklore rather than religious symbols.

Indian Mongooses: Among the six mongoose species present in the Indian subcontinent, the common Indian mongoose (*Herpestes edwardsi*) and the small Indian mongoose (*Herpestes auropunctatus*) are of particular interest due to their widespread distribution and the availability of extensive behavioural data.

The common Indian mongoose, also referred to as the grey Indian mongoose, comprises four subspecies and is widely distributed across the subcontinent. This species may exceed one meter in total length, with the tail alone measuring approximately 45–50 cm. Its coat is generally long and may reach a length of 60 mm during winter. The coloration varies depending on season and subspecies, ranging from black and brown to grey and white. A coarse grizzled appearance results from alternating bands of light and dark hair, which appears finer around the facial region and feet.

In contrast, the small Indian mongoose is relatively smaller, reaching a total length of approximately 55–56 cm. It has shorter fur and its coloration ranges from speckled grey to yellowish or brown. While *H. auropunctatus* is widely distributed from Arabia to China, within India it is mainly found in the northern hills and plains and is rarely observed south of the Narmada River.

Behaviourally, both species are quite similar. They typically inhabit hedgerows and scrublands, often near cultivated fields. Nesting sites include areas under rocks, bushes, hollow tree trunks, termite mounds, or even rooftops of buildings. The small

Indian mongoose is more likely to be found in close proximity to human settlements, showing higher commensality than *H. edwardsi* (Roberts, 1977).

These mongooses are predominantly diurnal, with peak hunting activity around midday. However, *H. auropunctatus* may also forage nocturnally, especially in urban environments. Unlike some social mongoose species, both tend to hunt alone or in pairs. Family groups consisting of adults and offspring may be seen, but cooperative hunting is rare.

Their diet is broad and opportunistic. Field studies in the Rajasthan desert have found remains of insects, scorpions, termites, lizards, rodents and birds in the stomachs of both species (Prakash, 1959). Additional dietary items include snakes, centipedes, beetles, wasps, frogs, poultry and eggs (Powell, 1913; Roberts, 1977). They are also known to scavenge carrion and consume the leftovers of larger carnivores, as well as plant material on occasion.

Although mongooses are popularly associated with snake-killing, snakes may not constitute a significant part of their diet. While they can kill venomous snakes such as cobras (*Naja naja*) and vipers (*Vipera russelli*), mongooses usually do so only when necessary or when other food sources are scarce. Juvenile mongooses, in particular, are not always successful in such confrontations (Hinton & Dunn, 1967).

The mongoose's success in snake encounters is largely due to its agility and speed, allowing it to evade strikes and deliver lethal bites to the snake's head or neck. When threatened, a mongoose raises its fur, providing both a visual deterrent and physical protection. Its thick skin and a degree of physiological resistance to venom also enhance its survival in such conflicts. Cobras, with relatively short, fixed fangs and a limited strike range, are less effective in combat, whereas vipers, with longer, movable fangs and faster strikes, pose a greater threat to the mongoose (Oliver, 1955).

Despite previous beliefs, mongooses are not immune to snake venom. However, they do show a higher tolerance compared to many other species. Experimental studies by Calmette (1878–1879) demonstrated that mongooses from regions

without venomous snakes, such as Guadeloupe, still survived injections of cobra venom that were fatal to other animals. The theory that mongooses seek out antidotal plants after snakebites lacks scientific evidence (Crooke, 1906).

Information on mongoose reproduction in South Asia is limited. One of the most comprehensive studies was conducted in Hawaii (Pearson & Baldwin, 1953). Anecdotal observations suggest that *H. auropunctatus* can reach reproductive maturity by 11 months and produce multiple litters in quick succession. Similarly, *H. edwardsi* has been observed producing several litters over a short period, with a gestation period of approximately 60 days (Powell, 1913; Frere, 1929). According to Roberts (1977), *H. edwardsi* breeds throughout the year. Offspring are born hairless and blind, with eyes opening by the 16th or 17th day and they typically remain with the mother for about six months unless a new litter is born.

Lifespan data indicate that while some mongooses can live up to 17 years in captivity (Osman Hill, 1955; Phillips, 1955), *H. edwardsi* generally survives for about two years in captivity and *H. auropunctatus* about seven years (Dover, 1932–1933). In the wild, their life expectancy is likely shorter due to predation by birds of prey, snakes, jackals, dogs and human-related threats. In Hawaii, where predators are minimal, some individuals have been found to live up to ten years (Pearson & Baldwin, 1953).

2.6. Jackal

Jackals are medium-sized canids belonging to the family *Canidae* and are widely distributed across Africa and parts of Asia and Europe. There are four recognized species:

1. **Golden jackal (*Canis aureus*)** – The most widespread and the largest among jackals, often referred to as the "golden dog." It is considered a true member of the dog lineage and is commonly found across the Indian subcontinent, Southeast Europe, and parts of the Middle East.

2. **Black-backed jackal (*Canis mesomelas*)** – Also known as the silver-backed jackal, this species is native to eastern and southern Africa.
3. **Side-striped jackal (*Canis adustus*)** – Found primarily in central and southern Africa, this species inhabits forested and moist savanna regions.
4. **Simien jackal or Ethiopian wolf (*Canis simiensis*)** – The rarest of all jackals, it is endemic to the Ethiopian highlands and is critically endangered. Despite its name, it is more closely related to wolves and coyotes than to other jackals.

Fig. 5. Black-backed jackal (*Canis mesomelas*). (Simon Hurry., Published on March 21, 2021). https://images.unsplash.com/photo-1616269941487-430554784c24?q=80&w=1332&auto=format&fit=crop&ixlib=rb-4.1.0&ixid=M3wxMjA3fDB8MHxwaG90by1wYWdlfHx8fGVufDB8fHx8fA%3D%3D

Thirteen subspecies of the golden jackal (*Canis aureus*) have been recognized to date (Wozencraft, 2005). More recently, a new subspecies was identified in the Gaza Strip, Palestine, and named *Canis aureus palaestina* (Khalaf, 2008). In the broader Palestinian region, three golden jackal subspecies are typically recorded: the Syrian golden jackal (*C. a. syriacus*), the Egyptian golden jackal (*C. a. lupaster*), and the Arabian golden jackal (*C. a. hardranauticus*).

The Palestinian golden jackal is both morphologically and geographically distinct from these subspecies, primarily due to its unique fur coloration and intermediate

body size. It is smaller than the Egyptian jackal and larger than the Arabian jackal. As a small form of the golden or Asiatic jackal (*Canis aureus*), it occupies a size range between that of a fox and a wolf. Distinguishing features include relatively small, reddish (rufous) ears, a short black-tipped tail, and a dorsal coat that ranges from black and yellowish-gray to brown-yellowish with rufous tinges. The back appears greyer and is grizzled with varying amounts of black.

A dark dorsal stripe extends from the nose to the tail, broadening along the back and flanks. Two dark bands are typically present across the lower throat and upper chest. A reddish phase is also occasionally observed. The underparts are pale—either whitish or yellowish-brown—and the winter coat tends to be longer and greyer. The tail is short with a black tip.

Interestingly, recent genetic studies have proposed that the Egyptian jackal (*C. a. lupaster*), found in North Africa, may be more accurately classified as a subspecies of the grey wolf (*Canis lupus*), rather than a golden jackal (Nassef. 2003 and Rueness et al. 2011).

In India, golden jackals (*Canis aureus*) have been observed taking over dens originally excavated by Bengal foxes (*Vulpes bengalensis*) (Jhala and Moehlman, 2008). In contrast, jackals tend to avoid areas dominated by the larger grey wolf (*Canis lupus*), which often displays territorial intolerance toward them. Wolves have been reported to respond swiftly to jackal call-playbacks, possibly as a means of expelling potential competitors from their range (Giannatos et al. 2005). Nevertheless, there are instances where jackals have scavenged from wolf kills without triggering aggressive behaviour from the wolves (Jhala and Moehlman, 2008).

Golden jackal remains have also been detected in the scat of spotted hyenas (*Crocuta crocuta*), although hyenas typically avoid consuming jackals and may only do so under starvation conditions (Kruuk, 1972). Despite being the most widespread wild canid in India, ecological studies focused on the golden jackal remain limited (Jhala and Moehlman, 2008).

2.7. Hair Morphology

Trichology**,** the scientific discipline concerned with the study of hair, has its origins in the mid-19th century. Hair is remarkably resilient and often remains intact even when exposed to moisture or when surrounding tissues decompose, making it a valuable form of physical evidence in forensic investigations. When analysing hair, examiners assess several features such as colour**,** thickness**,** texture**,** granule distribution**,** scale patterns**,** overall diameter and the presence or absence of a medulla**.** This chapter focuses on evaluating these characteristics to determine whether multiple hair samples may have originated from the same individual or source (Lisa Knecht. 2011).

Hair types

Hair types in mammals can be broadly classified into guard hairs, underfur (or underhairs), overhairs, vibrissae and bristle hairs, each with distinct structural and functional characteristics.

- Guard hairs: These are the most prominent and robust, often straight and coarse. Also referred to as *shield hairs*, they are subdivided into primary and secondary guard hairs. Both types have a narrower and more flexible basal region compared to their mid and distal portions. Secondary guard hairs are generally shorter than the primary ones. Of these, primary guard hairs are the most diagnostic, offering significant species-specific features valuable for identification.

- Underhairs: These are much finer, softer and shorter than guard hairs. They are nearly uniform in thickness along their length and hold minimal diagnostic value due to their lack of species-specific features. However, these hairs are better visualized using Scanning Electron Microscopy (SEM).

- Overhairs: These are typically longer than the surrounding body fur and usually possess a circular cross-section. These hairs, while structurally distinct, are not helpful in determining species identity.

- Vibrissae: These are commonly known as whiskers or tactile hairs, serve a sensory role. These stiff, elongated hairs are thickest at the base and gradually taper toward the tip. They are also referred to as sinus hairs due to their association with sensory nerves (Brunner and Coman, 1974).

- Bristle hairs: These are characteristic of animals such as wild and domestic pigs. These hairs are thick, uniformly wide along their length, and possess a narrow medulla. A notable feature is their bifurcated or trifurcated tips. The term *bristle* was introduced by Noback (1951) to describe this type of hair.

Hair structure

A strand of hair is composed of three main layers: the cuticle (outermost layer), cortex (middle layer), and medulla (central core) (Mathiak, 1938). Hair is made of keratin, a fibrous protein, and consists of cornified, dead cells. Melanin, the pigment responsible for hair colour, is found in the cortex.

The hair shaft can be divided into three regions: the proximal region near the root, the medial region in the middle, and the distal region toward the tip. These regions are significant because features such as scale patterns and medulla structures can vary along the hair length (Adorjan and Kolenosky, 1969). These divisions also help in identifying the positions of banding or other region-specific characteristics during examination.

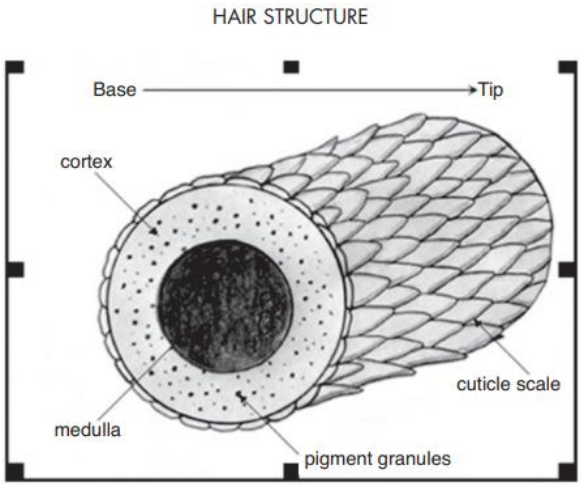


Fig. 6. Cross-section of hair shaft. Source: http://www.ecobyte.com.au/using_.html

Cuticle:

The cuticle is the translucent outer covering of the hair, made up of overlapping scale-like cells that point from the root (proximal end) toward the tip (distal end) of the shaft (Moore et al. 1974; Hicks, 1977). These scale patterns play a key role in species identification. Although detailed scale traits like crest height, spacing, and overlap are not covered here, their variability in mammals is documented (Moore, Spence and Dugnolle, 1974).

Classification:

Scale patterns can be regular or irregular and may shift along the shaft. Occasionally, patterns blend, forming combinations such as “diamond petal.” Variation may also be observed across body regions within the same individual. Typically, the dominant pattern in the proximal region of guard hairs is used for comparative analysis (Moore, Spence and Dugnolle, 1974).

The common scale pattern types include:

- Coronal: Crown-like scales completely surrounding the shaft.
- Diamond petal: Overlapping scales with diamond-like shapes aligned from base to tip.
- Double chevron: V-shaped, wave-like non-overlapping scales.
- Irregular mosaic: non-overlapping, unevenly sized, and shaped scales (Moore et al., 1974).
- Irregular petal: Overlapping petal-like scales, varied in size and shape (Brunner and Coman, 1974).
- Irregular wave: Wavy non-overlapping scales with inconsistent wave lengths and crest heights.
- Irregular-waved mosaic: A hybrid of wave and mosaic forms with broken wave continuity.
- Pectinate elongated: Overlapping, comb-like scales arranged in oblique wave-like rows (Moore et al., 1974).
- Regular mosaic: non-overlapping scales with uniform shape and spacing.
- Regular petal: Evenly shaped overlapping petal-like scales.
- Regular wave: Consistent, continuous wavy patterns.

- Single chevron: V-shaped scales showing either high crests or troughs.

- Streaked wave: Wavy scales interrupted by vertical columns with steep margins.

Cortex

The cortex is the main structural layer of hair, made of elongated, spindle-shaped (fusiform) cells. It may contain cortical fusi, pigment granules, and ovoid bodies (Hicks, 1977). These internal features are useful for microscopic comparison and species differentiation.

Medulla

The medulla, located in the center of the hair shaft, may appear dark and opaque if air-filled, or clear and translucent when filled with mounting media or fluid (Moore et al., 1974; Hicks, 1977). In humans, the medulla typically appears amorphous, while in non-human mammals it tends to be more structured and clearly defined (Deedrick and Koch, 2004a, 2004b; Hicks, 1977).

Classification-

The medulla can be classified into several types (Tumlison, 1983):

- Absent: No visible medulla; seen in human head hair and all bat hair (Hicks, 1977).
- Fragmented: Irregularly interrupted by the cortex; common in human hair.
- Uniserial ladder: A continuous column formed by a single row of discrete rectangular cells.
- Multiserial ladder: Multiple rows of rectangular cells, often found in Leporid species.
- Simple unbroken amorphous: A dark, tube-like structure lacking internal detail.
- Unbroken cellular: A tube-like core with distinct, irregular cells (Hicks, 1977).
- Unbroken vacuolated: Medulla containing large vacuoles, with clearly defined cell structures.

- Unbroken with cortical intrusions: A continuous medulla interrupted by inward extensions of cortical material (Hicks, 1977).
- Unbroken lattice: A fine network of polygonal cells, typically found in species with narrow cortices like cervids.

CHAPTER 3: AIM AND OBJECTIVE

Aim:

To comprehensively investigate the ecological and genetic dynamic of selected carnivore population through species identification of field-collected and chosen Tiger Reserve collected samples of scat, assessment of diet preferences and population genetic analysis of tigers, in order to inform and enhance conservation and management strategies.

Objectives:

1. Identification of species from scat samples collected from field and Corbett Tiger Reserve.
2. Molecular phylogeny and population genetics of *Panthera tigris* samples collected from Corbett Tiger Reserve.
3. Estimation of the diet preference of *Panthera tigris* samples collected from Corbett Tiger Reserve.

CHAPTER 4: MATERIAL AND METHODOLOGY

4.1. Identification of species of scat samples collected from field and Corbett Tiger Reserve.

- Few samples were collected from field, those include following species- Jungle Cat, Mongoose, Jackal and Rusty-spotted cat.
- Majorly, samples are of tigers which are collected from different ranges of Corbett Tiger Reserve.

Study Area

In Himalayan region, Corbett Tiger Reserve is considered as the best living area for tigers. This zone is being the exclusive distinction of being the very first and foremost National Park and Conservation Centre of wildlife in India. It is being created in throughout the British Regime of United Provinces in 1936. In 1973, Corbett national park achieved another milestone by becoming the starting point for India's tiger conservation initiative, Project Tiger. Consequently, it is also designated as the country's first ever Tiger Reserve (Singh et al. 2009).

The Corbett Tiger Reserve is located in Uttarakhand states of India at Latitudes 290 48' N- 290 15' N & Longitudes 780 39 E- 790 27' E, encircling area around 1386.91 km^2 with elevation of 385 to 1100 m. In terms of administration, it falls within the boundaries of three districts Nainital, Pauri (Uttarakhand) and Bijnore (Uttar Pradesh). It is encompassed by forest divisions of Bijnore, Pauri, Tarai West, Almorah and Ramnagar.

The region lies nestled between the Shivalik hills in the north and the Gangetic plains towards the south. Its landscape transitions from rugged, hilly areas with coarse, rocky soils and boulders in the northern parts to fertile, alluvial plains and clay-rich wetlands with a shallow water table in the southern areas. Prominent rivers such as the Ramganga, Palain, Mandal, and Sone Nadi traverse through the Corbett Tiger Reserve (CTR). The area experiences an average annual rainfall of approximately 1925 mm, predominantly received during the southwest monsoon season, which lasts from June to September.

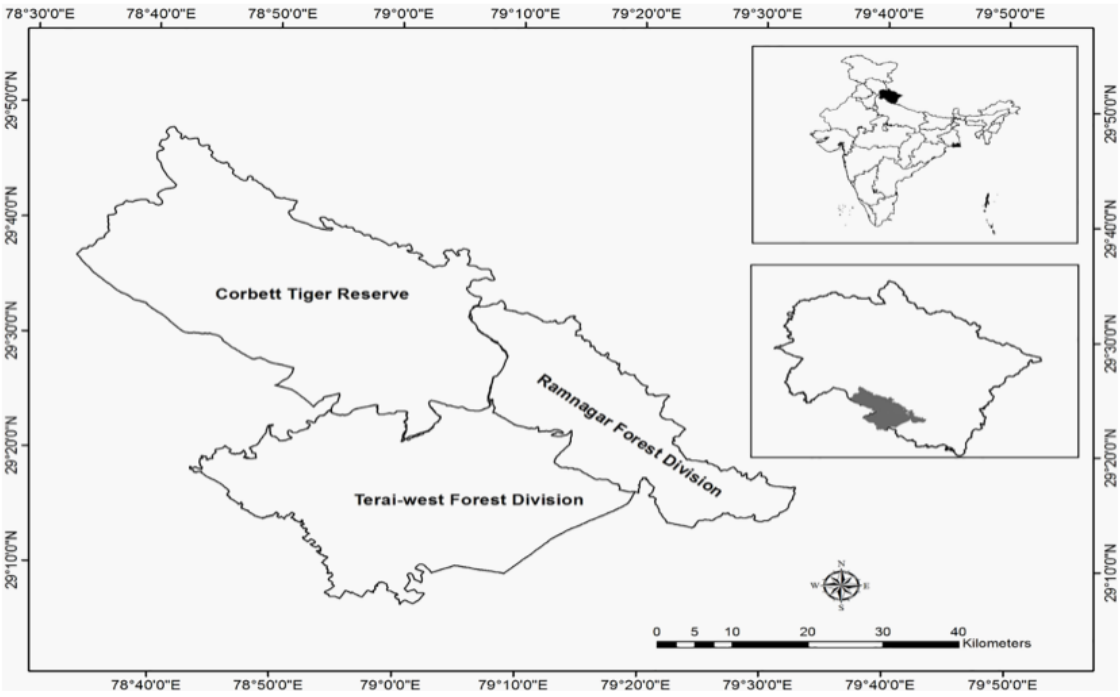


Fig. 7. Map of the study area showing Corbett Tiger Reserve and adjoining forest divisions.
Patterns of livestock depredation by tiger *(Panthera tigris)* and leopard *(Panthera pardus)* in and around Corbett Tiger Reserve, Uttarakhand, India. (https://doi.org/10.1371/journal.pone.0195612.g001)

The Corbett Tiger Reserve (CTR) and its surrounding areas exhibit a wide range of vegetation types. These include Moist Siwalik Sal Forest, Moist Terai Sal Forest, Western Gangetic Moist Mixed Deciduous Forest, Khair-Sissoo Forest, Northern Dry Mixed Deciduous Forest, Dry Siwalik Sal Forest, Dry Plains Sal Forest, Dry Deciduous Scrublands, Upper Himalayan Chir Pine Forest, Himalayan Subtropical Scrub, Oak Forest (*Quercus incana*), Western Mixed Coniferous Forest (with species like Spruce and Blue Pine), along with several plantation zones (Ministry of Environment & forest and climate change, Government of India. 2014). This ecological diversity sustains a rich array of biodiversity, consisting of 617 plant species and 1,013 animal species, which include 49 species of mammals, 685 species of birds, 39 species of reptiles and 36 species of fish (Zoological Survey of India, 2008). CTR is also notable for its high tiger density, with approximately 17.8 tigers per 100 km^2 and is recognized for hosting the largest population of Asian elephants (*Elephas maximus*), estimated at 1,035 individuals as of 2015 (Jhala et al. 2011; The Tribune. 2017).

Field Visits and Sample Collection in Corbett Tiger Reserve, Uttarakhand

To facilitate dissertation-based research, a field visit was conducted to Corbett Tiger Reserve, Uttarakhand, for a duration of 3–4 days. The study commenced in the Kalagarh region and subsequently continued through Dhikala Zone, Pateer Pani, Jhirna Range, Haldukheda Range, and Dhela Range. The primary objective of the visit was to collect faecal samples for species identification and analysis. Additionally, forest staff were provided on-site training for non-invasive scat sample collection so that further samples could be collected and sent post-visit.

Fig. 8. Forest Staff, during Sample Collection Training, Kalagarh, Corbett Tiger Reserve

The first visit was made to Kalagarh, with accommodation at Kalagarh Jungle Resort, located within the core zone of the reserve. Initial samples were collected from this region, with a focus on Pater Pani, a stony and swampy area characterized by eutrophic water bodies.


Fig. 9 and 10. Pater Pani Region, Eutrific water and stoney trail

Sample collection trails extended up to 2–4 km, following tiger trails in the presence of forest rangers for protection and research scholars for guidance. During tracking, tiger pugmarks were spotted, indicating recent movement of the species in the area. they told about the identification of male and female tiger pugmarks, they said that male tiger pugmark looks broader in comparison to female tiger pugmark and with practice it is easily identifiable whether the pugmark is of male or female tiger.


Fig. 11 and 12. Spotted pugmarks of animals and tiger pugmark respectively.

Subsequently, the team proceeded to the Dhikala Zone, often referred to as the heart of Corbett, known for its rich biodiversity and scenic landscape. The Ramganga River, which flows through this zone, was observed, along whose banks herds of elephants and deer were sighted. Few birds were also spotted, which are usually difficult to be viewed.

Fig. 13 and 14. Wise Brown Fish Owl (Least Concern) and Grey Headed Fish Eagle (Near Threatened) respectively.

The Dhikala Range is closely associated with the Ramganga River, particularly in the region where it forms the Ramganga Reservoir (also known as the Kalagarh Dam). The river enters Corbett Tiger Reserve from the northeastern boundary and flows through the core zones, notably enriching the Dhikala Chaur—a vast grassland area renowned for its biodiversity and frequent wildlife sightings.

Fig. 15. Elephant Spotted at Corbett Tiger Reserve

Fig. 16. Ram Ganga River Area, Dhikala Range, Corbett Tiger Reserve

Following this, the Dhela Range was visited. This area comes under the Sone Nadi Range of Corbett and is notable for the presence of the Saddle Dam. During the visit, a Fish Eagle *(Haliaeetus leucoryphus)*, an endangered species listed in the IUCN Red Data Book, was observed.

Additionally, in the dam waters, sightings of the Golden Mahseer *(Tor putitora)*, a large-sized and endangered fish species, were recorded.

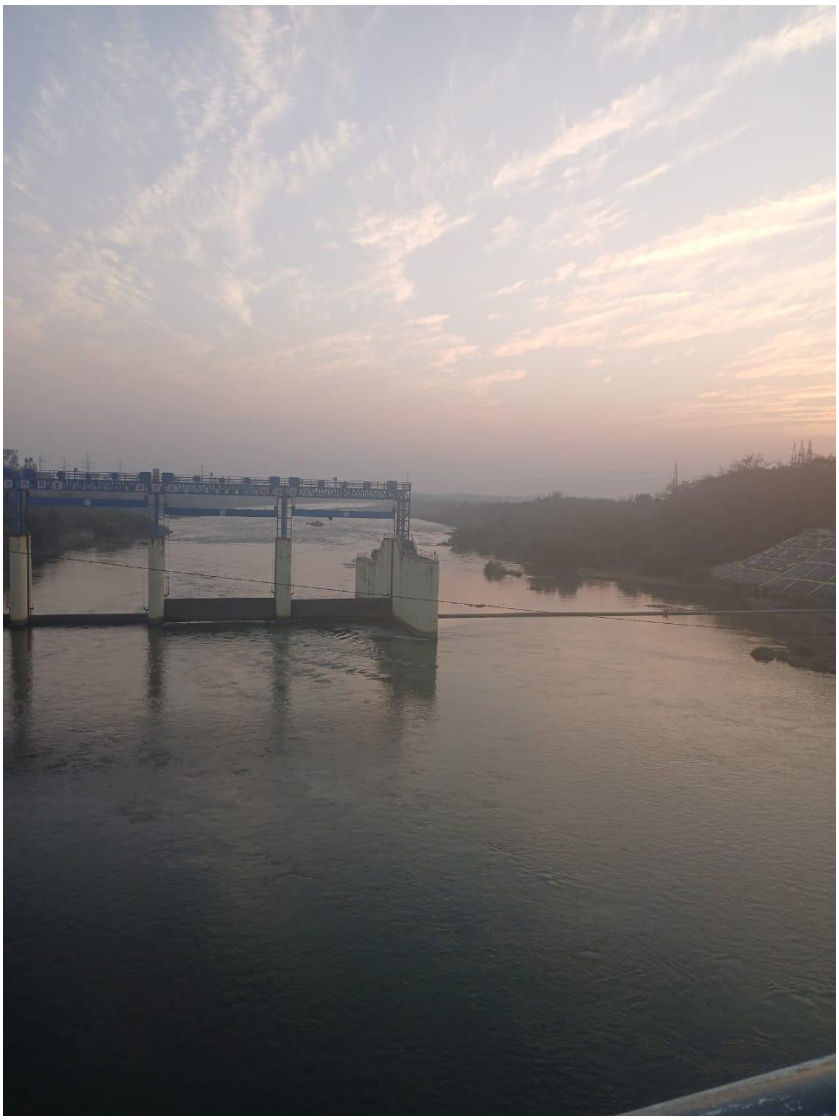


Fig. 17. Saddle Dam, Sone Nadi Range, Corbett Tiger Reserve

The next destination was the Jhirna Range, from where scat samples were successfully collected. Jhirna is one of the few zones in Corbett open to tourists year-round and supports diverse vegetation and wildlife.

Fig. 18. Jhirna Range Office, Corbett Tiger Reserve

Following this, the team visited the Haldukheda Range, where additional samples were collected. This area also presented an opportunity to observe the local ecological landscape and wildlife presence.

A significant site visit included the Corbett Rescue Centre, it comes under Dhela Range, where man-eater tigers and leopards are held in secure enclosures. Strict restrictions on photography and videography are maintained at this facility to ensure the welfare and security of the animals.

Additional exploratory visits were conducted to nearby areas where honeybee fencing was being trialed as a conflict mitigation strategy. The field trails during these explorations revealed camera trap installations and sightings of sambar deer, barking deer, and other herbivores.

Fig. 19 and 20. Honey Bee Fencing, near Corbett Rescue Centre Area, Dhela Range.

In total, 12 scat samples were collected during the field visit. Subsequently, an additional 28 samples were collected by trained forest staff and sent for analysis.

- Sample collection

  Requirement:

  - Zip-lock bags
  - Silica gel (laboratory grade)
  - Permanent marker
  - Hand gloves

  Methodology:

- Use new gloves to handle scat samples (if no gloves then we can use leaves, pebbles or twigs). Do not handle or touch scat with bare hands.

- Pick up the selected part of scat and put inside the zip-lock bag and put silica beads in the zip-lock containing scat. Ensure minimal damage to the morphology of the scat along with minimal transfer of other elements such as grass, sand etc.
- Label the zip-lock bag using permanent marker with the details of scat samples, I. e. suspected species, GPS location, date, name of researcher, micro and macro habitat details etc.
- If the scat sample is fresh and moist, add silica desiccant to the zip-lock bag in an approximate 4(silica):1(scat) w/w ratio. Reduce silica accordingly- no need to silica if scat sample appear very dry and old.
- Store samples bag in a cool and dry place and transport to the laboratory for long term storage at 4˚ C and subsequent downstream analysis.

- DNA extraction

1. GuHCl Method (Manual method)

Reagents required

- 6M GuHCl buffer (57.3 g for 100ml)

(NOTE: Usually GuSCN (Guanidine thiocyanate) is used in DNA extraction but it is replaced by GuHCl (Guanidine hydrochloride) because-

I. GuSCN is harmful and GuHCl is safe to use.
II. GuHCl is economically more worthy, it is 10 times cheaper than GuSCN.)

einase K

- Si solution (cover the tubes with aluminium foil as it reacts with light).
- 2x wash buffer

(50% ethanol, 20Mm, Tris-HCl, 1mM EDTA, 1M NaCl and dH_2O).

Table 1: GuHCl DNA Method Reagent composition:

S.no	Stock	Required conc.	Vol. of stock required (For 50 ml 2x wash buffer)	Vol. of dH_2O
1	1M Tris-HCl	20mM	0.05ml	23.2ml
2	0.5M EDTA	1mM	0.5ml	
3	1M NaCl	50mM	1.25ml	
4	Ethanol	50% of whole	25ml	

- AE/TE buffer

Methodology:

a) Cell lysis:

- Collect 100-200mg of faecal pallet (outer layer) in 2ml tube.
- Add 600µl-1ml of 6M GuHCl to it and mix gently.
- Add 20µl of Proteinase K, vortex and incubate at 56℃ for overnight.

b) DNA binding:

- After incubation, vortex and centrifuge the sample @10,000 rpm for 1 min.
- Collect the supernatant in new 1.5 ml MCT.
- Then add 40µl silica solution to each sample.
- Mix the sample inverting several times for 2-5 hours.

c) Precipitation:

Then centrifuge @10,000 rpm for 3 min and discard the supernatant.

d) Washing:

Add 500µl of 2x wash buffer to the pallet and mix it by pipetting in and out or by shaking, then again centrifuge @10,000 rpm for three min and discard the supernatant.

(Repeat this step 3-5 time accordingly till the pellet become clean).

e) Elution:

- Then place the Si column on block heater at 60℃ until it dry (15-20 mins).
- Then add 40µl of AE buffer in it and incubate for 15 min at room temperature.
- Then centrifuge the tube @14,000 rpm for 10 min and collect the solution in new MCT.

2. Qiagen kit DNA extraction method:

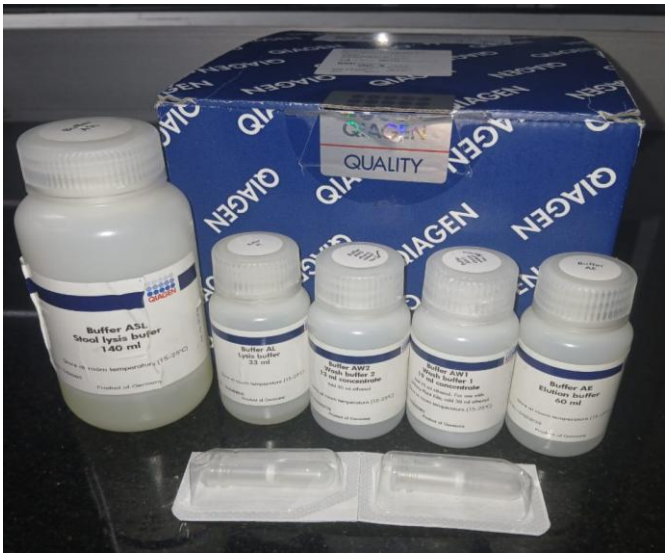


Fig. 21. Qiagen Kit Reagents and spin column

Requirement-

- Qiagen DNA extraction kit
- MCTs
- Spin column

Methodology-

- Step-1 Lysis of cell

Took tissue sample up to 50-80mg. 200µl of ATL buffer and 20 µl of Proteinase K was added into chopped tissue sample, vortexed and incubated at 56℃ in water bath and lysed for overnight.

- Step-2 DNA Release

In Lysed sample, 200µl of AL buffer was added, vortexed and incubated at 72℃ for 15min, 200 µl of 100% ethanol was added and mixed gently for the release of DNA in the solution.

- Step-3 DNA Binding

The mixture was then transferred into a spin column in a 2 ml collection tube and then centrifuged 8000 rpm for 1 min and then filtrate was discarded.

- Step-4 DNA Washing

The spin column was placed in a new 2 ml collection tube and 500 µl AW1 buffer was added and centrifuged for 1 min at 8000 rpm. Filtrate was discarded. Then the spin column was placed in a new 2 ml collection tube and 500µl of buffer AW2 was added again centrifuged for 1 min at 8000 rpm. Filtrate was discarded. Then the spin column centrifuged for 3 min at 14000 rpm for dry wash.

- Step-4 DNA Elution

The spin columns were transferred to a new 1.5 vials and 40 µl AE buffer was added for elution of DNA and kept it for 10 min at room temperature and centrifuged at 8000 rpm for 1 min.

➢ Quantification of extracted DNA

Gel electrophoresis: It is a technique used to separate substances based on the movement rate of charged particles under an electric field. Several factors influence DNA migration, including the strength of the electric field, the concentration of agarose in the gel and most importantly, the size of the DNA molecules. Smaller DNA fragments migrate faster through the agarose matrix compared to larger ones.

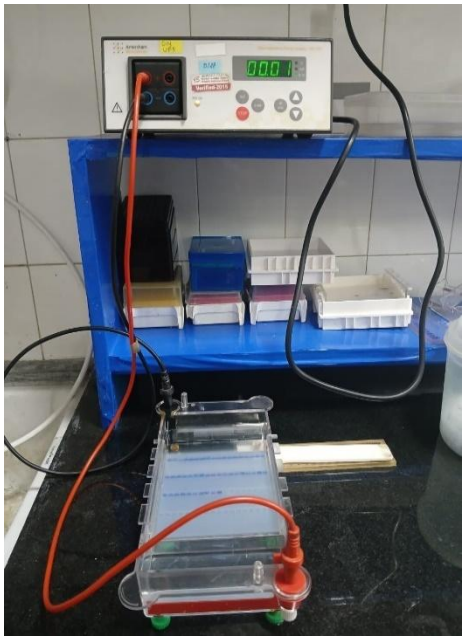


Fig. 22. Gel Electrophoresis System

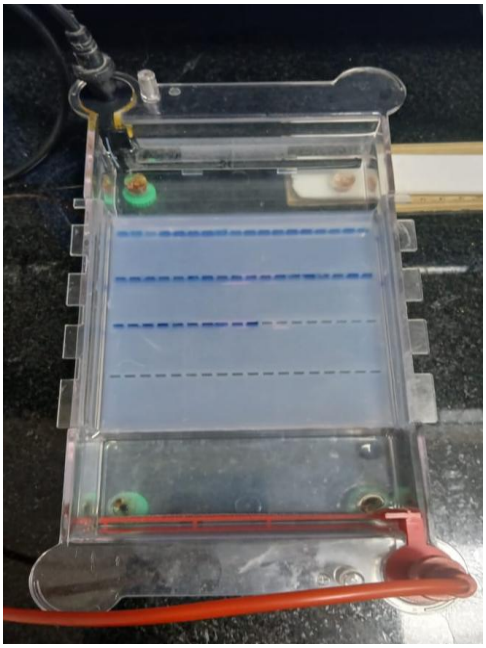


Fig. 23. Gel Electrophoresis Tank

Requirement:

Reagent composition-

1. 10x TAE buffer (1000 ml) contains:
   - Double-distilled water- 600 ml
   - Tris-base- 48.4 g
   - Glacial acetic acid- 11.4 ml
   - 0.5 M EDTA (pH=8.0)- 20 ml
2. 6x Loading Dye Composition
   - 0.1% xylene cyanol
   - 90% glycerol in water
   - 0.1% bromophenol blue

Methodology:

1. Preparation of gel:

- Clean the casting tray with 70% ethanol to remove grease.
- Prepare 0.8% agarose gel in 1x TAE buffer by dissolving 0.8 g of agarose in 100 ml buffer.
- Heat the solution in a microwave oven until the agarose completely dissolves.
- Allow the gel to cool to approximately 50°C, then add 2 µl of Ethidium Bromide (EtBr) per 100 ml of gel.
- Swirl gently to mix, ensuring no air bubble formation.

- Place a comb in the casting tray and pour the prepared gel into it, avoiding air bubbles.
- Allow the gel to polymerize and solidify for 20-30 minutes.

2. Loading of samples:

- Mix the sample with 6X gel loading dye.
- Carefully remove the comb to prevent deformation of wells.
- Place the casting tray in the electrophoresis tank and pour 0.5X TBE buffer into it.
- Load the prepared samples into the wells using a pipette.
- Run the gel at 100 volts for 30-60 minutes.

3. Visualization of gel: Observe the gel under a UV trans-illuminator when the dye reaches approximately 3/4th of the gel length.

- Amplification: Polymerase Chain Reaction-

Fig. 24. Thermal Cycler (PCR Machine)

Gene which we have used-

- *Cyt b*- for field samples
  Forward: 5′-CTATAG CCTCAC TATCAG CAC-3′
  Reverse: 5′- AGCACAGTT ATGTGTGAGC-3′

- *16S*- for Corbett Tiger Reserve samples
  Forward Primer (16Sar-L): 5'-CGC CTG TTT ATC AAA AAC AT-3'
  Reverse Primer (16Sbr-H): 5'-CCG GTC TGA ACT CAG ATC ACG T-3'

Requirement:

- Normal taq PCR-
  Buffer=1µl
  dNTPs=0.5µl
  F/R primers=0.30µl
  Taq polymerase=0.20µl
  Distilled water=6.75µl

- Multiplex PCR-
  Q solution=1 µl
  multiplex=4µl
  F/R primers=0.25µl
  Distilled water=3.5µl

Methodology:

(1). Preparation of Master Mix:

- Mix all reagents in a 2 ml tube according to the required PCR type (Normal Taq PCR or Multiplex PCR), here multiplex PCR used.
- Vortex the mixture to ensure proper mixing of components.

(2). Aliquoting: Dispense 9 µl of the master mix into each PCR tube.

(3). Addition of template DNA:

- Add the appropriate volume of template DNA into each tube.
- Perform a short spin to ensure proper mixing.

(4). PCR cycling conditions:

Place the tubes in a thermal cycler and set up the program according to the following general cycling conditions:

- Initial denaturation: 95°C for 3-5 minutes.
- Denaturation: 95°C for 30 seconds.
- Annealing: 50-65°C (depending on primers) for 30 seconds.

- Extension: 72°C for 30 seconds to 1 minute (depending on product size).
- Repeat steps 2-4 for 25-35 cycles.
- Final extension: 72°C for 5-10 minutes.
- Hold at 4°C until further processing.

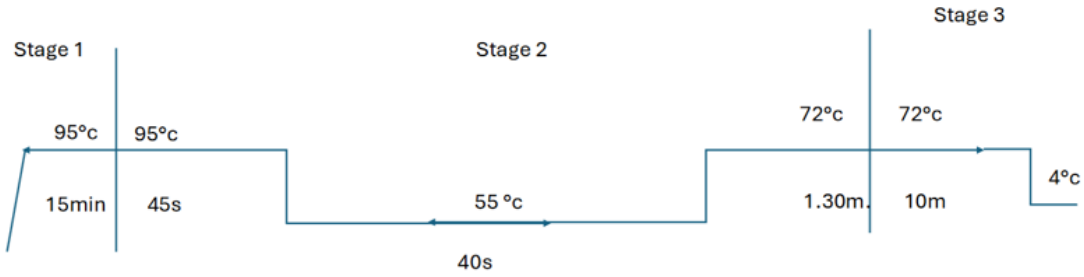


Fig. 25. PCR Cycling Conditions

(5). Post PCR processing: Store the amplified products at -20°C or proceed directly to gel electrophoresis for visualization.

➢ DNA Sequencing Process:

Fig. 26. 3200XL Genetic Analyzer (Applied Biosystem)

DNA sequencing determines the precise order of nucleotides within a DNA molecule. Several preparatory steps are required before samples can be processed for sequencing. At the Wildlife Institute of India, DNA sequencing is conducted using the 3200XL Genetic Analyzer (Applied Biosystem).

(1). Exo-SAP Treatment:

This step is crucial for purifying the PCR products by removing residual primers and unincorporated nucleotides. The reaction is set up as follows:

- 1.2 µl of PCR product is transferred into a PCR plate.
- 0.5 µl of Exo-SAP mix (containing Exonuclease I and Shrimp Alkaline Phosphatase) is added.

Function of Exo-SAP components-

- Exonuclease I: Degrades residual single-stranded primers and any non-specific single-stranded DNA present after PCR amplification.
- Shrimp Alkaline Phosphatase (SAP): Removes the phosphate group from the 5' end of DNA and RNA. This prevents re-ligation during cloning and aids in the removal of dNTPs, ensuring efficient downstream sequencing.

Exo-SAP Reaction Conditions: The Exo-SAP reaction is performed in a 2 µl total volume using 1.5 µl of amplified PCR product. The thermal cycling conditions are:

- Incubation at 37°C for 20 minutes (enzyme activation).
- Heat inactivation at 85°C for 15 minutes to deactivate the enzymes.

(2). Cycle Sequencing:

Cycle sequencing uses a thermostable DNA polymerase to repeatedly denature, anneal and extend the DNA template. This process amplifies the signal linearly, facilitating accurate nucleotide identification.

Table 2: Master Mix Composition:

Reagents	Volume (per reaction)
BigDye Terminator Mix	0.25 µl
5X Sequencing Buffer	1.0 µl
Primer	0.2 µl
PCR Product	1.0 µl

Sterile Water	6.55 µl
Total Volume	9.0 µl

Thermal Cycling Conditions:

- Initial denaturation: 96°C for 1 minute
- Denaturation: 96°C for 10 seconds
- Annealing: 50°C for 10 seconds
- Extension: 60°C for 4 minutes
- Final hold: 4°C or -20°C (for storage)

(3). Purification (Clean-Up Procedure):

The clean-up step removes unincorporated dyes and impurities from the sequencing reaction. The procedure is as follows:

1. Add 40 µl of a 24:1 ethanol: sodium acetate mixture to each well of the PCR plate.
2. Incubate at room temperature for 10 minutes.
3. Centrifuge at 4000 rpm for 30 minutes at 15°C.
4. Carefully discard the supernatant.
5. Add 100 µl of 80% ethanol to each well.
6. Centrifuge at 4000 rpm for 25 minutes at 15°C.
7. Discard the supernatant and allow the plate to dry for 10 minutes.
8. Rehydrate the samples by adding 9 µl of Hi-Di formamide to each well.
9. Centrifuge at 4000 rpm for 1 minute at 15°C.
10. Denature the samples at 95°C for 3 minutes.

Finally, the prepared samples are loaded onto the Applied Biosystem 3200XL Genetic Analyzer for sequencing analysis.

➢ Mitochondrial DNA Sequence Analysis

Species identification is done with help of BioEdit tool, sequences were matched with the reference in NCBI database.

4.2. Molecular phylogeny and population genetics of *Panthera tigris* samples collected from Corbett Reserve.

Microsatellite Primers used:

- PttF1
- PttF4
- FCA304
- PttC6
- PttA2
- PttG4

Requirement:

- PCR Master Mix (or individual components like Taq polymerase, dNTPs, $MgCl_2$, buffer)
- Species-specific or previously validated microsatellite primers (fluorescently labeled, if using capillary electrophoresis)
- Nuclease-free water
- Loading dye and DNA ladder (for agarose gel verification)
- Agarose and electrophoresis buffer (e.g., TAE or TBE)

Methodology:

1. DNA Extraction and Locus Selection

Genetically confirmed samples are subjected to microsatellite genotyping. A set of microsatellite loci is initially selected based on prior validation studies. Final loci used for analysis are chosen based on consistent amplification, clear allele profiles, and minimal genotyping errors.

2. PCR Amplification

PCR reactions are prepared using standard thermal cycling protocols. Amplification success is verified via agarose gel electrophoresis. Only clearly amplified products are carried forward for further analysis.

3. Genotyping and Allele Scoring

Fluorescence-based genotyping is conducted using a capillary electrophoresis system (e.g., Genetic Analyzer). Alleles are scored using dedicated software such as Gene Mapper, ensuring consistency and accuracy in allele calling.

4. Locus Filtering and Selection for Analysis

A subset of loci with high amplification success, low genotyping error, and suitable amplicon size is selected for individual identification. Loci are also assessed for polymorphism levels and discriminatory power.

5. Individual Identification and Genetic Diversity Estimation

The number of unique individuals is estimated by evaluating multilocus genotypes. Various genetic diversity indices are calculated, including:

- Observed alleles (Na)
- Effective alleles (Ne)
- Observed heterozygosity (Ho)
- Expected heterozygosity (He)
- Inbreeding coefficient (F)

6. Population Structure Analysis

To determine population structure, both Bayesian (e.g., STRUCTURE) and non-Bayesian (e.g., DAPC) clustering methods are employed. Bayesian analysis is performed with multiple assumed population clusters (K), and the optimal K value is determined using an ad hoc quantity method (ΔK).

7. Assignment of Individuals to Clusters

Individuals are assigned to inferred clusters based on their membership coefficient (q). A threshold (e.g., $q \geq 0.80$) is applied to distinguish purebred individuals from admixed ones.

4.3. Estimation of the diet preference of *Panthera tigris* samples collected from Corbett Reserve.

Requirement:

- Sieve
- Gelatin
- Forceps
- Dropper
- Xylene
- Slides
- Coverslips
- Ziplock

Methodology:

- Scat samples were taken and washed under water stream in tap water to remove the soil and debris from the samples and the diet content including bones, hairs, hooves are left in the sieve.
- Then the samples were spread on paper and kept in hot air oven to dry out the water from the samples.
- Then bones, hairs and hooves are separated and identified which could be visually identified.
- Hairs present in samples were visually observed on the basis of color, strength and texture.

Species identification on the basis of Hair morphology-

Cuticular pattern:

- Slides were kept on clean space and marked according to sample ID.
- Layer of gelatin spread over the slide with the help of spreader.
- Hairs were kept over it with the help of forceps.
- The slides were kept aside to dry out for some time (1-2 hours).
- After drying the hairs were plucked out form the slide.
- The slides were observed under microscope and images taken to match the references.

Medullary Pattern:

- The hair samples were chopped and dipped overnight in xylene in petri plates.
- Samples were placed on slides with the help of forceps and 2-3 drops of xylene dropped over hairs.
- Glycerin dropped over it with the help of dropper so that coverslip could adhere properly on the slide.
- Coverslip placed gently over the slide, ensuring that the bubbles do not form.
- The slides were observed under microscope and images taken to match the references.

CHAPTER 5: RESULT AND DISCUSSION

4.1. Identification of species of scat samples collected from field and Corbett Tiger Reserve.

Out of the 68 scat samples collected from various field locations, 36 of them were in condition from which DNA could be extracted, a significant number showed successful DNA amplification, indicating that the extraction protocol was efficient and the samples were in usable condition. From these, 22 samples were successfully sequenced and identified, representing multiple carnivore species.

From the Corbett Tiger Reserve, a total of 11 samples were positively identified as *Panthera tigris* (Tiger) through sequencing among 35 samples collected from different locations. This molecular confirmation of tiger presence validates the accuracy of field identification and the effectiveness of targeted scat collection strategies in protected tiger habitats.

Here, mitochondrial DNA sequencing is done for identification, *cytb* primer and *16S* primer is used for amplification of DNA for Field and Corbett Tiger Reserve collected samples respectively. Very few samples have given results among all samples from which DNA is extracted because these all are scat samples and they contain degraded DNA in them, as mitochondrial DNA sequences are large in size so many samples showing smearing as they are degraded. Later more samples showing positive results when amplified with microsatellite primers as microsatellite primer sequences are small in size so even in degraded DNA small sequences are easily amplifiable.

- Sample collection

  Table 3: Samples collected from field -

S. No.	ID	DOC	Latitude	Longitude	Elevation
1	JK01	20-01-2024	21.11415	71.00665	261m
2	JK02	31-12-2023	21.12151	71.023508	228m
3	JK03	02-01-2024	21.09006	71.0711	198m
4	JK04	31-12-2023	21.11886	71.01935	259m
5	JK05	11-01-2024	21.06671	71.00317	200m
6	JK06	31-12-2023	21.11969	71.02971	224m
7	JK07	31-12-2023	21.1198	71.02069	254m

8	JK08	31-12-2023	21.12124	71.02319	234m
9	JK09	08-01-2024	21.093	71.03847	189m
10	JK10	01-01-2024	21.0828	70.99505	231m
11	JK11	08-01-2024	21.093	71.03847	189m
12	JK12	08-01-2024	21.093	71.03847	189m
13	JK13	30-05-2024	21.0421	70.93199	142m
14	JK14	31-12-2023	21.11143	71.03703	264m
15	JK15	29-05-2024	20.99058	71.00824	
16	JC01	31-12-2023	21.11088	71.02943	231m
17	JC02	20-04-2024	21.11116	71.06584	200m
18	JC03	11-01-2024	21.06625	70.99714	217m
19	JC04	31-12-2023	21.11981	71.02066	253m
20	JC05	29-05-2024	21.0008	71.02319	134m
21	JC06	29-05-2024	21.00014	71.02882	130m
22	JC07	07-01-2024	21.12109	70.99109	268m
23	JC08	20-01-2024	21.11832	71.0318	223m
24	JC09	28-05-2024	21.0036	71.05324	96m
25	JC10	07-01-2024	21.12118	70.99081	25m
26	JC11	27-12-2023	21.07558	71.01125	238m
27	JC12	20-04-2024	21.11201	71.06551	194m
28	JC13	31-12-2023	21.11966	71.01696	234m
29	JC14	31-12-2023	21.11886	71.01935	259m
30	JC15	30-05-2024	21.06187	70.94917	183m
31	JC16	30-05-2024	21.06187	70.94917	183m
32	JC17	31-02-2023	21.11968	71.0167	238m
33	JC18	29-12-2023	21.09424	70.99286	243m
34	JC19	31-12-2023	21.12266	71.03492	267m
35	JC20	11-01-2024	21.06685	71.00095	201m
36	JC21	27-12-2023	21.07558	71.01125	238m
37	JC22	30-055-24	21.06187	70.94917	183m
38	JC23	08-01-2024	21.09274	71.04657	211m
39	JC24	08-01-2024	21.09188	71.04498	198m
40	JC25	07-01-2024	21.0965	70.99246	245m
41	JC26	29-12-2023	21.11027	71.04743	241m
42	JC27	31-12-2023	21.11981	71.01372	273m

43	JC28	31-12-2023	21.1198	71.02069	254m
44	JC29	20-04-2024	21.09325	71.06015	192m
45	JC30	20-04-2024	21.11162	71.06637	204m
46	JC31	20-04-2024	21.09404	71.05856	201m
47	JC32	19-04-2024	21.07951	71.04994	190m
48	JC33	20-04-2024	21.11148	71.06626	204m
49	JC34	20-04-2024	21.11148	71.06626	204m
50	JC35	03-01-2024	21.06548	71.02843	176m
51	JC36	19-04-2024	21.07924	71.05238	188m
52	JC37	31-12-2023	21.11173	71.0157	250m
53	JC38	31-12-2023	21.11889	71.01085	282m
54	C01	20-01-2024	21.114	71.06672	268m
55	C02	20-01-2024	21.114	71.06672	268m
56	C03	20-01-2024	21.1141	71.0066	260m
57	M01	11-01-2023	21.0692	71.02573	182m
58	M02	28-05-2024	21.00407	71.05438	108m
59	M03	20-01-2024	21.114	71.06672	268m
60	M04	20-01-2024	21.114	71.06672	268m
61	M05	02-01-2024	21.08645	71.03506	197m
62	RS01	27-12-2023	21.07573	71.00753	210m
63	RS02	18-04-2024	21.11994	71.1006	250m
64	RS03	11-01-2024	21.07927	71.02861	182m
65	RS04	20-01-2024	21.1141	71.0066	260m
66	RS05	01-01-2024	21.08481	71.03118	182m
67	RS06	19-01-2023	21.12084	71.01712	236m
68	RS07	08-01-2024	21.0848	71.03121	182m

Table 4: Samples of Corbett Tiger Reserve-

S.NO	UID	DOC	RANGE	BEAT	LAT	LONG
1	JC01	3.02.25	KALAGARH	LALDKHT	29.49167	78.78333
2	JC02	3.02.25	DHIKALA	PATERPANI	29.51685	78.82667
3	JC03	4.02.25	KALAGARH	BUXAD	29.54417	78.815
4	JC04	4.02.25	SARPDULI	DHANGADHI	29.51461	79.10505
5	JC05	5.02.25	DHELA	SAVALDEH	29.38612	79.04979

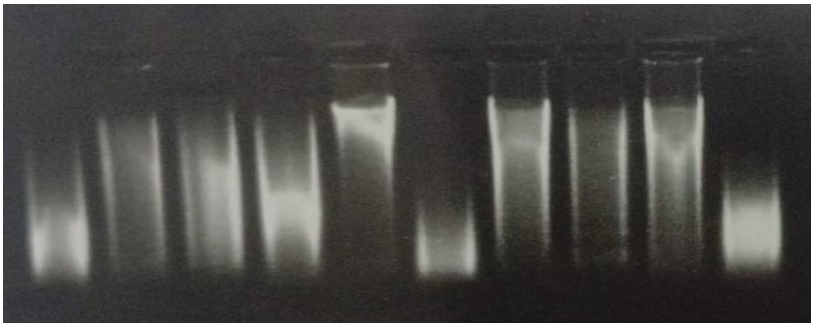
6	JC06	5.02.25	DHELA	HALDUKKHEDA	29.4535	79.01432
7	JC07	5.02.25	DHELA	HALDUKHEDA(E)	29.4462	79.00923
8	JC08	5.02.25	DHELA	HALDUKHEDA	29.45393	79.0148
9	JC09	5.02.25	DHELA	HALDUKHEDA(E)	29.4493	79.00994
10	JC10	5.02.25	JHIRNA	MACHIYAKHAL	29.45666	78.93896
11	JC11	5.02.25	JHIRNA	LALDKHT	29.44614	78.94316
12	JC12	5.02.25	DHELA	HALDUKHEDA(E)	29.44633	79.00927
13	JC13	25.02.25	DHIKALA	SHURNKHAL	29.56225	78.85261
14	JC14	25.02.25	DHIKALA	SHURNKHAL	29.56944	78.85333
15	JC15	25.02.25	DHIKALA	CHAURH	29.57783	78.85392
16	JC16	25.02.25	DHIKALA	CHAURH	29.58458	78.84503
17	JC17	18.02.25	DHIKALA	FULAI	29.62239	78.89156
18	JC18	25.02.25	DHIKALA	FULAI	29.60869	78.89658
19	JC19	18.02.25	DHIKALA	FULAI	29.60992	78.89761
20	JC20		SARPDULI	DHANGADHI	29.52989	79.06847
21	JC21		DHIKALA	SULTAN (2)	29.53889	79.03056
22	JC22		DHIKALA	SULTAN (2)	29.54183	79.00649
23	JC23		DHIKALA	SULTAN (1)	29.53583	79.06139
24	JC24	25.02.25	DHIKALA	DHANGADHI	29.49825	79.10589
25	JC25	12.02.25	SARPDULI		29.94178	80.49454
26	JC26	25.03.25	DHIKALA	SULTAN	29.50047	79.01522
27	JC27	25.03.25		KASREBA	29.46722	79.03894
28	JC28		BIJRANI	BIJRANI (S)	29.43202	79.09819
29	JC29		BIJRANI	BIJRANI (S)	29.44209	79.09347
30	JC30	25.03.25		JHABAR	29.46147	79.0565
31	JC31	25.03.25		TOWER CHAUKI (E)	29.48625	79.05299
32	JC32	25.03.25		TOWER CHAUKI(N)	29.49117	79.05256
33	JC33	25.03.25		TOWER CHAUKI (E)	29.48494	79.05489
34	JC34	25.03.25		TOWER	29.49328	79.04767

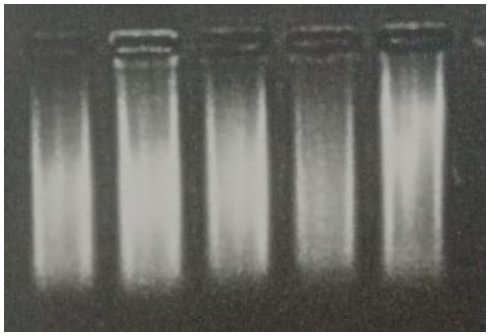
				CHAUKI(N)		
35	JC37				29.44209	79.09347

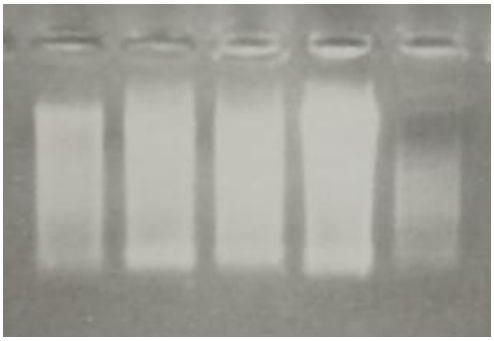
- DNA extraction and Quantification:

Samples collected from field

Among 68 samples, 36 samples were in condition whose DNA could be extracted. Fig. 27, 28, 29 and 30 showing results of gel electrophoresis of 36 samples whose DNA was successfully extracted.







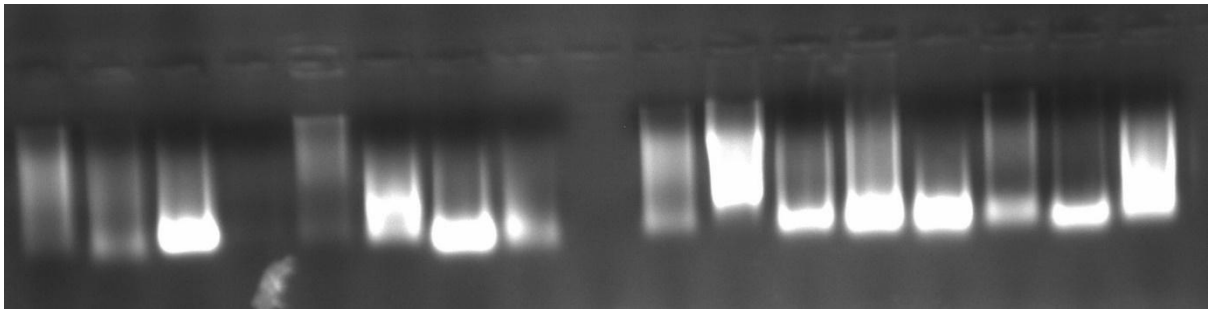


Fig. 27,28,29,30. Electrophoresis gel results of 36 samples extracted from samples collected from field

Samples collected from Corbett Tiger Reserve

Among 35 collected samples, 27 were in condition whose DNA could be extracted. Fig. 31 showing Electrophoresis gel results of 27 samples extracted from samples collected from Corbett Tiger Reserve

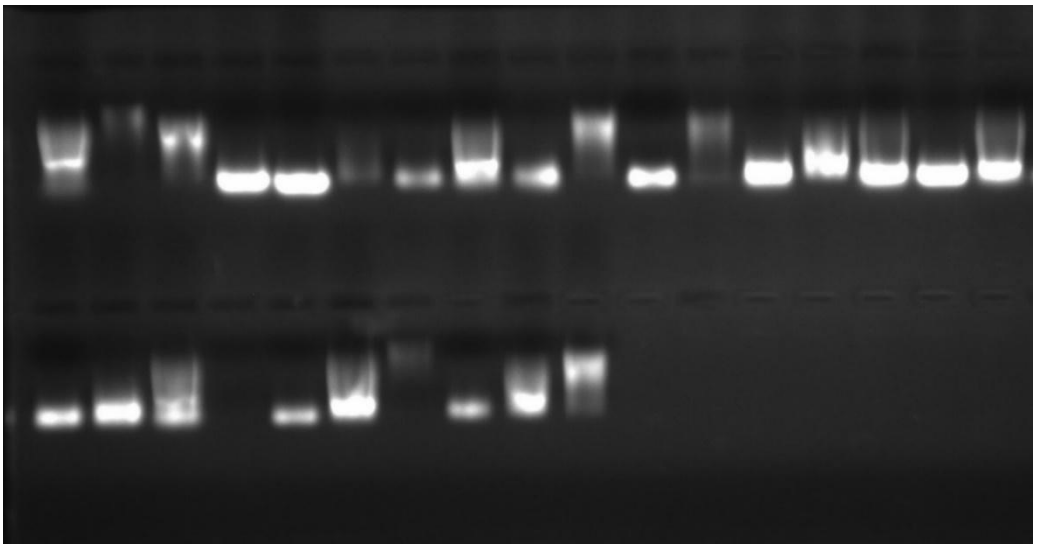


Fig. 31. Electrophoresis gel results of 27 samples extracted from samples collected from Corbett Tiger Reserve

- Amplification: PCR

Results of gel electrophoresis of PCR products of 36 DNA samples extracted from scat samples collected from field, among these 22 were identified in sequencing. Fig. 32 showing Gel electrophoresis results of PCR products of 36 DNA Samples extracted from field collected scat samples.

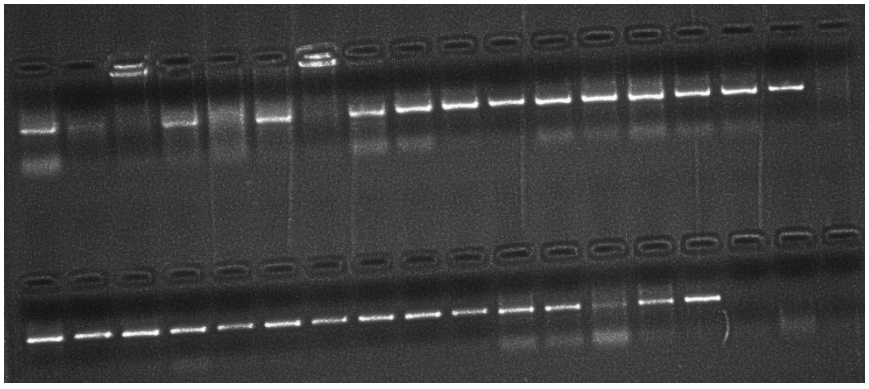


Fig.32. Results of gel electrophoresis of PCR products of DNA samples extracted from field collected scat samples.

Results of gel electrophoresis of PCR products of 27 DNA samples extracted from scat samples collected from Corbett Tiger Reserve, among these 11 were identified in sequencing.

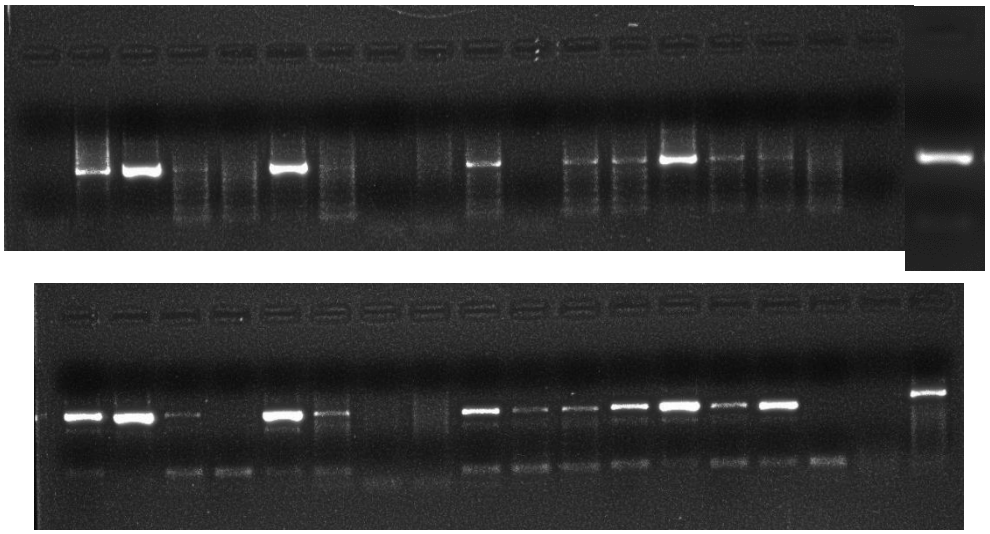


Fig.33. PCR results of gel electrophoresis of 27 DNA samples collected from Corbett Tiger Reserve

- DNA Sequencing

22 sample species were identified in sequencing of field collected samples among 36 of total extracted DNA samples and 11 samples species were confirmed to be tiger in sequencing of Corbett tiger reserve samples among 27 of total extracted DNA samples.

- Data analysis

Table 5: Species are identified with associated species ID's, these are field collected samples, Cyt *b* gene used:

Among 68 collected samples 36 were in condition to extract DNA from them and later following 22 were identified by sequencing.

S.NO	ID	SPECIES	COMMON NAMES
1	JC01	*Felis chaus*	Jungle cat
2	JC06	*Felis chaus*	Jungle cat
3	JC07	*Felis chaus*	Jungle cat
4	JC08	*Felis chaus*	Jungle cat

5	JC09	*Felis chaus*	Jungle cat
6	JC36	*Felis chaus*	Jungle cat
7	JC37	*Felis chaus*	Jungle cat
8	JC19	*Felis chaus*	Jungle cat
9	JC24	*Felis chaus*	Jungle cat
10	JC03	*Felis chaus*	Jungle cat
11	JC05	*Felis chaus*	Jungle cat
12	JC21	*Felis silvestris*	Jungle cat
13	JC25	*Felis chaus*	Wild cat
14	RS01	*P. Rubiginosus*	Rusty-spotted cat
15	RSO3	*P. Rubiginosus*	Rusty-spotted cat
16	JK05	*Canis aureus*	Golden jackal
17	JK19	*Canis aureus*	Golden jackal
18	JK15	*Canis aureus*	Golden jackal
19	MO3	*Herpestes smithii*	Mangoose
20	MO4	*Herpestes smithii*	Mangoose
21	C01	*Herpestes smithii*	Mangoose
22	C02	*Herpestes smithii*	Mangoose

Table 6: Species are identified with associated sample ID's, the samples were collected from Corbett Tiger Reserve, *16S* gene used

Among 35 collected samples 27 were in condition to extract DNA from them and later following 11 were identified by sequencing.

S.NO.	ID	SPECIES	COMMON NAMES
1	JC01	*Panthera tigris*	Tiger
2	JC02	*Panthera tigris*	Tiger
3	JC03	*Panthera tigris*	Tiger
4	JC04	*Panthera tigris*	Tiger
5	JC05	*Panthera tigris*	Tiger
6	JC06	*Panthera tigris*	Tiger

7	JC07	*Panthera tigris*	Tiger
8	JC10	*Panthera tigris*	Tiger
9	JC13	*Panthera tigris*	Tiger
10	JC17	*Panthera tigris*	Tiger
11	JC21	*Panthera tigris*	Tiger

4.2. Genetic diversity and population genetics of *Panthera tigris* samples collected from Corbett Reserve.

Partial 16S sequences were successfully obtained from 27 scat samples, 11 of which matched *Panthera tigris* in a BLAST search against the NCBI GenBank database, confirming species identity. Among all these, consistent amplification (≥60% success) was achieved for six microsatellite loci, which were retained for downstream analysis (Table. 7). These loci exhibited high informativeness, with polymorphic information content (PIC) values exceeding 0.5.

The genetic result indicated that the population of tiger had a modest amount of genetic diversity, as showed through a mean number of alleles per locus (Na = 4.833), a Shannon's index (I = 1.243). All loci were polymorphic (100%), then effective alleles (Ne = 3.177, which explained that there are a maximum of different alleles and a high of genetic variation that may showed. The observed heterozygosity (Ho = 0.248) was minimum than the predicted heterozygosity value (He = 0.656), which led to a high average fixation index (F = 0.643). There may be high of inbreeding in tiger population, substructuring (perhaps due to the Wahlund effect), or non-random mating in the population, as indicated by this lack of heterozygocity. Notably, two loci (PttF1-A and PttC6-A) exhibited extremely high fixation (F = 1.000), indicating the low absence of heterozygotes. F result were moderate to high for other loci as well. Followed by, a large amount of data was missing at loci FCA304-A (40%) and PttF4-A (45%) which may reduce the observed heterozygosity values and impair the

reliability of diversity estimations. It is preferable to eliminate loci with more than 25% missing data in order to improve the accuracy of downstream analyses. Although the overall the population is genetically diverse of tiger species, the statistics result suggested that there due to a genetic structure and potential inbreeding that warrants further investigation.

Bayesian clustering analysis in STRUCTURE information of the presence of three distinct genetic clusters of tiger samples, as indicated by the highest ΔK value at K = 3 (Fig. 34). The genetic structure of individuals analysis based on a model-based clustering method—likely produced through STRUCTURE software is showed by the bar plot. A single individual is represented by each vertical bar, and the colours inside each bar indicate the percentage of that individual's genome that is allocated to various genetic clusters. Three different colours—purple, orange and blue—indicate that K = 3 genetic clusters were used to analyse the dataset. The blue and orange clusters make up most of the mixed ancestry of individuals on the left and right sides of the plot. The purple cluster only adds a little bit. Individuals in the middle of the plot, on the other hand, have a strong genetic link to the purple cluster, which suggests that there is a separate population group. There is gene flow and admixture between the clusters because the colour proportions are different for each group. Overall, the plot shows that there are three genetically different groups of individuals and that there is some genetic mixing between them. Based on DAPC genetic clustering analysis shows that the individuals that were sampled fall into three different groups. The group at the bottom left (Cluster 1) has almost all of its members in one cluster, which means that the population is genetically similar and has very little mixing. Individuals on the bottom right (Cluster 2), on the other hand, are firmly linked to a different cluster, which suggests that they are part of a genetically diverse group. Individuals who are in the center or spread out throughout the plot, on the other hand, show evidence of mixing, meaning they belong to both Clusters 1 and 2 and may perhaps include genes from a third source called "All others." This pattern of distribution shows that there is both significant genetic separation and gene flow or historical mixing across groups.

Table 7: Results of Microsatellite Markers, the Species is Tiger-

S.no	ID	PttF1–A		PttF4-A		FCA304-A		PttC6-A		PttA2-A		PttG4-A	
1	JC01	125	125	0	0	130	132	174	174	244	244	120	120
2	JC02	122	122	0	0	130	138	172	172	244	244	117	117
3	JC03	122	122	0	0	130	138	172	172	240	240	120	120
4	JC04	122	122	0	0	0	0	170	170	240	240	117	117
5	JCO5	120	120	0	0	130	130	170	170	240	240	117	117
6	JC06	120	120	0	0	130	138	170	170	240	240	117	117
7	JC07	120	120	194	194	128	136	170	170	242	242	117	117
8	JC10	122	122	194	194	0	0	172	172	242	242	117	117
9	JC13	122	122	196	196	132	132	176	176	0	0	120	122
10	JC14	122	122	0	0	0	0	172	172	242	242	117	122
11	JC16	120	120	196	196	0	0	0	0	242	248	117	117
12	JC18	120	120	196	196	0	0	172	172	242	248	117	122
13	JC19	0	0	194	194	136	136	170	170	248	248	117	122
14	JC20	120	120	0	0	132	136	170	170	248	248	117	122
15	JC21	120	120	0	0	128	128	172	172	242	242	0	0
16	JC25	122	122	196	196	128	136	170	170	250	250	117	122
17	JC27	122	122	200	202	130	130	170	170	246	246	118	124
18	JC28	120	120	194	196	0	0	172	172	244	244	118	124
19	JC29	0	0	0	0	0	0	172	172	0	0	117	122
20	JC30	122	122	196	196	130	138	0	0	252	252	117	120
21	JC32	120	120	194	196	0	0	170	170	242	242	116	120
22	JC34	120	120	194	194	0	0	172	172	242	242	120	120

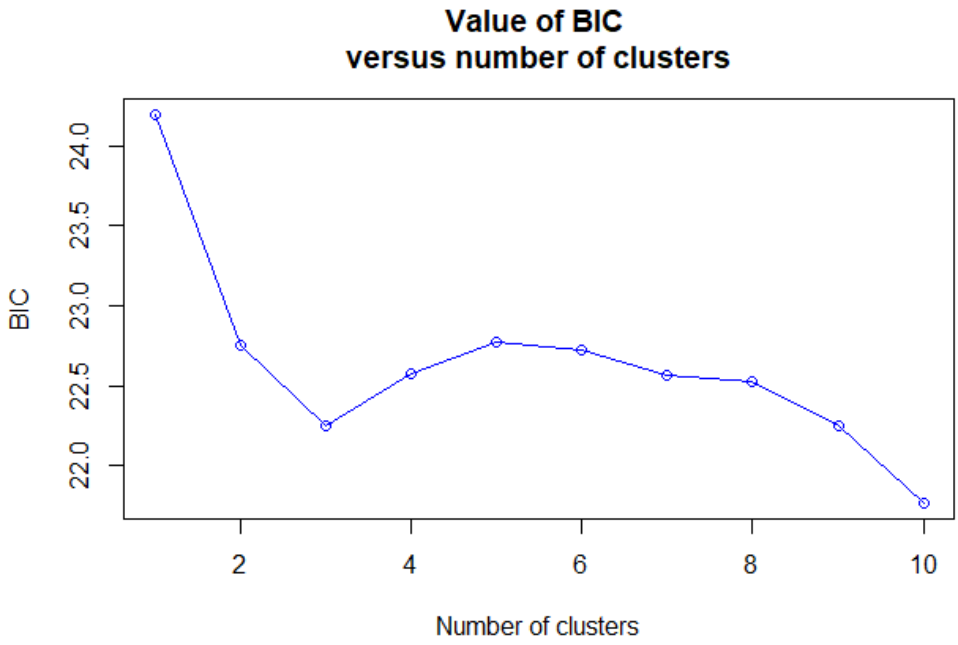


Fig. 34**.** BIC value of Non-Bayesian pattern of Tiger indicated the low BIC value in 3 used in DAPC.

Fig. 35. Tiger population clustering patterns using Bayesian clustering at K3 and population-level assignment; Mean L (K), A (i) (ii). For every K value, the ad hoc quantity (delta K) is calculated across 20 runs. STRUCTURE v 2.3.4 was used to conduct the structural analysis.

DAPC Structure-

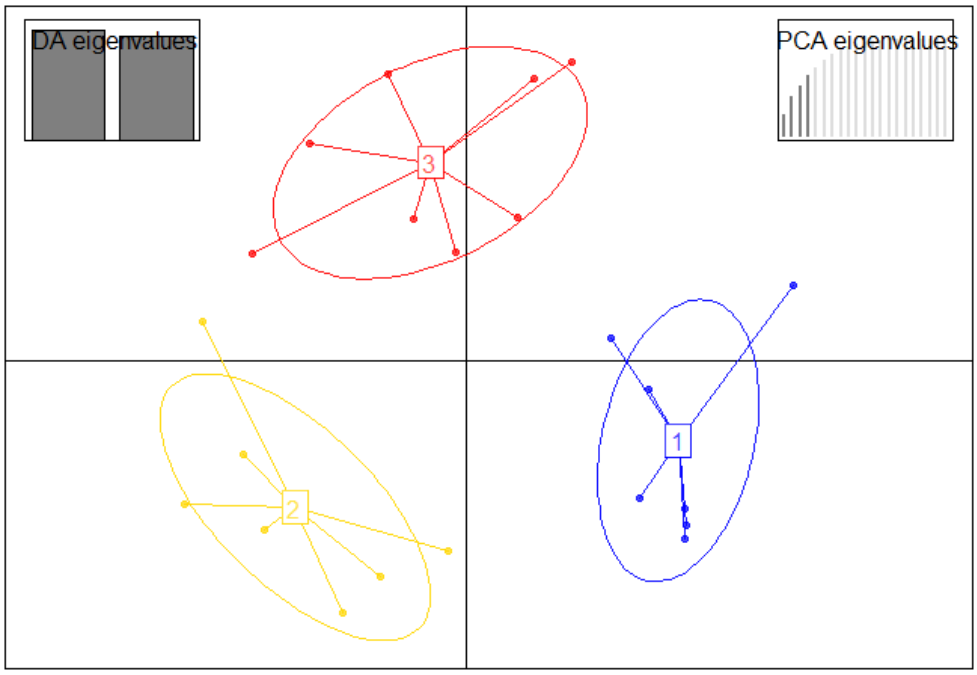


Fig. 36. Non-Bayesian pattern of Tiger with DAPC.

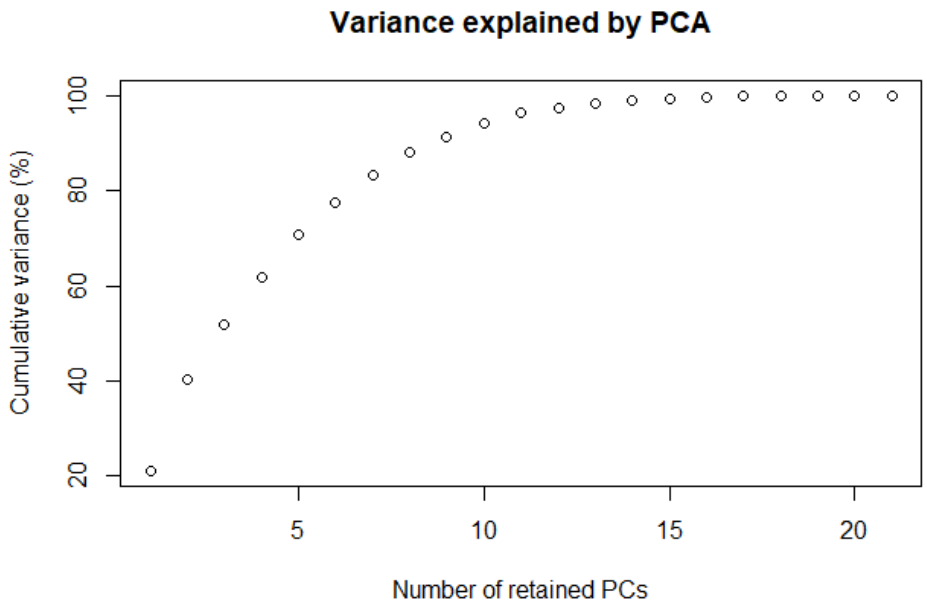


Fig. 37. DAPC result showed variance explained by PCA

Table 8: Sample Size, No. Alleles, No. Effective Alleles, Information Index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, and Fixation Index.

Locus	N	Na	Ne	I	Ho	He	uHe	F
PttF1 -A	20	3.000	2.198	0.856	0.000	0.545	0.559	1.000
PttF4-A	12	4.000	2.341	0.976	0.250	0.573	0.598	0.564
FCA304-A	13	5.000	4.390	1.548	0.615	0.772	0.803	0.203
PttC6-A	20	4.000	2.439	1.018	0.000	0.590	0.605	1.000
PttA2-A	20	7.000	4.651	1.708	0.100	0.785	0.805	0.873
PttG4-A	21	6.000	3.041	1.354	0.524	0.671	0.688	0.220
Mean	17.667	4.833	3.177	1.243	0.248	0.656	0.676	0.643
SE	1.647	0.601	0.442	0.141	0.109	0.042	0.044	0.151

Na = No. of Different Alleles

Ne = No. of Effective Alleles = 1 / (Sum pi^2)

I = Shannon's Information Index = -1* Sum (pi * Ln (pi))

Ho = Observed Heterozygosity = No. of Hets / N

He = Expected Heterozygosity = 1 - Sum pi^2

uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He

F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)

Where pi is the frequency of the ith allele for the population & Sum pi^2 is the sum of the squared population allele frequencies.

Result: Low Genetic Diversity (Ho>He), but moderate interbreeding found in genetic population.

4.3. Estimation of the diet preference of *Panthera tigris* samples collected from Corbett Reserve

Table 9: Scat content on the basis of percentage of Hairs, Bones, Hoofs and Others

S. NO.	UID	Image	Scat Content	Hairs %	Bones %	Hoofs %
1	JC01		Hairs/Others	99.5	-	-
2	JC02		Hairs/Bones/Others	95.4	4.1	-
3	JC03		Hair/Bones/Hoofs/ Others	94.3	2.6	2.6
4	JC04		Hairs/Others	62.3	-	-

5	JC05		Hairs/Bones/Others	96	3.8	-
6	JC06		Hairs/Bones/Others	95.6	-	3.9
7	JC07		Hair/Bones/Hoofs/ Others	94	4	1.5
8	JC08		Hairs/Bones/Others	95	2.5	2
9	JC10		Hairs/Bones/Others	94.8	4.7	

10	JC11		Hairs/Bones/Others	93.8	5.5	-
11	JC13		Hairs/Bones/Others	97	2.5	-
12	JC14		Hairs/Bones/Others	96.5	3	-
13	JC15		Hairs/Bones/Others	95.5	4	-
14	JC16		Hairs/Others	99.5		-

15	JC18		Hairs/Others	99.5	-	-
16	JC19		Hairs/Others	99.5	-	-
17	JC20		Hairs/Others	48.8	-	-
18	JC21		Hairs/Bones/Others	92.9	6.2	-
19	JC23		Hairs/Bones/Others	2.9	5.8	-

20	JC25		Hairs/Bones/Others	97.1	2.6	-
21	JC27		Hairs/Bones/Others	98	1.8	-
22	JC28		Hair/Bones/Hoofs/ Others	94	3.5	2.3
23	JC30		Hairs/Bones/Others	94.5	5	-
24	JC33		Hair/Bones/Hoofs/ Others	96	2.8	1

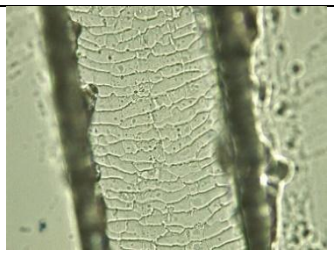
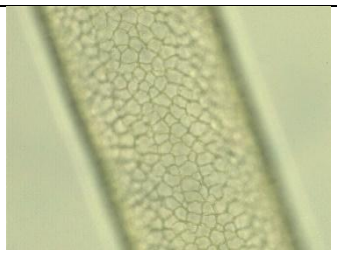
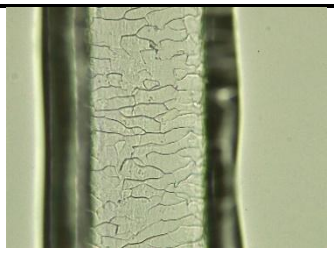
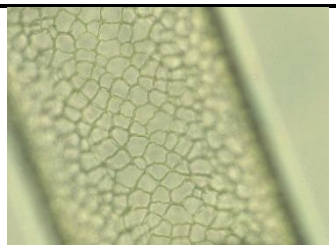
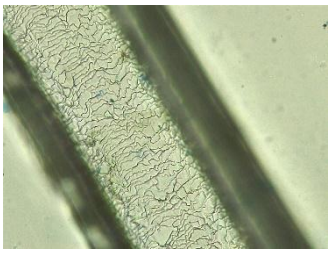
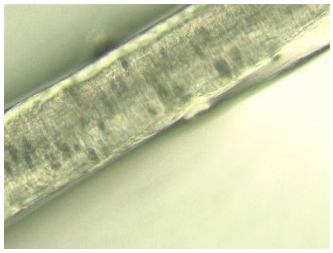
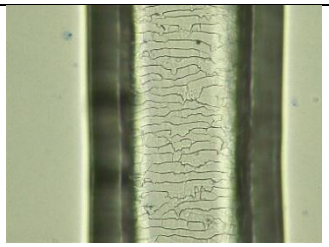
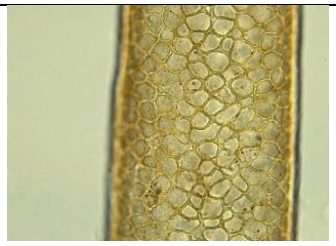
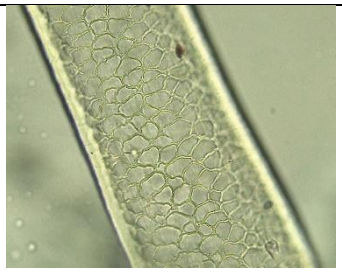
25	JC34		Hair/Bones/Hoofs/ Others	95.2	-	-
26	JC37		Hair/Bones/Hoofs/ Others	98.2	-	-

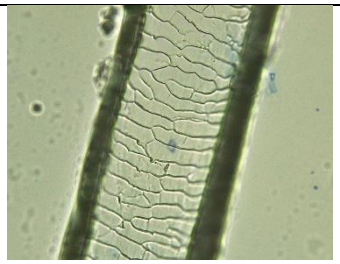
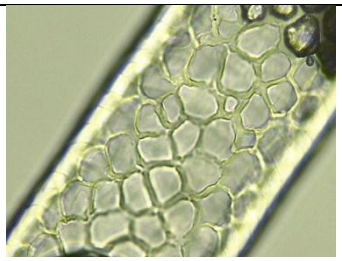
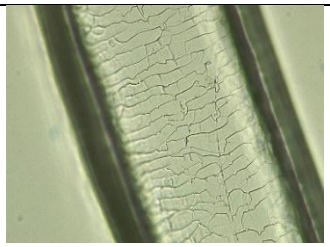
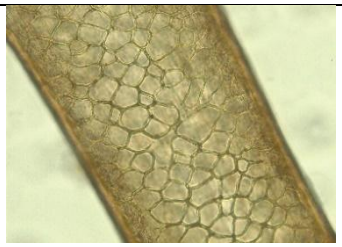
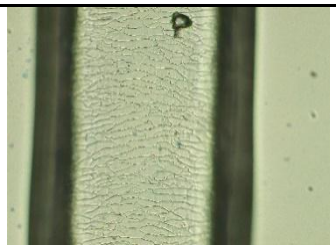
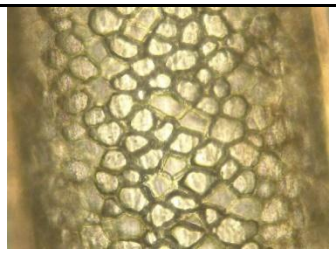
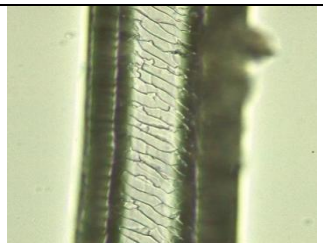
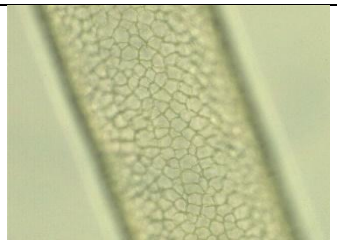
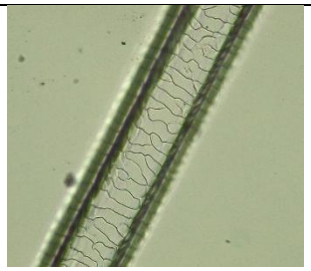
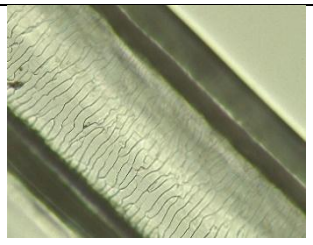
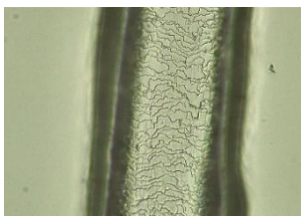
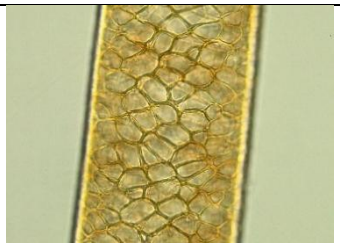
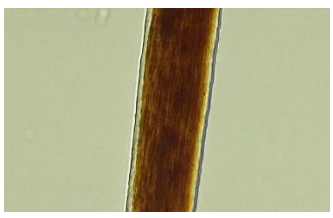
Table 10. Classification of hairs on the basis of Colour, Strength and Texture:

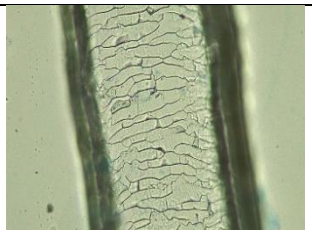
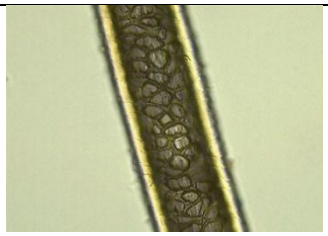
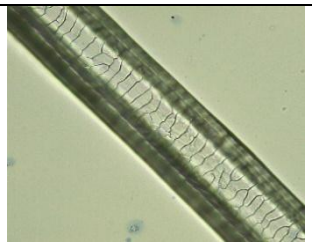
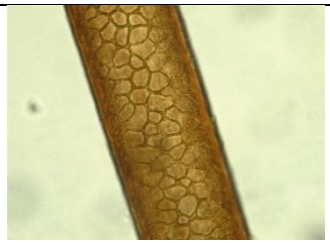
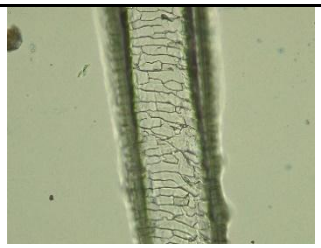
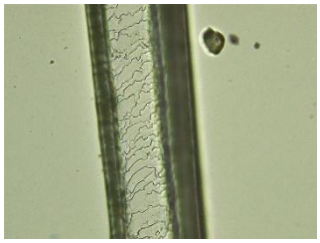
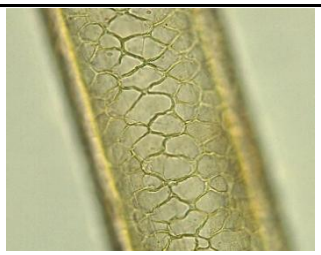
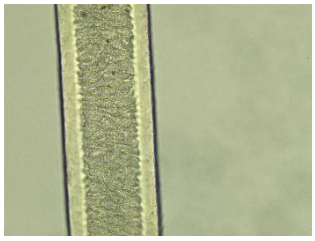
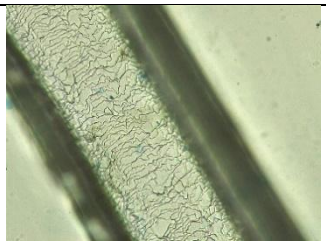
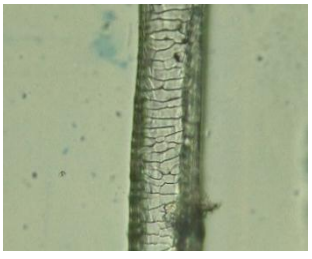
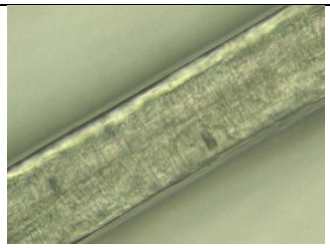
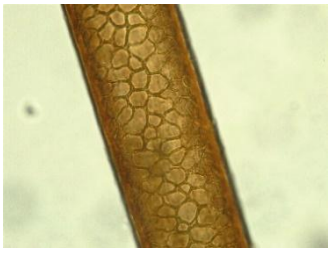
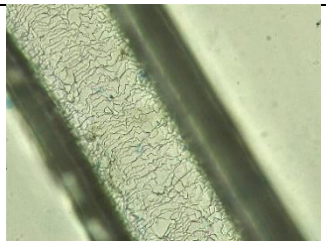
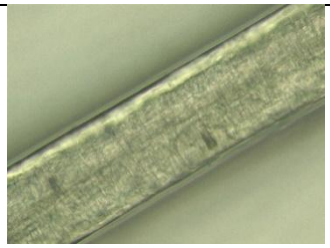
S. No.	Sample ID	Colour	Strength	Texture
1	JC01	White and brown mainly but few black also	Mainly medium and few very thin	Medium
2	JC02	White and brown mainly but few black also	Medium and very thick	Strong
3	JC03	White and brown mainly but very few black also	Mainly thin, few could be medium	brittle
4	JC04	Mainly white, few brown	Thin and few medium	Brittle to medium
5	JC05	Brown and white mainly, few black	Thin and few medium	Brittle
6	JC06	Brown and white mainly, few black	Mainly medium and very thin also	Brittle and medium
7	JC07	White and brown mainly but few black also	Medium and very thick	Strong
8	JC08	Brown and white mainly, few black	Thin and few medium	Brittle
9	JC10	Brown and white mainly, few black	Thin and few medium	Brittle

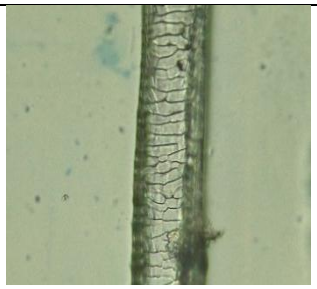
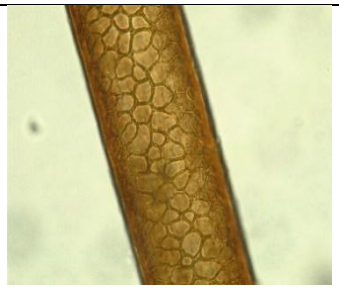
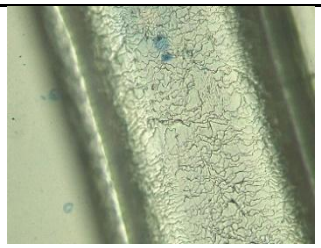
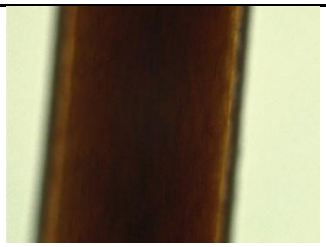
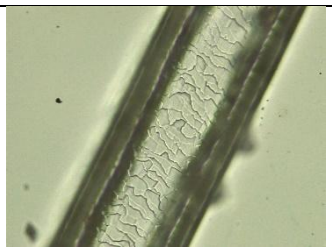
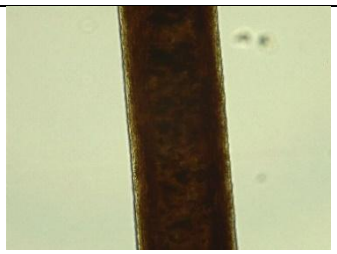
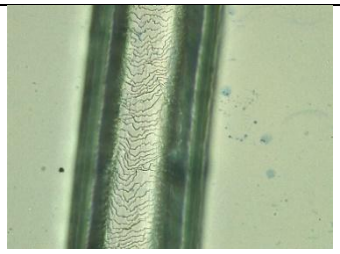
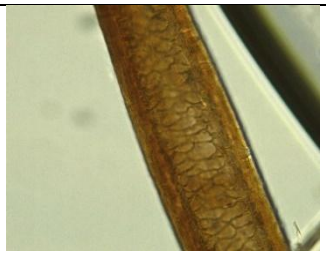
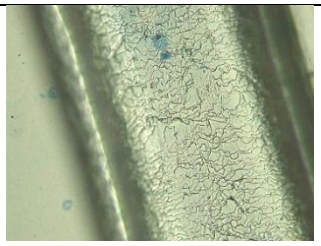
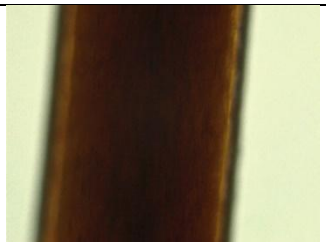
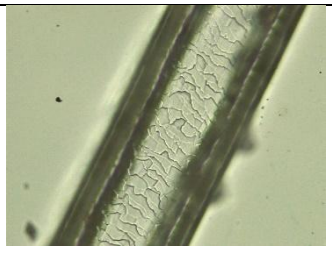
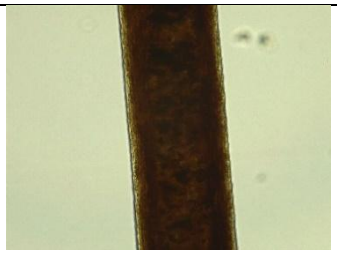
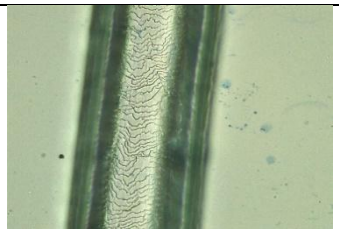
10	JC11	White, reddish brown and few black	Medium and very thin	Brittle
11	JC13	White and Brown	Thin and Medium	Brittle
12	JC14	White and brown mainly but very few black also	Mainly thin, few could be medium	brittle
13	JC15	White and brown mainly but few black also	Mainly medium and few very thin	Medium
14	JC16	Black, white and brown also	Very thick	Very strong
15	JC18	Black, white and brown also	Very thick	Very strong
16	JC19	Dark brown, very few white and black	Very thin	Medium
17	JC20	Mainly white, few brown	Thin	Brittle
18	JC21	White and brown mainly, but very few black also	Medium (Black one very thick)	Brittle and medium
19	JC23	Mainly white, brown, few black	Thin to medium	Medium and fragile
20	JC25	White and brown mainly but very few black also	Mainly thin, few could be medium	brittle
21	JC27	Brown and white mainly, few black	Thin and few medium	Brittle
22	JC28	Mainly brown and white	Thin, thick and few medium	Medium
23	JC30	White and brown mainly but very few black also	Mainly thin, few could be medium	brittle
24	JC33	White and brown mainly but few black also	Mainly medium and few very thin	Medium
25	JC34	Mainly brown and white also	Mainly thick, few medium	Brittle
26	JC37	Brown, Black and White	Medium and thick	Strong and brittle

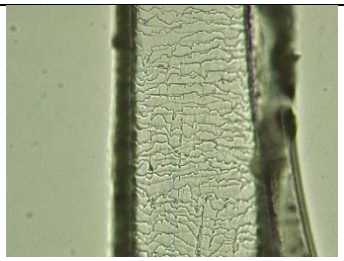
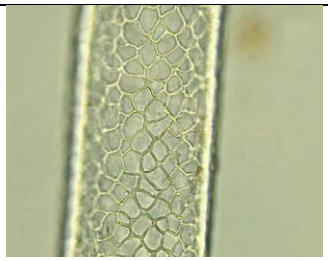
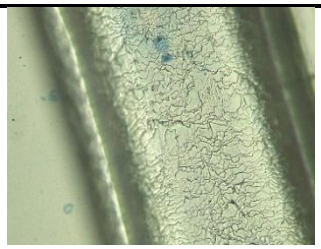
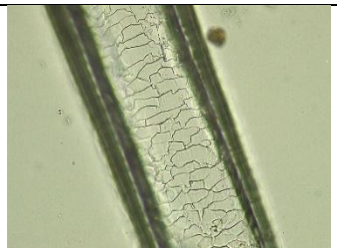
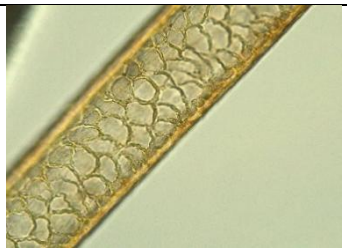
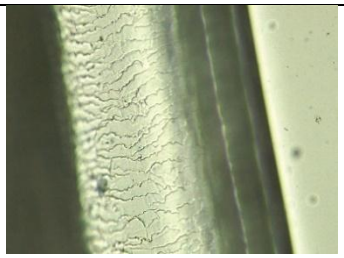
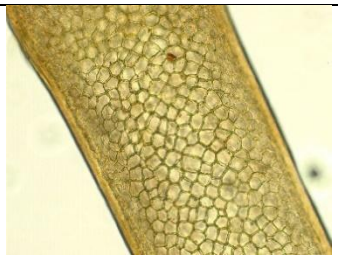
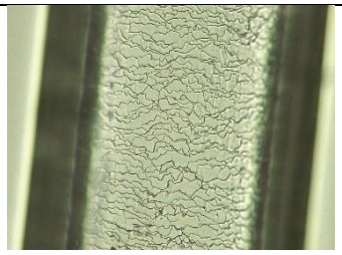
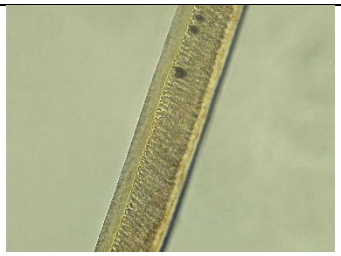
Table 11. Identification of species on the basis of Hair Morphology:

S. No.	Sample ID	Cuticlular Pattern	Medullary Pattern	Species
1	JC01			Sambar *(Rusa unicolor)*
2	JC02			Chital *(Axis axis)*
				Wild pig *(Sus scrofa)*
3	JC03			Barking deer *(Muntiacus muntjak)*
4	JC04			Chital *(Axis axis)*

5	JC05			Chital *(Axis axis)*
6	JC06			Sambar *(Rusa unicolor)*
7	JC07			Sambar *(Rusa unicolor)*
8	JC08			Chital *(Axis axis)*
9	JC10			Chital *(Axis axis)*
10	JC11	 	 	Barking deer *(Muntiacus muntjak)* Nilgai *(Boselaphus tragocamelus)*

11	JC13			Chital *(Axis axis)*
12	JC14			Chital *(Axis axis)*
13	JC15	 	 	Chital *(Axis axis)* Nilgai *(Boselaphus tragocamelus)*
14	JC16	 	 	Wild Pig *(Sus scrofa)* Sambar *(Rusa unicolor)*
15	JC18			Wild Pig *(Sus scrofa)*

				Sambar *(Rusa unicolor)*
16	JC19			Unidentified
17	JC20			Chital *(Axis axis)*
18	JC21			Chital *(Axis axis)*
19	JC23			Nilgai *(Boselaphus tragocamelus)*
20	JC25			Chital *(Axis axis)*
21	JC27			Chital *(Axis axis)*

22	JC28			Chital *(Axis axis)*
23	JC30			Sambar *(Rusa unicolor)*
24	JC33			Sambar *(Rusa unicolor)*
25	JC34			Sambar *(Rusa unicolor)*
26	JC37			Nilgai *(Boselaphus tragocamelus)*

Results were matched with Bahuguna et al (2010).

- Sambar= 7 samples =26.92%
- Chital =11 samples =42.31%
- Barking deer=2 samples =7.69%
- Wild Pig=3 samples =11.54%
- Nilgae=4 samples =15.38%

Diet shows preference for medium-sized ungulates (Few samples contains 2 species also).

Discussion:

The study adopted an integrative approach to assess carnivore diversity, genetic structure, and feeding ecology in and around Corbett Tiger Reserve using non-invasive techniques. Each objective offered valuable insights into the presence, behavior and conservation needs of the target species, particularly tigers and other sympatric carnivores.

Scat samples collected from multiple field sites, including Corbett Tiger Reserve, revealed a diverse carnivore presence. The most frequently identified species was *Felis chaus* (jungle cat), followed by *Felis catus* (domestic cat), *Prionailurus bengalensis* (leopard cat), and occasional detections of *Panthera pardus* (leopard) and mongooses (*Herpestes urva*, *H. edwardsii*). The prevalence of jungle cat scats suggests its adaptability across varied habitats, including human-influenced zones. The identification of domestic cat DNA hints at anthropogenic interference or feral presence, further emphasizing the need to monitor human-wildlife overlap.

From the Corbett Tiger Reserve specifically, 37 scats were processed, and 11 were genetically confirmed as tiger. The 30% success rate in species confirmation highlights both the efficiency of field staff in scat identification and the utility of non-invasive monitoring in dense forest habitats where direct sightings are rare.

The use of mitochondrial 16S rRNA markers effectively distinguished between closely related carnivores, confirming the robustness of the molecular techniques used. Gel electrophoresis supported the reliability of the extraction process, though a few samples exhibited smearing, likely due to environmental degradation or low DNA concentration.

Microsatellite genotyping across six polymorphic loci revealed moderate genetic diversity within the sampled tiger population. The observed mean number of alleles per locus (Na = 4.833) and Shannon's diversity index (I = 1.243) indicated a reasonably diverse gene pool, although a lower effective allele number (Ne = 3.177) suggested uneven allele distribution. Notably, the observed heterozygosity (Ho = 0.248) was significantly lower than expected (He = 0.656), and a high average fixation index (F = 0.643) indicated substantial inbreeding or population sub-structuring.

Two loci showed complete fixation (F = 1.000), hinting at possible genetic bottlenecks or limited gene flow. Bayesian clustering via STRUCTURE suggested the existence of three genetic clusters (K = 3), supported by DAPC analysis, which revealed two distinct groups and one admixed group. These findings suggest that while some tigers remain genetically isolated, others might still interbreed across home ranges or via ecological corridors.

The dietary analysis of 26 predator scats from Corbett provided insights into feeding behaviour, confirming a carnivorous diet dominated by medium to large herbivores. Microscopic analysis of undigested hair and other remains identified *Axis axis* (Chital) as the most frequent prey, followed by *Rusa unicolor* (Sambar), *Sus scrofa* (wild pig), *Muntiacus muntjak* (barking deer), and *Boselaphus tragocamelus* (Nilgai). The preference for Chital corresponds with its high abundance and availability in the area.

Hair morphology—such as medullary patterns, texture and colour—was critical in species identification, especially between morphologically similar species. The occasional presence of hoof fragments and entire limb remains suggested minimal selectivity for body parts, consistent with the feeding habits of apex predators. The detection of minor “other” materials may represent accidental ingestion or environmental contamination.

This scat-based dietary profiling proves valuable in understanding predator-prey dynamics and guiding conservation efforts. By identifying key prey species, such assessments help prioritize prey population management and habitat conservation strategies.

Overall, this study highlights the strength of non-invasive techniques in wildlife monitoring. From detecting elusive carnivore species to revealing their genetic health and dietary patterns, the multi-objective approach supports data-driven conservation planning in protected ecosystems like Corbett. These insights can aid in managing carnivore populations, maintaining prey availability, and mitigating human-wildlife conflicts.

CHAPTER 6: CONCLUSION

This study demonstrates the robustness and versatility of non-invasive scat analysis as a tool for multi-dimensional wildlife monitoring. Through successful DNA extraction and sequencing from field-collected samples, we were able to identify a diversity of carnivore species, with the Jungle cat emerging as the most commonly detected species across heterogeneous landscapes. These findings highlight both species richness and the adaptability of carnivores in mixed-use landscapes, reinforcing the effectiveness of mitochondrial DNA markers in distinguishing sympatric carnivores.

In the Corbett Tiger Reserve, molecular techniques proved especially valuable in confirming the presence of tigers, with nearly 30% of processed scats being positively identified as *Panthera tigris*. Microsatellite genotyping revealed that while the tiger population in Corbett maintains a good degree of allelic diversity, it also shows signs of inbreeding and population structure, as reflected by high fixation indices and reduced heterozygosity. The presence of three distinct genetic clusters suggests that some level of gene flow persists, possibly facilitated by habitat corridors, though certain groups remain relatively isolated. These insights are vital for shaping future conservation strategies focused on connectivity and population health.

Complementing the genetic data, dietary analysis from Corbett samples revealed a strong predation preference for commonly available herbivores, particularly Chital, followed by Sambar and Wild Pig. The prevalence of hair and bone fragments in scats confirms the carnivorous diet and ecological role of apex predators in the reserve. The identification of prey species through microscopic hair analysis underscores the effectiveness of this method in studying feeding behaviour non-invasively.

Together, these findings not only enhance our understanding of carnivore distribution, genetic health, and dietary preferences, but also emphasize the critical role of prey base availability, habitat integrity, and genetic connectivity in sustaining viable carnivore populations. Integrating such molecular and ecological tools offers a

holistic approach to long-term wildlife conservation and adaptive management in both protected and human-dominated landscapes.

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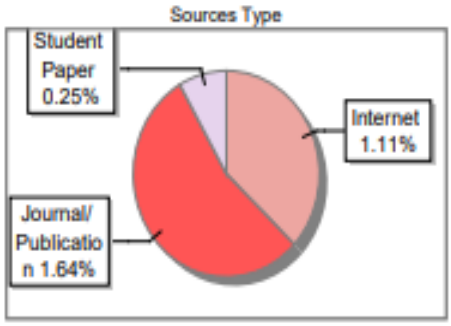

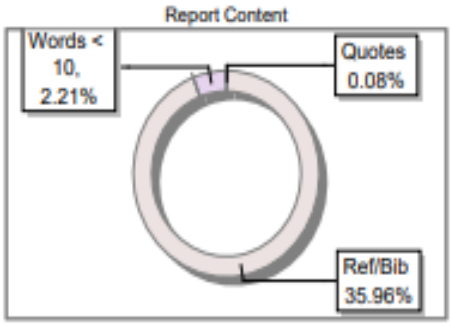

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