The Conservation of Musk Deer: Integrating Ecology and Genetics in North-western Himalaya

Thesis submitted to the
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IN
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Submitted by
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Under the guidance of
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&
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Declaration by the Candidate

I, Amira Sharief, hereby declare that the thesis entitled **“The Conservation of musk deer: Integrating Ecology and Genetics in North-western Himalaya”** submitted for the partial fulfilment of the requirements for the degree of Doctor of Philosophy at Saurashtra University Rajkot, Gujarat, is my original work which has been carried out by me during the period from January 2020 to August 2023 under the guidance of Dr. Ramesh Chinnasamy, Scientist-E, Wildlife Institute of India and Co-supervisors Dr. Lalit Kumar Sharma, Scientist-D and Dr. Mukesh Thakur, Scientist-D, (Zoological Survey of India) and has not been submitted previously for any degree or examination at any other institution. I further declare that all the sources used or referred to in the thesis have been duly acknowledged and cited. Any material used from other sources has been properly attributed and referenced. I also affirm that any contribution made by others to this research work, including research assistants, colleagues, and advisors, has been duly acknowledged in the thesis. I understand that any act of plagiarism or academic dishonesty is a serious offense and may lead to severe consequences, including the rejection of my thesis and disciplinary action by the university. I take full responsibility for the accuracy, originality, and authenticity of the research presented in this thesis. I also accept any responsibility for any errors or omissions that may exist in the work.

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Contents

List of Figures

List of Tables

Executive Summary

This dissertation comprises seven chapters, each contributing to a comprehensive understanding of the study species and its conservation in the North-western Himalayas. The initial chapter serves as a general introduction, providing essential background information about the study species, its ecological significance, and the current conservation challenges it faces. Additionally, the second chapter outlines the study area where the research was conducted, shedding light on the unique characteristics of the region and its importance as a habitat for the species under investigation. Furthermore, the third chapter details the general methodology adopted for conducting the study, highlighting the various tools and techniques employed to gather data and achieve the research objectives.

India, being one of the mega-biodiverse countries, is home to 39 species of ungulates, constituting approximately 15% of the global extant ungulate species (Wilson & Reeder, 2005). These ungulates play a crucial role in ecosystem functioning, contributing to forest structure maintenance, seed dispersal, nutrient cycling, and serving as a prey base for predators such as the snow leopard (McNaughton, 1979). Among the ungulates, musk deer, belonging to the family Moschidae, are distributed across thirteen range countries in Asia (Pan et al., 2015; Singh et al., 2022). The genus Moschus includes seven reported species of musk deer: Siberian musk deer (*Moschus moschiferus*), Chinese forest musk deer (*M. berezovskii*), Anhui musk deer (*M. anhuiensis*), Alpine musk deer (*M. chrysogaster*), Himalayan musk deer (*M. leucogaster*), Black musk deer (*M. fuscus*), and Kashmir musk deer (*M. cupreus*) (Pan et al., 2015; Ostrowski et al., 2016; Singh et al., 2019). However, there is still ambiguity and debate surrounding the number of species within the genus Moschus.

The Kashmir musk deer is a rare and elusive forest-dwelling species highly sensitive to anthropogenic disturbances, and it is already classified as endangered due to poaching and habitat loss. The fragile landscape of the Himalayas has been severely impacted by increased human expansion and anthropogenic activities, leading to an increased risk of species extinction (Pandit et al., 2007; Flynn et al., 2009; Corlett, 2015). The Himalayas are experiencing escalating human activities such as agriculture expansion, urbanization, development of linear features, and overexploitation of natural resources, all of which have detrimental impacts on wildlife species, including musk deer (Dixo et al., 2009; Cushman et al., 2018; Kaszta et al., 2019; Chandra et al., 2020). The musk deer population is facing three major threats: illegal hunting for musk pods, habitat loss, and intensive livestock grazing. Its territorial behavior and the use of laterine sites for territorial marking make it particularly vulnerable to hunting. The musk pod is highly valuable and is used for making perfumes, medicines, and for religious purposes. As a result of these threats, all species of musk deer are listed as endangered according to the International Union for Conservation of Nature (IUCN) (Timmincks and Duckworth, 2015).

Understanding the declining population of musk deer is critical for their long-term viability, necessitating both ecological and management perspectives. Given the current levels of habitat loss, anthropogenic activities, and rapid changes in land use patterns, it is essential to conduct a landscape-level study covering the North-western Himalayas of India to prioritize populations and regions for effective and meaningful conservation efforts. Therefore, this study highlights the combined use of ecological models and genetics of musk deer in the North-Western Himalayas. By employing both ecological models and genetics, this research aims to identify populations and regions that require high conservation priority to ensure the species' survival

Subsequent to the introduction, the following four chapters delve into the specific objectives of the study. My thesis seeks to explore and answers four *WH* questions *viz How many are they? Where are they distributed? How are they distributed*? *and What is there genetic structure?* These four questions helped me to resolve the taxonomy of musk deer, delineate its distribution, assess the habitat use and understand the population genetic structure of musk deer in Northwestern Himalayas. To address these questions the study was conducted in two states *viz* Himachal Pradesh (Lahaul valley) and Uttarakhand (Uttarkashi) of Northwestern Himalayas. Entire study area was divided into 10 x 10 km grids for conducting extensive study and 1 km × 1 km grids for intensive study adopting the methods from (Singh et al., 2018). Single time effort was given in the extensive grids to cover the entire landscape and to identify the intensive sites. Two intensive study sites were then selected in both districts of Western Himalaya. Govind Pashu-vihar National Park (GPVNP) in Uttarkashi and Pattan Forest range in Lahaul valley were selected as intensive study sites. The field survey was conducted using different conventional methods such as sign survey, camera trapping and non-invasive DNA sampling in the selected sites of musk deer covering different elevation ranges. Total of 134 camera traps in Uttarkashi district, 222 camera traps in Lahaul and spiti valley, Himachal Pradesh were deployed. Of which 79 camera traps in Uttarkashi, 105 in Lahual valley were installed within the range of Musk deer distribution between 2200 to 4200 meters elevational range. I also traversed 106 trails in Lahual valley, 99 in Uttarkashi of 2-7 Km in length to study the latrine sites, collect non-invasive samples and other habitat parameters. A total of 170 samples (116 Lahaul valley, 54 Uttarkashi) were collected from the study sites. I also identified 32 latrine sites of Musk deer and 11 latrine sites in Uttarkashi since no other overlapping species is found within that range especially in Lahaul valley. All the putative faecal pellets of KMD origin were air-dried in field conditions and stored in 50 ml sterilized Silica containing vials for DNA analysis.

Chapter four of this study aimed to address the taxonomic ambiguity surrounding musk deer in the Northwestern Himalayas. To achieve this, a combination of camera trapping, trail transects, and non-invasive genetics was employed. A total of 184 camera traps (105 in Lahaul and 79 in Uttarkashi) were deployed in different habitats of these regions, resulting in 103 independent captures of musk deer. All the images captured from both areas, Himachal Pradesh and Uttarakhand, and through genetic-based identification, confirmed the presence of Kashmir musk deer (*Moschus cupreus*), thereby resolving the taxonomic uncertainty and establishing the new record of this species in the area. Interestingly, no other species of musk deer, such as the Himalayan musk deer or Alpine musk deer, which were previously thought to occur in the landscape based on earlier studies, were captured in any of the studied areas. These findings emphasize the need for future conservation plans and management efforts to be tailored specifically for the protection of the Kashmir musk deer population in the Northwestern Himalayas.

Chapter five focuses on understanding the potential changes in habitat for the endangered Kashmir musk deer in the Western Himalayas. Human expansion and anthropogenic activities are threatening the natural habitats of various species, including the Kashmir musk deer. To address this issue, the study uses simulation techniques to predict the future landscape for the year 2020 and employs ensemble modeling to assess the impact of projected land use changes on the distribution of the Kashmir musk deer. The simulation results indicate a decline in croplands and shrublands, while mixed forests are expected to increase in the future scenario. There will be transformations from evergreen broad-leaf and needle-leaf forests to mixed forests, and from croplands and barren areas to savannas.

The study identifies precipitation, elevation, and mixed forests as the most significant factors influencing the distribution of the Kashmir musk deer. Currently, only a limited area of about 20,690 km^2 is considered suitable for the species out of a total landscape of 324,666 km^2. However, the projection indicates an increase in suitable habitat to around 22,701.47 km^2 in the future. While there is a potential habitat gain of approximately 2,722 km^2 in new areas, there is also a projected loss of 711 km^2 in existing habitats for the Kashmir musk deer by 2030. The state of Uttarakhand is expected to experience a significant decline in suitable habitat, while Jammu and Kashmir may offer new habitats for the species.

Notably, most of the suitable habitats identified for the Kashmir musk deer are currently outside the boundaries of protected areas (PAs), highlighting the inadequacy of the current PA network for conservation. To address this concern, the research suggests boundary rationalization of protected areas, encompassing currently unprotected suitable habitats. Such measures would be essential to safeguard the Kashmir musk deer and preserve its population in the face of human-induced land use changes. By considering these findings and taking appropriate conservation actions, wildlife managers and policymakers can make informed decisions to ensure the long-term survival of this iconic species in the Western Himalayas.

The *Chapter six* focuses on the assessment of habitat use by Kashmir musk deer (KMD) in Pattan Valley, Himachal Pradesh, and Govind Pashu Vihar National Park (GPVNP), Uttarakhand. Through sign surveys and camera trapping data analyzed with single species occupancy modeling, the study explores the impact of environmental factors on KMD's habitat preferences in the two regions. The research confirms that KMD predominantly occupies conifer forests at higher elevations (2946-4418 m) in Pattan Valley, while in GPVNP, mixed forests at lower elevations (2145-3641 m) are preferred. Elevation and conifer forests positively influence KMD's occupancy and detection probability in Pattan Valley, whereas in GPVNP, mixed forests and elevation have a positive effect, and conifer forests negatively impact KMD's detection probability.

The findings provide valuable insights into KMD's habitat selection patterns in the landscape, emphasizing the importance of preserving and protecting high-altitude forested areas, which serve as critical habitats for the species. It is essential to minimize poaching pressure and human disturbances in these regions to ensure the long-term survival of the endangered Kashmir musk deer population. By understanding KMD's habitat preferences, wildlife managers can develop effective conservation plans and make informed decisions to safeguard this iconic species in the face of environmental challenges. The research contributes valuable information to aid conservation efforts and protect the unique and vulnerable Kashmir musk deer population in the North-western Himalaya.

This dissertation's seventh chapter focuses on the population genetics of the endangered Kashmir musk deer in the North-western Himalayas. The study utilizes mitochondrial DNA and microsatellite markers to assess genetic diversity and population structure. Ten unique haplotypes were identified, with relatively high genetic diversity at the mitochondrial Cytb gene and moderate variability at microsatellites. The presence of genetic clusters within Lahaul Valley suggests admixture from neighboring areas. Bayesian skyline plots and mismatch distribution curves indicate stable population sizes with recent declines in effective population size. The study highlights the importance of considering genetic factors in conservation efforts to ensure the long-term survival of the species. The findings underscore the need to preserve local genetic variation and expand sampling to include additional regions for a comprehensive analysis. Overall, the study provides valuable insights into the genetic makeup and population dynamics of the endangered Kashmir musk deer, emphasizing the urgency of conservation efforts.

These objectives are designed to address key aspects related to the conservation and population dynamics of the study species in the North-western Himalayas. Each chapter focuses on distinct research questions, providing in-depth analyses and findings to contribute valuable insights to the field of wildlife conservation.

Together, these seven chapters offer a comprehensive and cohesive exploration of the study species, its habitat, and the crucial conservation efforts required to ensure its long-term survival in the face of mounting environmental challenges.

Chapter 1: Introduction

Musk deer (*Moschus* spp.) are elusive, solitary forest dwellers known for their crepuscular and nocturnal activity patterns (Sathyakumar et al., 1993; Groves et al., 1995; Ostrowski et al., 2016). These fascinating creatures are distributed across the mountains of Asia, inhabiting continuous to fragmented patches of forested and alpine scrub habitats in at least 13 range countries (Sathyakumar et al., 1993; Timmins & Duckworth, 2015; Pan et al., 2015). Recently, biologists have become increasingly concerned about the rapid decline in musk deer populations. There are seven described species of musk deer, including the Siberian musk deer (*Moschus moschiferus*), Chinese forest musk deer (*M. berezovskii*), Anhui musk deer (*M. anhuiensis*), Alpine musk deer (*M. chrysogaster*), Himalayan musk deer (*M. leucogaster*), Black musk deer (*M. fuscus*), and Kashmir musk deer (*M. cupreus*) (Pan et al., 2015; Ostrowski et al., 2016; Singh et al., 2019).

Within India, four species (*M. chrysogaster, M. cupreus, M. fuscus, and M. leucogaster*) are distributed in the fragmented patches across states such as Uttarakhand, Himachal Pradesh (North-Western Himalayas), Sikkim (Central Himalayas), Arunachal Pradesh (Eastern Himalayan), and the Union territory of Jammu and Kashmir (Western Himalaya), typically found at elevations ranging from 2500-3500 meters above sea level (IUCN, 2018). Although it is believed that the Himalayan Musk Deer (*M. leucogaster*) and Alpine Musk Deer (*M. chrysogaster*) are present in Uttarakhand, there is a lack of confirmed records through DNA-based techniques or Camera trapping. Some studies have even reported the presence of the Kashmir musk deer, which is usually restricted to Kashmir, in Nepal (Singh et al., 2019). The overlapping ranges of these species and their different body morphs have led to misidentification, causing ambiguity in the taxonomy of musk deer in India and other regions (Pan et al., 2015; Shukla et al., 2018).

Musk deer populations are facing numerous threats, particularly poaching and habitat degradation, leading to a significant decline in their numbers over the past few generations (Green, 1986; Timmins & Duckworth, 2015). The illegal exploitation of musk pods, found only in males, contributes to the high demand in the national and international market for traditional medicines and perfumes (Wangdi et al., 2019). Consequently, all species of musk deer are now listed as "Endangered" on the IUCN Red List of Threatened Species (except *M. moschiferus*, which is classified as "Vulnerable"), and they are also listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2015). In India, musk deer species also receive protection under Schedule I of the Wildlife Protection Act of 1972.

Apart from poaching, habitat destruction, degradation, climate change, and various anthropogenic pressures threaten the survival of musk deer species (Grumbine & Pandit, 2013; Ilyas, 2014). Consequently, suitable musk deer habitats have significantly reduced, and their populations are now confined to protected areas and some remote regions in the Himalayas. Urgent conservation efforts are necessary to protect these remarkable creatures and their habitats.

Despite limited ecological studies in India on musk deer (Sathyakumar et al., 1993; Ilyas, 2007; Sathyakumar et al., 2013) and genetics (Shukla et al., 2018), comprehensive landscape-level studies on their ecology and genetics are lacking.

Therefore, the proposed study aims to address the taxonomic complexities of musk deer in the North-Western Himalayas, investigate their spatial distribution patterns, and assess population genetic structure using ecological niche modeling and genetic tools.

The combined approach of ecology and genetics in this study will identify populations and regions requiring high conservation priority and will help determine the corridors necessary for gene flow. Molecular markers play a vital role in understanding taxonomy, population genetic structure, and phylogeography (Zhang and Jiang, 2006; Bärmann et al., 2013). They are particularly useful in defining "Evolutionary Significant Units" (ESU) of musk deer, especially in cases where different ectomorphs are considered separate species (Hajibabaei et al., 2007; Pan et al., 2015), or in situations where the taxonomy of musk deer species remains unresolved in India (Shukla et al., 2018; Singh et al., 2019).

While it is known that musk deer inhabit the Himalayan range, detailed studies on the fine-scale ecological covariates of their habitat have been lacking, and the genetics of these species at the landscape level remains unresolved, except for a single species study using mitochondrial DNA (Shukla et al., 2018). This proposed study aims to fill these gaps in knowledge and contribute valuable information to the conservation and management of musk deer populations in the North-Western Himalayas (**Figure 1.1**).

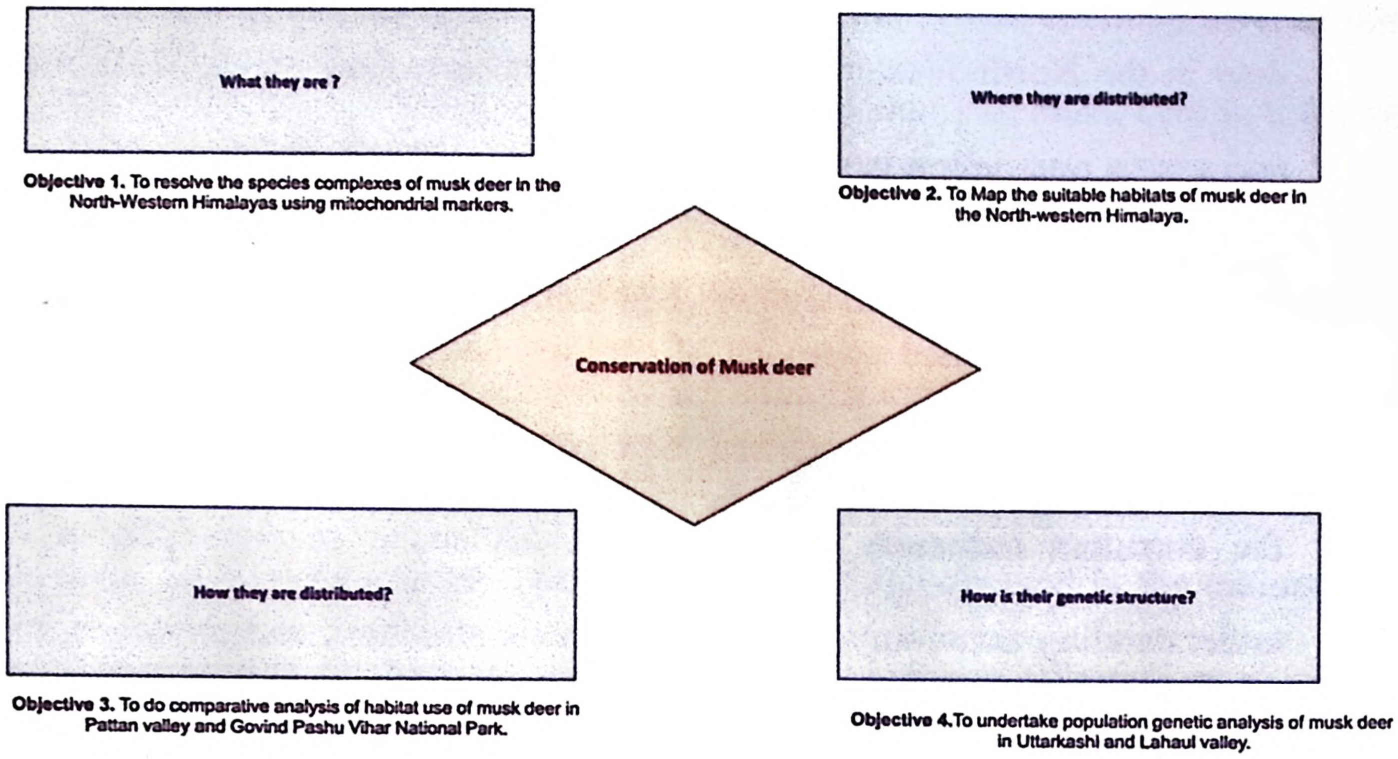

Figure 1.1. The figure displays the goal of the proposed study conducted in North-western Himalaya.

1.1. Literature review

Musk deer (Genus *Moschus*) belongs to the family Moschidae and is considered a primitive representative of the family Cervidae (Brooke, 1878). There are seven known species of musk deer distributed across thirteen range countries in Asia (Zhou et al., 2004; Singh et al., 2018). These unique creatures have been exploited for their musk pods, which are found in the preputial gland sac of male musk deer (Green, 1985). The extraction of musk for various purposes, including perfumes and medicinal uses, has led to the killing of approximately 30,000 musk deer annually, putting the species at risk of extinction (Oza, 1988; Homes, 1999). Globally, musk deer populations have experienced a significant decline, primarily due to poaching and habitat degradation (Green, 1986; Yang et al., 2003; Timmins & Duckworth, 2015; Wangdi et al., 2019). Despite their non-vocal nature, musk deer rely heavily on olfaction for communication (Green, 1987; Sathyakumar & Rawat, 2015; Singh et al., 2018). They communicate through marking behaviors, such as defecating or urinating at specific sites known as latrine sites (Sathyakumar & Rawat, 2015). Male musk deer use their musk secretions in urine and paste the secretions of the caudal gland on vegetation (Green, 1985, 1987). Latrine sites serve multiple purposes, including kin recognition, advertisement of reproductive success, predator-prey recognition, and conveying messages related to personality, maturity, sexual status, and territory marking (Singh et al., 2022). These elusive creatures often visit latrine sites, making them valuable for studying various ecological aspects, such as population estimation, habitat use, and distribution modeling (Green, 1985; Sathyakumar, 1994; Ilyas, 2014; Singh et al., 2018).

Once having a continuous distribution, musk deer populations are now restricted to isolated pockets in the Himalayas and other range countries due to large-scale poaching and extensive habitat destruction (Green, 1985, 1986; Sathyakumar et al., 1993a; Sathyakumar, 1994; Timmins & Duckworth, 2015). Studies on the natural history of musk deer in the northern Qinghai province (Zheng and Pi, 1987) and the ecology of Himalayan musk deer in Sagarmatha National Park, Nepal, using radio telemetry (Kattel, 1990), have revealed that musk deer have limited home ranges. For instance, Kattel (1992) estimated the average home ranges for males and females in Sagarmatha National Park, Nepal, as 14 and 13 hectares, respectively. Additionally, Harris and Guiquan (1993) estimated the autumn home range of musk deer in the Biazah forest in the Tibetan Plateau to be approximately 17.6 hectares. Green (1989) conducted a review of the status of captive musk deer worldwide from 1959-1980.

However, there are few studies on the conservation status of *Moschus spp.* in China, which have suggested that populations are declining due to over-hunting for musk pods and loss of habitats (Yang et al., 2003; Zhou et al., 2004). It is crucial to address the decline in musk deer populations and to implement effective conservation measures. Understanding their natural history, ecology, and genetic diversity is essential for devising conservation strategies. By studying their communication behavior at latrine sites and using advanced techniques like radio telemetry and genetic analysis, we can gain valuable insights into the ecological aspects and distribution patterns of these elusive creatures.

Studies on the habitat use and distribution of musk deer indicate a preference for forested to alpine scrub habitats (Green, 1985; Sathyakumar, 1994; Singh et al., 2018). These elusive creatures are commonly found at elevations above 2500 meters, with a primary presence above 3000 meters in the western Himalayas, and they rarely venture to lower levels (Green, 1985; Sathyakumar, 1994; Vinod and Sathyakumar, 1999). During the day, musk deer tend to utilize forested and scrub habitats at lower altitudes, while they seek alpine meadows and pastures at higher elevations during the night (Green, 1985).

Specific studies shed light on the habitat preferences of different musk deer species. For instance, Kattel (1992) suggested that musk deer prefer to inhabit birch-rhododendron forests and dwarf rhododendron shrubs in Sagarmatha National Park, Nepal. In the Neelam Valley, Pakistan, and Machiara National Park, the distribution of Himalayan musk deer was observed in the sub-alpine scrub forest and Himalayan moist temperate forest at higher elevations (Khan et al., 2004; Qamar et al., 2008).

Subedi et al., 2012 documented the habitat ecology of Himalayan musk deer in Manaslu Conservation Area, Nepal, and found that musk deer showed a significant preference for altitudes between 3601-3800m, with 26%-50% crown cover being preferred in the area.

Furthermore, camera trap-based studies play a crucial role in collecting photographic evidence and exploring the ecomorphology and habitat use of elusive species inhabiting small areas with cryptic behavior (Singh et al., 2019; Wangdi et al., 2019). For instance, Tobgay et al., 2017 documented the presence of musk deer in the Sakteng Wildlife Sanctuary, Bhutan, dispelling earlier assumptions of its extinction from the area. Similarly, Pal et al., 2021 utilized camera trap devices to estimate the density of musk deer in the Neelang Valley of Gangotri National Park, Uttarakhand. They found that the estimated density was about 0.4±0.1 individuals/km^2 during summer and 0.1±0.05 individuals/km^2 during winter. Sharief et al., 2023 also employed camera traps to document the presence of Kashmir musk deer in Uttarkashi and reported an overall abundance of 28.40 with an average density of 4.73/100 km^2. These studies demonstrate the effectiveness and applicability of such robust tools in exploring rare and elusive species like musk deer.

Camera trapping studies have also provided valuable insights into musk deer habitat use. In the Kala Pani Valley, Gilgit-Baltistan, the abundance of musk deer was recorded as 0.02 animals per square kilometer (Ali & Din, 2013). Khadka, 2017 evaluated the habitat ecology, trophic interactions, and distribution of the endangered Himalayan Musk deer in the Nepal Himalaya. The study revealed that *Abies spectabilis, Pinus wallichiana*, and *Berberis* species constituted the major portion of musk deer's diet, and habitat selection was significantly influenced by elevation, aspect, canopy cover, and tree species.

Other studies have shown the impact of human activities on musk deer habitat selection. For example, Slaght et al., 2019 found that intensive hunting and logging in Russia influenced the habitat selection of Siberian musk deer. Singh et al., 2020 documented that precipitation played a crucial role in governing the distribution of Kashmir musk deer in the greater Himalaya, South Asia. They also highlighted that Uttarakhand could potentially offer new places for Kashmir musk deer in the future. Zang et al., 2022 suggested that Alpine musk deer adjusted their spatial and temporal behavior to avoid overlap with livestock and other human activities in the human-dominated semi-arid mountain ecosystem of Xinglong Mountain Nature Reserve, China.

The taxonomy of musk deer has been a subject of long-standing debate and remains controversial (Wang, 1993). Various studies have proposed the presence of one to seven species across their range (Grove, 1975; Groves & Grubb, 1987; Grove, 1995; Pan et al., 2015; Singh et al., 2019). Sokolov and Prikhod'ko, 1997 initially considered all musk deer species as one (*M. moschiferus*) and later divided it into two groups: i) sibirica, which includes four subspecies of musk deer, and ii) himalaica, which comprises three subspecies. Groves and Grubb, 2011 refined this classification and identified seven distinct species of musk deer: Siberian musk deer (*Moschus moschiferus*), Chinese forest musk deer (*M. berezovskii*), Anhui musk deer (*M. anhuiensis*), Alpine musk deer (*M. chrysogaster*), Himalayan musk deer (*M. leucogaster*), Black musk deer (*M. fuscus*), and Kashmir musk deer (*M. cupreus*). Pan et al. (2015) conducted a study on the species delimitation within the genus Moschus and its origins in the high plateau of Tibet using mitochondrial full genome analysis. They proposed that only six species, namely *M. leucogaster, M. fuscus, M. moschiferus, M. berezovskii, M. chrysogaster,* and *M. anhuiensis*, are authentic members of the Moschus genus.

However, the exact number of species within the Moschus genus and their distribution still remain contentious. Recent genetic studies have shed light on the distribution of Kashmir musk deer in new areas within the Himalayan region (Singh et al., 2019; Kumar et al., 2020). Notably, Singh et al., 2018 confirmed that musk deer utilize latrines in forests located at higher elevations (3,200–4,200 m) based on data collected over multiple seasons and years. They observed that greater crown cover and shrub diversity were associated with the presence of musk deer, indicating their preference for specific habitat conditions. Moreover, recent research suggests that musk deer are distributed at higher elevations and latitudes due to the influence of climate change (Jiang et al., 2019). However, comprehensive taxonomic studies are needed to sample all species adequately and enable species-specific conservation planning at regional and global levels.

Musk deer species in India have been inadequately studied, with limited available information derived from intensive studies and some population surveys focusing on aspects such as ranging behavior, activity patterns, habitat use, feeding habits, and ecological relationships with other ungulates (Green, 1985; Green, 1987; Sathyakumar, 1994; Sathyakumar and Rawat, 2015; Syed & Ilyas, 2016). Existing literature suggests that musk deer exhibit a preference for warmer slopes (southern aspect) and areas with shrub cover in the Kedarnath Wildlife Sanctuary (Green, 1985; Sathyakumar, 1994). Similarly, Vinod and Sathyakumar, 1999 found that musk deer prefer areas with lower tree cover, medium to high shrub coverage, and low grass cover in the Great Himalayan National Park, Himachal Pradesh.

During the day, Musk deer are known to utilize forested and scrub habitats at lower altitudes, while they tend to occupy alpine meadows and pastures at higher elevations during the night (Green, 1985). Other studies have covered various aspects, such as the status review of captive musk deer in India (Sathyakumar et al., 1993b), status surveys in Langtang National Park (Green, 1978) and Sagarmatha National Park (Upreti, 1979), as well as studies in the Great Himalayan National Park and Nanda Devi National Park (Gaston & Garson, 1992; Sathyakumar, 1993).

Recently, there have been evaluations of the conservation status and ecology of musk deer (*Moschus chrysogaster*) in Kumaon and Garhwal Himalayas (Ilyas, 2007; Syed and Ilyas, 2012) and investigations into the habitat preference and feeding ecology of alpine musk deer (*Moschus chrysogaster*) in Kedarnath Wildlife Sanctuary, Uttarakhand. However, these studies lack comprehensive details on habitat ecology, distribution, and genetics, necessitating a more intensive sampling design and applied approaches (Singh et al., 2018; Shukla et al., 2018).

Genetic studies of musk deer species in India are still limited, with only one detailed study utilizing mitochondrial COI gene available (Shukla et al., 2018). This study indicated that the musk deer species found in Uttarakhand belong to a distinct genetic lineage and warrants further investigation with larger sample sizes. Kumar et al., 2022) also employed the COI gene to evaluate the phylogenetic relationship of musk deer from Jammu and Kashmir, Kedarnath Wildlife Sanctuary, and Nanda Devi Biosphere Reserve, Uttarakhand region, India. Their findings revealed that the musk deer lineage in the Uttarakhand region belonged to the Kashmir musk deer.

To better understand the species ecology and habitat use of musk deer and other mammals, latrines are used for territorial defense, established in both the periphery and core areas of their habitat (Wronski et al., 2006; Syed & Ilyas, 2016; Singh et al., 2018). Utilizing such methods allows for the study of fine-scale ecology, distribution, habitat association, preferences, and usage patterns (Ostrowski et al., 2016; Singh et al., 2018).

In addition, researchers often employ a combination of mitochondrial DNA (MtDNA) and microsatellite markers to study various aspects such as evolutionary history, biological identification, population genetics structure, gene flow, taxonomy, biogeography, and phylogeny, which ultimately aids in improving our understanding of genetic fitness and the conservation of musk deer species (Galtier et al., 2009; Singh et al., 2019).

1.2. Justification of the study

Among the four species found in India, the taxonomy remains complex, and evolutionary insights are inconclusive. Morphological and ecological studies are still in their early stages, and the population genetics structure is largely unknown. Musk deer, being victims of their own possession (the musk gland), face severe poaching pressure to acquire their musk pods, which are sought after for traditional medicines and perfumes since ancient times (Feng et al., 1981). Apart from poaching, habitat destruction and degradation pose significant threats to musk deer populations (Green, 1986; Ilyas, 2014; Yang et al., 2003). Deforestation leads to fragmented habitats, affecting the movement of various species, including musk deer. This fragmentation can cause inbreeding and loss of genetic variation due to restricted movements. The construction of roads and other developmental activities, such as urban expansion and extensive habitat loss, may further confine musk deer populations to fragmented habitats.

Given the current levels of habitat loss, fragmentation, and human activities, it becomes essential to conduct a landscape-level study encompassing the North-western Himalayas of India. This study aims to prioritize populations and regions for targeted conservation efforts in an effective and meaningful manner. While a few studies on musk deer ecology and status are available in India (Ilyas, 2007; Sathyakumar et al., 2013), they lack detailed landscape-level investigations on ecology and genetics.

Hence, the proposed study aims to utilize a combined approach of ecological modeling and genetics to study musk deer in the North-Western Himalayas. The integration of ecological models and genetics will aid in identifying populations and regions that require high conservation priority. Additionally, it will help identify corridors for gene flow, allowing for the maintenance of genetic diversity and genetic exchange between and within populations of this endangered species.

1.3. Objectives

1. To resolve the species complexes of musk deer in the North-western Himalaya using mitochondrial markers.
2. Mapping the habitat suitability of musk deer in the North-western Himalaya.
3. To understand habitat use pattern of musk deer in Lahaul Valley and Uttarkashi.

4. To undertake population genetic analysis of musk deer in Lahaul valley and Uttarkashi.

1.4. Research questions

1. What are the species/subspecies of musk deer distributed in the Uttarkahsi and Lahaul valley?
2. What are those factors that have shaped the distribution of musk deer species in these two study sites?
3. Does the habitat use pattern of musk deer vary in Uttarkashi and Lahaul valley?
4. Does the population genetic structure of musk deer vary between the study sites?

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Chapter 2: Study area

The study was conducted in the Lahaul valley of Himachal Pradesh and Uttarkashi district of Uttarakhand of the Western Himalayas (**Figure 2.1**).

Lahaul valley

The Lahaul and Spiti (L&S) district in Himachal Pradesh spans from latitude N 31°44'57" to 32°59'57" and longitudes E 76°46'29" to 78°41'34"E. It is situated between the Pir Panjal Mountain chains of the Greater Himalaya and the Trans-Himalaya, as described by Aswal & Mehrotra in 1994. Covering an extensive area of 13,841 km^2, L&S is the largest district in Himachal Pradesh, characterized by limited temperate forest on the left bank of the Chandrabhaga River. Much of the Lahaul Valley falls within the rain shadow region, giving it features typical of the Trans-Himalayas. The landscape exhibits diverse topography, with altitudes ranging from 2400 m to 6400 m above mean sea level (amsl). The Lahaul region consists of three major valleys - Tinan Valley, Pattan Valley, and Punan or Todh/Gahr Valley - and is divided into four forest ranges: Udaipur, Keylong, Tindi, and Pattan.

The climate in this region experiences distinct seasons: spring, summer, autumn, and a very cold winter with temperatures ranging from a maximum of 27°C in July to a minimum of –13°C in February, as documented by Wagnon et al., in 2007. The unique physiographic structure is characterized by snow-covered peaks and valleys, with high steep and undulating terrains covered in various land cover types, including coniferous forests, alpine and subalpine vegetation, grasslands, and agricultural lands, as noted by Joshi et al.,2006.

The mammalian fauna of this landscape includes the Snow leopard (*Panthera uncia*), Ibex (*Capra sibirica*), Musk deer (*Moschus leucogaster*), Himalayan thar (*Hemitragus jamlahicus*), and others. Due to the challenging climatic conditions and a short growing season, the Trans-Himalayan Mountains have relatively low vegetation cover, accounting for less than 20% of the basal area. Nevertheless, they are known for harboring a unique and diverse assemblage of wild flora and fauna.

Uttarkashi

Uttarkashi, situated in Uttarakhand, India, is predominantly recognized as a sacred town near Rishikesh. It lies on the banks of the Bhagirathi River, between 38°28′–31°28′N latitude and 77°49′–79°25′E longitude, encompassing mostly hilly terrain with an average elevation of 1,165 m. The district is blessed with several rivers, among which the Yamuna and the Ganges (Bhagirathi) hold immense religious significance, originating from Yamunotri and Gangotri (Gomukh), respectively.

Uttarkashi boasts abundant biological resources and biodiversity, making it a hub of rich religious, cultural, and eco-tourism aspects. The district is divided into three Forest Divisions: Uttarkashi Forest Division, Upper Yamuna Forest Division, and Tons Forest Division, with three Protected Areas - GNP, GPVNP, and GPVWLS. These forested habitats support a variety of top conservation priority species, including the Snow Leopard, Musk Deer, Asiatic Black Bear, Himalayan Brown Bear, Western

Tragopan, and Himalayan Monal. Additionally, the district is home to small mammals such as European Otter, Goral, Civet, Wild Boar, and Hodgson's Giant Flying Squirrel. The avian population is also noteworthy, including the Himalayan Monal and numerous other bird species.

The floral resources in the district exhibit diverse vegetation types, ranging from western Himalayan broadleaf woods at lower elevations to western Himalayan subalpine conifer forests and alpine shrublands and meadows at higher elevations. Tree species found in the lower regions include Pine, Deodar Cedar, Oak, etc.

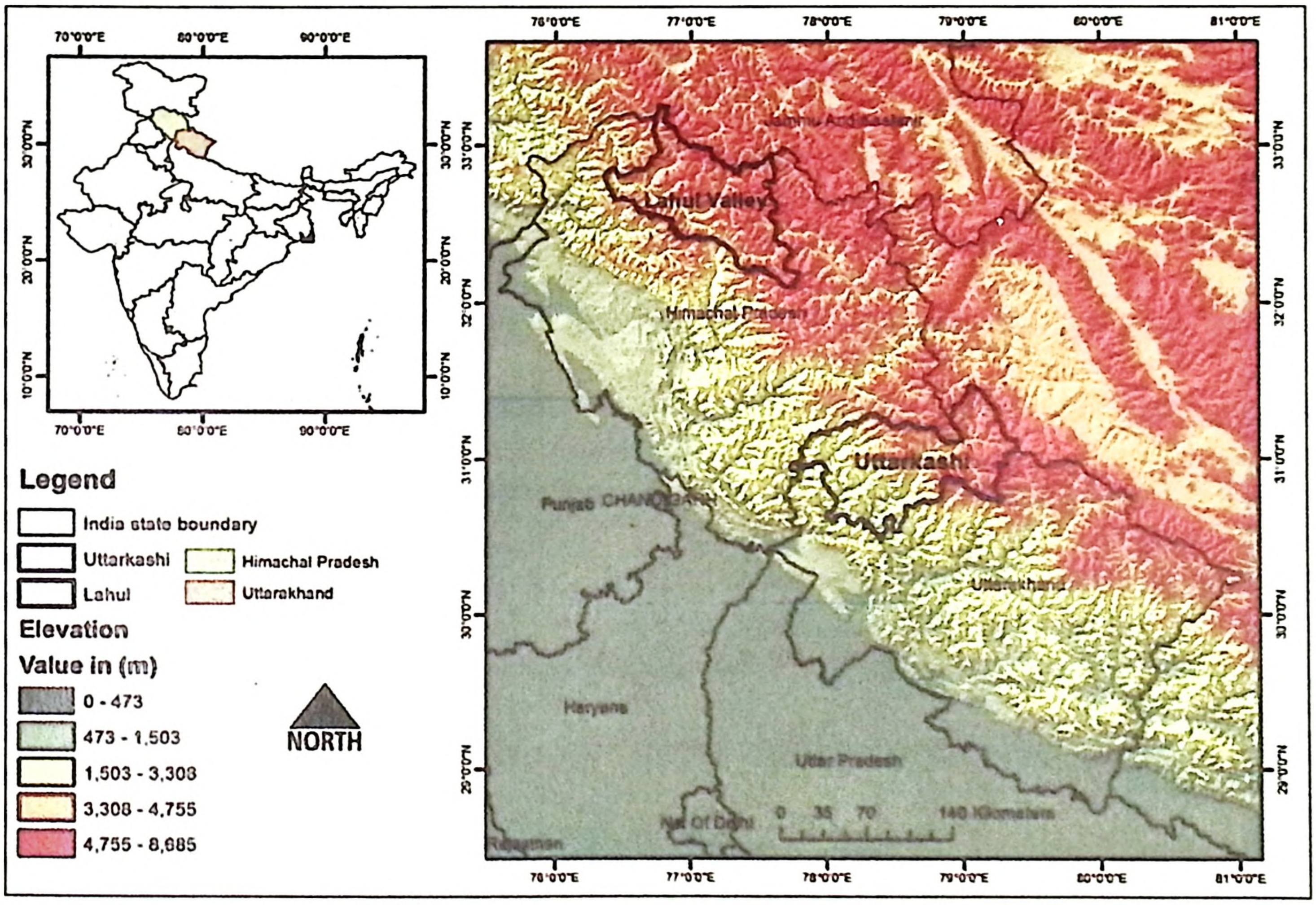

Figure 2.1. Map of the study area (Lahaul valley, Himachal Pradesh and Uttarkashi, Uttarakhand) showing state boundaries with elevation range (m).

2.1. Background

Lahaul-Spiti district in the Indian state of Himachal Pradesh is renowned for its breathtaking valleys and scenic beauty. It holds the distinction of being the largest district in Himachal Pradesh, established in 1960. The administrative headquarters, Keylong, is nestled in the greener and more fertile Lahaul valley, while the Spiti valley is predominantly barren and dry. The district encompasses three major valleys - Tinan Valley, Pattan Valley, and Punan or Todh/Gahr Valley - and is divided into four forest ranges - Udaipur, Keylong, Tindi, and Pattan.

Strategically located, Lahaul-Spiti serves as a gateway to popular tourist destinations in Northern India. To its south lies the Kullu valley, to the west are the Pangi and Churah areas of District Chamba, and to its north lie the valleys of Zanskar and Ladakh. The southeastern boundaries of the district stretch towards Western Tibet, beyond the Kunzom Pass. The weather in the district remains pleasant from May to October, with high mountain ranges covered in snow and glaciers throughout most of the year. The average annual rainfall is a mere 170 mm, and the district's highest mountain pass is the Kunzum La, standing at 4551 m near Chandrataal Lake and Batal. The tallest mountain peak in the district is Gya, towering at 6,794 m.

The Uttarkashi is located in Uttarakhand, India, is renowned for its religious significance, natural beauty, trekking opportunities, and adventure sports. The town is beautifully situated along the banks of the Bhagirathi River, a significant tributary of the river Ganges. The district is divided into three Forest Divisions: Uttarkashi Forest Division, Upper Yamuna Forest Division, and Tons Forest Division, with three Protected Areas - GNP, GPVNP, and GPVWLS.

Uttarkashi is surrounded by picturesque mountains, lush forests, and adorned with several waterfalls and hot springs, adding to its allure. It serves as a gateway to the Gangotri glacier and other captivating locations, accessible through various trekking routes. The region is also popular for mountaineering expeditions to peaks in the Garhwal Himalayas. Wildlife enthusiasts are drawn to the nearby Gangotri National Park, home to various rare and endangered species. Furthermore, Uttarkashi has evolved into an educational and research hub, with institutes offering courses in mountaineering, tourism, and environmental studies. Its captivating natural beauty and cultural significance attract tourists from across the globe.

2.2. History of the study area

Lahaul and Spiti are two remote valleys nestled in the Indian state of Himachal Pradesh, situated within the majestic Himalayas. These valleys boast a fascinating history that dates back to ancient times. Initially, they were inhabited by nomadic tribes, such as the Bhotias and the Monpas. Subsequently, the rulers of Tibet established strong cultural and economic connections with the region. Over time, different kingdoms, including Guge-Purang and Ladakh, invaded and conquered the area, introducing Buddhism and constructing several monasteries. Local chieftains governed the region until it was annexed by the rulers of Kullu during the 17th century. Lahaul was later annexed by Maharaja Ranjit Singh in 1840, along with Kulu, and remained under his rule until 1846, when it came under British control. From 1846 to 1940, Lahaul was a part of the Kulu sub-division in the Kangra district, with local jagirdars or thakurs governing the area. The Wizier of Lahaul was responsible for judicial and executive duties, while another thakur held the position of Revenue

Officer, wielding both traditional and government-authorized powers. Despite attempts by the Mughal and British empires to conquer the region, they were thwarted by the challenging terrain and harsh weather.

After India gained independence in 1947, Lahaul and Spiti became integral parts of the Indian state of Himachal Pradesh, witnessing substantial development in infrastructure, education, and healthcare. The region's rich history and cultural heritage, combined with its scenic beauty and abundant natural resources, have made it a popular destination for tourists and researchers.

Uttarkashi is a town situated in the Uttarakhand state of India, and it was officially established on February 24, 1960, located at the foothills of the Himalayas. The town boasts a rich and ancient history, believed to have been founded by Lord Shiva, and is even mentioned in the Ramayana epic. During the 8th century, the Katyuri dynasty ruled over Uttarkashi, known for their patronage of literature and art. Subsequently, the Gurkhas conquered the town in the early 19th century, and later, in 1815, the British took control of the region (Saxena, 2012).

Under British rule, Uttarkashi flourished as a significant center for trade and commerce, witnessing the establishment of schools and hospitals. The town played a noteworthy role in the Indian independence movement, attracting leaders like Mahatma Gandhi and Jawaharlal Nehru. After India gained independence in 1947, Uttarkashi became a part of Uttar Pradesh and was later included in the newly formed state of Uttarakhand in 2000.

In the present day, Uttarkashi remains a popular destination renowned for its cultural heritage and scenic beauty. It draws pilgrims, trekkers, and adventure enthusiasts alike, who are captivated by the town's charm and allure.

2.3. Cultural signficance

The language, people, and culture of Lahaul Spiti may appear similar, but they exhibit significant variations even among neighboring villages. Due to their proximity to Tibet, the people of Spiti share numerous Tibetan traits, language, and customs, while the Lahauli people have a blend of Indo-Aryan and Tibetan influences. Despite their linguistic differences, both languages belong to the Tibetan family. Religion plays a pivotal role in the culture of Lahaul Spiti, with Buddhism being the predominant faith practiced in the region. The monasteries or gompas hold immense importance in the cultural landscape, serving as centers of spiritual and cultural life for the local population (Rathore and Shashni, 2023).

Uttarkashi's culture is a unique amalgamation of tradition and modernity. Deeply rooted in spiritual and religious traditions, Hinduism is the dominant faith in Uttarkashi. The district attracts pilgrims from across the globe who seek to take a dip in the holy waters of the River Ganges and visit various temples. Among them, the Kashi Vishwanath Temple, dedicated to Lord Shiva, holds significant prominence. The people of Uttarkashi are known for their simplicity, hospitality, and deep reverence for nature. Agriculture and animal husbandry remain the primary occupations, and many locals continue to adhere to traditional farming practices. The local cuisine of Uttarkashi is simple yet flavorful, featuring popular dishes such as chainsoo, jholi, and kheer. Festivals play a vital role in Uttarkashi's culture, and they are celebrated with great enthusiasm. The Kandali Festival, honoring Lord Shiva, stands out as one of the most significant festivals in the district (Papnai et al. 2020).

2.4. Flora and fauna

Lahaul Spiti is a habitat for numerous extremely rare animals and plants, many of which are exclusive to this region and not found elsewhere. The snowline in this area begins at around 5000 meters, and most plants can only grow below this altitude. Due to the dry weather and high average altitude, trees are only found in lower areas, while the rest of the flora consists of hardy shrubs, herbs, and wildflowers, including *Crepis flexuosa, Krascheninikovia ceratoides, Caragana brevifolia, Seseli trilobum, Ausinia thomsonii*, and others (**Figure 2.2**). The locals utilize many of these plants for herbal medicine, fodder, and fuel.

In Lahaul and Spiti, people engage in farming wheat, cabbage, potatoes, iceberg lettuce, and other crops. The region is home to several important animal species, such as snow leopards (*Panthera uncia*), musk deer (*Moschus* sps), brown bears (*Ursus arctos isabellinus*), ibex, Himalayan wolves (*Canis vulpus*), and more. Some of the animals sighted by research teams include Brown bears (*Ursus arctos isabellinus*), Kashmir musk deer (*Moschus cupreus*), Blue Sheep (*Pseudois nayaur*), Himalayan ibex (*Capra sibrica*), Himalayan Tahr (*Hemitragus jemlahicus*), Stone martens (*Martes Fiona*), and pikas (*Ochotona* sps.).

Uttarkashi is renowned for its diverse and unique ecosystem, characterized by a wide range of plant and animal life. The district is covered with forests and situated in the Himalayan region, resulting in varied topography and climate that support a rich biodiversity. Dominant tree species in the area include Deodar (*Cedrus deodara*), Pine

(*Pinus roxburgi, Pinus wullachiana*), Oak (*Querques* sps.), and Rhododendron trees (*Rhododendron campanulatum*), found in abundance throughout the region (**Figure 2.3**). Uttarkashi is also well-known for its medicinal plants, such as Himalayan Yew, Kutki, and Atis. The district is home to a wide range of wildlife, including endangered species like the Snow leopard, Himalayan black bear, Musk deer (*Moschus* sps), Barking deer (*Muntiacus vaginalis*), Brown bear (*Ursus arctos isabelinus*), and Common Leopard (*Panthera pardus*).

Bird enthusiasts can also spot various species in Uttarkashi, including the Himalayan Monal (*Lophophorus impejanus*), Koklass Pheasant (*Pucrasia macrolopha*), Himalayan Griffon (*Gyps himalayensis*), and Bearded Vulture (*Gypaetus barbatus*). The region is also inhabited by other animals such as Langur (*Semnopithecus*), Wild boar (*Sus sucrofa*), Yellow-throated marten (*Martes flavigula*), and Red Fox (*Vulpes vulpes*). The unique ecosystem of Uttarkashi holds significant scientific importance, and efforts are being made to conserve and protect this biodiversity hotspot.

Figure 2.2. Landscape pictures of Lahaul valley, Himachal Pradesh showing mosaic of habitats.

Figure 2.3. Landscape pictures showing mosaic of habitats in Uttarkashi, Uttarakhand.

2.5. Landscape characteristics

Topography

The districts are situated in the high-altitude Himalayan region, giving rise to a distinct topography characterized by lofty mountains, deep valleys, and expansive plateaus. The name "LhoYul," meaning "Southern Country," signifies its location south of Ladakh. To the south of Lahaul lies the picturesque valley of Kullu, accessible via the Rohtang Pass at an altitude of 3,195 meters. Another beautiful valley, Bara Bangahal, in the Kangra district, can be reached across the Asakh Pass, positioned at an altitude of 5,051 meters. Lahaul's western boundaries touch the Pangi and Churah areas of District Chamba. To its north lie the valleys of Zanskar and Ladakh, accessible through the Shingola Pass at an altitude of 5,090 meters and Baralacha La Pass at an altitude of 5,450 meters, respectively. The eastern and southeastern boundaries of Lahaul coincide with those of Spiti and Western Tibet, accessible through the Kunzom Pass at an altitude of 4,500 meters (Mandal et al., 2016).

The geographical location of Lahaul and its proximity to neighboring valleys and regions make it a unique and significant area for scientific study and research. Its diverse geography, geology, climate, and ecology provide valuable opportunities for further investigation and exploration (**see Figure 2.4 and 2.5**).

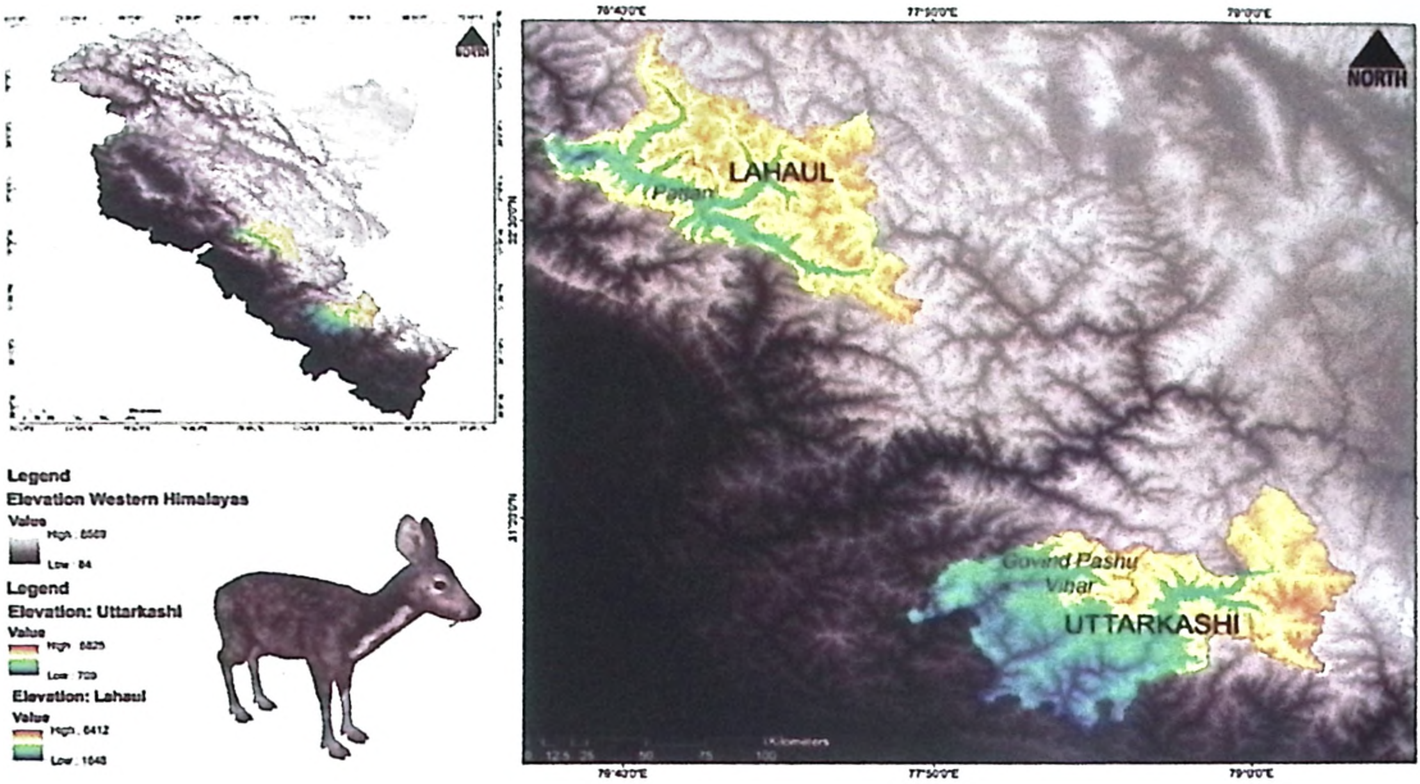

Figure 2.4. Map representing the study area sites at Lahaul and Uttarkashi with elevation range (m).

Contrastingly, the Uttarkashi District showcases a captivating fusion of diverse landscapes, encompassing verdant scenes adorned with abundant vegetation, crisscrossed by streams, creeks, and waterways, as well as towering and majestic rocky edges that gently slope down to lofty snow-capped peaks. The district's climate and geography are exceptionally varied, giving rise to a wide range of vegetation types and diverse habitats for wildlife. Forests cover a substantial portion of the region, and they showcase a rich variety of flora (see **Figure 2.4 and 2.5**).

Approximately 88 percent of the total district area is under the control of the Forest Department. Pine forests thrive at elevations between 900 meters to 2000 meters, while Deodar forests dominate the areas from 2000 meters to 3000 meters. Above 3000 meters, Fir and Spruce forests take over, and further up, between 3000 meters to 4000 meters, Kharshu, Birch, and Juniper forests can be found. The district's higher elevations, above the Fir and Spruce Forest zone, boast alpine meadows stretching from 3500 meters to 4877 meters above sea level. These alpine meadows host a unique and diverse array of vegetation and wildlife, including elusive snow leopards, graceful musk deer, and various species of birds.

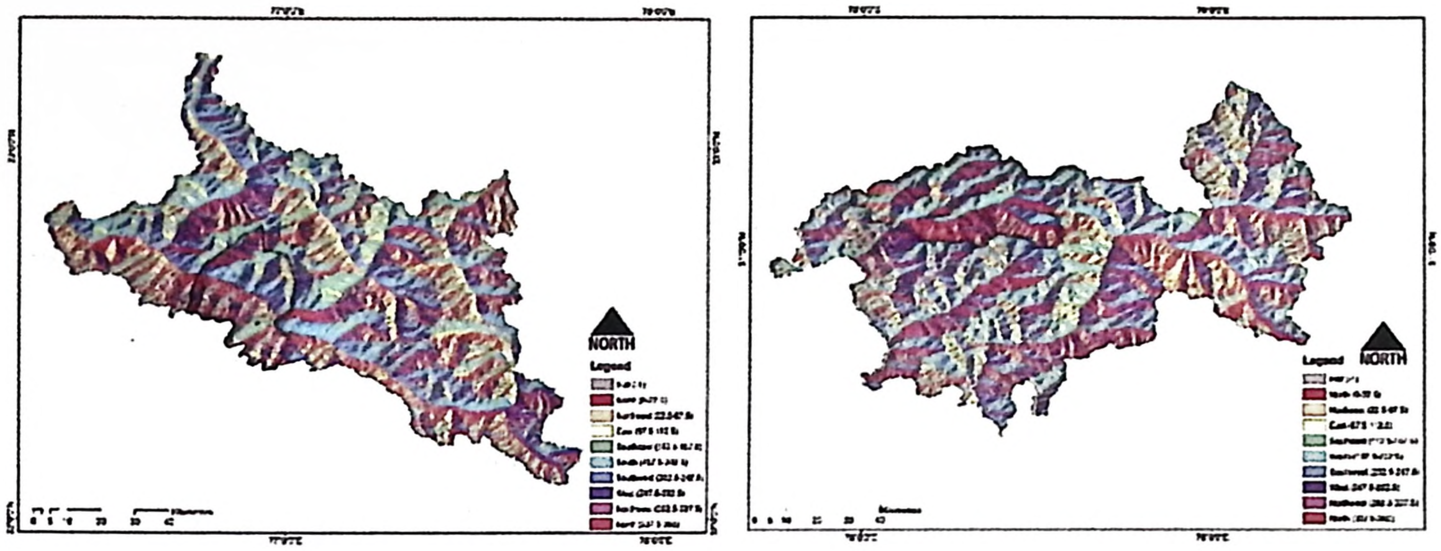

Figure 2.5. Map representing the aspect of study landscape.

2.6. Land Use Land Cover

The land use land cover of Lahaul exhibits various categories, including barren and rocky areas, forests, agricultural land, pasture land, and water bodies. Barren and rocky areas predominate the region, while forests are mainly concentrated in lower altitude zones. Agricultural land is utilized for cultivating crops like potatoes, peas, and barley, and a substantial portion of the area is dedicated to grazing livestock. The presence of water bodies, including rivers, streams, and glacial lakes, serves as a crucial source of water for irrigation and drinking purposes.

On the other hand, Uttarkashi boasts a diverse land use land cover. Extensive forests, mainly consisting of oak, pine, deodar, and rhododendron trees, cover a significant portion of the district. Agriculture plays a crucial role in Uttarkashi, with agricultural land used for cultivating crops such as wheat, rice, millet, and various vegetables. The district also features vast grasslands and meadows utilized for grazing livestock, along with several rivers providing essential water resources for irrigation and drinking. Additionally, higher altitudes in the district comprise some barren and rocky areas (**see Figure 2.6**).

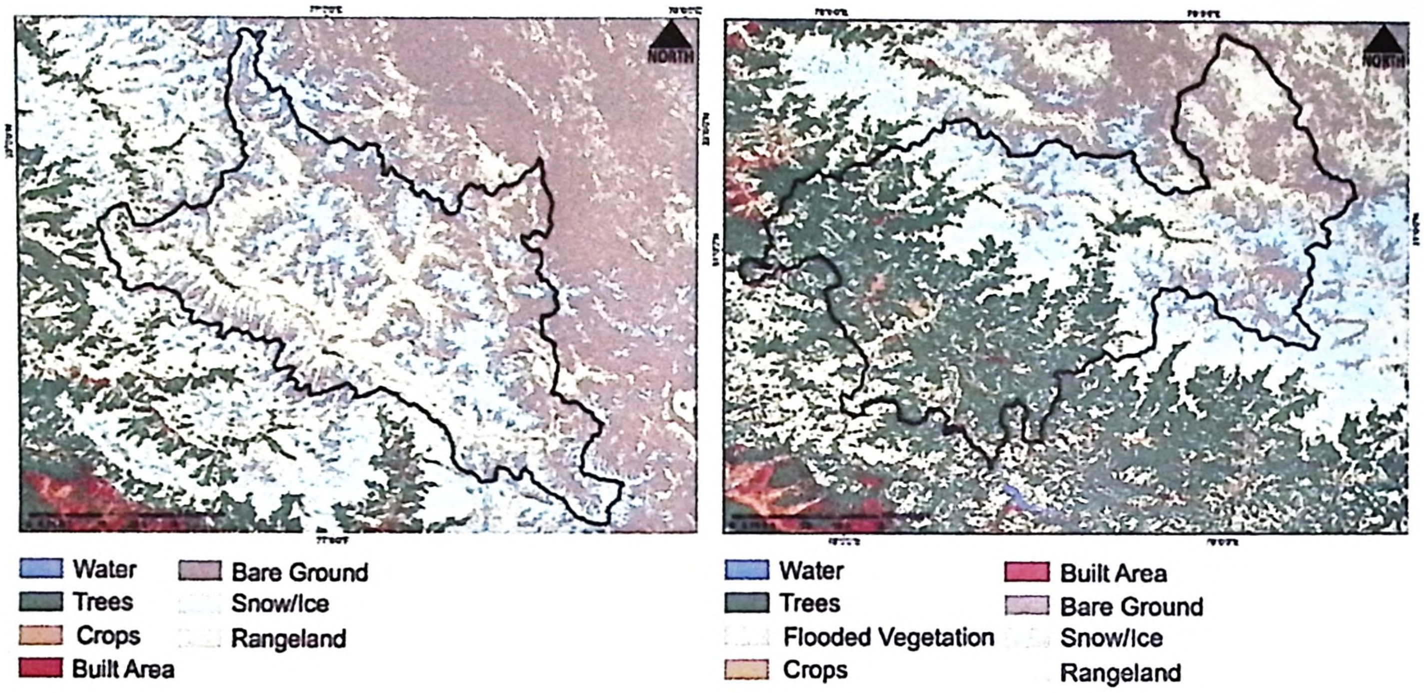

Figure 2.6. Map representing the spatial distribution of Landuse/landcover of both the study areas. Source: https://livingatlas.arcgis.com/landcover/

2.7. Geology

Lahaul and Uttarkashi, situated in the northernmost and central Himalayas, respectively, possess distinctive geologies owing to their distinct locations (Singh et al., 2021; Agarwal and Kumar, 1973). The geology of Lahaul is predominantly characterized by Precambrian metamorphic rocks such as gneiss, schist, and phyllite, alongside granite intrusions from the Paleozoic era, indicating intense tectonic activity. In contrast, Uttarkashi's geology comprises sedimentary rocks, including sandstone, shale, and limestone, suggesting the presence of a shallow sea that later subsided and was uplifted by tectonic forces. Additionally, some Mesozoic igneous rocks, such as granite and basalt, can also be found in Uttarkashi. The varying rock types in each region have given rise to distinct landforms and topography (**see Figure 2.7**).

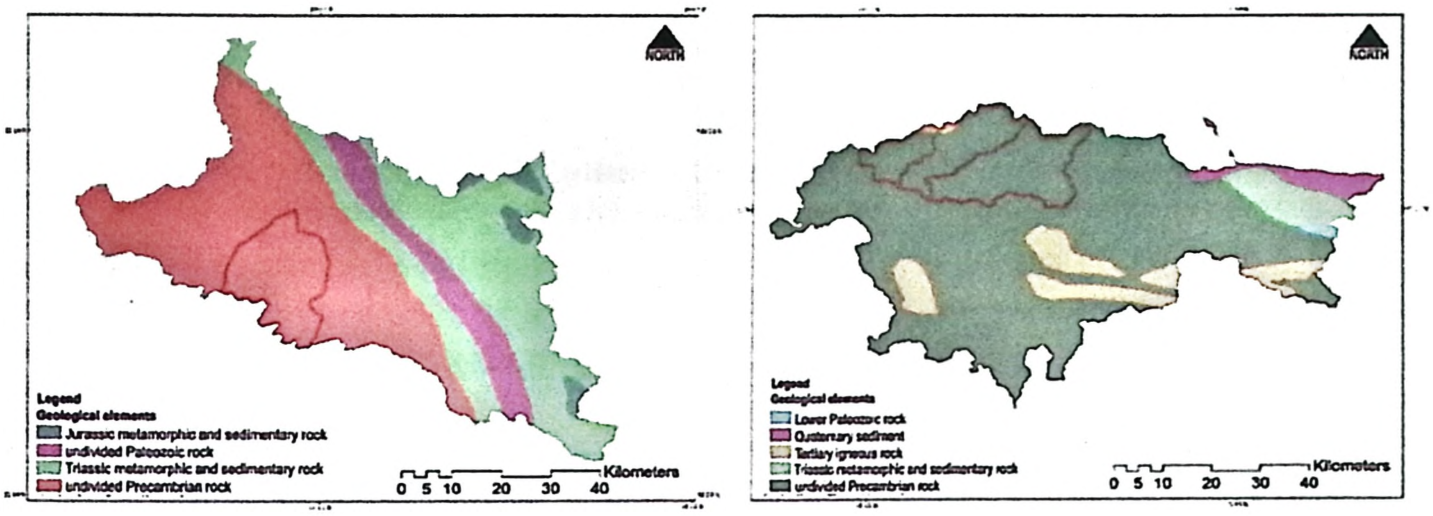

Figure 2.7. Map representing the geological characteristics of the study landscapes. Source: source: https://certmapper.cr.usgs.gov/data/apps/world-maps/

2.8. Soil

The soils in the Lahaul and Spiti valleys exhibit variability in fertility. While the soils in lower altitudes are relatively fertile, those found at higher elevations tend to be rocky and unsuitable for agriculture. The region's harsh climatic conditions and sparse vegetation cover contribute to lower soil organic matter content. As a result, essential nutrients like nitrogen, phosphorus, and potassium are deficient in the soils. However, the region's soil is enriched with micronutrients such as iron, copper, and zinc. Specifically, the soil of Lahaul and Spiti has been identified as silty clay loam, slightly alkaline, with a pH ranging from 7.2 to 8.1 and a soil organic matter content of 0.08 to 2.98% (Singh and Gupta, 1990). The nutrient content in these soils decreases in the order of Ca > K > Na > P.

In contrast, the soils of Uttarkashi comprise a mix of sandy, loamy, and clayey textures and are generally well-drained and mineral-rich. These soils also contain organic matter, contributing to their fertility. The soil acidity levels vary from slightly acidic to neutral, with some areas exhibiting slight alkalinity, and the pH ranges from 6.28 to 7.00 (Rathod et al., 2020). In the lower altitude regions of the district, the fertile soils support crop cultivation, including paddy, wheat, and various vegetables. However, as the elevation increases, the soils become less fertile, thinner, and are mainly utilized for grazing livestock (**see Figure 2.8**).

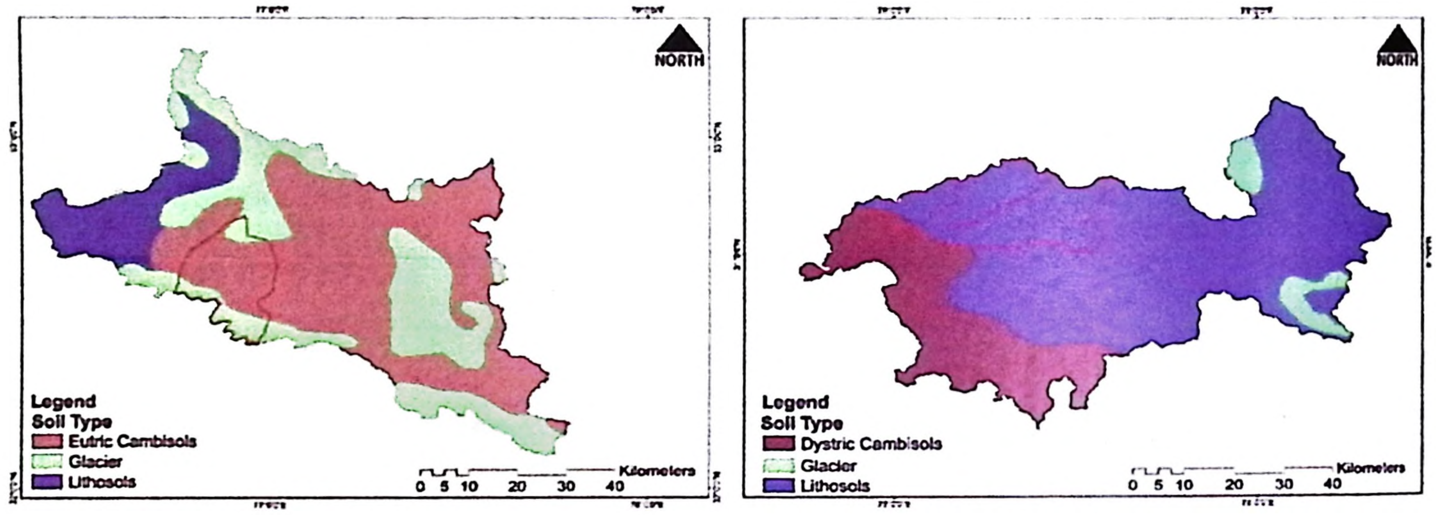

Figure 2.8. Map representing the soil characterstics of the study Landscape. Source: https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases

2.9. Rivers and Road connectivity

The Lahaul-Spiti district of Himachal Pradesh is blessed by the Spiti River, which originates from the Kunzum Range of the Himalayas, serving as a natural divider between the Lahaul and Spiti regions. On the other hand, the Chenab River flows through both India and Pakistan. It is formed by the confluence of the Chandra and Bhaga rivers, both of which arise in the upper Himalayas of the Lahaul region in Himachal Pradesh, India. The merging of these two rivers takes place at Tandi, situated

8 kilometers southwest of Keylong in the Lahaul and Spiti district. The Bhaga River originates from the Surya Taal lake, located a few kilometers west of the Baralacha La pass, while the Chandra River stems from glaciers to the east of the same pass, near Chandra Taal. The pass marks the water divide between these two rivers. The Chandra River flows for a distance of 115 kilometers, while the Bhaga River travels 60 kilometers through narrow gorges before their confluence at Tandi (Naqvi Saiyid Ali, 2012).

Conversely, the Yamuna River originates from the Yamunotri Glacier at an elevation of approximately 4,500 meters on the lower Himalayan slopes of the Bandarpunch peaks in Uttarakhand. It traverses a total distance of 1,376 kilometers, and its drainage basin covers an area of 366,223 square kilometers, constituting 40.2% of the entire Ganges Basin (Jain et al., 2007) **(see Figure 2.9)**

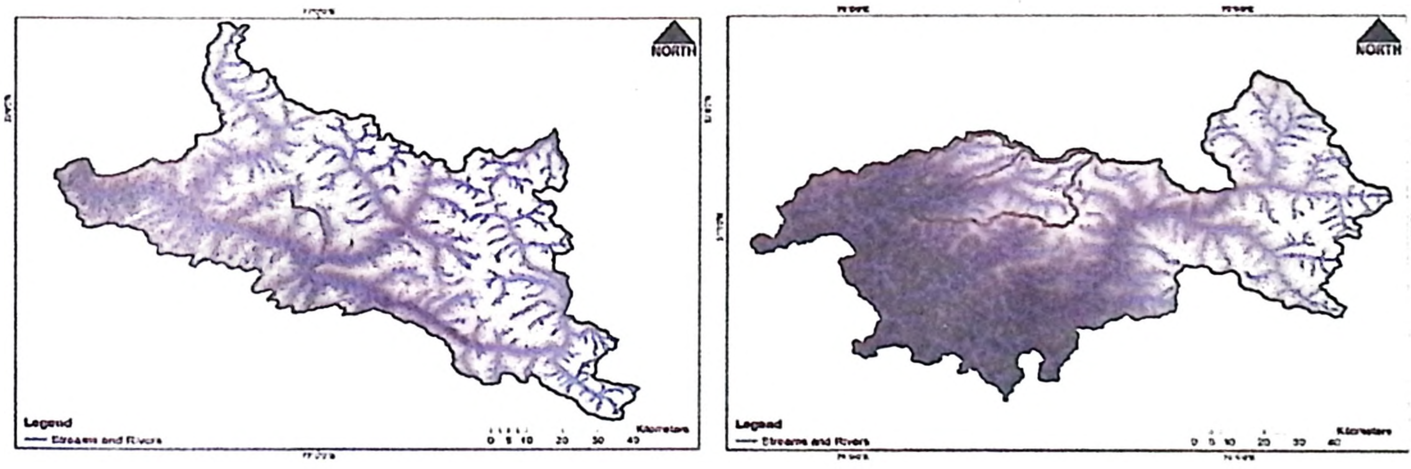

Figure 2.9. Map representing the spatial distribution of streams and rivers in the study area. Source: https://www.diva-gis.org/datadown

Lahaul Spiti, being a remote and mountainous region in the state, faces challenges in road connectivity due to its rugged terrain and harsh climate. The district is linked to the rest of the state primarily through two major roads - the Manali-Leh Highway and the Hindustan-Tibet Road. The Manali-Leh Highway is a high-altitude road connecting Manali in Himachal Pradesh to Leh in Ladakh via the Atal Tunnel. However, this road remains closed for several months during winter due to heavy snowfall and the risk of avalanches. Similarly, the Hindustan-Tibet Road, also known as National Highway 5, connects Shimla, the state capital of Himachal Pradesh, to the Tibetan plateau through Kinnaur and Spiti valleys. This road is also prone to landslides, particularly during the monsoon season.

On the other hand, Uttarkashi enjoys relatively better road connectivity, although certain parts of the district may present challenges due to its mountainous terrain. The National Highway 108, also referred to as the Char Dham Highway, runs through Uttarkashi and connects it to other significant towns in the region, including Gangotri, Yamunotri, and Badrinath. This highway holds great religious significance for Hindus, as it leads to the four sacred shrines known as Char Dham. In addition to the National Highway, Uttarkashi is served by several state highways and rural roads that facilitate connectivity to different parts of the district (**see Figure 2.10**).

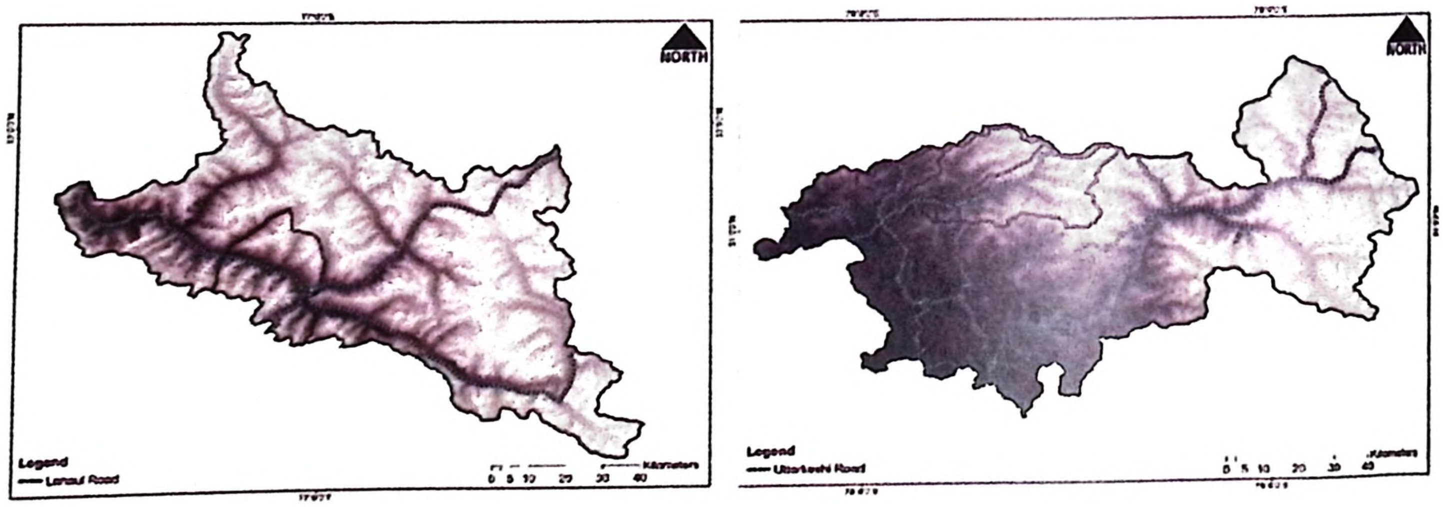

Figure 2.10. Map representing the road connectivity in the study area. Source: https://www.diva-gis.org/datadown

2.10. Climate

Temperature and Precipitation

Lahaul experiences heavy snowfall during the winter season, which poses challenges for transportation and movement. The temperatures during winters can drop to as low as -20 degrees Celsius, creating harsh and cold conditions. In contrast, the summers in Lahaul are mild, with temperatures ranging between 15 to 20 degrees Celsius. The monsoon season brings moderate rainfall and occasional thunderstorms.

Despite the difficulties imposed by its climate, Lahaul's rugged and scenic beauty attracts tourists from all over the world. Tourists are drawn to the region's picturesque landscapes, making it a popular destination despite the harsh weather conditions.

On the other hand, Uttarkashi has a humid subtropical climate with hot summers and cool winters. During the summer season, temperatures can rise to 30 to 35 degrees Celsius. The monsoon season, lasting from June to September, brings heavy rainfall and the risk of floods and landslides. The winter season, from October to February, can be cold with temperatures dropping to around 0 to 5 degrees Celsius. Occasional snowfall can occur in the higher reaches of the district.

Despite the challenges posed by extreme temperature variations and occasional natural disasters, Uttarkashi's scenic beauty continues to attract tourists from around the world **(see Figure 2.11)**.

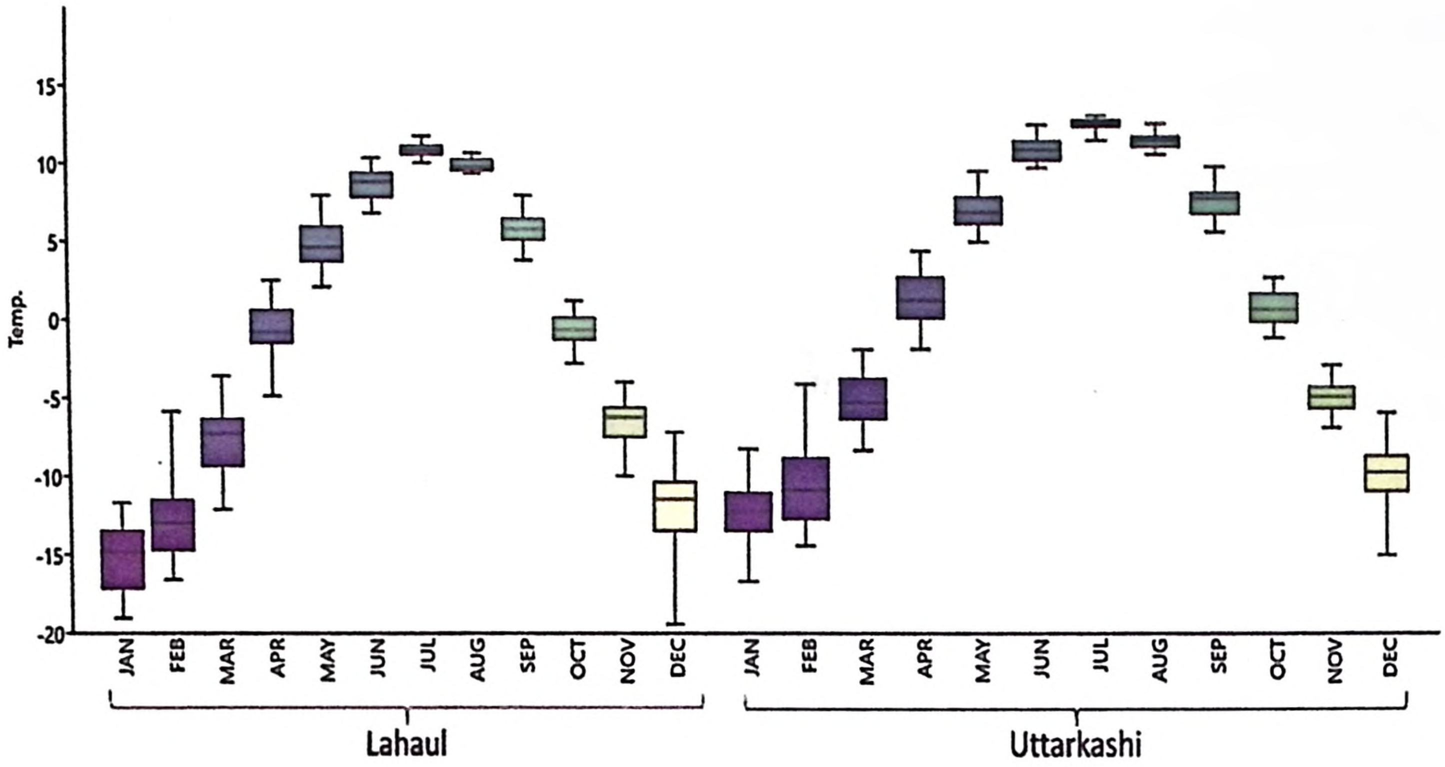

Figure 2.11. Graph depicts the annual temperature pattern in the study area from 2000 to 2021. Source: NASA POWER | Prediction Of Worldwide Energy Resources

In Lahaul, the average annual rainfall is relatively low, measuring around 170 mm. Most of the precipitation occurs during the monsoon season, lasting from June to September. In contrast, Uttarkashi experiences significantly higher levels of rainfall, with an average annual rainfall of approximately 1,400 mm. The majority of the rainfall in Uttarkashi also takes place during the monsoon season from June to September.

The heavy rainfall during the monsoon season in Uttarkashi often leads to challenges such as floods and landslides, impacting both local residents and tourists. In contrast, the winter season in Uttarkashi is relatively dry, with occasional snowfall occurring in the higher elevations of the district (Yadav et al., 2014) **(Figure 2.12)**.

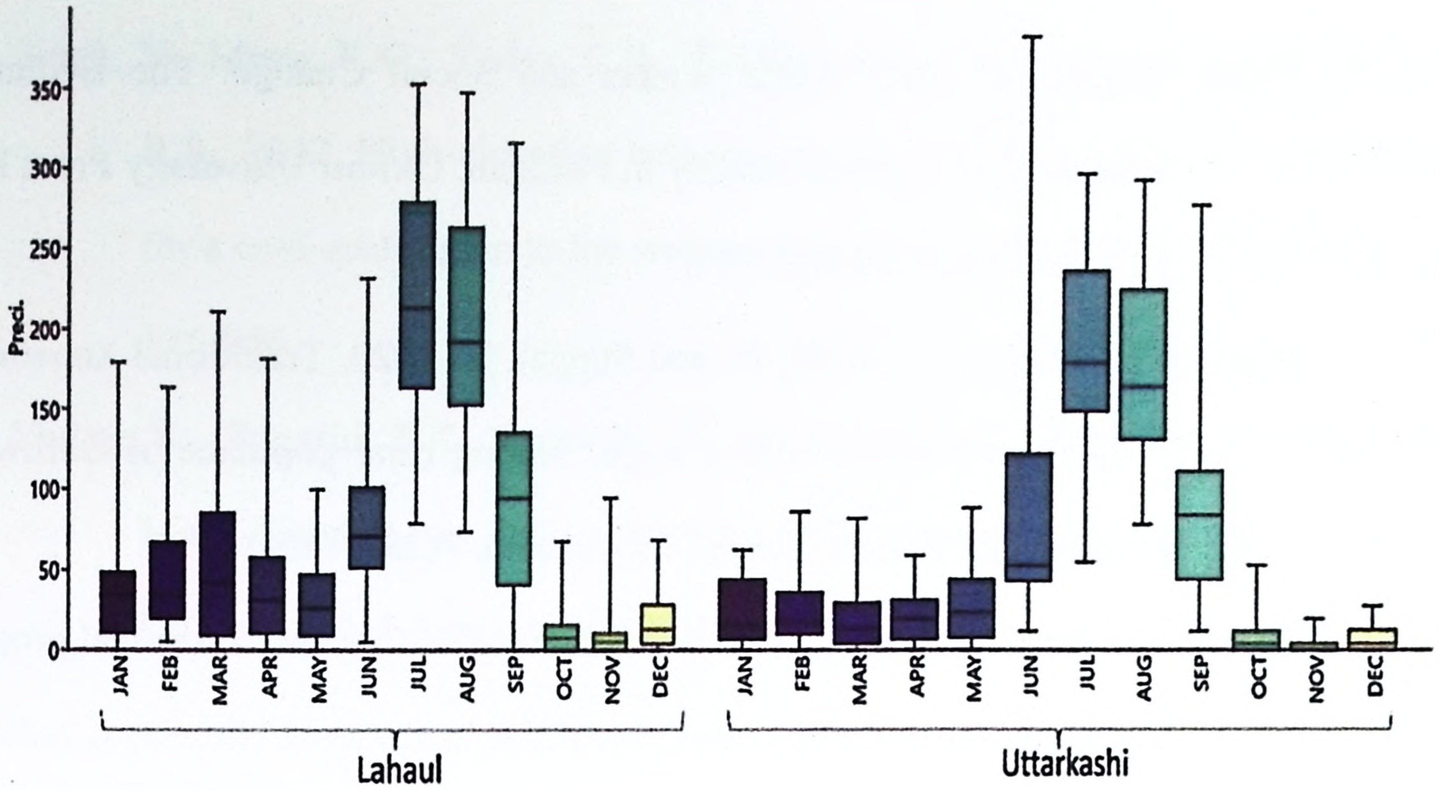

Figure 2.12. Graph depicts the annual precipitation pattern in the study area from 2000 to 2021. Source: NASA POWER | Prediction of Worldwide Energy Resources

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Chapter 3: General methodology

3.1. Study design

For the field survey, the entire landscape of the two study sites, Uttarkashi and Lahaul & Spiti, was stratified into different ranges. The study region was divided into 10 km × 10 km grids for extensive study, and further divided into 1 km × 1 km grids for intensive study (**see Figure 3.1 and Figure 3.2**), following the methods described in (Singh et al., 2018).

A single-time effort was made in the extensive grids to cover the entire landscape and identify the intensive study sites (Figure 3.1). Two intensive study sites were then selected in each district of the Western Himalaya. In Uttarkashi, the Govind Pashuvihar National Park (GPVNP) was chosen as an intensive study site, while in Lahaul Valley, the Pattan Forest range was selected.

The field survey was conducted using various conventional methods, including sign surveys, camera trapping, and non-invasive DNA sampling, specifically targeting the musk deer populations across different elevation ranges. These methods allowed for a comprehensive assessment of the musk deer distribution and behavior in the selected study sites. The use of non-invasive techniques ensured minimal disturbance to the wildlife and their habitats during the survey process.

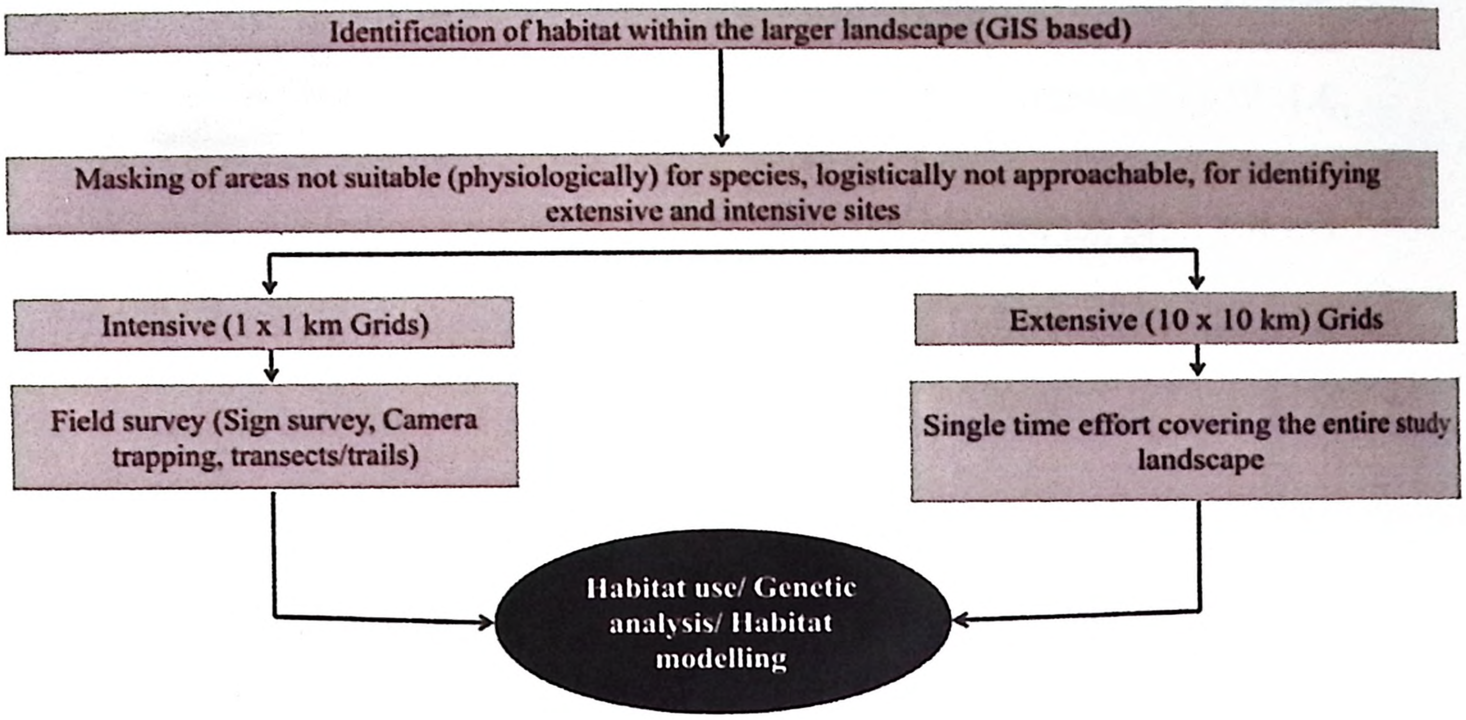

Figure 3.1. Study design flowchart for field work in North-western Himalayas.

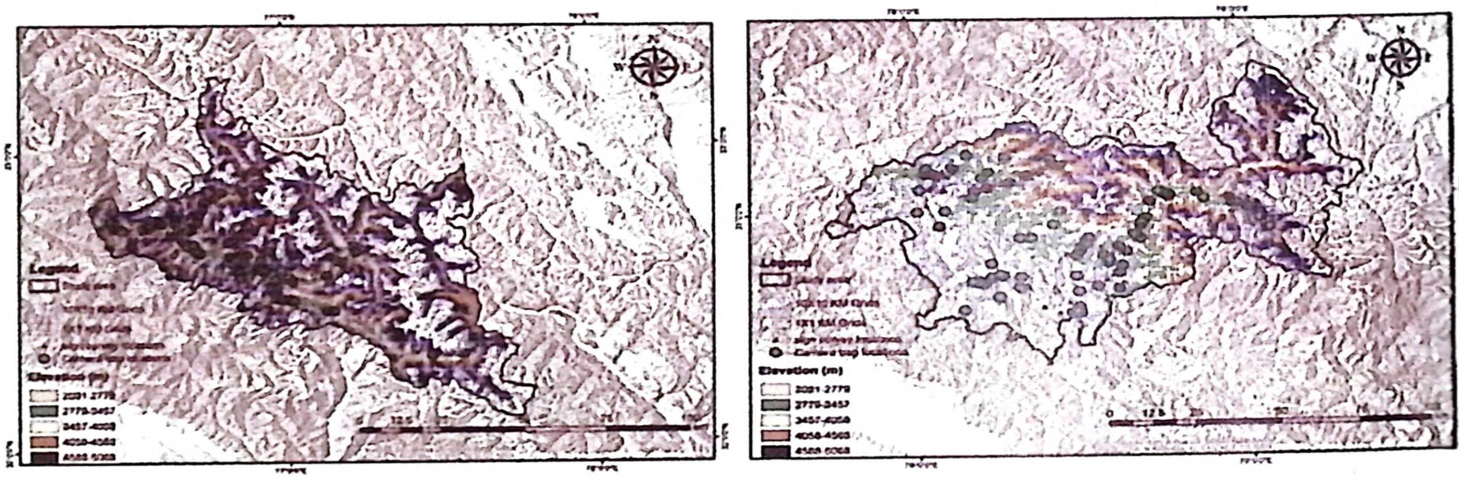

Figure 3.2. Mapping musk deer sampling locations in lahaul valley, Himachal Pradesh and Uttarkashi, Uttarakhand.

3.2. Sign survey

Sign surveys are considered a crucial method for assessing nocturnal and elusive species inhabiting high-altitude areas (Ali & Din, 2013). In this study, both direct and indirect evidence of musk deer presence were recorded through trail transects (**see Figure 3.3**). The trails were carefully monitored for any signs of musk deer, including direct sightings and laterine sites.

Sampling plots with a radius of 10 meters were established along the trail transects. For each sampling location, the spatial GPS coordinates were recorded, along with various habitat variables, such as forest type, tree and shrub species, herb species, sign types, and topographic variables (e.g., terrain, distance from water, slope, aspect, altitude).

On each sampling plot, the number of musk deer laterine sites within a 10-meter radius circular area was counted. Additionally, musk deer pellets were identified based on their distinctive shape, size, and color (Haleem, 2014). This comprehensive approach allowed for the systematic collection of data on musk deer presence and their habitat preferences in the study area. The use of GPS technology ensured accurate spatial data collection, and the analysis of habitat variables provided valuable insights into the ecological requirements of musk deer in their high-altitude environment.

Figure 3.3. Signs recorded during trail transect survey in natural habitat of musk deer.

3.3. Vegetation sampling

In addition to assessing musk deer presence, the same sampling plots were utilized to investigate the habitat utilization patterns of musk deer in the study areas. Vegetation sampling was conducted using nested circular plots with varying sizes: Trees were sampled in 10m x 10m plots, shrubs in 5m x 5m plots, and herbs/grasses in 1m x 1m plots. These sampling plots were laid along the trail transects in all available grids (**see Figure 3.4**).

To quantify the canopy cover, measurements were taken at four points within each sampling plot using a gridded mirror of 25x25 cm. The percentage of canopy cover for each sampling plot was then calculated based on the number of grids covered by foliage. Shrub species and their individual counts were recorded within 5m radius circular plots, while for herbs and grasses, four quadrates of 1m x 1m were laid within each circular plot.

This rigorous approach to vegetation sampling allowed for a comprehensive assessment of the habitat parameters and vegetation composition in the study areas. The use of nested circular plots helped capture information at different spatial scales, providing valuable insights into the habitat utilization patterns of musk deer and their interaction with the vegetation in the high-altitude environment.

3.4. Camera trapping

The use of camera traps in studying wildlife is of great importance as it provides a non-invasive and efficient method to monitor elusive and nocturnal species in their natural habitats (Tobler et al., 2008, Joshi et al., 2019). In our study, camera traps were strategically deployed along the grids used for the sign survey, targeting active natural trails, areas near forests, and water sources across various elevation ranges (**see Figure 3.2**). The placement of camera traps at knee height, approximately 2-3 meters apart from the trails (**see Figure 3.5**), allowed for optimal capture of wildlife activities.

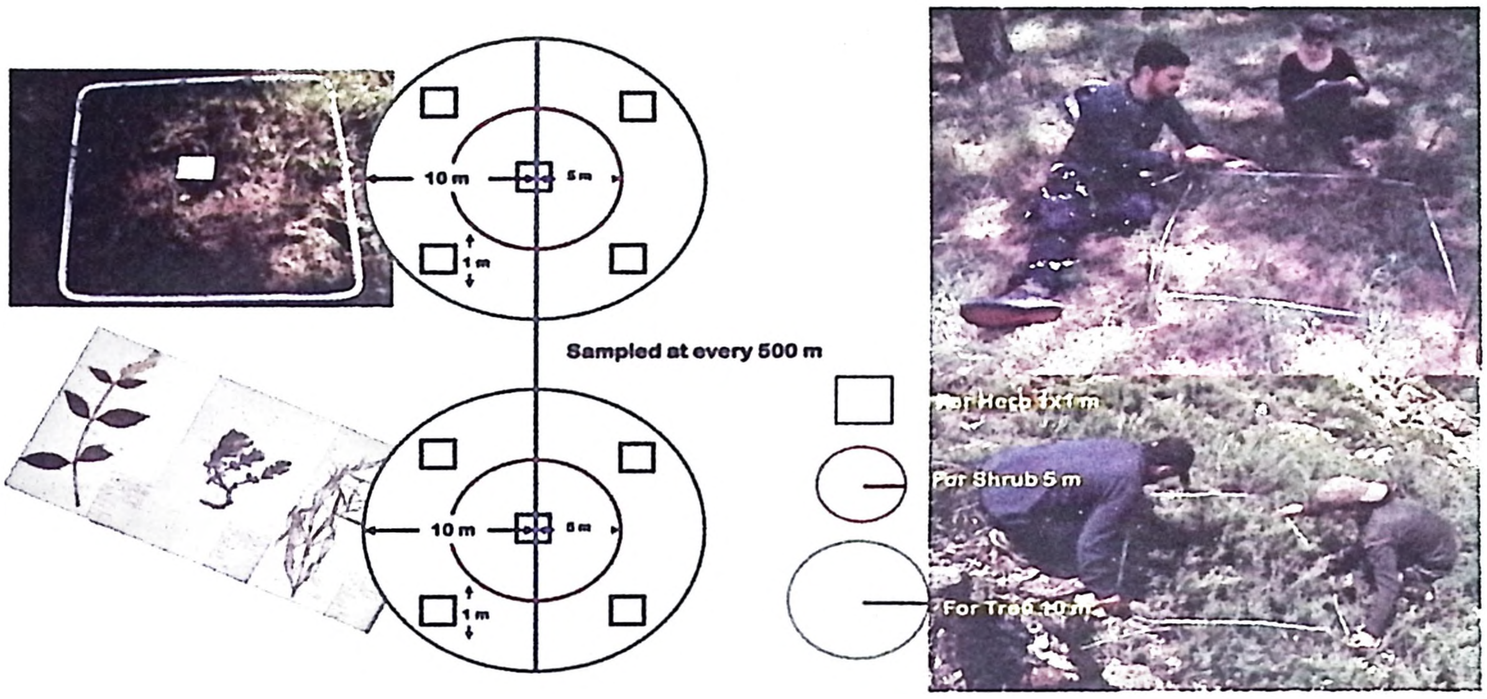

Figure 3.4. Vegetation sampling plots during our field survey in Lahaul, Himachal Pradesh and Uttarkashi, Uttarakhand.

For each camera trap, detailed information such as Camera No, SD-card No, location name, GPS coordinates, altitude, and habitat variables were recorded. The cameras were left active for a period of 24 hours (one trap night) for a duration ranging from 14 to 30 days. This extended monitoring period ensured sufficient data collection on the presence and behavior of wildlife species, particularly the elusive musk deer, within the study areas.

In conjunction with the sign survey and vegetation sampling, the camera trap method significantly contributed to our understanding of musk deer populations and their habitat utilization patterns. The camera trap data allowed us to obtain valuable insights into the temporal and spatial distribution of musk deer, their activity patterns, and their interactions with the surrounding environment. By combining the information from multiple survey methods, we were able to conduct a comprehensive assessment of the musk deer population and their ecological requirements in the high-altitude regions of Uttarkashi and Lahaul & Spiti.

Figure 3.5. Camera traps deployed in Lahual valley and Uttarkashi to unveil the elusive musk deer.

3.5. Non-invasive DNA sampling

The non-invasive DNA sampling method proved to be a valuable tool in the field survey, allowed me to gather genetic information from musk deer without direct contact or disturbance to the animals (Sharief et al.,2023). During the survey, pellet and hair samples of musk deer were collected systematically and non-invasively (**Figure 3.6**). These samples were carefully air-dried to preserve the genetic material and later transferred to sterilized vials containing silica gel for long-term storage following (Joshi et al.2019). This method ensured the integrity of the DNA samples and minimized the risk of contamination. All the collected samples were then brought back to the laboratory for further analysis, where genetic techniques were employed to study the genetic diversity, population structure, and relatedness among musk deer individuals. The non-invasive DNA sampling method has proven to be a crucial tool in conservation biology, providing valuable insights into the distribution, population size, and genetic health of elusive and endangered species like musk deer.

Figure 3.6. Non-invasive DNA sampling of musk deer in its natural habitat.

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Chapter 4:. Objective 1. To resolve the species complexes of musk deer in the North-western Himalaya using mitochondrial markers.

This chapter has been pulished as **Sharief et al.,2023.** Empirical data suggests that Kashmir musk deer is the one Musk deer distributed in Western Himalayas:An integration of ecology, Genetics and Geospatial modelling approaches.Biology. 12, 786. https://doi.org/10.3390/biology12060786.
This chapter has been presented in International conference, **Biology23 held in Geneva, Switzerland on 16-17 February 2023.**

Abstract

Resolving the taxonomic ambiguity of a species is important for the long-term viability. : Insufficient research has been conducted on Musk deer species across their distribution range primarily because of their elusive behavior, and it occupies remote high-altitude habitats in the Himalayas above 2500m. The available distribution records, primarily derived from ecological studies with limited photographic and indirect evidence, fail to provide comprehensive information on species distribution. Consequently, uncertainties arise when attempting to determine the presence of specific taxonomic units of Musk deer in the Western Himalayas. This lack of knowledge hampers species-oriented conservation efforts, as there needs to be more species-specific initiatives focused on monitoring, protecting, and combatting the illegal poaching of Musk deer for their valuable Musk pod. I used transect surveys (220 trails), camera traps (255 cameras), non-invasive DNA sampling (40 samples) and geospatial modelling (279 occurrence records) to understand the occurrence and identify suitable habitat of Kashmir musk deer (KMD) (*Moschus cupreus*) in the Uttarkashi district of Uttarakhand and Lahaul–Pangi landscape of Himachal Pradesh.

All captured images and DNA- based identification results confirmed the presence of only Kashmir musk deer) in Uttarakhand and Himachal Pradesh. Results suggest that KMD inhabits in a narrow range of suitable habitats (6.9%) of the entire Western Himalayas. Since all evidence indicates that only KMD is present in the Western Himalayas, we suggest that the presence of other species of musk deer (Alpine musk deer and Himalayan musk deer) was wrongly reported. Therefore, future conservation plans and management strategies must focus only on the KMD in the Western Himalayas.

4.1. Introduction

Musk deer are solitary, shy and crepuscular/nocturnal animals which are distributed in a continuous to fragmented patches of forested and alpine scrub habitats of mountain regions in 13 Asian range countries (Groves et al., 1995; Ostrowski et al., 2016; Pan et al., 2015; Shukla et al., 2018). Musk deer are least studied group among the ungulates which have a specific role in alpine scrub habitats, thus are considered as ecological indicators for the ecosystem stability (Zaitsev, 2006). Total, seven species of musk deer have been described *viz.*, Siberian musk deer *(Moschus moschiferus)*, Chinese forest musk deer (*M. berezovskii*), Anhui musk deer (*M. anhuiensis)*, Alpine musk deer (*M. chrysogaster*), Himalayan musk deer (*M. leucogaster)*, Black musk deer (*M. fuscus*) and Kashmir musk deer (*M. cupreus*) (Ostrowski et al., 2016; Pan et al., 2015; Sathyakumar et al., 2015; Singh et al., 2020). While their distribution status is yet to be delineated with the concrete evidences in support of their presence in the different geographic areas. Out of seven species, four species (*M. chrysogaster, M. cupreus, M. fuscus and M. leucogaster*) are supposed to be distributed in India in the fragmented patches all along the states of Uttarakhand, Himachal Pradesh, Union territory of Jammu and Kashmir (Western Himalaya), Sikkim (Central Himalayas) and Arunachal Pradesh (Eastern Himalayan) between the elevation range of 2500-3500 asl (IUCN, 2018). The species is recently gaining attention among the biologists after drastic population decline caused by intensive illegal hunting for musk pod (Khan et al., 2006; Wangdi et al., 2019; Yang et al., 2003,). Increased anthropogenic activities such as livestock grazing, fuel wood collection, habitat fragmentation and climate change are other key drivers responsible for population decline of the species (Green, 1986; Timmins & Duckworth, 2015; Wangdi et al., 2019). Consequently, all the species of musk deer have been listed as an "*Endangered*" according to IUCN Red List of Threatened species (Timmins & Duckworth, 2015) and also listed in *Appendix I* in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2015) and listed as *Schedule I* in the Wildlife Protection (Act) 1972 of India.

Literature suggests the presence of both Himalayan musk deer (*M. leucogaster*) and Alpine Musk deer (*M. chrysogaster*) in Uttarakhand, Himachal Pradesh, Sikkim and Arunachal Pradesh (Ilyas, 2014; IUCN, 2023; Pan et al., 2015; Pal et al., 2020; Shukla et al., 2018). However, confirmed records of presence of these two species from the Western Himalaya is not available either through the DNA based techniques or Camera trapping. Moreover, earlier existence of Kashmir musk deer (*Moschus cupreus,* henceforth KMD) was only reported from the western most limits of Hindukush Himalaya in Jammu and Kashmir, India and Afghanistan (Ali, 2014; Ostrowski et al., 2016). Recent genetic evidence revealed presence of distinct lineage of musk deer in Uttarakhand than all other known species (Shukla et al., 2018), which was subsequently confirmed as KMD in Uttarakhand and central Nepal (Kumar et al., 2022; Singh et al., 2019). The ambiguities still prevail in musk deer over the number of species/sub-species, taxonomy and distribution in India (Pan et al., 2015; Shukla et al. 2018). Resolving taxonomic ambiguity, uncertainty in distribution pattern of musk deer species in Western Himalayas is important to develop species-oriented conservation programme and provide specific protection under Wildlife Protection Act (WPA). Therefore, the present study was aimed to understand, how many species of musk deer are distributed with the possible distribution range of KMD in Western Himalayas.

We used combined approaches (camera trapping, sign survey, non-invasive DNA sampling and species distribution modelling) to understand the taxonomy and distribution of musk deer. Camera trapping is one of the most important non-invasive methods to detect elusive species for assessing habitat ecology, activity pattern and density estimation, inventorization (Bowkett et al., 2008; Rovero et al., 2014). While DNA based approaches provide the information about the taxonomy, distribution and evolutionary history (Joshi et al., 2022; Kumar et al., 2022). Confirmed species occurrence data from different methods accurately help to understand the distribution, delineate species boundary and identify possible suitable areas in the different habitats using species distribution modelling methods (Guisan & Thuiller, 2005; Tara et al., 2015). Therefore, present study integrates the different methods to delineate the species boundaries, assess the distribution range and predict the suitable habitat of musk deer using the mitochondrial DNA markers, camera trapping, sign survey and species distribution modelling in Western Himalayas.

4.2. Materials and Methods

4.2.1. Study area

The present study was conducted in Uttarkashi district of Uttarakhand, Pangi valley of Chamba district and Lahaul valley of Lahaul and Spiti district in Himachal Pradesh. We divided study landscape into 10 × 10 km to conduct the reconnaissance survey and further based on the species presence in different elevation ranges and forest types we intensified the study are into 1 × 1 Km grids. We covered all the logistically accessible grids in possible distribution range of musk deer in both the landscapes.

4.2.2. Camera trapping, sign survey and non-invasive genetics sampling

We placed total of 134 camera traps in Uttarkashi district, 111 camera traps in Lahaul valley, Himachal Pradesh. Of which 79 camera traps in Uttarkashi, 105 in Lahual valley were installed within the range of Musk deer distribution between 2200 to 4200 meters elevational range (**Figure 4.1**). We used Ultra-compact SPYPOINT FORCE-11D trail camera (SPYPOINT, GG Telecom, Canada, QC) and Browning trail camera (Defender 850, 20 MP, Prometheus Group, LLC Birmingham, Alabama, https://browningtrailcameras.com) camera traps to detect the presence/absence species. The cameras were mounted 30–45 cm above ground on natural trails, valley and in the identified latrine sites of Musk deer without lures. We also traversed 106 trails (592 km) in Lahual valley, 99 in Uttarkashi of 2-7 Km (1277 km) in length to study the latrine sites, collect non-invasive samples and other habitat parameters. We collected a total of 170 samples (116 Lahaul valley, 54 Uttarkahsi). We also identified 32 latrine sites of Musk deer in lahaul and 11 latrine sites in Uttarkashi since no other overlapping species is found within that range especially in Lahaul valley. All the putative faecal pellets of KMD origin were air-dried in field conditions and stored in 50 ml sterilized Silica containing vials for DNA analysis. Below is the workflow followed for resolving taxonomy of musk deer (**Figure 4.1**)

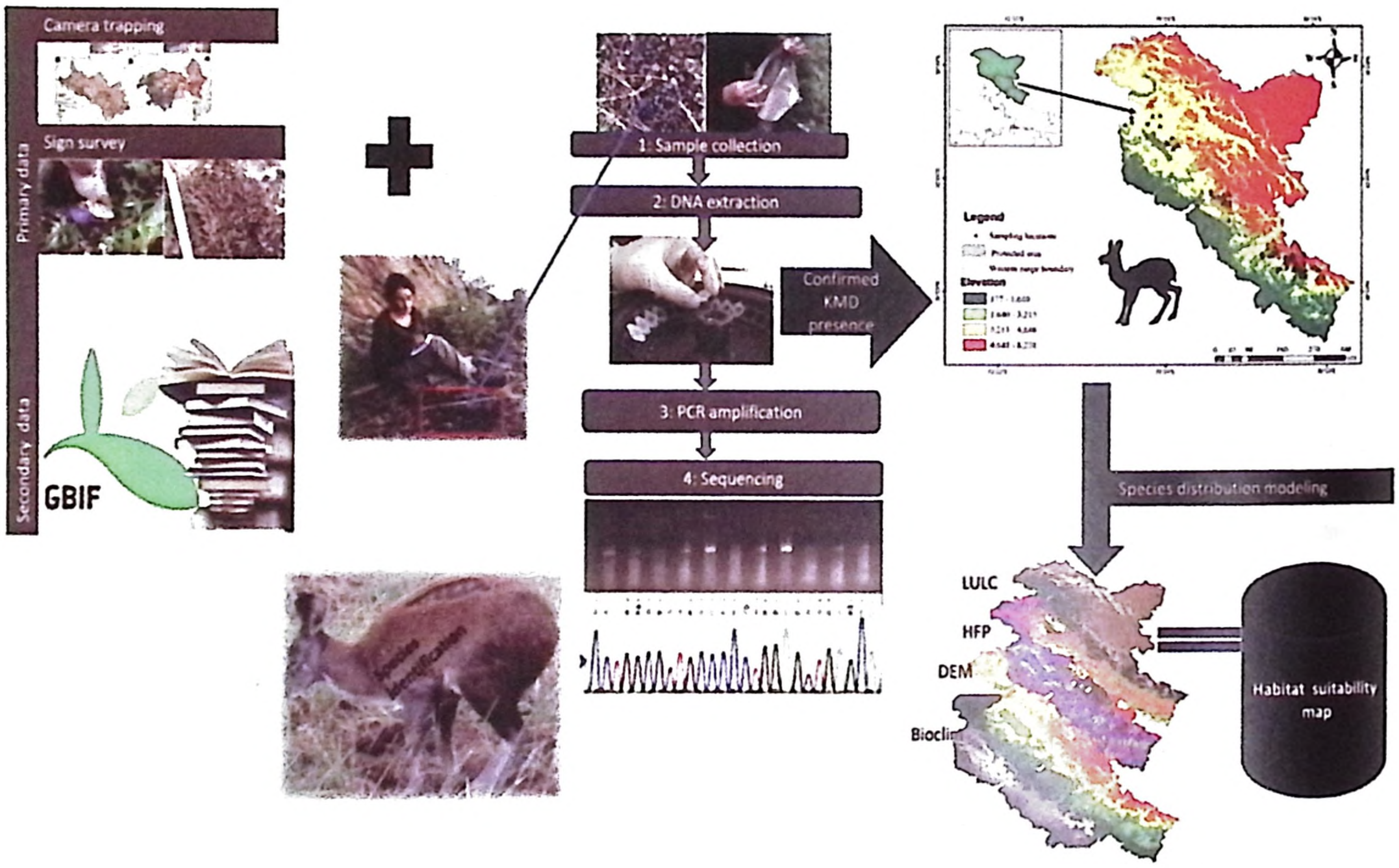

Figure 4.1. Workflow followed for resolving the taxonomic ambiguity and distribution of musk deer in Western Himalaya.

4.2.3. DNA extraction, PCR amplification and species identification

All the collected samples were further processed for the DNA extraction and genomic DNA was extracted using the QIAamp DNA Stool Mini Kit, (Qiagen, Germany), according to the manufacturer's instructions. Extracted DNA was checked on 1.0% agarose gel (**Figure 4.2**). The estimation of DNA band intensity was performed using gel electrophoresis. The band intensity was classified on a scale of 0 to 3, based on the visualization of the bands on the gel. A classification of 0 indicated that the band was not visible, 1 represented a band with poor intensity, 2 denoted a band with moderate intensity, and 3 indicated a band with good intensity. To ensure accurate measurements, DNA templates were appropriately diluted, taking into account the dilution factor determined from the 0 to 3 scaling of band intensity observed on the gel. This approach allowed for a reliable and quantifiable assessment of the DNA bands during the analysis. To detect any contamination in the DNA extraction procedure and reagents, we performed the extraction procedure with two independent negative controls. Since it is inherent problem with failure of amplifying larger amplicon size primers or uniformly amplify the same length of primers in all samples for species identity with faecal samples due to long exposers to environmental conditions, degraded DNA yield, poor samples quality and presence of inhibitors is major challenge (Joshi et al., 2022). Therefore, i ensured the species identification of all collected samples using the mitochondrial primers of different sizes to achieve maximum success and used five set of mitochondrial primer from higher to lower base pair size primer (**Table 4.1**). All the extracted samples of musk deer were PCR amplified using five set of primers *viz.,* two cytochrome b: MCB gene (Verma and Singh, 2003), BOV-CYTB (Joshi et al. 2019) and three control region primers: MD-CR1 and MD-CR2 (designed by present study) and BOV-CR (Joshi et al.2019) of 192-450 bp range size (**Table 4.1**). PCR reactions were carried out in a 10 µl PCR, containing 2.0 µl DNA template, 0.8 mM $Mgcl_2$, 2x Buffer, 0.3-unit Tag DNA polymerase, 2.0 µl dNTPs and 1.0 µM each primer. PCR cycling was performed with an initial denaturation for 5 minutes at 94 ˚C, followed by 40 cycles of denaturation for 45s at 94 ˚C annealing for 60 s at 55 ˚C and 1 min extension at 72 ˚C with a final extension of 72 ˚C for 10 min. After amplification through PCR, the resulting PCR products were purified using Exo-SAP treatment. This step was crucial to eliminate any remaining oligonucleotides and dNTPs, ensuring a clean template for the subsequent DNA sequencing process. For each mitochondrial gene (**as listed in Table 4.1**), both the forward and reverse primers were employed to set up the cycle sequencing PCR. The Big dye terminator cycle sequencing kit version 3.1, manufactured by Applied Biosystems in the USA, was utilized for this purpose.

The sequencing products underwent a purification step to remove any unbound ddNTPs, accomplished through an alcoholic precipitation method. Subsequently, all purified PCR samples were subjected to DNA sequencing using the ABI 3730 Genetic Analyzer (Applied Biosystems in the USA).

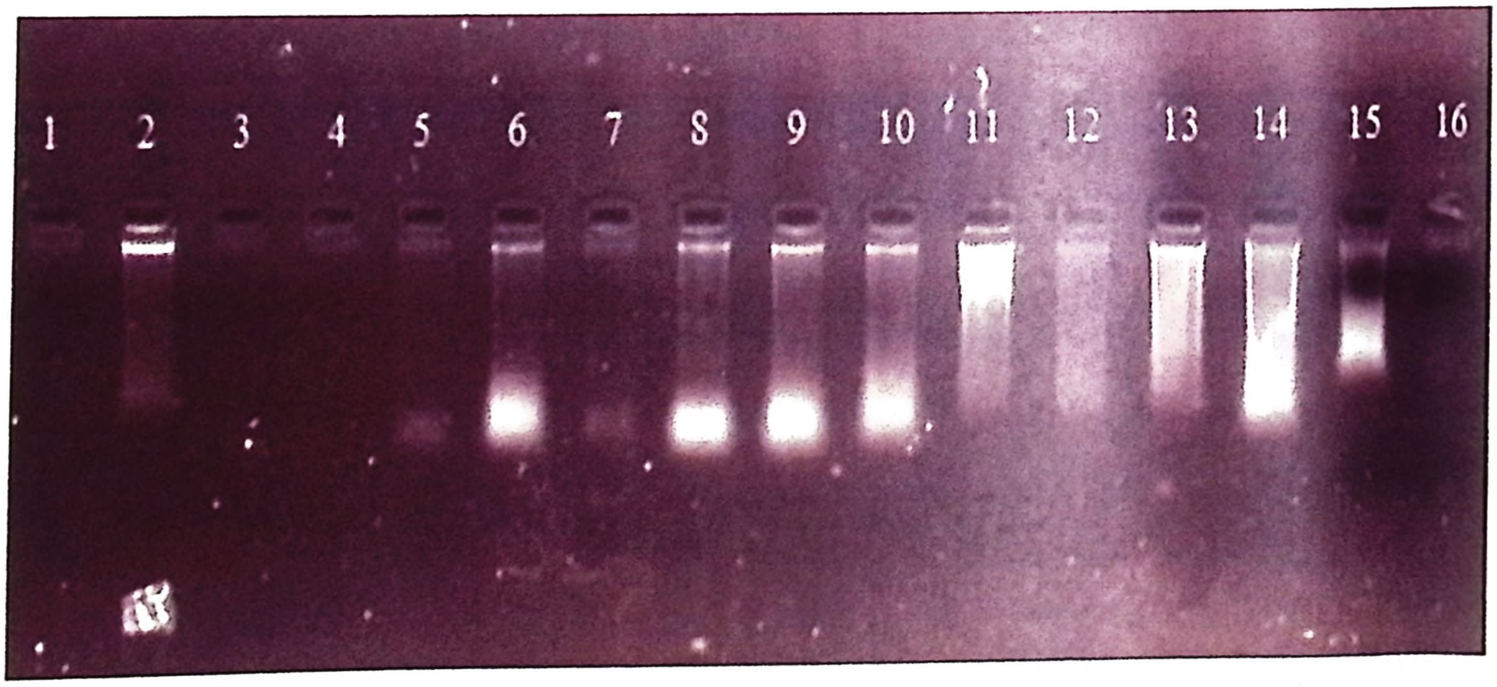

Figure 4.2. Gel image of DNA extracted from pellet samples of musk deer on 1.0% agarose gel.

Table 4.1. Details of mtDNA primers used for species identification.

SNT.	Primer name	SG*	Bp	Reference
Cytochrome b				
	MCB	36	401	Verma and Singh, 2003
	Bov-CYTB	67	192	Joshi et al. 2019
Control Region				
	MD_CR1	24	335	Present study
	MD_CR2	4	293	Present study
	Bov-CR	10	190	Joshi et al. 2019
	Total	**141**		

SG*: Number of sequences generated with each prmer for species identification.

4.2.4. Data Analysis

All the sequences of musk deer were examined and processed for the quality check using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Total of 141 sequences pass the quality check which were further used for species identification and subsequent analysis. For multiple sequence alignment (MSA), the CLUSTAL-W algorithm, implemented within the BioEdit v 7.0.9.0 software (Hall, 1999), was employed to align and compare the sequences for further analysis. Generated sequences were validated using reference data through the BLAST tool of GenBank (http://www.ncbi.nlm.nih.gov) and our reference database. The taxonomic units (TUs) were detected by having above 98% similarity of our sequences with reference sequences available in NCBI and with intra-species sequence divergence of <2%. The sequences of other Musk deer species were retrieved from the NCBI to understand the phylogenetic relationship (23 for cytb and 24 for control region, 19 complete mitogenome). Haplotype file was generated using the DnaSP and nucleotide position for each variable sites were identified using the MEGA 10 (Kumar et al., 2018). Sequence variation, number of polymorphic sites and sequence divergence, neighbour-joining and Maximum likelihood phylogenetic tree were calculated using the Kimura 2 parameters (K2P) in the MEGA 10 (Kumar et al., 2018). The Bayesian phylogenetic tree was generated using BEAST v.2.1.3 to reconstruct the phylogenetic relationship among musk deer species. I used the HKY nucleotide substitution model selected for 20 million generations, with trees sampled at every 1000th generation. Substitution rate was used following John and avise, 1998; Kumar et al., 2022. To ensure convergence, I discarded the initial 20% of trees as considered burn-in. The phylogenetic tree was then annotated and visualised using Tree-Annotator version 1.8.1 and FigTree version 1.3.1.

Camera traps and sign survey

All the images of camera traps were sorted species wise and species were identified with expert advice and species images which were not identifiable were discarded from the analysis. The capture rate (total number of independent captures/total trap nights) of Musk deer was calculated using identified number of camera trap images. The number of camera trap nights was computed from the camera's deployment to its retrieval. We only evaluated a second capture after a one-hour break for large mammals (Tobler et al., 2008).

4.3. Results

Based on the combined effort of 245 camera traps *(111 (2657 trap nights) in Lahaul, 134 (3563 trap nights) in Uttarkashi)* deployed in different habitats of Lahaul and

Uttarkashi, total of 103 independent captures of Musk deer were recorded. The image were validated with the available photographic records of musk deer. All the captured images of musk deer were identified as Kashmir musk deer (KMD; *Moschus cupreus*) **(Figure 4.3)**. The capture rate was 0.006 ±0.002 in Uttarkashi, 0.03±0.008 in Lahaul valley. Among the 220 trails walked we identified laterine sites of Kashmir musk deer. Both the independent captures of the Musk deer (103 captures) in Lahaul and Uttarkashi and 141 generated sequences confirmed the presence of Kashmir Musk deer in Western Himalaya. Thus, I confirmed the presence of Kashmir Musk deer in Himachal Pradesh and Uttarakhand, Western Himalaya using both DNA based techniques and photographic evidence through camera traps. Our results suggest that Kashmir musk deer has extended distribution from Northern Pakistan till central Nepal **(Figure 4.4)**. All the samples were identified as Kashmir musk deer with >98% similarity (**Figure 4.5 a**). Further, for the detailed phylogenetic and genetic diversity, a total of 69 sequences of cyt b (320bp) and 64 sequences of control region (335bp) were found comparable and compatible with unified length with data I obtained from NCBI. Both primer sequences were merged and a consensus sequence of 644 bp was further used for taxonomic classification and phylogenetic reconstruction in which a total of 10 haplotypes with 10 polymorphic sites were identified from 63 concatenated sequences of Kashmir musk deer and (to estimate the population genetics using mtDNA are described in details in Chapter 7) . In concatenated sequences, intraspecies sequence divergence ranged between 0.002-0.011 in Kashmir musk deer (**Table 4.2**). Whereas, the highest interspecies sequence divergence was found between Himalayan musk deer and Kashmir musk deer = 0.087 (**Table 4.3**). The least sequence divergence was found between Anhui musk deer and Alpine musk deer =0.026. The phylogentic tree is broadly splitted into two major clades, clade 1 representing KMD and clade 2 representing other species of musk deer. In the Neighbor-joining phylogenetic tree KMD form a separate basal clade when compared with other species having high bootstrap support (**Figure 4.5**) and is diverged from other species of musk deer over ~5.1 million years ago. Whereas other species have divergence time between 1.5-4.7 mya.

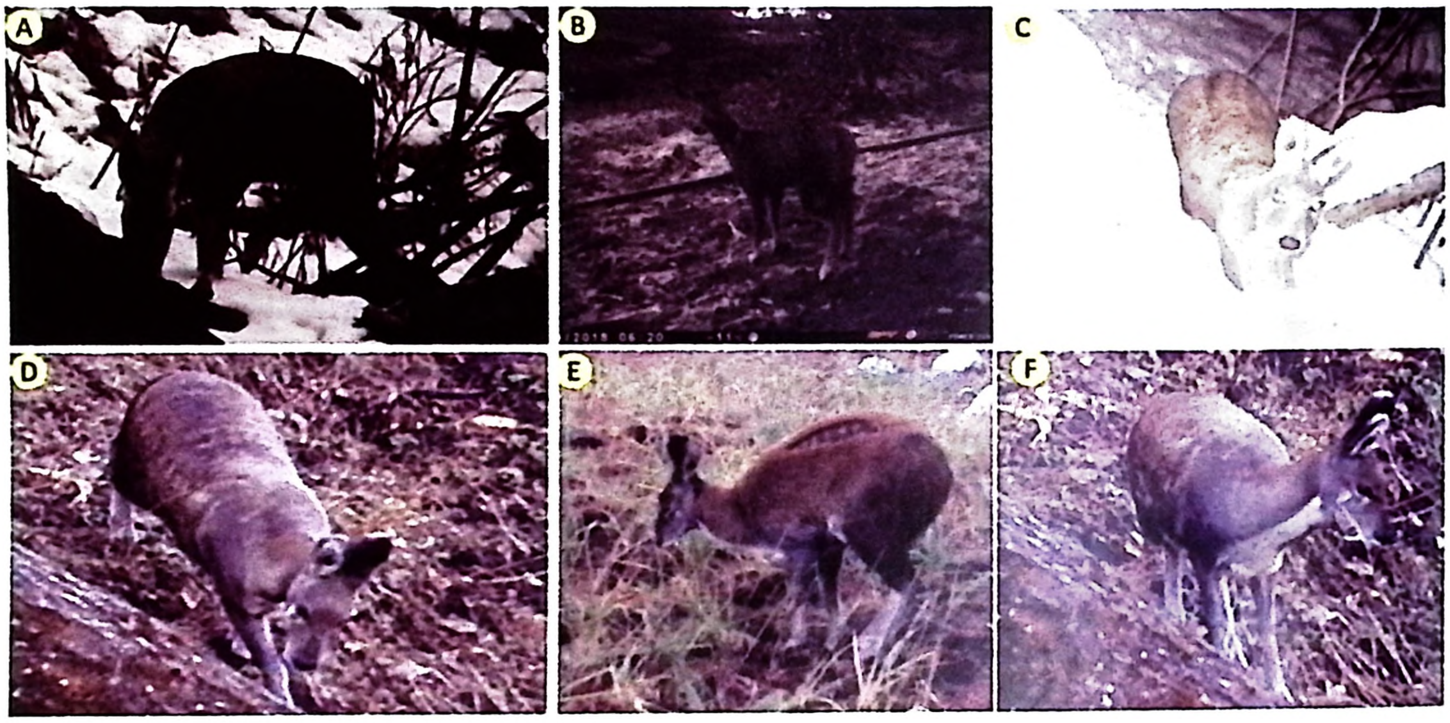

Figure 4.3. Camera trap captures of Kashmir musk deer: A–C in Lahaul Valley, and D–F in Uttarkhashi.

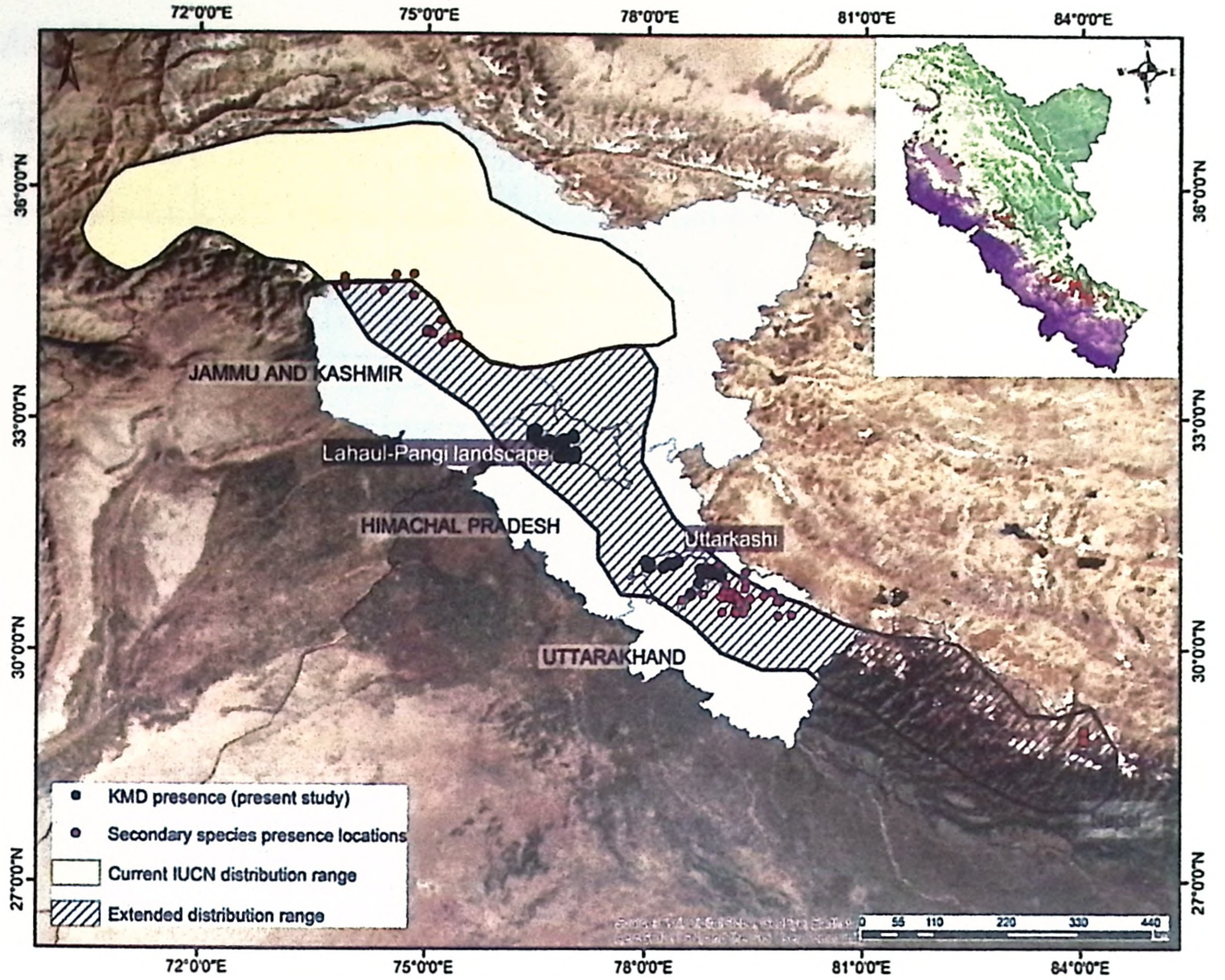

Figure 4.4. Confirmed presence of Kashmir musk deer in Western Himalaya.

Table 4.2. Table showing the intra species sequence divergence of Kashmir musk deer.

		N			P			H		
KMD		63			10			10		
H1	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
H2	0.003									
H3	0.005	0.002								
H4	0.006	0.003	0.005							
H5	0.008	0.005	0.003	0.002						
H6	0.009	0.006	0.008	0.003	0.005					
H7	0.011	0.011	0.013	0.008	0.009	0.005				
H8	0.009	0.009	0.011	0.009	0.011	0.006	0.005			
H9	0.008	0.005	0.006	0.002	0.003	0.002	0.006	0.008		
H10	0.005	0.005	0.006	0.002	0.003	0.005	0.009	0.011	0.003	

Table 4.3. Table showing interspecies sequence divergence in genus *Moschus*.

	M.anhuiensis	*M.Chrysogaster*	*M. beresovskii*	*M.moschiferus*	*M.leucogater*	*M.cupreus*
M.anhuiensis						
M.Chrysogaster	0.0263					
M. beresovskii	0.0387	0.0334				
M.moschiferus	0.0540	0.0558	0.0538			
M.leucogater	0.0648	0.0608	0.0626	0.0780		
M.cupreus	0.0723	0.0701	0.0710	0.0806	0.0874	

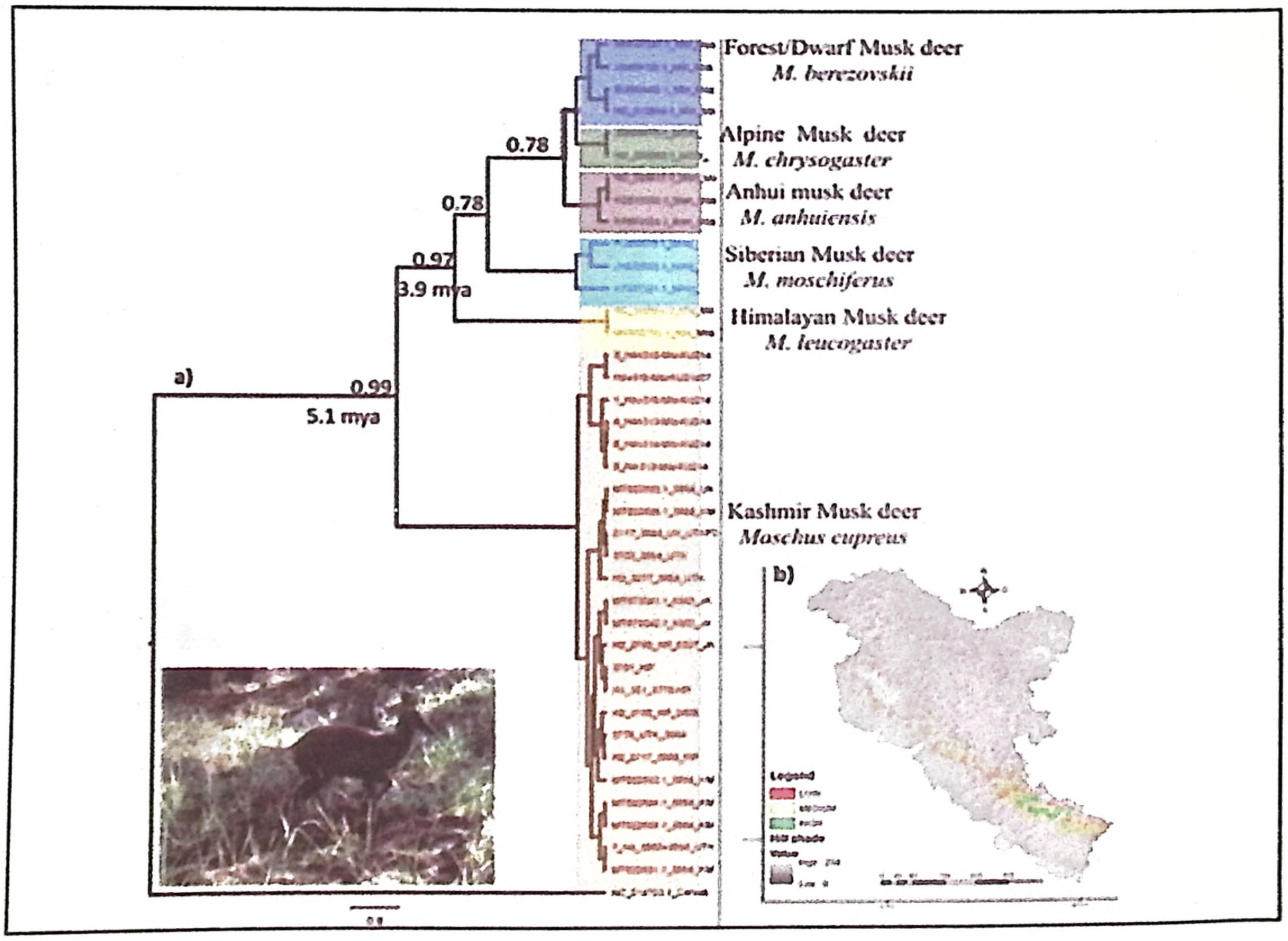

Figure 4.5. a) Phylogenetic tree (Neighbor-joining) showing relationship of Kashmir Musk deer with other musk deer species using mitochondrial Cytochrome b gene. b) Possible occurrence of KMD in Western Himalaya (detailed results and discussion in in Chapter 2)

4.4. Discussion

The current research resolved the taxonomic ambiguity of musk deer and observed presence of KMD only in both the studied sites and the suitable habitat of KMD is mostly confined to higher altitudes between 2500-4500 m (Singh et al. 2020). However, other two species Himalayan musk deer and Alpine musk deer were surprisingly not observed thus indicates the presence of only KMD in the landscape. We confirmed the presence of Kashmir musk deer in western Himalayas through camera trap images and DNA based identification. The main morphological difference between Kashmir musk deer and other species of musk deer is the presence of white patch underneath starting from throat to belly. Since the IUCN database suggests the distribution of other two Alpine musk deer and Himalayan musk deer in Western Himalayas. Hence, based on our results, we infer that, the presence of Himalayan musk deer and Alpine musk deer may be misinterpreted in the Western Himalayan, especially in Uttarakhand and Himachal Pradesh. A noteworthy discovery has been made in Mustang, Nepal, where the presence of KMD was recently reported. This is particularly significant as KMD was previously thought to be confined to the Himalayan region of Kashmir, Pakistan, and Afghanistan, according to earlier studies (Grubb et al., 2005; Singh et al., 2019). The confirmed extended range of KMD up to central Nepal (Singh et al., 2019) support our finding of presence of only KMD in Western Himalayas. Recent images and video captured on June, 2021 by Uttarakhand Forest Department after a span of 10 years was also identified as KMD from Chamoli district (Media report, 2021). Further, the phylogenetic tree shows that our sequences clustered with the sequences of Kashmir musk deer and formed a separate and basal clade from other species of musk deer. Our results infer that Kashmir musk deer forms a distinct evolutionary taxonomic unit from other species. Recent study by Kumar et al 2020, also suggest a distinct lineage of Kashmir musk deer in Kedarnath WLS and Nanda Devi biosphere reserve, Uttarkahand. Furthermore, KMD was found to be highly diverged from other species of musk deer over ~5.1 mya having highest interspecies divergence with Himalayan musk deer indicating a significant genetic difference between the two species **(Figure 4.5)**. On the other hand, the least sequence divergence was found between Anhui musk deer and alpine musk deer (0.026) suggesting a closer genetic relationship between these two species. Our results suggest a long and distinct evolutionary history of Kashmir musk deer from other species of musk deer.

The KMD clade is highly divergent, possibly due to geological and environmental changes that had a prominent impact on the species evolution, including musk deer in the Himalayas and the Tibetan Plateau (Pan et al. 2015). Considering the distribution of KMD in the western Himalayas eco-climatic zone (Pandit MK, 2017) which is geographically separated than its congeneric species distributed eastwards (eastern Himalayas) and experiencing variability in vegetation, temperature, precipitation, and topographic barrier may result in observed paraphyletic clades **(Figure 4.5.)**. Thus, the conservation, distribution, and status of KMD need to be extended throughout the western Himalayas up to central Nepal.

Our study suggests that KMD inhabit a narrow range of western Himalayas between 2500-4500 m (Singh et al., 2020), corresponding to only 6.9% of the western Himalayas; the rest of the region is likely a non-suitable habitat for the species. The conservation, distribution, and status of KMD needs to be extended throughout the western Himalayas. Since this species is found in higher elevation ranges and mostly inhabit inhospitable habitats thus limited information is available. Further, this species is also heavily poached for musk pod which is leading to rapid decline of its population (IUCN, 2023; Khan et al., 2006). I also observed during our survey and interacting with locals that poaching for musk pod and encroachment are the two major threats of declining population of musk deer. I found that, most of the habitat in Uttarkashi and Lahaul–Pangi landscape is restricted to a narrow range which is threatened by anthropogenic pressures such as intensive livestock grazing, fuel wood cutting, fodder collection. Conservation and management plans need information on various life history traits of KMD especially population estimation which is completely lacking. Conservation of this rare animal is of utmost importance today, as it is fast heading towards total extinction. Instead of lamenting for the past follies, adaptive conservation planning is required to conserve the Musk deer in and outside protected areas. The study has provided valuable information on the status, taxonomy and distribution of KMD using multiple approaches. These methods can be effectively applied in population estimation, studying habitat ecology, and assessing suitable habitats for the species. To enhance our knowledge, I recommend the implementation of long-term monitoring to assess musk deer occupancy, identify poaching hotspots, estimate population size, and study habitat utilization patterns within potential areas in the Western Himalaya region.

4.5. Conclusion

The present study confirms the new record of Kashmir musk deer from Lahaul valley, Himachal Pradesh and Uttarkashi district of Uttarakhand. No evidences of other two supposed species (Alpine musk deer and Himalayan musk deer) were found from the studied landscape. Thus, we infer that there is only presence of Kashmir musk deer in Western Himalayas and the other two species were misidentified earlier. Based on the phylogenetic tree, Kashmir musk deer is placed in a separate clade as an outer group and different from all other musk deer species. It is diverged from other musk deer species ~5 mya. Our study also predicts the suitable habitat of KMD in Western Himalayas and suggests precipitation and elevation are the top influential factors governing the distribution of KMD. Since the population of KMD is declining at an alarming rate, proper conservation and management is of utmost importance. The identified suitable habitats in and outside the protected areas should be prioritized for proper conservation and management planning. Although KMD is a top conservation priority species, no robust data is available on the population of KMD hence we urge further research on population estimation in its entire distribution range.

4.6. References

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Chapter 5: Objective 2. Mapping the habitat suitability of musk deer in the North-western Himalaya.

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Abstract

Human expansion and anthropogenic activities are causing the conversion of forests to other land uses in the Himalayas, which is threatening species with extinction. To address this issue, I simulated the future Landuse/Landcover for the year 2030 and then used an ensemble model to assess its impact on the Kashmir Musk Deer (KMD) distribution in the context of land use change. Our simulation suggests a decline in croplands and shrublands, while mixed forests increase in the future scenario. Evergreen broad-leaf and needle-leaf forests are likely to convert to mixed forests, while croplands and barren areas transform into savannas. Precipitation, elevation, and mixed forests were found to be the most significant factors influencing KMD distribution. Only 20,690 km^2 out of the total area of 324,666 km^2 is currently suitable for KMD, but this is projected to increase to 22,701.47 km^2 in the future. I predict a habitat gain of about 2,722 km^2 in new areas and a loss of 711 km^2 in existing habitats for KMD by 2030, with Uttarakhand state losing much of the suitable habitat. However, Jammu and Kashmir will offer new habitats for KMD in the future. Our landscape configuration investigation indicates a decline in the number of patches and aggregation index in the future scenario. Most of the suitable KMD habitats are outside

the current protected areas (PA), making the current PA network insufficient for long-term conservation. Therefore, I suggest boundary rationalization of the PA to include suitable habitats that are currently not protected to ensure the long-term survival of the KMD.

5.1. Introduction

Although the Himalayan ecosystem is identified as a global biodiversity hotspot, it is indubitable that this region is undergoing tremendous degradation because of growing anthropogenic pressure and climate change (Mukherjee, 2021; Penjor, 2022). The cumulative impact of land use and climate change on species distribution and the associated environmental predictors of the species are eye-catching topics that have gained attention from researchers in recent years (Singh et al., 2020: Bhagaria et al., 2021; Rathore et al., 2022). Previous studies have brought out that global warming is threatening the biological integrity of the Himalayan region by altering the climatic isotherms (Lamsal et al., 2018; Mukherjee et al., 2020; Bhagaria et al., 2021; Dutta et al., 2022). However, recently it has been established that forest to other land-use conversions, along with anthropogenic activities, has significantly altered the configuration of the fragile Himalayas ecosystem, thereby increasing the extinction risk to the species (Pandit et al., 2007; Flynn et al., 2009). Besides this, the growing anthropogenic activities, including agriculture expansion, growing urban sprawl, increasing linear projects (road, rails), and unviable utilization of ecosystem produces are detrimental to the local biological diversity (Pandit et al., 2007; Chandra et al., 2020; Chatterjee et al., 2021). To deal with these degradation drivers, it is imperative to recognize and project the impacts on the species habitat in the future scenario.

The Himalayan mountain ecosystem is considered the water tower region of Asia. It provides habitat for diverse flora and fauna, including ecologically specialist ungulates such as Kashmir musk deer (*Moschus cupreus*, hereafter = KMD), a conservation-dependent species throughout its range. The KMD is distributed in high-elevation mountain ranges of Afghanistan, Pakistan, India and Nepal in continuous-to-fragmented habitat patches (Grubb 2005; Ostrowski et al., 2016; Singh et al., 2020). In India, it is distributed in the Western Himalayan region (Jammu and Kashmir, Himachal Pradesh and Uttarakhand). KMD is a shy and solitary species which is mostly crepuscular and nocturnal in activity (Harris and Cai 1993). It plays an essential role as a herbivore by influencing the mountain ecosystem's vegetation composition and nutrient recycling (Green, 1987; Bagchi and Ritchie, 2010). However, climatic and land use changes in recent years have impacted many species, including KMD (Singh et al., 2020; Dutta et al., 2022) and the impacts may lead alternation of species composition as well as the habitat (Laws, 2017; Bagaria et al., 2020). It is well known that KMD, an ecological specialist, is most vulnerable to such changes due to the non-availability of new habitat refugia because it already occupies higher elevations between 2200-4300 m (Schaller, 1977; Singh et al., 2020).

Furthermore, the musk deer population in its entire range is dwindling because of the illegal killing for its pods, habitat fragmentation, and habitat loss as a result of land use change (Aryal and Subedi, 2011; Khadka et al., 2017). The over-exploitation and habitat destruction are significant factors behind its population decline throughout its entire distribution range. Considering the hastened declines in its population, the species is listed among the endangered species by the IUCN (Timmins & Duckworth, 2015), Appendix-I species by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2015), and Indian Wildlife Protection Act, (1972) provided it highest protection by listed musk deer among the Schedule-I species. Hence due to increasing threats and its endangered status, it becomes crucial to evaluate the habitat at the current as well as to predict anticipated changes in the future for developing a strategy so that the species can be conserved on long term basis in the Himalayas.

The rising transformation of forest to non-forest land use and increasing temperature in the Himalayan region are accelerating biodiversity loss (Brodie, 2016, Penjor, 2022). Although the cumulative impact of warming climate and land use change on species is little explored (Chatterjee et al., 2022; Rathore et al., 2022); however, future simulated landuse change is yet under-explored (Mukherjee et al., 2019; Chatterjee et al., 2022). For developing data-driven conservation and management decisions, there is a need to understand how land use change can affect species distribution. Therefore, I attempted to evaluate the change in land use using the ensemble modelling approach by modelling its impact on the distribution of KMD across its distribution range in the Western Himalayas. I projected the anticipated effects of land cover and land use (LULC) conversion on species range by simulating the LULC of 2030.

Additionally, I evaluated the current and future suitable habitats using the landscape configuration indices to recognize the habitat fragmentation extent and habitat patch intactness of the KMD in the Western Himalayas. Finally, I quantified the proportion of suitable habitats of KMD distribution inside and outside the Protected

Areas (PA) network in the Western Himalayas. Understanding the current and future suitable habitats of KMD and the change will help develop a data-driven strategy for this lesser known and conservation priority species.

5.2. Materials and methods

5.2.1. Kashmir musk deer presence location data

The occurrence GPS locations of KMD were collected from both the primary as well as secondary data sources. The species occurrence survyes were conducted during 2018-2020 in the range districts of Uttarakhand and Himachal Pradesh (**Figure 5. 1**), and the secondary data were collected from published studies for the KMD distribution range. However, A total of n=279 presence locations were obtained by combining primary as well as secondary sources, i.e., published articles (Syed and Ilyas, 2007; Ali et al., 2014; Shukla et al., 2018; Nandy et al., 2020; Pal et al., 2021; Kumar et al., 2022). Out of the total presence locations, only n=220 spatially independent occurrence records of KMD were used in performing distribution modelling of the KMD in the Western Himalayas. The SDM toolbox in ArcGIS 10.9 was used for testing special autocorrelation among the locations.

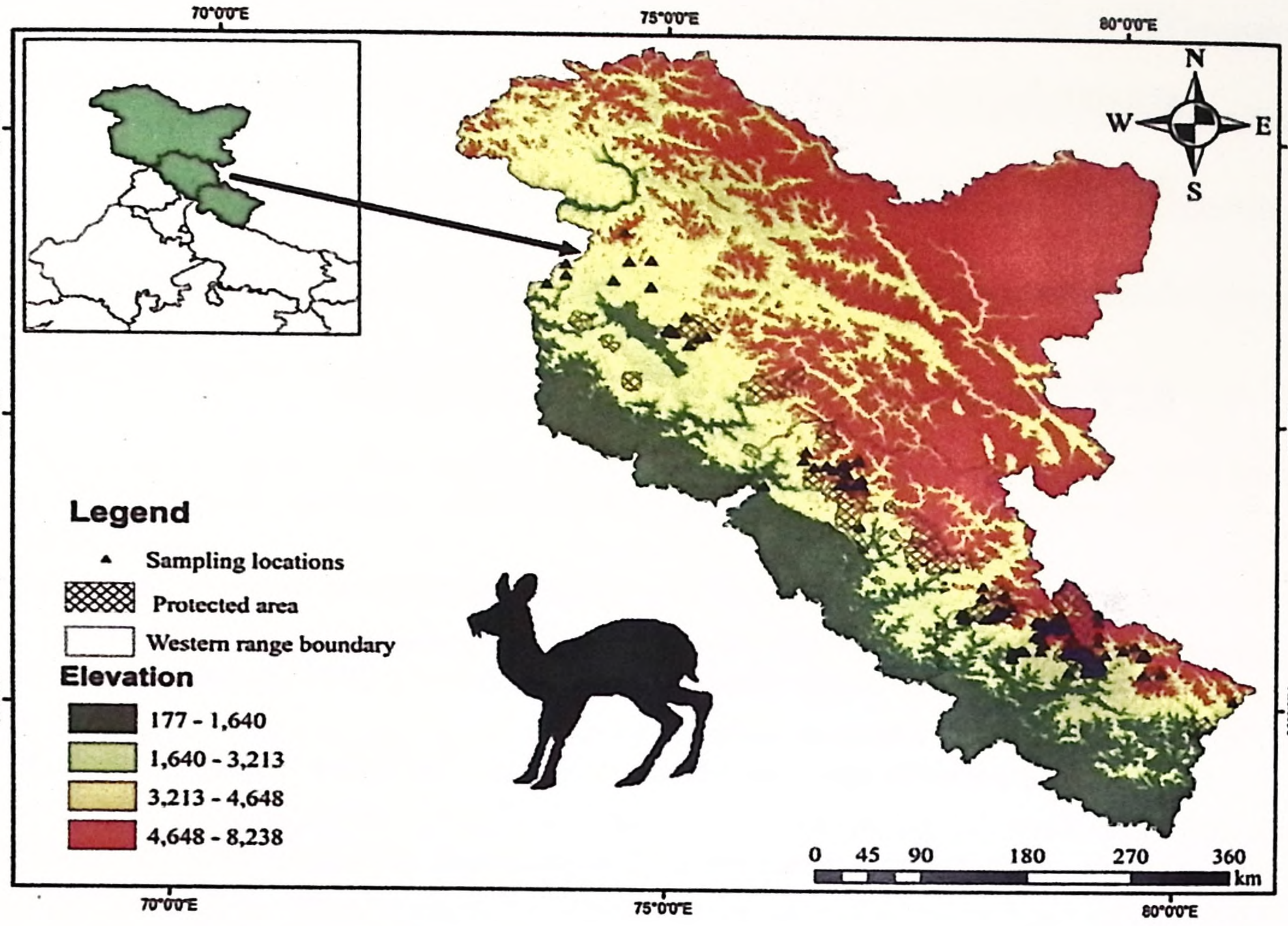

Figure 5.1. Study area map with presence locations of Kashmir musk deer.

5.2.2. Data Preparation

I used habitat variables considering the ecological needs and behaviour of the species. These variables were classified into four categories (bioclimatic, topographic, LULC and anthropogenic) for mapping the suitable habitats of the KMD. I extracted 19 bioclimatic variables from World Clim Ver. 2 (www.worldclim.org) with ~1km spatial resolution (Hijmans, 2005). The LULC for 2010 and 2020 was classified using the MODIS (Moderate Resolution Imaging Spectroradiometer) Land Cover Type Product (MCD12Q1) version 6 with a 500-meter resolution (https://earthexplorer.usgs.gov). After reclassifying the MODIS data, I classified the landscape into 11 different land use and land covers. The raster images were generated for all the landuse landcover classes available for the study extent using ArcGIS 10.9. The topographic predictors (elevation, slope and aspect) were generated by using the Shuttle Radar Topographic Mission image downloaded from Earth Explorer (https://earthexplorer.usgs.gov). The study species is susceptible to anthropogenic disturbances; hence I used the global human footprint data (downloaded from SEDAC, NASA; https://sedac.ciesin.columbia.edu). Further, I downloaded the linear features, such as road and water, from DivaGis (www.diva.gis.org). I rescaled all the predictors at 30 arc second spatial resolution using the spatial-analyst tool of ArcGIS 10.9. The collinearity among the predictor variables was removed using the Pearson correlation test, and the variables with a value higher than 0.8 (rs>0.8) were dropped from the analysis (**Figure 5.2**) (Warren et al. 2010). Finally, only 18 spatially independent variables were retained for modelling the habitat suitability of the KMD (**Table 5.2**).

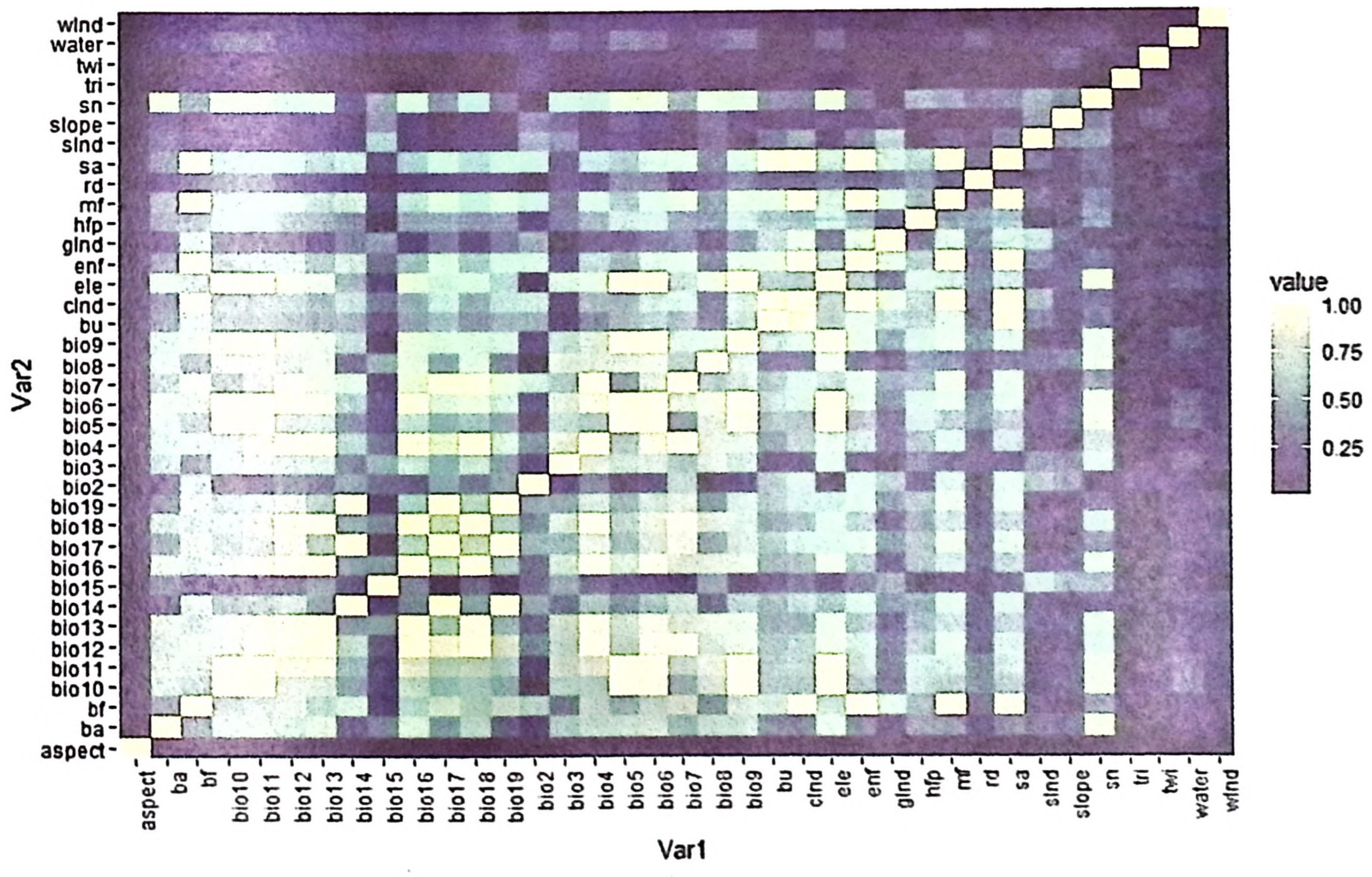

Figure 5.2. Graph showing the variables used for modelling the suitable habitat of Kashmir musk deer with pearson correlation coefficient greater than 0.8 (rs>0.8).

Table 5.1. List of environmental variables used for modeling the distribution of the species in the western Himalayas.

S.No	Variable	Code	Data	Source
1	Bio2 (Mean diurnal range)	Bio2	Worldclim	Worldclim
2	Bio5 (Maximum temperature of warmest quarter)	Bio5		
3	Bio9 (Mean temperature of driest quarter)	Bio9		
4	Bio11 (Maean temperature of coldest quarter)	Bio11		
5	Bio13 (Precipitation of wettest month)	Bio13		
6	Bio14 (Precipitation of driest month)	Bio14		
7	Bio15 (Precipitaion seasonality)	Bio15		
8	Bio19 (Precipitation of coldest quarter)	Bio19		
9	Grass land (dominated by herbaceous annuals)	Grass	MCD12Q1	USGS
10	Mixed Forest	MF		
11	Evergreen needle leaf forest	ENF		
12	Permanent snow and ice (at least 60% of area is covered by snow for at least 10 moths of a year)	SN		
13	Distance to water	WA	Calculated using log Euclidean distance (Arcgis 10)	Diva gis
14	Distance to road	RD		
15	Elevation	ELE	SRTM	USGS
16	Slope	SLP		
17	Aspect	ASP		
18	Human foot print	HFP	EARTHDATA	SEDAC

5.2.3. Data analysis

Landuse future simulation

The future LULC was simulated using the MOLUSCE plugin of QGIS (ver: 2.8.9) by following the cellular automata (CA) model (NEXTGIS, 2017). The acquired LULC data for 2010 and 2020 was used for simulating the future landscape for 2030 using the spatial transition matrix (Liping et al., 2018; Chatterjee et al., 2022). I used the transition probability matrix and the variables responsible for the transition to simulate the LULC of 2030 based on past patterns of LULC changes. (Ullah et al., 2019; Chatterjee et al., 2022). The transition probability matrix was calculated using the artificial neural network analysis of LULC data from 2010 and 2020 (Chatterjee et al., 2022) (**Table 5.2**). Further, I considered elevation, aspect, distance to road, topographic roughness index, topographic wetness index, surface temperature and human footprint as dependent variables for computing the transition matrix (Chatterjee et al., 2022). The sampling mode was random, and the neighbourhood pixels for the analysis were kept at nine cells with 1000 iterations. The accuracy of the model was tested using Cohen's kappa (k) following our previous study by Chatterjee et al.,2022).

Table 5.2. Table showing the transition probability surfaces of future simulated landuse/landcover classes in North-western Himalaya.

Transition period	Land use land cover classes	ENF	BF	MF	SLND	SA	GLND	WLND	CLND	BU	SN	BA
2010-2020	**ENF**	0.804	0.005	0.113	0.000	0.077	0.001	0.000	0.000	0.000	0.000	0.000
	BF	0.044	0.745	0.117	0.001	0.082	0.010	0.000	0.000	0.000	0.000	0.000
	MF	0.025	0.036	0.860	0.000	0.077	0.001	0.000	0.000	0.000	0.000	0.000
	SLND	0.001	0.000	0.000	0.492	0.002	0.418	0.000	0.000	0.002	0.008	0.076
	SA	0.018	0.003	0.042	0.000	0.893	0.032	0.001	0.010	0.001	0.000	0.000
	GLND	0.001	0.000	0.000	0.001	0.077	0.899	0.002	0.004	0.000	0.000	0.016
	WLND	0.002	0.000	0.000	0.000	0.009	0.015	0.939	0.000	0.000	0.010	0.025
	CLND	0.000	0.000	0.000	0.000	0.217	0.047	0.000	0.735	0.000	0.000	0.000
	BU	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
	SN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.938	0.061
	BA	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.024	0.959
2020-2030	**ENF**	0.605	0.000	0.000	0.000	0.395	0.000	0.000	0.000	0.000	0.000	0.000

Transition period	Land use land cover classes	ENF	BF	MF	SLND	SA	GLND	WLND	CLND	BU	SN	BA
	BF	0.000	0.826	0.006	0.000	0.167	0.001	0.000	0.000	0.000	0.000	0.000
	MF	0.000	0.000	0.952	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000
	SLND	0.000	0.000	0.000	0.974	0.026	0.000	0.000	0.000	0.000	0.000	0.000
	SA	0.000	0.000	0.012	0.000	0.987	0.001	0.000	0.000	0.000	0.000	0.000
	GLND	0.000	0.000	0.000	0.000	0.040	0.960	0.000	0.000	0.000	0.000	0.000
	WLND	0.000	0.000	0.000	0.000	0.139	0.001	0.860	0.000	0.000	0.000	0.000
	CLND	0.000	0.000	0.002	0.000	0.077	0.000	0.000	0.920	0.000	0.000	0.000
	BU	0.000	0.000	0.000	0.000	0.628	0.000	0.000	0.000	0.372	0.000	0.000
	SN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.997	0.003
	BA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000

ENF $^{\text{evergreen needle forest}}$, BF $^{\text{broadleaf forest}}$, MF $^{\text{mixed forest}}$, SLND $^{\text{shrubland}}$, SA $^{\text{savanna}}$, GLND $^{\text{grassland}}$, WLND $^{\text{wetland}}$, CLND $^{\text{cropland}}$, BU $^{\text{built-up}}$, SN $^{\text{snow}}$, BA $^{\text{barren area}}$

Model building

The SDMs are commonly used to model species-environment relationships and forecast species spatial distributions (Bagaria et al., 2020; Dutta et al., 2022; Elith et al., 2006; Guisan and Zimmermann, 2000; Hijman et al., 2005). However, current progress in advanced computing algorithms with varying ensemble procedures producing more reliable habitat suitability maps (Araújo and New, 2007; Buisson et al., 2010; Kindt, 2018). Hence, I implemented ensemble modelling in the R package ('biomod2') (Thuiller et al., 2009; Thuiller et al., 2016) for the distribution modelling of KMD in the Western Himalayas. All eight modelling algorithms for performing species distribution modelling in 'biomode2' of the R package were used for selecting the best model. I split the presence location data into 80% as training and the remaining 20% for testing the model with ten repetitions. In addition, I followed Zao et al. (2021) for better distribution output and to minimise spatial deviation. I randomly selected 1000 pseudoabsence points, and this process was repeated twice for model building. The Receivers Operating Curve (ROC) and True Skill Statistic (TSS) scores were used to understand the model performance and used the best-performing models for ensemble model building (Chatterjee et al., 2022; Dutta et al., 2022; Elith and Leathwick, 2009; Mukherjee et al., 2020). The variable importance scores of the models were obtained in-built function of the 'biomod2' package. The mean values of variable importance scores produced by the best model for the final ensemble model based on a TSS threshold were calculated (Allouche et al., 2006). The probability distribution surfaces were later converted into a binary where 0 value was 'absence' to 1 was 'presence' and considered the cut-off value as a threshold. I classified the probabilities above the threshold value as suitable habitat; otherwise non-suitable, and the same was used for the future scenario of species distribution (Bagaria et al. 2020).

Landscape Dynamics and habitat configuration analysis

Studies have brought out that habitat fragmentation can lead to a decline in abundance as well fitness of species in an impacted area (Kruess and Tscharntke, 1994). Therefore, the predicted probability surfaces were used for evaluating the fragmentation intensity and habitat patch placement in the mapped suitable habitats of KMD in the Western Himalayas. To understand the landscape configuration, I calculated the landscape metric and assessed the changes over study decades using FRAGSTATS version 4.2.1 (McGarigal, 2015). I calculated the total area (TA), number of patches (NP), Patch density (PD), largest patch index (LPI) and aggregation index (AI), which are used to understand the fragmentation intensity temporally (Bagaria et al., 2020; Chatterjee et al., 2022; Mukherjee et al., 2021). Further, the zonal statistics was calculated to quantify the available proportion of the KMD habitat distributed within the Protected Areas (PA) boundaries using ArcGIS 10.9 software. The KMD habitat falling under the PA boundaries was assessed using the percentage coverage of the KMD habitat area under each PA of the Western Himalayas to understand the PA representativeness in the present and future.

5.3. Results

5.3.1. Future simulated landscape

The simulated future LULC for 2030 has 82 % overall accuracy (kappa = 0.82) in the training model and the projected model with 93 % accuracy (kappa = 0.93). The multi-temporal scenarios comparison revealed the highest decline between 2010 and 2020 in the croplands (-5501.7 km^2), followed by barren areas (-4037.51 km^2), grasslands (-180.31 km^2) and shrublands (-44.22 km^2). In contrast, the highest gain was found in savannas (5148.16 km^2), followed by snow (2430.15 km^2) and mixed forest (1456.89 km^2) **(Table 5.3)**. However, between 2020-2030 all the land use classes except savanna (6822.71 km^2), mixed forest (162 km^2) and barren area (1.29 km2) declined in the future scenario. I found the highest decrease in evergreen needle forests (-2328.2 km^2), followed by grasslands (-2188.9 km^2) and croplands (-1448.3 km^2) (Table 1). The transition probability matrix suggests the highest conversion of shrubland (SLND) into grassland (GLND) (0.41), cropland (CLND) to Savana (SA) (0.21) and evergreen needle leaf (ENF) and broad leaf forests (BF) to mixed forests (MF) (0.11). However, in 2030 built-up (BU), ENF and BF will get converted into SA with values equal to 0.62, 0.39 and 0.16, respectively **(Table 5.3).**

Table 5.3. Land use pattern over the past, present and future scenarios. Classification accuracy: ANN training Cohen' s k = 0.82; Prediction Cohen' s k = 0.93.

LULC	Area (km^2)			Δ (km^2)		Area (%)			Δ (%)	
	2010	2020	2030	2010-2020	2020-2030	2010	2020	2030	2010-2020	2020-2030
ENF	5620.84	5891.52	3563.3	270.68	-2328.2	1.70	1.78	1.08	0.08	-0.70
BF	1397.43	1647.72	1361.4	250.29	-286.35	0.42	0.50	0.41	0.07	-0.08
MF	11301.8	12758.7	12921	1456.89	162.71	3.42	3.87	3.92	0.44	0.04
SLND	273.48	229.26	223.25	-44.22	-6.01	0.08	0.06	0.06	-0.01	-0.001
SA	53711	58859.2	65682	5148.16	6822.71	16.30	17.86	19.93	1.56	2.07
GLND	56021.8	55841.5	53653	-180.31	-2188.9	17.00	16.94	16.28	-0.05	-0.66
WLND	1187.92	1343.12	1155.7	155.2	-187.4	0.36	0.40	0.35	0.04	-0.05

	Area (km^2)			Δ (km^2)		Area (%)			Δ (%)	
LULC	**2010**	**2020**	**2030**	**2010-2020**	**2020-2030**	**2010**	**2020**	**2030**	**2010-2020**	**2020-2030**
CLND	23706.9	18205.6	16757	-5501.27	-1448.3	7.19	5.52	5.08	-1.66	-0.43
BU	702.58	754.53	280.34	51.95	-474.18	0.21	0.22	0.08	0.01	-0.14
SN	21070.7	23500.8	23433	2430.15	-67.4	6.39	7.13	7.11	0.73	-0.02
BA	154506	150468	150469	-4037.51	1.29	46.89	45.66	45.66	-1.22	0.0004

5.3.2. Species distribution modelling

Model performance

The ensemble model of the KMD was selected using the TSS values greater than 0.80 (**Figure 5.3**). Out of eight modelling algorithms used for building ensemble model, the best performing models were RF, MARS, GBM, and GAM respectively. RF had the highest average TSS value (0.86) amongst the other algorithms (**Figure 5.3**). The TSS weighted mean value of the ensemble model was equal to 0.83, which depicts the best performance of all the participating models used for mapping the suitable habitats of KMD. The response curves of all the models used for developing the ensemble model are provided in (**Figure 5.4 (a-d)**).

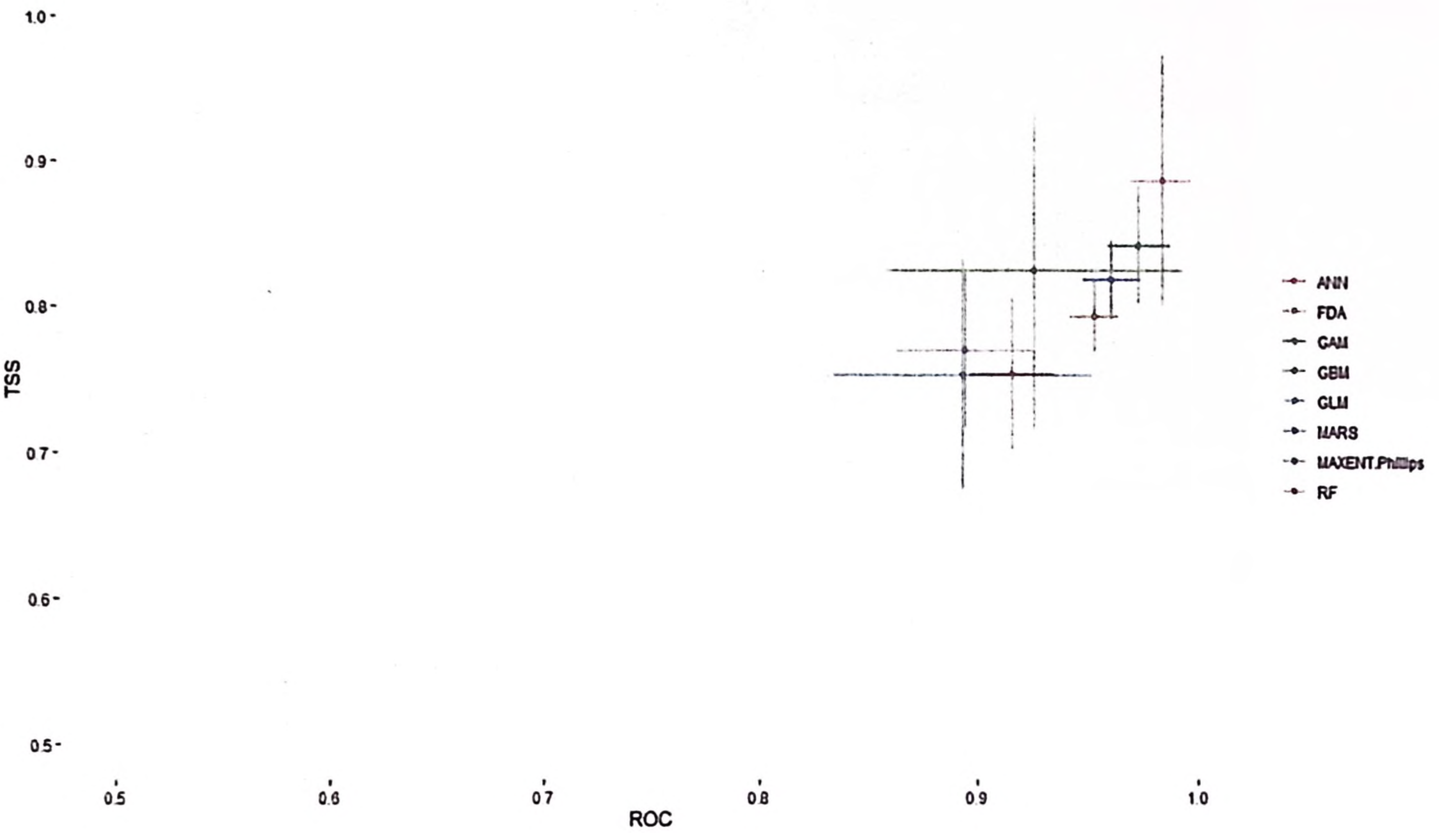

Figure 5.3. Plots depicts the selection of models by True skill sensitivity (TSS).Models with TSS value above 0.8 were used for ensemble modeling of Kashmir musk deer in Western-himalaya

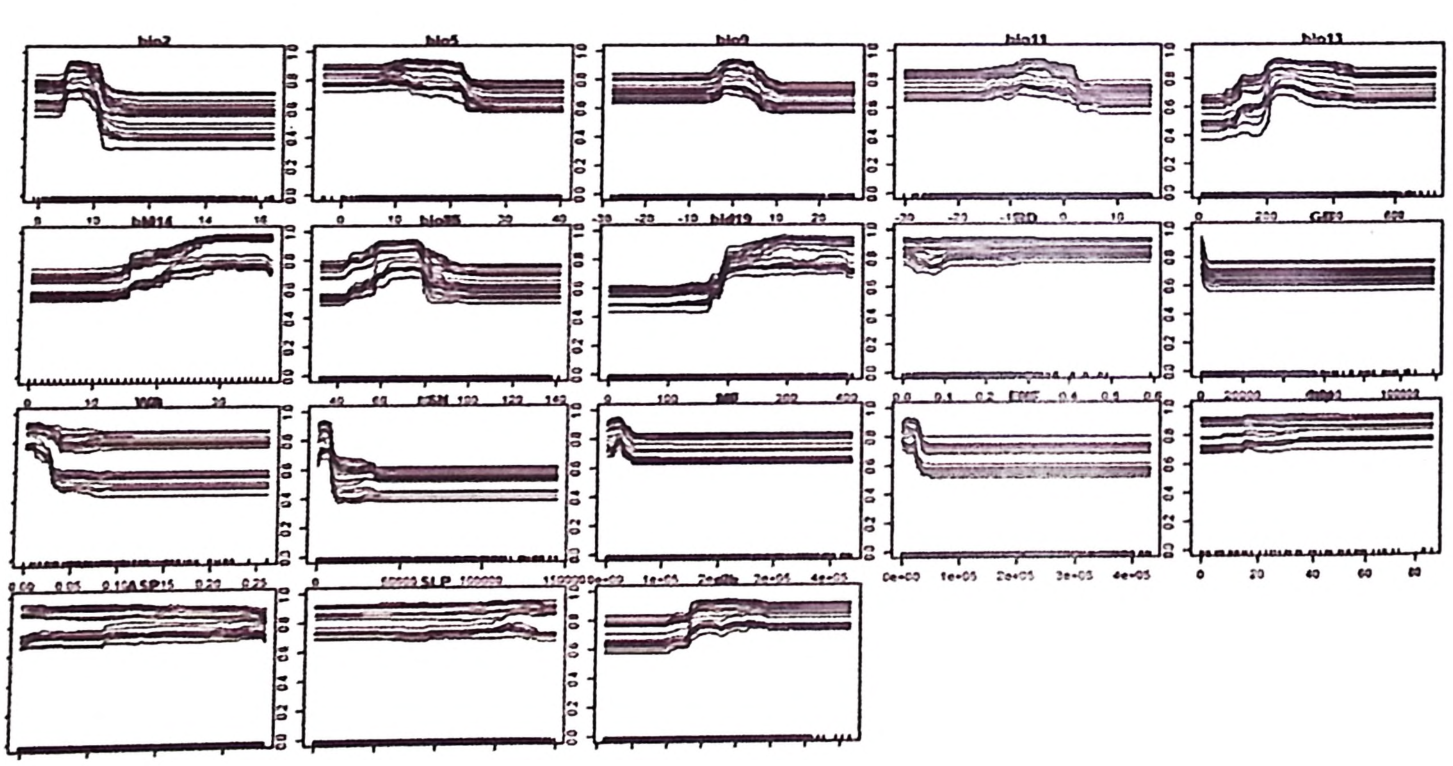

Figure 5.4 (a). Response curve of random forest used for ensemble modelling of Kashmir musk deer.

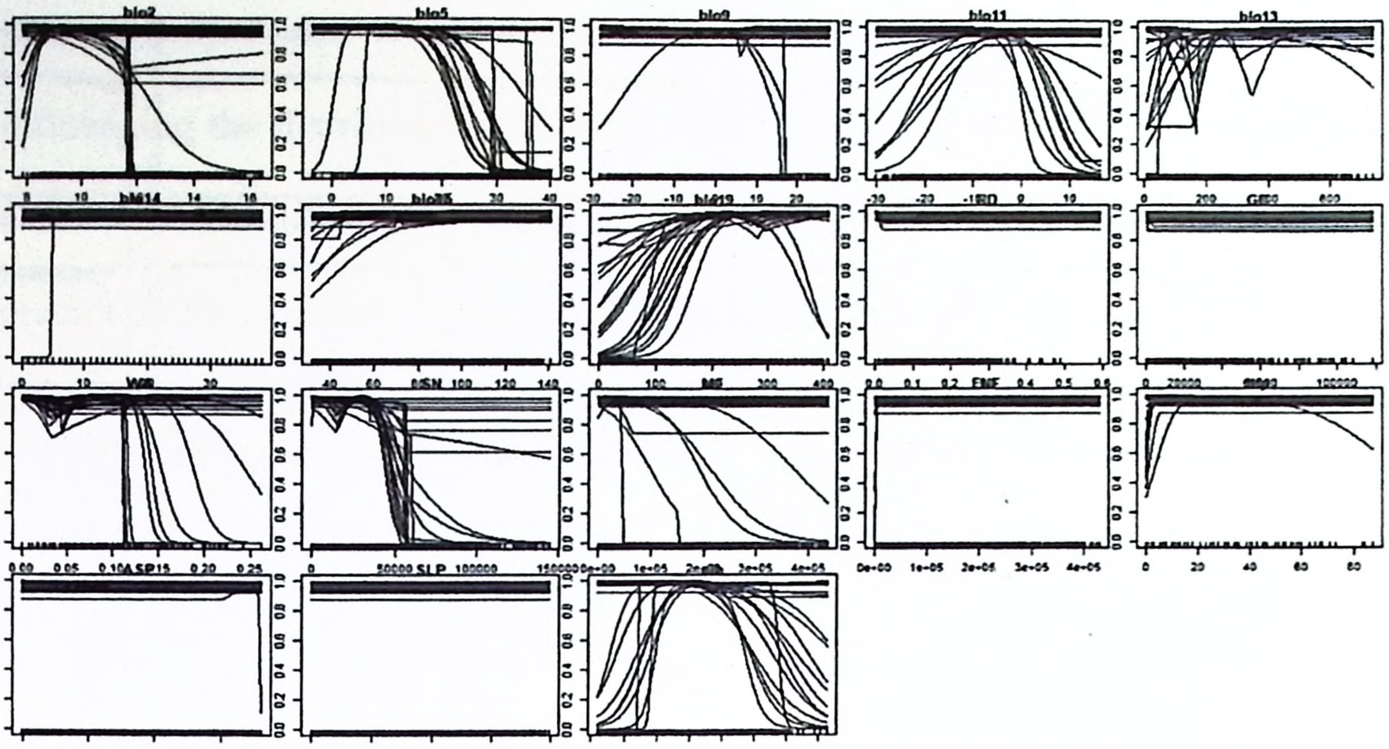

Figure 5.4 (b). Response curve of MARS used for ensemble modelling of Kashmir musk deer.

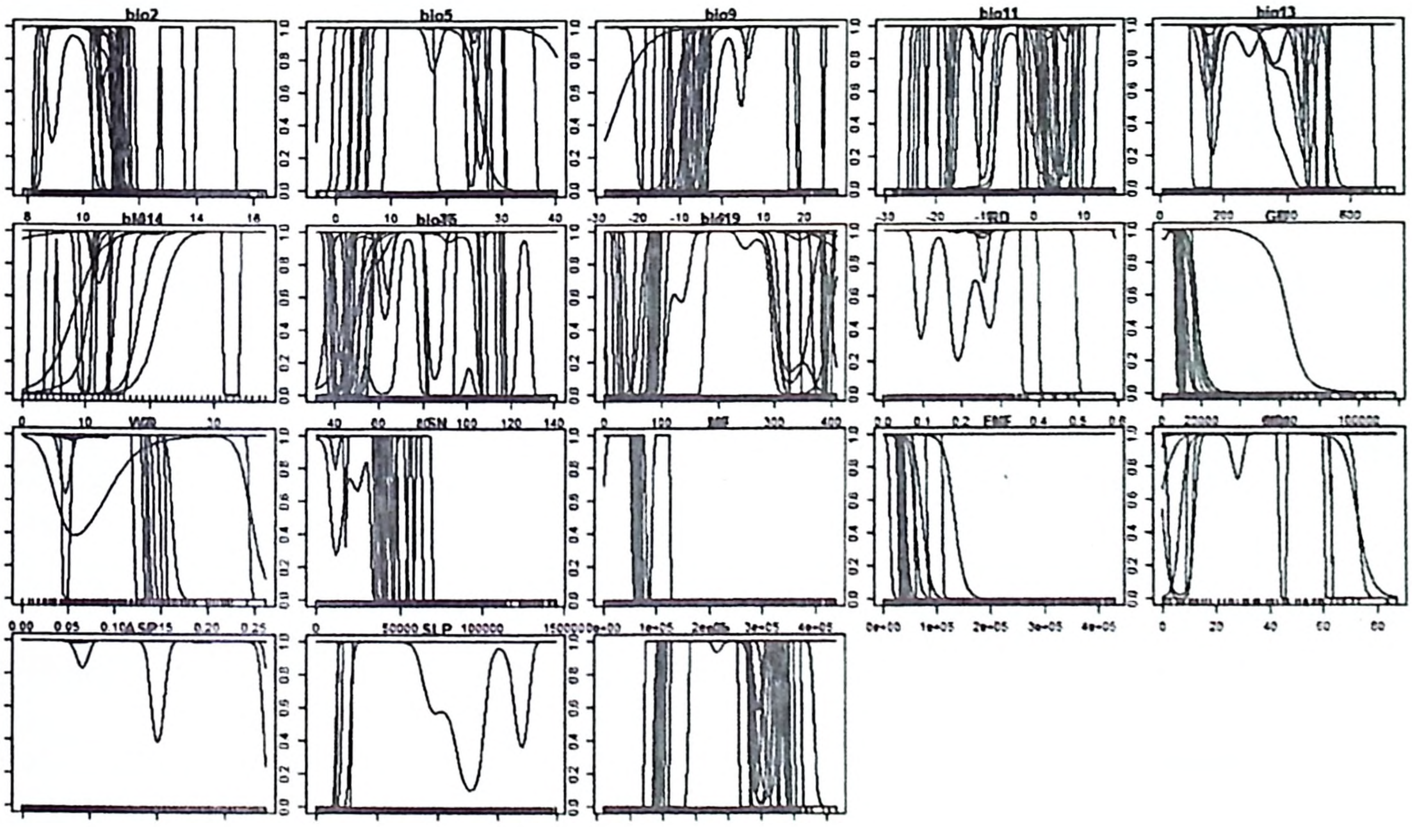

Figure 5.4. (c). Response curve of GAM used for ensemble modelling of Kashmir musk deer.

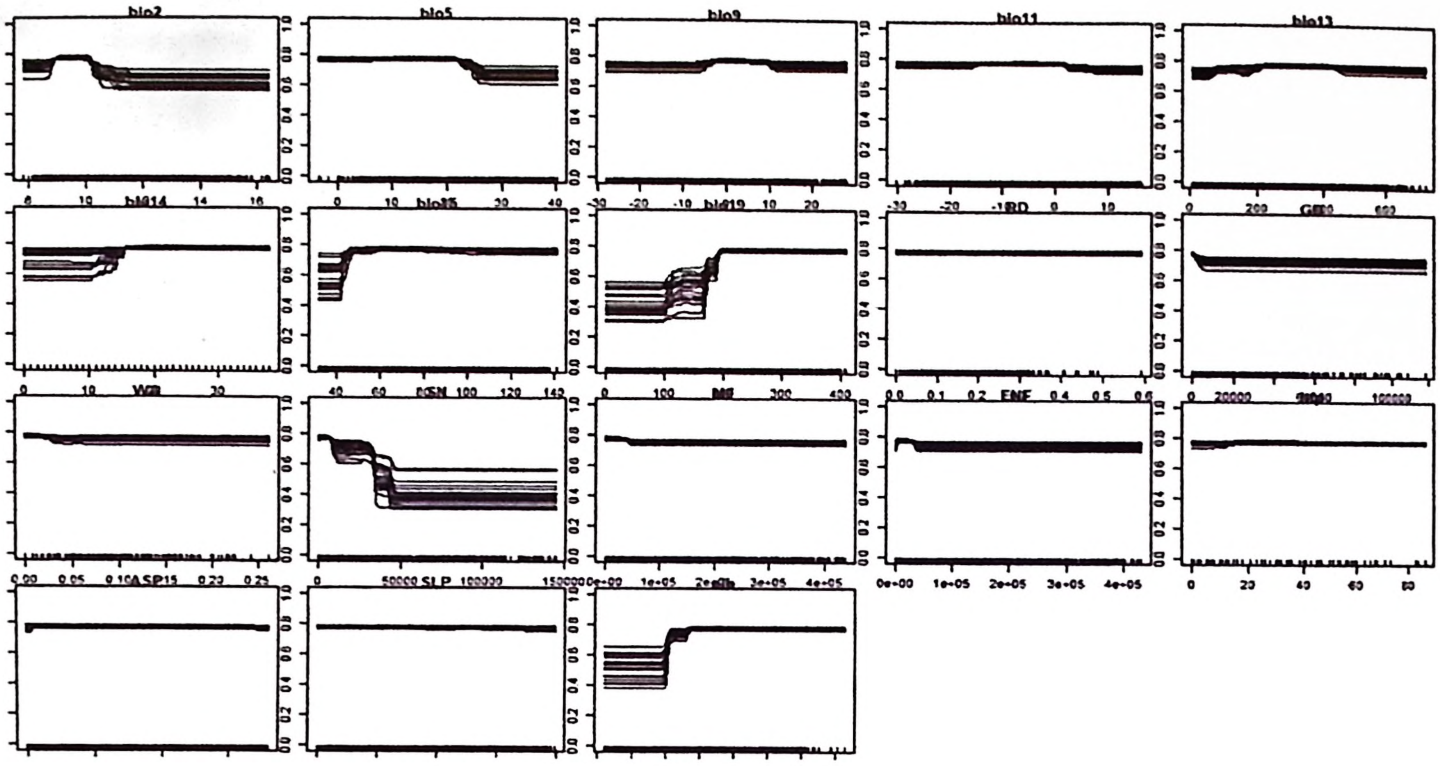

Figure 5.4. (d). Response curve of GBM used for ensemble modelling of Kashmir musk deer.

The KMD is predicted to inhabit in a narrow belt of high altitudes of Western Himalayas. The present study revealed that out of 3,24,666 km^2, only 20,690 km^2 (6.97%) is the suitable habitat. Our results revealed that out of the total suitable habitat (20,690 km^2), 9921 km^2 is low suitable, 6118 km^2 medium suitable and 4650 high suitable area for KMD (**Figure 5.5**). However, in a future scenario, 22,701.47 km^2 is the suitable habitat of KMD in the western Himalayas covering 10,503 km^2 as low suitable areas, 7,104 km^2 medium suitable area and 5,094 km^2 high suitable area (**Figure 5.5**). Approximately 2,722 km^2 will become available for the species in 2030 (**Table 5.4, Figure 5.6**). Furthermore 711 km^2 will be lost in 2030, out of which 77 km^2 falls inside the protected areas and 634 km2 outside the protected areas (**Table 5.5, Figure 5.6**). Two variables were found to be the most significant among all 18 participatory variables for the distribution of KMD: the precipitation of the coldest quarter (bio19) and elevation, with variable importance of 0.26 and 0.11, respectively

(Figure 5.7). These variables were identified as the most critical factors positively influencing the distribution of KMD in the Western Himalayas. Furthermore, mixed forests and snow were the most significant contributors among the land cover type predictors for determining the distribution of KMD (**Figure 5.7**).

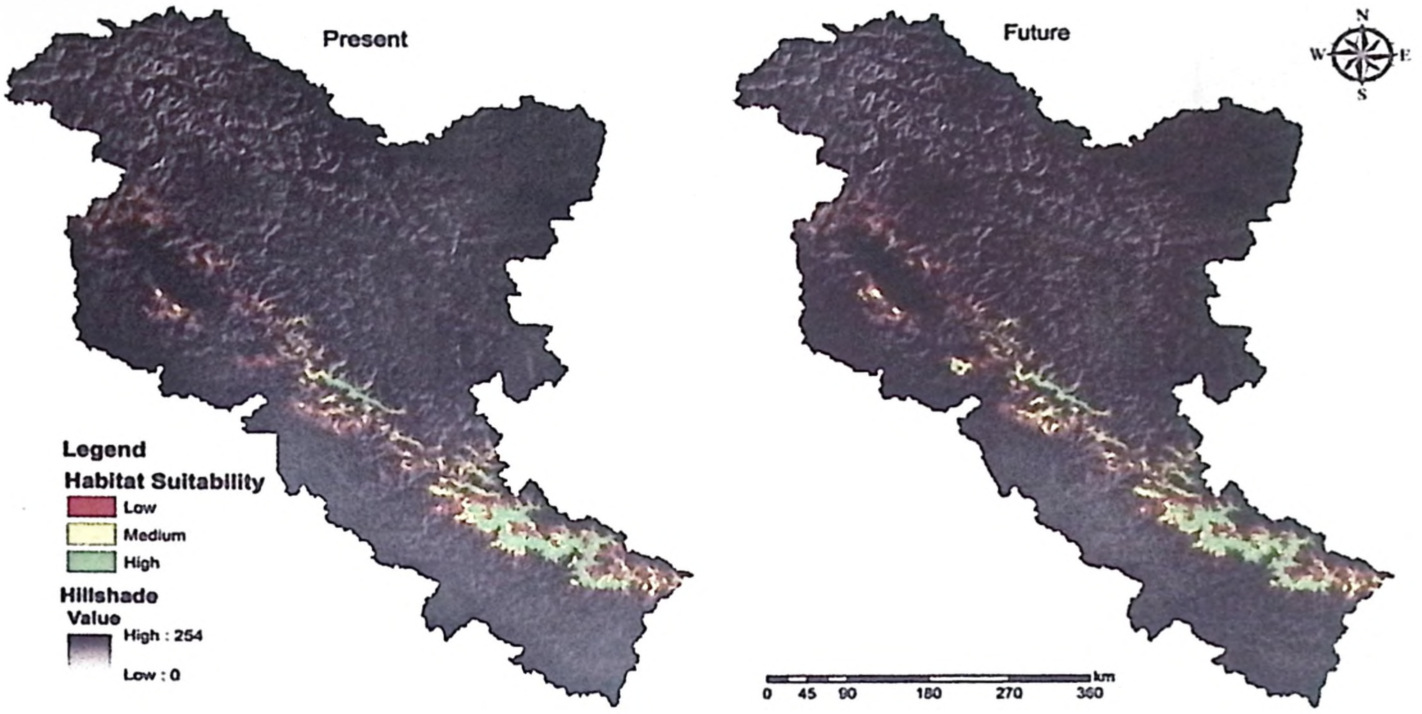

Figure 5.5. Predicted suitable habitat of KMD at present and in future scenario 2030.

Table 5.4. Suitable habitat of KMD in present and future scenario and estimated habitat change in and outside protected areas (estimation is in km^2).

Scenario	Suitable habitat (km^2)	Unsuitable habitat (km^2)	Habitat loss(km^2)	Habitat gain (km^2)
Present	20690	303976	-	-
Future	22701.5	301964	711	2722
Suitability inside PAs				
Present	4726	9911	-	-
Future	5140	9497	77	490

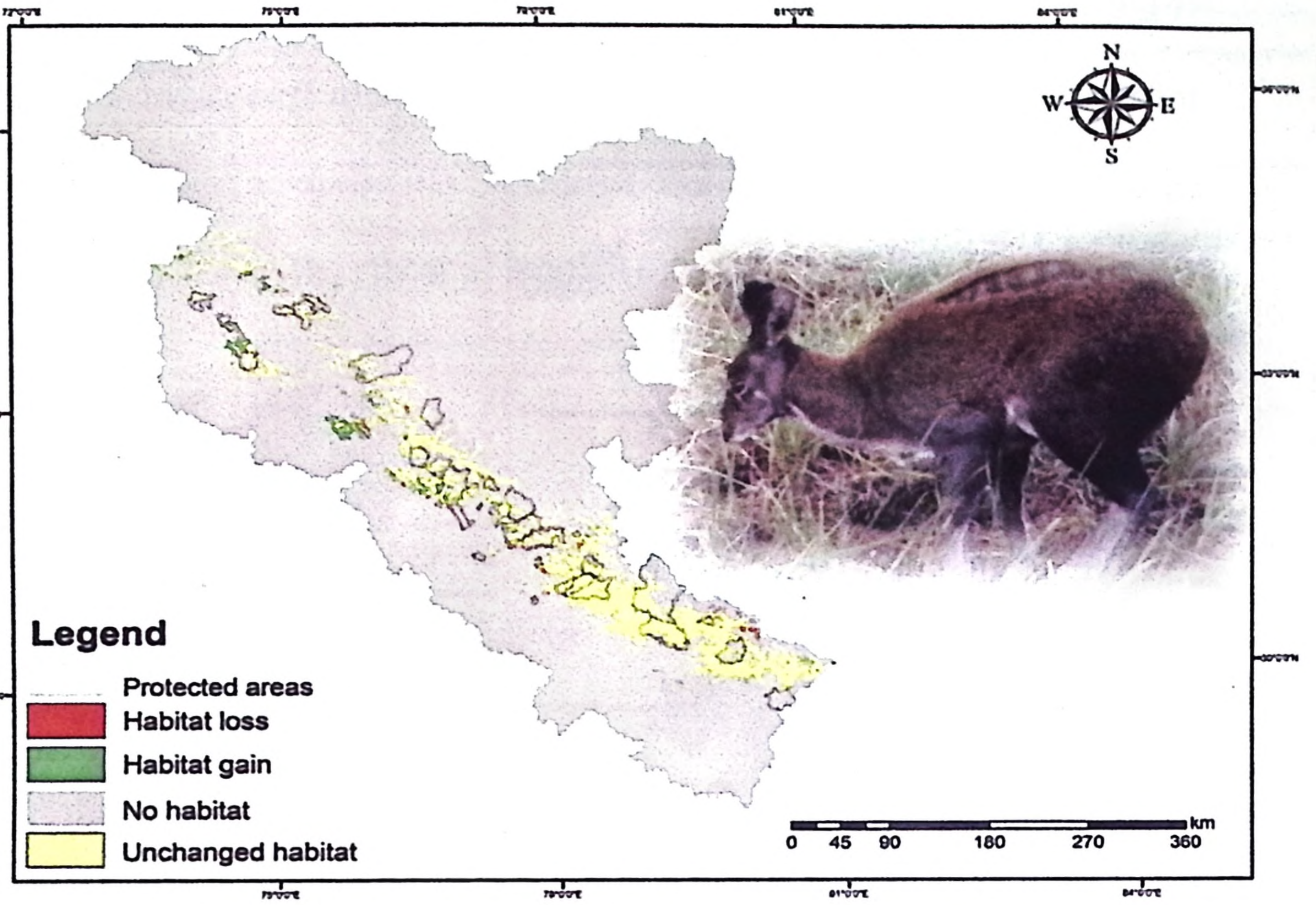

Figure 5.6. Predicted habitat change of KMD in and outside the protected areas.

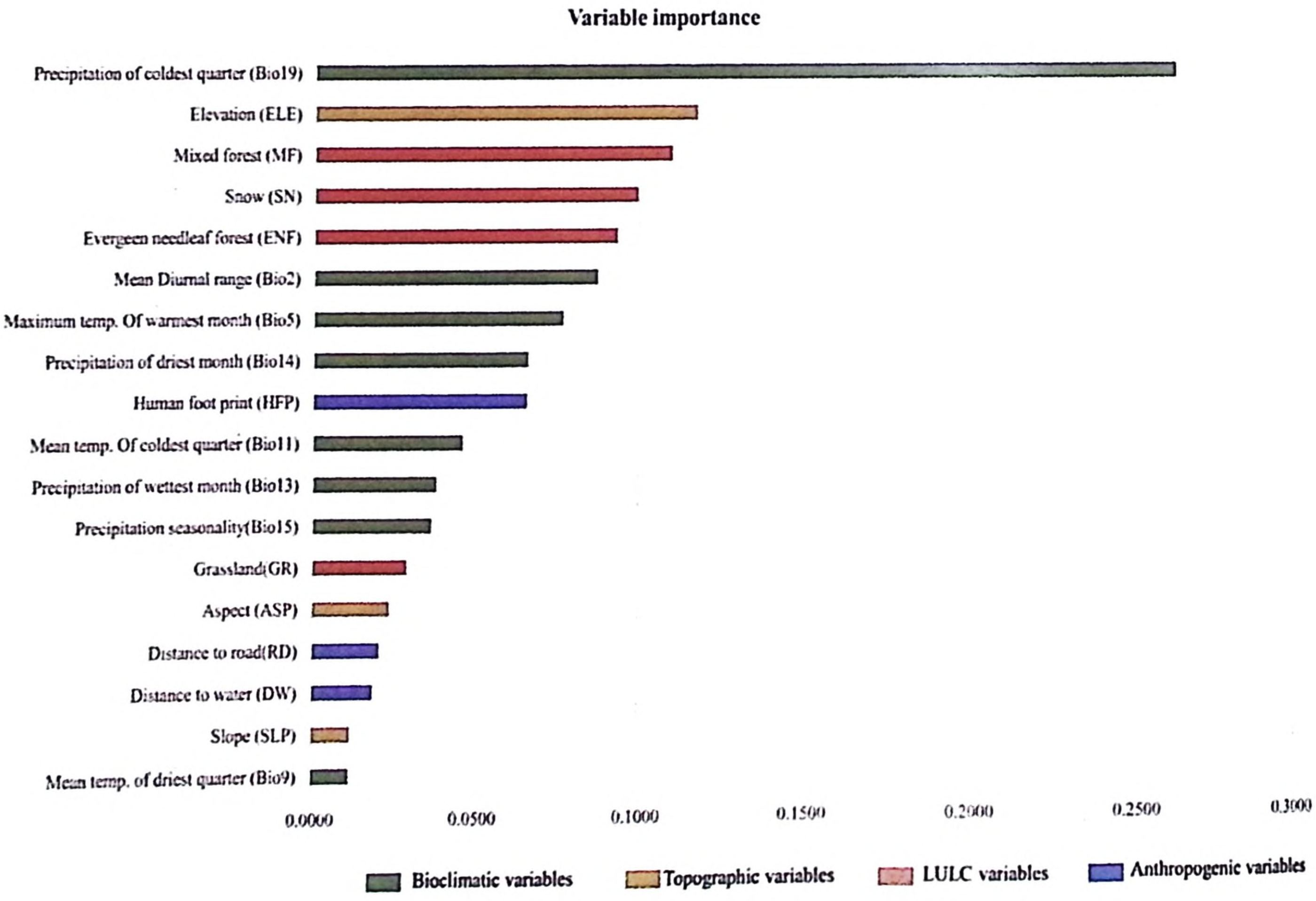

Figure 5.7. Variable importance of predictors used for ensemble modelling of Kashmir musk deer

Protected Area representation

Only around 22.6% of the total KMD habitat is distributed within the PA network, which is projected to decrease to 10.8% by 2030 in the future scenario (**Table 5.4**). Additionally, the future simulated habitat loss for KMD is expected to occur mainly outside the PA network. Our results also indicate that, in the present scenario, the Valley of Flowers Wildlife Sanctuary (WLS) ($\mu = 0.82$) and Govind Pashu Vihar ($\mu = 0.76$) have the highest mean suitability, while Askot Musk Deer Wildlife Sanctuary (WLS) ($\mu = 0.01$) has the lowest mean suitability. However, in the future scenario, the Valley of Flowers WLS ($\mu = 0.84$) is expected to have the highest proportion of its habitat, followed by Kedarnath WLS ($\mu = 0.79$) and Nargu WLS ($\mu = 0.005$), which exhibited the lowest mean suitability (**Figure 5.8**).

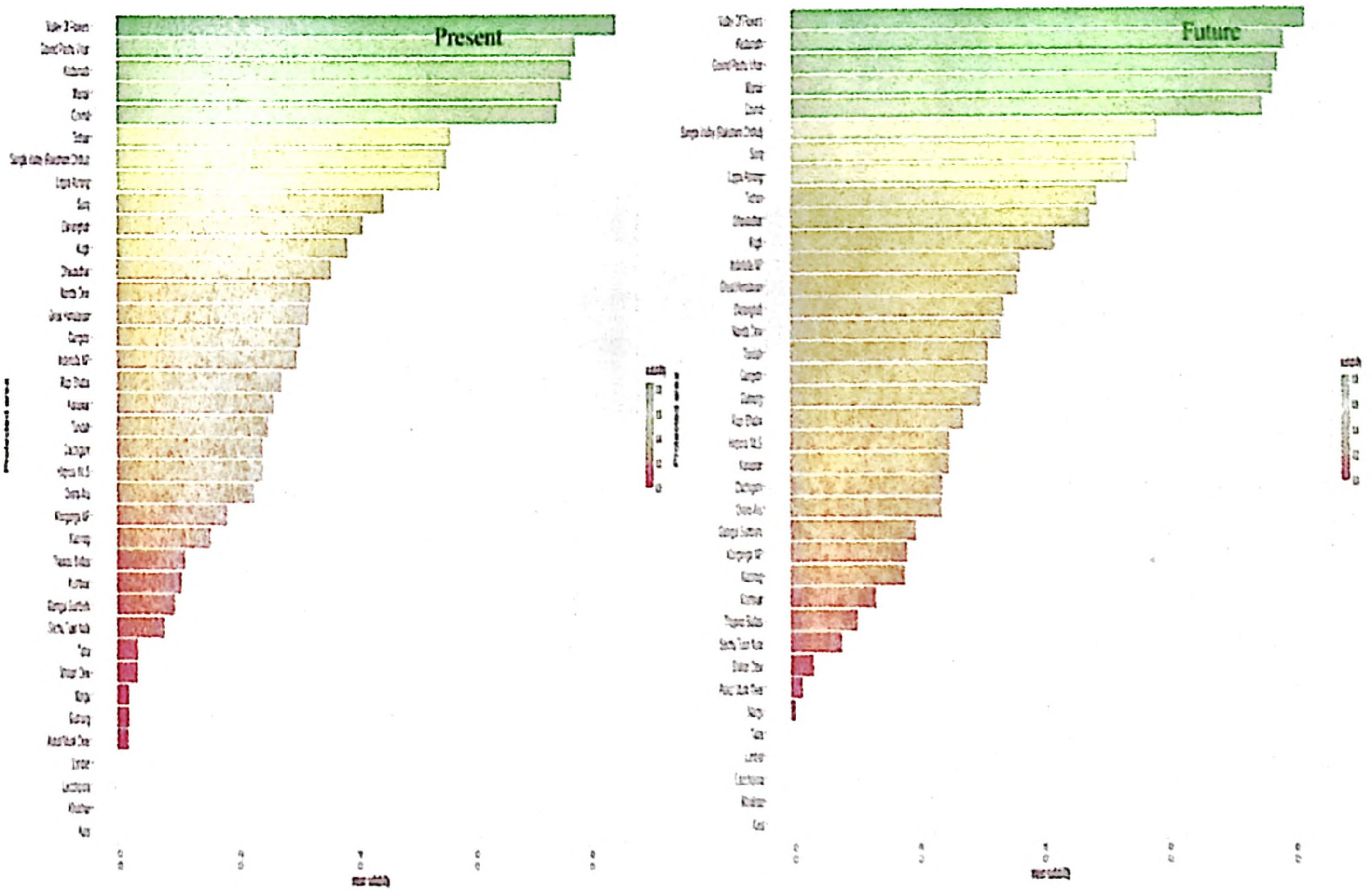

Figure 5.8. Mean suitability of protected areas in the present and future land use land cover change scenarios.

Landscape configuration

We observed changes in the landscape dynamics over three decades with decreased levels of fragmentation in the suitable habitat of the KMD. The decline in the suitable patch numbers indicates reduced fragmentation in the habitat of the species in the study landscape by 2030 (**Table 5.5, Figure 5.9**). Moreover, an increase in the aggregation index and the largest patch index was also observed in the future scenario (**Figure 5.9**). The landscape configuration matrices of the suitable habitat of KMD show an increasing trend in the future scenario (**Table 5.5, Figure 5.9**).

Table 5.5. Landscape configuration metrics of suitable habitats in the present and future scenario.

Scenario	PLAND	NP	PD	LPI	ED	AI
Present	6.37	755	0.0023	3.79	0.61	89.19
Future	6.99	721	0.0022	4.01	0.63	89.75

PLAND **percentage of land**, NP **number of patches**, PD **patch density**, LPI **largest patch index**, ED **edge density**, AI **aggregation index**

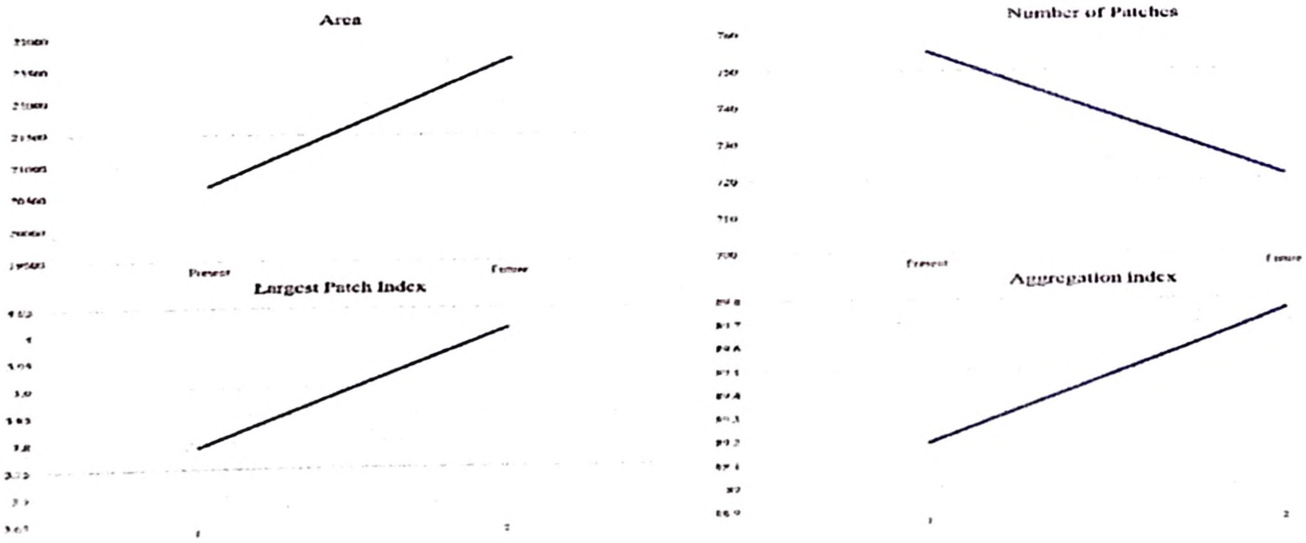

Figure 5.9. Graphs depicting landscape configuration matrices of KMD in the western Himalayas.

5.4. Discussion

Land use change has altered the landscape configuration of all the ecosystems, which has resulted in a wholesale replacement and fragmentation of habitats in the Himalayas (Penjor, 2022). In the Himalayan region, habitat fragmentation and degradation because of LULC change, agriculture expansion, industrialization, and untenable utilization of natural resources are the primary threats biodiversity faces (Pandit et al., 2007; Dutta et al., 2022; Chandra et al., 2020). The KMD is an endangered species distributed in the Himalayan range covering parts of Pakistan, Afghanistan, India, and Nepal (Kumar et al., 2022; Ostrowski et al., 2016; Singh et al., 2020). The habitat utilization patterns of an species can hint about the environment they are best adapted for; hence, it is pertinent to evaluate the LULC changes and examine the species' habitat requirements to make relevant conservation strategies. Therefore, I simulated the future landscape and predicted the present as well as the future distribution ranges of KMD through ensemble modelling for identifying the ecological requirements of the species and its responses to LULC change. The future simulated LULC scrutiny revealed that the croplands (5,501.7 km^2) followed by barren areas (4,037.51 km^2) had shown a significant decline, whereas the mixed forests have increased (1,456.89 km^2).

The results of this study are consistent with previous research conducted by Singh et al. (2018, 2021), indicating that KMD prefers mixed forests dominated by Himalayan birch, Himalayan fir, and blue pine tree species. At present, only 20,690 km^2 (6.97%) of the western Himalayas is deemed suitable for KMD, with a marginal increase of 2,722 km^2 projected by 2030 (**Table 5.4**). The majority of the suitable habitats will be located in Jammu and Kashmir, with only a few patches in Himachal

Pradesh (Fig. 3). However, suitable habitats in Uttarakhand will remain unchanged in the future scenario (**Figure 5.6**). Additionally, the ensemble modelling predicts a loss of 711 km^2 in 2030, with 77 km^2 falling inside the PAs and 634 km^2 outside the PAs. Future LULC simulations support the results of the ensemble modelling, indicating an increase in mixed forests in the future scenario.

Additionally, the transition probability matrix indicates that SLND was converted to grassland while ENF & BF to MF, as presented in (**Table 5.2**). These results suggest that an increase in grasslands and mixed forests within the landscape could provide more feeding habitats and suitable areas for the species in future scenarios. This finding is consistent with the study of Shrestha and Meng (2014), which identified mixed forests as the preferred habitat for musk deer. This is likely due to the territorial nature of musk deer, which leads them to occupy forests that provide shelter, canopy cover to avoid predation and space for latrine sites.

In addition, the mountain ecosystem's vegetation composition is largely influenced by precipitation and altitude, as demonstrated in studies by Salick et al. (2014) and Zuo et al. (2021). The present study found that precipitation of the coldest quarter (bio19) and elevation were the primary variables positively influencing the distribution of KMD in the Western Himalayas (**Figure 5.4 (a-d)**). This positive association with precipitation is consistent with the limiting factor for vegetation growth, which is the availability of moisture during the pre-monsoon season (Singh et al., 2020). Moreover, the predicted KMD distribution range of 2500-4500 m suggests that KMD prefers to inhabit higher elevations. This preference may be due to the species' sensitivity to anthropogenic disturbances, as KMD may seek to avoid human activity by inhabiting areas less accessible to humans. Previous studies have also reported a distribution range for musk deer between 2500 and 4200 m (Sathyakumar, 1994; Vinod & Sathyakumar, 1999; Ilyas, 2014; Subedi et al., 2012).

Of the land cover variables, mixed forests were found to be the most influential factor positively determining the distribution of KMD. This finding is in agreement with observations by Singh et al. (2018), who suggest that the canopy cover of mixed forests provides shade and allows the feacal pungency to remain intact for longer, enhancing effective territorial marking and chemical communication. Snow was also found to be a significant factor governing the distribution of KMD in the study area. KMD is well adapted to high-altitude environments, which typically have low temperatures, and this may be due to the species' pneumatic hair, which acts as insulation against the cold (Futuyma & Moreno, 1988; Green, 1985; Kattel, 1993).

In the current scenario, Valley of Flowers, Govind Pashu Vihar, and Kedarnath WLS offer much of the suitable habitats for KMD among the 34 protected areas. However, Kedarnath WLS is expected to become the most suitable after Valley of Flowers WLS in the future (**Figure 5.8**). This is because these protected areas are already under protection and offer more suitable areas for the species hence are important for KMD population viability in the Himalayas.

Habitat configuration analysis indicates decline in the number of patches in the simulated future land use change scenario. Furthermore, I also noticed an increase in the aggregation index by 2030 which suggests that the small patches are aggregating to form larger patches, ultimately resulting in the decline of patch number (**Table 5.5, Figure 5.9**). Our results are supported by the observations of Mukherjee et al., (2021), which suggests the Western Himalayas may show a decline in the patches density as well as fragmentation in the future scenario. Furthermore, the landscape configuration of the suitable habitats within protected areas remained unchanged in the future land use change scenario suggesting better management of suitable habitats within protected areas. Notably, the KMD populations decline is largely attributed to illegal hunting, anthropogenic pressure such as Non-Timber Forest Produce collection, habitat degradation and climate change (Khadka et al., 2017; Singh et al., 2018; Singh et al., 2020). Therefore, the conservation of KMD is critical since it plays a vital role in regulating the vegetation composition and sustaining ecosystem integrity (Green, 1987). The suitable habitats of KMD in the current and future scenarios may aid managers and other stakeholders in taking informed management actions. This will be valuable in identifying and prioritizing areas which can meet their ecological needs of the KMD in the future land use change scenario.

5.5. Conclusion

Our findings insinuate that the KMD suitable habitats may get reduced and shifted to new areas. The predicted suitable habitat gain is more than the loss of suitable areas in 2030 (**Table 5.4, Figure 5.6**). In the future land use change scenario, a significant portion of the KMD's suitable habitats in Uttarakhand is predicted to be lost, while new suitable habitats are expected to emerge in Jammu and Kashmir, followed by Himachal Pradesh states (**Figure 5.6**). The shift to new areas may negatively affect the population viability of KMD, especially given that the majority of suitable habitats are not protected, and the existing protected areas in the Western Himalayas are insufficient for protecting the endangered species. Thus, it is imperative to bring suitable habitats located in close vicinity to the existing PAs into the protected area network for effective protection and conservation planning. The findings of our study on the anticipated impacts of land use change on KMD can inform decision-making related to the implementation of habitat management activities

Although KMD is a conservation-dependent species, very little is known about its ecological aspects, such as population, habitat and behavioural ecology, which is vital for its long term conservation. Therefore, further research is urgently needed to estimate the population of KMD in its entire distribution range and determine its current status. Furthermore, the population of KMD is threatened due to poaching for musk pods, unsustainable livestock grazing, habitat destruction and climate change (Singh et al., 2020; Nandy et al., 2020). Hence, for KMD's long-term viability species conservation action plan for its entire distribution range should be the top priority. Finally, it is noteworthy that most of the threats to KMD are anthropogenic; an integrated conservation program comprising awareness among local people through capacity-building programmes, involvement of local communities, and habitat management seems to be an exigency.

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Chapter 6: Objective 3. To understand habitat -use of musk deer in Pattan valley, Lahaul and Govind Pashu Vihar National Park, Uttarkashi.

Part of this chapter has been pulished as **Sharief et al.,2023**. Estimating abundance and occupancy of Kashmir musk deer (*Moschus cupreus*) in Uttarkashi, Uttarakhand. Indian Journal of Ecoloy, 50(1), 272-275.

Abstract

The least studied Kashmir musk deer (*Moschus cupreus*, henceforward KMD) is a conservation priority species which is facing population decline primarily due to illegal hunting for musk pod and habitat loss. With the realization of the threats to KMD, the identification and management of its suitable habitat thus becomes vital for proper conservation of this rare creature. Therefore, conservation of KMD requires identification of habitats that support their survival and persistence of the species. Occupancy models are emerging as a useful tool for management interventions thus, are considered essential in the field of wildlife conservation. Hence the present study used sign survey and camera trapping data employing single species occupancy modelling to understand the influence of environmental predictors on the habitat use of KMD in pattan valley, Himachal Pradesh, and Govind Pashu Vihar National Park (GPVNP), Uttarakhand. I deployed a total of 43 camera traps and surveyed 54 trails in pattan valley whereas 23 camera traps and 43 trails were surveyed in GPVNP, in the selected grids of the landscape. Present study confirmed that KMD occupies conifer forests mainly comprising of (*Pinus wullachiana, Picia simithiana*, *Abies spectabilis*, *Cedrus deodara,* intermixed with *Betula utilis*) at higher elevation between 2946-4418 m in pattan valley whereas in GPVNP mixed forests (*Pinus wullachiana, Picia*

simithiana, *Cedrus deodara*, *Betula utilis*, *Abies spectabilis, Taxus wullachiana*, *Quercus semecarpifolia*) were used by the species between 2145-3641 m. I had a clear-cut discrimination of use of habitat by KMD in the landscape. Elevation and conifer forests had positive influence on the occupancy and detection probability of KMD in Pattan valley. Mixed forests and elevation had positive influence on the occupancy and detection probability of KMD whereas the supporting model suggests that conifer forests had negative influence on the detection probability of KMD in GPVNP. Since the high-altitude forested areas is an essential criterion for musk deer habitats, hence it is recommended these habitats should be maintained, protected, and poaching pressure as well as anthropogenic disturbances should be minimised. The findings of the present study may aid wildlife managers in better conservation planning and making informed management decisions.

6.1. Introduction

India is one of the mega biodiverse countries, representing 39 species constituting approximately 15% of the global extant ungulate species (Wilson & Reeder, 2005). Ungulates are essential for maintaining the structure and functioning of ecosystems, facilitating seed dispersal and nutrient cycling, and forming the prey base for predators such as the snow leopard (McNaughton, 1979). However, human-induced landscape alteration poses a significant threat to wildlife in the Himalayas and increases the risk of extinction (Flynn et al., 2009; Corlett, 2015). Understanding the declining population of these species is crucial for their long-term viability and is essential from both ecological and management perspectives. Effective conservation and management planning for species on the verge of extinction requires knowledge of how they use their habitat and interact with their environment (Singh et al., 2022). For decades, ecologists have explored the idea that the distribution/habitat use of ungulates depends on vegetation, which, in turn, is dependent on various factors such as altitude, aspect, slope, and precipitation (Green, 1985; Vinod & Sathyakumar, 1999; Sharma et al., 2010). Therefore, identifying the factors that govern habitat use, support survival, and ensure the persistence of the wildlife species is imperative for their effective management and conservation (Hutchinson, 1957; Levins, 1968).

The Kashmir musk deer is a conservation-dependent ungulate species occupying higher elevation areas of the Himalayan ecosystem and plays an essential role in regulating the vegetation structure (Green, 1987; Bagchi and Ritchie, 2010). The species is distributed across four Himalayan range countries: Afghanistan, Pakistan, India, and Nepal (Ali et al., 2014; Ostrowski et al., 2016; Singh et al., 2020; Sharief et al., 2023). The Nuristan in Afghanistan is documented to be the western range limit of the species, and the Mustang Valley, Nepal, confirms to be the eastern limit of KMD (Singh et al., 2020). It occupies mature coniferous to alpine scrub habitats of the Himalayas at an altitude between 2200-4500m (Khadka et al., 2017). KMD is an elusive and secluded forested species with a crepuscular/nocturnal activity pattern. The species is territorial and marks its territory through the selection of latrine sites, which makes them vulnerable to hunting. The KMD throughout its distribution range is imperilled due to habitat loss and human-induced pressures such as poaching for musk pods, habitat degradation, and extensive livestock grazing (Aryal and Subedi, 2011; Timmins & Duckworth, 2015; Syed and Ilyas, 2016; Khadka et al., 2017). The KMDs over-exploitation for musk pods and habitat destruction are substantial reasons for the population decline of KMD throughout its entire distribution range (Green, 1986; Singh et al., 2018). Subsequently, the illegal killing of KMD for musk pod has impelled the KMD to the verge of extinction.

Given the escalating decline in its population, KMD has been designated as an endangered species by the International Union for Conservation of Nature (IUCN) (Timmins & Duckworth, 2015). It is also classified as an Appendix-I species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2015) and a Schedule-I species under Indian Wildlife Protection Act, 1972. Moreover, overexploitation of natural resources, such as exhaustive grazing of livestock, timber/fuelwood cutting, and fodder and medicinal plant extraction are other existing threats to the habitats in the Himalayas (Vinod & Sathyakumar, 1999; Ilyas, 2014; Sathyakumar, 2015; Syed and Ilyas, 2016; Sharief et al., 2022). Hence, due to its threatened status, it becomes imperative to understand the factors governing the habitat use of KMD for effective conservation and management of the species (Khadka et al., 2017).

Limited research has been conducted on various aspects of KMD, including distribution modelling (Ali, 2014; Singh et al., 2020), ecology (Syed and Ilyas, 2012, 2016; Ilyas, 2014; Ostrowski et al., 2016), and genetics (Kumar et al., 2022). The rugged terrain and elusive nature of KMD make this species difficult to study. However, obtaining information on the ecological aspects of KMD is crucial for making informed management strategies. Hence, to overcome these challenges, camera traps were used to detect the presence of the species in the high altitudes of the Himalayas (Rovero et al., 2009; Joshi et al., 2019).

KMD's potential habitats are also located outside protected areas and are fragmented, highlighting the urgent need for conservation and protection efforts. Conservationists aim to understand how species respond to environmental resources across different spatial scales, and occupancy models have emerged as a reliable strategy to investigate the status of these species (Mackenzie et al., 2006). These models allow for testing hypotheses related to the impact of different variables on species presence while estimating the likelihood of species presence in a given location. According to the literature, occupancy modelling has been demonstrated to be an effective method for studying habitat use of single or multiple species (e.g., McHugh D et al., 2019; Sharief et al., 2020; Sharief et al., 2022), estimating the co-existence of two or more species (e.g., Bailey et al., 2009, Sharief et al., 2022), and quantifying the abundance of a species (Sharief et al., 2022; Sharief et al., 2023).

The current study hypothesized that vegetation type and topography are key determinants of KMD's habitat use in the landscape, given the importance of these habitat variables. Thus, this study employed occupancy modelling to evaluate the habitat use of KMD and recognize the factors controlling the species distribution in the Pattan Valley, Himachal Pradesh, and Govind Pashu Vihar National Park, Uttarakhand. The findings will be crucial for the development of effective conservation and management plan for the species (Sharief et al., 2023).

6.2. Materials and methods

6.2.1. Study area

The study area encompasses landscapes of two states in the western Himalayas, namely Pattan Valley in Himachal Pradesh and Govind Pashu Vihar National Park in Uttarakhand **(Figure 6.1)**. Pattan Valley is situated in the trans-Himalayan district of Lahaul & Spiti in Himachal Pradesh, while Govind Pashu Vihar National Park is located in the Greater Himalayas. Both sites feature diverse topography and climatic conditions that support unique biodiversity in the region. The vegetation is dominated by Himalayan Temperate, Sub-alpine and Alpine forests, Tropical and Sub-tropical broad-leaved forests, Tropical Coniferous Forests, Dry and Moist Deciduous Forest, and is characterized by an elevation range of 177 to 8569 meters (Champion & Seth, 1968). The prominent tree species in the study area include *Pinus wallichiana, Cedrus deodara, Picea smithiana, Pinus roxburghii, Quercus* sps, *Abies pindrow, Aesculus indica, Betula utilis*, *Taxus wallichiana*. The climate is harsh, with temperatures ranging from 32.5°C in the summers to -24.1°C during severe cold winters. The study landscapes provide habitat for some of the top conservation-priority large mammalian species, including *Panthera uncia, Ursus thibetanus, Canis lupus*, and *Moschus cupreus*.

6.2.2. Study design and Data collection

The study landscape was first stratified into different forest types in-order-to collect data on KMD **(Figure 6.1)**. Field surveys were conducted during 2020-2021 in all habitats, including mixed forests, conifer forests, broadleaf forests, shrubland, and grassland covering different elevation ranges. A preliminary survey was conducted in selected grids to determine KMD presence/absence. Transect surveys and camera trapping were used in a two-pronged strategy in seven selected grids measuring 10x10 km in both sites (**Figure 6.1**). Transect surveys and camera trapping were used as part of a two-pronged strategy to conduct the study in the seven selected grids, measuring 10 x 10 km in both the sites (**Figure 6.1**). All direct/indirect signs of KMD were recorded during the survey, such as sightings, pellets/laterines, and hoof marks. Grids with KMD signs were further divided into 1x1 km grids for intensive sampling, considering their movement ecology (Singh et al., 2019, Zhang et al., 2022) (**Figure 6.1**). A total of 61 and 43 grids were surveyed in pattan valley and GPVNP respectively which were logistically feasible to survey. However, some of the grids were dropped out due to the settlement areas and non-forested habitats (**Figure 6.1, 6.2**). Systematic surveys for direct/indirect signs of the KMD were conducted on 54 (206 km) and 43 (198 km) transects of approximately 1.5-4 km in length. To detect the presence of the elusive species, 43 and 23 camera trap devices were also installed in selected grids, of Pattan Valley and Govind Pashu Vihar NP, respectively covering elevational gradients from 2200-4500 m.

The Browning Trail Camera (Defender 850, 20 MP, Prometheus Group, LLC Birmingham, Alabama, <https://browningtrailcameras.com>) and Ultra-compact SPYPOINT FORCE-11D trail camera (SPYPOINT, GG Telecom, Canada, QC) were deployed on natural trails and at about 0.3-1 m above the ground to detect KMD presence. Each and every camera trap station remained active for a window of 30 days. Two repeated camera trapping surveys of 30 days each were conducted at both the sites during the study period. Hence camera–trapping survey was divided into two temporal replicates (considering 30 days as one occasion). Species capture rate was estimated using the total number of photographs captured/total number of camera trap days).

Local in the study area are mostly agrarian and are dependent on forested resources. Hence human disturbance such as livestock grazing, fuel and fodder collection was also recorded in the sampled grids. For habitat characterization high resolution LISS IV imagery was used to extract variables of land use land cover types in the landscape. To understand habitat characterization of KMD, 10m radius circular plots were placed around each surveyed location (camera location /signs recorded, which was later used as ground truthing points for classifying the imagery). The GPS location, along with the habitat variables such as altitude, slope, aspect, distance to water, and distance to village, were recorded for all the sampled locations.

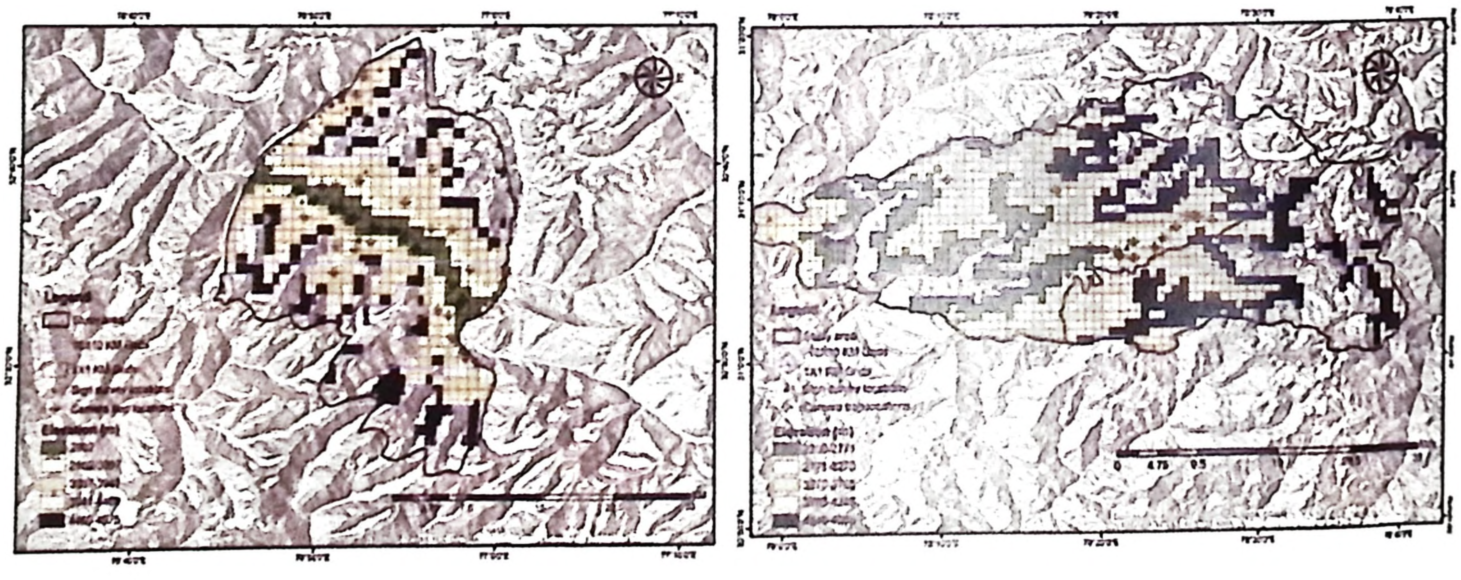

Figure 6.1. Sampling locations of Kashmir musk deer in Pattan valley, Lahaul and Govind Pashu Vihar National Park, Uttarakhand

6.2.3. Satellite data for landuse landcover analysis

The Land use/land cover (LULC) is the fundamental conception for understanding the landscape characteristics and the accessibility to high-resolution satellite imagery has made it possible to better understand their spatial distribution at any point of time (Zhang et al.2022). We used high resolution LISS IV (Linear imaging Self-Scanning Sensor) data comprising of three bands (b2: Green, b3: Red, b4: near infra-red (NIR) (obtained from National remote sensing centre Hydrabad) to understand the landscape more precisely and accurately (**Table 6.1**).

Table 6.1. Metadata of the LISS IV satellite imagery data procured from National Remote Sensing Agency.

Site	Satellite ID	Sensor	Path/Row	Acquisition Date	Resolution
Pattan valley	IRS-R2A	LISS-4 FX	097/049	09th December 2022	5.8m
	IRS-R2A	LISS-4 FX	094/048	09th December 2022	5.8m
GPVNP	IRS-R2A	LISS-4 FX	094/047	23rd Feb 2023	5.8m
	IRS-R2A	LISS-4 FX	094/047	23rd Feb 2023	5.8m

High resolution LISS IV image classification

Remote sensing data has been increasingly used for land use/land cover classification for better understanding of the landscape characteristics such as spatial and spectral features (Dhaka S 2016). Landuse/Landcover classification involves extracting distinct classes, from remote sensing imagery and grouping them based on their spectral characteristics. To achieve this I began by mosaicking of all bands of the LISS IV satellite images and creating a false color composite (FCC) (**Figure 6.2**). The images were then processed for atmospheric correction (Sobrino, Jiménez-Muñoz & Paolini, 2004). Next, the geometrically corrected and atmospherically calibrated LISS IV image was utilized to identify and classify unique land use land cover classes.

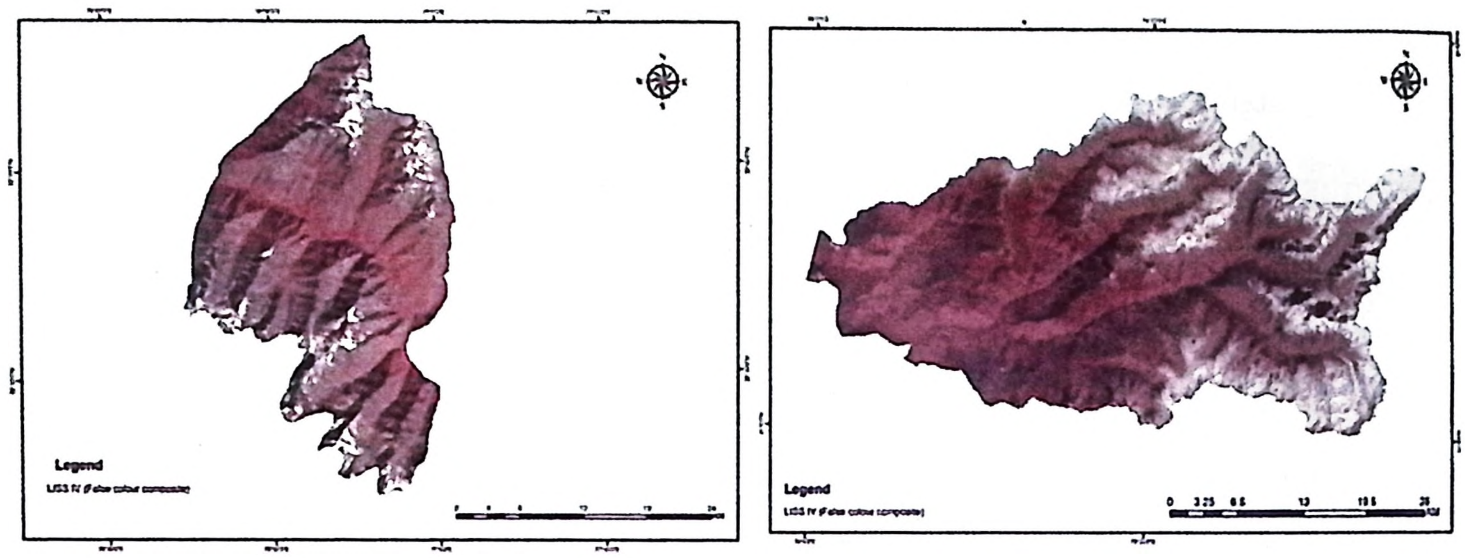

Figure 6.2. False color composite (FCC) of LISS IV satellite image: left- Pattan valley, right-Govind Pashu Vihar National Park.

The extensive training data for LISS IV imagery was collected during the vegetation sampling for all land use/ land cover category and marked as ground truth points. Hence a total of nine distinct categories as 1) barren area, 2) snow, 3) water bodies, 4) juniper forest, 5) conifer forest, 6) subalpine and alpine shrubland, 7) grassland, 8) builtup, & 9) agriculture, were identified in pattan valley. Whereas ten distinct classes as broadleaf forest, 1) barren area, 2) snow, 3) water bodies, 4) builtup, 5) conifer forest, 6) mixed forest, 7), 8) broadleaf forest, 9) agricultural land, 10) scrubland were identified in Govind pashu vihar national park. A minimum of 40-70 points were marked on each land use/land cover category in the study area as representative samples for classification.

Both the satellite images are then classified using one of robust supervised classifiers known as MLC Classifier (Maximum Likelihood Classifier) (Sun et al 2013, Shakya et al 2023). Classification is done based on spectral signatures which is obtained from representative samples of all land cover classes from the satellite imagery to be used for classification known as training samples. Thereafter identification and labelling of these clusters into a desired ground cover types representing a particular class is done using a trained classifier. Hence the caliber of training samples determines the quality of a supervised classification.

Supervised classification involves a sequence of steps to generate a better classified image which are as follows: a) generation of training samples & creating signature file, b) classification, c) recoding misclassified polygons, d) post classification smoothening, and e) classification accuracy assessment. The details of LULC classification and accuracy assessment are shown in the form of a flowchart **(Figure 6.3.)**. Generation of training samples is the most important step to proceed with supervised classification, because this step promises the classification accuracy and the reliable interpretation of the classified image (Ramezan et al.2021). More the number of training samples selected; more accurate the results can be achieved. In pattan valley a total of 520 training polygons were selected for nine land use land cover classes, whereas 600 polygons were selected for ten lulc classes in GPVNP. This step is followed by generation of signature files which records the spectral signatures of different classes and classes with similar land cover types were combined. We obtained nine classes in pattan valley and ten different land use/land cover classes in GPVNP by merging different LULC classes based on spectral signatures. Maximum likelihood algorithm-based classification makes use of these training signatures to train and classify the satellite imagery (Bakr et al., 2010). Obstruction during image classification was the spectral similarity among some land use/land cover classes which resulted in misclassification of these classes. Since roof tops of the builtup areas matches with the barren area hence builtup was misclassified as barren area and vice-versa. Likewise, the spectral signature of grassland matches with the agriculture, hence grasslands were misclassified as agriculture. Therefore, the classified images were then recoded for correcting the misclassified classes. Recoding was then followed by post classification smoothing tools in ArcGIS *viz.,* majority filter tool and boundary clean tool for better representation of the image.

The increased demand and usage of remote sensing data has made geospatial analysis more speedy and powerful, but the complexity has also increased the likelihood of error, thus making accuracy evaluation more crucial than ever (Congalton RG, 1991; Peacock rex, 2014).

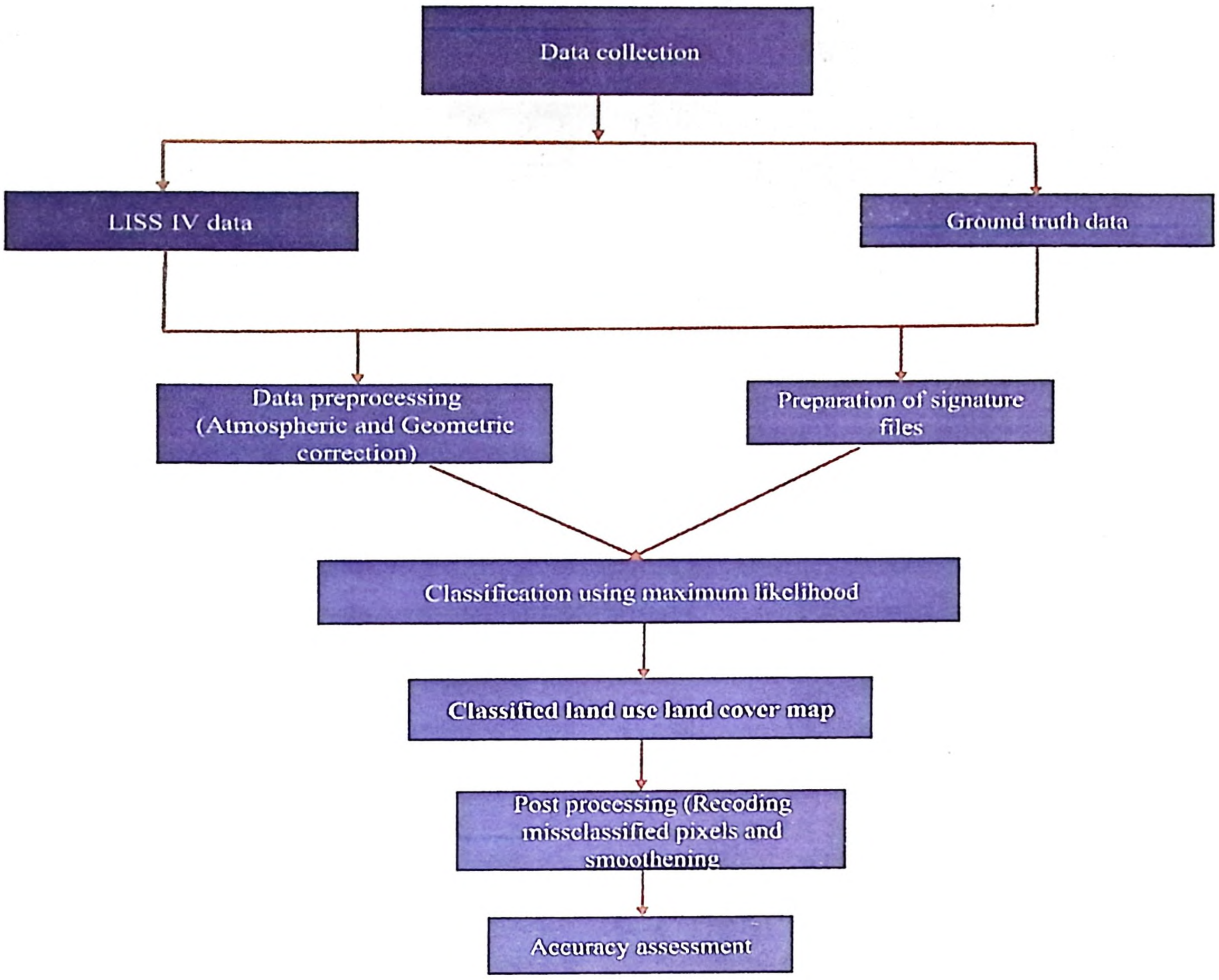

Figure 6.3. Flowchart for classifying LISS IV data in Pattan valley and Govind pashu vihar national park.

Accuracy assessment

In order to offer the impartial reference essential to conduct accuracy assessments, classification needs ground truth data. The classified image was then subjected to assess the accuracy on the basis of the ground-truthing data collected during the field visit. We used an error matrix which is a standard technique to evaluate the accuracy of the classified image (Foody, 2004; Jensen, 2005). This technique compares pixels/polygons of a classified image to ground truthing data (Jensen, 2005; Congalton and Green, 2019). The error matrices were used to derive the kappa coefficient, user and producer accuracy, and total accuracy (Keshtkar, Voigt & Alizadeh, 2017). The columns contain ground truth point values of landcover/use types and the rows represent classified point values of land cover/use types. For each study area, 312 and 352 points of different land use land cover classes were used as ground truth points and appropriate land cover type were identified from the classified image (**Table 6.2, 6.3**).

The accuracy results are evaluated by four major components *viz;* overall accuracy, producer's accuracy, user's accuracy, and kappa statistics ($\hat{k}$).

Overall accuracy: The total classification accuracy is represented by the overall accuracy. Overall accuracy is obtained by total number of correctly classified pixels/total number of reference pixels.

User accuracy: is the number of correctly classified pixels / total number of pixels classified in this category. It compares the classified data with the field data.

Producer accuracy: is the number of correctly classified pixels / total number of ground truth points. It compares field data with the classified image, indicating the likelihood that a randomly chosen point from ground truth (e.g., a location in field) falls on an exact class on the map.

Kappa statistics: A discrete multivariate approach for evaluating accuracy is the kappa coefficient. It is the (total number of pixels x number of correctly classified pixels) - sum of correctly classified pixels /total number of pixels^2 - sum of the products between ground truth data and classified pixels for each class It incorporates the influence of chance and therefore it is used to measure the reliability of the map (**Table 6.2, 6.3.**).

Table 6.2. Table showing error matrix of LULC classification of Pattan valley using maximum likelihood approach.

Landcover	BA	SN	BU	AG	GR	JF	SLND	CF	USER
BA	40	0	1	2	0	0	0	0	43
SN	1	43	0	0	0	0	0	0	44
BU	2	0	25	2	0	0	1	0	30
AG	1	0	0	30	3	0	0	0	34
GR	0	0	0	4	26	1	0	0	31
JF	1	0	0	0	0	35	2	0	38
SLND	2		1	0	0	3	38	0	44
CF	0	0	0	0	0	4	0	44	48
PRODUCER	47	43	27	38	29	43	41	44	312

Table 6.3. Table showing Error matrix of LULC classification of Govind Pashu vihar National Park using maximum likelihood approach.

Landcover	BA	SN	BU	AG	GR	BF	SLND	CF	MF	USER
BA	40	0	2	1	0	0	0	0	0	43
SN	2	40	3	0	0	0	0	0	0	45
BU	3	0	28	2	0	0	1	0	0	34
AG	0	0	0	27	4	0	0	0	0	31
GR	0	0	0	3	30	1	1	0	0	35
BF	0	0	0	0	0	36	1	0	4	41
SLND	3	0	2	0	0	4	27	0	2	38
CF	0	0	0	0	0	1	0	38	3	42
MF	0	0	0	0	0	4	0	3	36	43
PRODUCER	48	40	35	33	34	46	30	41	45	352

6.2.4. Analysis

Prepration of Habitat Covariates

Given that habitat variables such as altitude, slope, habitat types might influence the habitat use of KMD, hence a set of 20 variables were initially used which were recorded during the field survey or extracted from satellite imageries using ArcGIS 10.8.1 software (ESRI, Redlands, CA). These variables were grouped into three classes, i.e., Topographic variables, LULC (Land use land cover classes) and anthropogenic variables **(Table 6.4.)**. Different LULC classes were extracted after classifying the LISS IV imagery (5.8 m resolution) procured from National Remote Sensing agency, India. In the pattan valley a total of nine different LULC classes were identified 1) barren area, 2) snow, 3) water bodies, 4) juniper forest, 5) conifer forest, 6) subalpine and alpine shrubland, 7) grassland, 8) built-up, & 9) agriculture **(Figure 6.4.)**. In the Govind Pashu Vihar NP, ten LULC classes were extracted and classified 1) barren area, 2) snow, 3) water bodies, 4) built-up, 5) conifer forest, 6) mixed forest, 7) broadleaf forest, 8) agricultural land, 9) scrubland, 10) Grassland **(Figure 6.4.)**. Since the species is sensitive to anthropogenic disturbances hence predictors such as distance from village, distance to road, distance to water, and the Human footprint (HFP) were included as anthropogenic variables. These variables were documented during the field survey, except human footprint which was retrieved from (https://sedac. ciesin.columbia.edu) (https://sedac. ciesin.columbia.edu) (WCS 2005). Subsequently, the values for all the variables were extracted within each sampling location. Before performing any analysis, the variables were tested for collinearity and the variables with values <0.7 (Brun et al. 2020) were used for further analysis **(Figure**

6.7). Therefore, a total of 14 variables in pattan valley and 10 variables in GPVNP were used to model occupancy of KMD.

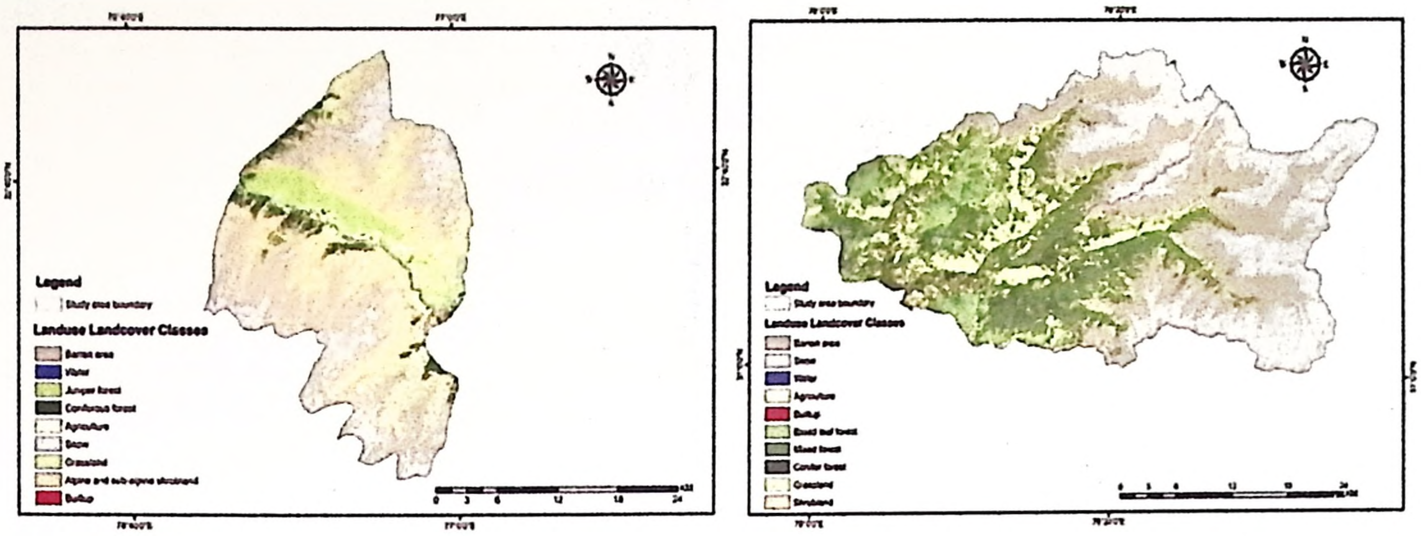

Figure 6.4. Classified images of the study area: leftside-pattan valley, Himachal Pradesh, rightside-Govind pashu vihar national park, Uttarakhand.

Table 6.4. Predictors used for occupancy analysis of Musk deer in Pattan valley, Himachal Pradesh and Govind pashu vihar national park, Uttarakhand.

S.No.	Predictor	Dummy Code	Data	Source
LULC/Land Use Land Cover Type				
1	Mixed forest (Querques species, birch, fir)	MF	LISS IV	USGs
2	Conifer forest (*Pinus sps, Picia sps*, blue pine)	CF		
3	Broad leaf forest (Ban oak forest)	BF		
4	Shrubland (*Rhododendron, Hippophea, Myricaria*)	SH		
5	Juniper forest (represented by juniper trees)	JF		
6	Grassland	GR		

7	Agriculture (cultivated area represented by agricultural and horticultural crops)	AG		
8	Snow (permafrost area)	SN.		
9	Builtup (Dark grey, purple, yellow, light pink, light green, light blue and structured areas)	BU		
Variables recorded from field				
10	Distance to village	DV	Calculated using log Euclidean distance (ArcGISx)	Field
11	Distance to water	DW		
12	Distance to road	DR		
Topographic variables				
13	Aspect	ASP	SRTM	Field
14	Slope	SLP		
15	Elevation	ELE		
Anthropogenic variables				
16	Low disturbance (Fodder collection)	LD	Field	
17	Medium disturbance (Fodder and firewood collection)	MD		
18	High disturbance (Fodder, firewood and timber collection, livestock grazing)	HD		
19	Anthropogenic disturbance (Fodder, firewood and timber collection, livestock grazing, hunting)	AD		
20	Human foot print	HFP	SEDAC	

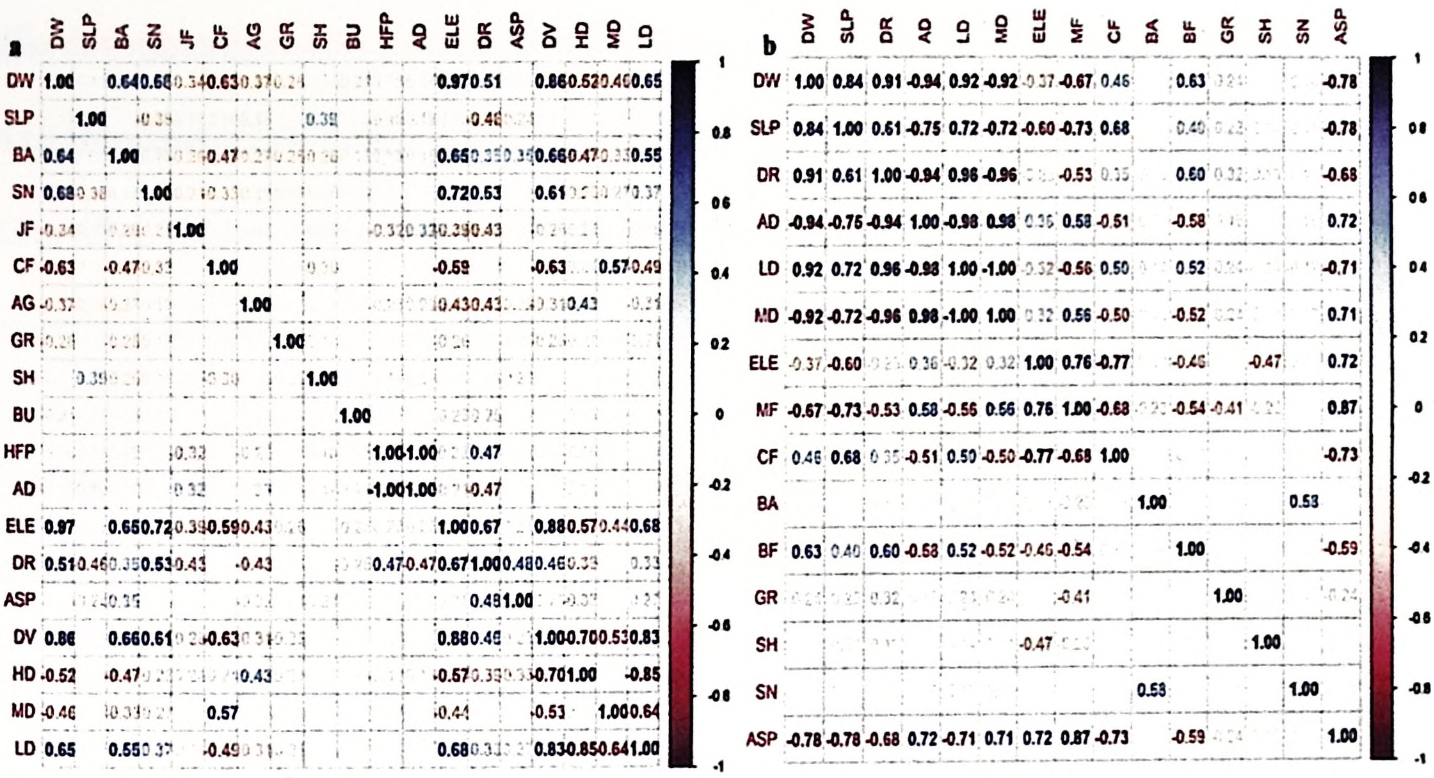

Figure 6.5. Correlation plot for testing collinearity among variables used for modelling occupancy in Pattan valley, Himachal Pradesh and Govind Pashu Vihar National Park, Uttarakhand.

Furthermore, images from camera traps were sorted and examined to identify the KMD from the landscape and doubtful pictures were excluded from the analysis. The camera trap nights were calculated from date of camera deployment until the camera was retrieved. Independent capture of KMD was considered only if the second capture was after an interval of an hour (Tobler et al., 2008). Then the images were used for calculating the capture rate of KMD (capture rate=total captures of KMD / total camera trap days (Joshi et al., 2019).

Photographic capture rate = No. of detections of a species/ Total number of operational days.

Since musk deer marks its territory through selection of laterine sites it was easier to identify the pellets of KMD. Hence for sign survey encounter rate was calculated as the number of pellets encountered/total km walked.

Occupancy framework

To understand the habitat use of KMD, we applied single-season occupancy modeling using data obtained from camera trapping and sign surveys as indicators of KMD habitat use (MacKenzie et al., 2002). Given the substantial snow coverage in both landscapes, conducting a multi-season analysis was not feasible. Therefore, the adoption of a single-season analysis strategy was necessary to explore the habitat use of KMD effectively.

The land use and land cover (LULC) variables were utilized as binary variables in our analysis. Where, a value of '1' denoted that a survey location fells within a particular land cover type, while '0' indicated otherwise. Prior to further analysis, the other non-binary predictors underwent standardization.

To establish standardized detection records for each site, we amalgamated data from all the locations within the respective site (MacKenzie et al., 2006). A site was deemed occupied ('1') if the KMD species was recorded during any of the two sampling occasions; otherwise, it was considered unoccupied. This approach enabled us to capture the presence or absence of KMD at each site in a consistent and standardized manner. Thus, we obtained detection/non-detection histories representing the presence/absence of KMD for each site using the two-pronged method over a 5-month study period each year. Since most camera traps operated for 30 days, we divided the surveys into two sampling periods of 30 days each. Single-season occupancy analysis was conducted using the "unmarked" package in R (Fiske and Chandler, 2011) to determine the influence of predictors on KMD habitat use at each sampling location.

Multiple models were created, with each variable modeled individually as well as in potential combinations. The models incorporated predictors as functions of detection probability while keeping occupancy constant (ψ(.)), and vice versa. The "MuMIN" package in R (Barton, 2015) and the 'dredge' function were used to develop models with all possible variable combinations. This allowed exploration of a greater number of potential predictors, automatically excluding variables based on user-defined exclusion criteria. All the models were ranked based on the Akaike Information Criterion (AIC), and models with a delta AIC (ΔAIC) of less than 2 were considered equally plausible (Akaike, 1973). The AIC values were used to determine the ranking of candidate models (Burnham, 2002). The sign of the beta estimates for each predictor (positive or negative) indicated the influence of the predictors on KMD occupancy and detection probability in the landscape.

6.3. Results

6.3.1. LULC Classification

High-resolution liss-4 images were classified into nine distinct categories viz 1) barren area, 2) snow, 3) water bodies, 4) juniper forest, 5) conifer forest, 6) subalpine and alpine shrubland, 7) grassland, 8) builtup, & 9) agriculture, in pattan valley (Figure 5). Whereas ten distinct classes as broadleaf forest, 1) barren area, 2) snow, 3) water bodies, 4) builtup, 5) conifer forest, 6) mixed forest, 7), 8) broadleaf forest, 9) agricultural land, 10) scrubland (Figure 6) were identified in GPVNP. For Patten Valley, a total of 312 ground truthing points were used to verify the accuracy of the results, of which 218 points were sampled from the appropriate classes. Likewise, among 352 ground truthing points, 302 points were sampled in correct land cover classes in GPVNP. In pattan valley and GPVNP the overall accuracy of the classified images is 90.06% and 85.5% with a kappa statistics of 0.88 and 0.83 respectively **(Table 6.5)**. The user's accuracy for different lulc classes in pattan valley is as follows: barren area-93.02%, snow-97.72%, builtup-83.33%, agriculture-88.23%, grassland-83.87%, juniper forest-92.10%, shrubland-86.36%, conifer forest-91.6%. The producer's accuracy for different lulc classes in pattan valley is: barren area-85.10%, snow-100%, builtup-92.59%, agriculture-78.94%, grassland-89.65%, juniper forest-81.39%, shrubland-92.68%, conifer forest-100%. Similarly, the user's accuracy for different lulc classes in GPVNP is as follows: barren area-93.02%, snow-88.88%, builtup-82.35%, agriculture-87.09%, grassland-85.71%, broadleaf forest-87.80%, shrubland-71.05%, conifer forest-90.47%, mixed forest-83.72%. Producer's accuracy is barren area-83.33%, snow-100%, builtup-80%, agriculture-81.81%, grassland-88.23%, broadleaf forest-78.26%, shrubland-90.0%, conifer forest-92.60%, mixed forest-87.80%. These values suggest a better agreement of ground truthing points with the classified map.

Based on the combined effort of 105 camera traps (43 in pattan valley, 61 in GPVNP) deployed in different habitats of KMD, we obtained a total of 53 and 8 independent captures of KMD in pattan valley and GPVNP respectively. This yielded a total of 61 independent captures of KMD. Total trap nights were 798 in pattan and 679 in GPVNP. The capture rate is 0.06±0.02 in pattan valley and 0.01 ±0.02 in GPVNP. Among the 97 trails traversed i identified 32 latrine sites of KMD in the landscape. Mean encounter rate of KMD was found to be 0.41±0.55 in the pattan valley (85 signs: 54 trails/ 206 km) and 0.18±0.03 (37 signs: 43 trails/198 km) in GPVNP.

Table 6.5. Accuracy estimates of the classified images of Pattan valley, Himachal Pradesh and Govind Pashu Vihar National Park, Uttarakhand.

Estimates		Pattan valley	GPVNP
Overall accuracy	Total no of correctly classified pixel(Diagonal)/Total No of Reference pixel *100	**90.06%**	**85.79%**
User Accuracy	no of correctly classified pixel in each category/Total no of classified pixels in that category (The Row Total)*100	89.525	85.56%
Producer Accuracy	No of correctly classified pixel in each category/Total no of Reference pixels in that category (The column Total)*100	90.04%	86.89%
Kappa Coefficient	(TS*TCS)-Σ(Column Total*Row Total)/TS2-Σ(Column Total-Row Total) (TS=Total Sample, TCS=Total Corrected sample)	**0.88**	**0.83**

6.3.2. Occupancy analaysis

Our occupancy modeling revealed the influence of habitat predictors on the habitat use of KMD in the two studied sites. Among the various models considered, the top four models were selected based on the lowest AIC values **(Table 6.6)**.

In the Pattan Valley, our best model showed that KMD occupancy was positively correlated with elevation and detection probability varied with the presence of conifer forests (**Table 6.7**). Specifically, higher elevations and a greater extent of conifer forests had a positive influence on both KMD occupancy (β = 5.20 ± 1.94) and detection probability (β = 1.44 ± 0.25) (**Table 6.7, Figure 6.7 a**). Moreover, the supporting model ψ (CF), p(JF) indicated that conifer forests positively impacted the occupancy of KMD, while juniper forests negatively affected the detection probability of the species (**Figure 6.7 a**).

Contrarily in case of Govind Pashu Vihar NP, the top model revealed a positive association between KMD occupancy (ψ) (β= 8.44 ±2.33) as well as detection probability (p) (β= 1.22 ±0.29) with the mixed forests as well as elevation (**Table 6.7, Figure 6.7 b**). In Govind Pashu Vihar NP, the supporting model also indicates a positive impact of elevation and mixed forest on the occupancy of KMD. However, the presence of conifer forests in Govind Pashu Vihar NP has a negative effect on the detection probability of KMD, indicating that as the extent of conifer forests increases, the likelihood of detecting the species decreases.

Interestingly, our findings show a contrasting pattern between the two study sites. The Pattan Valley exhibits a higher predicted occupancy of KMD compared to Govind Pashu Vihar NP (**Figure 6.6**). The detection probability of KMD in the Pattan Valley increases with an increase in conifer forest cover, while in Govind Pashu Vihar NP, the detection probability decreases with an increase in conifer forests (**Figure 6.7**). These results highlight the importance of considering the specific characteristics of each study site when assessing the habitat use of KMD.

Table 6.6. Summary of top four models selected for understanding the habitat use of Kashmir musk deer in Pattan valley, Himachal Pradesh and Govind Pashu Vihar National Park, Uttarakhand.

Site	Model	AIC	Delta AIC	AIC weights	K	Loglikelihood
Pattan valley	ψ (ELE),p(CF)	448.18	0.00	0.78	4	-220.01
	ψ (CF),p(JF)	464.80	16.62	0.16	4	-228.76
	ψ (.),p(CF)	469.32	21.14	0.05	3	-231.61
	ψ (CF),p(ELE)	470.33	22.15	0.01	4	-230.49
GPVNP	ψ (MF),p(ELE)	206.89	0.00	0.69	4	-99.21
	ψ (MF),p(ELE+CF)	208.99	2.11	0.24	5	-99.14
	ψ (.),p(ELE)	213.70	6.81	0.02	3	-103.71
	ψ (ELE+MF),p(CF)	214.98	7.87	0.01	5	-102.02

Table 6.7. Beta coefficient values of top two models influencing habitat use of musk deer in pattan valley, Himachal Pradesh and Govind Pashu Vihar National Park, Uttarakhand.

Site	Model	Covariate	β estimate	SE	z	P(>|z|)
Pattan valley	ψ (ELE),p(CF)	ψ (ELE)	5.20	1.94	2.68	0.007
		P(CF)	1.44	0.25	5.73	9.87e-09
	ψ (CF),p(JF)	ψ (CF)	8.98	2.76	0.34	0.73
		p(JF)	-7.54	4.62	-0.55	0.57
GPVNP	ψ (MF),p(ELE)	ψ (MF)	8.44	2.33	0.14	0.678
		p(ELE)	1.21	0.29	4.15	3.24e-05
	ψ (MF),p(ELE+ CF)	ψ (MF)	9.75	3.65	0.24	0.80
		p(ELE)	1.16	0.31	3.66	0.0002
		p(CF)	-0.26	0.72	-0.36	0.71

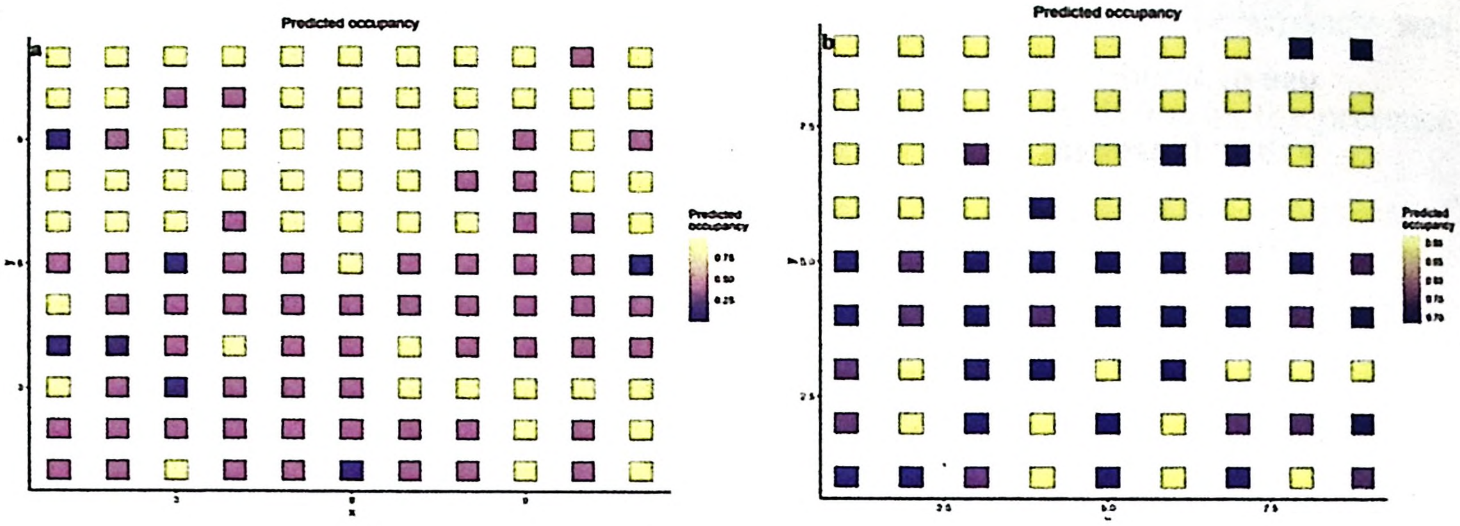

Figure 6.6. Predicted occupancy plot of KMD in a) Pattan valley and b) Govind Pashu Vihar National Park, Uttarakhand. Blue color indicates low occupancy pink color indicates medium occupancy and yellow indicates the high occupancy sites.

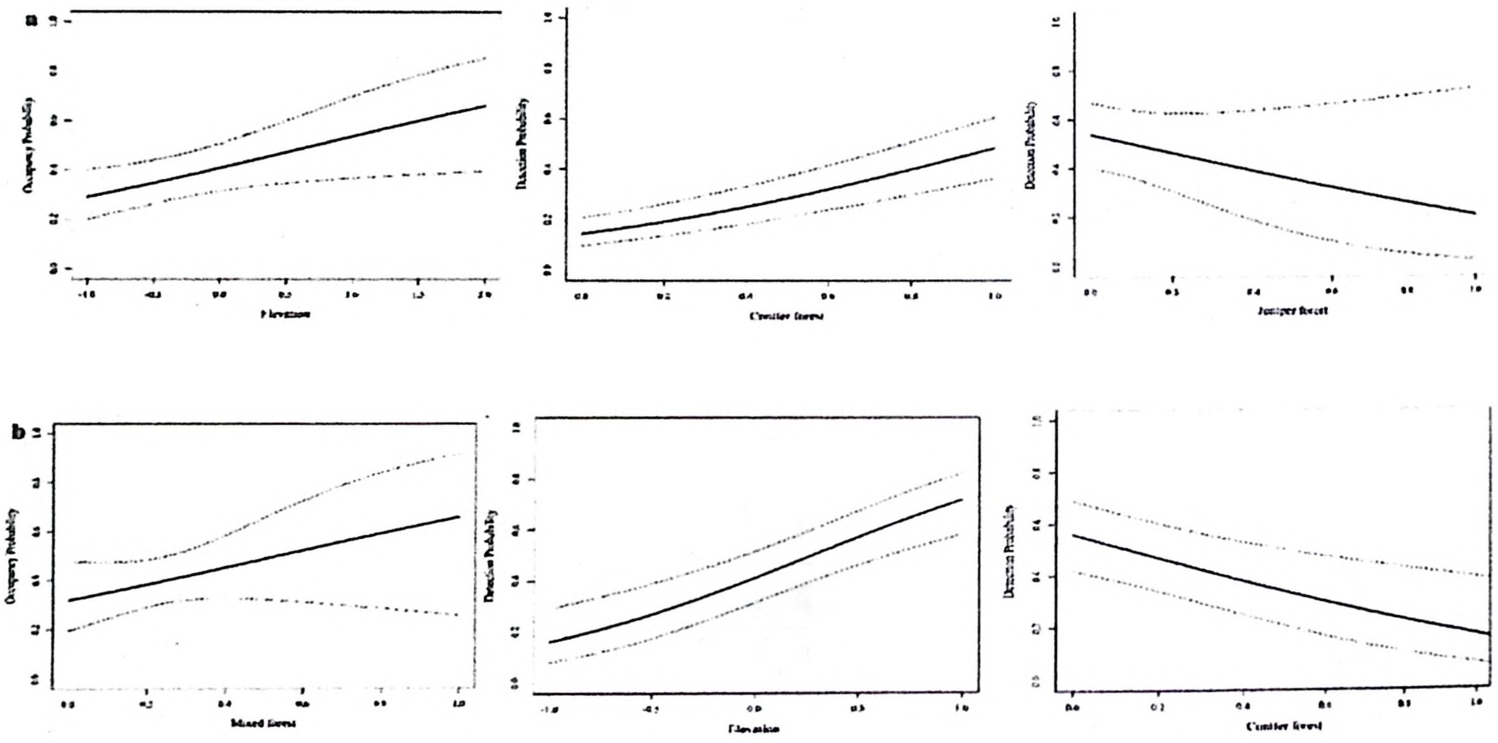

Figure 6.7. Predicted plots depicting influence of different predictors on occupancy and detection probability of KMD in a) Pattan valley, Himachal Pradesh and b) Govind Pashu Vihar National Park, Uttarakhand.

6.4. Discussion

Monitoring and understanding the distribution & habitat use of the Kashmir musk deer (KMD) is crucial for effective conservation efforts (Singh et al., 2018). Environmental factors, such as forest type, slope, and elevation, play a significant role in influencing the habitat preferences of this rare and elusive species (Singh et al., 2018). To study the wildlife habitat use while considering imperfect detection, ecologists commonly employ occupancy models (Mackenzie et al., 2002; Sharief et al., 2022).

In this study, I utilized single-season occupancy models to investigate the influence of environmental factors on the habitat use of KMD in two landscapes: Pattan Valley and Govind Pashu Vihar NP. My findings indicate that the presence of KMD is influenced by elevation, while the likelihood of detecting (detection probability) them is affected by the presence of conifer forests in the Pattan Valley. Both elevation and conifer forests positively impact the occurrence (ψ) and detection probability (p) of KMD **(Table 6.7)**. Therefore, KMD is more likely to be found and detected in higher elevations and areas with conifer forests **(Figure 6.7)**.

In the study landscape, KMD tends to inhabit areas characterized by the presence of Pinus spp. and Abies spp. trees within the elevational range of 2946-4418 m. This preference for higher elevations can be attributed to the species avoidance of human disturbances in the landscape (Subedi et al., 2012; Khadka KK, 2017; Singh et al., 2018). These findings align with previous studies that have reported the altitudinal range of musk deer between 2500 and 4200 m (Sathyakumar, 1994; Vinod & Sathyakumar, 1999; Ilyas, 2014; Subedi et al., 2012; Singh et al., 2018). The positive influence of conifer forests on the detection probability of KMD can be linked to the thermal benefits provided by the dense foliage in conifer forests, which reduce thermoregulatory costs for KMD in the high elevation areas of the Himalayas (Dussault et al., 2004; Maloney et al., 2005; Khadka and James, 2016).

The preferred habitat for KMD includes tree species such as *Picea smithiana, Abies pindrow, Taxus wallichiana, Pinus wallichiana, Betula utilis,* and *Rhododendron campanulatum*, which are commonly found in the study area (Khadka and James, 2016). The species longer hind limbs compared to forelimbs enable it to thrive in the rugged terrains of high elevation areas. Furthermore, our results suggest that KMD tends to avoid juniper forests in the Pattan Valley, possibly due to their proximity to human habitation. In Govind Pashu Vihar NP, mixed forests and elevation were identified as the most influential factors affecting KMD occupancy (ψ) (β= 8.44 ±2.33) and detection probability p) (β= 1.22 ±0.29). Mixed forests primarily composed of Himalayan fir (*Abies spectabilis*) and blue pine (*Pinus wullachiana*), along with Himalayan birch *(Betula utilis*) trees, positively influenced the occurrence and detection probability of KMD within the elevation range of 2145-3641 m (**Table 6.7**). These findings are consistent with previous studies indicating KMD's preference for mixed forests with specific tree species (Singh et al., 2018; Singh et al., 2021).

Furthermore, our supporting model revealed that conifer forests had a negative influence on the detection probability of KMD, indicating that the likelihood of detecting KMD decreases with an increase in conifer forests in Govind Pashu Vihar NP. I confirmed the presence of KMD in mixed forests at higher elevations (2146–3641 m) and their avoidance of conifer forests in Govind Pashu Vihar NP. The majority of the KMD's pellet/laterine sites were recorded in mixed forests composed of *Betula utilis*, *Quercus* species, *Abies spectabilis*, and *R. campanulatum*. These mixed forests provide easy access to food resources and offer good cover for escaping predators, such as the common leopard. Our findings align with Singh et al. (2018), who also reported that KMD prefers mixed forests composed of Himalayan birch, Himalayan fir, and high elevation oak trees over conifer forests in the landscape. As musk deer are territorial and mark their territory through latrine sites, we conclude that mixed forests might offer shelter, ample canopy cover to avoid predation, and space for latrine sites (Shrestha and Meng, 2014). The shade provided by the canopy cover of mixed forests helps maintain the pungency of faecal markings for a longer duration, facilitating effective territorial marking and chemical communication.

The present study clearly distinguishes the habitat use of KMD in two studied landscapes. I found that conifer forests positively influence the detection probability of KMD in the Pattan Valley, while the detection probability decreases with an increase in conifer forests in GPVNP **(Figure 6.7)**. However, the negative influence of conifer forests does not imply that KMD completely avoids these habitats. Instead, they tend to use mixed forests more frequently than conifer forests in GPVNP. I infer that KMD utilizes high altitude habitats as its preferred habitat depending on the forest conditions in the landscape. I findings are supported by previous literature indicating that musk deer inhabit different habitat types, including fir forests and birch forests in Sagarmatha National Park (Aryal et al., 2010), mixed forests and rhododendron forests in the Gaurishankar Conservation Area, Nepal (Shrestha and Meng, 2014), pine and fir forests in Nepal (Khadka and James, 2016), and fir forests in Annapurna Conservation Area, Nepal (Singh et al., 2018). KMD was found to occupy areas between 2946-4418 m in the Pattan Valley and 2145-3641 m in GPVNP. Musk deer typically inhabit high altitudinal ranges between 2500 m to 4800 m (Timmins and Duckworth, 2015; Singh et al., 2018). Previous studies in the Himalayas have also reported the preferred altitudinal range of musk deer between 2,500 and 4,200 m (Ilyas, 2014; Vinod & Sathyakumar, 1999), 3600-3800 m (Subedi et al., 2012), and 3200-4200 m (Singh et al., 2018). The insulation provided by their hollow hair and longer hind limbs, compared to forelimbs, allows them to adapt and thrive in higher elevation areas (Green, 1985; Futuyma & Moreno, 1988). Therefore, I conclude that KMD inhabits conifer to mixed forests in higher elevation areas between 2146-4418 m, depending on the availability of forest types in the landscape. High altitude habitats are essential for KMD in the Himalayas. Based on the study's findings, I recommend that conservation managers in Uttarakhand and Himachal Pradesh develop robust plans to create the best possible habitat for the species.

Primarily due to poaching and habitat loss, KMD has been categorized as an Endangered species on the IUCN Red List (Timmins and Duckworth, 2015). In addition to poaching, anthropogenic disturbances such as medicinal plant extraction, fodder collection, and exhaustive livestock grazing pose potential threats to KMD in the landscape. Govind Pashu Vihar NP, having a higher human habitation compared to the Pattan Valley, experiences greater anthropogenic pressure in the forested areas. The presence of tourism camping sites, such as Harki Doon, Kedar Kantha, Chansil Bugyal, and Robin Sara, in KMD habitats further exacerbates the disturbance in the area. To our observations, illegal hunting was found to be a major threat to KMD in the Mooling area of the Pattan Valley, which is situated on the left side of the river Chandra Bhaga. In contrast, Naingarh, Chokhang, and Gauri, located on the top corner of the left side of the river Chandra Bhaga, receive less poaching pressure than the Mooling area. This difference could be attributed to the easy access to the Mooling area due to its proximity to the national highway. Additionally, poaching and smuggling activities have increased in tandem with the rising price of musk pod in international markets across various regions of the world (Aryal and Subedi, 2011; Subedi et al., 2012; Sathyakumar and Rawat, 2015; Wangdi et al., 2019). The lack of sufficient control measures for poaching is primarily due to the absence of protected areas in the Lahaul Valley. In the Pattan Valley, where the majority of the residents are Buddhist, we observed less involvement of locals in poaching. Conversely, in Govind Pashu Vihar NP, where Hinduism is followed, locals are involved in hunting. Poachers in the Pattan Valley are mainly Nepalis and have been encountered in the forested areas upon returning from the field. During interviews with locals in GPVNP, we found that people have also observed a significantly lower population of KMD compared to previous years. This decline can be attributed to the high involvement of locals in poaching, further threatening the species in the area. Snares placed to kill musk deer were frequently encountered and destroyed by the research team. Given the poor involvement of residents in poaching, it suggest to initiate and strengthen community involvement in the Pattan Valley and GPVNP to combat poaching through regular participation in wildlife department surveillance. Building wildlife conservation stewardship within the local community will encourage community-based conservation efforts with appropriate incentives and awareness campaigns. If the existing threats to KMD in both study sites are left unmanaged, the species will be pushed further towards the brink of extinction.

To maintain a sustainable population of KMD, conservation strategies should include sustainable resource extraction practices and controlled grazing. In GPVNP, camping sites should be situated away from KMD habitats to minimize disturbance. Furthermore, considering the significance of the Pattan Valley as a habitat, we strongly recommend designating it as a protected area to ensure proper management of the species. Strengthening vigilance efforts will help combat illegal hunting in the landscape. Updating the notification in GPVNP to incorporate potential special protected zones for KMD habitat will also contribute to the continued persistence of the species. Based on the study's findings, we advise conservation managers in Uttarakhand and Himachal Pradesh to develop appropriate plans to create the best possible habitat for the species.

6.5. Conclusion

To ensure the protection and conservation of KMD in the landscape, it is crucial to utilize existing resources properly and wisely. In the Pattan Valley, KMD occupies conifer forests at elevations between 2946-4418 m, while in GPVNP, the species prefers mixed forests at elevations ranging from 2145-3641 m. Maintaining and protecting these high-altitude forests is essential as they serve as vital habitats for musk deer. Minimizing poaching pressure and anthropogenic disturbances in these habitats is necessary for the long-term viability of the endangered KMD. Poaching remains a persistent issue due to inadequate protection measures and the absence of a comprehensive management plan focused on conservation. A primary conservation action should involve developing a strategic community plan that includes public education, protection of KMD's forest habitats, and the implementation of effective patrolling and anti-poaching measures, such as utilizing advanced equipment like camera traps. The findings of this study can contribute to better resource management and wise allocation for the conservation of KMD. Considering the species' endangered status and its significance as the state animal of Uttarakhand, it is recommended to establish a long-term monitoring program spanning at least ten years. This program would assess the causes of population decline of KMD in the landscape and aid in implementing targeted conservation efforts for the long-term viability of this species.

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Chapter 7: Objective 4. To undertake population genetic analysis of musk deer in Uttarkashi and Lahaul valley.

Abstract

Population genetics is a crucial field of study especially for threatened species, that unravels the genetic variation, structure, and demographic history of species. Due to habitat loss and illegal hunting for musk pod Kashmir musk deer has been listed as an endangered species. Hence, in this chapter, i explore the population genetics of the endangered Kashmir musk deer (*Moschus cupreus*) in the North-western Himalayas using mitochondrial DNA (mtDNA) and microsatellite markers. Genetic diversity estimates of 63 consensus sequences revealed 10 unique haplotypes of Kashmir musk deer from North-western Himalayas with a select panel of 8 loci. Relatively high genetic diversity (Hd = 0.906± 0.031 and π = 0.00539± 0.004) at mitochondrial Cytb gene and a moderate genetic variability at microsatellites (Ho = 0.445±0.049, He = 0.764 ± 0.039) was observed in the present study. Data analysis reveals both shared and unique haplotypes found in different regions. The presence of three potential genetic clusters within Lahaul Valley suggests genetic admixture from neighboring areas. Bayesian skyline plots and mismatch distribution curves indicate stable population sizes with recent declines in effective population size. The microsatellite analysis confirms moderate genetic diversity within populations and highlights the importance of preserving local genetic variation. Our findings emphasize the need for conservation efforts to consider genetic factors, such as population structure and gene flow, to ensure the long-term survival and adaptability of this iconic species in the face of environmental challenges. As human activities continue to impact wildlife habitats,

understanding and preserving the genetic diversity of endangered species through population genetics research is becoming increasingly vital for effective conservation planning. We also suggest covering the samples from the wide geographic region like from the Chamba and Kinnaur district, HP, Jammu and Kashmir, possible areas in Uttarakhand for fine population level analysis.

7.1. Introduction

Over the past three decades, significant advances in DNA-based technology have propelled conservation genetics into the forefront of scientific research (Allendorf et al., 2008; Frankham, 2010). These cutting-edge techniques have greatly enhanced our understanding of genetic diversity and relationships, particularly at lower taxonomic levels, enabling the identification of cryptic species (Hebert et al., 2003). Conservation genetics, by utilizing DNA-based tools, plays a pivotal role in formulating effective strategies for species conservation. Knowledge of relatedness between individuals is of paramount importance in various conservation plans, such as captive breeding and stock identification, which aim to minimize inbreeding and prevent the loss of genetic variation (Frankham, 2010; Leary et al., 2015). Extensive research has demonstrated that the decline in genetic variation poses severe threats to a population's ability to adapt to its environment and compromises its long-term survival (Lande and Barrowclough, 1987). For small populations, which are particularly susceptible to genetic changes due to their small effective population size and increased risk of rare allele loss, both mitochondrial and nuclear markers have been employed to understand the genetic structure (Hoban et al., 2013).

Mitochondria, present in every cell of the body, are often referred to as the "powerhouses" due to their involvement in metabolism, apoptosis, disease, and aging. The mitochondrial genome, known as mitochondrial DNA (mtDNA), is separate from the nuclear genome and is maternally inherited, making it a valuable marker for analyzing maternal population structure and conducting phylogenetic studies (Kocher et al., 1989; Johns and Avise, 1998; Hajibabaei et al., 2012). Among different mtDNA regions, the D-Loop or control region exhibits greater variation than protein-coding genes, as it experiences reduced functional constraints and relaxed selection pressure (Iraldo, 2004). Consequently, mitochondrial control region variation provides insights into intraspecific diversity at the population level, while more conserved sequences are better suited for assessing biodiversity among vertebrates (Taberlet and Fumagalli, 1996; Avise and Walker, 1998.

Genetic markers, such as mtDNA, have been pivotal in species identification, understanding genetic diversity, and unraveling phylogenetic and phylogeographic patterns in various species (Castro et al., 2007; Bernard et al., 2016; Toha et al., 2016). The mitochondrial control region and cytochrome b are considered fundamental genetic markers for investigating phylogeographic and genetic variation at both intra- and interspecific levels and have been extensively employed in studies involving shark species, including the whale shark (Castro et al., 2007; Ramírez-Macías et al., 2007b; Bernard et al., 2016; Toha et al., 2016).

Microsatellite DNA, also known as short sequence repeats (SSRs), represents another revolutionary tool in conservation biology (DeSalle and Amato; Arruga et al., 2001). Microsatellites are co-dominant and PCR-based markers that offer valuable insights into bi-parental genetic structure. Due to their high polymorphism, microsatellites have emerged as powerful genetic tags for applications in aquaculture, such as parentage analysis, stock discrimination, population genetics, and genome mapping (Clarke et al., 2015; Smith et al., 2015; Williams et al., 2015). While isolation of microsatellites was previously a time-consuming task, advancements in next-generation sequencing technologies have accelerated this process significantly. Microsatellite primers are often conserved in closely related species, allowing for cross-species amplification in new species (Clarke et al., 2015; Smith et al., 2015; Williams et al., 2015). As markers of high variability, microsatellites efficiently aid in detecting population differentiation, and they have been routinely employed in conservation genetics to understand population genetic structure, phylogeography, and inform stock management and reintroduction plans (Smith et al., 2015; Williams et al., 2015). The use of microsatellite primers to infer population structure, phylogeography, and genetic diversity is widely practiced, particularly in marine species (Schmidt et al., 2009; Leary et al., 2015).

Kashmir musk deer populations face various threats, including habitat loss, poaching, and climate change (Sharief et al., 2023) and has been listed as an endangered species according to IUCN, (Timmins & Duckworth, 2015). Studying their population would be important for providing crucial information for conservation efforts, such as identifying genetically distinct populations, understanding gene flow, and determining effective population sizes. This knowledge is essential for formulating conservation and management strategies to ensure the long-term survival of musk deer populations. The population genetics is a vital tool for conservation biologists and wildlife managers to make informed decisions, protect genetic diversity, and ensure the long-term survival of threatened species. Ultimately, population genetics provides essential insights for conservation efforts, ensuring the long-term survival and adaptive potential of populations in the face of various threats and challenges. Hence in this chapter I aim to understand the population genetic structure of Kashmir musk deer in North-western Himalaya using mtDNA and microsatellite markers.

7.2. Material and Methods

7.2.1. Sample collection and DNA extraction

A total of 170 faecal samples from Lahaul and Uttarkashi and 3 samples from Jammu and Kashmir were used for understanding the population genetic structure of KMD. The GPS coordinates for all the sampling locations were recorded. Faecal samples of Musk deer were first air dried or using the hot air oven in field conditions. Subsequently, these samples were transferred in the 100 ml sterilized vials and transported to ZSI, Kolkata for the DNA analyses. All the samples were catalogued with ZSI ID and stored in silica containing vials. DNA was extracted from the faecal samples using the Qiagen Stool DNA extraction kit following manufacturer recommendations (Qiagen, Germany) with minor modifications. Extracted DNA was checked on 1% agarose gel. DNA band intensity was estimated using a gel, with bands being rated on a scale of 0 to 3 depending on how visible they were on the gel (0 being invisible, 1 being a poor intensity band, 2 being a moderate intensity band, and 3 being a good intensity band). DNA templates were diluted using a dilution factor selected using a 0 to 3 band intensity scaling on a gel. These samples were identified based on the mitochondrial markers Cyt b gene and D-loop (as discussed in Chapter 1).

7.2.2. PCR amplification and DNA sequencing

To amplify the mitochondrial gene, the polymerase chain reactions (PCR) were conducted in 10 µl volume containing 1X PCR buffer, 1.2 mM $MgCL_2$, 0.40 mM of each dNTP, 0.40 µM forward and reverse primer, 0. 5 unit of Taq DNA polymerase (Takara), 0.10 µg/µl of Bovine Serum Albumin, 1-2 20 µl DNA. The PCR profile included an initial denaturation step at 95°C for 5 min followed by 40 cycles at 95°C for 30 sec, annealing at a given temp 55 °C for 40 sec, primer extension at 72°C for 55 sec, and a final extension step at 72°C for 10 min with holding the cycle at 4 °C. The amplified PCR products were cleaned up using Exo-SAP treatment to remove residual oligonucleotides and dNTPs prior to DNA sequencing. Forward and reverse primer of each mitochondrial gene were used for setting up the cycle sequencing PCR using the Big dye terminator cycle sequencing kit version 3.1 (Applied Biosystems, USA). Qualities of sequences were determined using Sequence Analysis v 5.2 software (Applied Biosystems, USA) and validated by Sequencher v 4.7 software (www. gene code. com. Multiple sequence alignment (MSA) was performed using CLUSTAL-W as implemented in BioEdit v 7.0.9.0 software (Hall, 1999).

7.2.3. Selection and PCR amplification of microsatellite markers

Total 19 microsatellite loci were selected (10 musk deer specific and 9 cervid-bovid) (Moore et al., 1992; Georges & Massey 1992; Solinas-Toldo et al., 1993; Steffen et al. ,1993; Kaukinen & Varvio, 1993; Vaiman et al., 1994; Bishop et al. 1994;

Thieven et al., 1995; Maudet et al., 2002; Zou et al., 2005) and 141 genetically identified KMD samples were individually amplified using the PCR master mix in 10 µl volumes with the following PCR mix: 4 µlMultiplex master mix (Qiagen, Germany), 1 µM each primer **(Table 7.1).**

Several techniques have been employed for achieving the most efficient amplification results. The markers were chosen based on two main criteria: a high degree of polymorphism and their wide distribution across the genome. To ensure their reliability and accuracy, all selected markers underwent initial testing using a uniplex independent PCR with 10 known samples from each species. In order to optimize the cost and time involved in our study, various sets of multiplex panels for microsatellites were standardized. Firstly, gradient PCR experiments were conducted, varying the annealing temperature from 48°C to 62°C. Additionally, I utilized touchdown PCR, which involved starting with a higher annealing temperature and then gradually reducing it by 1°C per cycle. Furthermore, the effects of different final extension temperatures on the amplification process were explored. By implementing these optimization approaches, I aimed to streamline the PCR process and obtain the most reliable results for my multiplex panels of microsatellite markers. The details of the selected markers can be found in **(Table 7.2).**

After the initial testing phase, the primers were combined into multiplexes, taking into account factors such as annealing temperature, dye chemistry, and amplicon size. To facilitate the differentiation of alleles during sizing, the forward primer of each locus was labeled with one of four fluorescent dyes: FAM, VIC, PET, or NED, at the 5' end. In cases where markers were labeled with the same dye color, they were pooled only if they had distinct size ranges. Detailed information about the selected microsatellite markers can be found in **(Table 7.2)**. Finally, Microsatellite markers were selected based on similarities in annealing temperature, amplification success rate, and had different dye colors and amplicon sizes.

The thermal conditions were used from the original protocol suggested by Maudet et al., 2002. PCR amplification was checked on 2 % (w/v) agarose gel by loading a mixture of 3 µl PCR product and 1 µl loading dye. The bands of amplified product were observed under the UV light. The amplified products of microsatellite markers were pooled with different dyes in a single tube and run for genotyping in the ABI 3500 Genetic Analyzer (Applied Biosystem, USA). All the raw data were analyzed on the GeneMapper 4.1 (Applied Biosystem,USA).

Table 7.1. Characteristics of microsatellite markers used for the population genetic analysis of Kashmir musk deer.

S.No.	Locus	Repeat Motif	Sequence (F) (5'-3')	Sequence (R) (5'-3')	Allele Size Range (bp)	References
1	Mber103C	$(CA)_{28}$	TCCACCTCCCAAGTCCCT	GAATAAAGCAATGTGTAAAAGAACC	120-156	Zou et al., 2005
2	Mber18	$GT)_{15}$	CTCCAGGCAAGAACACTG	GCAAGAAGTTATGCAATCAA	256-286	Zou et al., 2008
3	MB33	$(GT)_{26}$	TCCTCGCTGATTATTTGG	CGGATTCGTAAAGTGGGT	226-220	Zhao et al., 2008
4	MB39	$(GT)_{34}$	ATCAAACCCACATCTCCT	TGCCCTGGTTAGAACTCC	257-305	Zhao et al., 2008
5	MB43	$(GT)_{22}$	TGGTGGCTGTTACCCTAT	AAACCTGCATCTCCTGAA	119-157	Zhao et al., 2008
6	MBA1	$(CA)_{16}$	ATTTTGCTTGATAACTGC	AATCCCCTATTACTGTGG	213-231	Zhao et al., 2008
7	MB14H	$(AC)_3$AGC(AC)8AGC$(AC)_{12}$	GTCAAGGGACCAGAACACAA	CGTCTGTCAAGGTGGGTTT	162-256	Zou et al., 2005
8	MB06	$(AC)_{12}$	GATAAGCAGGCAGCAACG	TGTCCAGGAAGAGGAGGG	294-302	Zhao et al., 2008
9	MB10	$(AC)_{50}$	GTGGGAAGGCAGCACAGA	AAGGCTCAGGTACAGTCAAGAA	290-340	Zhao et al., 2008
10	MB30	$(CA)_{22}$	TAGACCATGACGCCAGAT	GCTACACTGAGCCACCTAA	150-168	Zhao et al., 2008
11	Haut14	GT	CCAGGGAAGATGAAGTGACC	TGACCTTCACTCATGTTATTAA	120-157	Thieven et al., 1995

12	INRA35	GT	TTGTGCTTTATGACACTATCCG	ATCCTTTGCAGCCTCCACATTC	112-120	Vaiman et al., 1994
13	CSSM14	GT	AAATGACCTCTCAATGGAAGCTTG	GAATTCTGGCACTTAATAGGATTCA	127-134	Moore et al., (1992,
14	BM2113	(TG)	CGT GCC TTC TAC CAA ATA CCC	CTT CCT GAC AGA AGC AAC ACC	133-152	Georges & Massey, 1992
15	ETH10	AC	GTT CAG GAC TGG CCC TGC TAA CA	CCT CCA GCC CAC TTT CTC TTC TC	188-215	Vaiman et al., 1994
16	TGLA122	ACnATn	AAT CAC ATG GCA AAT AAG TAC ATA C	AAT CAC ATG GCA AAT AAG TAC ATA C	160-171	Bishop et al., 1994
17	ETH3	$(GT)_nCA(GT)_6$	GAACCTGCCTCTCCTGCATTGG	ACT CTG CCT GTG GCC AAG TAG G	148-162	Maudet et., 2002
18	ETH225	$(GT)_4CG(TG)(CA)_n$	GATCACCTTGCCACTATTTCCT	ACA TGA CAG CCA GCT GCT ACT	188-197	Steffen et al., 1993
19	ETH152F	TA	TACTCGTAGGGCAGGCTGCCTG	GAGACCTCAGGGTTGGTGATCAG	104-131	Georges & Massey, 1992

Table 7.2. Eight multiplex panels designed for the multilocus genotyping of Kashmir musk deer.

Species specific primer	Multiplex panel (MP)	Marker Name	Dye	Size Range (bp)	Temp. (°C)
Musk deer	MP1	**Mbr103C**	VIC	90.02-160	55
		MB18	FAM	250.02-310	
		MB33	NED	180.02-300	
	MP2	**MB39**	VIC	90-111	57
		MB43	FAM	122-128	
		MBA1	FAM	180.02-250	
	MP3	**MBr14H**	FAM	170.02-250	60
		MB06	FAM	270.02-320	
		MB10	NED	250.02-360	
		MB30	FAM	120.02-165	
Cervid-bovid	MP4	TGLA122	FAM	160.02-171.0	53
		ETH152F	FAM	104.02-131.0	
		ETH225	NED	188.02-197	
	MP5	ETH10	FAM	188.2-215	57
		BM2113	VIC	133.2-152.0	
		CCSM14	FAM	127.2-134	
		INRA35	FAM	112.0-120	
	MP6	ETH3	NED	148-162	53
		HAUT14	FAM	120.02-157	

7.2.4. Data analysis

Mitochondrial DNA analysis

Species identification and genetic diversity

Samples identified as musk deer origin were used for population genetics as following methods described in Chapter 1. Out of 141 samples identified of musk deer origin, 69 sequences of cyt b (320bp) and 64 sequences of control region (335bp) were found comparable and compatible with unified length with sequences obtained from NCBI (23 for cytb and 24 for control region and 19 were complete mitogenome). Both regions (Cyt b gene and control regions) sequences were then merged and a consensus sequence of 644 bp was further used for taxonomic classification, population genetics and phylogenetic reconstruction. Using the 63 concatenated sequences of uniform length, genetic diversity parameters i.e., number of haplotypes (H), haplotype diversity (Hd), the number of segregating sites (s) and nucleotide diversity (π) were estimated using following software viz., MEGA 10 (Kumar et al., 2018), DnaSP 5.10 (Librado and Rozas 2009), and BioEdit (Hall 1999).

Population demography

The population dynamics of Kashmir musk deer was investigated, aiming to understand whether the populations experienced expansion, equilibrium, or decline. To achieve this, I employed several statistical tests, including Tajima's D and Fu's FS, as well as mismatch distribution tests. Tajima's D and Fu's FS were computed using Arlequin v3.1 (Excoffier and Lischer, 2010), while mismatch distribution tests were conducted using DnaSP v.5.10 (Librado and Rozas 2009). Tajima's D allowed me to compare the observed genetic variation to the expected variation in a randomly mating population at mutation-drift equilibrium. On the other hand, Fu's FS measured the discrepancy between observed nucleotide differences and the distribution expected in a selectively neutral sample evolving randomly at population equilibrium. Significant departures from the null hypothesis in both neutrality tests indicates potential population expansion or decline. A positive Tajima's D value indicates a population decline, while a negative value suggests a population in equilibrium. Moreover, mismatch distribution tests were used to identify patterns of sequence variation in the population, with unimodal peaks indicating recent population expansion and ragged, multimodal distributions pointing to populations at demographic equilibrium using a DnaSP v.5.10 (Librado and Rozas 2009).

To examine the relationships among haplotypes and their geographic affinities, we utilized the median-joining (MJ) networks (Bandelt et al., 1999) constructed in NETWORK 4.5.1.0 (www.fluxus-engineering.com). This approach allowed us to gain insights into the genetic connections and distribution patterns of the haplotypes. Nucleotide substitution model were selected in MrModel test v2.3 (Nylander, 2004). Additionally, I employed a coalescent-based approach to further explore the demographic history of Kashmir Musk deer. Bayesian skyline plots were generated using mtDNA data, providing trajectories of the mitochondrial effective population size (mtNe) over time. This approach, implemented in the program BEAST, allowed me to estimate gene genealogy and demographic history simultaneously, presenting past population size fluctuations with 95% credibility intervals and implemented in BEAST 2 (Bouckaert et al., 2014).. The present study employed the HKY (Hasegawa-

Kishino-Yano) substitution model and empirical base frequencies for genetic analysis. To analyze the data, the Markov Chains Monte Carlo (MCMC) simulation was executed with 100 million iterations, sampling at every 10,000th step, and applying 10% burn-in steps. These measures ensured effective sample sizes (ESS) under a strict molecular clock. Visualization output of BSP results were employed in Tracer version 1.7 (Rambaut et al. 2018). The MCMC process allowed me to obtain effective sample sizes (ESS) while considering a strict molecular clock and a stepwise skyline model.. For this analysis, I used a substitution rate of 2.9×10^{-8} substitutions per site per year, as previously reported in previous studies (Pan et al. 2015; Kumar et al. 2022; Singh et al. 2022). To optimize the performance, all operators were automatically optimized. The output of the analysis was then visualized using Tracer version 1.6 (Rambaut et al. 2018) specifically designed for the exploration and visualization of MCMC results (John and avis 1989 and Kumar et al. 2022).

Microsatellite Analysis

The microsatellite electropherograms were scored using GENEMAPPER v. 5.0 after manual scrutiny of each allele call. To detect genotyping errors, specifically allele drop out (ADO) and false alleles (FA), multiple tube approach was used for repeat data, as per Johnson and Haydon, (2002). This process allowed us to identify and account for any potential inaccuracies in the initial genotyping results. We used MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al. 2004) to examine the presence of null alleles in the markers and GENEPOP v. 4.2 (Rousset 2008) was used to detect the presence of linkage disequilibrium.

Selection of Microsatellites for individual Identification

Identifying unique individuals may seem like a simple task, but it can become quite complex, especially when dealing with situations where multiple samples may be present for a few individuals. Additionally, the challenge is exacerbated when data sets contain missing values at certain loci or are prone to even minor genotyping errors. These factors can lead to variations in the genotypes of the same individual due to allele dropouts, false alleles, or missing information. Moreover, using a large number of loci in non-invasive samples can increase the chances of including silent genotyping errors or scoring biases, leading to an overestimation of the number of individuals in the population (Thakur et al., 2015a & 2015b). To address these complexities and ensure accurate identification of individuals, I adopted a careful approach. Limited number of loci were used for individual identification based on several criteria, including minimal genotyping errors, shorter amplicon size, high amplification success, and high discriminating power.

For the identification process, locus-wise and cumulative probability of identity for unrelated individuals (PID) and siblings (PID sibs) were calculated using the identity analysis module in GenAlEx version 6.5 (Peakall and Smouse 2012). By evaluating the PID and PID sibs, I was able to pinpoint unique genotypes from the vast amount of multi-locus genotype data, employing the GenAlEx version 6.5 software (Peakall and Smouse 2012). The software's algorithm facilitates the detection of similarities between samples, grouping similar samples together at a specified threshold of dissimilarity, typically considering one or two mismatches.

Estimation of Genetic diversity

To assess the genetic diversity within the populations, the numbers of observed alleles (Na) and effective alleles (Ne) provides insights into the allelic richness and accounting for variations in sample size were calculated in GenAlEx version 6.5 (Peakall & Smouse 2012). Additionally, the observed (Ho) and expected (He) heterozygosity were calculated in GenAlEx version 6.5 (Peakall & Smouse 2012), which helped to evaluate the level of genetic variability in the populations. Deviations from Hardy-Weinberg Equilibrium (HWE) were also examined to detect any potential departures from the expected genotypic frequencies. To explore the linkage disequilibrium (LD) between loci and understand the extent of distortion from independent segregation using GENEPOP version 4.2 (Raymond and Rousset in 1995). This involved conducting 10,000 dememorizations, 500 batches, and 10,000 iterations per batch, following the method proposed by Raymond & Rousset in 1995.

Population genetic structure

The present study used Structure software version 2.3.2.1 (Pritchard et al.2007) to estimate the number of probable populations (K) of Kashmir musk deer in Northwestern Himalayas. Structure employs a Bayesian clustering method to infer population structure and probabilistically assigns individuals to specific populations based on their genotypes and allele frequencies at each locus. To ensure robust results, we performed ten analyses for each number of clusters (K), ranging from 1 to 10. The parameters were set to 500,000 iterations with a 50,000 burn-in period. The method assumes the existence of K individuals, characterized by allele frequencies. For this analysis, we used individual genotypes to determine population assignments while estimating population allele frequencies.

To visualize and determine the most appropriate K value, different K values and likelihoods on a graph were plotted using Structure Harvester (web version 0.6.92) following Evanno et al. (2005). Each individual were assigned to the inferred clusters based on a threshold proportion of membership (q) of ≥0.80 and individuals with a q value less than 0.80 were classified as admixed (Thakur et al., 2013, Joshi et al., 2022).

7.3. Results

7.3.1. Mitochondrial DNA

Sequences generated with different primers were validated in NCBI with the references data. A total of 141 were found Kashmir Musk deer origin out of 172 samples collected from Western Himalayas (Lahaul valley, Himachal Pradesh and Uttarkashi, Uttarakhand). However, out of 141 samples identified of musk deer origin, 69 sequences of cyt b (320bp) and 64 sequences of control region (335bp) were found comparable and compatible with unified length with sequences obtained from NCBI. Both regions (Cyt b gene and control regions) sequences were then merged and a total 63 consensus sequence of 644 bp were found suitable population genetics analysis. The 63 consensus sequences resulted in overall 10 haplotypes with nucleotide diversity = 0.00053± 0.004 and haplotype diversity =0.906± 0.031 **(Table 7.3)**.

Table 7.3. Summary of overall molecular genetic diversity and neutrality tests of Kashmir musk deer in North-western Himalayas.

Diversity estimates CR+CYTB					**Neutrality Test**		
Populations	N	P	H	π	Hd	Tajima's D	Fu's Fs
KMD	63	10	10	0.0053±0.004	0.906±0.031	0.99268	-0.552

N- Number of samples; P- polymorphic sites; H- Number of Haplotypes; Hd-Haplotypes diversity; π-Nucleotide diversity.

Network displayed both shared and unique haplotypes from lahaul valley and Uttarkashi indicating population structing. Haplotype H1 is the unique haplotype from Jammu & Kashmir, haplotypes H2 & H5 are the unique haplotypes from Lahaul, Haplotype H3 is shared between Jammu and Kashmir and Lahaul, H4 is shared between Lahaul and Uttarakhand, H6, H9 and H10 are the unique haplotypes from Uttarakhand, H7 & H8 are the unique haplotypes from Nepal **(Figure 7.1).**

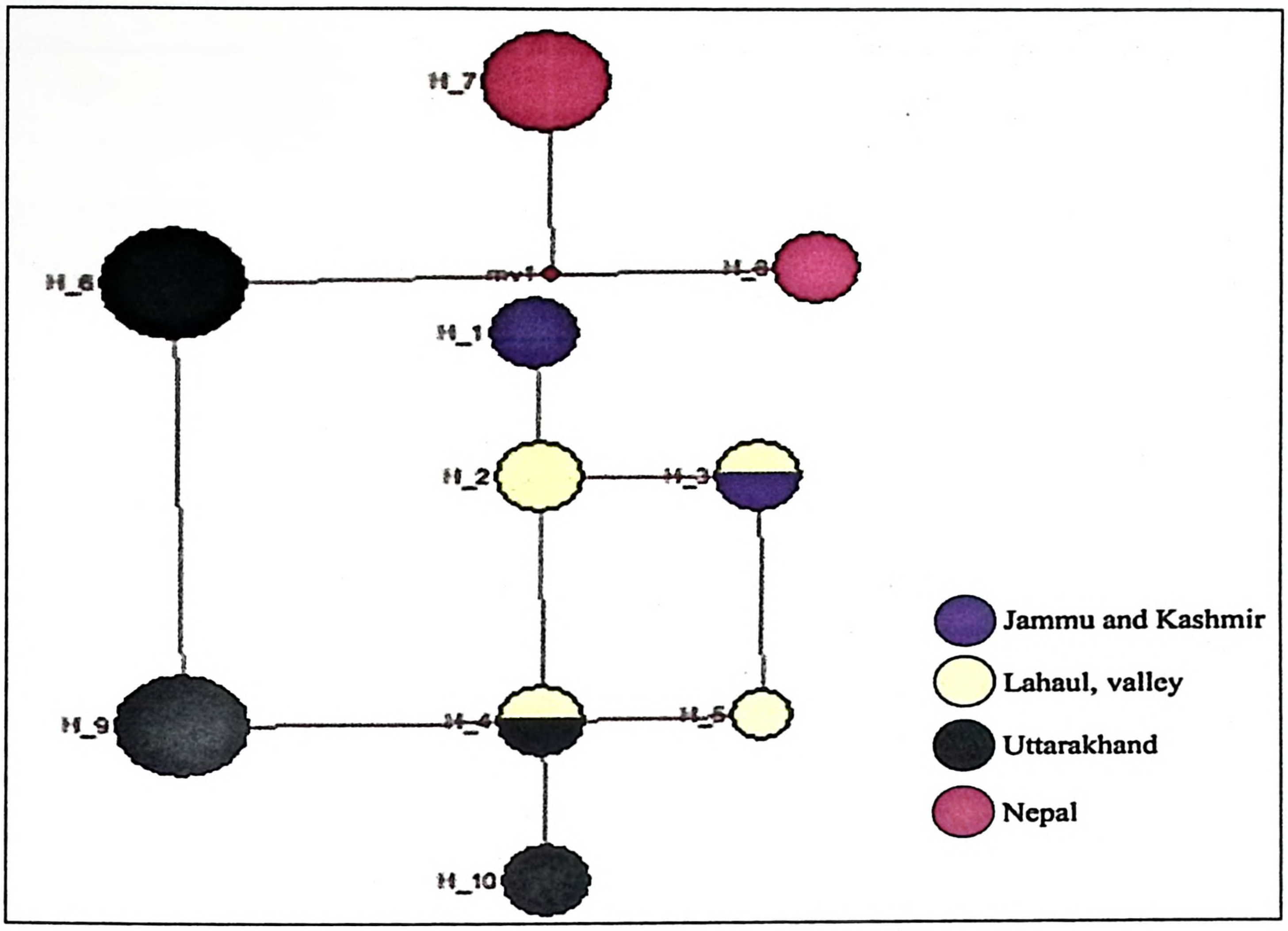

Figure 7.1. Median joining network of Kashmir musk deer (size of circles represents the relative frequency of that haplotype present in the population).

The Bayesian skyline plot revealed stable population of Kashmir musk deer during (1000-6000 years ago) and evidences a recent decline in the effective population size over the last 500 years (**Figure 7.2**). The population of Kashmir musk deer exhibited a multimodal pattern in the mismatch distribution curve, which indicated that the population is likely in demographic equilibrium (**Figure 7.2**). The estimates of neutrality tests corroborated this observation, as shown in (**Table 7.3**). Positive values of Tajima's D (0.99268) and negative value of Fu's Fs statistic tests (-0.552) (statistically not significant) suggested that historically the population of Kashmir Musk deer was demographically stable and did not undergo an expansion or population bottleneck.

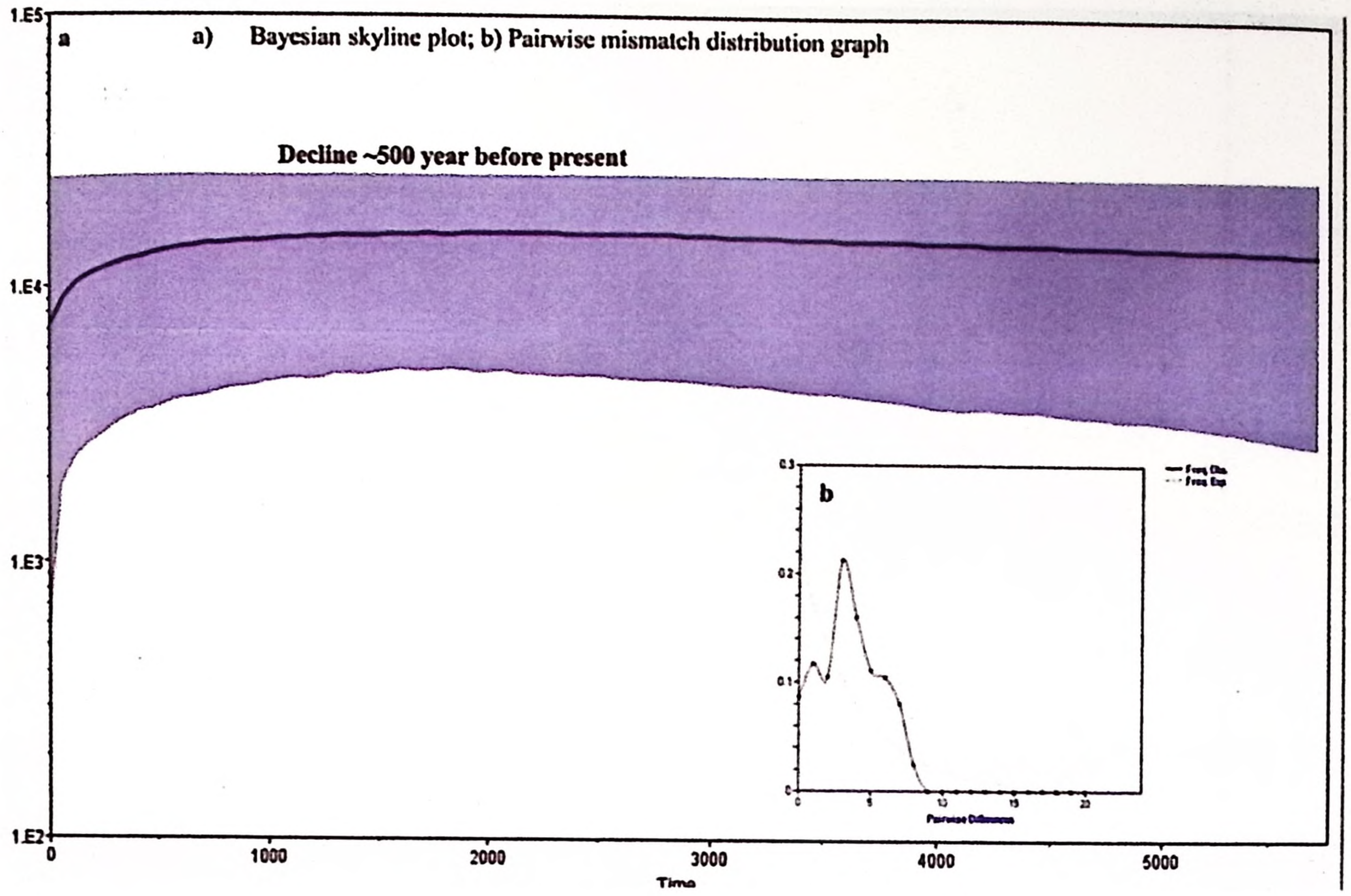

Figure 7.2. Demographic history of Kashmir musk deer estimated using Bayesian skyline plot and mismatch distribution. a) Bayesian skyline plot showing an overall stable population size during 1000-6000 years ago and a recent decline over the last 500 years. The solid line is the median estimates of Ne τ (Ne = effective population size; τ = generation time), and the grey lines around median estimates is the 95% highest posterior density (HPD) estimate of the historic effective population size; b) pairwise mismatch distribution graph.

7.3.2. Genetic diversity and Genotyping success rate

Nineteen microsatellite markers were pooled in seven multiplexes amplified with 141 genetically faecal pellets of Kashmir musk deer. No significant genotyping error was observed at all loci (Table 4). Of the 141 faecal pellets genotyped, 63 samples did yield consensus genotype with >76% amplification success with eight loci out of 19 used and used for further analysis (**Figure 7.3**).

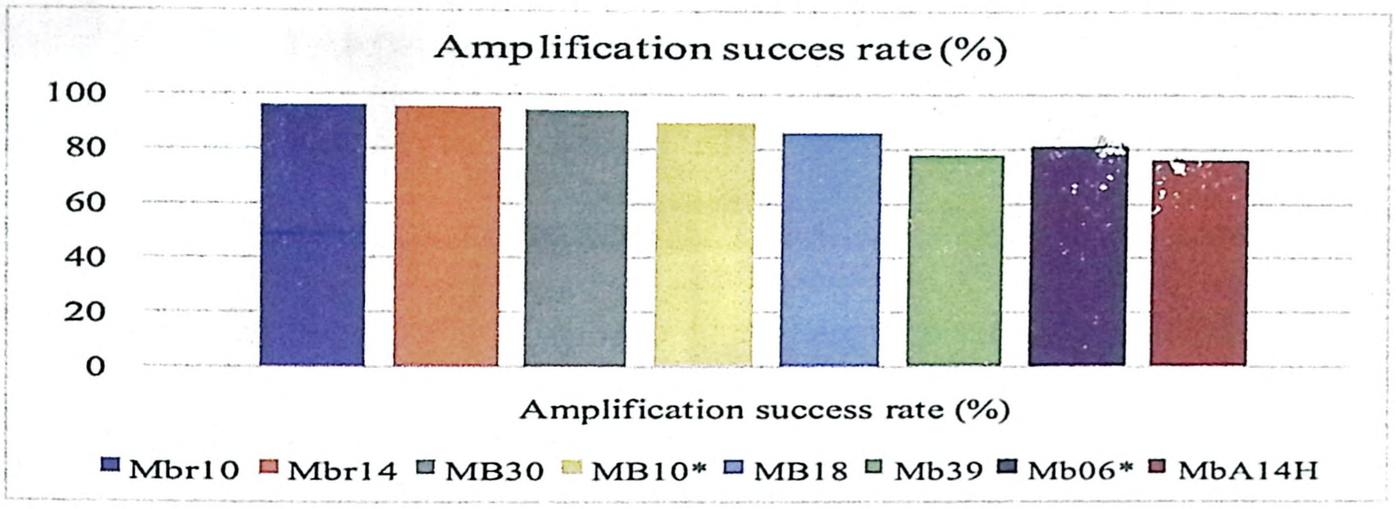

Figure 7.3. PCR amplification success rate of 9 microsatellite markers used for understanding the population genetic structure of Kashmir musk deer.

The overall mean observed number of alleles (Na) was found =12±0.491 (10-14) and mean effective number of alleles (N_e) was 5.2±0.921 (2.9-10.2) (**Table 7.4**). The range of observed number of alleles were 10-14 and effective number of alleles were 2.9-10.2. (**Table 7.4**). Data revealed the mean observed heterozygosity and mean expected heterozygosity were 0.445±0.049 and 0.764±0.039 respectively in KMD. The highest observed heterozygosity and expected heterozygosity was found in loci Mbr14 (Ho=0.623) & MB18 (He=0.902), while the least observed heterozygosity and expected heterozygosity was found in loci in Mb06(Ho=0.240) and MbA1 (He=0.577) (**Table 7.4**). Null alleles ranged from 0.11 to 0.475, with relatively high frequency in case of two loci Mb06 and Mb18.

In Lahual valley, the overall mean observed number of alleles (Na) was found =12.375±0.68 and mean effective number of alleles (Ne) was 5.204±0.809 (**Table 7.4**). The highest observed number of alleles and effective number of alleles was found in locus Mb18 (Na=14) and (Ne=8.791), while the least observed number of alleles and effective number of alleles was found in locus in MbA1 (Na=8, Ne=2.208) (**Table7.5**).

Data revealed the mean observed heterozygosity =0.45±0.046 and mean expected heterozygosity =0.769±0.041. The highest observed heterozygosity and expected heterozygosity was found in loci Mbr14 (Ho=0.646) & Mb18 (He=0.886), while the least observed heterozygosity and expected heterozygosity was found in loci in Mb06(Ho=0.297) and MbA1 with mean F_{is} value 0.404± 0.053 (He=0.547) (**Table 7.5**).

Whereas in Uttarkashi, the overall mean observed number of alleles (Na) was found =5.875±0.398 and mean effective number of alleles (Ne) was 3.466±0.310 (**Table 7.5**). The highest observed number of alleles and effective number of alleles was found in locus Mb30 (Na=8) and Mb18 (Ne=4.939), while the least observed number of alleles and effective number of alleles was found in loci in Mb06 (Na=5), Mb10 (Ne=2.600) (**Table 7.5**). Data revealed the mean observed heterozygosity was 0.409±0.062 and mean expected heterozygosity was 0.696±0.025. The highest observed heterozygosity and expected heterozygosity was found in loci Mbr14 (Ho=0.538) & Mb18 (He=0.798), while the least observed heterozygosity and expected heterozygosity was found in locus in Mb06(Ho=0.077) and (He=0.618) with mean observed F_{is} value of 0.404± 0.054 (**Table 7.5**).

Selection of microsatellite markers for individual identification

Following the criteria selected as discussed earlier, a panel of 8 loci were opted (**Figure 7.3**), based on cumulative probability of identity and the observed values for the PID siblings (P_{ID} sibs) was 4.7E-04 (4.7 mismatch in 1000 genotypes) (**Figure 7.4, Table 7.4**). The locus wise probability of matching genotypes among unrelated individuals (P_{ID}) and siblings (P_{ID} sibs) varied from 8.7E-02 to 1.3E-01 and 3.3E-01 to 5.1E-01, respectively (**Table 7.4**).

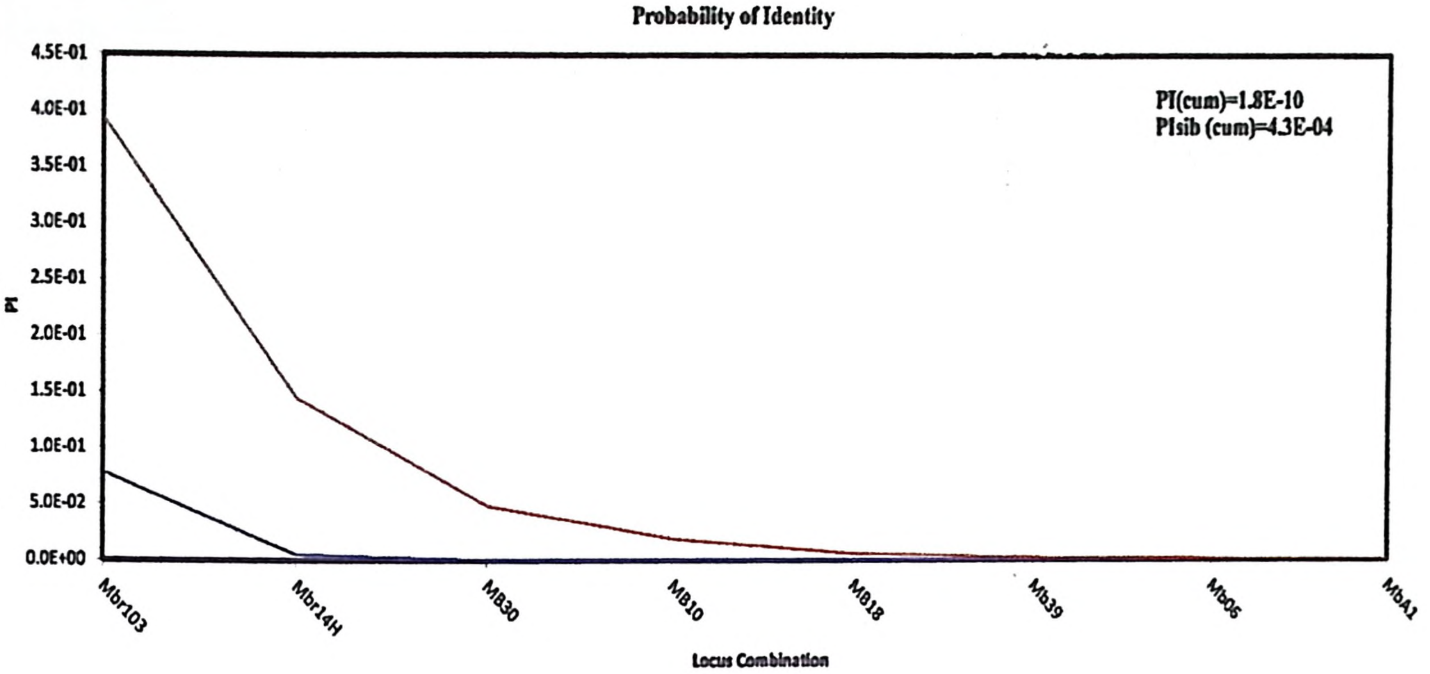

Figure 7.4. Select panel of eight polymorphic microsatellite loci used for individual identification of Kashmir musk deer.

While focusing on 8 specific genetic loci, only 2 (MB06 and MB10) showed significant deviation from Hardy-Weinberg Equilibrium (HWE) with a P-value less than 0.05, the other 6 loci followed HWE (**Table 7.4**). Furthermore, among the 63 pairwise comparisons of loci, two pairs were found to exhibit significant linkage disequilibrium with a P-value less than 0.05. The analysis of the population's genetic diversity revealed an estimated inbreeding coefficient index (FIS) of 0.41±0.057 (**Table 7.4**), indicating the presence of marginal inbreeding within the Kashmir musk deer population

Table 7.4. Genetic polymorphism of Kashmir musk deer at 8 microsatellite loci.

Locus	Amplification success rate (%)	N	Na	Ne	Ho	He	UHe	Fis (W&C)	PIC	P_{ID} (locus)	P_{ID} sibs(locus)	P_{ID} (Cum)	P_{ID} sib (Cum)	F(Null)	Genotyping error	
															ADO	FA
Mbr10	96.3	60	13	3.9	0.567	0.743	0.749	0.237	0.722	8.7E-02	4.0E-01	8.7E-02	4.0E-01	0.111	0.01	0.02
Mbr14	95.4	61	**14**	4.7	**0.623**	0.786	0.792	0.207	0.76	7.2E-02	3.8E-01	6.3E-03	1.5E-01	0.111	0.02	0.01
MB30	94.2	60	12	6.7	0.533	0.850	0.857	0.373	0.835	3.7E-02	3.3E-01	2.3E-04	5.0E-02	0.227	0.01	0
MB10*	90	56	14	3.9	0.339	0.744	0.751	0.544	0.723	8.7E-02	4.0E-01	2.0E-05	2.0E-02	0.388	0	0.01
MB18	86	51	14	**10.2**	0.353	**0.902**	0.911	0.509	0.893	1.8E-02	3.0E-01	3.6E-07	6.1E-03	0.425	0.03	0.01

Locus	Amplification success rate (%)	N	Na	Ne	Ho	He	UHe	Fis (W&C)	PIC	P_{ID} (locus)	P_{ID} sibs(locus)	P_{ID} (Cum)	P_{ID} sib (Cum)	F(Null)	Genotyping error	
															ADO	FA
Mb39	78	48	12	7.1	0.542	0.859	0.868	0.369	0.843	3.6E-02	3.3E-01	1.3E-08	2.0E-03	0.226	0.04	0
Mb06*	81	50	13	2.9	**0.240**	0.654	0.661	0.633	0.641	1.3E-01	4.6E-01	1.7E-09	9.2E-04	0.475	0.02	0.03
MbA14H	76	47	**10**	**2.4**	0.362	**0.577**	0.583	0.373	0.553	2.0E-01	5.1E-01	**3.5E-10**	**4.7E-04**	0.236	0.01	0.01
Mean			**12**	**5.2**	**0.445**	**0.764**	**0.771**	**0.418**								
SE (±)			**0.491**	**0.921**	**0.049**	**0.039**	**0.039**	**0.057**								

Na- observed number of alleles; **Ne**- effective number of alleles; **Ho**- observed heterozygosity; **He**-expected heterozygosity; UHe, PIC polymorphic information content; Fis- inbreeding coefficient index; **PID** (locus)- probability of identity (locus); **PID sibs** (locus)- probability of identity for sibs (locus); **PID (cum)**- probability of identity (cumulative); **PID sibs(cum)**- probability of identity for sibs (cumulative) **FNULL**- predicted frequencies of null alleles. ^{H}locus used for individual identification; *HWE deviation (P value <0.05).

Table 7.5. Genetic diversity indices of Kashmir musk deer in Lahual valley, Himachal Pradesh and Uttarkashi, Uttarakhand.

Pop	**Locus**	**N**	**Na**	**Ne**	**Ho**	**He**	**UHe**	**F**
Lahaul	Mbr10	48	13.000	4.081	0.583	0.755	0.763	0.227
	Mbr14H	48	14.000	4.971	**0.646**	0.799	0.807	**0.192**
	MB30	48	12.000	7.280	0.542	0.863	0.872	0.372
	MB10	43	13.000	4.376	0.326	0.771	0.781	**0.578**
	MB18	40	**14.000**	**8.791**	0.375	**0.886**	**0.897**	0.577
	Mb39	37	12.000	7.003	0.514	0.857	0.869	0.401
	Mb06	37	13.000	2.919	**0.297**	0.657	0.666	0.548
	MbA1	36	**8.000**	**2.208**	0.361	**0.547**	**0.555**	0.340
	Mean	**42.125**	**12.375**	**5.204**	**0.455**	**0.767**	**0.776**	**0.404**
	SE	**1.884**	**0.680**	**0.809**	**0.046**	**0.041**	**0.041**	**0.054**
Uttarkashi	Mbr10	12	5.000	3.097	0.500	0.677	0.707	0.262
	Mbr14H	13	5.000	3.347	**0.538**	0.701	0.729	0.232
	MB30	12	**8.000**	4.056	0.500	0.753	0.786	0.336
	MB10	13	6.000	**2.600**	0.385	0.615	**0.640**	0.375
	MB18	11	7.000	**4.939**	0.273	**0.798**	**0.835**	0.658
	Mb39	11	5.000	4.321	0.636	0.769	0.805	**0.172**
	Mb06	13	**5.000**	2.620	**0.077**	**0.618**	0.643	**0.876**
	MbA1	11	6.000	2.750	0.364	0.636	0.667	0.429
	Mean	**12.000**	**5.875**	**3.466**	**0.409**	**0.696**	**0.727**	**0.417**
	SE	**0.327**	**0.398**	**0.310**	**0.062**	**0.025**	**0.027**	**0.084**

Na- observed number of alleles; **Ne-** effective number of alleles; **Ho-** observed heterozygosity; **He-** expected heterozygosity; **Fis-** inbreeding coefficient index.

7.3.3. Population genetic structure

The highest mean log-likelihood [L(K)] and ad hoc quality (ΔK) estimate at K-3 suggesting three potential clusters of Kashmir musk deer (**Figure 7.5**). However, Bayesian structure analysis inferred majorly two clear population distinction of Lahual and Uttarkashi (**Figure 7.5**). Delta K value represents third genetic clusture of Kashmir musk deer within Lahual Valley which suggests possible admixing of individuals from the adjoining area of Kugti and Tunda WLS. The assignment percentage of individuals across 2 to 4 K values were further examined and noticed that the highest percentage of individuals (69.3%) was assigned to K3. The graphical clustering of populations and the contribution of the individuals are shown in **Figure 7.5.**

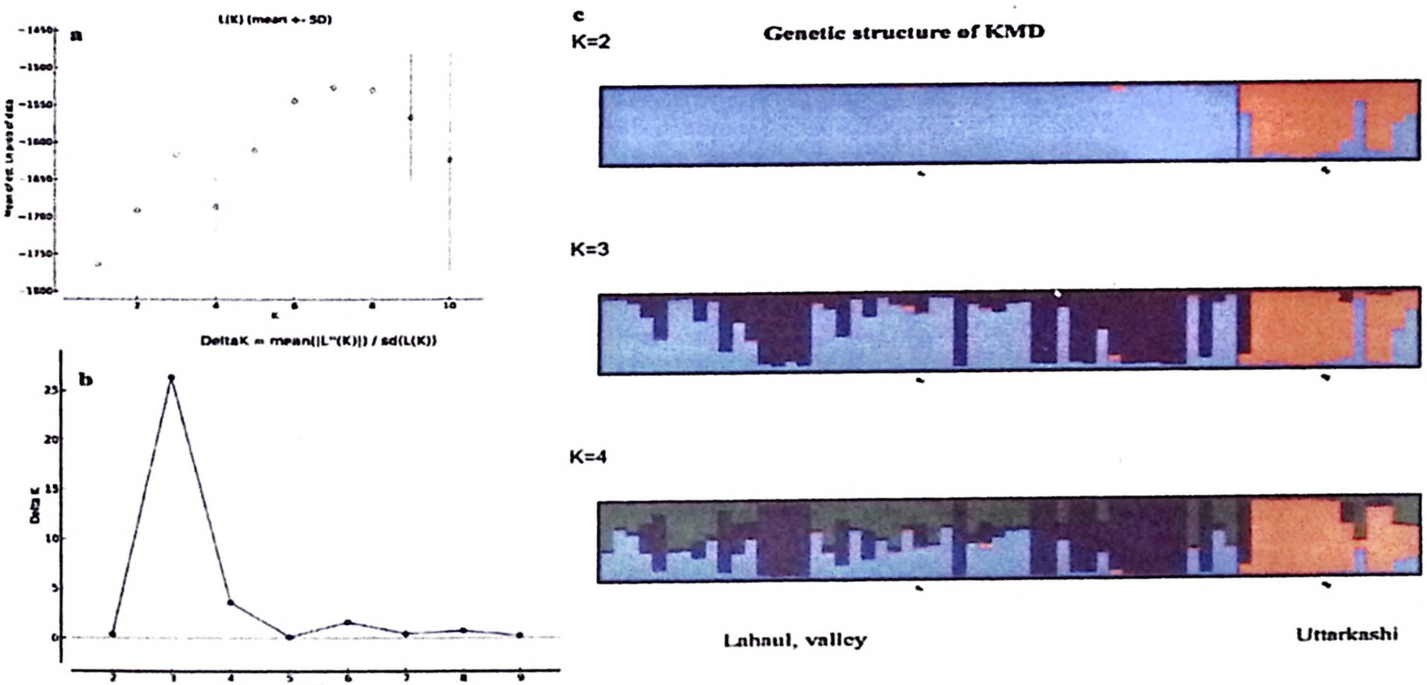

Figure 7.5. Graphical representation of the probable genetic clusters in Kashmir musk deer Population a) Mean L(K) over 10 runs for each K value, b) ad hoc quantity (delta K), c) Bayesian clustering patterns of Kashmir musk deer.

Table 7.6. Assignment of populations through Bayesian clustering analysis of Kashmir musk deer population at K=3

Population	**Cluster 1(%)**	**Cluster 2(%)**	**Cluster 3(%)**	**Assigned individuals (%)**	**Unassigned individuals (%)**
LS (49)	44.3 (16)	0.9 (0)	54.8 (18)	69.3(34)	30.7(15)
UK (14)	0.091 (1)	0.725 (8)	0.184 (0)	64.2(09)	35.8(05)

7.4. Discussion

Population genetics is a fascinating field of study that explores the genetic variation and evolutionary processes within populations. It provides invaluable insights into the dynamics of heritable traits, the mechanisms of evolution, and the interactions between populations over time. In this discussion, I delve into the key findings and implications of population genetics analysis, shedding light on the genetic structure, diversity, and demographic history of the Kashmir musk deer in North-western Himalayas. By examining various genetic parameters, such as nucleotide and haplotype diversity, neutrality test, allele frequencies, heterozygosity, and population differentiation, I aim to decipher the forces shaping the genetic diversity in a landscape and uncover potential factors influencing the observed patterns. Additionally, I discuss the relevance of these findings in the broader context of evolutionary biology and conservation efforts.

The genetic diversity estimates of the Kashmir musk deer (Ho-0.44; He-0.76) were found to be comparable (Ho-0.45-0.87, He-0.62-0.84) to forest musk deer (*Moschus berezovskii*) and other similar ungulate species, such as the Siberian ibex (*Capra sibrica*), Himaliyan tahr (*Hemitragus jemlahicus*), Hangul (*Cervus hanglu hanglu*), and blue sheep (*Pseudois nayaur*) (Zhao et al., 2008, Mukesh et al., 2015, Yang et al 2015, Singh et al., 2023, Jabin et al., 2023). The low nucleotide diversity (0.00053± 0.004) and high haplotype diversity (0.906± 0.031) (**Table 7.3**) suggests that the genetic variation in the population is due to differences between haplotypes, rather than differences at the individual nucleotide level.

Network displayed both shared and unique haplotypes from Lahaul valley and Uttarkashi indicating population structing. Haplotype H1, being unique to Jammu & Kashmir, indicates the presence of a distinct genetic signature in this region, possibly resulting from historical isolation or limited gene flow with neighbouring populations. On the other hand, the occurrence of unique haplotypes H2 and H5 in Lahaul Valley points to the presence of genetic variation unique to this specific region. These unique haplotypes may have been shaped by local environmental factors and population history. The shared haplotype H3 between Jammu & Kashmir and Lahaul Valley suggests some degree of gene flow or historical connectivity between these two regions. Genetic exchange could have occurred through movements of individuals or through historical connections between these populations. Similarly, haplotype H4, shared between Lahaul Valley and Uttarkhand, indicates possible gene flow and connectivity between these two areas. The unique haplotypes H6, H9, and H10 observed in Uttarakhand reveal the presence of genetic diversity specific to this region **(Figure 7.1)**. The possible reason might be due to local adaptations to the unique ecological conditions found in Uttarakhand, which could have driven the evolution of distinct haplotypes in the population.

Furthermore, the unique haplotypes H7 and H8 detected in Nepal indicate a separate genetic lineage in this region. Nepal's geographical features, such as high mountain ranges, may have contributed to genetic isolation and the evolution of distinct haplotypes in the Kashmir musk deer population there.

The observed population structuring, and the presence of unique haplotypes indicate the influence of various factors shaping the genetic landscape of Kashmir musk deer populations. Geographical barriers, environmental differences, and historical isolation may have contributed to the genetic divergence observed between different regions. Additionally, factors such as habitat fragmentation and human activities may have impacted the gene flow and genetic connectivity among populations. The population structuring observed in this study underscores the importance of considering local conservation efforts and the need to preserve genetic diversity within distinct subpopulations.

The results of the Bayesian skyline plots and mismatch distribution curves provide valuable insights into the historical demographic trends and population dynamics of the Kashmir musk deer (*Moschus cupreus*). The Bayesian skyline plot indicates a stable population of Kashmir musk deer from approximately 1000 to 6000 years ago (**Figure 7.2**). However, the plot also reveals a recent decline in the effective population size over the last 500 years. The multimodal pattern observed in the mismatch distribution curve (**Figure 7.2**) further supports the notion of demographic equilibrium, suggesting that the population is maintaining a relatively stable size without any major population expansions or contractions. The estimates of neutrality tests, such as Tajima's D and Fu's Fs, provide additional evidence of demographic stability, as positive Tajima's D and negative Fu's Fs values (though not statistically significant) suggest a lack of significant population expansion or bottleneck events.

The microsatellite analysis of the Kashmir musk deer populations from Lahual Valley and Uttarkashi provides valuable insights into the genetic diversity and population structure of this iconic species in the North-western Himalayas. The use of 19 microsatellite markers in 7 multiplexes allowed to screen and select 8 loci with high success (>70%) and which further used for a comprehensive assessment of genetic variation within and between populations. The overall mean observed number of alleles (Na) and mean effective number of alleles (Ne) were found to be 12 and 5.2, respectively (**Table 7.4.**). These values indicate a relatively high level of allelic diversity in the Kashmir musk deer populations, which is consistent (6-18) with other ungulate studies (Zou et al., 2005; Zhao et al. 2008; Jabin et al., 2023). Comparable levels of genetic diversity have been observed in other similar ungulates such as the Nubian ibex (*Capra nubiana*), Himalayan tahr (*Hemitragus jemlahicus*), Hangul (*Cervus hanglu hanglu*), and blue sheep (*Pseudois nayaur*) (Zhao et al., 2008; Thakur et al., 2015; Singh et al., 2023). This suggests that the Kashmir musk deer shares similar levels of genetic diversity with other ungulate species in the region.

The presence of highly polymorphic loci, such as Mbr14 and Mb18, which showed the highest number of observed and effective alleles, indicates that these markers have a higher capacity to capture genetic variation within the populations. This finding aligns with previous studies on musk deer (Zou et al.,2005; Zhao et al., 2008), where these microsatellite markers have been identified as highly informative and valuable for population genetic analyses.

The mean observed heterozygosity (Ho) and mean expected heterozygosity (He) values provide insights into the genetic diversity within populations. The moderate levels of genetic diversity observed in the Kashmir musk deer populations (Ho=0.445 and He=0.764) suggest that these populations have retained a considerable amount of genetic variation (**Table 7.4**). The findings are consistent with Zhao et al., 2008 and are comparable to those reported in other ungulate studies (Thakur et al., 2015; Singh et al.,2022; 2023, Jabin et al., 2023), indicating that the Kashmir musk deer exhibits similar levels of heterozygosity to other related species.

The F_{IS} values provide insights into the level of inbreeding within populations. The moderate mean F_{IS} value of 0.404 suggests some degree of inbreeding in the Kashmir musk deer populations. This finding aligns with other ungulate studies, where inbreeding has been observed in various populations (Singh et al., 2022; Jabin et al., 2023).

Comparing the genetic diversity estimates among the populations of Lahual Valley, and Uttarkashi), Lahual Valley exhibited slightly higher genetic diversity compared to Uttarkashi. This difference could be attributed to differences in available habitat size & type, landscape features, human disturbance, poaching pressure, and historical population dynamics between the two regions (Sharief et al., 2022, 2023). Larger population sizes or gene flow from neighboring areas (Kugti, Tunda WLS) may have contributed to the higher genetic diversity observed in Lahual Valley.

The Bayesian structure analysis reveal important insights into the population structure and genetic clustering of the Kashmir musk deer populations in Lahual and Uttarkashi regions. The analysis identified two clear population distinctions between Lahual and Uttarkashi, indicating some level of genetic differentiation between these two regions. This finding is consistent with the geographical separation and limited gene flow between populations residing in different regions.

However, the presence of three potential genetic clusters of Kashmir musk deer is also suggested by the highest mean log-likelihood (L[K]) and ad hoc quality (ΔK) estimates at K=3 **(Figure 7.5)**. The third cluster is identified within the Lahual Valley, indicating possible admixture of individuals from the adjoining area of Kugti and Tunda Wildlife Sanctuary (WLS). This result suggests that individuals from these nearby areas are contributing to the genetic composition of the Kashmir musk deer population in Lahual Valley, leading to a more complex population structure in this region.

The assignment percentage of individuals across different K values provides additional evidence for the presence of substructure and migration in the population. The highest percentage of individuals (69.3%) assigned to K=3 further supports the existence of the third genetic cluster within Lahual Valley **(Table 7.6)**. As the number of K values increases, indications of hidden substructure emerge, which can be attributed to the presence of migrant individuals from neighboring areas. These migrant individuals can introduce genetic variation from other populations, contributing to the observed substructure in the Bayesian structure analysis.

The graphical clustering of populations and the contribution of individuals depicted in **(Figure 7.5)** visually represent the genetic clustering and admixture patterns within the Kashmir musk deer populations. This graphical representation further supports the results obtained from the Bayesian analysis and highlights the presence of distinct genetic clusters and patterns of gene flow within the study area.

Overall, the population structure analysis indicates that the Kashmir musk deer populations in Lahaul and Uttarkashi regions are genetically distinct, but there is also evidence of genetic admixture within Lahaul Valley. This finding is valuable for understanding the population dynamics, migration patterns, and gene flow of this endangered species in the North-western Himalayas. The presence of substructure and migration in the population has important implications for conservation efforts. The identified genetic clusters and migration patterns can influence the exchange of genetic material between populations and impact the overall genetic diversity of the species. Conservation strategies should consider these patterns of population structure to ensure effective management and preservation of the genetic variation in the Kashmir musk deer populations. Additionally, further investigation into the specific factors driving genetic admixture and migration within Lahaul Valley can provide valuable insights into the ecology and behavior of this enigmatic species, aiding conservation efforts and promoting its long-term survival.

Through this exploration of population genetics, a deeper understanding of the intricate mechanisms that govern the genetic makeup of Kashmir musk deer populations has been gained. Since Kashmir musk deer is listed as an endangered species according to IUCN, understanding the genetic diversity and population structure can aid in formulating effective conservation strategies to preserve the unique genetic heritage of this iconic species. The population structuring observed in this study underscores the importance of considering local conservation efforts and the need to preserve genetic diversity within distinct subpopulations. Maintaining connectivity between populations and implementing measures to protect corridors for gene flow is crucial for ensuring the long-term survival and adaptability of the Kashmir musk deer. The presence of substructure and migration in the population has important implications for conservation efforts. The identified genetic clusters and migration patterns can influence the exchange of genetic material between populations and impact the overall genetic diversity of the species. Conservation strategies should consider these patterns of population structure to ensure effective management and preservation of the genetic variation in the Kashmir musk deer populations.

Over the past few decades, the Himalayan regions have undergone significant transformations due to rapid infrastructure development, climate change, and human population expansion (Chandra et al., 2020; Sharief et al., 2023). These changes have resulted in substantial habitat loss and fragmentation for numerous key ungulate species. The impacts of these environmental shifts on wildlife populations, including the endangered Kashmir musk deer, are a matter of growing concern. As these pressures continue to exert their effects, understanding the genetic structure and population dynamics of the Kashmir musk deer becomes essential for effective conservation strategies. Identifying how these factors influence the species' genetic diversity and gene flow can aid in developing targeted conservation measures to safeguard this unique and vulnerable species in the face of ongoing environmental challenges.

7.5. Conclusion

In conclusion, population genetics has provided valuable insights into the genetic diversity, structure, and demographic history of the endangered Kashmir musk deer in the North-western Himalayas. The analysis revealed moderate genetic diversity in the population, with unique and shared haplotypes specific to different regions. Geographical barriers, environmental differences, and historical isolation have contributed to the genetic divergence observed between populations. Furthermore, the presence of three potential genetic clusters within Lahaul Valley suggests genetic admixture from neighboring areas (Kugti and Tunda WLS). The Bayesian skyline plot and mismatch distribution curve provided evidence of stable population sizes over time, with recent declines in effective population size observed in some genomic regions. The microsatellite analysis highlighted moderate genetic diversity within populations, indicating the importance of preserving local genetic variation. The presence of substructure and migration in the population has implications for conservation strategies, emphasizing the need to consider connectivity and corridors for gene flow to ensure the long-term survival and adaptability of the species.

Given the endangered status of the Kashmir musk deer and the growing threats from habitat loss and fragmentation, understanding its population genetics is crucial for developing effective conservation plans. The identified genetic clusters and patterns of gene flow can guide management efforts to preserve genetic diversity and promote resilience in the face of changing environmental conditions. Additionally, insights into the historical demographic trends provide a better understanding of the species' evolutionary history and can help predict its future prospects.

This study underscores the importance of considering genetic factors when formulating conservation strategies for endangered species. By incorporating population genetics into conservation efforts, we can better safeguard the genetic heritage of the Kashmir musk deer and ensure its survival for future generations. As human activities continue to impact wildlife habitats, continued research in population genetics will play an ever more significant role in understanding and preserving the genetic diversity of endangered species in our changing world.

7.6. References

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8. Academic progress

Research paper

SN	Title of Paper	Journal	Status
1	Estimating occupancy and abundance of endangered Kashmir musk deer (*Moschus cupreus*) in Uttarkashi, Uttarakhand	Indian journal of Ecology	Published
2	Environmental predictors may change at fine scale habitat modelling: Implications for conservation of Kashmir musk deer in three protected areas of Uttarakhand, India.	Environmental science and pollution research	Published
3	Future simulation predicts better tomorrow for Kashmir musk deer in western Himalayas	Frontiers in ecology and evolution	Published
4	Empirical data suggests that Kashmir musk deer is the one Musk deer distributed in Western Himalayas: An integration of ecology, Genetics and Geospatial Modelling Approaches	Biology	Published

- **PhD work presented in international conferences**

SN	**Title of Talk**	**Conference organizer**	**Date**
1	Taxonomic delineation of musk deer species in western Himalayas, India	Young systematic forum 2022	11, November, 2022
2	Future simulation predicts better tomorrow for Kashmir musk deer in western Himalayas	British ecological society, 2022	18-21, December, 2022
3	One or three: Empirical data suggests only one species of musk deer in Western Himalayas.	Biology23	16-17 February, 2023

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9. Publications

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Estimating Occupancy and Abundance of Endangered Kashmir Musk Deer (*Moschus cupreus*) in Uttarkashi, Uttarakhand.

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Abstract: The present study aimed to assess the occupancy and abundance of musk deer in Uttarkashi using camera traps from October 2018 to October 2019. Musk deer was detected in 9 out of 24 grids yielding a naïve occupancy estimate of 0.29. By using the null model, the estimates of the site occupancy was 0.39 and detection probability was 0.19. The overall abundance of musk deer was 28.40 in the study area, with an average density of 4.73/100 km^2. This study is the first attempt to estimate occupancy and abundance using camera traps, providing baseline information for future management and conservation strategies within the landscape.

Keywords: Occupancy, Elusive, Camera trap, Habitat degradation, Threatened

Mammals which occur in low densities at high altitudes, are globally threatened due to habitat loss and anthropogenic disturbances (Woodroffe 2000, Sharief et al 2020). Monitoring the population of these mammals is pivotal for their long-term viability and is essential from both ecological and management perspectives. For effective conservation and management planning, especially for those species which are under increasing threat or on the verge of extinction. It is imperative to identify their habitats and estimate their abundance. Among these species, musk deer (*Moschus* spp.) is a globally threatened species and needs top priority conservation action (Singh et al 2020). Recent studies have highlighted that Kashmir musk deer (*Moschus cupreus*, hereafter KMD) is coverings parts of Afghanistan, Pakistan, India, and Nepal (Singh et al 2020). The species is distributed in continuous to fragmented patches of forested and alpine scrub habitats in the western Himalayas, which is experiencing a rapidly changing climate (Syed and Ilyas 2016, Singh et al 2020). Worldwide, the population of musk deer have dramatically dwindled to half of the original size in three generations (approximately 21 years) primarily because of poaching and habitat degradation (Green 1986, Homes 2004, Timmins & Duckworth 2015). Therefore, musk deer have been categorized as endangered (Timmins and Duckworth 2015) in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2015). In addition to poaching, the other prevailing threats to musk deer are habitat destruction and degradation (Yang et al 2003, Ilyas 2014). Habitats in the Himalayas are threatened by anthropogenic pressures such as intensive livestock grazing, fuelwood cutting, and fodder collection (Vinod & Sathyakumar 1999, Ilyas 2014, Sathyakumar 2015). The suitable habitat for musk deer is mainly confined to protected areas with fragmented habitats and therefore demands the urgent need for protection and conservation efforts. Information is documented on various aspects of the ecology of musk deer (Green 1986, Sathyakumar 1994, Vinod & Sathyakumar 1999, Syed 2014, Ilyas 2014, Sathyakumar & Rawat, 2015, Singh et al 2018, Wangdi et al 2019). However conservation and management planning need information on species' different life history traits, especially population estimation. Hence the present study assessed the occupancy and abundance of KMD in Uttarkashi using camera traps. It has been documented that camera trapping is particularly useful by allowing population densities of the species to be estimated when the identification of individuals is possible (Singh et al 2014b). In some mammals, including musk deer, individuals cannot be identified positively because of the lack of a distinct spot or stripe pattern; however, abundance estimates can be made with occupancy surveys that rely on a species being detected, or not, at a particular site (MacKenzie et al 2002). Occupancy yields unbiased maximum likelihood estimates of numerous variables relevant to a wide variety of conservation and management applications research. (Mackenzie et al 2006, Singh et al 2015). The objective was to estimate the site occupancy and abundance of KMD in Uttarkashi district using remotely triggered cameras and to provide information

biology

Article

Empirical Data Suggest That the Kashmir Musk Deer (*Moschus cupreus*, Grubb 1982) Is the One Musk Deer Distributed in the Western Himalayas: An Integration of Ecology, Genetics and Geospatial Modelling Approaches

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Simple Summary: Resolving the taxonomic ambiguity of a species is important for their long-term conservation. We used non-invasive DNA sampling and camera trapping to address the taxonomic ambiguity in musk deer species in the Western Himalayas, and species occurrence locations were used to model the species distribution to identify suitable habitats. The combined results confirm the presence of only Kashmir musk deer (KMD) in the Western Himalayas. During our survey, no other musk deer species were found in the study area. This finding suggests that the presence of other species, such as Himalayan musk deer and Alpine musk deer, were incorrectly reported in the past. The predicted distribution (6% of the total area) of Kashmir musk deer is a narrow belt of the Western Himalayas between 2500 and 4500 m. Based on this study, we recommend long-term monitoring and assessment of KMD throughout its distribution range for its conservation and management.

Abstract: Insufficient research has been conducted on musk deer species across their distribution range, primarily because of their elusive behaviour and the fact they occupy remote high-altitude habitats in the Himalayas above 2500 m. The available distribution records, primarily derived from ecological studies with limited photographic and indirect evidence, fail to provide comprehensive information on the species distribution. Consequently, uncertainties arise when attempting to determine the presence of specific taxonomic units of musk deer in the Western Himalayas. This lack of knowledge hampers species-oriented conservation efforts, as there need to be more species-specific initiatives focused on monitoring, protecting, and combatting the illegal poaching of musk deer for their valuable musk pods. We used transect surveys (220 trails), camera traps (255 cameras), non-invasive DNA sampling (40 samples), and geospatial modelling (279 occurrence records) to resolve the taxonomic ambiguity, and identify the suitable habitat of musk deer (*Moschus* spp.) in Uttarkashi District of Uttarakhand and the Lahaul–Pangi landscape of Himachal Pradesh. All the captured images and DNA-based identification results confirmed the presence of only Kashmir musk deer (KDM) (*Moschus cupreus*) in Uttarakhand and Himachal Pradesh. The results suggest that KMD inhabit a narrow range of suitable habitats (6.9%) of the entire Western Himalayas. Since all evidence indicates that only KMD are present in the Western Himalayas, we suggest that the presence of other species of musk deer (Alpine musk deer and Himalayan musk deer) was wrongly reported. Therefore, future conservation plans and management strategies must focus only on KMD in the Western Himalayas.

Keywords: Kashmir musk deer; Western Himalayas; endangered; DNA; camera trapping; species distribution modelling

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RESEARCH ARTICLE

Environmental predictors may change at fine scale habitat suitability modelling: implications for conservation of Kashmir musk deer in three protected areas of Uttarakhand, India

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Abstract

The Kashmir musk deer (*Moschus cupreus*, hereafter KMD) is one of the top conservation priority species which is facing population decline due to poaching, habitat loss, and climate change. Therefore, the long-term survival and viability of KMD populations in their natural habitat require conservation and management of suitable habitats. Hence, the present study attempted to assess the suitable habitat of KMD in three protected areas (PAs) of the Western Himalayan region of Uttarakhand using the Maxent modelling algorithm. Our results suggest that Kedarnath wildlife sanctuary (KWLS) possesses the maximum highly suitable habitats (22.55%) of KMD, followed by Govind Pashu Vihar National Park & Sanctuary (GPVNP&S; 8.33%) and Gangotri National Park (GNP; 5%). Among the environmental variables, altitude was the major contributing factor governing the distribution of KMD in KWLS. In contrast, human footprint in GPVNP&S and precipitation in GNP were the major contributing factors governing the distribution of KMD in these respective PAs. The response curve indicated that habitats with less disturbance falling in the altitudinal zone of 2000–4000 m were the most suitable habitat range for the distribution of KMD in all three PAs. However, in the case of GNP suitable habitat of KMD increases with an increase in the value of variables bio_13 (precipitation of wettest month). Further, based on our results, we believe that the predictors of suitable habitat change are site specific and cannot be generalized in the entire distribution range of the species. Therefore, the present study will be helpful in making proper habitat management actions at fine scale for the conservation of KMD.

Keywords Kashmir musk deer · Environmental predictors · Uttarkashi · Suitable habitat · Spatial scale

Introduction

Globally, habitat loss, fragmentation, and degradation are the key threats to species decline and, therefore, elucidating species-habitat relationships becomes imperative in making informed actions for the effective conservation planning (levin 1992; Marzluff and Sallabanks 1998; Lamsal et al. 2018). The spatial extent (broad or fine scale) to which the ecological analysis is being carried out can immensely affect the inferred species–habitat relationships (levin 1992; Holland et al. 2004; de Knegt et al. 2010; Jackson and Fahrig 2015). Further, environmental predictors play a vital role in determining the distribution of species (Abolmaali et al. 2018; Ahmad et al. 2021). Hence, explicit attention to identifying environmental predictors is critical for understanding the habitat suitability patterns of species at a fine or broad scale. Thus, determining which particular environmental predictor influences habitat selection of a species is essential for developing effective conservation and management strategies. The Himalayan ecosystem is home to a diverse range of flora (~ 16,000) and fauna (~ 30,000), including some of the top conservation priority species (Aryal et al. 2014; Chandra et al. 2018; Joshi et al. 2019). In the Himalayan ecosystem,

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Towards effective conservation planning: integrating landscape modelling to safeguard the future of the endangered Kashmir musk deer in the face of land use change

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Human expansion and anthropogenic activities are causing the conversion of forests to other land uses in the Himalayas, which is threatening species with extinction. To address this issue, we used an ensemble model to simulate the future landscape and assess its impact on the Kashmir Musk Deer (KMD) distribution in the context of land use change. Our simulation suggests a decline in croplands and shrublands and increase of mixed forests in the future scenario. Evergreen broad-leaf and needle-leaf forests are likely to convert to mixed forests, while croplands and barren areas transform into savannas. Precipitation, elevation, and mixed forests were found to be the most significant factors influencing KMD distribution. Only 20,690 km^2 out of the total area of 324,666 km^2 is currently suitable for KMD, but this is projected to increase to 22,701.47 km^2 in the future. We predict a habitat gain of about 2,722 km^2 in new areas and a loss of 711 km^2 in existing habitats for KMD by 2030, with Uttarakhand state losing much of the suitable habitat. However, new habitats in future will become available for the species in Jammu and Kashmir. Our landscape configuration investigation indicates a decline in the number of patches and aggregation index in the future scenario. Most of the suitable KMD habitats are outside the current protected areas (PA), making the current PA network insufficient for long-term conservation. Therefore, we suggest forest managers to rationalize the boundary of the PAs to include suitable habitats that are currently not protected for the long-term survival of the KMD.

KEYWORDS
land use simulation, habitat suitability, fragmentation, Kashmir musk deer, Western Himalayas

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