ASSESSMENT OF AFRICAN LEOPARD AND SPOTTED HYENA DIET AND PREY BASE ALONG BURGURET TRAIL IN MT. KENYA FOREST, KENYA

BY

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A Thesis Submitted in Partial Fulfillment of the requirements for the Award of the Degree of Master of Science (Biology of Conservation) in the School of Biological Sciences

UNIVERSITY OF NAIROBI

DECEMBER, 2018

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for academic credit.

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DEDICATION

This thesis is dedicated to my dear parents Elosy Mukwanjeru and late Hyacynth Gitari. I in a special way dedicate it to my Uncle Nicholas Mugambi, Aunt Idah Kanini and my siblings. I am eternally indebted to you for your encouragement and support. I also dedicate it to my husband Godfrey Kimathi, our son Ethan and daughter Molly for their prayers, love, unlimited support and understanding during the study period. I couldn't have done this without you. Thank you all for your support along the way.

ACKNOWLEDGEMENT

I thank the almighty God for taking me this far in my education. This work is a product of cooperation, guidance and support of many mentors, institutions and individuals. I am particularly indebted to my immediate advisors and supervisors, Prof. Nathan Gichuki (University of Nairobi), Dr. Robert Chira (University of Nairobi), and Dr. Molly McDonough (Assistant Professor of Biology, Chicago State University) for their guidance and assistance towards accomplishment of this research work. I also in a special way acknowledge Smithsonian Institution for funding my studies, facilitating my travel to the US to present this research work at the University of Idaho during an American Society of Mammalogists Annual Meeting in 2017, facilitating molecular analysis of samples at Bioscieces Eastern and Central Africa (BecA-ILRI) lab, and additional support through their on-going Smithsonian Roosevelt Resurvey Project in Mt Kenya that provided me with the required field work equipment and assistance. Special thanks to Mr. Bernard Agwanda (Research Scientist, National Museums of Kenya) for fund raising for the study and great assistance during the field work and laboratory work. I thank the American Society of Mammalogists for their award of African Graduate Student in Aid of Research Fund 2017 that aided data processing. Thanks to National Museums of Kenya for providing reference materials and offering their laboratory for sample analysis. Thanks to Kenya Wildlife Service and Kenya Forest Service for granting access to the study area more so to their rangers who provided security during data collection. I appreciate the various researchers who took the time to share their experiences with an insight into the research and focused assistance. For field assistance, am grateful to porters of Naro Moru and all members of the Smithsonian Roosevelt resurvey field party. For laboratory assistance, I thank Nancy McInerny and the team at the BecA-ILRI lab (International Livestock Research Institute), thanks to Mrs. Veronica Joash and Dr. Ogeto Mwebi (Osteology section-National Museums of Kenya) for their assistance in scats bone remains identification. For building the hair reference collection, I acknowledge Griphin Ochieng's (National Museums of Kenya) assistance. Special acknowledgements goes to Prof. Roland Kays (North Carolina State University) for his assistance in study area GIS mapping, guidance in camera trapping technology and its statistical analysis. I appreciate Dr. Rene Domain for plants species identification not forgetting Dr. Adam Ferguson's subsistence assistance while working. I am deeply thankful to the spirit of comradeship that I received from other members of the University of Nairobi too.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ACRONYMS AND SYMBOLS

^{0}C _ Degree Celsius

ACCNNR – African Convention on the Conservation of Nature and Natural Resources

CBD – Convention on Biological Diversity

CDV _ Canine Distemper Virus

CITIES – Convention on International Trade in Endangered Species

GEF – Global Environment Facility

GPS – Global Positioning System

Ha _ Hectares

IPCC _ Intergovernmental Panel on Climate Change

IPPC – International Plant Protection Convention

IUCN – International Union for Conservation of Nature

KARI – Kenya Agricultural Research Institute

KEPHIS _ Kenya Plant Health Inspectorate Service

KFS – Kenya Forest Service

KWS _ Kenya Wildlife Service

NEMA – National Environment Management Authority

NMK _ National Museums of Kenya

UNEP – United Nations Environment Programme (UNEA)

WHS – World Heritage Site

ABSTRACT

Mount Kenya has known populations of both African leopard and spotted hyena. However, based on habitat alone; one would expect the prey base available on the mountain to differ from savannah systems where the two apex predators are most commonly found. Considering climate change impacts, such as decline in prey base, or range shift of species, then leopards and hyenas may also shift to different areas within the mountain or may not continue to inhabit the mountain. Therefore, this study sought to assess the diversity of leopard and hyena diet and available prey base, along Burguret trail of Mt. Kenya. It also investigated the differences in the diversity of leopard versus hyena prey species, variation in diet of both leopard and hyena across various habitat types, and determine dietary overlaps between the two carnivores across habitat types. Systematic sampling design was used to lay the sampling points across altitudinal gradient at 100 m intervals while establishing a 20 by 20 m plot on each sampling point where thorough search and collection of the scats was done. Leopard and hyena prey base was documented using 50 camera traps (Reconnyx game trail camera) installed at 300 m intervals along the same transect and during the same timeframe as the scat collection. A total of 28 mammal species were documented in the study area within the different vegetation zones. The leopard diet did not vary significantly with the habitat type ($F = 1.508$; $df = 5$; $P = 0.49$). A paired t-test to examine differences in consumption rates of different prey consumed by both leopard and spotted hyena within the various habitat types confirmed that there was significant difference in the consumption of Mt. Kenya rock hyrax ($t_{0.05,2,8} = 5.7$, $P = 0.009 < 0.05$), tropical vlei rat ($t_{0.05,2,8} = 1.89$, $P = 0.04 < 0.05$), Suni ($t_{0.05,2,8} = 2.06$, $P = 0.006 < 0.05$) and bush pig ($t_{0.05,2,8} = 1.5$, $P = 0.03 < 0.05$). However the two predators had a dietary niche overlap index of 0.19. DNA metabarcoding was crucial in classification of predator scat samples. DNA metabarcoding results revealed that *Crocuta crocuta* scat samples contained a majority of Suiidae sequences (42.3%), followed by Cercopithecidae (25.6%), Bovidae (15.5%), Soricidae (8.6%), Procaviidae (4.8%), Muridae (2.4%), Lorisidae (0.5%), and Equiidae (0.2%).Within the leopard scat samples, the highest proportion of reads were *Dendrohyrax* (32.2%), followed by *Colobus* (23.0%), *Myosorex* (14.9%), *Procavia* (14.0%), *Syncerus* (10.6%), Murid rodent (3.0%), *Potamochoerus* (0.5%), and *Cercopithicus* (0.5%). There were also a few reads detected for *Tragelaphus*, *Equus*, *Phacochoerus*, *Crocidura*, *Neotraous*, *Loxodonta*, *Cephalophus*, *Ovis*, and *Orycteropus.* The leopards utilizing the various vegetation zones within the study area confirms their plasticity of feeding behavior that allows them to occupy structurally diverse home ranges. Hyenas feeding on genets is an indicator that top predators have impacts on diverse populations of small carnivores whenever they coexist. Application of good conservation strategies towards the apex carnivores, can promote conservation of other species within an area, and diet results assist in prediction of diet in other mountain areas where dietary information is lacking as well as assist wildlife managers understand predator-prey interactions.

Key words: *Panthera pardus pardus, Crocuta crocuta*, fecal hair microscopy, scats, prey, vegetation zones, DNA metabarcoding

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

Carnivores, especially the 245 terrestrial species are declining worldwide (Hunter, 2011). This event can be attributed to hunting and direct persecution due to conflict with humans, climate change, depletion of prey as well as loss and alteration of habitats and shrinking geographical ranges around the world (Weber and Rabinowitz, 1996; Beschta and Ripple, 2009). Both the leopard and spotted hyena are declining across their distribution just as in ranges of other large carnivores (Ripple *et al*., 2014; Ogara *et al*., 2010) where the International Union for Conservation of Nature classifies leopards as vulnerable by loss of prey and habitat and exploitation. Jacobson *et al*., (2016), conclude that it is listed as near threatened. The leopard (*Panthera pardus)* and spotted hyena (*Crocuta crocuta*) are sympatric, apex carnivores in Mt. Kenya ecosystem.

The leopards are solitary terrestrial carnivores. However, they are deemed successful in areas with diversity of habitats that give a variety of small to medium sizes of mammalian prey (Estes, 1991). Leopard is the most wide spread felid extending from Middle East to the Pacific Ocean and across Africa but despite their broad geographic range they are critically endangered. The loss of their range is greater than that of other large terrestrial carnivores. They currently occupy 65 % of their historic range (Ripple *et al*., 2014; Jacobson *et al*., 2016; Hunter *et al*., 2013). They have the broadest diet of all large oblate carnivores with their behavioral plasticity allowing them to persist in areas where other big cats have been extirpated from large portions of their historic range (Hayward *et al*., 2006; Athreya *et al*., 2016).

Leopards are quintessential ambush and stalking predators and seek to pounce before the prey can react. The leopards consume proteins in almost any form, ranging from beetles to mammal

preys twice their own weight (Estes, 1991). Leopards prefer large trees with big branches that are 2 – 3 m from the ground. They use their feline scent-marks and feces to communicate with one another. Males spray the bushes with their urine as they rub their heads and cheeks to mark a territory. Leopards deposit their feces along paths and roads where other leopards are likely to encounter those (Estes, 1991). However, knowledge of the species which they prefer or avoid consuming is lacking along with the reasons as to why such preferences occur (Hayward *et al*., 2006).

Spotted hyenas live in larger and complex societies when compared to other mammalian carnivores (Holekamp *et al*., 2012). They are gregarious and have group territories that they defend against encroachment by neighboring conspecifics. Their mating system is polygynandrous since both male and female mate promiscuously without developing any lasting pair bond among the sexes (East *et al.*, 2003). The females are larger and more masculine in both morphology and behavior (Van Meter, 2009). They therefore, take almost complete control over mating. Territorial clans are dominated by females, but variations occur in social organization. Territorial behavior is diminished where prey and hyena densities are low. The group organization tends to change with both short term and seasonal oscillations in abundance of prey (Smith *et al*., 2008; Holekamp e*t al*., 2012). In areas where the ungulate populations are migratory, the movement and social organization of hyenas depend on ungulate prey distribution and density (Mills, 1990).

Cooperative hunting behavior of hyenas enhances their hunting success or capture of large prey animals and also in the defense of the kill against intraspecific and interspecific kleptoparasitism (form of feeding which an animal takes food that was prepared by another animal) (Boydston *et al*., 2001). While other carnivores waste up to 40% of their kills; the spotted hyenas eats virtually

everything, eating castings of up to 20 by 8 cm containing hair and other indigestible material such as; bone & horn fragments, entire hooves, stones, grass and wood fragments which are often found around their dens (Estes, 1991. Spotted hyenas were previously perceived to be total scavengers but comprehensive research has shown that they are capable predators (Hayward *et al.,* 2006). They scavenge whenever possible and during hunting, they always select the easiest prey to capture. Instead of spotted hyenas fighting one another once the prey is captured, they compete by eating as quickly and as much as possible. A hyena can consume up to 1/3 its own weight. Hyenas are known to deposit their large white scats at prominent latrine sites.

Although there are various models available to predict Leopard diet, which are based on prey abundance, some researchers have shown that prey abundance may not be the only factor that affect their prey selection. Size of prey is also important. Leopard impact on the ecosystems, mesopredators, herbivores, predators, and other leopards is little known (Pitman, 2012). Therefore, the present research can help in refining some of the models, especially in the understanding of their prey selection strategy in mountainous terrains. Leopards may also affect the diet of hyenas since they are partly scavengers. That is why the present research sought to understand the diet of these two apex predators within Mt. Kenya ecosystem. Previous research in Mount Kenya revealed that leopard's most frequent prey was the rock hyrax, groove-toothed rats (*Otomys orestesels*) /*Otomys tropicalis,* African climbing mouse (*Dendromus insignis),* colobus monkey (*Colobus polykomos),* and common duiker (*Sylvicapra grimmia*)(Rödel *et al*., 2004). However, the potential prey species for the two carnivores within Mt. Kenya ecosystem include: Fish (3 species), birds (58 species), amphibians (2 species), reptiles (6 species) and 43 species of mammals (Young and Evans, 1993).

A suite of approaches have been applied in determining predators dietary components and range as mechanistic processes geared towards understanding community ecology and ecosystems functions through analysis of food webs and their dynamics. Carnivore diet studies through scat analysis have been used previously and proven important in ecological conservation studies in various ways like; estimating the diet composition of particular predators where it has been proven viable (Long *et al*., 2012), determining functional responses of carnivores like investigating the impacts of predators on the prey, which is important for conservation areas management as it provides quantitative data on how much ungulates are lost to the predators. This would assist in making decisions regarding prey stocking densities and reintroduction, the reflection of potential prey and its availability, as well as a suite of behavioral, morphological adaptations that allow individuals to locate, capture, ingest and digest a variety of prey taxa (Ogara *et al*., 2010). Scat analysis has also been used to determine interactions between humans and wildlife, especially where there is predation of livestock. The results obtained can be used to develop workable solutions in human wildlife-management. These include changing people's perception about carnivore conservation by determining their diet so as to determine whether the wild ungulates are consumed more by the carnivores than the livestock (Ogara *et al*., 2010). Scat analysis is more preferable in the study of carnivore diet because they are dangerous to handle. The method is good because target animals do not have to be directly observed or handled by the surveyor. It also helps in minimizing disturbance to the target animals but again it depends on the surveyor goal of the study.

Carnivores, small or big are too important to be lost from an ecosystem. They have mega charismatic status within ecosystems, which underpins both conservation and tourism development. Since they require large area to survive, conserving these apex carnivores will lead

to conserving a large amount of biodiversity. More importantly, they have intrinsic variability and significant effects on the structure and functioning of the diverse ecosystems that they occupy (Gittleman, 2001). It is in view of these traits that different species are referred to in different contexts as Keystone species, Umbrella species, Indicator species, Flagship species and Vulnerable species (Bauer *et al.,* 2015; Ripple *et al*., 2014); just to underline their ecological and conservation role and value. Hyena and Leopard's position as apex carnivores within the study area, provides an essential stability to Mt. Kenya ecosystem.

Furthermore climate change is a major threat to global diversity as it is already causing range shifts of species, hence disrupting existing species interactions (Keith *et al.,* 2008; Rinawati and Lindner, 2013). There is also uncertainty on the factors that determine the number of species that can coexist in an ecosystem. This study has provided useful insights into the prey base, distribution and habitat use of the two sympatric megacarnivores: Leopard (*Panthera pardus*) and Spotted Hyena (*Crocuta crocuta*). According to Ogara *et al.,* (2010) these species are threatened with extinction. Understanding the dietary habits of the two apex carnivores in Mt. Kenya ecosystem aids conservation efforts by clarifying prey preferences within the montane ecosystem. This study used non-invasive scat analysis method to determine the dietary behavior of the two carnivores.

1.2 Justification of the Study

There has been decline of large carnivores as stated by Weber and Rabinowitz, (1996); Beschta and Ripple, 2009). There is also limited data on the status, ecology and dispersal of carnivore species as stated by Ray *et al*., (2005) with most of their study concentrating on savannah ecosystems (Mwebi, 2013; Ogutu *et al.,* 2005; Boydston *et al*., 2001; Watts *et al.,* 2011; Karanth and Sunquist, 1995; Holekamp *et al*., 2002). Long *et al.,* (2012) concluded, that the low-density

populations, elusive and wide-ranging nature of most carnivores render them difficult to study with traditional capture-based or observational methods. Therefore many species are characterized by marking behaviors, territoriality, curiosity and traveling along routes which results in conspicuous placement of sign—traits that lend themselves well to noninvasive survey methods which were applied in the present research. The camera traps set in the study area recorded the pray base while the collected scats were analyzed to provide the diet results.

The predation events of top carnivores also affect the trophic cascades within an ecosystem hence contribute to ecosystem processes and species diversity, while if consumer-resource relationship is considered; key stone species of predators maintains the community stability. Therefore it was important to understand what supports the livelihood of these two sympatric carnivores within the study area as well as their habitat utilization. A factor such as climate change is a major threat to global diversity and can cause range shift of species with great effects on tropical forests ecosystems such as Mt. Kenya hence the urge to understand what species supports the existence of these apex predators in Mt. Kenya ecosystem and the habitats they occupy (Heller and Zavaleta, 2009; Keith *et al.*, 2008; Bailey, 1993).

A research by Rödel *et al*., (2004) indicated that there were only four prey types present in the scat samples collected. The analysis indicated that the most common prey for the leopards on the alpine zone of Mt. Kenya were the rock hyraxes. However, they were not able to determine whether the leopards present on the upper alpine zones had home ranges in the lower zones of the mountain. Therefore the present study builds on the prey base and dietary choices of both leopards and spotted hyenas being the top predators within the study area and helps to determine whether there has been a change on the dietary prey preference. The research has expanded on what they found since the rock hyraxes could be susceptible to impacts of climate change and

therefore determined that leopard's home ranges extended to the lower altitude of the mountain ecosystem. The results of the study has also expanded the existing knowledge base on what constitutes the diet of these two carnivores in montane ecosystem and their potential effects on the structure and functioning of the ecosystem, especially the pressure they exert on existing prey species. The results are therefore expected to reduce the uncertainty on what factors determine the number of species that can coexist in an ecosystem by revealing competition for the same wildlife prey species which has also presented major conservation problems to carnivores.

Moreover determining spatial overlap of sympatric carnivores is also important in understanding the potential costs of diseases on carnivore populations. Diseases like canine distemper virus (CDV) and rabies for instance, can be transmitted horizontally among taxa (Murray *et al.,* 1999). Therefore it was important to identify conditions like dietary overlaps and home ranges that are conducive for spreading wildlife diseases. This can help in implementing control measures that can prevent or moderate such occurences in Mt. Kenya ecosystems. The findings of this study can also aid in the development of management strategies and formulation of better conservation policies, especially in the context of expected changes in species home ranges due to climate change impacts. The local communities can benefit from the results through education and awareness so as to change their attitude towards the two apex carnivores and their perception that the predators rely only on domestic animals as their main source of food since larger portion of the diet constitute of wild preys. This will change their perception towards carnivores and in return conservation shall be promoted.

1.3 Statement of the Problem

Spotted hyena and leopard are apex carnivores in Mt. Kenya ecosystem and they occur throughout sub-Saharan Africa (Jacobson *et al*., 2016; Watts *et al.,* 2011). Their diet in savannah habitats is well known but their diet in mountain ecosystems is not well investigated. In fact little is known about their diet and behavior in montane ecosystems. Considering the fact that large carnivores live in small isolated populations, they are prone to extinction through stochastic events and habitat disruption (Ripple *et al*., 2014).

Crocuta crocuta and *Panthera pardus* being the apex predators in Mt. Kenya ecosystem have the potential to have great ecological value since they affect the structure and functioning ecosystems (Ripple *et al*., 2014). Empirical studies show that large carnivores have substantial effects on the structure and functioning of ecosystem with their influence cascading down to other species (Estes *et al.,* 2011; Ripple *et al.,* 2014; Sergio *et al*., 2008). This creates the various ways in which carnivores contribute to species diversity and other ecosystem processes (Estes *et al.,* 2011; Letnic *et al*., 2012).

By understanding the diversity of the two megacarnivores' diet and prey base within a montane ecosystem, management strategies can be developed for sustainable conservation of the two species. The diet and foraging behavior of the two apex predators can provide valuable insights on the impacts of climate change on biodiversity of tropical mountain ecosystems. This is vital as Mt. Kenya ecosystem doesn't contribute to local climatic conditions only, but also offers a suitable climate refuge for biodiversity. This also reduces short-sighted management strategies that can result to wide and long-term modifications of ecosystem structure and functioning which may increase the rate of extinction to threatened carnivore species.

1.4 Research Questions

1. Which prey species constitute the leopard and hyena diet in Burguret trail in Mt. Kenya?
2. How does the leopard and hyena diet change with habitat type and altitudinal gradient?
3. Are there diet overlaps between the leopard and hyena in different habitats and altitudinal gradient?

1.5 Objectives

1.5.1 Broad Objective

The aim of the study was to assess the leopard and hyena's prey base and diet and how they are influenced by habitat and altitudinal changes, along Burguret trail in Mount Kenya forest ecosystem.

1.5.2 Specific Objectives

The specific objectives were:

1. To assess diversity of hyena and leopard prey base along the Burguret trail of Mt. Kenya ecosystem.
2. To determine diet composition of spotted hyena and leopard and its variation across various habitat types.
3. To determine dietary overlaps between leopard and spotted hyena in Burguret trail of Mt. Kenya ecosystem.

1.6 Research Hypothesis

This study was guided by the following two hypotheses:

i. The prey base of the spotted hyena and leopard were not different.
ii. The leopard and hyena diet did not vary across various habitat types that they used.

CHAPTER TWO: LITERATURE REVIEW

2.1: Large carnivore diets relative to prey availability and habitat types

Ecosystems of Africa are rich in biodiversity and famous for their largest wild places on planet. As stated by Ray *et al*., (2005), large carnivores have been the focus of most research since they are assumed to be having greatest impacts on other components of biodiversity. They structure ecosystems due to their impacts on each other and their herbivorous prey. The selection of prey patterns by predators indicates resource partitioning, which may be governed by factors, such as hunting strategies, habitat requirements, predator – prey dynamics and morphology (Husseman *et al*., 2003). The predation impact on the prey population depends largely on how the predator's consumption rates vary with prey density. The social structure and abundance of prey affects the carnivore's spatial and temporal use of space (Ramesh *et al*., 2012) while high diet overlap could promote frequency of encounters when species share similar habitat or prey (Buskirk, 1999).

However, prey selection patterns of large carnivores particularly in tropical forests are not well understood (Karanth and Sunquist, 1995). It is difficult to study the carnivore diet, especially if the species is solitary and its prey indefinable due to the elusive predator's feeding and hunting behaviour. Since they are secretive, and not easy to see, it is rare to have a direct view of prey. Hence, many researchers result to the use of indirect methods, such as scats analysis (Karanth and Sunquist, 1995; Reynolds and Aebischer, 1991). The choice of prey by the predator connects the species dynamics in different trophic levels, which depends on habitat, activity patterns, different prey size, and differential space usage. Competitive selective pressures of carnivores are brought about by predator species operating within the same. (Van Valkenburgh and Wayne, 1994).

As a result of competition for prey species in different habitats or in different habitats, predators evolve different survival tactics (Gittleman, 2001). Such differential activity patterns separate niches, reduce competition, allowing the coexistence of sympatric species in a given ecosystem.

Predatory tactics are mostly controlled and shaped by natural selection through a wide range of ecological constraints and is affected by factors such as prey density within an area, different habitat types or altitudes that may be different within same species especially at the limits of carnivore geographical distributions (Sunquist and Sunquist, 1989). As stated by Ramesh *et al.*, (2012), sympatric large carnivores in Indian subcontinent share forested habitats and hence share similar prey. Other felids are nocturnal where they hunt under the cover of darkness while diurnal canids hunt during the day, in open places as they share prey species of small to medium sizes (Ramesh *et al.*, 2012).

Despite the fact that spotted hyenas were once considered as mere scavengers, Hayward (2006) showed that they are efficient predators mostly preferring prey within 56 – 182 kg body size range. Spotted Hyenas are social predators. They occur throughout sub-Saharan Africa, alpine areas and out in tropical forest (Holekamp and Engh, 2002). Pienaar as cited by Hayward (2006) states that spotted hyena use a flexible searching strategy. However their catholic tastes implies that they can consume any animate and inanimate objects. This means that no matter where prey animals' live, they are at risk of hyena's predation.

Predation rates may be affected by the habitat type through vegetation density which affects prey detectability. Geist as cited in Hayward (2006) stated that prey animals that resides in dense vegetation, adopt a solitary and silent hunting technique that helps them to evade detection by predators. Prey living in open grasslands are always detectable through sight when compared to

sound or smell and therefore can exist in large herds. Compared to the four large felids belonging to the genus Panthera, leopard is the smaller. It inhabits diverse ecosystems including; rain forests, semi arid environments, mountains and desert of sub-saharan Africa. Even though the Leopard is widely distribution, it is listed as nearly threatened (Stein and Hayssen, 2013). Therefore the present research was important as it determined the two carnivores prey base on a mountane ecosystem. Studies of leopards and spotted hyenas in mountain areas are uncommon in Africa.

2.2 Carnivore Conservation Efforts and Strategies

Despite their importance, carnivores have greatly decreased in numbers due to their exposure to different unfavorable factors (Weber and Rabinowitz, 1996). Referring to Heller and Zavaleta (2009); Keith *et al.,* (2008), is a major threat to biodiversity especially tropical forest ecosystems like Mt. Kenya that experience overall greater impacts globally. Other ecosystems and regions of the world receive lesser effects of climate change when compared to tropical forests (IPCC, 2012). The impacts have caused shifts of many species ranges, rising to higher elevations in the mountain areas, Parmesan (2003). That shift of range is predicted to affect all levels of biodiversity: genetic, species and habitat diversity.

There is also uncertainty regarding what factors determine the number of species that can coexist within an ecosystem (Keith *et al.,* 2008). Considering consumer-resource relationship, keystone species of predators maintains the community stability. Moreover, most carnivore conservation efforts in different parts of Africa and World are affected by limited data on the status, ecology and distribution of carnivore species (Ray *et al*., 2005). Therefore, it is more critical than ever before for scientists to produce relevant and sound data pertaining distribution, habitat use, and other biological and ecological measures relating carnivores. The present study involved

microscopic analysis of hair shaft morphology, macroscopic remains from scats and DNA metabarcoding to determine the diet of the target predators and reveal whether there is competition for the same wildlife prey species, which has also presented major conservation problems to carnivores (Keith *et al.*, 2008).

As stated by Woodroffe (2001), conservationists of carnivores have applied two approaches: ecomomic approach and ecological approach. Ecological approach helps in determining whether modification of habitat and its initial size can affect species. This approach also verifies if habitat modification is required if re-introduction of species is to be made, in cases where population has become extinct. In economic approach, the local communities are inolved in conservation practices which can change their livelihood positively, especially through sharing of revenues obtained from protected areas. This is an example of a social approach where different interventions are also applied to minimise human wildlife conflicts especially through community participation in conservation. Despite the human-wildlife conficts, communities are backing carnivore conservation through development of wildlife-ranching by private land owners over vast areas which has created significance potential for the conservation of carnivores out side protected areas. The outreach programs have had communities develop a positive perception towards carnivores which has achieved co-existence between carnivores and people which is the key to successful conservation. The rural communities are also willing to adopt management practices that help conserve carnivores (e.g. investment in infrastractures development that protects domestic animals).

Considering the fact that most carnivores occupy wide ranges, protected areas have also been established to promote their conservation despite the fact that there are many animals that exist outside these protected areas. The extensive habitat requirements of large carnivores requires that

management occur not only in public reserves, but also involve private landowners. This is because most of the wildlife corridors go through private lands while connecting public conservation areas. In general these approaches have revealed that it is important to understand the historical changes in distribution of a certain species. Significant strides have been made in private land management through the use of incentive programs that promote conservation of carnivores through dedicated reserve designs. In terms of ecological approach, research have been previously carried out on carnivores with a view to promote their conservation. Howerver Ray *et al*., (2005) suggested that conservation action research should be focused on addressing threat impacts and develop appropriate mitigation measures for carnivore species.

Darwin as cited by Allendorf and Luikart (2007), states that genetics is also an important factor in species management. It was first considered through expressing concerns about the loss of robustness in a deer population resulting from isolation and small-sized populations. In comparison, such implications are voiced today regarding carnivore populations, like the lion (*Panthera leo*) and cheetah (*Acinonyx jubatus*) Barnett *et al.,* (2006), which is a prove on how genetic information has been used in conserving species.

In Kenya, protected areas have been established to support conservation of wildlife including national parks, national reserves, marine parks, marine reserves, conservancies and sanctuaries. Most of the conservation areas incorporate adjacent communities in conservation practices as most wildlife co-exists with humans. These different management strategies are employed to promote conservation, especially to carnivores which are wide ranging.

Many studies in the past and even current ones on Mt. Kenya have been conducted on established trails such as Chogoria (Eastern slope), Naromoru (Western slope), Kamweti

(Southern slope) and Timau (Northern slope) (KWS, 2010). Some of the previous studies include: “The Distribution of C3 and C4 grasses by (Tieszen *et al*., 1979), Reconstruction of a subalpine grass-dominated ecosystem by (Ficken *et al*., 2002), Impact of monsoons, temperature, and CO2 by (Konecky *et al.,* 2014), Notes on the feeding habits of the leopard by (Rödel *et al.*, 2004), Ecosystem Services to the Community by (Gichuhi *et al*., 2014). Frequent studies along specific routes around the mountain has resulted into habitat disturbance through transects and sub trails used by mountaineering visitors (KWS 2010).

CHAPTER THREE: STUDY AREA, MATERIALS AND METHODS

3.1 Description of the Study Area

3.1.1 Location and Size

Mount Kenya was formed as a result of volcanic activity nearly 100 to 400 million years ago. The mountain is located in the central part of Kenya, nearly 180 km north of Nairobi and on the eastern side of the Great Rift Valley. It is the second highest mountain in Africa, after Mt. Kilimanjaro, with its highest peaks, Batian and Nelion, reaching 5198 m and 5188 m, above sea level (a.s.l.) respectively. The mountain is normally cone-shaped with exceptionally incised U-shaped valleys on the upper parts, which shows ancient glaciations. It has a base width of 120 km, where the foothills to the north reaches the equator (Bussmann, 2006). Most of the mountain especially on the north and northeastern slopes, is covered by pyroclastic rocks and volcanic ash which originated from various secondary eruptions. Mt. Kenya Ecosystem comprises of Mt. Kenya National Park, Mt. Kenya Forest Reserve and the adjacent environs including Ngare Ndare Forest and the Lewa Wildlife Conservancy (KWS, 2010).

3.1.2 Burguret Trail

The study area was purposively selected along an old infrequently used Burguret trail on northwestern slope of Mt. Kenya. There are minimal anthropogenic activities on the trail and thereby reducing research bias. The trail shown in Figure 1 was established in 1901 but was rarely used by both tourists and researchers as shown by KWS visitor use zonation in their management plan of 2010 and reconnaissance reports by NMK and Smithsonian Institution in 2015. I also found that the trail was heavily vegetated and traverses through Mt Kenya Forest Reserve and Mt Kenya National Park from the foot (2200m a.s.l towards the peak (4700m a.s.l.) of the mountain. Mt. Kenya ecosystem has a total of 2,700km^2 of protected area. The forest

reserve which is now gazetted as a National Reserve, covers about 74% of the protected area (2,000km^2) while the National Park covers about 700km^2.

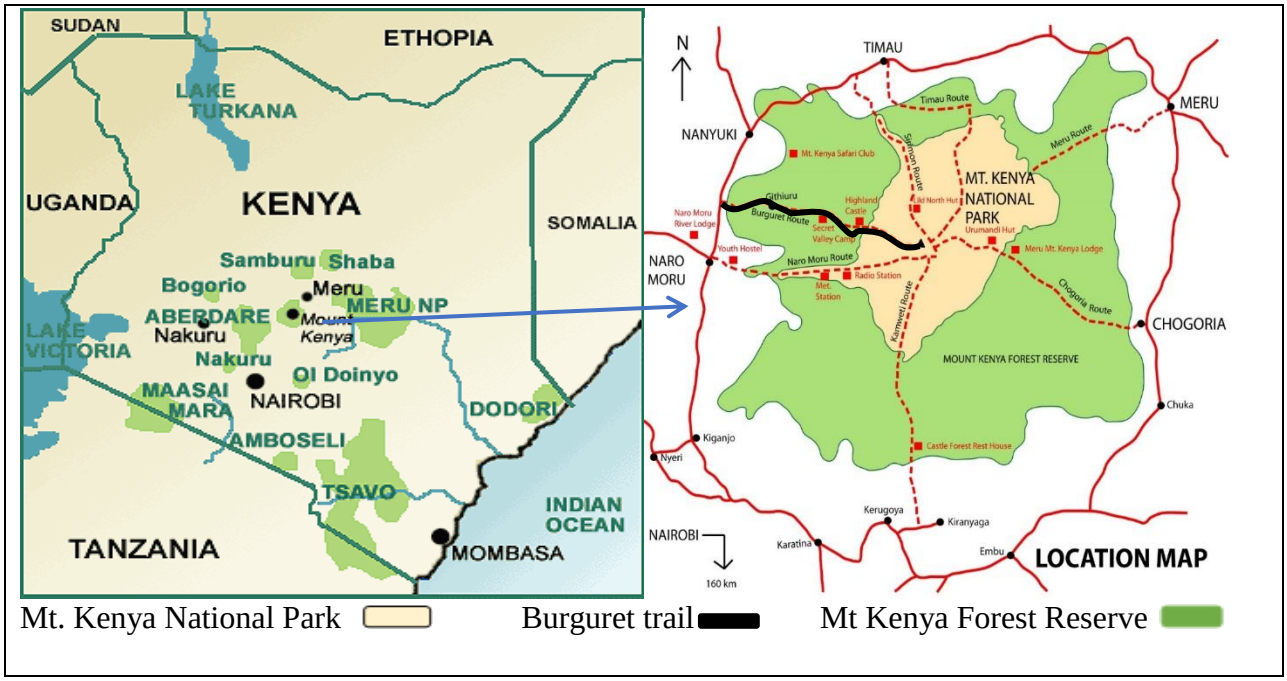


Figure 1: A composite map of Kenya (Source; Google Maps) showing location of study area

3.1.3 Climatic conditions

Mt. Kenya is an important watershed which experiences two rainy periods, long rains in March-June and the short rains in October-December which fluctuates every year. The mountain climate varies considerably with different zones of influence across the mountain. The rainfall variations per annum on the leeward north western side is 900mm to 2,300mm on the wind ward south eastern side. The rainfall generally increases with altitude up to 2,500-3,200m then afterwards decreases with altitude. Temperature on Mt. Kenya varies considerably with time of the day and -altitude. This fluctuation is largest on the lower slopes of the moorland zone of the mountain. The mean range in temperature at an altitude of 3,000m is 11.5^{0}C which goes down to 7.5^{0}C at 4,200m and at an altitude of 4,800m to 4^{0}C. Therefore, the temperatures on the summit are well

below freezing. Precipitation is about 700mm per year at 4500m above the sea level and subsequently decreases with altitude.

3.1.4 Topography and Soils

Speck (1982), conclude that the soils on the northwestern slopes of Mt. Kenya occur in four main physiographic units: foot slopes, valleys, foot ridges and mountains which were mainly developed from intermediate igneous rocks (trachytes). However, the soils have diverse chemical and physical characteristics ranging from poorly to well drained, silty loam to clay, shallow to deep, brownish black (10YR 3/2) to dark reddish brown (10YR 4/6) and are moderately fertile with moderate moisture storage capacity. The soils on the foot slopes are cambisols and andosols; those on the foot ridges are alisols, luvisols and andosols while those of the mountains and valleys are mainly leptosols and cambisols. The temperature regime of the soils is isothermic on the lower slopes (mean annual temperature of 15 to 22^0C) and isomeric on the upper slopes (mean annual temperature of 8 to 15^0C) while the moisture regime is ustic in the lower part and udic in the upper part of the mountain (mean annual rainfall of 700 to 900mm). The soil pH of top and sub soils varies from slightly acid to neutral (5.3 to 7.2). The topsoil base saturation is between 57% and 93%, with a cation exchange capacity (CEC) between 15.5 and 23.5 cm o/’’/ l kg-1 while organic carbon lies between 1.6% and 12.5%.

3.1.5 Flora

Like typical mountain ecosystems, Mt. Kenya consists of various vegetation structures primarily underpinned by altitude, precipitation and temperature. Species distribution and vegetation zones in Mt. Kenya ecosystem are distinguished according to different altitudes and climatic zones

with variations in vegetation cover, structure and composition as shown in fig 2 and habitats description in fig 9.

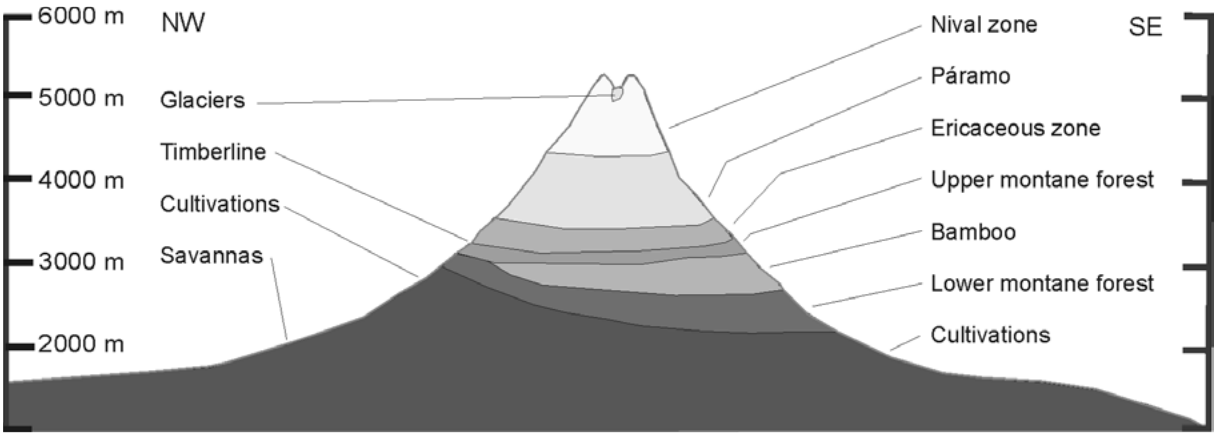


Figure 2: Vegetation Zones of Mt. Kenya (Source, Niemella and Pellikka, 2004)

Within Mt. Kenya ecosystem, 880 plant species belonging to 479 genera and 146 families have been recorded (Bussmann, 2006). However, some of the species of higher plants are strictly endemic to Mt Kenya e.g. *Lobelia deckenii/kenienisis*, *Lobelia telekii, Cardulus keniensis*, *Dendrosenecio keniodendron, Dendrosenecio johnstonii* where more than 150 species are near endemic. The greatest part of this mountain ecosystem is covered with forest, roughly up to 3400 m in the South and 3000 m in the North, with a strident boundary splitting the forest from the lower alpine zone. The location and shape of upper tree line is severely influenced by fires. The present-day lower timberline is a result of extensive agriculture and forest clearance reaching up to 1800 m on the Southern, up to 2400 m on the Western and Eastern and on the Northern slopes, rising up to 2900 m. Mt. Kenya is a characteristic, example for the altitudinal zonation of tropical African mountain vegetation. If it were not for human impacts, the mountain would be almost entirely surrounded by dense forests. In the last seven decades, however, the mountain has been

bounded by densely populated farming communities thereby culminating in forest loss on the foot of the mountain (Bussmann, 2006).

3.1.6 Fauna

At least 31 mammalian families are known to occur in Mt. Kenya. These alpine vertebrates include at least 43 species of mammals, 58 species of birds, 6 species of reptiles, 2 species of amphibians and 3 species of fish (Young and Evans, 1993). At least six rare or threatened mammal species occur: Leopard (*Panthera pardus*), Black-fronted duiker (*Cephalos nigrifrons*), Black rhinoceros (*Diceros bicornis*), Giant forest hog (*Hylochoerus meinertzhageni*), Mountain bongo (*Tragelaphus euryceros*) and, African elephant (*Loxodonta africana*). As an Important Bird Area, Mt. Kenya is home to the threatened and little-known Abbotts's starling *(Poeoptera femoralis)* (Bird Life International, 2016). At least 35 forest specialist species and six of the eight species belonging to Kenyan Mountains Endemic avifauna occur on Mt. Kenya.

3.1.7 Conservation value of Mt. Kenya ecosystem

Mt. Kenya is recognized as one of the key conservation areas in Kenya. It was first designated as a National Park in 1949 and later as a Man and Biosphere Reserve in 1978. In the year, 2000 it was described as a World Heritage Site by UNESCO (United Nations Educational, Scientific and Cultural Organization), and in 2001 it was designated as an Important Bird Area (IBA) by Birdlife International (Bennun and Njoroge, 2001) due to its rich biodiversity, cultural and biophysical significance. Both Mt. Kenya National Park and Mt. Kenya Forest Reserve represent one of the world's pristine tropical mountain ecosystems. The World Heritage Commission described Mt. Kenya as "one of the most impressive landscapes in East Africa with its Afro-

alpine moorland, rugged glacier-clad summits and its diverse forest that illustrate outstanding ecological processes."

Mt Kenya is a principal water tower in the country. It is encircled by gently sloping agricultural lands. Within Mt. Kenya region, 17% of the land surface is classified as agriculturally productive by FAO (Kenya Wildlife Service, 2010). Only few other places in Kenya experience such intense land use and have dense population existing so close to protected areas and wildlife as around Mt. Kenya.

3.1.8 Social-economic activities

The fertile slopes (in the lower slopes) of the mountain attracted settlement of farmers though the early inhabitants of the mountain slopes were wildlife and hunters. Due to fertile soils, high rainfall and water availability, subsistence and small-scale cash crop farming dominates around Mt Kenya conservation area. Tea bushes cover a major share of the land that is available with an exemption of small plots on valley bottoms where horticultural crops and vegetables are grown.

The variety of crops produced in the area include beans, maize, bananas, coffee, tea, arrowroots, yams, pawpaw, macadamia nuts, passion fruits, cassava, millet and cotton. Local community rears livestock (zero grazing) and more extensive ranching and pastoralism in the drier north arid and semi-arid lands. Surrounding communities also practice agro forestry comprising both exotic and indigenous tree species. Mt. Kenya area is an important tea and coffee growing zone with its tea being one of the finest in the world.

Mt Kenya forest provides a stream of goods and services, and therefore its conservation ensures that these economic benefits and activities are maintained. Despite this, economic activities, such as unsustainable exploitation of forest resources contribute to the degradation and loss of

biodiversity. Many of the threats to the forest convert, degrade or pollute forest land and displace species and habitats. The key threats within Mt. Kenya ecosystem are illegal poaching of wildlife and logging of hard wood for timber, forest fires, invasive species, human wildlife conflict and illegal water abstraction. This has led to loss of total forest cover by 25% (KWS, 1999). Human encroachment has also exerted pressure on the conservation area, where population has turned to the protected area for livelihood resources, such as harvesting of firewood, medicinal plants and wild honey.

Mt. Kenya ecosystem also forms one of the major water catchment areas in Kenya. It is here where parts of the perennial Tana and Ewaso Nyiro rivers rise. Destruction of forest has impaired the water catchment, hence reducing water supply downstream. Mt. Kenya provides more than 40% of the country's water requirement. Water from Mt. Kenya is important for electricity generation, tourism activities, livestock, industry and commercial purposes. Many river tributaries originating from Mt Kenya ecosystem feed river Tana basin, which is home to Kenya's hydro power production and if they dry up, then there will be reduction in power production.

Tourism is also an important economic activity within the mountain as Kenya is known for its unique wildlife but most visitors have a primary objective of climbing the mountain. Tourism around the mountain generates substantial income through activities, such as trout fishing, bird watching, climbing and walking along its wilderness trails. Revenue for the National Park and National Reserve are generated from those activities for mountain ecosystem protection and management.

3.2 Materials and Methods

3.2.1 Research Design

Stratified sampling design was adopted for the purpose of conducting vegetation survey and sampling the distribution of the scats. The strata used were based on established vegetation zone boundaries (Bussmann, 2006). Systematic sampling (A type of probability sampling method in which samples from a larger population are selected according to a random starting point but with a fixed, periodic interval) was conducted within the distinct vegetation zones (strata). A perpendicular transect line (the trail) from baseline (forest edge) was established along the altitudinal gradient. Following this design, the sampling points were established along Burguret trail from the bottom towards the top of the mountain. Along the established transect, sampling was carried out at 100 m intervals, where plots of 20 by 20 m were established at each sampling point. Each sampling point was marked using GPS handset and delimited temporarily using flagging tape during the sampling. The transect baseline was set at the foot of the mountain at around 2200m a.s.l., where transect was laid perpendicular to the baseline across the various vegetation strata along the mountain and terminated at around 4700m a.s.l., where walking was possible. Other sections of the mountain peak were not accessed because they required specialized mountaineering skills and equipment to access.

Within the belt transect, camera traps (Reconnyx rapid fire and hyper fire game trail cameras) were installed at an interval of 300m, which were then moved 100m up the transect monthly after being deployed for two consecutive months. The main transect had 233 camera traps set having an effective sampling area of 22.55 km^2. The camera trap data were used to develop an inventory of mammal species using the study area. The data subsequently assisted to establish the reference hair library for these mammals which acted as either competitors or potential prey

items of the two target predators. The reference hair collection was ultimately used to identify hair samples recovered from scats.

3.2.2 Habitat Analysis

The main vegetation zones within Mt. Kenya ecosystem were; 1. Forestry / plantation forest zone, 2. Indigenous forest / lower montain forest zone, 3. Bamboo forest zone, 4. Transition / mixed vegetation/upper montain forest zone, 5. Grassland / Moorland / Afro alpine zone, 6.Rock / Nival / Paramo zone as shown in figure 2 for zone correspondence. Systematic random sampling technique was applied in collection of plant specimens from the established 20 by 20 plots in every zone following the same standardized transect as in scat sampling (i.e. the perpendicular transect across the study area with a baseline at the forest edge starting at 2200 m a.s.l. at the mountain base). This was to ensure that plant samples were obtained from the six vegetation zones at least twice. Using a GPS, the plots from which the plant samples were collected were marked and areas with gradual changes in vegetation community types noted. Plant identification was partially done while in the field and further verified in the herbarium purposively to note the changes in plant communities. Classification was based on the International Vegetation Classification (IVC) following the dominance approach which considered broad groups of vegetation with similar dominance of trees in the upper plant canopy but often with heterogeneous under stories (Grossman *et al*., 1998). Habitat type and altitude data recorded in the field were fed into Geographical Information System (GIS) (Arc View 3.2) for establishing the vegetation zones and mapping the distribution of scats. The vegetation zones around the mountain were then interpreted from Land sat TM scenes and the records obtained were compared to the previously published accounts.

3.2.3 Assessment of the leopard and hyena prey base

Wildlife camera trap were set at strategic locations, ready to take photo graphs of any moving humans and wild animals along an identified track. The presence of the animal in front of the camera, automatically activated the shutter of the camera, which took one or more photos or video sequences in absence of the photographer. Application of camera traps in the present study in Mt Kenya forest was efficient since cameras were non-intrusive, did not require to be operated by the researcher, had continuous data collection, gave the time of contact as they took pictures of the animals, and were weather hardy hence, were not affected by changes in weather conditions, such as rainfall or sunlight. The images taken can be retain for years since they are saved in a memory card (Kays *et al*., 2010). Figure 3 shows the camera trap images of an African leopard and spotted hyena captured during the study.

Figure 3: Camera trap images of (a) African leopard, (b) Spotted hyena in Mt. Kenya forest.

Placement of Camera Traps

Camera locations. **-** The primary aim of the camera traps was to obtain photographic images of the mammalian species moving on the ground in Mt. Kenya ecosystem. The success of obtaining

these crucial data depended on the camera location and behavior of the target species in the area. This was important for the study as the focus was to identify potential prey species as well as establish a check list of species found within the study area. Therefore, it was important to have basic knowledge of the behavior of target species so as to determine the best site to place the cameras. Setting the camera traps along the animal trails and other existing human trails was easier for better image capture.

Considering the fact that Mt. Kenya ecosystem has different vegetation zones, with different terrains, it was necessary to make adjustments to camera locations when on the ground. Therefore, while approaching the location stored in the GPS unit prior to the study; it was important to observe the surrounding areas so as to identify habitat features, such as streams and ridge lines which may not have been captured on the map during the reconnaissance. This required a walk beyond the provisional camera location to assess the habitat condition. The deviations from the proposed GPS locations depended on the desired camera spacing and density of vegetation. In this study, the cameras were placed at an interval of 300m ranging from the lowest (2200m a.s.l) to the highest altitude (4700m a.s.l.) across the different vegetation types. Previous studies had shown that placing a camera 50m-100m away from the GPS point where one expected to lay the camera was acceptable; especially where the sample grid was small (Kays *et al*., 2010). In this study cameras were fitted on trees to enable tighter camera placing (fig 4). Alternative devices for camera attachment, such as poles were also employed. After setting the camera, it was very important to store that actual camera location in GPS and in a note book record and update the map of the predetermined locations as well.

Figure 4: A Strategically Set Camera Trap within a cleared forest patch in the study area

Camera height. -The target species partly determined the height of the camera attachment. In this study, passive infra-red cameras, which relied on sensing the body heat of the animal as it passed within the camera range were used. Since the aim of using cameras in the study was to obtain records of animal species, the cameras were set at 'knee height' or approximately 45cm off the ground which was more useful to obtain data on a range of species simultaneously within the study area.

Camera Angle. -The target area was a very important aspect when setting the camera, particularly the target trail so that the camera angle was parallel to the ground. This was a time

consuming but essential step to get right. Therefore, considering the fact that the camera area of sensitivity was horizontal, then the camera was angled in such a way that it remained horizontal in relation to the trail. The angle was set in relation to the camera sensor so as to capture reliable photos. Tests were made by moving in front of the camera to determine the distance at which the camera was able to detect and take a good photograph of some moving animal. A flashing RED light on the camera provided an indication of an active trigger to the camera. This was followed by clearing vegetation, such as grass in the camera's field of view, so as to remove obstructions and improve on the quality of photos obtained fig 3. The last step was to ensure that the camera took a photo before leaving it to ensure that it was working correctly. (Kays *et al*., 2010).

3.2.4 Scat Collection and Preservation

A non-invasive sampling approach, which focused on collecting carnivore scats and mammal detection using camera trap images was used to quantify both the abundance of carnivores and their mammalian prey items.

Leopard and hyena scat collection along transect followed systematic sampling design. The sampling points were established systematically at 100 m intervals along the altitudinal gradient. At each sampling point, 20 m by 20 m plots were established where thorough search of the scats was carried out. The opportunistically encountered scats between the sampling points were also collected. The entire altitudinal transect was walked twice in search of carnivore scats in every sampling period which was carried out monthly for six months.

The data collected, including, GPS location, altitude, habitat type, date of collection, time of collection, provisional species identification, diameter of the scat, the collector's name, and a unique number assigned to each scat which was linked to the data, were recorded in a field note

book. The scats were then placed in zip lock bags marked with the unique number then stored in cool boxes and ultimately transferred to -20^0 C freezers after field work.

3.2.5 Microscopic Approach of Scat Analysis for diet determination

This approach was based on reference key development, specifically for use in the study area. The mammalian species reference collection was used in conjunction with other published dichotomous keys, field guides as well as reference collections of teeth, bones and hair for identification of prey species found in the scats. The scat samples were sub sampled to include only those samples that had definitive mtDNA molecular identification. It was guided by molecular analysis of all collected samples to determine the actual predator which was crucial in microscopic analysis as it set a clear record of whose scat sample was being worked on. This was to avoid potential bias that could have potentially arose from relying solely on field identification that typically relies on subjective scat morphology contents and size for initial predator identification as stated by Zuercher and Stewart (2003). The assumption made during analysis was that each scat sample represented a different individual prey as they were from different locations and collection points. In the instances where scats were obtained from a den, they were of different ages and individuals foraging in different sites of the study area.

Establishment of Hair Reference Library

The hair collection for the reference library was obtained from the database and mammal specimens housed in the mammalogy section of the National Museum of Kenya. In establishment of hair reference library, guard hairs (the straightest, largest and most robust of all the hair types) from the back, hip, belly and shoulder of a potential prey (especially if observed from camera trap data), were mounted on glass slides using fine forceps after coating the slide

thinly with the mounting medium. This was because cuticular scales under normal microscopy were not visible on the hairs. For this reason, imprints of hairs were used to reveal scale patterns. The mounted hairs were left overnight to dry before removal for observation of scale imprints (See Appendix IV A to IV D).

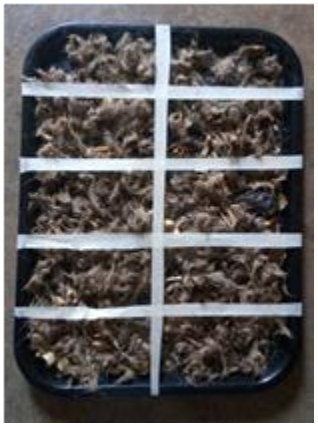
However, Bahuguna *et al.,* (2010) concluded that the structural characteristics alone cannot be used as the basis for development of an identification key due to the variations of cuticular pattern along the length of the hair and among hairs from different body parts. The hairs used in establishment of imprints were the same hairs used to establish permanent slides that facilitated observation of the cortex and the medulla. Details, such as cortex and medulla width, were measured through a calibrated eye piece. Therefore, the cuticular hair scale patterns, arrangement of medulla and scale margins of mammalian hair were incorporated since they are important features for identification (Keogh, 1983). In this case the scale patterns along the hair and the medulla patterns of the hairs were established under 10X and 40X magnifications followed by taking of microphotographs using AmScope microscope camera.

Preparation of scats for microscopic examination so as to identify prey items followed a number of steps. Figure 5 below shows the steps followed in scat processing, from raw carnivore scat to mounted hair slides ready for microscopy work.

(i)

(ii)

(iii)



(iv)

(v)

(vi)

Figure 5: Scat processing from (i) raw scat on site (ii) through thawing of scat (iii) Scat washing with a water jet (iv) drying and establishing grids (v) sorting of undigested fragments (vi) and mounting of hair slides for microscopy.

Each collected scat was carefully cleaned from attached plant debris after allowing it to thaw for at least 30 minutes. The scats were washed in running tap water through sieves with 0.5-mm meshes to gain all macroscopic scat reminders. After washing, the remains were then air dried followed by their spread on the dissecting pan after drying. The bones and the hairs were then

sorted separately to begin identification process as each followed a different identification procedure. After all the dry hairs were obtained from the scat, they were spread in the dissecting pan.

The dissecting pan had 10 grids of equal proportions. Then at least 10 hairs were randomly selected from every grid and used in identification process. To ensure satisfactory impressions from the hairs, they were cleaned with ether before attempting an impression and then dried thoroughly. The laid hair on a slide was left overnight to obtain an impression that clearly showed the cuticular patterns. During hair identification, the comparison was always based on the hair cortex, medulla and cuticle as shown in Figure 6.

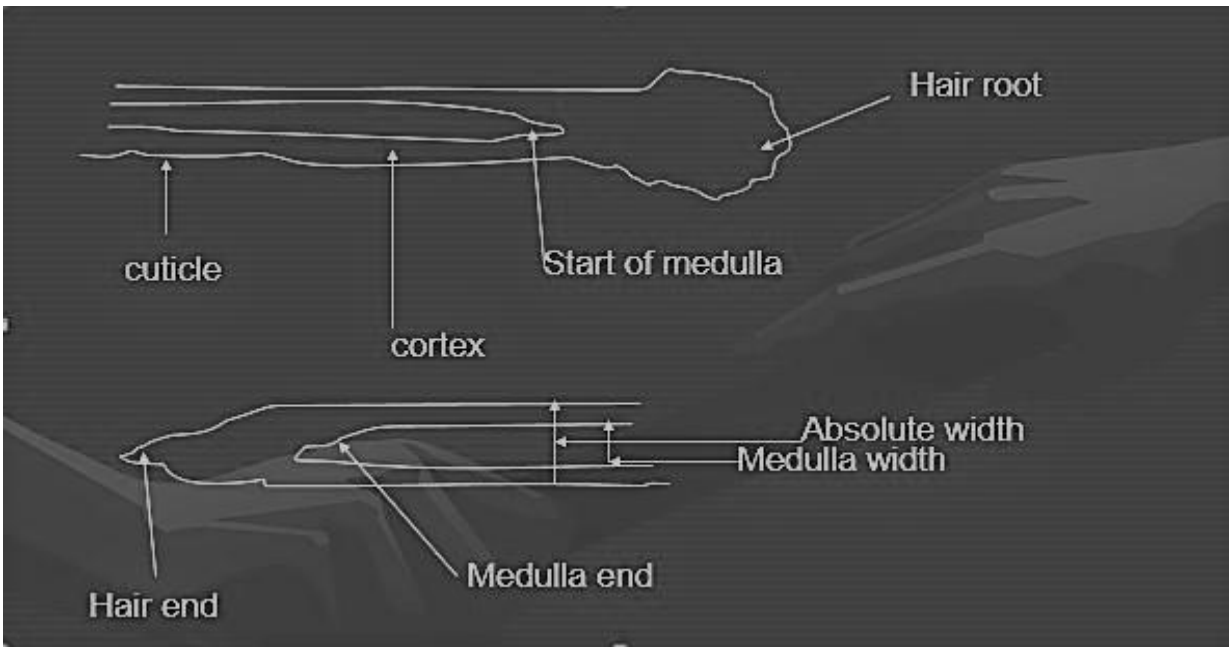
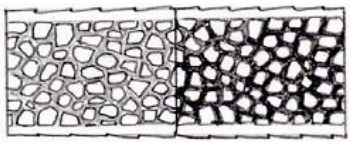
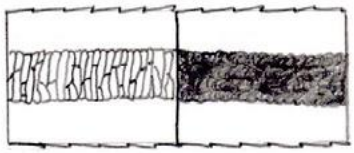
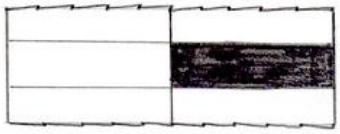


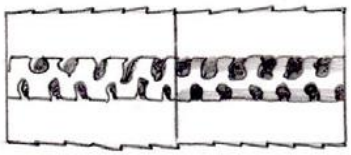
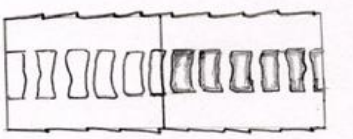
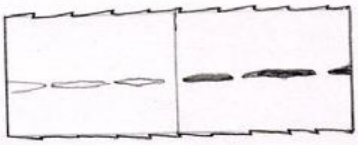
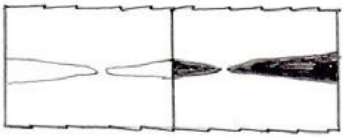
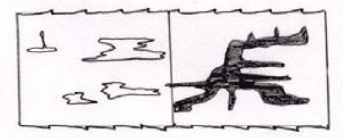
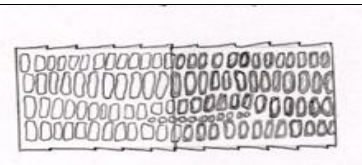
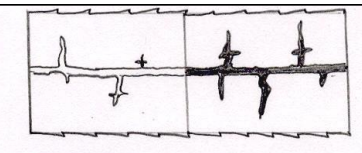
Figure 6: Basic structural features of mammalian hair (Source: Bodendorfer, 2006).

In the system of hair identification, the cuticle and the medulla are most important features because they are consistent in each species. It was important to note details such as cortex and

medulla width which were measured through a calibrated eye piece and hair roots to check whether they were clear or dotted though complete hairs often missed from the samples. This was established under 10X and 40 X magnifications. The impressions of the scale patterns form the basis for the system of hair identification. These scales form patterns along the length of the hair cuticle with different types of margin shapes and sizes. In this case the same hairs that were used to establish the imprints were the same hairs used to view the cortex and the medulla (Table 1) using a light microscope to observe their structure.

Table 1: Mammalian hair medulla patterns used as reference material (Source: Keogh, 1983; Bahuguna *et al.*, 2010).

1	Wide medulla lattice	The medulla is wide and fills most of the hair. It's thickness more than half the total thickness of the hair.	
2	Narrow medulla lattice	The thickness of the medulla is less than half the total thickness of the hair.	
3	Simple medulla(amorphous)	This medulla type lacks a specific pattern and has amorphous appearance. It may be narrow or wide.	
4	Wide aeriform lattice	The medulla is wider than that of a narrow aeriform lattice covering more than half of the total thickness of the hair. The hair spaces form a lattice.	

5	Narrow aeriform lattice	This medulla type comprises of air spaces arranged in the form of a lattice. The medulla thickness is less than half the total thickness of the hair.	
6	Uniserial ladder medulla	This medulla type has a single row of hair spaces along its length which could be rounded, flattened, angular, or cup shaped.	
7	Fragmental medulla	The medulla is interrupted by a long section of cortical material along its length.	
8	Interrupted medulla	This medulla type is interrupted along the length of the hair by short sections of cortical material.	
9	Globular medulla	This medulla shows an aggregation of globular air spaces.	
10	Intruding medulla	This medulla consists of narrow irregular air spaces that projects into the cortex in several directions and essentially may not be found at the center of the air.	
11	Multiserial ladder	This medulla comprises of two or more distinct rows of mostly uniform air spaces.	
12	Stellate medulla	This medulla resembles a simple medulla with an exception of the finger-like projections that radiates into the cortex along the length of the medulla.	

The hairs were also examined for color i.e. whether multicolored or bicolored, color band pattern i.e. number and position of bands following guidelines from Moore *et al*., (1974). However, these characteristics are too variable especially considering the seasonal changes so they were not considered as key identification features as they may be misleading causing bias to the study.

Therefore following Hickey & Fenton (1987) and Benedict (1957) agreement, only the scales in the mid-region of a hair shaft are the mature and uniform types. The study, therefore, emphasized on the central mid-region of specimen's medulla and cuticular structure of the hair shaft for comparison to the reference hair catalogue for identification purposes. However, other parts of the hair were also not ignored as a difference could differentiate between species. The patterns were identified using the scale identification nomenclature by Keogh (1983) and Bahuguna *et al*., 2010, followed by taking of scale and medulla pattern microphotographs using AmScope microscope camera.

Features Applied in the Study to Examine the Scats

As described by Lovari *et al*., (2009); Luca and Mpunga (2018), examination of undigested remains in a scat other than hairs, grass, bones and horn fragments, entire hooves and hair, were vital in defining the dietary habits in carnivores. However, hair recuperated from the carnivore scats alone cannot act as the base for comprehensive diet determination as it only accounts for between 18 and 48% of total diet consumed. Nevertheless, scat analysis has been used before to study diets of carnivores (Ogara *et al.*, 2010; Mwebi, 2013; Mutoro, 2015). Many times, carnivores, especially felids, ingest their own hair as they groom, hence scat analysis in some instances helps to determine the producer of the scat (Wacher *et al.,* 2006).

Since it was difficult to accurately identify carnivore species on the basis of scat morphology (i.e. shape, color, odor, mass, length, diameter) while in the field (Shehzad *et al*., 2012), further scat analysis in the laboratory for accuracy was important. To achieve this, measurement of scat, other forensic evidence noted in the field, coupled with scat analysis was necessary. Identification of scat was confirmed through DNA metabarcoding approach and the research was augmented with camera trap data. The research required establishment of reference hair collection. The reference hair specimens were obtained from National Museums of Kenya mammals' collection. These reference specimens were used for comparison with the samples obtained from the scats.

The study of hair structure followed standard methods outlined by Keogh (1983) using characters of the two main forms of hairs: 1) Guard hairs: the outer long and thick hairs with examples of awns, spines, and bristles and 2) Under fur hair: fine and short hairs with examples of fur, wool and vellus. The guard hairs are the commonly used in species identification where they are further categorized into primary and secondary hairs. The primary type was used in identification as they are always specific to the species (Bahuguna *et al*., 2010; Keogh, 1983). The external wall of the hair stem, the cuticle comprises of keratinous scales which are arranged in various patterns. The features used in this study on the hair structure were the cuticular and medulla structure patterns, which are illustrated in figure 6. There are five main cuticular structure patterns: Petal, chevron, coronal, pectinate and mosaic. These structures are unique for each species mammal species and that is why they were used as diagnostic tools in this study.

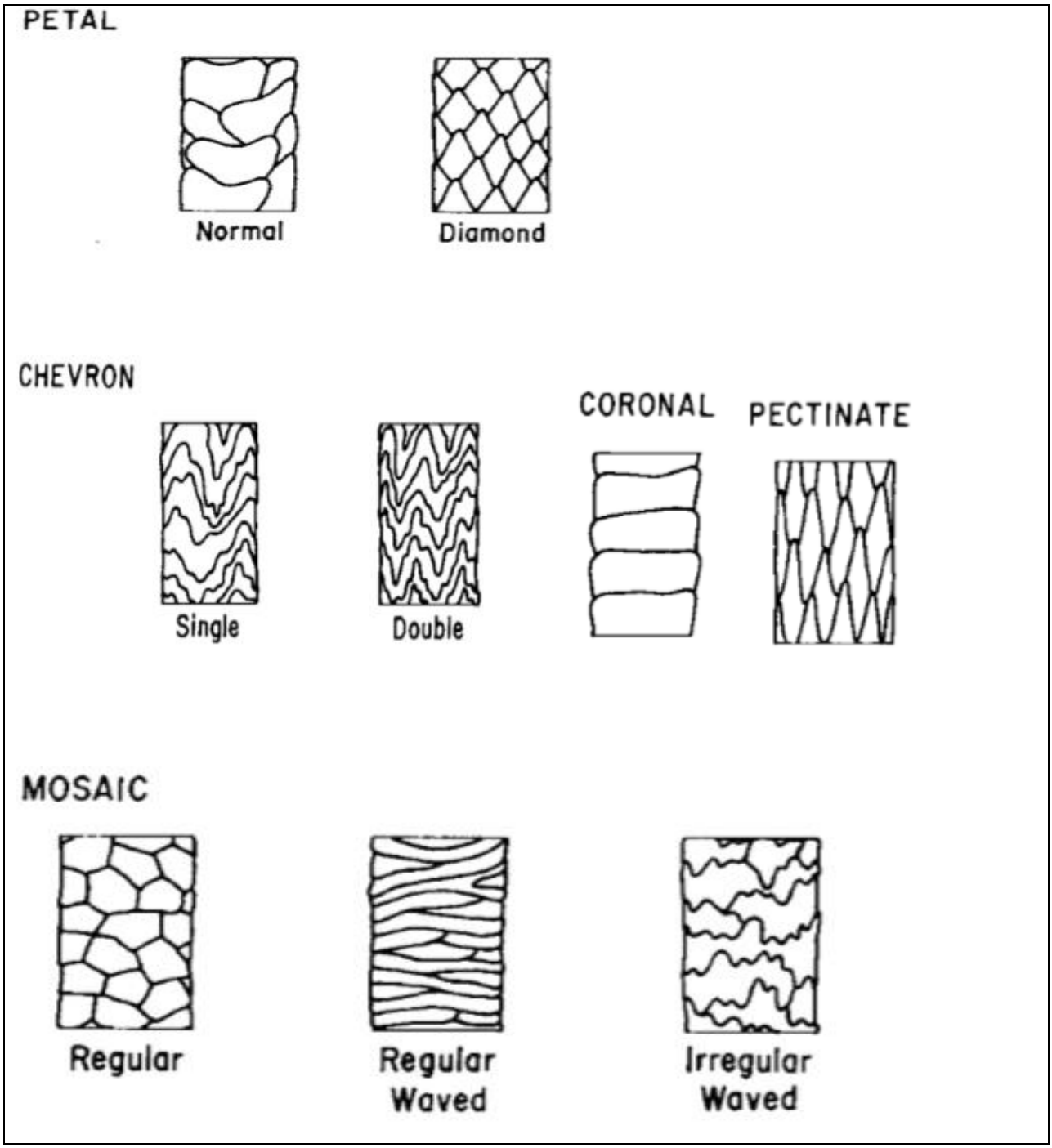
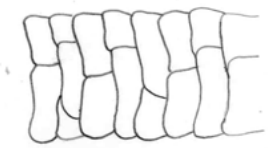
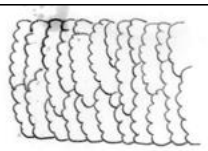
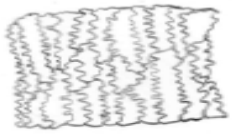
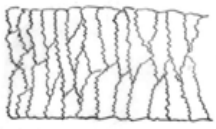
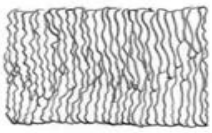
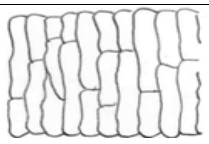
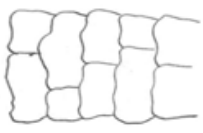


Figure 7: Cuticular scale patterns (imprints) of mammalian hair applied as reference to study the hair structure (Source: Keogh, 1983).

Table 2: Illustrations and descriptions of various forms of scale margins and distances between scale margins of a mammalian hair (Source: Keogh, 1983 and Bahuguna *et al.,* 2010).

FORM OF SCALE MARGINS			
1	Dentate Margin	The scale margin have toothlike projections.	
2	Smooth Margin	The margin of the scales is smooth wthout any serrations, indentations or ripples.	
3	Scalloped Margin	The margins comprises of series of culves with rounded peaks and pointed troughs.	
4	Rippled Margin	When compared to crenate pattern, the indentations are deeper and rounded.	
5	Crenate Margin	The scale margin comprises of shallow and pointed identations	
DISTANCES BETWEEN SCALE MARGINS			
1	Close	Scale margins close to each other	
2	Near	Scale margins near each other	
3	Distant	Scale margins distant to each other	

3.2.6 Molecular analysis for prey determination

Primer Design— DNA Metabarcoding requires comparison of unknown sequences to a DNA reference library. In this case a 200 base pair region of the mitochondrial 16S rRNA gene was amplified with primers modified from previously published primers (Kitano *et al.* 2007; Table 3).

Table 3: Primers used in this study

Published Primer Name	Primer Sequence	Novel Primer Name	Novel Primer Sequence (blue) with Illumina adapter sequence (red)
16SrRNA Kitano et al. 2007			
L2513-F	GCCTGTTTACCAAAAACATCAC	L2513-Fmod	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTGTTACCAAAAACATCRC
H2714-R	CTCCATAGGGTCTTCTCGTCTT	H2714-Rmod	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCAYRGGTCTCTTCTCGTCTT
Cytochrome oxidase I Meusnier et al. 2008			
Uni-MinibarF1	TCCACTAATCACAARGATATTGG	COI-MiniFmod	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCYACNAAYCAYAAAGAYATYGGYA
Uni-MinibarR1	GAAAATCATAATGAAGGCATGA	COI-MiniRmod	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAARATTATNAYRAANG

These primer sequences were compared with sequences of prey species known to occur on the mountain that were publically available on GenBank and therefore primers were modified to incorporate degenerate bases in the primer design (Table 3; L2513-Fmod and H2714-Rmod). In addition to the locus-specific sequence, the primers also contained the Illumina overhang adapter sequence (Table 3: red versus blue nucleotides).

Reference species database was set up using the 'Spatial Query' function in QGIS 2.8.1. In this, polygons were selected representing mammalian ranges (IUCN) that intersected polygons of either Mount Kenya National Park or Mount Kenya National Forest Reserve, which surrounds the park. This query resulted in 195 ranges of mammals (including several domestic animal species). We further reduced this database using expert opinions regarding what would logically occur on the western slopes of Mt. Kenya given that the habitat type hosted 135 taxa.

DNA extraction: To obtain a broad spectrum of potential prey, samples were first homogenized by hand in their plastic collection bags, then a sterile wooden stick was used to scrape samples along the length of the plastic and placed in the extract collection tube. Genomic DNA was extracted from fecal samples using the ZR Fecal DNA Mini prep kit (Zymo Research) following manufacture's protocols (Zymo Research). In usual scat samples, isolation of DNA is low and abundance so careful laboratory procedures were applied to obtain reliable individual genotypes. Genomic Library sets a collection of total genomic DNA from a single organism, therefore it was the most preferable in this study for the often dilute and degraded DNA in the scats samples.

Library Preparation: The 16S rRNA gene region was amplified following Illumina's metagenomics library preparation protocol that includes two PCR reactions. An initial amplification PCR was set up using 10-15 ng/μL of genomic DNA, 5 μM of each primer, and 12.5 μL of 2X HiFi Hot Start Ready Mix (Kapa Biosystems) in a 25 μL reaction. Reactions were denatured at 95°C for 3 minutes followed by 25 cycles of: 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec; followed by a final extension at 72°C for 5 min. Products were visualized on a 1.5-2% agarose gel stained with gel red using a 100 bp ladder. AMPure XP (Agencourt Bioscience) beads were used to purify PCR products and remove primer dimer following

manufactures protocol. A second indexing PCR was performed to attach Nextera-style dual indices and Illumina sequencing adapters (i.e. i5 and i7 primers). A 50 μL reaction included 5 μL of each indexing primer, 25 μL of 2X HiFi HotStart Ready Mix, 5 μL of DNA (from previous reaction), and 10 μL of water. Reactions were placed on the thermal cycler with the same conditions as above, except 8 cycles were performed, rather than 25 cycles. Products were visualized on a 1.5-2% agarose gel stained with gel red using a 100 bp ladder. AMPure XP beads were used to purify PCR products and remove primer dimer following manufacturer's protocol.

Quantification, pooling, and sequencing: A subset of samples were validated using an Agilent High Sensitivity DNA Kit (Agilent) where the correct size for each of 16S and COI gene regions were amplified for verification. Samples were quantified using Qubit (Life Technologies) fluorometric quantification method. DNA concentrations in nM were calculated for each library following the Illumina protocol. Each library was diluted to 4 nM using 10 mM Tris pH 8.5. Finally, 5 μL of each diluted library was combined for the sequencing run. Sequences were generated on a single MiSeq run using a 300 cycle v2 PE kit (Illumina).

Quality Control for Sequencing Reads: The reads are the numerical representation of sample composition. The paired-end reads were merged using PEAR version 0.9.4. Illumina adapter sequences were trimmed using Trim Galore version 0.4.1. Quality filtering of reads was performed using Prinseq-lite version 0.20.4 to remove reads with a minimum quality score mean below 20 and all exact duplicates, 5' duplicates, and reverse complement duplicates. The obtained sequence read counts are converted to occurrence where the number of reads required for each taxon to be tailed as an occurrence had to reach the required threshold (i.e. present / absence of a taxa). To provide an overall diet summary, relative read abundance (RRA) values

that are sample – specific are averaged focusing on samples with sufficient target DNA and checking replicates hence giving the diet as an approximation for the relative biomass consumed.

3.3 Data Analysis

Data for statistical analysis was tested for normality using Shapiro-Wilk Test. Comparisons were made on means for samples obtained from a normal distribution. Statistical tests were performed using PAST software. One-way ANOVA test was used to examine whether prey consumption rates of the leopard diet varied with the habitat types. A paired t-test was also conducted to examine the differences in consumption rates of different prey items consumed by both the leopard and spotted hyena within the various habitat types. In all the statistical tests conducted, the differences were deemed statistically significant if $\alpha < 0.05$. The mean values were reported as means ± SE. Habitat type and altitude data recorded in the field were fed into Geographical Information System (GIS) (Arc View 3.2). This was then interpreted from Landsat TM scenes to show the vegetation boundaries within Mt. Kenya ecosystem following dominance species approach. Relative abundance of hyena and leopards' scats were estimated through the number of scats collected every 10 km of survey (Wilson and Delahay, 2001).

As a result of deployment of camera traps 221 times, for a total of 5,721 trap nights, the wild animal detections were revealed covering the sampling area (22.5km^2) through camera trap data analysis. SECR(Spatially Explicit Capture-Recapture) version 3.1.6 program in R was used in data analysis. The model was used to estimate the mammal species densities in the study area. The data obtained were summed up and used to estimate the number of various mammal species within each habitat zone and the total number of trap-nights per zone. This was further reduced to get the number of animals per day for each zone. The area covered by various vegetation zones was indicated by an editted version of Bussmann (2006) through GIS mapping using Arc

View X-Tools extension to show the total area covered by each zone from Landsat images. This was important in revealing the foraging ranges of potential prey species of the two carnivores within Mt. Kenya ecosystem. Corresponding coordinates of the scats sample collection points were also overlaid on the map of the study area. This helped to map out the distribution of the two carnivores scats in the study area. That scat distribution was superimposed on the habitat types along altitudinal gradient.

In metabarcoding scats analysis, a perl script was used to split individual scat samples into file folders that contained 10,000 sequencing reads each. All of the reads for each individual scat sample were then compared to the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) version 2.2.29 and an .xml file was saved for each scat sample. Files were imported into MEtaGenome ANalyzer (MEGAN) version 6.7.18 with the following lowest common ancestor (LCA) parameters: minimum support 1, minimum score 35, top percent 5, win-score 0 and no minimum complexity filter. Samples were analyzed at the family and genus levels to determine the diet of these two carnivores. It was also important in confirming the predator owner of each analyzed scat.

The diet was also established through microscopic examination. In this, undigested remains from the washed scats such as the hairs, bone and horn fragments, entire hooves, teeth, and craws were used to identify the prey species of the respective carnivores. Diet analysis was carried out after positively identifying the predator owner of the scat sample through molecular analysis. This was important in calculating frequency of occurrence of diet items (percentage of scats containing a specific food item), relative percent occurrence of diet items, estimation of dietary niche breadth and deriving dietary niche overlap.

Frequency of occurrence was calculated as the number of times a diet category was present in a sample over the total number of scat samples for that predator. Ideally one scat sample may contain multiple food items; hence, frequency of occurrence of all diet items for a predator species can sum to >100%. In this case, the relative percent occurrence was also calculated by dividing frequency of occurrence for each category of diet by the sum of all frequency of occurrences for each predator species to provide proportional occurrence indication of diet items in leopard and hyena diets as described by Pianka (1974).

The dietary niche breadth was estimated by considering the number of different diet items together with the proportions in which those items were found in the scats. It can range from 1 to the total number of diet substances found in individual predator species as defined in Morin *et al.,* (2016). An estimate that is close to the total number of possible diet items, suggests that all possible diet items are being consumed in equal proportions. Where the estimate is close to 1 it's an indication that there is a narrow dietary niche with a large proportion of diet consisting of just a few items.

$$\text{Niche breadth (B)} = \frac{1}{\sum \%\text{occurrence for each diet item}^2.} \qquad \text{Equation 1}$$

The obtained measures were standardized on a scale of 0 to 1 using the formula below;

$$B_A = \frac{B-1}{n-1} \qquad \text{Equation 2}$$

Where B_A is the standardized niche breadth, and n is the total number of food items found in scats of the species of interest.

Niche overlap (C) was derived from the dietary-niche breadth estimates for the two predators (leopard and hyena). An index of niche overlap (C) was used to assess similarity of food

composition and habitat utilization between leopard and spotted hyena. That is the Pianka's niche overlap index (C) Pianka (1974), which has a distribution ranging from 0 (indicating that no diet items are being shared between the 2 predators) to 1 (indicating a complete diet overlap between the 2 species)

$$C = \frac{\sum \%\text{occurrence for each diet item for predator A} \times \%\text{ occurrence for each diet item for predator B}}{\sqrt{\sum \%\text{ occurrence for each diet item for predator A}^2 \times \sum \%\text{ occurrence for each diet for predator B}^2}} \quad (3)$$

The habitats used for hunting of prey in Burguret trail of Mt. Kenya were treated as equally available to leopard and hyena. Thus, the two carnivores were equally constrained by prevailing ecological conditions.

CHAPTER FOUR: RESULTS

4.1 Diet, availability of prey and utilization

Scat analysis for the leopard and the spotted hyena involved both morphological and molecular analysis. Findings from the two types of analysis revealed the diet composition of these two key mammal species as well as the rates of consumption of the prey species. Of the 135 taxa known to occur on Mt. Kenya, 110 had 16S rRNA gene sequences available on GenBank. Despite amplifying only 200 base pairs of the 16S rRNA gene, this region appeared to exhibit enough variation to distinguish between most species and all genera.

For the molecular analysis, a total of 85 scat samples were extracted, including two negative controls. Out of these, three of the scat samples did not produce any reads (numerical representation of sample composition). The remaining 80 scat samples resulted in 4,059,999 good quality reads after quality filtering. The overall proportion of reads per sample was used to detect the host species from which the scat sample was derived. Leopards comprised 61 samples representing 3,079,067 of the total reads. The next most numerous host was serval (6 samples, 223,159 reads), followed by genet (5 samples, 239,363 reads), and hyena (2 samples, 225,820 reads). Metabarcoding studies uses the sequence read counts, to record the occurrence of food species within a sample based on sequences threshold number (i.e. present/absence of a taxa) or to calculate the DNA percentage belonging to each food species as an approximation for the relative biomass consumed (i.e. the relative abundance of a taxa) hence giving the variety of diet species within a sample (scat).

Of the 31 mammalian families of potential prey that were known to occur within the Mt. Kenya ecosystem, mammals belonging to fourteen families were detected in the scat samples. In addition, out of the 102 potential genera known to occur on Mt. Kenya, 33 genera were

successfully identified across the scat samples for both leopard and the spotted hyena.

Based on the morphological analysis of scats using the hair reference collection, an average of 3.8 items were recorded per scat and 22 different prey items were identified from the analyzed scat samples. The prey species identified from leopards' scats were fourteen while the rest of the species were from spotted hyena scats.

The scats were distributed at different altitude levels and vegetation types (Fig. 8). Most of the scats (67%) were found in Afroalpine and Lower montane forest zones while 33% of the scats were in the Upper montane and Bamboo forest zones. The leopard scats were found to be distributed in all elevation zones and vegetation types along the Burguret trail indicating that the leopard moved over a wide range of elevation and used all the habitats for foraging. In contrast, hyena scats were found in the Lower montane forest and the Bamboo forest zones. This distribution indicates that foraging hyenas were restricted to the lower altitudinal levels and only in two vegetation zones. The low number of scats and their absence in the upper vegetation zones suggests that foraging hyenas did not utilize the upper altitude zones. Of the two predators, only the leopard that appears to have the ability to expand its foraging range up to the top of the mountain.

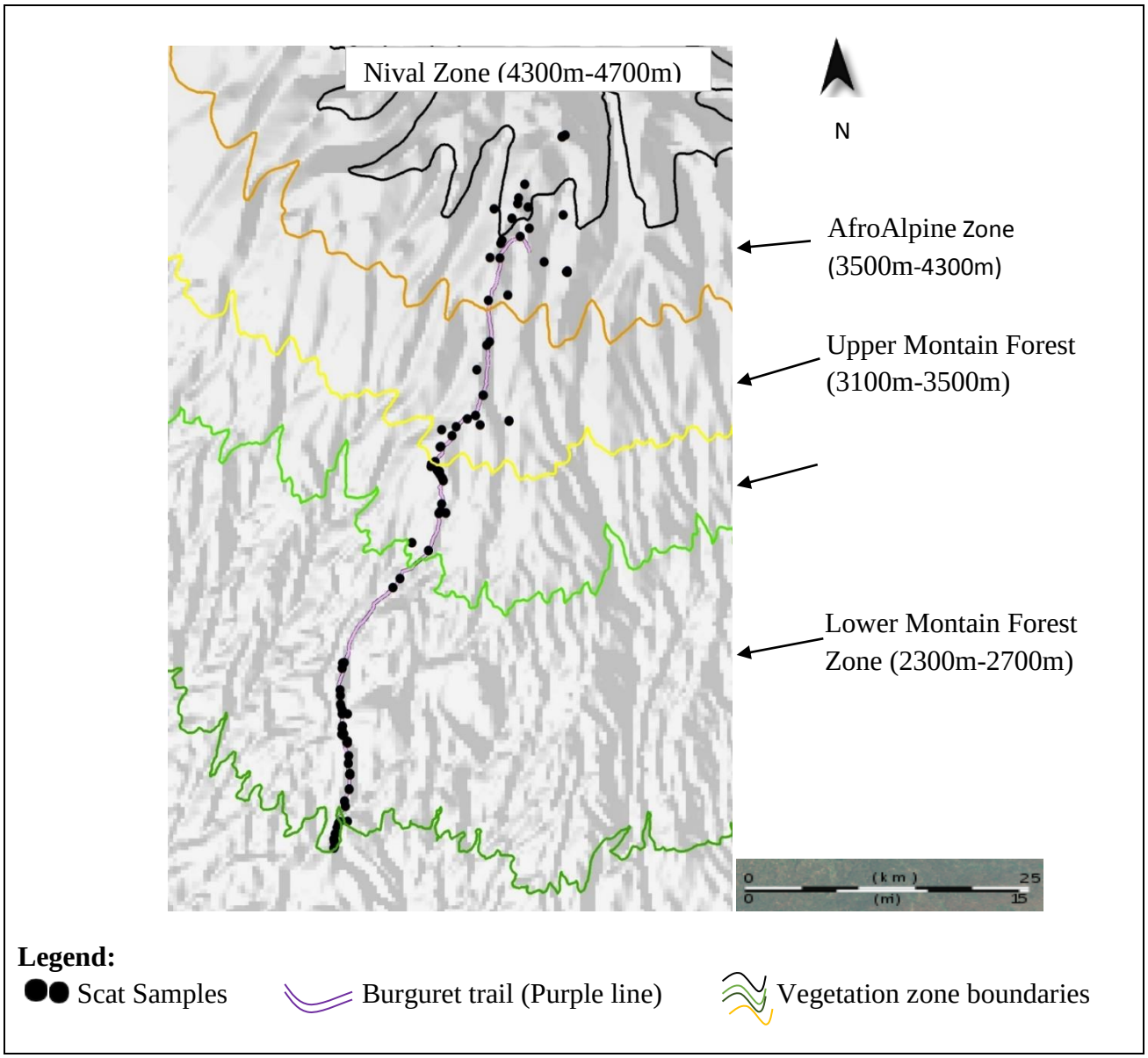


Figure 8: Surveyed vegetation types and the distribution of carnivore scat samples along Burguret trail, Mt Kenya ecosystem.

4.2 Habitat types in the study area

Within the study area, six vegetation types were identified, which were characterized by different dominant tree species. The sampled area was within the protected area (Mt. Kenya NP and Mt. Kenya Forest) and adjacent this area laid an Australian cedar *(Toona ciliata)* plantation/forestry zone where camera traps were also installed. The mountain's vertical vegetation characterization

can be described as follows; a lower montain forest at its base where *Podocarpus latifolis* dominated. Above this is a bamboo forest with uniform thickets where *Arundinaria alpine* was dominant with dense culms. This was followed by upper montain forest/ cloud forest comprising of thick evergreen vegetation dominated by East African Cedar *(Juniperus procera)* and African rosewood *(Hagenia abbysinica).* Above it is the Ericaceous zone which is mainly dominated by elephant grass (*Eleusine jaegeri), Erica arborea*, and other *Erica* species, such as *Festuca pilgeri* and tussock grass, which dominated the ground cover. Above this zone is the Afroalpine zone / Paramo which has wet sites, dry sites and other sites with baren soil / rock. It is mainly characterized by low vegetation comprising of common tussock grasses, such as *Festuca pilgeri*, sedge *Carex monostachyus*, herbs growing a few centimeters from the ground, Giant rosette plants (*Senecio* and *Lobelia*) and dwarf shrubs often growing like hemispherical cushions. The other zone is the cold desert/Nival zone. This zone doesn't have continuous vegetation, but occasional shrubs of *Dedrosenecio brassica* and *Lobelia telekii and Lobelia keniensis*. The soil is mostly bare moraine, gravel and stones with generally less than 10% plant cover. Figure 9 below clearly indicates the habitat types (represented by the dominant species), their boundaries and changes in altitude. The key to various vegetation zones are:

F- Forestry zone

LMF- Lower Montain Forest

UMF- Upper Montain Forest

AA- AfroAlpine Zone

N- Nival Zone

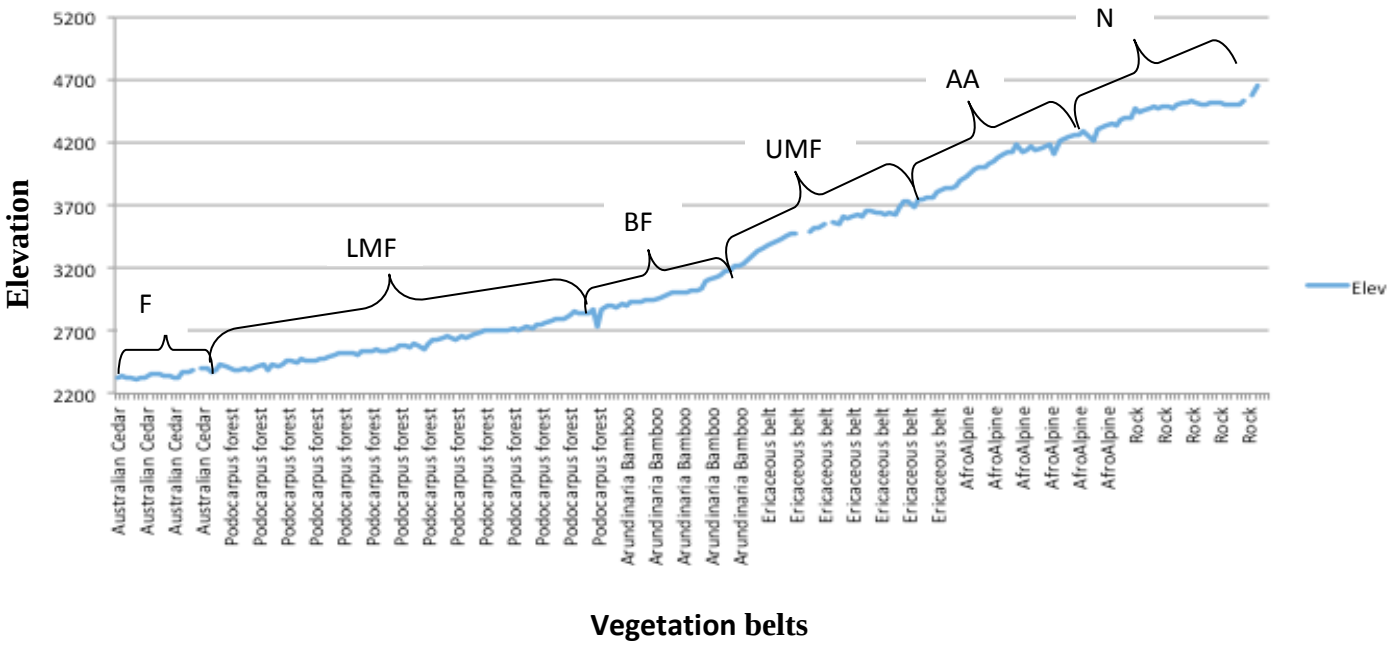


Figure 9: Habitat types in relation to altitude along Burguret Trail in Mt. Kenya

4.3 Diversity of the leopard and spotted hyena prey base in the various vegetation zones

Following deployment of camera traps 221 times in a 23 km belt transect covering an area of 22.5km^2, there were a total of 2566 wild ungulates detections. The SECR program in R produced a density estimate of 0.24 animals/km^2 (-/+ 0.01) as the 95% confidence limit. Suni *(Neotragus moschatus*) and rock hyrax (*Procavia capensis*) were the most abundant species whose images were captured by the camera traps, each having a detection rate of 1.63% and 1.58% respectively. A total of 28 mammal species were detected in the study area using the camera traps. (Figure 10)

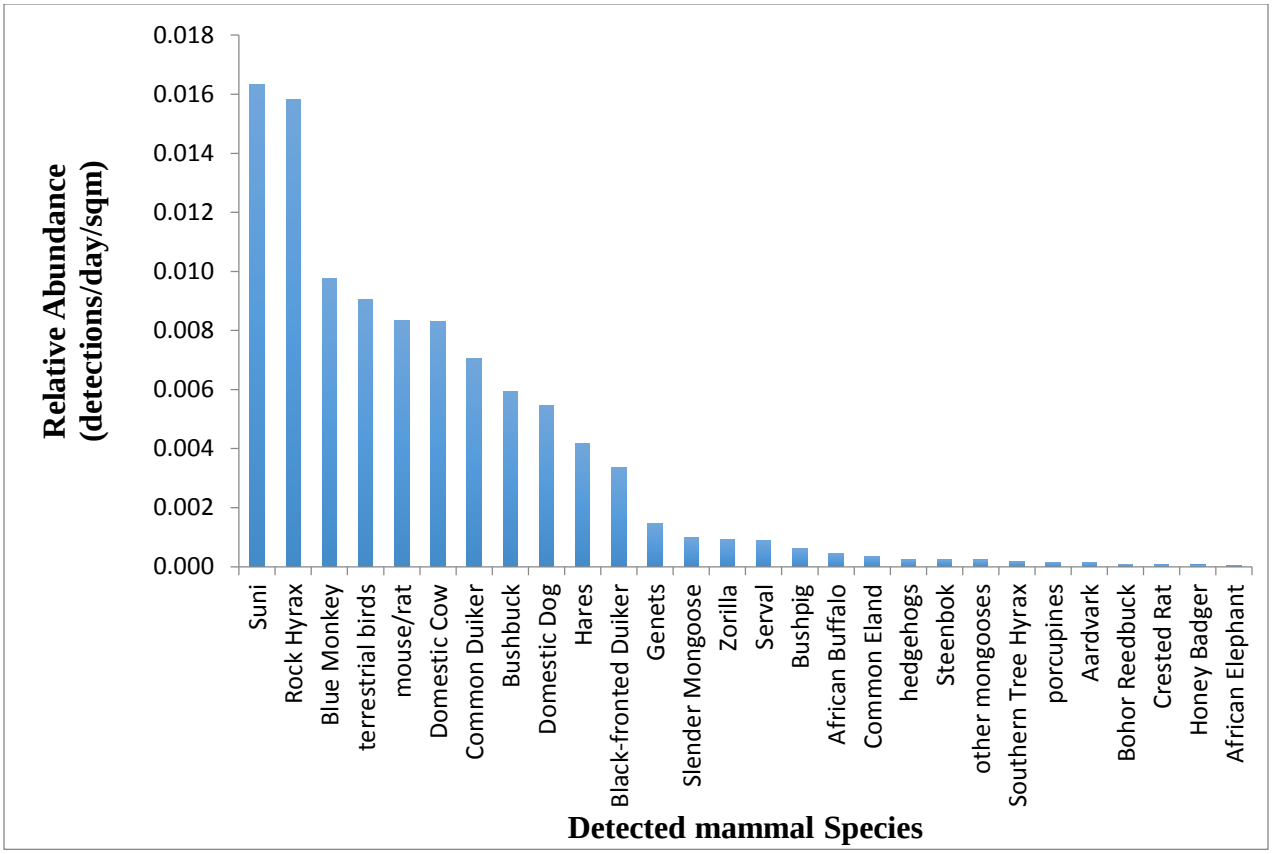


Figure 10: The leopard and hyena prey base in Burguret trail, Mt. Kenya ecosystem in Kenya.

4.4 Diet composition of the spotted hyena and utilization of prey species

The total number of Spotted hyenas' scats confirmed by both morphological and molecular analysis were two (n = 2). Results of the molecular analysis of the two scat samples showed that majority of sequences were those of prey species belonging to the families Suiidae (Artiodactyla) (42.3%), Cercopithecidae (Old world monkeys) (25.6%), Bovidae (Bovid) (15.5%), Soricidae (Shrews) (8.6%), Procaviidae (Hyraxes) (4.8%), Muridae (Rodents) (2.4%), Lorisidae (Primates) (0.5%), and Equiidae (0.2%) (Fig. 11). Clearly hyenas had a very diverse diet but dominated by duikers, vervet monkeys and large antelopes.

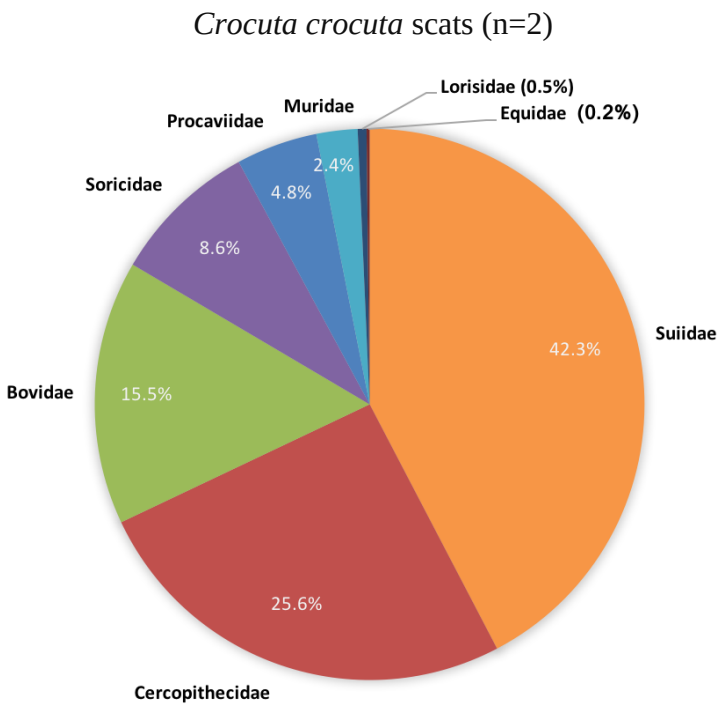


Figure 11: Proportion of sequencing reads sorted by family from *Crocuta crocuta* scat samples.

Morphological analysis of the spotted hyena scats revealed that the spotted hyenas consumed eight mammalian prey species; Bush pig (*Potamochoerus larvatus)* (22.2%), Mount Kenya Potto *(Perodicticus potto)* (11.1%), Sykes' (blue) Monkey (*Cercopithecus mitis)* (11.1%), Suni (*Neotragus mastatus)* (11.1%),Tropical Vlei (groove toothed) rat (*Otomys tropicalis)* (11.1%), Olive Baboon (*Papio Anubis)* (11.1%), Common Genet (*Genetta genetta)* (11.1%), and Mount Kenya Rock Hyrax (*Provavia johnstoni)* (11.1%). Out of the eight prey species consumed by the spotted hyena, bush pig (*Potamochoerus larvatus)* had the greatest frequency of occurrence. Other prey species had almost similar frequency of occurrence as revealed by morphological analysis (Fig. 12).

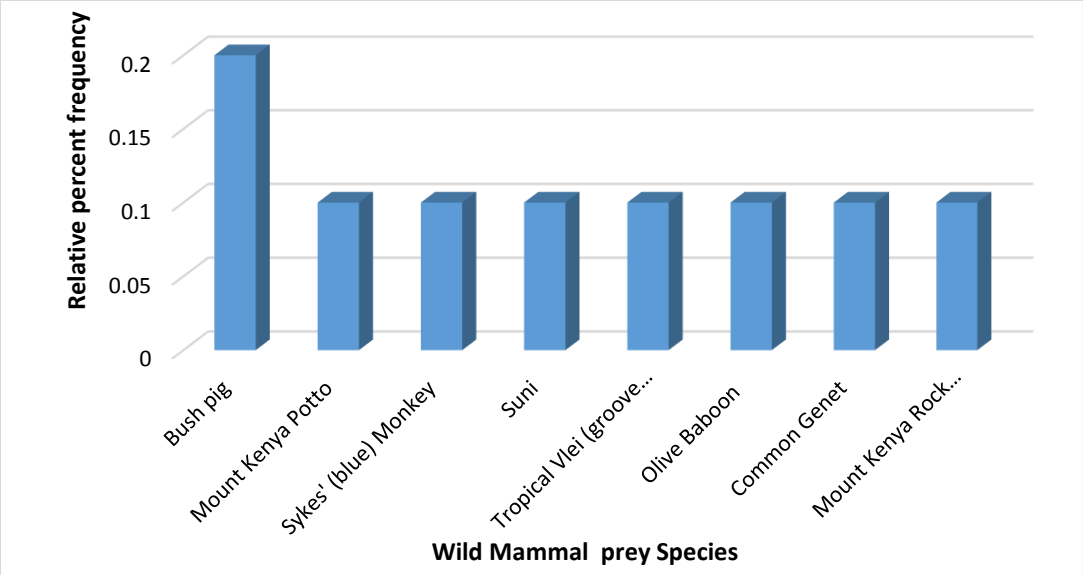


Figure 12: Relative frequency of occurrence of diet items in the spotted hyena scats in Burguret trail, Mt. Kenya ecosystem.

4.5 Diet composition of the leopard and its utilization of prey species

Morphological (n=59) and molecular analysis (n=61) of the leopard scats was conducted. Molecular analysis of the leopard scat revealed that the highest proportion of sequences reads were for prey species in the family's Procaviidae (hyraxes) (63.5%), followed by Cercopithecidae (Old world monkeys) (11.8%), Bovidae (Bovids) (11.5%), Soricidae (Shrews) (7%), and Muridae (Rodents) (5.6%). The remaining sequences included a low number of Suiidae (Artiodactyla), Equidae (Zebra family), and Sciuridae (Squirrels) (combined 0.6%) (Figure 13).

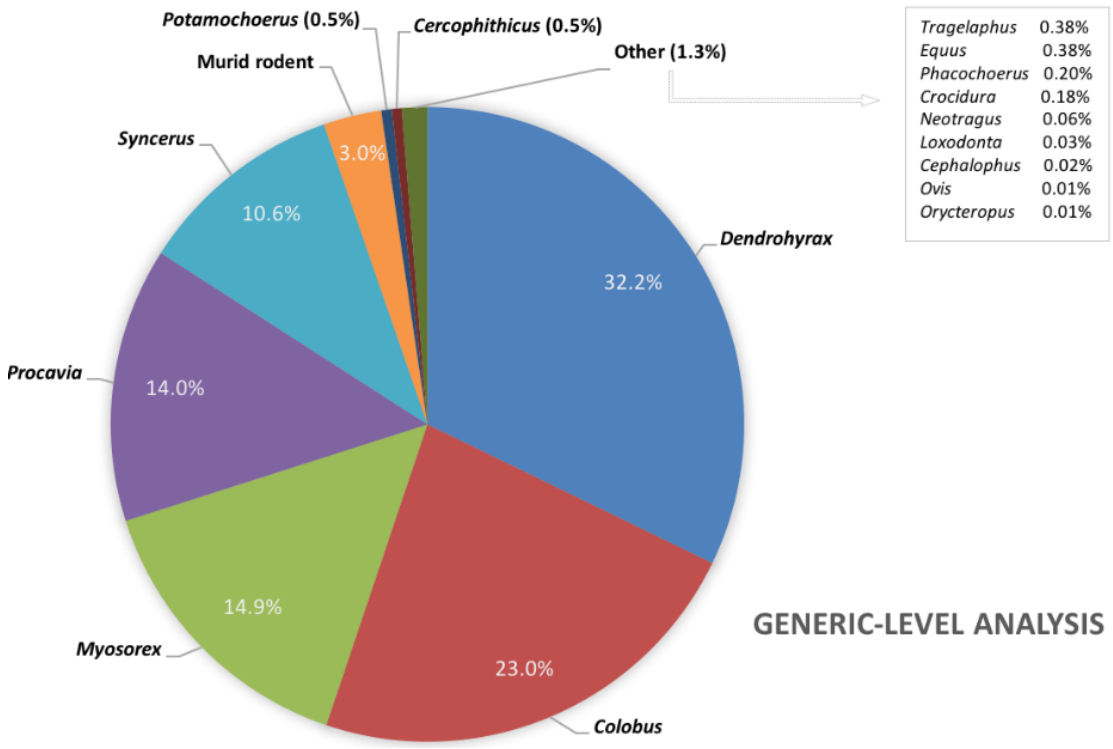


Figure 13: Proportion of sequencing reads sorted by genera for 61 *Panthera pardus* scat samples.

Of the 28 identified potential prey species, 19 prey species were identified in leopard scat through morphological analysis as indicated in Figure 14 below. The most important prey species for the leopard were Mt. Kenya rock hyrax (*Provavia johnstoni*), Tropical Vlei (groove toothed) rat (*Otomys tropicalis),* and Suni (*Neotragus mastatus),* as they occurred in nearly 50% of the scats that were collected and analyzed. Within the leopards' scats there was also an unidentified rodent that had a relative percent occurrence of 0.04. The Rufous elephant shrew (*Elephantulus rufescens),* Kirk's/ Gunther's dikdik (*Madoqua guentheri / kirkii)* and Olive baboon (*Papio anubis)* had the lowest occurrence rate.

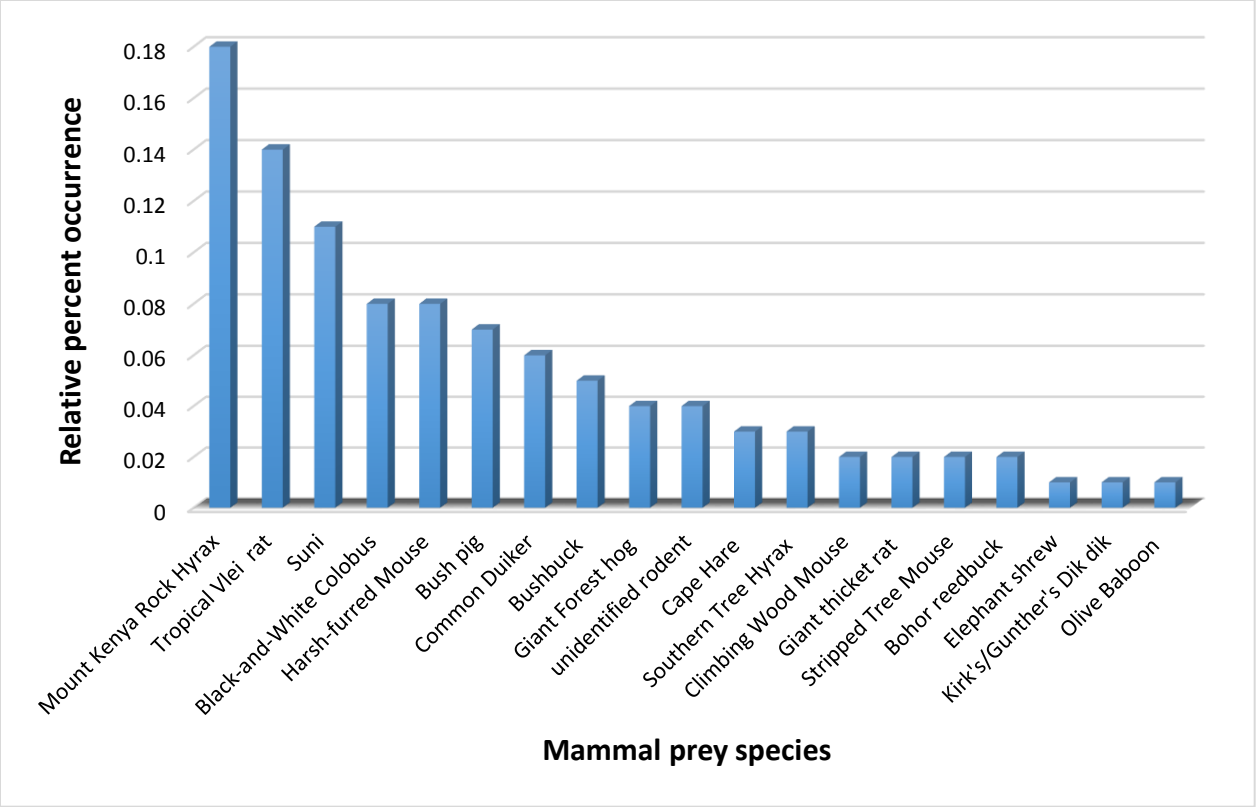


Figure 14: Relative frequency of occurrence of prey species in leopard scats collected in Burguret trail Mt. Kenya ecosystem

4.6 Variation in diet of the leopard and spotted hyena across various habitat types

The prey species consumed by leopards in various vegetation zones distributed along the altitudinal gradient of Burguret trail in Mt Kenya ecosystem are shown in Table 4. In the lower montane forest zone, leopards consumed 13 species of prey just as they did in the Afroalpine zone. However, the prey consisted of larger species in the lower montane forest zone and smaller prey species in Afroalpine zone. In the bamboo forest and Upper Montane zones, the leopards consumed eight species consisting of small and medium size animals. In the Nival zone, the leopards consumed seven species of medium and small sizes.

Table 4: The occurrence (Yes) and absence (No) of prey species consumed by leopards across various habitat types along Burguret trail, Mt. Kenya ecosystem.

Species	**Elevation (m)**				
Scientific Name	Lower Montain Forest Zone (2300-2700)	Bamboo Forest Zone (2700–3100)	Upper Montain Forest Zone (3100–3500)	AfroAlpine Zone (3500–4300)	Nival Zone (4300–4700)
Lophuromys flavopunctatus	Yes	No	Yes	Yes	Yes
Neotragus mastatus	Yes	Yes	Yes	Yes	No
Tragelaphus scriptus	Yes	No	Yes	Yes	No
Colobus guereza	Yes	No	Yes	No	Yes
Dendrohyrax arboreus	Yes	Yes	No	Yes	No
Procavia johnstoni	Yes	Yes	Yes	Yes	Yes
Hylochoerus meinertzhageni	Yes	Yes	No	No	No
Papio anubis	Yes	No	No	No	No
Redunca redunca	Yes	No	No	Yes	No
Praomys (Hylomyscus) denniae	Yes	No	No	No	No
Grammomys ibeanus	Yes	No	No	No	No
Otomys tropicalis	Yes	Yes	No	Yes	Yes
Sylvicapra grimmia	Yes	Yes	Yes	Yes	No
Potamochoerus larvatus	No	No	Yes	No	Yes
Praomys (Hylomyscus) denniae	No	Yes	No	No	No
Potamochoerus larvatus	No	Yes	No	Yes	No
Madoqua guentheri/ kirkii	No	No	Yes	No	No
Dendromus insignis percivali	No	No	No	Yes	No
Elephantulus rufescens /brachyrhynchus	No	No	No	Yes	No
Lepus capensis	No	No	No	Yes	Yes
Lemniscomys striatus/zebra	No	No	No	Yes	No
Cercopithecus mitis	No	No	No	No	Yes

A one-way ANOVA test was used to examine whether consumption rates of prey by the leopard varied with the habitat types. The results revealed that the leopard diet did not vary significantly with the habitat type (F = 1.508; *df* = 5; P = 0.49). The mean consumption rates of prey in the lower montane forest zone was 1.5 ± 0.2, 0.39 ± 0.1 in the bamboo forest zone, 0.27 ± 0.1 in the upper montane forest zone, 0.5 ± 0.1 in Afroalpine zone and 1.1 ± 0.4 in Nival zone (Figure 15).

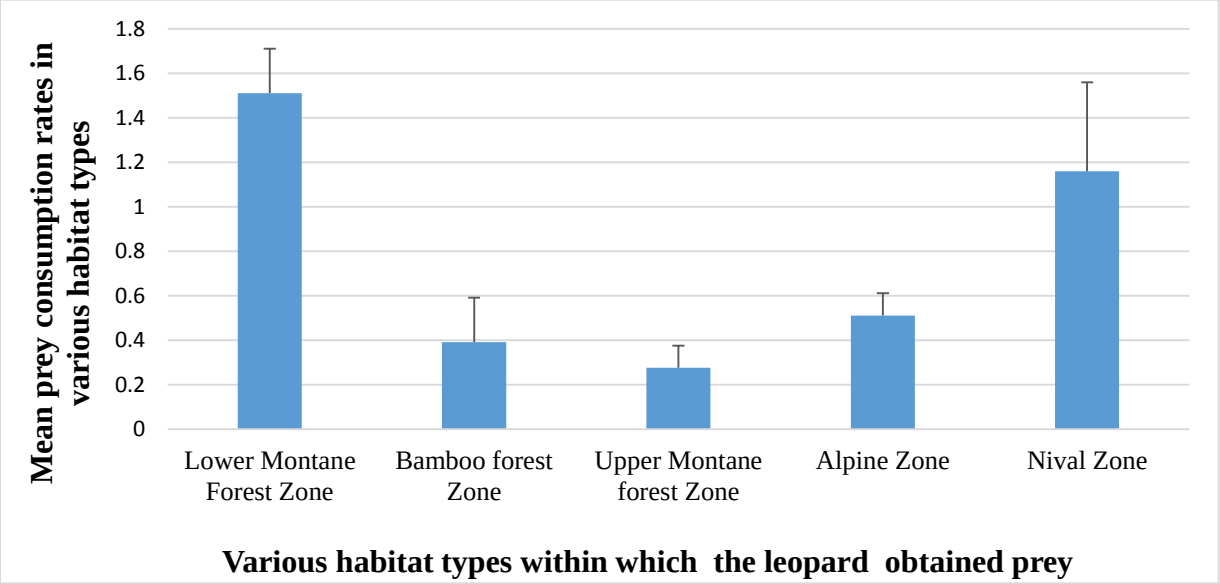


Figure 15: Mean consumption rates (± SE) of prey by leopard in various habitat types

For the spotted hyena, the two scats that were analyzed were only collected in two habitat types; one in bamboo forest zone and the other in lower montane forest zone. Table 5 below shows the various prey types identified in hyena scats found in the two vegetation zones. The prey types were predominantly of larger animals than those consumed by the leopard in the same zones.

Table 5: Species composition in spotted hyena's diet across the habitat types.

Vegetation Type	**Elevation Band (m)**	**Type of prey**	
		Scientific Name	**Common Name**
Lower Montain Forest Zone	2300 - 2700	*Otomys tropicalis*	Tropical Vlei (groove toothed) rat
		Perodicticus potto	Mount Kenya Potto
		Cercopithecus mitis	Sykes' (Blue) Monkey
		Neotragus mastatus	Suni
		Potamochoerus larvatus	Bush pig
Bamboo Forest Zone	2700 - 3100	*Procavia johnstoni*	Mount Kenya Rock Hyrax
		Potamochoerus larvatus	Bush pig
		Genetta genetta	Common Genet
		Papio anubis	Olive Baboon

4.7 Dietary overlap between the two species

Prey items that were consumed both by the leopard and the spotted hyena included the Mt. Kenya hyrax (*Procavia johnstoni),* tropical vlei rat (*Otomys tropicalis),* Suni (*Neotragus mastatus),* Olive baboon (*Papio anubis),* and bush pig (*Potamochoerus larvatus).*

A paired t-test to examine differences in consumption rates of different prey consumed by both leopard and spotted hyena within the various habitat types confirmed that there was significant difference in consumption of Mt. Kenya rock hyrax ($t_{2,8} = 5.7$, $P < 0.05$), tropical vlei rat ($t_{2,8} = 1.89$, $P < 0.05$), Suni ($t_{2,8} = 2.06$, $P < 0.05$) and bush pig ($t_{2,8} = 1.5$, $P > 0.05$) (Fig. 15).

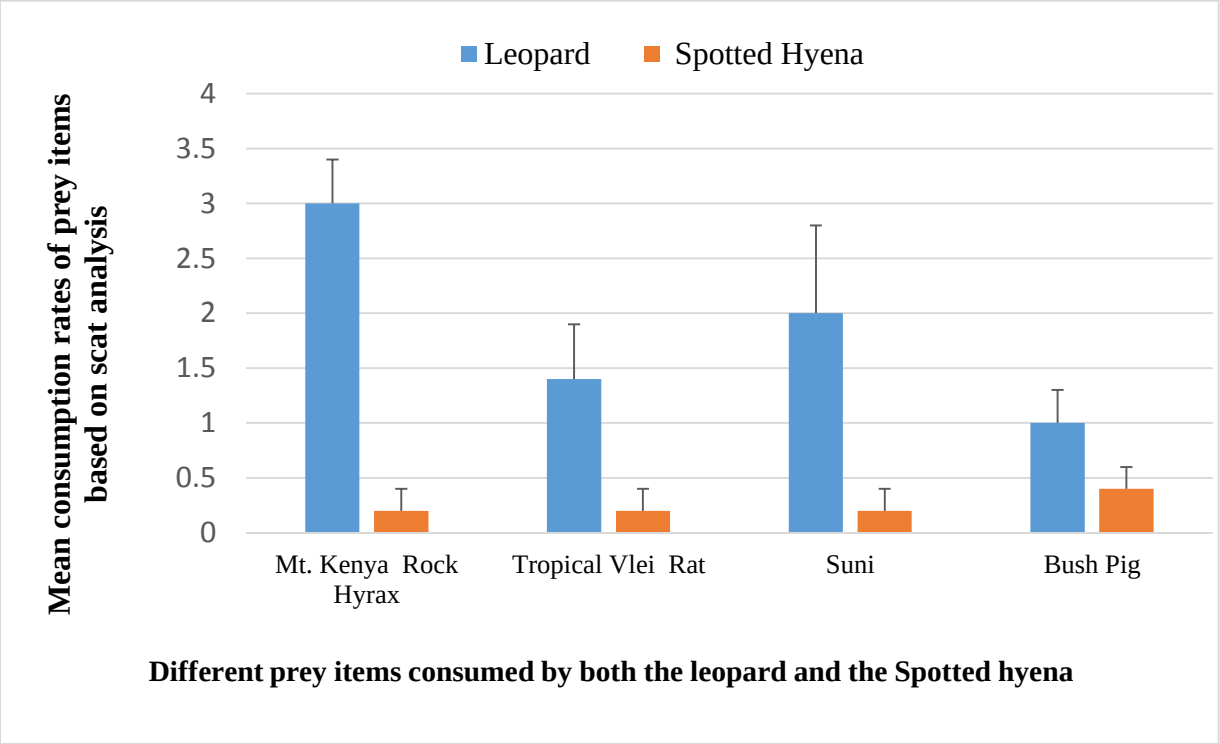


Figure 16: Mean consumption rates (± SE) of different prey items by the leopard and the spotted hyena based on morphological scat analysis.

Of the nine species of mammalian prey consumed by the hyena in the lower montane bamboo forest zones, seven species were also consumed by the leopard in the same zones. Only two

species (Mt. Kenya Potto and common genet were found in the hyena scats but not in the leopard scats. The estimated dietary niche breadth for hyena and leopard was 9.09 and 10.54 respectively with hyenas having a standardized niche breadth of 1.16 while the leopards' standardized niche breadth was 0.5. These niche breadth estimates suggest that, even though there was a broad variety of prey items in the diet of the two predators, they were just feeding on a few prey items but in large proportions. As a result, the two predators shared diet items in different proportions. The dietary niche overlap index between the two predators was low (Jaccard index, J = 0.19). This is an indication that the two predators never had a complete dietary overlap but still shared many food items despite the distribution of the scats indicating that they shared foraging habitat. However, prey became smaller and less abundant with increasing land elevation up the mountain. This apparent prey scarcity coincided with notable diversification of diet, especially by the leopard.

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMEDATIONS

5.1 Discussion

5.1.1 Distribution of the scats along the Burguret trail

Feeding strategies of predators can be controlled and shaped by environmental conditions in the foraging area such as prey density within an area, different habitat types or altitudes, which differ among different species (Sunquist and Sunquist, 1989). During this study, the leopard scats were found to be distributed in all elevation zones and vegetation types along the Burguret trail indicating that the leopard moved over a wide range of elevation and used all the habitats for foraging. Most of the scats, however, were found in lower montane forest and in the Afroalpine zone indicating that the leopard spent more time in these habitats than in the Bamboo forest, Upper montane and Nival zones.

In contrast, hyena scats were found in the Lower montane forest and the Bamboo forest zones. This distribution indicates that foraging hyenas were restricted to the lower altitudinal levels and only in two vegetation zones. The low number of scats and their absence in the upper vegetation zones suggests that foraging hyenas did not utilize the upper altitude zones. These predators may not have been adapted to the cold environment on the higher areas of the mountain. Of the two predators, only the leopard that appears to have the ability to expand its foraging range up to the top of the mountain.

5.1.2 Dietary composition in the target predators

Diet studies are frequently used to improve understanding of predator ecology, potential effects of carnivores on prey populations, and competition among predators. The dietary results of the two top predators in Mt. Kenya ecosystem gave an indication that the leopard and hyena are

partitioning food resources within this ecosystem. This also suggests that there is a potential for competition between these two species as dietary similarity increases. Their combined pressure may in turn affect the diversity of mammal species present in the study area. However, there was no direct evidence that the presence of one predator influenced the population dynamics of the other predator. The leopards could be residents of Mt. Kenya ecosystem as more of their scats (n=59) were collected in the various vegetation zones: Lower montane forest zone (36%), bamboo forest zone (19%), upper montane forest zone (10%), Afroalpine zone (27%) and the nival zone (8%). Only two hyena scats collected in the lower montane forest zone (50%) and bamboo forest zone (50%) which possibly suggests that hyenas could be transients in this ecosystem as they only foraged on the lower altitudes of the mountain.

The fact that leopards frequently consumed Mount Kenya rock hyrax and the tropical vlei rat, implies that the leopards in the mountain zones can subsist on small mammals, such as the rodents and hyraxes. This research revealed the importance of small mammals in the diet of leopards as predicted by Rödel *et al*., (2004) and Hayward *et al*., (2006). Both leopard and the spotted hyena consumed prey in terms of preference and not the prey abundance. Irrespective of the fact that suni had the greatest abundance in the study area it was not the highly consumed prey by both the spotted hyena and leopard.

The wide distribution of leopard scats along Burguret trail proved that leopards moved widely between the different vegetation zones of Mt. Kenya ecosystem. Noticeably, the southern tree hyraxes and the black-and-white colobus monkeys appeared in the diet from scats collected way above the tree line. The Mount Kenya rock hyraxes were found as prey in the scats collected way below the lower montane forest zone. In this case, this research fills the gap identified by Rödel

et al., (2004) on whether the leopards in the upper alpine zone of Mount Kenya have home ranges in the lower altitudes. While leopard scats had wide altitudinal distribution range (2300m - 4700m), hyena scats had a narrower altitudinal range (2300m - 3100m). It appears that leopards can withstand the environmental stress of reduced temperature and oxygen and forage for food in Afroalpine and Nival zones, where competition for food was mainly from birds of prey. This knowledge gap was filled by this study as it found that dietary constituents were derived from various habitat types. It is clear from the present research that the home ranges of leopards extended through the upper alpine zone to lower altitudes, such as the bamboo forest zone and the lower montane forest zone.

5.1.3 The importance of reference hair library

Species identification procedure using hairs, involved assignment of taxonomic names to unknown specimens using reference hair library of voucher specimens obtained from known species within the specific study area. In this study, the voucher specimens were obtained from museum collections from Mt Kenya area. This was important as the established reference library makes comparisons with other specimens easier, saving on time and resources. Hair collection of potential prey in the specific locality was used to identify diet of specific predators within the study area through microscopy scats analysis. These advances have boosted mammological research as they provide a basis for future research. This is because the reference libraries are made available to other scientists interested in a similar field studies. They also help to meet the role of museum in univocally labelling, storing and sharing reference biological materials.

5.1.4 Importance of keystone predators (leopard and spotted hyena) in Mt. Kenya ecosystem

Keystone species have special impacts on natural environment irrespective of their abundance (Miller *et al.,* 2001). The two apex predators in Mt Kenya ecosystem affects populations of prey species and other predators generally affecting the species population. This way they control the ecosystem functions. Leopards' diet in present research, mostly consisted of small mammals with greater consumption of the rock hyraxes and the tropical vlei rats. These predators are therefore controlling the populations of small mammals or else exerting lots consumption pressure on them generally affecting the ecosystem functions. It is also clear that the hyenas are consuming mesocarnivores e.g. genets, thus controlling both prey density and restriction of smaller predators which in turn affects the soil, floral and hydrological systems. These apex predators have noticeable dietary overlap and similar interaction structure which is an indication that they can easily replace each other in case one becomes extinct (Woodroffe and Ginsberg, 1998). Therefore they are of great value in an ecosystem.

5.1.5 Importance of molecular analysis in the study

Molecular identification of scats was important in this study as it ascertained the correct identity of the predator from the collected scat samples. This was crucial in selecting the number of scats to be used in the study from the total number of scat samples. In this study the number of scat samples that had high confidence for use in field identification were (30) with a couple (12) that had misidentification, compared to (80) scats that were successfully identified through molecular analysis and had good quality molecular reads. Field misidentification of most leopard scats to be hyenas' scats was evidence that there could have been a high potential bias in diet analysis if field identifications were not confirmed through molecular investigation prior morphological

analysis. This was because molecular results had high identification accuracy. The use of field identification results alone could have brought unintentional bias in diet analysis of the scats, thereby resulting in erroneous conclusions. In this study, the highest probability of bias could have arisen from misclassification of predator scat samples following field identification. Noninvasive genetic sampling advances allowed molecular identification of predator scats, eliminating many issues associated with field methods of identification. Therefore, molecular analysis is always important, especially if there are two sympatric carnivores within the study area. In this study, it was also essential to increase the total sample size for diet analysis. Another added advantage was that molecular approach helped in identifying foods that do not leave any hard remains (completely broken food particles) or simply prey types that lack the diagnostic taxonomic features of predators consuming them.

5.2 Conclusion and Recommendations

5.2.1 Conclusion

During this study, the leopard scats were found to be distributed in all elevation zones and vegetation types along the Burguret trail. This finding implied that the leopards moved over a wide range of elevation and used all the habitats for foraging. In contrast, the hyena scats were found in the Lower montane forest and the Bamboo forest zones. This distribution indicated that foraging hyenas were restricted to the lower altitudinal levels and only in the two vegetation zones. Of the two predators, only the leopard appeared to have the ability to expand its foraging range up the mountain. This could be related to changes in food supply as a result of the impacts of climate change on the mountain ecosystem.

The most important prey species for the leopard in Mt. Kenya ecosystem, were Mt. Kenya rock hyrax (*Provavia johnstoni*), Tropical Vlei (groove toothed) rat (*Otomys tropicalis)*, and Suni

(*Neotragus mastatus)*, as they occurred in nearly 50% of the scats. The spotted hyena scats revealed that the predator consumed both large and small mammalian prey species including mesocarnivores such as the genets. These included Olive Baboon (*Papio anubis)*, Bush pig (*Potamochoerus larvatus)*, Sykes' (blue) Monkey (*Cercopithecus mitis)*, Suni (*Neotragus mastatus)*, Common Genet (*Genetta genetta)*, Mount Kenya Potto *(Perodicticus potto)*, Mount Kenya Rock Hyrax (*Provavia johnstoni)* and the Tropical Vlei (groove toothed) rat (*Otomys tropicalis)*. Of the eight prey species, the bush pig (*Potamochoerus larvatus)* had the greatest frequency of occurrence with the other prey species being consumed almost equally.

The prey species consumed by leopards did not change significantly with altitude and various vegetation zones. In the lower montane forest zone, leopards consumed 13 species of prey just as they did in the Afroalpine zone. However, the prey consisted of larger species in the lower montane forest zone and smaller prey species in Afroalpine zone. In the bamboo forest and Upper Montane zones, the leopards consumed eight species consisting of small and medium size animals. In the Nival zone, the leopards consumed seven species of medium and small sizes. In contrast, the hyena consumed a wide range of species of herbivores and occasionally other predators but only two vegetation zones where scats were collected during this study.

There was little overlap in the overall diet of the leopard and the spotted hyena in the study area. Of the nine species of mammalian prey consumed by the hyena in the lower montane and bamboo forest zones, seven species were also consumed by the leopard in the same zones. Only two species (Mt. Kenya Potto and common genet were found in the hyena scats but not in the leopard scats. The dietary niche overlap index, between the two predators was low. This is an indication that the two predators never had a complete dietary overlap but still shared many food items with notable diversification of diet, especially by the leopard.

5.2.2 Recommendations

The following recommendations were made from the research findings.

- DNA analysis is essential for verifying species identity and ensuring reliable data collection, especially in predator identification during scats analysis. This is because the ability to distinguish scats accurately in the field is limited especially where there are sympatric carnivores.
- Future research is recommended to determine the impact of leopards on small mammal populations, especially the rock hyraxes and Mount Kenya potto. It will also be important to examine the spatial and temporal dynamics of leopards' diet in a mountain ecosystem.
- Further research on spotted hyenas can give a true picture of their diet in Mt. Kenya ecosystem as only two spotted hyena scats were available for analysis in the present research. Hence if a greater number of samples can be obtained and analyzed then a more accurate assessment of the predators' diet and habitat utilization can be obtained to give a true picture of their diet.
- A comprehensive hair reference Library of wild mammals of East Africa can facilitate easier analysis of carnivore scats. This would ensure hair reference library is available to other researchers engaging in a similar field of research, making hair analysis work less strenuous and less time consuming.
- Education and awareness is essential so as to change the perception of the local communities towards conservation of leopards and hyenas. Especially where human-wildlife conflict is prevalent and people have perceived that the wild animals only prey on their domestic animals which has not been the case in the present research. Therefore, community awareness is important to ensure their involvement in conservation activities as one of the key stakeholders.

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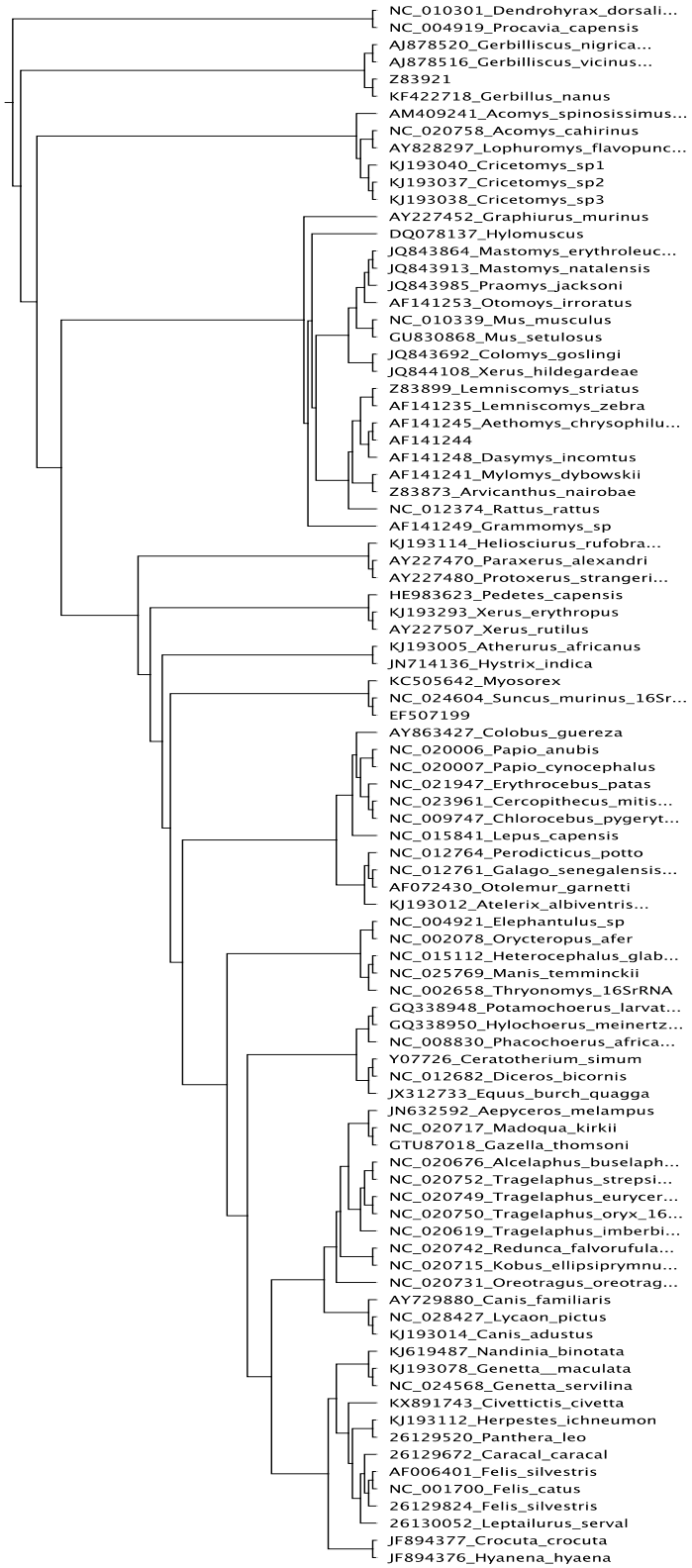
APPENDICES

Appendix I: List of animal species in the study area included in the hair reference library catalogue.

Common Name	**Scientific Name**	**Order**	**Family**
Mount Kenya Rock Hyrax	*Procavia johnstoni mackinderi*	Hyracoidea	Procaviidae
African Buffalo	*Syncerus caffer*	Artiodactyla	Bovidae
Suni	*Neotragus moschatus*	Artiodactyla	Bovidae
Bohor reedbuck	*Redunca redunca*	Artiodactyla	Bovidae
Olive Baboon	*Papio anubis*	Primates	Cercopithecidae
Giant Forest hog	*Hylochoerus meinertzhageni*	Artiodactyla	Suidae
Common Duiker	*Sylvicapra grimmia*	Artiodactyla	Bovidae
Mountain bongo	*Tragelaphus eurycerus*	Artiodactyla	Bovidae
African Elephant	*Loxodonta africana*	Proboscidea	Elephantidae
Southern Tree Hyrax	*Dendrohyrax arboreus*	Hyracoidea	Procaviidae
Spotted hyena	*Crocuta crocuta*	Carnivora	Hyaenidae
Bushbuck	*Tragelaphus scriptus*	Artiodactyla	Bovidae
African Leopard	*Panthera pardus*	Carnivora	Felidae
African Crested Porcupine	*Hystrix cristata*	Rodentia	Hystricidae
Abyssinian Black-and-White Colobus Monkey	*Colobus polykomos*	Primates	Cercopithecidae
Sykes' (blue) Monkey	*Cercopithecus mitis*	Primates	Cercopithecidae
Brush-furred Mouse	*Lophuromys flavopunctatus*	Rodentia	Muridae
Climbing Wood Mouse	*Praomys (Hylomyscus) denniae (Hylomyscus) denniae*	Rodentia	Muridae
Groove-toothed rat	*Otomys tropicalis tropicalis / O. orestesels orestesels*	Rodentia	Muridae
Four-striped Grass Mouse	*Rhabdomys pumilo diminutus*	Rodentia	Muridae
White-toothed shrews	*Crocidura spp*	Eulipotyphyla	Soricidae
Mount Kenya Mole Rat	*Tachyoryctes splendens*	Rodentia	Spalacidae
Giant thicket rat	*Grammomys ibeanus*	Rodentia	Muridae
Aardvark	*Orycteropus afer*	Tubulidentata	Orycteropodidae
Stripped Tree Mouse	*Dendromus insignis percivali*	Rodentia	Muridae
Bush pig	*Potamochoerus larvatus*	Artiodactyla	Suidae
Hare	*Lepus sp.*	Lagomorpha	Leporidae
Common Genet	*Genetta genetta*	Carnivora	Viverridae
Potto	*Perodicticus potto*	Primates	Lorisidae

Appendix II: Neighbor-joining tree constructed using GenBank sequences containing the 200 base pair 16S rRNA region used in the metabarcoding analysis demonstrating the utility of the gene region to discern differences between various taxa on Mt. Kenya.



Appendix III: List of animal species known to occur in Mount Kenya ecosystem

Scientific Name	Common Name	Scientific Name	Common Name
Cephalophus harveyi	Harvey's Red Duiker	*Lepus microtis*	African Savanna Hare
Cephalophus nigrifrons	Black-Fronted Duiker	*Elephantulus brachyrhynchus*	Short-snouted Elephant Shrew
Eudorcas thomsonii	Thomson's Gazelle	*Elephantulus rufescens*	Rufous Elephant Shrew
Kobus ellipsiprymnus	Waterbuck	*Equus quagga*	Plains Zebra
Madoqua guentheri	Gunther's Dik-dik	*Ceratotherium simum*	White Rhinoceros
Perodicticus potto	Potto	*Diceros bicornis*	Black Rhinoceros
Graphiurus murinus	Woodland Dormouse	*Cercopithecus mitis*	Sykes' (blue) Monkey
Heterocephalus glaber	Naked Mole Rat	*Chlorocebus pygerythrus*	Vervet Monkey
Atherurus africanus	African Brush-tailed Porcupine	*Colobus guereza*	Mantled guerza
Hystrix cristata	African Crested Porcupine	*Erythrocebus patas*	Patas Monkey
Acomys wilsoni	Wilson's Spiny Mouse	*Papio anubis*	Olive Baboon
Aethomys chrysophilus	Red Rock Rat	*Papio cynocephalus*	Yellow Baboon
Crocuta crocuta	Spotted hyena	*Galago senegalensis*	Senegal Bushbaby
Hyaena hyaena	Striped Hyena	*Otolemur garnettii*	Nothern Greater Galago
Proteles cristata	Aardwolf	*Orycteropus afer*	Aardvark
Madoqua kirkii	Kirk's Dik-dik	*Tachyoryctes splendens*	Mount Kenya Mole Rat
Nanger granti	Grant's Gazelle	*Canis adustus*	Side-stripped Jackal
Neotragus moschatus	Suni	*Canis aureus*	Golden Jackal
Oreotragus oreotragus	Klipspringer	*Canis lupus*	Domestic Dog
Ovis aries	Domestic Sheep	*Canis mesomelas*	Black-backed Jackal
Raphicerus campestris	Steenbok	*Lycaon pictus*	African Wild Dog
Redunca fulvorufula	Mountain Reedbuck	*Otocyon megalotis*	Bat-eared Fox
Redunca redunca	Bohor reedbuck	*Caracal aurata*	African Golden Cat
Sylvicapra grimmia	Common Duiker	*Caracal caracal*	Caracal
Syncerus caffer	African Buffalo	*Felis cattus*	Domestic Cat
Tragelaphus eurycerus	Bongo	*Felis silvestris*	African Wild Cat
Tragelaphus imberbis	Lesser Kudu	*Leptailurus serval*	Serval cat
Tragelaphus oryx	Common Eland	*Panthera leo*	African Lion
Tragelaphus scriptus	Bushbuck	*Aonyx capensis*	African Clawless Otter
Tragelaphus strepsiceros	Greater Kudu	*Ictonyx striatus*	Zorilla
Hylochoerus meinertzhageni	Giant Forest hog	*Lutra maculicollis*	Spotted-necked Otter
Phacochoerus africanus	Common Warthog	*Mellivora capensis*	Honey Badger
Potamochoerus larvatus	Bush pig	*Poecilogale albinucha*	African Striped Weasle
Capra aegagrus	Domestic Goats	*Nandinia binotata*	African Palm Civet
Loxodonta africana	African Elephant	*Civettictis civetta*	African Civet
Crocidura spp	White-toothed shrews	*Genetta genetta*	Common Genet
Suncus	Suncus Shrew	*Genetta maculata*	Rusty Spotted Genet
Surdisorex polulus	Mt. Kenya Mole Shrew	*Genetta servalina*	Servaline Genet
Sylvisorex granti	Grant's Forest Shrew	*Genetta tigrina*	Large Spotted Genet
Atelerix albiventris	Four-toed Hedgehog	*Panthera pardus*	Leopard
Dendrohyrax arboreus	Southern Tree Hyrax	*Atilax paludinosus*	Marsh Mongoose
Heterohyrax brucei	Yellow Spotted Rock Hyrax	*Bdeogale jacksoni*	Jackson's Mongoose
Procavia capensis	Rock Hyrax	*Helogale parvula*	Common Dwarf Mongoose
Lepus capensis	Cape Hare	*Herpestes ichneumon*	Egyptian Mongoose
Aepyceros melampus	Impala	*Herpestes sanguineus*	Slender Mongoose
Alcelaphus buselaphus	Hartebeest	*Ichneumia albicauda*	White-tailed Mongoose
Bos taurus	Domestic Cow	*Mungos mungo*	Banded Mongoose

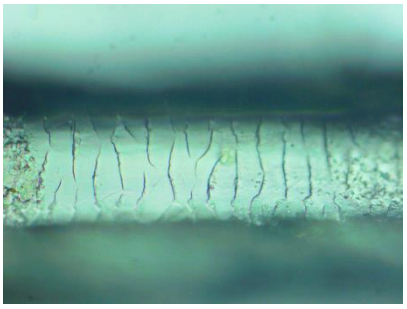
Appendix IV: Examples of medulla and scale patterns from respective animals in the reference hair catalogue

Harsh-furred mouse (*Lophuromys flavopunctatus*) reference hair description.

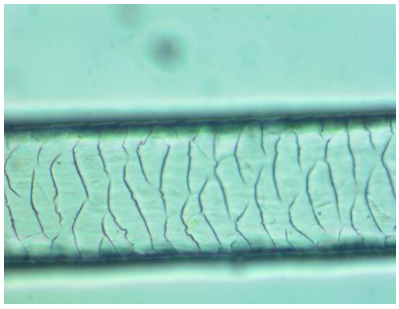
A. Description of the back hair of *Lophuromys flavopunctatus* (harsh-furred mouse)

i) Cuticular Characteristicsy

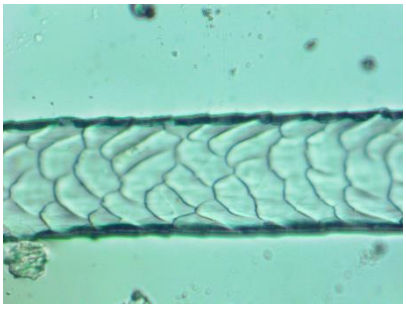
Region	**Scale distance**	**Scale margin**	**Scale pattern**
Distal	Near	Smooth	Regular waved mosaic
Medial	Near	Smooth	Regular waved mosaic
Proximal	Near	Smooth	Diamond petal



(a) Distal



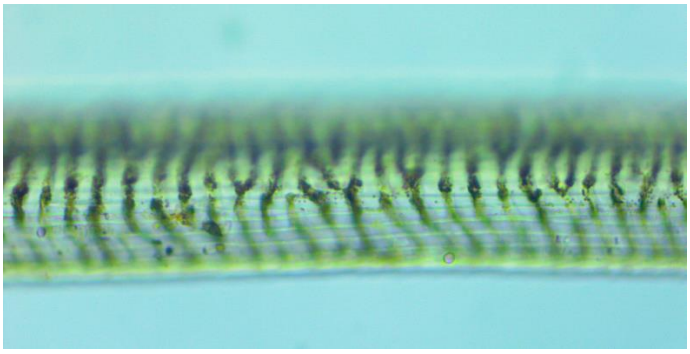
(b) Medial



(c) Proximal

ii) Categorization of the medulla structure

The medulla is a nodose type, discontinuous, compound, flattened, has air spaces forming a lattice filling more than half the hair (wide aeriform lattice)

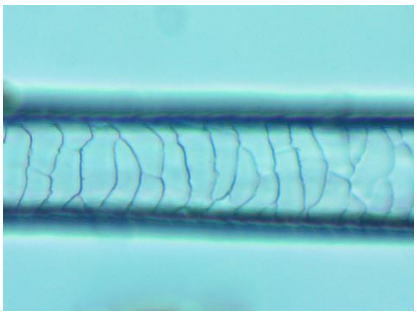


(d) Medulla pattern

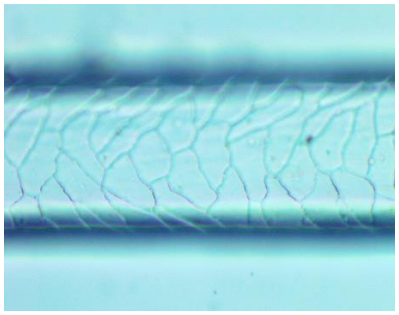
B. Description of belly hair of *Lophuromys flavopunctatus* **(**harsh-furred mouse)

i) Cuticular Characteristics

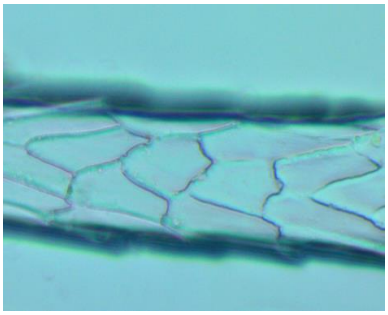
Region	**Scale distance**	**Scale margin**	**Scale pattern**
Distal	Near	Smooth	Regular waved mosaic
Medial	Near	Smooth	Regular waved mosaic
Proximal	Distant	Smooth	Diamond petal



(a) Distal



(b) Medial



(c) Proximal

ii) Categorization of the medulla structure

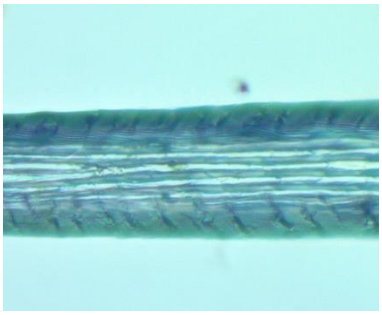
The medulla is a nodose type, discontinuous, simple, flattened, has single row of air spaces along its length. (Uniserial ladder medulla)

(d) Medulla pattern

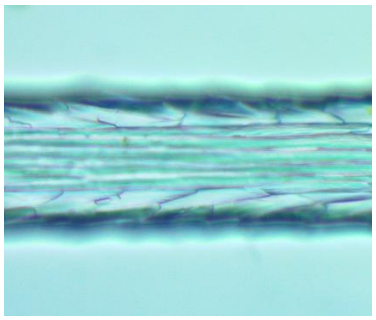
C. Description of shoulder hair of *Lophuromys flavopunctatus* (harsh-furred mouse)

i) Cuticular Characteristics

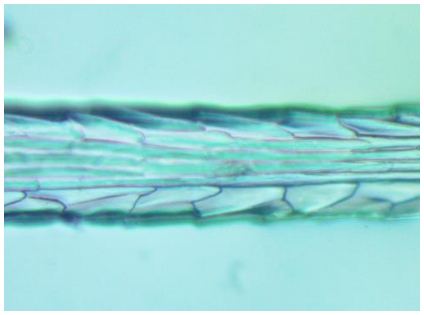
Region	**Scale distance**	**Scale margin**	**Scale pattern**
Distal	Near	Smooth	Regular wave
Medial	Distant	Smooth	Broad petal
Proximal	Distant	Smooth	Broad petal



(a) Distal



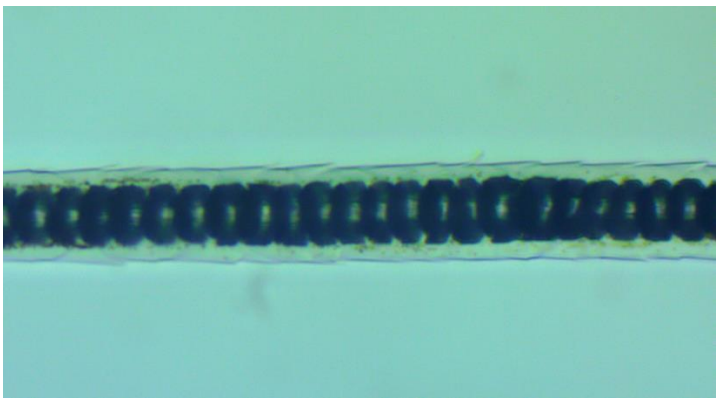
(b) Medial



(c) Proximal

ii) Categorization of the medulla structure

The medulla is a nodose type, discontinuous, simple, ovate, has single row of air spaces along its length. (Uniserial ladder medulla)

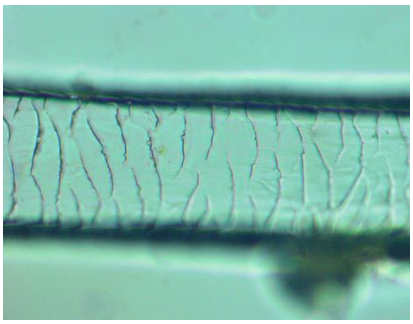


(d) Medulla pattern

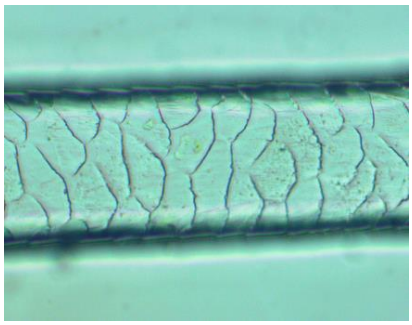
D. Description of thigh hair of *Lophuromys flavopunctatus* **(**harsh-furred mouse)

ii) Cuticular Characteristics

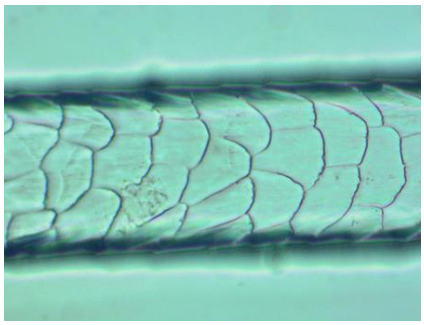
Region	**Scale distance**	**Scale margin**	**Scale pattern**
Distal	Near	Crenate	Regular waved mosaic
Medial	Near	Crenate	Regular waved mosaic
Proximal	Near	Smooth	Diamond petal



(a) Distal



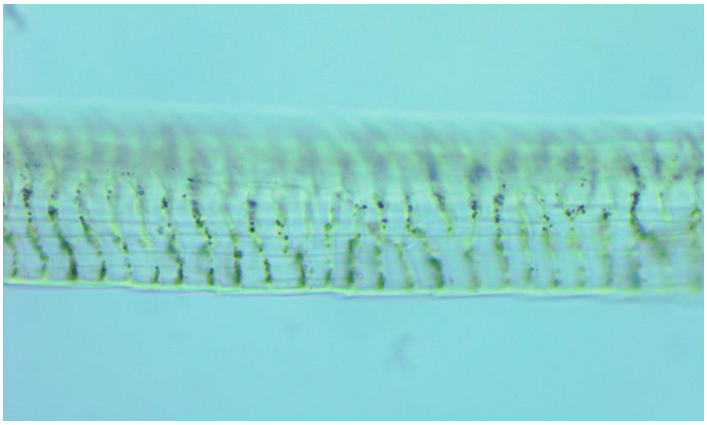
(b) Medial



(c) Proximal

ii) Categorization of the medulla structure

The medulla is a nodose type, discontinuous, simple, flattened, has air spaces forming a lattice filling more than half the hair (wide aeriform lattice).



(d) Medulla pattern