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RESEARCH PROPOSAL FOR THE DEGREE OF MASTER OF SCIENCE BY COURSEWORK AND DISSERTATION.

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5.0. Title. Assessment of Genetic diversity and Delimitation of Giraffe variants (*Giraffa camelopardalis)* in Tarangire - Manyara Ecosystem Northern Tanzania.

6.0 INTRODUCTION

6.1 General introduction

Giraffes (*Giraffa camelopardalis)*, the tallest land animals, are distributed across sub-Saharan Africa, primarily in southern and eastern regions, with smaller populations in central and western regions (Raw et al. 2016). The habitats of these charismatic African megafauna primarily range from deserts to savannas, but their populations have become fragmented across sub-Saharan Africa (Niekerk 2018), and believed to have undergone extinction in several African countries, including: Nigeria, Mali, Senegal, and Eritrea (Raw et al. 2016). Giraffes are listed as vulnerable by the International Union for Conservation of Nature Red List of Threatened Species, with declining populations due to habitat fragmentation, poaching, disease, and climate change (Muller et al 2018) and also listed in appendix II on the Convention on International Trade in Endangered Species (CITES).

Globally, there was one species of giraffe (*Giraffa camelopardalis*) with nine recognized subspecies classified based on morphological traits and geographical location (Dagg 1971). Recent molecular studies based on DNA analysis across African populations, has reclassified the nine subspecies into four distinct giraffe species with five subspecies (Fennessy et al. 2016). The four recognized species are: Masai giraffe (*G. tippelskirchi*), Reticulated giraffe (*G. reticulata*), Southern giraffe (*G. giraffa*), and Northern giraffe (*G. camelopardalis*). Evolutionarily, giraffes are most closely related to the okapi and are classified within the family Giraffidae and order Artiodactyla (Kümpel 2015).

Tanzania harbors the Masai giraffe (*G. tippelskirchi*), one of the four distinct giraffe species recognized in recent taxonomic studies (Kiula et al. 2021).This species is broadly distributed throughout central and northern Tanzania (Bolger et al. 2019). The Masai giraffe has experienced

population decline by almost 50% since the 1980s, with current estimates at approximately 35,000 individuals (Bolger et al. 2019). This decline has been attributed to habitat loss, poaching, human-wildlife conflict, predation and diseases (Raw et al. 2016). The decrease in population size could have led to reduced dispersal or gene flow between or among populations leading to reduced genetic diversity (Allendorf et al. 2010). Reduced genetic diversity has been implicated with inbreeding depression, which diminishes reproductive fitness and limits the species' ability to adapt to environmental changes (Frankham 2002)

The taxonomic classification of giraffes has traditionally been simplistic, grouping all giraffes under a single species *G. camelopardalis* (Dagg 1971). The affordability and simplicity of this method makes it the preferred choice by many scientists (Wiens 2004). However, this method is limited by variability in physical traits like coat patterns influenced by environment and development factors (Rakov 2020). To address the challenges of morphological taxonomy Hebert et al. (2003) recommended the use of mitochondrial DNA Cytochrome c oxidase subunit 1 (COI) gene as a reliable method for identifying and distinguishing animal taxa. The gene's high variability among closely related species coupled with minimal differences within the same species, makes it particularly effective for species identification (Hebert et al. 2003).

The Tarangire-Manyara Ecosystem (TME) is an ecological region in northern Tanzania that support a rich array of wildlife and plant species. The Makuyuni Wildlife Park (MWP) in Arusha is one of the wildlife areas within the TME that provide crucial habitat for giraffes in Tanzania similar to Tarangire and Lake Manyara national parks. The Masai giraffe (*G. tippelskirchi)* is the current known species of giraffe found in TME (Kiula et al. 2021). However In MWP, one morphological giraffe variant that is endemic to the area have been spotted, representing a potentially significant discovery (Nkwame 2024). Morphological analysis suggests that the

giraffe variants differ in coat patterns from the known Masai giraffe (Nkwame 2024). Given the hitches associated with morphological species identification, it is possible that the variants could be a subspecies of the Masai giraffe or separate species when molecular taxonomy is used. However, the molecular insights on the classification of this variant remain unknown. In addition, recent declines in giraffe population size in the TME, where MWP is part of (TAWIRI 2016), could implicate decline in giraffe's genetic diversity which could be detrimental to the continued existence of the species. However, the knowledge on the genetic diversity of giraffes in the area still limited, this limit conservation efforts. Thus, the present study aims to perform genetic analysis to depict the phylogenetic relationships among the variants and assess the genetic diversity of giraffes in TME.

6.2 Statement of the Research Problem

The accurate identification and classification of wildlife species are vital for effective conservation (Mace 2004). Historically, giraffes were classified under one species, *G. camelopardalis* (Dagg 1971). However recent DNA studies have revealed four distinct giraffe species (Fennessy et al. 2016). In TME, the Masai giraffe (*G. tippelskirchi*) is the known species (Kiula et al. 2021). However, one giraffe variant, differing in coat patterns from the known Masai giraffes has been observed in MWP, potentially representing a significant discovery in giraffe taxonomy (Nkwame 2024). Identifying this variant based solely on morphology can be problematic, as giraffes have various coat patterns that may not reflect genetic difference (Rakov 2020). Molecular identification is therefore necessary. However, the molecular taxonomy of these variants remains unknown.

In addition to taxonomic uncertainties, a report from the Tanzania Wildlife Research Institute (TAWIRI) indicates a decline in giraffe populations in the TME, including MWP, due to human activities (TAWIRI 2016). This decline could have resulted in reduced population sizes, isolation, limited gene flow, and increased inbreeding, all of which reduce genetic diversity and reproductive fitness, making the species more vulnerable to environmental changes (Allendorf et al. 2010). However, the knowledge on genetic diversity of giraffes in TME is limited, hindering conservation efforts. This study aims to analyze the genetic diversity and phylogenetic relationships of giraffes in TME to inform conservation strategies and enhance tourism activities in the region.

6.3 Objective of the Study

6.3.1 General Objective

The main objective of this study is to assess genetic diversity and phylogenetic relationship among variant giraffe species in TME.

6.3.2 Specific Objectives

i. To evaluate the phylogenetic relationship among giraffe variants in TME.
ii. To determine genetic diversity of giraffe variants in TME
iii. To genetically determine the barcode gap among the giraffe variants of TME

6.4 Research Question

i. To what extent are the giraffe variants of TME phylogenetically related?
ii. How genetically diverse are the giraffe variants in TME?
iii. To what extent are the giraffe variants of TME distinct from each other?

6.5 Significance of the Study

This study will come up with molecular data that will be archived in biological databases particularly the NCBI GenBank and the Barcode of Life Database (BOLD). Deposition of these sequences will help scientists worldwide to access and compare them with other giraffe populations or even with different species. This will further help in understanding evolutionary relationships, migration patterns, and the genetic diversity within giraffe species**.** The present study will determine the phylogenetic relationships and delimit giraffe variants. This will be essential for classification and understanding evolutionary trends within the giraffe lineage. Additionally, information on genetic diversity of giraffes will provide insights on whether the population of giraffes in TME is genetically stable or not which will be crucial for conservation strategies. On a broader scale, the discovery of new giraffe variants will significantly enhance tourism in MWP as a part of TME where new giraffe variants will become a unique attraction, drawing wildlife enthusiasts, researchers, and photographers.

7.0 LITERATURE REVIEW

7.1 Classification and Distribution of Giraffes

Giraffes belong to the order Artiodactyla, family Giraffidae, and genus *Giraffa* (Giraffe Conservation Foundation 2022). All giraffes were previously classified into a single species *G. camelopardalis*, with nine sub species (Dagg 1971). The subspecies includes the Angolan giraffe (*G. c. angolensis*), Kordofan giraffe (*G. c. antiquorum*), Masai giraffe (*G. c. tippelskirchi*), Nubian giraffe (*G. c. camelopardalis*), Reticulated giraffe (*G. c. reticulata),* Rothschild's giraffe (*G. c.rothschildi*), Thornicroft's giraffe (*G. c. thornicrofti*), West African giraffe (*G. c. peralta*), and South African giraffe (*G. c. giraffa)* (GCF 2022). However recent genetic studies have revealed significant differences among giraffe populations, suggesting the existence of four

distinct species rather than subspecies (Fennessy et al. 2016) These species include the Southern giraffe (*G. giraffa)* that comprises the Angolan giraffes (*G. g. angolensis)* and South African giraffes (*G. g. giraffa)* subspecies, Masai giraffe (*G. tippelskirchi)* that comprises the Luangwa giraffe (*G. c.thornicrofti*) and the masai giraffes (*G. c. tippelskirchi)* subspecies, Reticulated giraffe (*G. reticulata),* Northern giraffe (*G. camelopardalis*) that comprises the West African giraffes *(G. c. peralta*), Kordofan giraffes (*G. c. antiquorum*), Nubian giraffes (*G. c. camelopardalis*) and Rothschild's giraffe (*G. c.rothschildi*) subspecies (Fennessy et al. 2016).

Giraffes are distributed throughout southern and eastern Africa, with smaller, isolated populations in central and western regions (Raw et al. 2016). These iconic species inhabit a variety of environments, from deserts to woodlands and savannas, across sub-Saharan Africa (Niekerk 2018). Recent studies have updated the geographical distribution of the four recognized giraffe species. *G. camelopardalis* covering nine countries from southwestern Niger to Uganda, with smaller populations in western Kenya (O'connor et al. 2019). *G. reticulata* occupies northern Kenya, with small populations in southern Ethiopia and western Somalia. *G. tippelskirchi* is found in southern Kenya, much of Tanzania, and a small population in Zambia (O'connor et al. 2019). *G. giraffa* ranges across southeastern Angola, Namibia, Botswana, South Africa, and parts of Zimbabwe, Mozambique, Zambia, and Malawi. In Tanzania, Masai giraffes live in both protected and unprotected areas across the north, central, western, and southern regions, including ecosystems like Serengeti, Tarangire-Manyara, and Nyerere National Park. In the Selous-Mikumi ecosystem, they are confined to Nyerere National Park, as the Rufiji River blocks access to the southern section (Ramos 2016)

7.2 Conservation Status and Charismatic Value of Giraffes.

Worldwide giraffes face a significant population decline of about 30% over recent decades due to threats like habitat loss, poaching, climate change, and diseases (GCF 2022, Lohay et al. 2023). As a result, currently IUCN has listed giraffes as vulnerable in the IUCN Red List of Threatened Species (Muller et al. 2018). In East Africa, Masai giraffe populations have dropped by 52%, with Tanzania's numbers declining from 31,000 in 1986 to a recent estimate of 28,850 (TAWIRI 2016). These figures underscore the urgent need for conservation efforts to safeguard this iconic species (Muller et al. 2018). The Masai giraffe holds deep cultural and national significance in Tanzania, symbolizing grace, wisdom, and the country's rich wildlife heritage (Linzmayer 2011). Its role as a national symbol was retained after independence as its historical presence is reflected on Tanganyika's flag, Tanzanian banknotes (1961–2011), and its protection under the Wildlife Conservation Act No. 5 of 2009, which prohibits harm to the species (MNRT 2009).

7.3 Ecology of Giraffes.

Giraffes, native to sub-Saharan Africa, are iconic herbivores found in savannas, grasslands, and open woodlands. They prefer area with scattered trees, particularly acacias, which make up a significant portion of their diet (Seeber et al. 2012b). With long necks and prehensile tongues, giraffes feed on leaves, flowers and fruits, consuming up to 30 kilograms of diet daily. Feeding at higher levels in the canopy minimize competition with smaller browsers showing that resource partitioning can be influenced by competition, where smaller foragers push larger ones away from shared resources (Cameron and Du Toit 2007). Their social structures and behaviors vary, with some populations forming stable groups and dominance hierarchies (Seeber et al. 2012a). Males are generally more solitary, with fewer males moving in larger groups (Muller and Harris

2022). Male giraffes use reliable cues to assess female reproductive status and adjust their mating efforts accordingly, male giraffes adopt a roaming reproductive strategy, leveraging their size to search for and guard fertile females, which helps minimize energy costs while maximizing reproductive success (Bercovitch et al. 2006).

7.4 Species Delimitation (SD).

SD is the process of identifying and defining species boundaries using various methods based on specific criteria (De Queiroz 2007). One common approach is morphological identification, which classifies organisms based on observable physical traits like size, shape, color, and structure (Yang et al. 2022).This method is cost-effective and requires minimal specialized equipment (Wiens 2004). However, it has limitations, as physical traits can vary significantly due to environmental factors or developmental stages (Rakov 2020). To address these challenges, molecular techniques particularly DNA sequencing have become essential in modern taxonomy. These techniques provide precise species identification by analyzing genetic material and can uncover cryptic species that are morphologically similar but genetically distinct (Hebert et al. 2003). However, accurate species identification depends on comprehensive reference sequence databases, which can be a limiting factor (Lauri and Mariani 2009).

For giraffes, studies using mitochondrial DNA (mtDNA) and nuclear DNA markers, such as microsatellites, have been instrumental in understanding genetic diversity and phylogenetic relationships (Brown et al. 2007). For example, Brown et al. (2007) revealed significant genetic divergence among giraffe populations in Africa, while Fennessy et al. (2016) identified four distinct giraffe species using nuclear intron markers and mtDNA cytochrome b analysis, challenging earlier classifications of one species and nine subspecies.

7.5 DNA Barcoding and Genetic Diversity.

DNA barcoding refers to a system in taxonomy that uses information from short region of standardized mitochondrial DNA (mtDNA) sequence to distinguish specimens and uncover undisclosed species (Valentini et al. 2009). DNA barcodes are currently being used for two distinct purposes which are species identification and species description (DeSalle et al. 2005). DNA barcoding uses standardized gene regions as internal species tag to delimitate species (Hubert and Hanner 2015). The mitochondrial Cytochrome Oxidase 1 (COI) gene has been chosen as a universal marker for barcoding of life (Hebert et al. 2003). The gene is variable among conspecifics but highly conserved among congeneric, allowing accurate species identification (Hebert et al. 2003).. In DNA barcoding, DNA sequences from unknown specimen are matched with sequences of known identity in the barcode of life (BOLD) database to enable specific identification (Hubert and Hanner 2015). The method has received acceptance because of its simplicity and affordability (Casiraghi et al. 2010).

Genetic diversity (GD) encompasses the heritable variations within and between populations, serving as a key driver of biodiversity at species and ecosystem levels (Frankham 2002). It enables species to adapt to environmental changes and is influenced by factors such as genetic drift, mutation, migration, natural selection, and population size (Frankham 2002). GD can be assessed using parameters like nucleotide diversity, polymorphic sites, haplotype diversity, and heterozygosity through tools like DnaSP (Rozas et al. 2017) and Arlequin (Excoffier and Lischer 2010). Studies on giraffes show moderate genetic diversity levels (Brenneman et al. 2009). Geographic barriers, such as Tanzania's Gregory Rift Valley, can hinder gene flow, isolating populations like the Masai giraffes and increasing inbreeding coefficients, which may reduce genetic diversity and lead to inbreeding depression (Lohay et al. 2023).

7.6 Phylogenetic Analysis (PA).

PA investigates evolutionary relationships among species by constructing phylogenetic trees using morphological or molecular data (Horner and Pesole 2004). Methods like Maximum Likelihood, Bayesian Inference, and Neighbor-Joining help estimate evolutionary connections, with techniques such as the HKY85+I+G model and bootstrap resampling enhancing clade reliability (Brown et al. 2007). In giraffe studies, PA has clarified evolutionary history, species differentiation, and genetic structure (Mitchell et al. 2003). Whole-genome sequencing revealed four distinct giraffe lineages diverging 230,000–370,000 years ago, challenging the idea of giraffes as a single species and highlighting their unique genetic traits (Coimbra et al. 2021). Despite their distinctions, historical gene flow, particularly between reticulated and Nubian giraffes, indicates past interbreeding (Coimbra et al. 2021).Tools like MEGA, BEAST, MrBayes, and RAxML are commonly used for reconstructing phylogenies and analyzing evolutionary patterns (Horner and Pesole 2004).

8.0 MATERIALS AND METHODS.

8.1 Study Area.

The Tarangire-Manyara Ecosystem (TME) is an ecological region in northern Tanzania that support a rich array of wildlife and plant species. TME covers an area of 16,521 square kilometers. Located between latitude 3° 22' 00" to 5° 12' 20" S and longitude 35° 40' 53" to 37° 5' 22" E (URT 2018)(Figure 1).. The area covers the Tarangire National Park, Lake Manyara National Park, Mkungunero Game Reserve, Lolkisale Game Controlled Area (and Randilen Wildlife Management Area), Simanjiro Plains, Mto wa Mbu, Kwakuchinja Open Area, Kibaoni Open Area, Burunge Wildlife Management Area, Makuyuni Wildlife Park, Manyara Ranch,

Makame Wildlife Management Area (TAWIRI 2016). TME is characterized by semi-arid climate (Foley 2000) with March to April characterized with precipitation, while June to October is typically very dry, with little to no rain. The ecosystem comprises of floristic diversity, featuring primarily *Combretum-Dalbergia* and *Acacia-Commiphora* woodlands, along with grasslands and floodplains as well as *Acacia-Themeda* wooded grasslands. The predominant grass species in the area are *Sporobolus spicatus, Sporobolus robustus, Sporobolus marginatus, Cyperus laevigatus, Themeda triandra, Panicum species, Hyparrhenia species, Digitaria species,* and *Pennisetum* species.(TAWIRI 2016).

8.2 Study sites.

So far, the unique giraffe variant with distinct coat pattern has been spotted in MWP, located in Monduli District within TME, northern Tanzania (Nkwame 2024). Covering 49 km², MWP lies between 3°24'01" S to 3°24'37" S latitude and 36°10'03" to 36°55'29" E longitude (URT 2018)(Figure 1). The area was previously used for large-scale farming of beans and peas by South African farmer Hermanus Philipus Steyn in the 1960s and 1970s, then was nationalized in the 1980s and left idle, allowing wildlife and natural habitats to recover (Massawe 2023). In 2022, the area was transferred to the Tanzania Wildlife Management Authority (TAWA) to promote eco-tourism and conservation (Nkwame 2024). Some wildlife species found in the area include mammals such as the African elephants (*Loxodonta africana*), Masai giraffes (*G. tippelskirchi,* Buffalo (*Syncerus caffer),* Oryx (*Oryx gazelle),* Wildebeest (*Connochaetes taurinus), pictus),* Lion (*Panthera leo),* Zebra (*Equus quagga),* Impala (*Aepyceros melampus)*. Sampling will also be conducted in other protected areas near MWP occurring in TME including Tarangire National Park and Lake Manyara National Park.

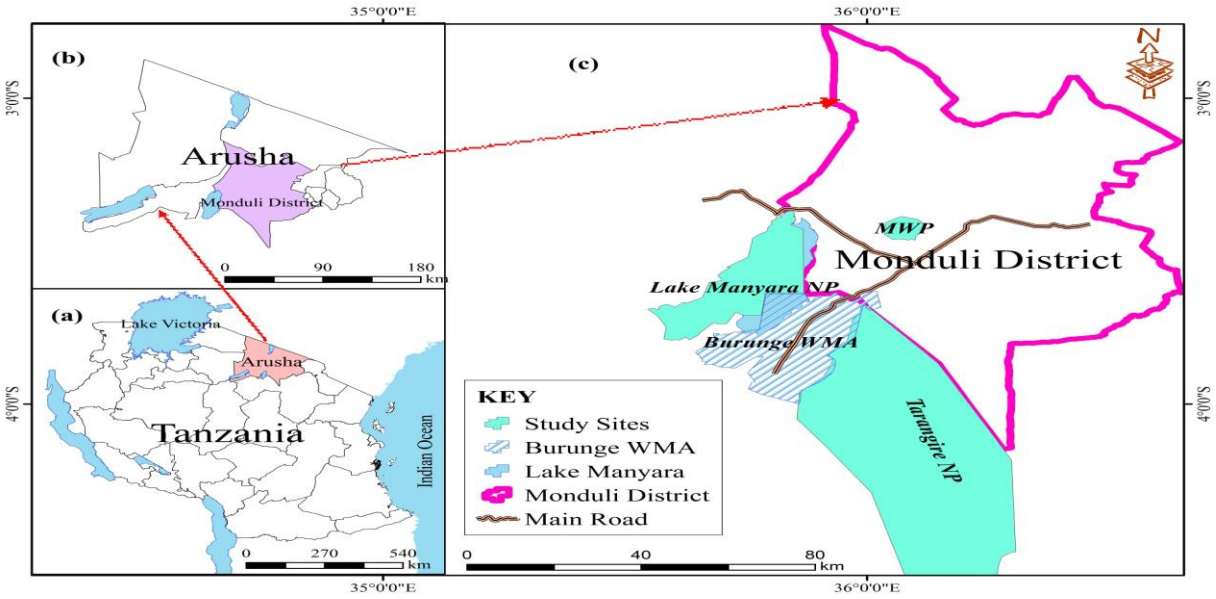

Figure 1. Map of Tanzania showing (a) Arusha region in relation to other Tanzania regions (b) Location of Monduli district in Arusha region (c) Location of Tarangire-Manyara Ecosystem.

8.3 Sample Collection.

Non-invasive sampling will be conducted to minimize stress on the individuals, that will enhance cost-effectiveness while adhering to ethical standards (Rojas et al. 2024). Fecal samples from at least 50 giraffes will be collected. An opportunistic sampling approach will be employed, beginning with the identification of giraffe groups within the park. The protocol at each site will proceed as follows: upon locating a giraffe, observation will commence, when the giraffe defecates, the precise location will be recorded using GPS, each giraffe will be photographed (Strauss et al. 2015). Fecal samples will be collected immediately after defecation as the pellets dry quickly. Fecal samples will be processed according to Niekerk (2018) preserved in 15ml Eppendorf tubes containing absolute ethanol. Each tube will be labeled with the sample number, date of collection and locality.

8.4 DNA Extraction and Genotyping.

DNA will be extracted from the fecal sample preserved in 15ml Eppendorf using the Zymo Research ZR Fecal DNA MiniPrep™ kit, following the manufacturer's protocol (Niekerk 2018).The purity and concentration of the extracted DNA will be assessed using a NanoDrop spectrophotometer, and the integrity will be checked by agarose gel electrophoresis. Genotyping will be achieved by amplifying both the mtDNA COI (for barcoding) and the D- loop region (for genetic diversity). The PCR will be done according to Niekerk (2018) for D- loop region and (Lah et al. 2015) for COI. PCR will be performed in a total volume of 25 µl consisting of 6.25 µl of Ready-mix, 0.5 µl of each primer (10 pmol), nuclease free water, Taq DNA Polymerase and DNA template. Thermocycling will begin with pre-heating at 94°C for 1 minute, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at an optimized temperature, and elongation at 72°C for 1 minute. A final extension at 72°C for 10 minutes will complete the process. PCR products will be verified using 1% agarose gel electrophoresis stained with ethidium bromide and compared to a 1 kb reference ladder. Purification will be done with the ExoSAP-IT kit, and bi-directionally Sanger sequenced at Psomagen, USA.

8.5 Data Analysis.

The forward and reverse sequences for each specimen will be manually aligned and edited using BioEdit software (Version 7.7.1) (Hall 1999) to resolve ambiguities and generate consensus sequences. These sequences will then be analyzed using BLAST against the BOLD and NCBI nucleotide databases to identify giraffe variants in comparison to existing GenBank and BOLD records. Giraffe variants will be delimited through DNA barcode analysis using the Automatic Barcode Gap Discovery (ABGD) method (Puillandre et al. 2021), the barcode gap will be validated using the 10-fold criterion proposed by Hebert et al. (2004). Genetic diversity

parameters, including: nucleotide diversity, haplotype diversity, polymorphic sites, nucleotide differences, and observed haplotypes, will be calculated with DnaSP software (Version 6.2) (Rozas et al. 2017). Genetic distances among variants will be assessed using Arlequin software (Version 3.5.1.2) (Excoffier and Lischer 2010). Phylogenetic relationships will be analyzed by combining the study's sequences with orthologous giraffe sequences from GenBank. Neighbor-joining and Bayesian phylogenetic trees will be constructed using MEGA (Version 11) (Tamura et al. 2021), with bootstrap analysis (1000 replicates) to evaluate tree robustness and Mr. Bayes software (Ronquist et al. 2012). Tamura 3-parameter model of nucleotide substitution will be used in MEGA software with an *Okapia johnstoni* used as an outgroup. Additionally, a TCS haplotype network will be constructed using PopART (Version 1.7) (http//popart.ontago.ac.nz.) to visualize genetic divergence among haplotypes.

8.6 Ethical Considerations.

All animal-related procedures in the field will adhere to Tanzania's wildlife ethical guidelines. Required permits for sample collection will be secured from the Tanzania Wildlife Research Institute (TAWIRI) and the Commission for Science and Technology (CoSTECH).

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10. OTHER RELEVANT INFORMATION.

10.1 Detailed budget.

S/N	Item	Amount (Tshs)
University fees		
1	Tuition fees	4,250,000
2	University Direct Cost	230,000
Sub total		**4,480,000**
Research budget		
1	Field costs	5,100,000
2	Transportation	668,186
3	Stationery and thesis production	700,000

4	Laboratory assistance	1,800,000
5	Reagents and consumables	7,573,678
6	Sequencing	7,280,000
7	Allowances (student and supervisors)	6,000,000
Sub total		**29,121,865**
Grand total		**33,601865**

10.2 Time Frame for the Study.

Activity	**Year**	**2024**						**2025**								
	Month	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**
Literature review																
Proposal writing & presentation																
Data collection, and lab work																
Data analysis																
Dissertation writing & submission																

CANDIDATE.
Name. Mr. Meshack J. Madata Reg No. 2023-06-00322
Signature. Madata Date. 10/02/2025

SUPERVISOR (1)
Name. Dr. Y.J CHUHILA Signature. Date. 10/02/2025
Comments. The proposal is ready for implementation.

SUPERVISOR (2)
Name. Dr. S.E. Temu Signature. Date. 10/02/2025
Comments. The candidate has addressed the comments

CHAIRPERSON- HDRPC DEPARTMENT COMMITTEE.
Name. Dr. M. KIBANJA Signature. Date. 10/02/2025
Comments. Recommended for approval

HEAD OF DEPARTMENT.
Name. Dr. Flora Stephen Signature. Date. 10/02/2025
Comments. Approved!