POPULATION STRUCTURE, PARASITIC ASSOCIATIONS, DISTRIBUTION DRIVERS, AND GENETIC DIVERSITY OF *OSYRIS LANCEOLATA* (Hochst. & Steud.) IN UGANDA AND KENYA

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Thesis submitted in Fulfilment of the Requirements for the Award of the Degree of Doctor of Philosophy in Plant Ecology

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2023

DECLARATION

I **Ben Belden Mugula**, declare that this research thesis is my original work and it has never been submitted to any institution of higher learning for research leading to any academic award. I therefore take personal responsibility for the content of this work.

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DEDICATION

I dedicate this thesis to my dear mother **Barbara Kirangi**, and my late father **Sylvester Mugula**, who worked tirelessly to shape me into the person I am today.

ACKNOWLEDGEMENT

I thank God for giving me this opportunity to accomplish this study. My dear supervisors; Prof. Samuel Kiboi, Prof. James I Kanya, Prof. Manuel Curto, I cannot thank you enough! It was your tireless efforts that enabled me to complete this thesis. May God bless you abundantly. My external advisory team, Dr. Paul Okullo, Prof. Anthony Egeru, Dr. Stephen F. Omondi, and Prof. Harald Meimberg, thank you so much for your assistance throughout this study. Prof. Egeru and Dr. Okullo, you have been so inspiring to me even when everything was tough. May God bless you.

Prof. John RS Tabuti, thank you for setting the foundation for my research career during my undergraduate studies. Please continue nurturing young researchers. Dr. Stephen Omondi, you worked so hard to ensure that I complete my molecular work, I cannot forget your parental guidance, love and encouragement all the time. Prof. Meimberg, you provided invaluable guidance to me and initiated several options for me to complete my thesis. Thank you so much for your interest in developing young researchers. The entire staff of KEFRI Biotechnology department; Asenath, Charles, Susan, Priscilla, Mutai, Rebecca and Makatiani, thank you so much for your technical and moral support during my lab work. Special thanks to Gorret, Chief lab technician Soil and Water laboratory, at Makerere University, for the guidance during analysis of the soil samples.

Special thanks to Bugema University and Uganda Union Mission for sponsoring my studies. Iam grateful to Prof. Patrick Manu who started this PhD journey, the DVC Academics, the late Prof. Paul Katamba, DVC Finance and Administration, Ms. Susan Nambi, not forgetting the UUM Treasurer Eld. Kiggundu, Mrs. Lillian Mugerwa, Mrs. Sarah Nyende and Dr. Moses Maka for the support. May God bless you. Special thanks to Mr. Henry Mukisa for your love and inspiration throughout my studies. God bless you. All my family members, relatives and friends thank you so much for your support and encouragement.

To the people of Karamoja I thank you for the warm reception and cooperation that you offered to me during field work. Special thanks to Mr. Kiyonga, thank you so much for opening the doors for research in Amudat district. My field assistants, Mzee Daniel, Mr. Ronald Kaliamoi and James Oziri, thank you for helping me to collect data in a very difficult environment. Finally, conducting this research in Karamoja would have been a nightmare without the great sacrifice of our dear officers of the Uganda People's Defense Forces (UPDF), Uganda Police and all security agencies in Amudat, Nakapiripirit, and Moroto in maintaining the peace that enabled me to traverse very fragile ecosystems without any fear. When I sought your protection, indeed, it was provided during very difficult times. I also wish to appreciate the International Foundation for Science (IFS), Sweden, for supporting young researchers in Africa. It is through this kind of support that enabled me to conduct part of the field work for my PhD project. Iam indebted to Prof. Catherine W Lukhoba, Florence, Judith and staff in the Department of Biology, University of Nairobi, for their professional assistance throughout my PhD studies. God bless you.

The permission to conduct my doctoral studies in Uganda was granted by Uganda National Council for Science and Technology (UNCST) under Ref: A585. The National Commission for Science, Technology & Innovation (NACOSTI) granted me permission to conduct part of my studies in Kenya, under License number: AC24057.

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ACRONYMS

SSR	Simple Sequence Repeats (Microsatellites or Short Tandem Repeats STR)
SCD	Size Class Distribution
UNCST	Uganda National Council of Science & Technology
UWA	Uganda Wildlife Authority
NFA	National Forestry Authority
IUCN	International Union for Conservation of Nature
DNA	Deoxy-Ribonucleic Acid
AFLP	Amplified Fragment Length Polymorphisms
KWS	Kenya Wildlife Service
ANOVA	Analysis of Variance
AMOVA	Analysis of Molecular Variance
CTAB	Tri-methyl Ammonium Bromide
EDTA	Ethylene-diamine tetra acetic acid
PCR	Polymerase Chain Reactions
PCQ	Point Centered Quarter Method
PPL	Percentage of Polymorphic Loci
PD	Power of Discrimination
HWEs	Hardy-Weinberg equilibrium
BAP	Bayesian Analysis Program
IBD	Isolation by Distance
UoN	University of Nairobi
RCF	Research Conceptual Framework
SGS	Spatial Genetic Structure
RAPDs	Randomly amplified polymorphic DNA (RAPD)
AFLPs	Amplified Fragment Length Polymorphisms
UUM	Uganda Union Mission
KEFRI	Kenya Forestry Research Institute
LOT	Lotemwoyes
KAN	Kangisa
NGA	Ngaram
RUW	Ruwotokech
KOP	Kopedur
LON	Lonyilik
AKA	Akariwon
CHP	Cheporon
KOR	Korenyang
KAR	Karengepoche
CE	Capillary Electrophoresis
SSR	Short Sequence Repeat
NACOSTI	National Commission for Science, Technology and Innovation
CITES	Control of International Trade in Endangered Species
FLT	Full light Illumination
MLT	Medium light
DSD	Dense habitat.
LCG	Litter cover on ground
ES	Exposed soil
AT	Animal trails seen on ground
RSS	Rock at soil surface
MDP	Mammal droppings
GLG	Gulley on ground
WCG	Dry water courses on ground
FFD	Fallen plant matter decomposing on ground
TMD	Termite mounds
OGU	Open grass understory
OSU	Open shrub understory
DIC	Dense impenetrable climbers

ABSTRACT

Osyris lanceolata is highly valued in Africa. The species is endangered due to exploitation and yet among the least studied. Information on the species ecology and genetics is limited in literature. Previous studies revealed knowledge gaps on the species ecology and population genetics which undermines its conservation and threatens livelihoods. To address the existing knowledge gaps, this study was designed to: determine the population structure of *O. lanceolata* in Uganda; analyse its hosts, habitat qualities and morphology; identify the species' distribution drivers; and characterize its genetic diversity and structure in Uganda and Kenya. Using the nearest neighbor methods, eleven sites were inventoried and 112 soil samples collected in Karamoja. A total of 210 genetic samples, 96 from Uganda and 114 from Kenya were analysed. A regression analysis of 388 individuals revealed an irregular and poorly regenerating population structure (*slope = -4.7058, $r^2 = 0.2617$*) with more coppiced individuals (58.7%), fewer seedlings (4%), saplings (46%) and adults (50%). The species density ranged from 5.095 trees ha^{-1} (Amudat), 27.306 trees ha^{-1} (Nakapiripirit) and 48.3 trees ha^{-1} in Moroto District. The species distribution was influenced by highly clustered hosts namely; *Euclea racemosa*, *Rhus natalensis*, *Maytenus senegalensis*, *Ozorea insignis*, and *Terminalia browni* as well as habitat qualities such as moderate illumination, rocky surfaces, water gullies and mammal droppings. Multiple regression analysis inferred phosphorus ($r^2 = 0.6534, p<0.001$); nitrogen ($r^2 = 0.2123$, $p<0.001$), sodium ($r^2 = 0.3282$, $p<0.001$) and calcium ($r^2 = 0.3719$, $p <0.001$) as distribution drivers. Kenyan populations showed higher genetic diversity (0.587 - 0.681) than Ugandan populations (0.49 - 0.677). AMOVA revealed greater genetic divergence among individuals (91%), populations (1%), regions (3%) and within individuals (4%). Ugandan populations showed more deviations from Hardy -Weinberg Equilibrium (HWE) than Kenyan populations. STRUCTURE analysis revealed two genetic clusters (K = 2) suggesting emerging evolutionary trends within *O. lanceolata* taxon that might lead to full allopatric speciation. The findings provide a foundation for developing conservation action strategies to halt further decline in the population and genetic erosion of *O. lanceolata* and save the species from impeding extinction.

***Key words**: population ecology; population genetics; microsatellites; multivariate analysis, African sandalwood;*

CHAPTER ONE

INTRODUCTION

1.1.1 Background of the study

Tropical ecosystems provide habitats to many economically important plants (Osborne, 2000; Richter et al., 2009). Most cultivated crops and plant species originate from tropical ecosystems. Besides having higher species diversity, tropical ecosystems store over 40% of terrestrial carbon stocks in the world hence having huge capacity to mitigate climate change impacts and safeguard livelihoods in developing countries (Neale and Kremer, 2011; Gould et al., 2024). Human disturbance to ecosystems over the years has caused substantial changes in population structure of plant species leading to their population decline, loss of genetic diversity, and, hence compromising their evolutionary potential for survival (Hawkins et al., 2008). The decline in plant populations and loss of genetic diversity is escalated by over-exploitation, habitat fragmentation, climate change and inadequate strategies for species conservation (Frankham, et al., 2002; Rocky and Mligo, 2012; Weronika, 2015; Chang et al., 2015). Also, in response to climate change impacts, most plant populations experience significant reduction in growth rates with profound shifts in genetic diversity patterns, (McClean et al., 2005; Pauls et al., 2013; Lyam et al., 2018; Wróblewska and Mirski, 2018). Genetic diversity is the basis for evolutionary change for adaptation to climate change related impacts or fitness in a changing environment (McLaughlin et al., 2002; Frankham et al., 2002). In addition, genetic diversity is the basis for developing breeding programmes for adaptation to climate change related impacts. Understanding the ecological traits of plants and factors that determine their survival including

edaphic variables and habitat characteristics provides useful insights to the genetic adaptive potential of plant species (Graudal et al., 2014; Prunier et al., 2016).

1.1.2 The biology and ethnobotany of *Osyris lanceolata*

Osyris lanceolata Hochst. & Steud**.** is an ever-green, hemi parasitic tree species known for its aromatic wood that contains essential oils (CITES, 2013; Teixeira et al., 2017). The species grows up to 1-7 meters depending on soil type, climate, and genetic composition (Breintenbach, 1963; Palmer and Pitman, 1972; Mwang'ingo, 2002; Texeira et al., 2016), and has a wide geographical distribution in Africa, Europe and Asia (Breitenbach, 1963; Teklehaimanot et al., 2004; Gathara et al., 2014). In East Africa, *O. lanceolata* occurs in Kenya, Tanzania, and Uganda, particularly in dry areas on rocky and stony soils (Kokwaro, 2009; CITES, 2013). The distribution of *O. lanceolata* is poorly documented in Uganda though scanty information indicates its occurrence in Sango-bay, Kigezi, Mbale, and Karamoja (CITES, 2013; Muhoozi; 2013; Tajuba, 2015; USAID, 2015 and UIA, 2016).

The species is used as local medicine to treat candidiasis, malaria, diarrhea, chest pain, and fever in Ethiopia, Uganda, Kenya, and Tanzania (Njoroge and Bussmann, 2006; Ochanda, 2009; Orwa, et al., 2009; Masevhe et al., 2015). Sandalwood oils have chemo-preventive properties used to manage eruptive skin and inflammatory diseases such as dysuria, bronchitis, gonorrhea, and urinary infections. The bark and roots provide red dye which is useful in skin tanning (Mbuya et al., 1994) while its shoot provides antipyretic agents for cattle. It has the ability to accumulate heavy metals and is thus useful in phytoremediation (Xiaohai et al., 2008). The oil has blending and anti-septic properties suitable for making fixatives in other fragrances (Coppen, 1995; Shyaula, 2012).

1.1.3 Utilisation of *O. lanceolata* in East Africa

Osyris lanceolata oils have been used for a long time in perfumery and fragrance when populations of *Santalum alba* (Indian sandalwood) and *Santalum spicutum* (Australian sandalwood) declined in the 1900s (Mbuya et al., 1994). Since then, the species has become a substitute source of sandalwood oil to supply the perfumery and fragrance industry which has led to its over-exploitation in East Africa (CITES, 2013; Otieno et al., 2016; Bunei, 2017). The demand for sandalwood oil has also put much pressure on *O. lanceolata* populations in East Africa (Gathara et al., 2014) leading to over-exploitation (CITES, 2013; Bunei, 2017) and consequential decline in natural populations. For instance, domestic demand for Sandalwood in India was over 4950tonnes/year in 2006 (Teixeira et al., 2017) but only 3000 tonnes were imported due to limited supply (USAID, 2015). The illegal harvesting of *O. lanceolata* trees in Karamoja (Tajuba, 2015; USAID, 2015; Teixeira et al., 2017; Bunei, 2017) implies the total destruction of future livelihoods in East Africa. This raises a concern over the species' population stability amidst illegal harvesting if no action is taken. Amidst the continuous reduction in habitat area, land fragmentation and unsustainable harvesting of valuable plant resources, the spatial heterogeneity of the overexploited species will be significantly reduced (Jensen and Mellby 2012).

The 2007 presidential decree that banned *O. lanceolata* trade in Kenya (Ochanda, 2009) also intensified pressure on *O. lanceolata* populations in the Ugandan Karamoja sub-region (Tajuba, 2015; USAID, 2015) hence leading to population decline. Secondly, the establishment of a factory to extract essential oils from *O. lanceolata* in eastern Uganda is a threat to existing populations of *O. lanceolata* (Tajuba, 2015) if strategies for commercial production are not implemented. In fact, some populations of *O. lanceolata* have completely disappeared while

others are rapidly declining as a consequence of illegitimate harvesting and trafficking of trees even when the species is already listed under Appendix II of CITES in East Africa (CITES, 2013; Muhoozi; 2013; Tajuba, 2015; USAID, 2015; Bunei, 2017).

After realizing the economic potential of *O. lanceolata,* and the faster decline in the species populations, emphasis has been placed on establishment of commercial plantations for the species to boost commercial harvesting (USAID, 2015; UIA, 2016). However, such initiatives cannot be successful without adequate information on the species' population dynamics and survival strategies. Particularly the lack of understanding of the population structure, genetic diversity, and environmental variables influencing the species' survival makes it difficult to strategize for the species' commercial production and conservation.

Although studies on local uses, genetic variation, host-plant associations, oil yield, land suitability and conservation of *O. lanceolata* have been done in Tanzania and Kenya (Njoroge and Bussmann, 2006; Ochanda, 2009; Orwa et al., 2009; Mwang'ingo et al., 2003; Mwang'ingo et al., 2015; Gathara et al., 2014; Otieno et al., 2016; Andiego et al., 2019) there are knowledge gaps in understanding the species distribution drivers, population structure, and host composition in the natural habitats (Mugula et al., 2021). Also, the species morphological response to altitude gradient in semi-arid ecosystems is poorly understood which hinders its responsible management in the semi-arid ecosystems. It is believed that the use of molecular markers has been implemented in restricted areas, and on the other side, there is lack of understanding and disengaging of farmers and policy makers towards this type of data (Antonovics, 2014; Otieno, et al., 2016; Teixeira et al., 2016).

The plant genetic studies provide a basis for assessing the impact of disturbance on random distribution of genes within a population. This information is necessary to control genetic erosion and enhance plant survival (Kalisz et al., 2001; Muchugi et al., 2005; Curto et al., 2015). Information on the species population structure helps to determine the strength and stability of species populations in future amidst ecological and human disturbances (Tabuti & Mugula, 2007). This study was designed to generate knowledge for understanding the population ecology, survival conditions, and genetic diversity of *O. lanceolata* in the semi-arid ecosystems of Uganda and Kenya.

1.2 Statement of the Problem

Osyris lanceolata is valued for its scented wood and essential oils useful in cosmetics and pharmaceuticals industries. However, the species is now endangered due to over exploitation and yet one of the least studied amongst African tree species. Information regarding populations ecology, genetic diversity and structure of the species across eco-physiological gradients to enhance the development of suitable conservation measures, which is lacking for *O. lanceolata* in Uganda and Kenya. In fact, earlier studies revealed knowledge gaps in the species taxonomy, population ecology and genetic diversity. The lack of adequate scientific data on the species population status and genetics hinders conservation efforts and value addition to *O. lanceolata* resources which expose the species to environmental risks that could lead to extinction and loss of livelihoods. For instance, understanding the species' population genetic structure helps to explain the genetic variations in space and time, which also information on the species' mode of dispersal, mating behaviors, and the delimitations of the species and population boundaries. However, the data to understand the genetic structure of economically important species like *O.*

lanceolata is generally lacking in East Africa. Although the species' genetic markers have been developed in East Africa, and existence of some ecological data on its population status, a huge gap in knowledge exists to explain how the *O. lanceolata* populations in Uganda and Kenya vary genetically in and between populations that are already subdivided and the extent to which the species genetic structure has been influenced by anthropogenic, abiotic and biotic factors across their natural range. This study sought to assess the population structure, distribution drivers, genetic diversity and structure of *O. lanceolata* populations in Uganda and Kenya as a basis for developing informed conservation strategies for the species.

1.3 Study Objectives

1.3.1 Main Objective

The main objective was to characterise the population status, distribution drivers and genetic diversity and structure of *O. lanceolata* in Uganda and Kenya.

1.3.2 Specific objectives

Specifically, the study sought to:

1. Determine the population structure of *O. lanceolata* in the Karamoja sub-region of Uganda.
2. Analyse the hosts, habitat qualities and morphology of *O. lanceolata* in Uganda
3. Identify edaphic variables influencing the distribution and density of *O. lanceolata*
4. Characterize patterns in genetic diversity and structure of *O. lanceolata* populations in Uganda and Kenya.

1.3.3 Hypotheses

i. H_O: The population structure of *O. lanceolata* is stable, strong and sufficiently regenerating in Karamoja. H_A: The population structure is weak, unstable and poorly recruiting in Karamoja

ii. H_O: There are no specific hosts and habitat qualities that influence the distribution of *O. lanceolata*. H_A: The distribution of *O. lanceolata* is influenced by specific hosts and habitat qualities

iii. H_O: The morphological traits of *O. lanceolata* are not affected by environmental gradients and species exploitation. H_A: Environmental gradients and species exploitation influence the morphology of *O. lanceolata*

iv. H_O: Edaphic variables have no influence on the distribution and density of *O. lanceolata* in Karamoja. H_A: The distribution and density of *O. lanceolata* is influenced by edaphic variables in Karamoja

v. H_O: There are no distinct patterns in genetic diversity and structure of *O. lanceolata* among the Ugandan and Kenyan populations. H_A: Distinct patterns in genetic diversity and structure exist among populations in Uganda and Kenya.

1.4 Justification

The analysis of population structure, plant-host associations, and edaphic distribution drivers of *O. lanceolata* helps to identify suitable ecological zones and appropriate host species for restoration strategies, commercial production, and conservation of the species in East Africa. In particular, the understanding of plant-host associations and edaphic distribution drivers provides

an insight on suitable conditions for the species survival which helps to inform appropriate conservation strategies.

Analysis of the species genetic diversity and structure reveals genetically diverse provenances to enhance populations in the natural range. But, achievement of these goals, requires ecological and genetic data to understand the status of existing populations. Without such data, conservation efforts can be misguided hence leading to eventual depletion of the species populations given the numerous anthropological and environmental threats to the species survival.

At the species management level, understanding the species population structure should be considered in crafting strategies for responsible management. There is need for urgent conservation interventions to save the declining population of *O. lanceolata* in Uganda including further inventorying of the unknown populations for better management. Thus, contributing knowledge towards understanding the population ecology and patterns in genetic diversity and structure of *O. lanceolata* will strengthen the species' conservation efforts in East Africa and beyond.

1.5 Thesis organisation

This thesis is structured into nine chapters. Chapters one, two, three, and four provides the introduction and methodology. Chapter four highlights knowledge gaps in the taxonomy, ecology and genetics of *Osyris lanceolata*. Chapter five, six, seven and eight address objective one, two, three and four. The published and prepared manuscript (s) from this thesis include:

Published Papers

1. Mugula, B.B. Kiboi, S.K., Kanya, I.J., Egeru, A., Okullo, P., Curto, M., Meimberg, H. (2021) Knowledge gaps in taxonomy, ecology, population distribution drivers and genetic diversity of African sandalwood (*Osyris lanceolata* Hochst. &Steud.): A scoping review for conservation, *Plants,* 10(9). Available at: https://doi.org/10.3390/plants10091780.

2. Mugula, B.B., Omondi, FS., Curto, M., Kiboi, S.K., Kanya, I.J., Egeru, A., Okullo, P., and Meimberg, H. (2023) Microsatellites reveal divergence in population genetic diversity and structure of *Osyris lanceolata* (Santalaceae) in Uganda and Kenya. *BMC Ecology and Evolution* (2023). https://doi.org/10.1186/s12862-023-02182-2

Prepared Manuscripts

1. Mugula, BB., Kiboi, SK., Kanya, IJ., Curto, M., Meimberg, H., Egeru, A., Salamula, JB., Omondi, FS., and Okullo, P., (2024) Unraveling the population status of *Osyris lanceolata* (Santalaceae) in the Karamoja sub-region, Uganda.
2. Mugula, BB., Kiboi, SK., Kanya, IJ., Curto, M., Meimberg, H., Egeru, A., Omondi, FS., and Okullo, P., (2024) Edaphic distribution drivers of *Osyris lanceolata* revealed in the semi-arid habitats of Karamoja, sub-region, Uganda

1.6 Definition of terms

Population dynamics: changes in population structure (size class distribution), density, spatial distribution and abundance.

Ethnobotany: different uses of *Osyris lanceolata*.

Distribution drivers: soil variables and habitat characteristics that favor the distribution and survival of *O. lanceolata* in natural habitats.

East/African sandalwood: *Osyris lanceolata* and its scientific synonyms.

Parasitic associations: the relationship between *Osyris lanceolata* and the hosts including level of proximity, frequency of clustering between the host species and *O. lanceolata*

Associated species or Associates: The species whose distribution is highly linked to the distribution and occurrence of *Osyris lanceolata.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Population patterns of *Osyris lanceolata* in East Africa

The species loss in the tropical ecosystems is largely due to anthropogenic disturbance of habitats (Jimu et al., 2012; IUCN, 2013; Piotti et al., 2013). Such disturbances alter the micro-climatic conditions needed for the survival and fitness of plant species (Graudal et al., 2014). *Osyris lanceolata* is one of the most valuable species whose populations are believed to be declining due to over exploitation in East Africa (Wambua, 2010; CITES, 2013; Tajuba, 2015; Otieno et al., 2016; Bunei, 2017). However, information on the species population status is limited in literature (Mugula et al., 2021).

Disturbed populations are usually characterised by low species abundance, density, poor recruitment and irregular distribution of individual size classes. The determination of plant population structure involves analysing the size class distributions (SCDs) (Hall and Bawa, 1993; Lykke, 1998), density and regeneration potential (Obiri et al., 2002; Jim et al., 2012) which helps to predict the species stability and conservation priorities (Henderson and Wood 2016). The population structure can further be used to explain population decline, identify population threats for action and evaluate the impact of human disturbance on species genetic potential and survival which facilitates action for management responses to specific ecosystem threats (Lindenmayer & Laurance, 2016).

Information on species population structure is also needed to complement genetic studies in understanding evolutionary processes and their interaction with environmental forces to influence species selection and adaptation in an ecosystem (Vellend, et al., 2005). But, the limited information on the population dynamics of *O. lanceolata* in in most semi-arid ecosystems in east Africa is a bottleneck to the sustainable management of the species.

Population assessment for rare plant species is one of the most challenging tasks for ecologists due to the irregular and unpredictable distribution of rare species. This challenge has further complicated the possibility of obtaining accurate information on the population status of rare species. Parameters of plant population structure include; density, abundance, dispersion, height, size class, crown cover, weight, proximity among individuals, and morphological traits such as leaf length, leaf width, and the number of stems. The use of distance methods is recommended in the assessment of absolute density, especially for irregularly distributed species like *O. lanceolata* (Mueller-Dombois & Ellenberg., 1974; Jensen and Meilby, 2012). The application of distance sampling methods in population assessments is explained further in chapter three of this thesis.

2.1.1 The need to assess populations of rare plants

The determination of plant population structure involves analysis of the species size class distributions (SCDs) (Hall and Bawa, 1993; Lykke, 1998), density and regeneration potential (Obiri et al., 2002; Jimu, et al., 2012) which helps to predict the species stability and conservation priorities (Henderson and Wood 2016). The population structure can further be used to explain population decline, identify population threats for action and evaluate the impact of

human disturbance on species genetic potential and survival which facilitates action for management responses to specific ecosystem threats (Lindenmayer & Laurance, 2016).

2.1.2 Distance methods

Distance sampling technique helps to absolutely assess the density of species populations based on accurate distance measurements of detected objects in the locality of a sampling point (Jensen and Meilby, 2012). The principle behind distance methods is to compute the density of trees per unit area using the regular distance among trees (Mueller-Dombois & Ellenberg., 1974). Secondly, tree density can be analysed from average distances between the trees rather than counts in quadrats, plots or strips (Mueller-Dombois & Ellenberg., 1974). Bowering et al., (2018) indicated that rare species are usually clustered in space and thus delineation of sample plots non-randomly increases accuracy in abundance estimates. Thus, density is computed from the mean area (MA) = plot area/number of trees as follows; Density = $\sqrt{MA}$. The method saves time and no boundaries are needed which are difficult to measure. To determine the mean distance between tree individuals in a natural population, random numbers may be assigned on trees and then closest individuals (nearest neighbor method) are randomly selected for sampling the distance between them. Here the mean distance (d) is then determined with correction factors in the nearest neighbor method as 1.67 x (d), and for closest individual method as 2x (d), and for random pairs method as 0.8 x (d) (Mueller-Dombois & Ellenberg, 1974).

2.2 Distribution drivers of *O. lanceolata*

The factors influencing the distribution and abundance of *O. lanceolata* continue to dominate discussions among plant researchers all over the world. A variety of theories have been established to explain what drives the distribution of parasitic plants with emphasis on aerial hemiparasites (mistletoes) than root hemiparasites (Dean et al., 1994; Watson et al., 2007; Jiang et al., 2008; Watson, 2009; Těšitel et al., 2010; Scalon and Wright, 2015; Dueholm et al., 2017). Dean et al., (1994), indicated that mistletoes abundance is influenced by nitrogen levels of a biome. Similar observations were emphasised by Rodrigues et al., (2019) who indicated that soil texture, the macro and micro nutrients also regulate the distribution and abundance of plant species in tropical habitats. In other studies, the distribution of hemi parasitic plants is explained by the host quality hypothesis (HQH) and the abundant center hypothesis (ACH) (Fox 1997; Pfenninger et al., 2011). The latter highlights water availability and edaphic variables as key drivers in spatial distribution. In this case, *O. lanceolata* grows in habitats with suitable conditions for the survival of host species (Watson et al., 2007; Irving and Cameron 2009). The HQH suggests that the abundance of *O. lanceolata* is positively correlated with suitable conditions for reproduction and population growth (Watson, 2009) within an arrangement that favors plant hemi-parasitism. Thus, the distribution of *O. lanceolata* seem to be governed by major factors such as parasitism, altitude, and edaphic variables.

The current theories advanced to explain the abundance and distribution of *O. lanceolata* point towards a combination of biotic and abiotic factors or drivers classified into three major categories; parasitic associations (plant-host associations); altitude; and edaphic variables. These drivers are further shaped by genetic and anthropogenic factors (Jensen and Mellby 2012; Hahn

et al., (2017). The in-depth understanding of the species distribution drivers helps to identify suitable conditions for the species survival within the habitat and plan strategies for responsible species management.

2.2.1 Edaphic distribution drivers of *O. lanceolata*

Understanding specific soil variables that influence the distribution of plant species remains a hot topic among researchers to date and several attempts have been made towards obtaining adequate information to correlate soil variables with the distribution of plant species (Dean et al., 1994; Watson, 2009; Buri et al., 2017; Rodrigues et al., 2019). Edaphic variables are measurable soil parameters, or characteristics that determine the nature and function of a particular type of soil. The converging conclusion from several studies is that a combination of soil variables influences the spatial distribution and survival of plant species (Rodrigues et al., 2019; Chitiki, 2020). Therefore, an investigation into soil variables that potentially influence the presence and survival of a plant species is important in strategising plans for the long-term species conservation.

Past studies have established that *O. lanceolata* has an inconsistent relationship with edaphic variables, especially in humid highland and dry lowland forest ecosystems (Gathara, et al., 2014). However, its correlation with soil nitrogen is significant in humid highland ecosystems (Gathara et al., 2014). A number of other studies have also linked the distribution of *O. lanceolata* to the presence of edaphic variables. Edaphic drivers can be understood by considering the species distribution and abundance as a linear function of soil nutrient variables interacting with biotic and abiotic factors to influence the species' survival within a habitat (Scalon and Wright, 2015; Rodrigues et al., 2019). Ordination techniques such as canonical

ordination helps to analyze relations between plant species and edaphic variables (Oksanen, et al., 2016; Flesch, 2017). These detect variation patterns in species data explained by environmental factors (ter Braak and Verdonschot, 1995).

Considering *O. lanceolata* distribution, suitable areas for the species survival should have specific nutrients that support plant growth. Secondly, because edaphic variables are unequally distributed within the habitats, some variables exert more influence on the species' survival than others (Zhang et al., 2018). Thus, some areas will support survival than other areas even within the same ecosystem. To demonstrate this observation, analysis of soil nutrient levels between areas where the species survives and those where the species is absent helps to reveal key nutrients that influence the species distribution. This has implications on the sampling strategy for the samples to be analysed.

2.2.2 Parasitism in *O. lanceolata*

Parasitism in plants could have evolved in dry environments where water and nutrients influenced the development of haustoria in root parasitic plants (Tenakoon et al., 1997; Okubamichael et al., 2016). *Osyris lanceolata* is a root hemi parasitic species in the family Santalaceae. The species may require or may not require any host to grow until maturity (Bell and Adams, 2011; Furuhashi et al., 2012). However, the seed germination of *O. lanceolata* does not require hosts at early stages (Kuijt, 1969; Mwang'ingo et al., 2005) except further development of seedlings which require hosts to progress (Rao, 1942; Metcalfe and Chalk, 1950; Kuijt,1969; Herrera, 1988; Mbuya et al., 1994).

Angiosperms that are parasitic are categorized into two groups as hemi parasites or holo parasites. Hemi parasites contain their chlorophyll and need water and mineral salts acquisition from their hosts (Tenakoon et al., 1997; Tenakoon and Cameroon, 2006). However, holo parasitic plants lack their own chlorophyll and thus require access to water, mineral nutrients and carbohydrates (Bell and Adams, 2011). Some plants are in-between hemi and holo-parasites; for instance, genus Cuscuta which depends on hosts for carbon at a certain stage of growth (Těšitel et al.,2010). Among the hemi and holo parasites, there are two broad forms of parasitism which include; aerial parasites which parasitise on stems and these constitute about 40% and the root parasites which are the majority constituting over 59% (Musselman and Press, 1995). The only exception to these two broad forms is the genus Tripodanthus where one species attaches to both roots and stems of the host plants (Mathiasen et al., 2008). Further classification of plant angiosperm parasites includes xylem or phloem feeders (Irving and Cameron, 2009). Some parasitic plants have evolved to even parasitise on other parasitic angiosperms in a relationship called epi-parasitism and hyper parasitism (Mathiasen et al., 2008).

Some plants parasitise over 440 hosts and other species are very host specific Matthies, (1999). Therefore, hosts tend to influence the general distribution of angiosperm parasites because they facilitate the acquisition of essential nutrients required by parasitic plants to survive hence influencing their distribution. However, much of the ecology, biology, and determinants of host-parasite relationships is poorly understood (Irving and Cameron, 2009; Marvier, 2014) including which host compositions characterise the different life stages of *O. lanceolata* and how such composition varies across altitude gradients in the natural habitats (Mugula et al., 2021).

Essentially, *O. lanceolata* parasitizes roots of plants categorised as hosts (Qasem, 2006). *O. lanceolata* is thus believed to use hemiparasitism as a survival strategy in acquiring nutrients where it occurs (Tenakoon et al., 1997; Tenakoon and Cameroon, 2006; Mwang'ingo et al., 2005; Nge et al., 2019). These essential growth nutrients include; K^+ P & Mg^{2+}. Marvier, (2014) indicated that interactions between parasite-hosts may have strong direct and even indirect effects on both parasites and hosts performance.

Among parasitic plants, the determinants of host specificity and preference remains a complex question among plant ecologists and researchers to-date. As indicated by Westwood et al., (2010) host species are recognised by parasitic plants through chemicals or contact signals which initiates haustoria development catalysed by inducing factors including flavonoids. Also, the host inducing factors may be specific to hosts by stimulating specific receptors in specific parasites (Tormilov et al., 2006). Some parasitic plants that survive as generalists may prefer specific abundant hosts in a given locality (Mwang'ingo et al., 2005; Tenakoon and Cameroon, 2006). Previous studies also established that most hemiparasites survive as generalists with a wide range of hosts, though they perform better when specific host species are present than others (Sandner et al., 2022). This implies that in diverse communities of host species, the hemiparasitic plants exhibit preference to some hosts (Sandner et al., 2022). However empirical data to ascertain this is parasitic associations is lacking for *Osyris lanceolata***.** To understand the similarity in host composition of a root parasitic plant the Sorensen index (SI) is usually applied to calculate the similarity index as follows:

Sim = $^{2D}/_{B+C}$, where B is the species number in site B, C is the species number in site C, and D = species number common both in site B and C. If the indexes between sites are less than 0.5, then study sites are dissimilar in host species composition and *O. lanceolata* have no host preference

2.2.3 Effect of altitude on morphology of *O. lanceolata*

O. lanceolata occurs in a variety of altitudes ranging from as low as 900M. asl to very high altitudes (over 2000M.asl (Mwangi'ngo, 2002; FSSD, 2021). However, the species morphological response to altitude is not well documented. A proper characterisation of the species morphological traits across environmental gradients is a good strategy to identify suitable environmental conditions that boosts its survival and fitness. This is necessary for planning suitable sites and habitats for species conservation (Lomolino, 2001). Previous studies on shifts in plant morphologies have largely focused on understanding how plant morphologies respond to climate change (de Groot et al., 2019) with less attention to altitude variations. Thus, altitude gradient is a suitable variable for assessing a species fitness to habitat conditions (Nepali et al., 2021). The plant response to altitude variation can be manifested in measurable morphological attributes such as crown cover, height, stem diameter, leaf size and number of stems.

2.3 Genetic diversity of *O. lanceolata* in East Africa

O. lanceolata is one of the least genetically studied species compared to other members in family Santalaceae and a huge gap in knowledge exists in understanding the species population ecology and genetics in East Africa (Mugula et al., 2021). Nevertheless, the few genetic studies on the species, although restricted in Kenya, have yielded commendable benefits such as identification and development of microsatellite markers, polymorphic and monomorphic markers, and delineation of genetically diverse populations for in-situ conservation (Otieno et al., 2016;

Andiego et al., 2019). Among the seven *Osyris lanceolata* populations studies in Kenya, Gwasii population was the most genetically diverse, followed by Mt. Elgon, while Baringo population was genetically distinct from the rest of the populations (Andiego et al., 2019). The conservation implication from the findings was; that genetically diverse populations (Gwasii and Mt. Elgon) would be conserved in-situ, while ex-situ conservation would target good individuals from all populations. In related studies, morphological analysis of *O. lanceolata* in selected Kenyan populations distinguished Gwasii population from populations in Kitui, Kabarnet, and Marigat areas. However, all individuals revealed population genetic interrelationships (Andiego et al., 2022). For other sandalwood species like Santalum sp, genetic advances have provided further benefits including better understanding of phylogenetic relationships among populations, molecular identification of *Santalum sp* and adulterant species, assessment of genetic diversity and differentiation, clonality, marker-assisted breeding, disease control through quantitative trait loci identification, and changes in gene expression through RNA sequence analysis (da Silva et al., 2018).

The understanding of genetic diversity enhances long-term conservation of threatened species (Zong et al., 2015) Genetic diversity as the number of alleles present in the gene pool of a population measured through parameters including; observed heterozygosity (Ho), expected heterozygosity (He), and allelic richness (Frankham et al., 2002). However, there are other parameters such as nucleotide diversity, the number of polymorphic loci that can be used as well. Genetic diversity enhances species survival and adaptation through natural selection (Alfaro et al., 2014; Govindaraj et al., 2015; Ellegren & Galtier, 2016; Fuentes-pardo, 2017).

Among populations of threatened species like *O. lanceolata,* characterisation of genetic diversity helps to understand population dynamics, origin and trends in evolutionary processes which facilitate development of strategies for conservation of genetic resources (Zong et al., 2015). However, due to population bottlenecks, useful tree species are experiencing higher rates of loss in genetic diversity and thus genetic erosion in tropical ecosystems (Neale & Kremer, 2011; Fuentes-pardo et al., (2017). The importance of genetic studies and ecology has been emphasised in past studies (Kahilainen et al., (2014) to understand anthropogenic impacts on the genetic adaptation potential of a plant species.

The effects of anthropogenic disturbances such as habitat fragmentation have been highlighted to reduce geneflow levels and contribute to further decline in effective population size and loss of genetic diversity (Curto et al., 2015). The decline in genetic diversity for seedlings and saplings has also been indicated resulting from habitat alterations (Graudal et al., 2014). For instance, Farwig et al., (2008), showed reductions in allelic richness and heterozygosity of an overexploited plant species in Kakamega forest and reduced regeneration potential was also reported by Owiny and Malinga (2014) within *Prunus africana* populations due to habitat disturbance in Kibale forest. These observations imply that human disturbances lead to habitat degradation and loss of genes from plant species due to destruction of individuals. Thus, it is necessary to understand the population genetic structure of threatened species so as to detect gene dispersal distances and the effect of ecosystem disturbances on the non-random distribution of genes within populations (Volis et al., 2016).

2.3.1 Analysis of genetic diversity

Analysis of genetic diversity is necessary to forecast changes in genetic structure and document genetic diversity loss in plant populations (Frankham et al., 2002). In such cases the genetic diversity measures include number of; observed alleles (Na), effective alleles (Ne), the private alleles (A_p), the percentage of polymorphic loci (PPL) per population, Shannon's Information Index (*I*), coefficient of inbreeding (*Fis*), observed heterozygosity (Ho), fixation index (*Fst*), expected heterozygosity (He) and gene flow (Nm) (Toro et al., 2005; Zong et al., 2015; Yang et al., 2016). Heterozygosity is relative to the level of genetic variability at a locus in a population (Frankham et., al., 2002) and also describes the chance that two randomly chosen alleles from the population are dissimilar (Frankham et al., 2002). Higher levels of heterozygosity show high genetic variability attributed to natural selection occurring for a long time and leading to species adaptation to the environment. The low heterozygosity indicates little genetic variability, attributed to isolation leading to loss of genetic potential in a population (Frankham et al., 2002).

The Nei's (1973) formula is used to compute heterozygosity as:

$H = n\ (n-1)\ *(1-\sum p^2_i)$, where P_i is the alleles i frequency in the population and n as the alleles number.

Measuring genetic diversity with molecular markers includes comparing observed heterozygosity with expected heterozygosity under the principle of Hardy-Weinberg Equilibrium (Toro and Caballero, 2005). When observed heterozygosity is lower than expected, the variance is attributed to inbreeding or the Wahlund effect, which is the increased frequency of homozygotes in a subdivided population (Frankham et al., 2002). When observed heterozygosity is higher than expected, the variance may be attributed to the hybridisation or mixing of two

populations that were previously isolated from each other, and balancing selection or gene duplication (Frankham et al., 2002). The value of heterozygosity ranges from 0 indicating no heterozygosity to 1.0 for populations with equally frequent alleles.

The observed heterozygosity is the proportion of heterozygous loci per individual or the number of individuals who are heterozygous at each locus.

The expected heterozygosity (He) for one locus is calculated as:

$$He = 1 - \sum_{h=i}^{k} \left(P_1{}^2\right)$$ Where *pi* is the i^{th} frequency of k alleles.

The observed and expected heterozygosity can be used to estimate the extent of inbreeding in a population. Inbreeding can be estimated as:

$F = 1 - {}^{Ho}/_{He}$ per population using Gene pop software (Mehes et al., 2009), where, Ho, refers to the observed heterozygosity, and He refers to the expected heterozygosity.

The Wright's F-statistics and Analysis of Molecular Variance (AMOVA) are further used to estimate the genetic diversity in populations and among populations (Mehes et al., 2009; Yang et al., 2016). The Wright's F-statistic or Fixation Index (FST) calculates the rate of decline in heterozygosity attributed to subdivision within a population. The values of fixation index usually range from 0 which implies no differentiation between the overall population and the subpopulation, to a maximum of 1.0 (Fernanda et al., 2017). AMOVA describes the extent of division of genetic dissimilarity or variation between and in groups, and these measures can be computed by Arlequin, and GeneAlex programs (Zong et al., 2015; Soorni et al., 2017).

Genetic differentiation is defined as the gradual increase in differences in allelic frequencies between populations that are entirely or moderately isolated because of evolutionary forces including selection and genetic drift (Frankham et al., 2002). The F-statistics, analysis of molecular variance (AMOVA) and Fixation index are used to measure genetic differentiation (Verity and Nichols, 2014).

To measure Nei's genetic differentiation, the Nei's genetic similarity is first calculated as:

In $=\sum p_{ix}.\, p_{iy} / [(\sum pix2)\,(\sum piy2)]^{1/2}$: and then log transformed as:

D_N = - In (I_N). The $p_{ix,}$ refers to frequency of allele *i* in the population or species *x*. The p_{iy} refers to the frequency of allele i within population *y,* and m to the number of alleles at the locus.

When two populations have related allele frequencies as ($p_{ix=}p_{iy),}$ then, the genetic similarity tends towards 1 while the genetic distance tends towards 0 implying that the two populations have no shared alleles, and the index of genetic similarity between populations is 0 and genetic distance is infinity (Frankham et al., 2002).

2.3.2 Gene flow in natural populations

Gene flow describes a situation where previously isolated populations begin to interbreed with each other hence bringing in "foreign" genotypes which may delay local adaptation. Gene flow (N_m) is assessed from the extent of genetic differentiation between populations (Frankham et al., 2002). Gene flow or gene migration is said to be influenced by factors such as; the number of fragments of a population, spatial patterns of population or geographical distribution, the ability of the species to disperse, the distance between fragments, migration rates between fragments,

and time since fragmentation (Frankham et al., 2002). The Pop-gene software is used to estimate gene flow (N_m) among populations (Zong et al., 2015).

2.3.3 Tests for linkage disequilibrium (LDE)

Frankham et al., (2002), describes the association of alleles non-randomly between loci as linkage disequilibrium. It occurs usually in threatened species with small population sizes (Toro and Caballero, 2005) and is caused by the mixing of different populations, selection, and population size. Linkage disequilibrium is usually measured as a coefficient of linkage disequilibrium (D), which is the extent of association of alleles non-randomly at different loci (Frankham et al., 2002). If a population is found to be in linkage disequilibrium and not linkage equilibrium, the implication is that such a population must have been affected by various disturbances. The genetic perturbations causing linkage disequilibrium between loci include; bottlenecks in populations, selection and mixing of populations. The presence of linkage disequilibrium implies presence of genetic admixture which is the mixing of genes within populations which are differentiated.

Testing for linkage disequilibrium is important because it helps to ascertain whether the species is threatened or not, provides insight about the size of a population, indicates occurrences of bottlenecks in a population, and helps to establish whether evolutionary processes of a population are altered or not (Frankham et al., 2002). The Gene Pop and FSTAT programs can be used to test for linkage disequilibrium (Mehes et al., 2009). Gene Pop program formulates contingency tables for all loci pairs for each population and the pooled sample of populations

and then performs a test of probability to find p-values for each table by the Markov chain (Hey and Nielsen, 2007; Uleberg and Meuwissen, 2011; Ocampo et al., 2021).

Estimates of geneflow in other hemiparasites such as *Rhinanthus serotinus* (Scrophulariaceae) has been done in recent studies and revealed the virulence of the parasitic plants to be highly varied among populations, which suggests genetic variation (Mutikainen et al., 2000). In similar studies, a climatic niche shift was detected in hemiparasitic species; *Thesium canariense* (Gran Canaria) which showed high differentiation, while *T. subsucculentum*, *T. retamoides* (Tenerife), and La Palma populations, showed different distributions across the environmental gradients of temperature (Rodríguez-Rodríguez et al., 2022). Specific studies have investigated the possible criteria for selecting seed sources or provenances from useful plant species in semi-arid habitats, and found out that the selecting geographic origins of provenances or seeds is complicated by climate change; seed sourcing strategies are dependent on habitats and species' genetic and biochemical attributes (Walters et al., 2022). However, much as the geneflows and possible genetic attributes for selection of better provenances for conservation have been studied in other hemiparasitic plant species, such investigations are currently lacking for *Osyris lanceolata* in Uganda and Kenya. Thus, it is necessary to undertake comparative studies on the species genetic diversity and structure to contribute towards narrowing the existing knowledge gaps on the species ecology and genetics.

2.3.4 Genetic structure

Genetic structure is the amount and distribution in genetic variation in and between populations and this is determined by the interaction of genetic and ecological processes (Frankham et al.,

2002) Population genetic structure involves understanding the variations genetically in space and time. The investigation of population genetic structure provides information on the species' mode of dispersal, mating behaviors, and the delimitations of the species and population boundaries (Volis et al., 2016). There is a general lack of genetic data to understand the genetic structure of economically important species like *O. lanceolata* in Uganda and Kenya. Despite some efforts to develop genetic markers and evidence of ecological data on the population structure of *O. lanceolata*, a huge gap in knowledge exists to explain how populations vary genetically in and between populations that are subdivided and how the genetic structure has been influenced by anthropogenic, abiotic and biotic factors across their natural range (Mugula et al., 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area (s)

The study was conducted in Moroto, Nakapiripirit and Amudat district (Karamoja sub-region) in Uganda, and selected areas in the rift-valley region of Kenya (Baringo, Mau, and Laikipia) and in the areas of Mt. Elgon in Kenya (Figure 2). Karamoja is located between 1°.4'- 4.24°N and 33°50'- 35°E and it borders South Sudan to the north, Kenya to the east and Acholi, Lango, and Teso to the west (KRSU, 2018). Karamoja occupies over 9.5% of Uganda's land area and is divided into seven districts including; Kabong, Kotido, Abim, Moroto, Napak, Amudat and Nakapiripirit. The population ranges between 1-1.3 million people of which 75-80% live in absolute poverty (KRSU, 2018). The region is characterized by distinct dry and wet seasons (Egeru et al., 2014) with an average rainfall of 400-1200 mm, and temperatures ranging between 28°C and 33°C (Wanyama et al., 2024) with a monomodal type of rainfall. The rains usually begin in late February to September with its peak in April to May and a break in June (KRSU, 2018). The largest human population in Karamoja are mainly pastoralists, with few individuals involved in crop production, practicing on a communal land tenure system. *Osyris lanceolata* is illegally harvested by local people to obtain fencing poles, firewood, and herbal medicine. On many occasions the local people use destructive methods to harvest the tree species. The host plant species are also harvested for mainly charcoal burning, firewood and timber harvesting and

poles for construction. On a commercial scale *O. lanceolata* has been illegally harvested to a tune of over *500 tonnes* lost since 2011(Tajuba, 2015).

3.1.1 Selection of populations

The Karamoja sub-region was purposively divided into two study blocks namely; Central and Southern block. The Central block consisted of Moroto district while Amudat and Nakapiripirit districts constituted the Southern block. Study populations were identified from different altitude zones between 1200M.asl and 1800M.asl. The sites were selected after extensive surveys conducted between 2018 and 2020 and by CITES reports and articles on *O. lanceolata* (CITES, 2013). The selection of target populations was also based on the species availability and accessibility. A further criterion was to select populations from a spectrum of altitudes. Subsequently, eleven sites were selected in Uganda for sampling and these include: Akariwon, Lonyilik (Moroto); Lotemwoyes, Kopedur and Lolupe (Nakapiripirit); Cheporon, Kangisa, Karengepoche, Korenyang, Ngaram, and Ruwotokech (Amudat) (Figure 3.1). In Kenya, four study sites were selected for sampling, based on accessibility, representation in central, western Kenya, rift valley region, and higher altitude areas >1800M.asl. The sites include; Mt. Elgon, Baringo, Mau and Laikipia (Figure 3.1). The sites were selected basing on their accessibility and availability of *O. lanceolata* individuals, and their locality in western Kenya, rift-valley, and the central Kenya region, in higher altitude areas.

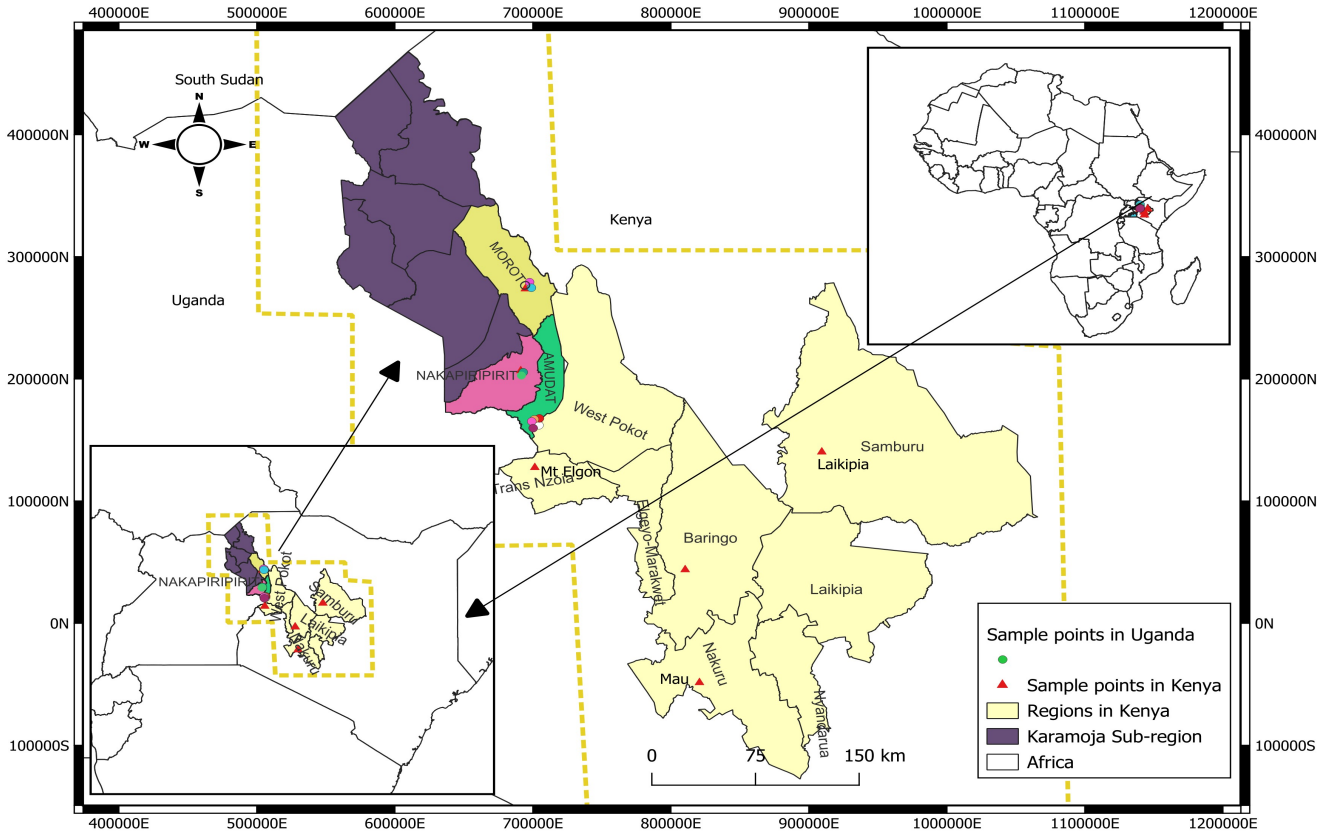


Figure 3.1: Location of Sampled populations of O. lanceolata in Kenya and Uganda

3.1.2 Sampling and data collection

The population and morphological variables of *O. lanceolata* were assessed by sampling using the nearest neighbor method (NNM). This is a plotless sampling technique widely used to assess the density of plant species with patchy distributions (Mueller-Dombois & Ellenberg, 1974; Mwang'ingo et al., 2002). The NNM can be used to estimate the density of a species with small proportions of individuals in the sampled area (Mueller-Dombois & Ellenberg, 1974). In this method the first population measurements are made at a randomly selected initial sampling point, followed by choosing the nearest individual from the initial point (Anthony and Catana, 2012). A pre-determined criterion is used to identify the first sampling point and the next nearest individual of the species is then identified following the nearest neighbor approach (Mueller-

Dombois & Ellenberg., 1974, Cogbill et al., 2018). The NNM technique is effective in estimating density of rare and sparsely distributed species across large geographical areas (Jensen and Meilby, 2012; Thomas et al., 2010; Flesch, 2017). The species density is determined from the mean area occupied by individuals (Jensen & Meilby, 2012; Flesch, 2017). Finally, the dispersion pattern is determined by squaring the sum of distances between a randomly selected sampling point and the nearest tree individuals over squared sum of distances between one tree and its nearest neighbor as follows:

$A = \Sigma (d_1^2) / \Sigma (d_2^2)$; where A = pattern of dispersion; d_1= the distance from randomly selected sampling point to nearest tree individual; d_2 = the distance to nearest neighbor. If $A<1$ = the dispersion is regular; if A = 1, dispersion is random; and if $A>1$, dispersion is clumped (Talvitie et al., 2006).

For each site, the first sampling point was obtained after encountering the first *O. lanceolata* in a north-south direction. The sampling intervals were not specified due to the patchy distribution of the species. The general movement followed a square-like pattern during sampling so as to encounter more individuals of *O. lanceolata* as they could be easily spotted from all directions. At each sampling point, *O. lanceolata* was identified, all measurements and recordings were done within a radius of 5m. The altitude and GPS coordinates for each sampling point was recorded by a GPS (Garmin 64s). The species attributes, measured at every sampling point included; population variables, morphology, regeneration, and soil samples. The subsequent sampling and measurements at each site continued until at least ten individuals of *O. lanceolata* were encountered.

3.1.3 Species morphological characteristics

The study assessed different morphological traits of *Osyris lanceolata* including; number of stems; height; crown cover; leaf length; leaf width; and proximity among individuals. The number of stems was recorded to check the species' extent of branching and how it varies with altitude. Analysis in the branching patterns of the species with altitude provides an insight on how altitude affects the species pattern of branching. The species height was measured by estimating the total height of the tree from the ground to the last tip of the shoot using the halving method. Tree height provides an indicator of the species' stress due to water and nutrient deficiency in the study area (Wonn, 1998). Further, the species height also helps to determine the height to diameter ratio which is important in predicting the species' stability to wind damage across altitudes (Wonn, 1998). In this study, the height attribute was used to construct height class distribution curve and explore whether the species displays any bonsai effect which could affect its growth capabilities in the semi-arid ecosystem (Cousins et al., 2014).

The leaf length and width were measured to examine the extent of variation in leaf size across the altitude gradient and its implications for species' survival and fitness in the ecosystem. The crown cover was obtained after measuring the crown diameter in two cardinal directions of the crown (Ibrahim et al., 2014), and crown size measured by a tape measure. The crown cover assessment helps to explain the effect of overexploitation on the crown size of *O. lanceolata* through analysis of variations between coppiced and non-coppiced juveniles and adult individuals. The species regeneration strategy was assessed by examining the existing form of growth/vegetative / coppiced / reproductive) (Mligo and Kikoti, 2015) and availability of seedlings exhibited by *O. lanceolata*. The species growth mode was categorised into two classes

as; growth after coppicing (vegetative); and independent growth from seedlings without coppices (uncoppiced individuals).

3.1.4 Hosts and habitat characteristics

The hosts of *Osyris lanceolata* were identified around the species within a radius of 5 meters. For each sampling point, the host identify, host proximity to *O. lanceolata*, and life stage of *O. lanceolata* were recorded. The voucher specimens of unidentified hosts were wrapped in newspapers, pressed and sent to Makerere University Herbarium for identification and further reference. The identity of host species was recorded, and their families identified and recorded at each sampling point. The following attributes were used for habitat assessments: habitat type based on levels of illumination such as; full light habitats (FLT), medium light (MLT), and dense habitat (DSD). The habitat conditions were described as follows: litter cover on ground (LCG), exposed soil (ES), animal trails seen on ground (AT), rock at soil surface (RSS), gulley on ground (GLG), dry water courses, water courses on ground (WCG), mammal droppings (MDP), fallen fruits decomposing on ground (FFD), termite mounds (TMD), open grass understory (OGU), open shrub understory (OSU), and dense impenetrable climbers (DIC). All habitat attributes were checked to establish any relationship between the presence of hosts and habitat qualities (Fig.3.3A-F). Habitat assessment helps to identify suitable conditions for the species habitats that could influence distribution, abundance.

3.2A 3.2B 3.2C

3.2D 3.2E 3.2F

Figure 3.2 A-F: Field work activities during collection of ecological data in Karamoja

3.1.5 Soil sampling

The soil samples were collected at two points; within 5m radius around *O. lanceolata*, and at 10m away from *O. lanceolata* (sampling point). One hundred and twelve (112) soil samples were collected randomly from eleven populations to assess edaphic distribution drivers of *O. lanceolata*. Two locations were sampled at each site: one location within the 5m radius of *O. lanceolata* and another location at 10m away from *O. lanceolata.* The samples collected within the 5m radius of *O. lanceolata* were referred to as "*Osyris* samples" while non-*Osyris* samples were collected at 10m away from *O. lanceolata*. A soil auger was used to collect all samples at two depths, so as to explore how nutrients vary with depths between *Osyris* and non-*Osyris*

samples. The topsoil was collected at 0-20 cm while bottom soil at 20-40cm. All samples were packed in tight black polythene bags and labeled using non-erasable ink on a masking tape to indicate the study site, sampling point, and sample category. The labeled samples were temporarily stored under room temperature at NARO-Nabuin Zonal Agricultural Research and Development Institute (ZARDI) in Karamoja and later transferred to Makerere University, for analysis.

3.1.6 Soil processing and analysis

Prior to analysis of soil samples in the laboratory, all samples were dried by air at room temperature, grinded, and sieved through a 2mm sieve. Samples were analyzed for pH, measured on a ratio of 1:2.5 soil and distilled water used to make a suspension, and later pH meter used to take the pH readings. Soil texture was assessed by the bouyoucos or hydrometer method. Exchangeable cations (K^+ Na^+ and Ca^{2+}) were extracted with neutral ammonium acetate solution and then determined directly from emissions measured by a flame photometer. The nitrogen content (N) was determined by Kjeldahl method, and Bray 1 method used to measure phosphorus (P) and determined by a spectrophotometer (JENWAR 6405UV/vis) (Olsen et al., 1982; Okalebo et al., 2002). The oxidation method was used to determine organic matter content in the samples (Olsen and Summers 1982). The soil processing and analysis is illustrated in Fig.3.3A-H

3.3A *3.3B* *3.3C* *3.3D*

3.3E *3.3F* *3.3G* *3.3H*

Figure 3.3 A-H: Field soil sampling, laboratory processing and analysis.

3.2 Selection of methods for genetic diversity assessment

The developments in genetic technology have improved our understanding of genetic diversity among different plant species (Szczecińska et al., 2016). Specifically, the use of molecular markers and microsatellites is a more preferred method in assessment of the species genetic diversity (Frankham et al., 2002; Porth and Kassaby, 2014). According to Holliday et al., (2017), suitable genetic methods can be selected based on their cost of assay; degree of polymorphism, multiplex ability, ease of assay use, sample amounts per assay, reliability, null allele detection, dominance, and a theoretical number of loci present. The higher levels of genetic diversity per locus can be revealed by microsatellites than allozymes while much more loci can be surveyed by RAPDs, AFLPs and DNA fingerprints than allozymes (Frankham et al., 2002). In light of

second-generation sequencing techniques such as *Illumina microsatellite,* discovery became an easy process (Castoe et al., 2012). Recently, these technologies were applied for microsatellite genotyping and exhibited a higher throughput which makes it easier and more reproducible (Tibihika et al., 2018; Neophytou et al., 2018). These approaches retrieve a higher number of alleles than traditional fragment length analysis, which is a consequence of the possibility of genotyping SNP variation together with the repetition motif (de Barba et al., 2017; Neophytou et al., 2018; Tibihika et al., 2018). This decreases the degree of homoplasy which is characteristic to SSR markers, and also improves the statistical power of the markers (Vartia et al., 2016). In this study, the SSR markers were used to genotype the selected populations.

3.2.1 Collection and processing of genetic samples

Fresh leaf material was collected from mature individuals of *O. lanceolata* at each study site and used for genetic analysis. A genetic sample was composed of fresh leaves obtained from adult trees of *O. lanceolata*. A total of 96 samples were collected from populations in Amudat, Moroto and Nakapiripirit. The origin of samples was clearly marked. The collected samples were labeled for easy identification in the following order; country of origin, district, population, and sample number. After labeling, all samples were placed in top sealed paper bags and later inserted and sealed in plastic dishes containing silica gel. The labeled samples were temporarily stored at room temperature at Nabuin-NARO-ZARD laboratory store for Ugandan samples and Kenya Forestry Research Institute labs (KEFRI) for Kenya samples until DNA extraction and further genetic analysis. Storage of samples under room temperature is cheaper and useful especially when laboratory facilities are located far away from study sites (Morgante et al., 2013). The process of genetic analysis from sample collection, DNA extraction and quantification are summarized in Fig. 3.4A - I.

3.4A 3.4B 3.4C

3.4D 3.4E 3.4F

3.4G 3.4H 3.4I

Figure 3.4A-I: Genetic sample collection and Laboratory analysis

3.2.2 DNA extraction and amplification

A total of seven populations, three from Uganda and four from Kenya were sampled for genetic analysis. Karamoja populations were; Moroto, Nakapiripirit and Amudat, while the Kenyan populations included Mt. Elgon, Baringo, Mau, and Laikipia (Figure 3.2). the samples of leaves were collected from mature, young individuals of *O. lanceolata*. The collected samples were later dried on silica gel in paper bags, briefly stored under room temperature before DNA

extraction. The isolation of DNA from dried leaf samples followed the CTAB (acetyl-trimethyl-ammonium-bromide) protocol described by Hanaoka et al., (2013). A total of 0.2g of dry sample were ground into fine powder at a frequency of 30 turns per second for 5 minutes by a steel ball grinder (Retsch MM400, Hamburg Germany). Five hundred microlitres (µL) of the isolation buffer (IB) consisting of 0.1M Tris-HCl, 0.35M Sorbitol[9], and 0.5%β-Mercaptoethanol was added to the fine powdered leaf fine sample. The mixture was vortexed for one minute and centrifuged for 3 minutes at a frequency of 10,000 rotations per minute (rpm) using a Mikro 12-24 model centrifuge at 4^0C. This was done to separate secondary metabolites from the sample mixture. After centrifuging, the supernatant was discarded. The process was repeated two times with 800 µL of IB to ensure thorough washing of the sample mixture. The remaining sample solutions were pipetted and transferred to separate Eppendorf tubes. 800 µL of CTAB extraction buffer containing, 1ml of 1% CTAB solution, 2.8mls of 0.7M NaCl, 0.4mls of EDTA, 1ml of 0.05M Tris-HCl, 0.05mls of 0.5% β-Mercaptoethanol and 2.75mls of distilled water, and RNase enzyme added to the sample solution. Then, solution was vortexed for one minute, followed by incubation for one hour at 65^0C. After one hour, the temperature was reduced to 37^0C to allow the action of enzyme RNase, and incubation was continued for one hour. After incubation, 800 µL of Chloro-isoamyl alcohol (CIA) was added to precipitate DNA molecules. CIA was added two times to ensure complete precipitation of proteins and polysaccharides. The sample solution was then shaken using a shaker (Edmund Buhler,7400 Turbingen, SM 25) at a frequency of 250turns per minute (tpm) for 3 minutes to allow thorough mixing of the CIA with the sample solution. The samples were centrifuged for 3minutes at 60rpm to separate the supernatant from residues and CIA. The resulting supernatant was pipetted off and placed into separate clean Eppendorf tube and centrifuged for the second time, at 60rpm for 3 minutes. This was done to

allow separation of chloroform, polysaccharides and the supernatant. 70 µL of Sodium acetate was added to the supernatant followed by 800 µL of Isopropanol and slowly mixed by inversion. The sample tubes were inverted 50 times to allow proper mixing of the supernatant with Sodium acetate and Isopropanol. The sample solution was then deep frozen at -20^{0}C for 5 minutes to allow precipitation of the DNA. The residual solution was then poured off and DNA molecule precipitated as white pellets. 800 µL of 70% ethanol was added to wash the DNA molecules. The solution was finally centrifuged at 60rpm for 3 minutes to allow settling of pure DNA molecules at the bottom of the sample tubes. This was followed by pouring off the supernatant and leaving the settled DNA molecules to air dry for 24 hours.

3.2.3 DNA Quantification

The quantification of DNA samples was done by a nanodrop spectrophotometer (BioSpec-nano, Shimadzu Biotech, Hamburg Germany) to check the concentration and purity. Then, samples were diluted to a concentration of 10ng/ µL and stored under -21^{0}c until PCR analysis.

3.2.4 Dilution and reconstitution of primers

Ten primer pairs consisting of di or tri-nucleotide phosphate repeats were used to characterise the populations of *O. lanceolata*. The primer sets, consisted of reverse primers, forward primers and one set of forward radio-labeled or fluorescently labelled primers. Before dilution, the primers were briefly vortexed. 20 µL of each primer was pipetted into a fresh microtube. Eighty microliters (80 µL) of PCR water were added to make 100mls of solution and stored under -4^{0}C before multiplexing. The primer pairs were reconstituted into three primer mixes (1, 2, 3) as follows: Mix 1 consisted of $KFOL_2$; $KFOL_{13}$; $KFOL_{16}$; $KFOL_{17}$; KR_1, KR_2, KR_3, KR_4. Mix 2: $KFOL_{19}$; $KFOL_{24}$; $KFOL_{28}$; $KFOL_{48}$; $KR_{5\text{-}8}$ Mix 3: $KFOL_{37}$; $KFOL_{42}$; $KFOL_{48}$; and $KR_{9\text{-}12}$.

Each primer mix was made by adding 1.8 µL of the forward fluorescently labeled primer, 25.2 µL of the forward nonfluorescent labeled primer, and 27 µL of the reverse primer.

3.2.5 PCR Multiplexing

The PCR master mix consisted of 3 µL of PCR multiplex (comprised of Taq-polymerase, dNTPS and buffer solution), 0.52 µL of PCR water, 1 µL of Q-solution, 0.48 µL of the primer mix, and 1 µL of the DNA sample. The master mix was briefly centrifuged before PCR multiplex reactions. The multiplex reactions of PCR were performed in a total volume of 6 µL with 1 µL of QIAGEN multiplex PCR kit (QIAGEN), 0.48 µL primer mix, 0.52 µL of PCR water, and 1 µL of diluted DNA. The primer mix consisted of a 1.8 µL of fluorescently labeled forward primers, 25.2 µL of non-fluorescently labeled primers, and 27 µL of a reverse primer. The touchdown thermocycling program was used in PCR reactions, programmed as follows: initial denaturation at 95^0C for 15 minutes, 30 seconds denaturation at 94^0C, 90 seconds annealing at 57^0C, and 1 minute elongation at 72^0C for 35 cycles. Final cycle occurred at 60^0C for 30 minutes. The amplified PCR products were briefly stored under -20^0C before the DNA fragment analysis.

3.2.6 DNA Fragment analysis

The analysis of fluorescently labeled DNA fragments was done by capillary electrophoresis (CE) using a genetic analyzer (Applied Biosystems Japan). The technique helps to differentiate alleles for overlapping loci through labelling of the locus with specific primers that have different colored dyes (Pergiovanni, 2012; Wang et al., 2022). Because of this, even more than 20 loci can be analysed in a single reaction due to the use of multiplexed primers. CE is very sensitive to variation in DNA fragment sizes, and can accurately size fragments that differ by only one base pair. To perform fragment analysis on a CE system, the fluorescently labeled primers were used

to flank the *O. lanceolata* region or loci of interest and amplified by PCR before electrophoresis. To prepare the CE, a special calibration with the corresponding matrix standard for the selected group of dyes was performed on the genetic analyzer (Biosystems) to allow accurate detection of the dye-labelled primers. Also, each unknown sample was mixed with the size standard and formamide before CE to allow sizing of the sample peaks and correct for injection variations. During CE, the PCR products were injected electrokinetically into capillaries filled with low-viscosity polymer (POP-4) (Wenz et al., 1998). High voltage is applied so that the fluorescent DNA fragments are separated by size and are detected by a laser/camera system. The data analysis software provides a profile of the separation, precisely calculates the sizes of the fragments, and determines the microsatellite alleles present in the sample. This is manifested in an electropherogram, which is a plot of DNA fragment sizes. The fluorescently labelled fragments are separated by capillary electrophoresis and sized according to an internal standard. The peaks correspond to the different color dyes that are all resolvable and sized along the x-axis. The red line indicates low-level signals (noise) between the peaks. The fragment analysis is a powerful tool that provides relative quantitation, sizing, and genotyping information and enables a wide array of genetic analysis applications. CE is a simple technique to prepare and does not require cleaning of DNA fragments as opposed to sequencing. Data analysis especially fragment analysis is done by use of software and it does not require knowledge of the sequence of the fragments. The quantification information for fragments is easily discerned from peak intensities using the Gene Mapper 5.0 software (Applied Biosystems, California, USA) that captures the genotypic data.

3.3 Data analysis

3.3.1 Population structure

The collected data was analysed to answer questions on the species' distribution and population structure, host composition, habitat characteristics, edaphic distribution drivers, and genetic diversity across the natural range. The population structure was assessed in terms of species distribution, dispersion, density per unit area, size and height class distributions, and regeneration potential. The method of species continuity was used to evaluate the regeneration potential of *O. lanceolata*, while the size class distribution curves and density helped to assess the population stability and strength of *O. lanceolata*. Further, the intensity of the species exploitation was investigated by determining the proportion of coppiced individuals to non-coppiced individuals expressed in percentages of saplings and adults per site (Osman and Idris, 2012).

To understand the species density and abundance, the recorded GPS coordinates (bearings) were used to establish the total area sampled and calculate the species abundance which is the total number of individuals per hectare or in a unit area sampled (Hall and Bawa, 1993; Obiri et al., 2002; Tabuti and Mugula, 2007). The total area sampled per site was computed by Quantum-GIS software from recorded coordinates of *O. lanceolata* as follows: The GPS coordinates were first saved in a compatible format (comma delimited text file) using the "add delimited text" command, followed by conversion of the CSV point data shapefile to enable its editing. After this conversion, the distribution points of *O. lanceolata* as displayed in point data were joined into a polygon, and the distances between data points (natural spacing) were measured using the "measure line tool. The area of the resulting polygon (area sampled) was determined by a field calculator tool in the attributable table, and later used to calculate the species density as follows:

$Osd = {}^{nol}/_{tas,}$ where; '*Osd'* = *O. lanceolata* population density; '*nol'*= number of *O. lanceolata* individuals encountered in a sampled population; and '*tas'* = total area sampled in a study site/population.

A species density for a stable population structure was considered to be ≥45 trees per hectare, while densities ≤45trees per hectare were considered to characterise poor, weak, and unstable population structures with low capacity for recruitment and survival (Tabuti and Mugula 2007; Maua et al. 2020).

To reveal the population structure, stem diameters were arranged into three major size classes of seedlings, saplings, and adults as follows: <1cm (seedlings); 2.5-6.0cm (saplings), and >6.5cm(adults). The frequency of individuals in each size class and their proportions in each life stage were graphically represented in a stem diameter curve to characterise the species' population structure (Tabuti and Mugula, 2007). The density diameter curves were used to analyse the levels of the species disturbance where populations heavily disturbed were expected to show curves which are reverse J-shaped or curves which are exponentially negative while the less disturbed populations could show sigmoid to bimodal-shaped mound curves (Rao et al., 1990). The dominance of *O. lanceolata* was assessed to ascertain the relative importance of the species in the habitat and thus its influence in the entire ecosystem (Burak et al., 2011). This was calculated as follows: Dominance (Do) = mean BA per tree x number of tree individuals of *O. lanceolata;* where BA is the basal area. The number of trees = density x average BA.

3.3.2 Edaphic distribution drivers

The descriptive statistics was performed for edaphic variables using the vegan package in R version 4.1.2. The canonical correspondence analysis (CCA) was applied to explain the relationships between the species presence and edaphic variables. Permutations for CCA under reduced model were performed at 999 iterations to test for the significance of variations in edaphic variables across altitude. All data was condensed and transformed by detrended correspondence analysis (DCA) then CCA was applied to explain the relationship between the species transformed variables and edaphic variables (Carleton, 1984; Anderson and Willis, 2003). The DCA analysis had 26 segments and rescaling of axes with 4 iterations. A combination of multivariate analysis techniques, ANOVA and regression were used to analyse the significance of edaphic variables with a focus on predicting and explaining the relationship between *O. lanceolata* distribution. Subsequently, direct ordination techniques (Canonical Correspondence analysis (CCA) were applied to explain the relationships between *O. lanceolata* distribution, edaphic variables, and altitude (ter Braak and Verdonschot, 1995) using recommended steps (Carleton, 1984; Anderson and Willis, 2003) to enhance the identification of edaphic variables influencing the species distribution.

A multiple regression interaction model (MRIM) was developed and implemented using R to predict specific edaphic drivers influencing the species density and also to show the relationship between species density and edaphic variables. In this model, the response or predictor variable was *O. lanceolata* density *(Osd)* per site/population against edaphic variables (pH, electroconductivity (EC), Na^{+}, Mg^{2+}, Ca^{2+}, K^{+}, N, P, and organic matter), as independent variables.

3.3.2 Host composition analysis

The *Osyris lanceolata* hosts were assessed in terms of host range, abundance, frequency, and closeness to *O. lanceolata*. The host proximity to *O. lanceolata* was assessed by measuring the distance between the host and *O. lanceolata*. Data on host abundancies was used to compute the Sorenson index and reveal the levels of host variation among different sites as follows:

Sim = $^{2D}/_{B+C}$, where *B is the species number in site B, C is the species number in site C, and D = species number common both in site B and C*

Usually, the calculated indexes between sites that are less than 0.5, indicate dissimilar sites in host species composition and hence *O. lanceolata* have no host preference. In addition, the host proximity to *O. lanceolata* was determined by estimating the closest and widest distance between *O. lanceolata* and hosts and also identifying specific host species which are closer and those associating at wider distances. The relative frequencies of habitat characteristics were assessed to identify those conditions that dominate *O. lanceolata* habitats and which support the species' survival.

3.3.3 Molecular data analyses

The estimation of the number of observed alleles (N_a) was used to assess the genetic diversity of *O. lanceolata*; and other parameters such as effective alleles (N_e), observed heterozygosity (H_o), and expected heterozygosity (H_e) were also used in genetic diversity assessment. The fixation index (F*st*), number of private alleles (A_p), inbreeding, and the percentage of polymorphic loci (PPL) per population were calculated using GeneAlex or Gene Pop software (Zong, et al., 2015). Heterozygosity was calculated from the Neis (1973) formula. The power of discrimination (PD) for each locus is calculated as;

PD =1-$\sum p^2_i$ *where, p_i refers to the frequency of genotype $_i$* (Kloosterman et.al., 1993).

Inbreeding was estimated as;

F = 1 - $^{Ho}/_{He}$ per population, *where Ho = observed heterozygosity, and He = expected heterozygosity.*

In addition, GeneAlex 6.501 was used to assess the genotypic linkage imbalances between loci pairs and deviations from Hardy-Weinberg equilibrium (HWEs) at each locus (Peakall and Smouse, 2012). The Ne's estimates of heterozygosity for all populations at each locus, genetic diversity (h), Shannon's Information index (*I*), level of gene flow (Nm), total gene diversity (H_t), variability within populations (H_s) or mean genetic diversity (H_s) and coefficient of genetic differentiation (G_{st}) were also calculated using Gene pop version 1.32 and GenAlEx version 6.4.1 (Peakall and Smouse, 2012). Analysis of molecular variance (AMOVA) and the components of variations or variance within and between populations was done using GeneAlex6.51b2 (Peakall and Smouse, 2012; Zong et al., 2015). F - statistics for all loci was calculated using GenAlEx version 6.4.1 (Excoffier et al., 1992; Peakall and Smouse, 2012). Geographic trends in distribution of genetic diversity were investigated by testing the null hypothesis of independence between pairwise F_{ST} and geographical distance using Mantel test (Hahn et al., 2017).

3.3.4 Genetic structure analysis

Bayesian methods for analysis of population genetic structure were used to estimate hidden population organization through collecting all populations into panmictic groups (Corander et al., (2003). The Bayesian analysis program (BAP) was run twelve times with repetitions of 10^5 and a

burn-in period of 10,000 generations to allow populations to mix randomly (Fuentes-Pado et al., 2017). Subsequent partitions or genetic clusters were presented in a plot of delta K values. The largest delta K value was considered to correspond to the number of major genetic clusters among the sampled *O. lanceolata* populations. Groups of genetically similar individuals were identified using Model based clustering algorithm and this also tested for proportions of admixtures among populations using STRUCTURE software, version 4.5.7 (Weir, 1996; Zong et al., 2015; Muriira et al., 2018).

To avoid overrating the number of clusters in all allele frequencies the factor of individual admixture alpha was considered similar (Heuertz et al., 2004). The $F_{ST}/1\text{-}F_{ST}$ was regressed based on F_{ST}-values by pairwise among all sampled populations, against the log-transformed geographic distance separating the populations to test for isolation by distance (IBD) at population level (Slatkin, 1985; Rousset, 1997). Also, genetic differentiation among populations were also computed to characterise the species genetic relationships among and within populations.

3.3.5 Genetic relationships among populations

The genetic relationship within and among populations was assessed by determining genetic identity and genetic distance (G_{ST}) through illustrating genetic patterns of relationships and linkages or genetic similarity among individuals through computing pair wise relatedness coefficients between individuals in a population. This comparison provides an insight on whether distance or landscape has an effect on genetic similarity between tree individuals of the same species using GeneAlex version 6.51b2 (Peakall and Smouse, 2012). The STRUCTURE program was used to analyse spatial genetic structure (SGS) of *O. lanceolata* across the Uganda and Kenya populations by computing the measures of genetic identity, and genetic distances among individuals or populations using pairwise comparisons.

CHAPTER FOUR

KNOWLEDGE GAPS IN TAXONOMY, ECOLOGY, POPULATION DISTRIBUTION DRIVERS AND GENETIC DIVERSITY OF (*Osyris lanceolata* Hochst. & Steud.)

4.1 Introduction

The high global demand for ornamental, cosmetic and pharmaceutical products is driving exploitation of plant species all over the world (da Silva et al., 2018). Sub-Saharan Africa harbors an important stock of unique and valuable plant resources, and therefore is a target of expanding plant resource exploitation (Ola et al., 2019). African sandalwood (*Osyris lanceolata* Hochst. & Steud.) is a multipurpose, drought-tolerant and hemi parasitic tree, well known for its essential oils used in perfumery industries (da Silva et al., 2018). It emerged as a potential commercial species in Africa due to significant decline in original sources of sandalwood oil, e.g., *Santalum album* L. (Indian subcontinent) and *Santalum spicatum* (R.Br.) A. DC. (Australia) in the 1990s, and the increasing demand for sandalwood oil over the years (Mbuya et al., 1994; Page et al., 2012; Thomson, 2020).

Dwindling of the species populations in Africa is attributed to overexploitation and lack of robust management strategies (Thomson, 2020; Rao et al., 2007; Mwangi'ngo et al., 2005; CITES, 2013). Some populations in Uganda, Kenya, Tanzania and South Sudan have completely disappeared due to illegal harvesting and smuggling of tree logs despite the species being protected under Appendix II of the Convention on International Trade in Endangered Species (CITES) (CITES, 2013; Muhoozi, 2018; Tajuba, 2015; Bunei, 2017). *O. lanceolata* is assigned

an automated status of least concern (LC) (Wilson, 2018) with an unknown population trend but acknowledging decline in East Africa due to over exploitation (CITES, 2013; Wilson, 2018).

Apparently, the lack of adequate information to reliably manage a sound resource base for *O. lanceolata* makes it very difficult to implement informed strategies for in situ and ex situ conservation in Africa (Thomson, 2020). Previous emphasis on plantations (ex situ strategy) and in situ measures for conservation have not succeeded due to information gaps on the species ecology, population dynamics and genetics (USAID, 2015; UIA, 2016; Neuhauser et al., 2003). Additionally, identification of suitable sources for genetic resource improvement is difficult without adequate scientific information on the species (Neuhauser et al., 2003; Mwang'ngo et al., 2007). Knowledge of non-random distribution of genes from these studies may be even more important for conservation of the species (Kalisz et al., 2001; Curto et al., 2015).

Information on species population structure and demographic data help to predict the future stability of a species population amidst environmental and anthropogenic disturbances (Tabuti and Mugula, 2007; Virillo et al., 2011). Whereas the ecology, population genetics and phylo-geography of other economically important species like *Prunus africana*. (Hook.f.) Kalkman. Are well documented in Africa (Kadu et al., 2012), similar information is lacking for *Osyris lanceolata* (Qasem, 2006). There are peculiar ecological and genetic aspects of *O. lanceolata* which need to be understood and aligned with strategies for responsible management, in particular hemi parasitism, complex distribution patterns and low survival rate (Nurochman et al., 2018; Gathara et al., 2014). These attributes raise the following questions which require critical analysis: (i) What is the distribution, taxonomy and ethnobotany of *O. lanceolata*? (ii)

Which environmental factors influence the species distribution and hemi parasitic relationships across habitats? and (iii) How do such factors impact on characteristics of the species population structure, genetic diversity and conservation status in Africa? Understanding these questions contributes to informed conservation strategies. This review analyses the missing links in population dynamics, ecology, taxonomy and genetic diversity of *Osyris lanceolata* using the available literature with a special interest in populations in Sub-Saharan Africa. The species taxonomy and ethnobotanical uses are presented and the role of hemi parasitism is discussed while identifying emerging questions for further research. A global scope of the species distribution is provided and factors influencing its spatial distribution are explored. Further, the role of population structure assessment and general trends in the species population in Africa is discussed. Finally, the relevance of genetic diversity assessment, the extent of genetic studies on the species and implications for further research and conservation of *O. lanceolata* in Africa are proposed.

4.2 Methodology

4.2.1. Study Review Design

Research on African sandalwood is not yet extensive and thus a scoping approach was adopted to map the available literature (Munn et al., 2018; Arksey and O'Malley, 2005; Lauwers et al., 2020). The review process began with formulating a general research question as follows: "What is known in the literature about the ecology, taxonomy, population dynamics, hemiparasitism, ethnobotany and genetic diversity of *Osyris lanceolata*?" and which gaps exist in literature on the same topics? The following guiding questions were developed to explore the general question. (1) What is the distribution of *O. lanceolata* and its synonyms? (2) Which species are

accepted in the genus Osyris and what are the synonyms for *O. lanceolata*? (3) Which ethnobotanical uses are reported for *Osyris lanceolata*? (4) What is the role of hemi parasitism in *Osyris sp*? (5) What are the suitable habitats, population trends and patterns of *O. lanceolata* across the range of distribution? (6) Which factors influence the species distributions? (7) Are there theories to explain distribution drivers for *Osyris sp*.? (8) What is the role of genetic diversity and to what extent has it been studied for *O. lanceolata* in Africa? (9) Which conservation implications could enhance informed strategies for responsible management of *O. lanceolata* in Sub-Saharan Africa?

4.2.2 Literature review

Relevant studies were identified through searching for evidence in electronic databases like SCOPUS, Web of Science and Google Scholar, websites, use of reference lists, manual searching of key journals, species taxonomy databases, USAID and CITES reports using the search terms: African sandalwood, *Osyris lanceolata*, Osyris species, plant species distribution drivers, population structure, population trends, Osyris taxonomy, hemi parasitism, reproductive biology and genetic diversity. Further information on genetic diversity was obtained through specific searches in molecular science journals. Specific papers were then sorted from different sources according to search topics and summarised into tables, figures and short paragraphs Munn et al 2018; Arksey and O'Malley, 2005; Lauwers et al., 2020). While analysing information, the main focus was on study aims, methods, findings, controversies, recommendations, conservation inferences and knowledge gaps for further studies.

4.3 Results

Fewer studies on *Osyris sp*. were retrieved compared to other sandalwood species such as Santalum sp. after conducting extensive searches on different study fields as indicated in Table 4.1.

Table 4.1. Summary of results on sandalwood species.

Search Topic	Google Scholar		Web of Science		Scopus		Screened papers		
	Santalum sp.	*Osyris* sp.	*Santalum* sp.	*Osyris* sp.	*Santalum* sp.	*Osyris* sp.	General Papers	*Santalum* sp.	*Osyris* sp.
Population dynamics	4760	833	2250	11	347	50	17	07	06
Ethnobotanical uses	-		06	01	71	18	16	11	05
Genetic diversity	304.8	538	70	02	101	15	43	07	03
Hemi parasitism	-	-			01	00	22	04	03
Distribution drivers	-	-	00	01	-	-	09	-	03
Propagation methods	-	-			44	11	-	-	02
Reproductive biology	4030	01	01	393			-	-	01
Species taxonomy	-	-	12	01	125		32-	-	05

(Source: Mugula et al., (2021)

4.3.1 Taxonomy of *Osyris sp.*

Parasitic plants have been the most difficult plant groups to classify due to their specific adaptations in biology and morphology (Fay et al., 2010). Most members of the genus *Osyris* are hemi parasitic plants, with complex physiognomy, physiology and morphology (Nurochman et al., 2018). The genus belongs to the angiosperm order Santalales, family Santalaceae. The family has over forty genera and 400 species distributed in the tropics and temperate ecosystems (Fay et al., 2010). The three African genera in this family include *Thesium*, *Osyridicarpus* and *Osyris* (CITES, 2013). Genus Thesium is the largest with over 200 species native to Africa and regions with a Mediterranean climate (CITES, 2013).

According to the webpage of the International Plant Names Index (www.ipni.org-accessed on 25 June, 2021) five species of the genus *Osyris* are accepted: *O. alba* L. with Mediterranean distribution in south Europe and north Africa, *O. daruma* Parsa with a range in southern Iran and *O. compressa* (P. J. Bergius) A. DC. and *O. speciosa* (A.W. Hill) J.C. Manning & Goldblatt, both with a main distribution in the Cap provinces. All remaining described species are not accepted to date and treated as one taxon, *Osyris lanceolata* Hochst. & Steud., resulting in a very large and inhomogeneous range for the species, with areas in south and East Africa, in parts of southern Europe and Asia. In the Catalogue of life (COL, www.catalogueoflife.org (accessed on 25 June, 2021) the taxonomy that forms the base for the Global Biodiversity Information Facility (GBIF), and World Flora Online (WFO), which is a global online compendium of the world's plant species, *O. lanceolata* is subdivided into *O. quadripartita* Salzm. ex Decne with the European and south and East African populations and *O. wightiana* Wall. ex Wight with the Asian populations. In this study, the name "*Osyris lanceolata*" is used in accordance with previous treatments of African sandalwood (Polhill, 2005).

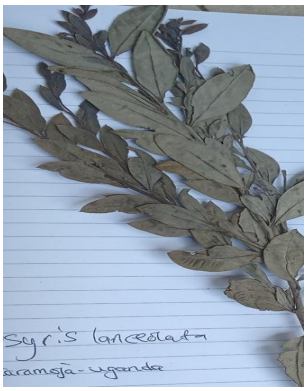
In consequence, the species *Osyris lanceolata* Hochst. & Steud. (1832) is represented by various synonyms (Polhill 2005; Fay et al., 2010) and multiple independent classifications (Plant list, 2016). The species (Figure 1) is commonly known as African sandalwood, East African sandalwood, Nepalese sandalwood, or false sandalwood. *O. lanceolata* is highly variable in morphology, especially leaf size and shape depending on climate, altitude, edaphic variables and sex type (Fay et al., 2010), which may account for the occurrence of various synonyms. For instance, in some field work activities in Uganda, specimens of *Osyris lanceolata* were identified

as *Osyris compressa* (Berg.) A. DC due to variations in leaf size and thickness leaves as shown in Figure 4.1a-f

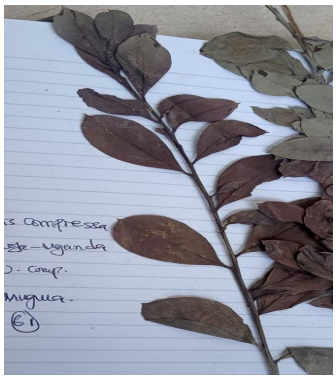
4.1(**a**)

4.1(**b**)

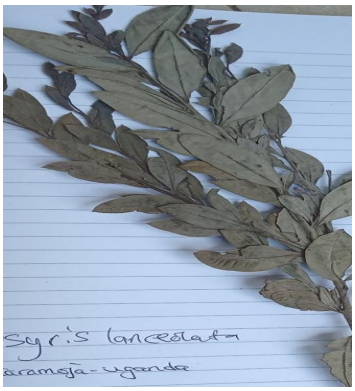
4.1(**c**)



4.1(d)



4.1(e)



4.1(f)

Figure 4.1a-f: (a) Adult tree of O. lanceolata in Uganda; (b) "O. compressa" (adult tree); (c) O. lanceolata (sapling); (d) specimen of O. lanceolata from Karamoja; (e) "O. compressa" (Karamoja); (f) O. lanceolata (Karamoja) (Source; Mugula et al., 2021).

4.3.2 Global distribution of *O. lanceolata*

O. lanceolata is widely distributed with different native ranges in the Canary Islands in Spain, southern Iberian Peninsula, Baleares, Sahara to South Africa, Socotra, Indian subcontinent to south China and Indo-China (Polhill, 2005; Harbaugh and Baldwin, 2007). Nevertheless, information from public databases like GBIF is prone to curation errors and, most importantly, it can be incomplete. For example, although not present in the GBIF database, there are reports

indicating the occurrence of *O. lanceolata* in Tunisia, Libya and Egypt. Hence, there is a need for further exploration of the global distribution of this species.

As *Osyris* sp. occurs in diverse habitats, specific environmental variables influencing its distribution and population structure across isolated habitats are not known (Gathara et al., 2014; Ndangalasi et al., 2014). For instance, the Socotra Islands have been isolated from large landmasses for a long time which may have caused *Osyris sp*. to evolve multiple genetic lineages different from *Osyris* species in Africa. Similarly, *O. lanceolata* includes an island population on the Canary Islands.

The *O. lanceolata* distribution indicates the possibility of local adaptation of single populations. In addition, multiple lineages or even additional subspecies of sandalwood with distinct morphological and genetic characteristics could exist. Currently, available taxonomic solutions in the reviewed literature on the species do not reflect this complexity. Thus, characterising the genetic diversity and structure of populations from different regions to understand the origins of lineages within this widespread species is necessary to avoid translocations and artificial admixture and hybridisation effects due to commercial exploitation that may compromise conservation efforts, hence enhancing possible extinction of subpopulations with unique characteristics.

The country range distribution of *O. lanceolata* populations in Sub-Saharan Africa is shown in Table 4.2. Local distribution of populations for *O. lanceolata* synonyms is also reported as follows. *O. compressa* occurs in Uganda, *Osyris quadripartita* Decne. in Algeria, *Osyris*

abyssinica A. Rich and *Osyris parvifolia* Baker. occur in Ethiopia, *Osyris pendula* Balf.f. is distributed on the Socotra Islands, *Osyris rigidissima* Engl. occurs in northern Somalia, while *Osyris tenuifolia* Engl., *Osyris oblanceolate* Peter., *Osyris laeta* Peter. and *Osyris densifolia* Peter. occur in Tanzania, and *Osyris urundiensis* De Wild occurs in Burundi. *Osyris quadrifida* Kirk. and *Osyris quadrifida* Salzm. ex DC. occur in Morocco. *Osyris quadrifida* Salzm and *Osyris quadripartita* Salzm. ex Decne. occur in Algeria (Polhill, 2005).

Table 4.2. Country range distribution of O. lanceolata in Sub-Saharan Africa (Mugula, et al., 2021).

African Region	Country Ranges	Local Populations (Reported)
East Africa	Uganda, Kenya, Tanzania, Rwanda, Burundi, South Sudan	Uganda: Karamoja subregion, Mbale (Kaburorun), West Nile, Bukwo, Kween and Kapchorwa (Polhill 2005) Kenya: Turkana, Baringo, Bogoria, Narok, Amboseli, Pokot, Samburu, Laikipia, Kajiado, Kitui, Taita hills, Chyulu hills, Gwasi hills, Marsabit, Makueni, Kikuyu escarpment forest, Mbeere, Narok, Ol-donyo Sabuk, Oloitokitok and Mt. Kulal (Polhill, 2005) Tanzania: Ufipa, Mbulu district, Mbisi, Lake Manyara, Songea, Ihang'ana forest, Kilimanjaro region, Masai Boma, Ol-donyo Sambuk (Schueler & Hemp 2016) Rwanda: Akagera, Eastern Province Burundi: Near Lake Shohoho and Rugweru region
Southern Africa	Namibia, Zambia, Zimbabwe, Mozambique, South Africa, Malawi, Swaziland, Botswana	South Africa: Eastern Cape; Free state, Gauteng, Kwazulu-Natal
Central Africa	Chad, Lesotho	Not reported in literature
North Africa	Algeria, Ethiopia, Somalia, Morocco, Tunisia, Gibraltar	Ethiopia: Shoa, Domak, Efat, Roth, Adua, Mt. Sholoda, Tigray Somalia: Surud, Mt. Maydh, Mt. Hildebrandt Algeria: Tangiers (Polhill 2005)
Cross-border Islands	Socotra	Socotra: Yemen, Haghier Hills (Polhill 2005)

A revision of the genus *Osyris* describing morphological variation and outlined potential subdivision into intraspecific taxa of the widely distributed species *O. lanceolata* was found to be lacking. Synonymisation of described species had rather been carried out in regional revisions where the status of the material might not have been questioned or investigated in a wider context of the whole range of the species. This results in a situation that one species exists in very different areas, from the Mediterranean to the tropics, at high and low altitudes and in very different precipitation regimes. Even though it is possible that a species can be widely distributed

by effective dispersal mechanisms, it is likely that individuals in the different hemispheres have been isolated from each other for long time and faced different selection pressures during long time spans. Thus, even if morphologically no species subdivision of *O. lanceolata* is suggested and no supporting characters can be found, very diverse adaptation patterns could exist in the species. For instance, analysis of genetic structure among members of the Ancistrocladaceae revealed multiple species in different ranges but having almost similar morphological variations that correspond to local adaptation (Turini et al., 2014; Meimberg et al., 2010). This possibility is underlined not only by the large amount of different ecological conditions the species can be found in, but also life history traits like dioecy that can counteract colonisation, even though zoochory could provide a vector to connect distant populations.

The patchiness of the distribution in Kenya and Uganda indicates possible genetic differentiation of populations and a requirement of management of the species at a local scale and difficulties to restore locally extinct populations. Morphological variation and the diverse ecological conditions the species can occupy indicate that the current species circumscription underestimates species numbers. A close evaluation of morphology, ideally combined with molecular data, could suggest the recognition of additional taxa. The species that are poorly represented as herbarium specimens might be prone to underestimation of the real species diversity. One example is the Ancistrocladaceae where investigations on genetic structure showed the existence of multiple species with overlapping ranges, even though morphological variation did not allow a clear subdivision (Turini et al., 2014; Meimberg et al., 2010).

The possibility that multiple species could be recognised in *O. lanceolata* is underlined by recognition of different morphological forms. For instance, there is a general tendency to identify some East African sandalwood specimen as *Osyris compressa* (Figure 4.1b), when they show leaf characteristics which differ from the typical *O. lanceolata* habitus. However, it is unlikely that these specimens belong to *O. compressa* because they differ from this species in other characters and a critical examination of morphological characteristics is lacking. This highlights that morphological forms can be recognised and are used in the field but are not reflected by current taxonomic treatment. This calls for a revision and harmonisation of *Osyris* to resolve such anomalies in taxonomy, and avoid treatment of different species of *Osyris* as one species in experimental studies (Bhat et al., 2006).

Molecular investigations can help to resolve confusion in the taxonomy of species with overlapping ranges, especially where morphological variations cannot provide an accurate basis for identification (Turini et al., 2016; Meimberg et al., 2010). For instance, molecular and biochemical studies have proved to be of immense help to distinguish sandalwood oils obtained from different species (*Santalum* sp. vs. *Osyris lanceolata*/*Osyris weightiana*) (Bhat et al., 2006). Another example is when molecular data were used to differentiate the genus *Colpoon* from *Osyris* in South Africa (Nickrent, 2017). Using morphological and phylogenetic data, it was established that the genus *Colpoon* is distinct from *Osyris*, and hence the two taxa are not congeneric, as was considered before. Resolving taxonomic uncertainty would help to conserve species diversity because exploitation can lead to overuse of specific populations and unnoticed extinction of parts of the diversity, hence decreasing the overall availability of the resource.

4.3.3 Ecology of *Osyris lanceolata*

Plant parasitism is suggested to have evolved in arid environments where water and nutrients are scarce (Kuijt, 1969; Kokwaro, 2009; Tennakoon and Cameroon, 2006; Okubamichael et al., 2016; Bell and Adams, 2011; Nge et al., 2019; Furuhashi et al., 2012), to help parasitic plants access carbohydrates, water and mineral nutrients through hosts (Tennakoon and Cameroon, 2006; Bell and Adams, 2011). These hemiparasites make their own chlorophyll, but also need hosts to obtain water and nutrients to boost their survival. Without hosts, the hemi parasitic growth rate declines rapidly, especially in later stages due to poor acquisition of nutrients such as Ca^{2+}, K^{+}, P and Mg^{2+} (Bell and Adams, 2011). For instance, seed germination of *Osyris lanceolata* does not require any host influence in the early stages (Kuijt 1969; Okubamichael et al., 2016) but further development of seedlings requires hosts (Watson, 2009). A detailed analysis of the role of hosts on life stages of hemiparasites and mechanisms for interaction between hemiparasites and their hosts is already documented (Yoder 1999; Nilsson and Svensson, 1997; Matthies, 1999; Irving and Cameron, 2009; Westwood et al., 2010; Tomilov et al., 2006).

Some hemiparasites co-exist with a wide range of host species (over 440) (Nilsson and Svensson 1997) while others are extremely host specific (Matthies and Egli, 1999; Okubamichael et al., 2016). Host specificity is not static but dynamic depending on levels of plant diversity within an ecosystem (Tenakoon and Cameron, 2006). Thus, hemiparasites that are generalists across the entire range can be specific to particularly abundant hosts at a local scale (Matthies and Egli, 1999). For instance, a study in Tanzania observed common hosts for *Osyris lanceolata* in controlled environments to include: *Rhus natalensis*, *Dodonaea viscosa* Jacq., *Tecomaria*

capensis (Thunb.), *Catha edulis* (Vahl) Forssk. Ex Endl., *Apodytes dimidiata* E. Mey. ex Arn., *Brachystegia spiciformis* Benth., *Maytenus acuminata* var. acuminata and *Aphloia theiformis* (Vahl) Benn (Mwang'Ingo et al., 2004). As hosts of hemiparasites differ geographically (Qasem, 2006), it is also necessary to explore if this is due to their adaptation to local flora or because they are generalist plants. If they adapt to local flora, are there similar attributes in the biology of host plants in different regions of distribution? What role do such attributes play in the survival of hosts and hemiparasites?

As hemiparasites rely on hosts to acquire nutrients, their survival is also tied to the degree of conservation of host plants (Nge et al., 2019; Tenakoon and Pate, 1996). However, hosts have other immediate socio-economic values, depending on the needs of local communities, hence the survival of hemiparasites becomes more fragile due to competing threats in the form of ethnobotanical or industrial plant uses. Secondly, the presence and absence of certain hosts will affect genetic diversity, and thus the evolutionary potential of a hemiparasite. If hemiparasites are generalists, then species composition may not have comparable influence on genetic diversity, but rather the main factors shaping their genetic diversity should depend upon the presence or absence of any hosts. Equally, if it is a specialist, its fitness will be dependent on the presence of certain key species and thus its diversity is also affected by their presence or absence.

What is lacking in the biology of *O. lanceolata*, in particular, is an understanding of the factors influencing the use of available potential hosts at a local scale (host specificity), and the hierarchical ranking of host use (host preference). The identity of suitable hosts and their ecological roles in the survival of *O. lanceolata* at different stages of growth and development

require further scrutiny. It is important to understand the trade-offs and variations in proximity levels between *O. lanceolata* and different hosts in natural habitats. This information is key in developing strategies for in situ and ex situ conservation. Further, we know much less about the factors influencing the distribution of hemiparasites and their hosts, and how host composition influences genetic diversity of hemiparasites (Bell and Adams, 2011; Irving and Cameron, 2009). Host abundance alone has been disputed to influence the distribution of hemiparasites, but rather there may be a combination of environmental factors interacting to determine definite distribution (Tennakoon and Cameron, 2006). In addition, to narrow this gap in the ecological science of hemiparasites and to enhance species conservation, a thorough investigation of the relationship between edaphic, biotic and other environmental factors in spatial distribution of *O. lanceolata* in natural populations is necessary. These analyses could improve understanding of suitable conditions for species survival and adaptation to enhance conservation strategies.

4.3.4 Genetics of *Osyris lanceolata*

There was a handful of genetic studies found on *Osyris lanceolata* unlike other species such as *Santalum* sp. For instance, three studies were found focusing on the genetics of *O. lanceolata* (Bhat et al., 2006; Andiego et al., 2019; Otieno et al., 2016). Two studies (Andiego et al., 2019; Otieno et al., 2016) were focused on developing microsatellite markers to assess genetic diversity of *O. lanceolata* in the Kenyan populations. The study (Otieno et al., 2016) identified and developed 12 polymorphic and five monomorphic markers for population genetic studies including assessment of gene flow levels in different populations. The second study by Andiego et al., (2019) assessed genetic diversity and population genetic differentiation among seven populations in Kenya. As a result, the most genetically distinct populations were identified for conservation strategies. In this case, assessment of genetic diversity is crucial in identification of

populations for conservation priority and creating baseline data for informed conservation strategies at the local scale. Such decisions cannot be made without genetic data on species populations. However, these studies have not been carried out on other populations in East Africa which creates a huge gap in the knowledge on the species. Another study focused on identification of *Santalum album* and *O. lanceolata* using biochemical characteristics and molecular markers to check adulteration in Asia (Bhat et al., 2006). This study highlighted the importance of using biochemical characteristics and nucleotide sequence dissimilarities in the rRNA genes to distinguish between *Santalum alba* and *O. lanceolata*, and also provided a molecular framework and methodology for checking adulterations in sandalwood oils.

The limited number of studies on genetics of *Osyris lanceolata* creates a huge gap in the understanding of the genetic adaptive potential of the species in Africa, given the changing environmental conditions affecting the survival of the species. Analysis of genetic diversity involves assessment of genetic variation in time and space, to understand the species dispersal, mating behavior, delimitations and population boundaries (Vellend, 2005). This helps to obtain information about the species population structure and degree of connectivity and identify barriers to gene flow within and among populations (Miller et al., 2013) so as to design informed strategies for conservation. High genetic diversity provides more alleles to increase genetic adaptive potential and fitness of populations in response to environmental changes (Gacheri et al., 2016).

Unlike habitat loss and fragmentation which can have an extreme impact on genetic diversity of plant species (Gacheri et al., 2016; Ellegren and Galtier, 2016; Ratnaningrum et al., 2015),

domestication was established to have minimal impacts on plant genetic diversity in the short term (Miller et al., 2013; Gacheri et al., 2016; Ellegren and Galtier, 2016; Ratnaningrum et al., 2015; Farwig et al., 2008). Despite this assertion, the studies to show how asexual reproduction (through sprouting) influences the genetic diversity of *O. lanceolata* were not found. On the contrary, human activities were reported not to impact genetic diversity of species such as *Scaphium macropodum* (Miq.) Beumee ex. K. Heyne., *Dryobalanops aromatica* C.F. Gaertn. and *Shorea curtisii* Dyer ex. King. across generations (Zhang et al., 2017). Although regeneration of *O. lanceolata* is more successful through coppicing or root stocks, rather than seed germination (Mwangi'ngo et al., 2005), the mother source which contains higher genetic diversity in subsequent generations is not known. The factors accelerating seed germination failure in *O. lanceolata* need to be explored further to improve recruitment programs in natural populations. Thus, further studies to understand variations in genetic diversity across life stages, and between asexually produced individuals (root stocks/sucker/coppicing) and sexually propagated individuals of *O. lanceolata* are necessary to guide conservation actions such as restoration of overexploited habitats.

Genetic diversity assessment is also necessary to forecast changes in genetic structure and document loss of genetic diversity in populations of plant species (Zhang et al., 2017; Graudal et al., 2014; Frankham et al., 2002). Understanding fine-scale spatial genetic structure helps to describe the non-random distribution of genotypes in space within populations due to genetic drift, selection and gene flow (Yang et al., 2016; Vekemans and Hardy, 2004). It also helps to detect gene dispersal distance and the extent to which ecosystem disturbance can influence non-random distribution of genes in a population, leading to inbreeding and loss of genetic diversity

(Petkova et al., 2016). Based on tremendous advancements in genetic technology (Volis et al 2016; McQuillan et al., 2008; Szczecińska et al., 2016; Porth and Kassaby, 2014; Holliday et al., 2016; Castoe et al., 2012; de Barba et al., 2017; Neophytou et al., 2018; Vartia et al., 2016; Šarhanová et al., 2018; Parchman et al., 2018; Campbell et al., 2015), future studies on *O. lanceolata* should consider this focus, to evaluate populations and their suitability as provenances for in situ conservation, commercial propagation, restoration and further genetic improvement of the species.

4.3.5 Distribution drivers of *Osyris lanceolata*

Osyris lanceolata occurs in a diverse range of habitats including upland dry evergreen forests and mist forests characterised by bushland and grassland that usually extend downwards to rivers and slightly into deciduous woodlands at 900–2700M above sea level (Dennenmoser et al., 2017). Other suitable habitats for *O. lanceolata* include: dry savanna forests and woodlands, moist woodlands, thicket edges and dry submontane *Hyparrhenia* grasslands at an elevation range of 1000 m to 1730 m above sea level (Fox, 1997). However, the species also occurs in rocky and non-rocky habitats (Amundrud, 2020; Pfenninger et al., 2011) at even higher altitudes ranging from 900 m to 2250 m and with mean annual rainfall of 600 to 1600 mm with well-drained soils, but it cannot tolerate frost conditions (Kamondo et al., 2014).

Despite the reported habitats for the species, little is known about suitable survival conditions, and factors that would influence the species distribution in natural habitats. Understanding plant species distribution drivers helps to analyse the species survival conditions and strategies in habitats which is important in conservation planning. Although scientific evidence suggests *Osyris lanceolata* to exhibit a clumped or patchy distribution (Kuijt, 1969), little is known about

drivers of the species distribution. *O. lanceolata* is typically rare throughout its distribution range and also has a non-uniform pattern of distribution even in areas with abundant suitable hosts (Watson, 2009). The highly patchy nature of distribution clearly suggests the influence of specific factors in determining the species distribution. A recent study by Fox (1997) proposes the presence of hosts and habitat attributes as key determinants for the distribution of parasitic plants.

In other studies, the importance of habitat quality (Fox, 1997; Pfenninger et al., 2011) is stressed, while seed dispersal capability could also influence species distribution (Amundrud 2020). Host quality includes water availability, edaphic variables and nutrients (Watson, 2009). According to the host quality hypothesis (HQH) *O. lanceolata* can only establish and grow if they parasitise a host with sufficient quality such as one with low water stress (Watson, 2009). In areas where water is limited, parasitic plants are likely to establish on hosts with greater access to water (Watson, 2009). Proper illustration of this hypothesis requires a detailed field assessment of habitat quality for *O. lanceolata* populations and their hosts in natural populations. In addition, the abundant center hypothesis (ACH) suggests that a species will be more abundant where conditions for reproduction and population growth are most suitable (Fox, 1997). A further implication of this hypothesis is that population density of a species declines towards areas with less suitable environments (Watson, 2009). Therefore, if the spatial distribution of a species is correlated with corresponding environmental variables, an insight into drivers of species distribution and survival can be obtained as an indication of desirable survival conditions of a species in natural environments. Although studies suggesting specific germination requirements, seed vectors and site–microsite preference (Fox, 1997; Kamondo et al., 2014; Mortelliti et al.,

2010; Laurance, 2010) as key drivers for species distribution were found, these conditions cannot account for the highly patchy spatial structure of *O. lanceolata* (Kuijt, 1969). Thus, empirical data are required to understand key drivers of the distribution of *O. lanceolata* in natural habitats.

4.3.6 *Osyris lanceolata* populations in sub-Saharan Africa

There was overall support in the literature for a declining trend in populations of *Osyris lanceolata* which is believed to be endangered in Africa due to overexploitation (CITES, 2013; Chase et al., 2020), habitat loss (Mortelliti et al., 2010; Laurance, 2010) and accidental destruction of host plant species for fuel wood, timber, charcoal burning and building materials. For instance, the species is smuggled by uprooting the whole plant in Uganda and Kenya, hence leading to loss of genetic variability and population decline (CITES, 2013). Habitat loss is fueled by human activities such as deforestation, urbanisation, logging and mining, leading to land cover change, conversion and land use intensification with eventual loss of ecosystem services (Mortelliti et al., 2010; Laurance, 2010). Other activities threatening *O. lanceolata* habitats include overgrazing and bush burning. The loss of natural habitats reduces local species abundance and diversity which leads to population decline and extinctions (Mortelliti et al., 2010).

The lack of informed conservation strategies in Africa also exacerbates the decline in *Osyris lanceolata* populations (Laurance, 2010). With the rapid depletion of tropical forests, over 125,000 km^2 per year (Mortelliti et al., 2010), which form a significant portion of the habitats for *O. lanceolata*, and lack of informed conservation measures, urgent action is needed to save the species from total depletion. Some African governments have responded to address overexploitation by instituting restrictive policy actions such as presidential decrees prohibiting trade in the species products (Kenya, Uganda, Tanzania), sanctions on illegal traders and by-laws

(CITES, 2013). These are commendable practices, but further strategies are needed to promote sustainable harvesting, production and conservation in Africa.

To establish robust management strategies for *Osyris lanceolata*, studies to generate scientific data on the species population status and genetics are necessary. The lack of empirical data makes it difficult to design measures for effective species management, including monitoring population trends of *O. lanceolata* in Africa. Assessment of a species population structure helps to detect reasons for population decline, threats and human impact on species genetic diversity which is necessary to guide habitat management responses for declining species (McKinnell and Levinson, 2008; Okiror et al., 2012; Wiegand et al., 1999; Lindenmayer and Laurance, 2016). Secondly, information on population structure complements genetic studies towards understanding the interaction between evolutionary processes and environmental forces in shaping species adaptation in ecosystems (Tennakoon and Pate, 1996). Additionally, comparing demographic data with genetic diversity data helps to assess threats and identify genetically diverse populations of plant species (Rao et al., 2007).

4.3.7 Ethnobotany of *O. lanceolata*

Documented uses of *Osyris lanceolata* can be divided into categories that include: cosmetics, emergency food, pharmaceutical industries, crafts, cultural/spiritual uses, local medicine, timber and ecological services (phytoremediation) (Coppen, 1995; Shyaula, 2012; Njoroge and Bussmann, 2006; Moy and Levenson, 2017; Dwivedi and Abu-Ghazaleh, 1997; Jain et al., 2019) (Table 4.3).

Table 4.3. Ethnobotanical uses of *O. lanceolata*.

Use Category	Plant Resource	Description
Cultural/spiritual uses/perfumery	Wood and oil	Oils are used to make perfumes and cosmetics while the wood is burnt during ceremonies in Asia (Shyaula, 2012)
Pharmaceutical/ local medicine	Leaves, bark, root	Oils are used in pharmaceutical industries, local decoctions to treat malaria and for sexually transmitted diseases (STDs), chest pain, hepatitis B, fever, diarrhea, chronic mucus infections, cough and asthma (CITES, 2019)
Tanning and dyeing	Roots	Roots contain red dye for tanning leather (Moy and Levenson, 2017)
Food	Root, bark oil extract; fruits	Roots and bark provide flavored powder for tea and tonics The root extract is used in preservation of milk, while the fruits are eaten as emergency foodstuffs by children and herdsmen
Ecological services	Root system	Root haustoria can accumulate heavy metals for phytoremediation (Liu et al., 2008)
Timber	Wood	Hardwood makes carvings and fencing homesteads (CITES, 2013)
Ethnoveterinary uses	Leaves	The leaves provide fodder, and contain antipyretic agents for cattle in east Africa (Jain and Nair, 2019)

Extensive uses attached to *O. lanceolata* include essential oils, being the most commercially valuable and tradeable resource (Page et al., 2012; Thomson, 2020). The oils are naturally contained in the bark, lower stem and roots of sandalwood species, they contain α, β and epi-β-santalols as active ingredients (Shyaula, 2012) and are used in the production of perfumes, toiletries, mouth fresheners, incense, cosmetics, aromatherapy (Shyaula, 2012) and flavoring agents (Page et al., 2012). The oils are reported to have blending and antiseptic properties suitable for making fixatives in other fragrances (Jain and Nair, 2019). The same oils have chemo-preventive properties used to manage eruptive and inflammatory skin diseases (Coppen, 1995). Other diseases such as dysuria, bronchitis and gonorrhea can be treated with sandalwood oils (Hemp et al., 2009). *O. lanceolata* products are used to treat candidiasis (Masevhe et al., 2015), malaria (CITES, 2019), diarrhea (Ochanda, 2009; Bhowmik et al., 2011), chest pain and fever in Africa (Shyaula, 2012). The oil and wood are burnt during spiritual and cultural ceremonies by Muslims, Hindus and Buddhists (Wang and Kim, 2015). The bark and roots provide a red dye for skin tanning (Ellegren and Galtier, 2016) while its shoots provide antipyretic agents for cattle in Africa (Moy and Lovenson, 2017). The root system can be used to

accumulate heavy metals and is hence useful in phytoremediation strategies (Xiaohai et al., 2008). Irrespective of sandalwood species, the major tradable products include oil, powder and wood logs, and these have significant markets in Germany, the United Kingdom, France, South Africa, the United States, India, the United Arab Emirates and China (CITES, 2013; Page et al., 2012).

Although a large variety of uses for *Osyris* sp. are reported, the majority of studies cover medicinal or pharmaceutical and perfumery uses of the species. Only a handful of studies focus on other uses such as phytoremediation and ethnobotanical uses. Among medicinal uses, limited studies were focused on assessing the efficacy of concoctions from *Osyris* species in the treatment of human and veterinary diseases. There is need to document detailed ethnobotanical uses and indigenous knowledge associated with *Osyris* species so as to guarantee conservation of traditional knowledge on the species. Understanding the multiple alternative and local uses of a slow-growing species such as *Osyris lanceolata* helps to improve the attitudes of local communities towards conservation of that species (Tabuti and Mugula, 2007). These communities can derive socio-cultural and ecological benefits from the species in the short term, in addition to economic benefits which could be gained later if sustainable populations are conserved.

4.3.8 Implications for conservation of *O. lanceolata* in Africa

This paper highlights four major issues with significant implications for conservation of *O. lanceolata* in Africa. First, the taxonomy of *O. lanceolata* is still complex due to over synonymisation, country range distribution and ambiguity in species ranking. Second, the population dynamics of *O. lanceolata* across its range of distribution are anecdotal, though

CITES reports indicate significant population declines, particularly in east Africa due to overexploitation. Third, the drivers of the spatial distribution of *O. lanceolata* in natural habitats are not understood. The species is highly patchy and exhibits an irregularly clustered pattern of spatial distribution which requires further analysis. Fourth, the species genetic diversity and ethnobotany are barely studied and hence not understood. These issues affect conservation of *O. lanceolata* as follows: the confusion in the taxonomy of *O. lanceolata* leads to continuous treatment of different species of *Osyris* as one taxon which may lead to loss of unnoticed populations with diverse morphological and genetic attributes. Secondly, continuous harvesting and utilisation of *O. lanceolata* with unknown population dynamics puts the species at a greater risk of depletion since the absence of population data complicates species monitoring and management. In addition, poor understanding of drivers of the distribution of *O. lanceolata* is a hindrance to conservation in Africa.

Drivers of spatial distribution correlate strongly to suitable conditions for survival and fitness of a species in natural habitats and hence such information is necessary in planning for conservation approaches. Additionally, limited understanding of the genetic diversity of the species and structure hinders conservation efforts. For instance, suitable provenances cannot be identified easily to boost conservation programs. Equally, limited documentation of the ethnobotanical uses of the species also hinders conservation initiatives. Local communities may be reluctant to appreciate conservation of a species whose value and benefits are not understood.

The three approaches needed for continued survival of *O. lanceolata* populations include: conservation, restoration and sustainable commercial use. In particular, conservation of

threatened habitats for the species population is necessary (Cogon et al., 2021). As different populations exhibit different population dynamics, conservation planning ought to be undertaken at the population level and reinforced by local investigations which are more informative than global studies (Sulis et al., 2021). Additionally, locally adapted monitoring protocols that consider different stakeholders at local and regional levels are key in tracking populations of threatened species (Fenu et al., 2020). However, these actions cannot be realised without adequate scientific information as a basis for informed policy actions. Finally, the risk of extinction of a species without adequate scientific data is high and impacts are extreme if resource extraction continues without planned strategies. Thus, our findings will stimulate constructive debate and more focused research towards responsible management of *Osyris lanceolata* in the long run, to avert the looming threat of extinction of the species in Sub-Saharan Africa.

4.4 Conclusion

The purpose of this study was to survey relevant research on the taxonomy, ecology, population dynamics, ethnobotany and genetic diversity of *Osyris lanceolata*, and highlight knowledge gaps for further research. It was established that *O. lanceolata* is distributed in Africa, Asia, Europe and the Socotra Islands with no identified center of origin. The species has a relatively confusing taxonomy, with unresolved issues in nomenclature, country range distribution, oversynonymisation and uncertainty in biological form (shrub or tree), which calls for a deliberate global revision and harmonisation to resolve anomalies in taxonomy. Information on the species population dynamics across its entire range of distribution is anecdotal. Secondly, several use categories are reported for *O. lanceolata*. There are a handful of studies on the

genetics and ecology of *O. lanceolata* in Africa. The available studies help little to understand the underlying factors for the species distribution and its survival in natural habitats. There are no scientific data to explain how the species genetic diversity varies across life stages and between modes of propagation (seed and asexual). Our review suggests that, currently, (i) species distribution drivers which are possible factors for survival of *O. lanceolata* in natural populations are invariably barely studied and (ii) despite the vital role of genetic diversity assessment in the conservation of plant genetic resources, and the availability of molecular techniques for its investigation, it is the least studied area for *O. lanceolata*, which partly underpins the slow progress in improvement in the species and its conservation in Africa. Therefore, a deliberate focus to understand detailed ethnobotanical uses and the ecological, population dynamics and genetic characteristics of *O. lanceolata* is urgently needed in present and future studies to enhance informed strategies for sustainable management of the species in Africa.

CHAPTER FIVE

POPULATION STRUCTURE AND REGENERATION OF OSYRIS LANCEOLATA IN KARAMOJA SUB-REGION, UGANDA

5.1 Introduction

Osyris lanceolata Hochst. &Steud. (East African sandalwood) is a root hemi parasitic plant and one of the most culturally and commercially recognized plant species in East Africa (Kamondo et al., 2007). The species is a drought tolerant in family Santalaceae and genus *Osyris* (Kamondo et al., 2007) and widely distributed in diverse habitats in Africa, Asia, and parts of Europe (Breitenbach, 1963; Mwang'ingo, 2002; Kokwaro 2009; CITES, 2013; Teixeira, 2016) and provides multiple uses and products including essential oils used as a raw material in the manufacture of pharmaceuticals, perfumes, and cosmetics (Coppen, 1995; Shyaula, 2012). The oils have blending and anti-septic properties used in making fixatives in fragrances (Coppen, 1995; Shyaula, 2012). Locally, the bark and root extracts are used to treat different ailments including candidiasis, malaria, diarrhea, chest pain, and fever (Orwa et al., 2009; Masevhe, 2015), and also provides a red dye for skin tanning (Mbuya et al., 1994). This species is also used in phytoremediation (Mbuya et al., 1994; Xiaohai et al., 2008) and the shoot contains antipyretic agents for cattle.

The shift to *O. lanceolata* as an alternative source of essential oils gained importance after the sandalwood resource base from India and Australia was over exploited leading to population decline in the 1990s (Mbuya et al., 1994). As a result, the preferential exploitation has exerted sustained and extensive pressure on the *O. lanceolata* populations in East African countries

particularly in Kenya, Tanzania and Uganda (Otieno et al., 2016; Bunei, 2017) leading to over-exploitation. The same situation applies to Karamoja sub-region where the species population has been extensively degraded through illegal harvesting (Tajuba, 2015; FSSD, 2021) hence weakening the population structure and density. Previous studies suggested an estimate of 80 tons of *O. lanceolata* trees lost annually in 2011 through illegal harvesting (Tajuba, 2015). These statistics raise serious concerns over the sustainability and stability of the species populations in the wild if no action is taken to ensure sustainable management. For instance; what has happened to the species' population structure, regeneration strategy, and density after several years of over-exploitation? Addressing such questions provides an informed basis for designing sustainable management strategies for the useful plant species.

There has been emphasis over the years on establishment of commercial plantations for *O. lanceolata* to boost the species conservation in developing countries (Winterbottom and Eilu 2006; UIA, 2016; FSSD, 2021) and safeguard people's livelihoods. The ex-situ populations are considered to be reliable sources for sustainable commercial harvesting to sustain the high demand for the species' raw materials for industries and local use (Page et al., 2012; USAID, 2015; Texeira et al., 2017). Despite the high value and demand for *O. lanceolata* (Njoroge and Bussmann, 2006; Muhoozi, 2015; Orwa et al., 2009; Masevhe et al., 2015) initiatives to commercially produce the species have not succeeded in Africa largely due to limited studies on the species' population ecology and survival conditions (Mugula et al., 2021). For instance, reports in Uganda indicate *O. lanceolata* to be scattered in Kigezi, Mt. Elgon, Karamoja (CITES, 2013; Tajuba, 2015) and recently in Arua, but no empirical data is available to understand the species population structure to guide sustainable commercial harvesting and monitor the species

trends in distribution an abundance. The absence of baseline information creates information gap for the species sustainable management.

The sustainable management of valuable plant species is usually complicated by poor understanding of the species population structure and ecological conditions (Mugula et al., 2021). Sustainable management of *O. lanceolata* amidst the increasing anthropogenic and climatic change impacts is important and requires a good understanding of the species' population autecology to provide valuable insights on specific favorable conditions that can be considered in designing strategies for conservation (RMS) (Lomolino, 2001). Indeed, the analysis of *O. lanceolata* population structure, stem density and regeneration potential help to guide sustainable species management against the rapid loss of habitats and overexploitation. The information from population analysis helps to identify suitable habitats for restoration and monitoring the trends in species abundance (Lomolino, 2001).

Population structure of aerial hemiparasites (Mistletoes) and non-hemiparasites is well documented (Dean et al., 1994; Watson et al., 2007; Jiang et al., 2008; Watson, 2009; Těšitel et al., 2010; Scalon and Wright, 2015; Dueholm et al., 2017) than root hemiparasites. The effect of anthropogenic disturbances on population structure, and distribution of non-hemiparasitic species is also well documented (Condit et al., 1998; Lykke, 1998; Tabuti and Mugula, 2007). Condit et al., (1998) established that a stable and healthy species population reflects an inverse - J shaped curve of the size class distribution (SCD) with more seedlings, saplings and few adults. However, a poorly regenerating and highly disturbed population shows a J-shaped curve of the size class distribution with more adults and very few seedlings or juveniles (Condit et al., 1998;

Lykke, 1998; Tabuti and Mugula, 2007). These observations hold true for most plant species except a few long-lived and slow growing species such as *Aloe plicatilis* whose stable populations usually show a bell-shaped curve of the SCD (Cousins et al., 2014). *Aloe plicatilis* shows a bonsai effect due to growth suppression to individuals that survive in stressed environments, hence creating an adult-persistence survival strategy without adequate regeneration or recruitment (Cousins et al., 2014).

Osyris lanceolata is a long-lived and slow growing species which survives in rocky and stressed habitats. However, it is not clear whether the species population structure exhibits a bell-shaped or inverse-J-shaped SCD curve in the semi-arid ecosystem and such information is worthy to be explored. Secondly, *O. lanceolata* survives in very stressed semi-arid ecosystems hence making it necessary to establish its stand stability through estimating the height to stem diameter ratio. Another aspect of importance to sustainable management of *O. lanceolata* is understanding the relationship between *O. lanceolata* density and the proportion of fruiting individuals as this tends to influence the natural regeneration success of the species.

Assessment of the species density, and size class distribution helps to evaluate the strength and stability of the species population structure (Jensen and Meilby, 2012). A strong and stable population structure shows an inverse J-shaped curve, a significant number of independently growing saplings and adults and the adequate number of seedlings with potential for regeneration through natural seedlings (Lykke, 1998; Obiri et al., 2002; Tabuti and Mugula, 2007). Poor regeneration potential is shown by higher number of saplings and adults and extremely few seedlings (Maua et al., 2020).

Despite the ecological and economic importance of *O. lanceolata* and the need for responsible management in diverse ecosystems, the species population structure and regeneration strategy have been little studied and thus poorly understood in the semi-arid savanna ecosystems. It is in this context that this study was designed to characterise the population structure, density, and regeneration strategies of *O. lanceolata* in the semi-arid savannas of Karamoja where the largest population occurs in Uganda.

However, the *O. lanceolata* populations in Karamoja are interesting because first; they have been protected by prolonged and extensive insecurity in the region which deterred large-scale commercial traders from exploitation. As such, the illegal harvesting of *O. lanceolata* in Karamoja is recent (CITES, 2013; Muhoozi, 2015; Gathara et al., 2014; Tajuba, 2015). However, the empirical evidence to understand the impact of overexploitation on the species population structure is lacking, yet populations of *O. lanceolata* offer an excellent opportunity to diversify the livelihoods of local people in one of the poorest regions in Uganda.

Therefore, the study undertook to understand the population structure, parasitic associations, distribution drivers and genetic diversity of *O. lanceolata* using the following hypotheses: (i) the population structure of *O. lanceolata* is stable, strong and sufficiently regenerating in Karamoja; (ii) there are no specific hosts and habitat qualities that influence the distribution of *O. lanceolata*; (iii) the morphological traits of *O. lanceolata* are not affected by environmental gradients; (iv) soil nutrients have no influence on the distribution of *O. lanceolata*; and (v) there

are no distinct patterns in the species genetic diversity and structure among populations in Uganda and Kenya.

5.2 Material and Methods

For description of the study area, climate, local population, and selection of study sites and general sampling procedure refer to chapter III (3.1, 3.1.1 and 3.1.2).

5.2.1 Data collection

To assess the population structure and density of *O. lanceolata*, the sampling plot was considered to be circular with 5m radius and *O. lanceolata* at the center. Although the absolute size of plant niches fluctuates with the patchy distribution of survival resources, the 5m radius was considered a suitable microhabitat and realised niche for *O. lanceolata* survival (Terradas et al., 2009). The conditions beyond 10m away from *O. lanceolata* were thus different from ideal conditions for the species survival (Terradas et al., 2009; Zhang et al., 2018). These spatial measurements were also based on previous studies which established that most root hemiparasites usually have ideal zones where growth occurs in association with the host species, and this implies, not too near to allow space for their fine roots and also evade competition for light resources (Herrera, 1988) and also not too far away to provide the appropriate association with the hosts especially in the earlier phases of establishment (Dueholm et al., 2017).

A total of 388 plots were inventoried using the nearest neighbor method. This method was suitable given the patchy distribution of the species (Mueller-Dombois and Ellenberg, 1974) and it enabled to capture more *O. lanceolata* individuals than would be for quadrat sampling (Jensen and Meilby, 2012). This number (388) was adequate and within the range of individuals needed

to build a population model (300-400) (Ramula et al., 2009). At each site, the initial sampling point was established after encountering the first individual of *O. lanceolata* in a north-south direction. The sampling intervals were not specified due to the patchy distribution of *O. lanceolata* but the movement pattern in sampling followed was a square-like and this increased chances of encountering more *O. lanceolata* because it could be spotted from all directions. For every sampling point, *O. lanceolata* was identified and categorised into three life stages (seedlings, saplings, and adults) (Tabuti and Mugula, 2007). The seedlings were considered to have ≤1cm (seedlings); 1.2cm - 6.5cm (saplings); and >6.5cm diameter (adults) (Mohammed et al., 2021). Because *O. lanceolata* is a multi-stemmed plant and it's branching usually starts at lower heights approximately 55cm above the ground (Mugula, Pers.observ, 2021), the species stem circumference was measured at 55cm above the ground using a measuring tape, and later converted to diameter by the formula:

$D = {}^{circ}/_{\pi}$. Where, D, is the diameter; circ is the circumference, and π, is pi, equivalent to 3.14.

For seedlings, the stem circumference was measured at ≤ 20cm above the ground within the 5m radius.

The altitude for each sampling point was recorded in the field by a GPS (Garmin 64s). The species regeneration status was assessed by examining the mode of growth (coppicing/independent growth), number of individuals growing from coppices, and proportion of seedlings and saplings within the sampling point (Mligo and Kikoti, 2015). The proportion of coppiced individuals, it was possible to estimate the intensity of destruction due to illegal harvesting, expressed as the proportion of coppiced individuals to the independently growing stems of saplings and adults per site (Osman and Idris, 2012). The proximity among *O.*

lanceolata individuals was also measured and recorded to establish the level of natural spacing exhibited by the species in the natural range along the altitude gradient.

5.2.2 Data analysis

The species density (number of individuals per unit area) was calculated per site using two variables: (i) the number of *O. lanceolata* individuals in each site; and (ii) the total area sampled in a site (Tabuti and Mugula, 2007). The sampled area for each site was calculated in Quantum-gis software(Q-gis) using *O. lanceolata* coordinates. To achieve this, GPS coordinates were first saved in a compatible format (comma delimited text file) using the "add delimited text" command, followed by conversion of the CSV point data shapefile to enable its editing. After this conversion, the distribution points of *O. lanceolata* as displayed in point data were joined into a polygon, and the distances between data points (natural spacing) were measured using the "measure line tool.

The area of the resulting polygon (area sampled) was determined by a field calculator tool in the attributable table, and later used to calculate the species density as follows:

$Osd = {}^{nol}/_{tas,}$ where; '*Osd'* = *O. lanceolata* population density; '*nol'*= number of *O. lanceolata* individuals encountered in a sampled population; and '*tas'* = total area sampled in a study site.

To reveal the population structure, stem diameters were arranged into size classes of seedlings, saplings, and adults as follows: ≤1cm (seedlings); 1.2cm - 6.5cm (saplings); and >6.5cm (adults). The frequency of individuals in each size class were graphically represented in a stem diameter curve to characterise the species' population structure (Tabuti and Mugula, 2007). The levels of the species disturbance and stability were analysed using stem diameter curves where disturbed

populations were expected to show irregular curves, reverse-J-shaped curves and exponentially negative curves while the less disturbed populations reflect an inverse-J-shaped, sigmoid to bimodal-shaped curves (Rao et al., 1990). The species stand stability was analysed by determining the height-to-diameter ratio of individuals (saplings and adults).

The effect of coppicing on stem diameter and height was tested using regression analysis and ANOVA to find out whether there were interaction effects between altitude and coppicing on the stem diameter and height of *O. lanceolata*. If interaction effects were found to be present, the simple main effects of the independent variables were then investigated among subset data of coppiced and non-coppiced individuals to identify the actual effect of coppicing on the species' stem diameter and height. The status of population structure in each site was inferred by analysis of the slope of the linear regression of stem diameter classes (Hall and Bawa, 1993; Lykke, 1998; and Obiri et al., 2002). The midpoint of each stem size class was used as the independent variable (Midpt), while the density of individuals in each class (D/ha) was used as the dependent variable. D/ha was transformed by In(D/ha+1) to avoid zero individuals in some classes. The regression was made between In(D/ha+1) and Midpt. Steep negative slopes indicate stable and self-replacing populations (Hall and Bawa, 1993; Lykke, 1998; and Obiri et al., 2002). The weak negative slopes indicate limited recruitment and populations that are declining (Hall and Bawa, 1993; Lykke, 1998; and Obiri et al., 2002).

5.3 Results

5.3.1 Patterns in Population structure

Three hundred ninety-four (394) individuals of *O. lanceolata* were inventoried across all altitudes (low - 1275 - 1416 masl and high 1721 - 1800masl). Out of the 394 individuals, 196 were adults, 184 saplings, and 14 seedlings. The populations showed an overall irregular population structure pattern with missing individuals in the higher mature size classes. The population structure was thus characterised by 50% adults, 46% saplings, and 4% seedlings.

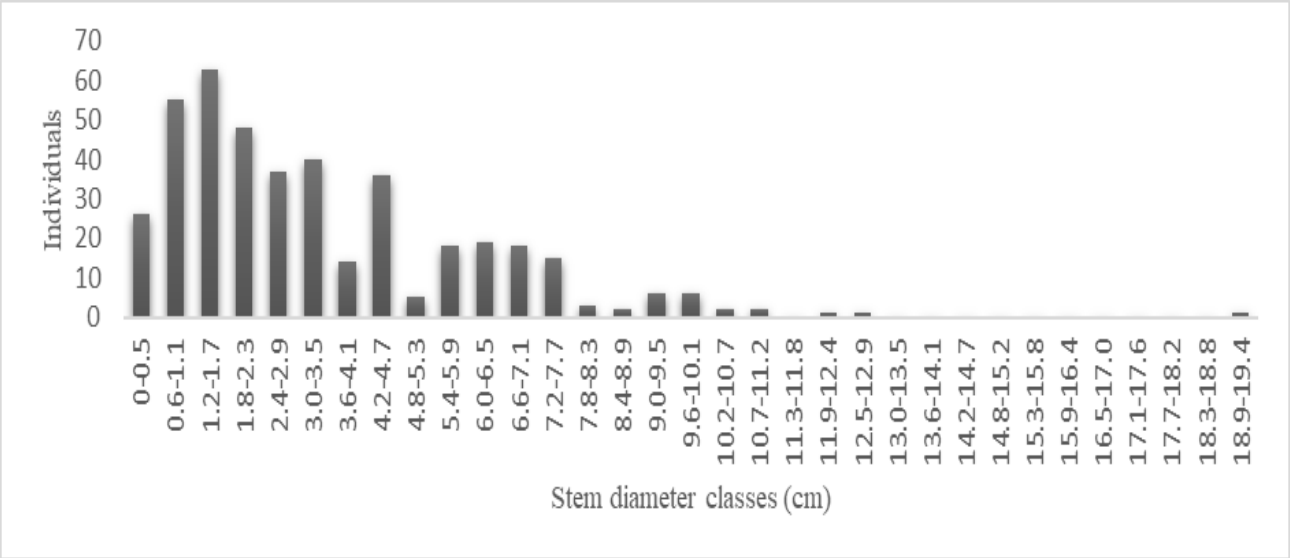


Figure 5.1: Overall size class distribution of O. lanceolata in Karamoja

5.3.2 Population structure of *O. lanceolata* in Uganda

Regression analysis indicated most populations to be undergoing recruitment except one population in Cheporon (CHP) in Amudat district (Table 5.1).

Table 5.1: Regression slope analysis of *O. lanceolata* population structures

Population	Slope coefficient	r^2 values
Akariwon (AKA)	-0.5608	0.2499
Lonyilik (LON)	-27.126	0.1598
Karengepoche (KAR)	-0.3863	0.2392
Cheporon (CHP)	0.103	0.0021
Kangisa (KAN)	-240.12	0.1688
Korenyang (KOR)	-1.5706	0.0776
Ngaram (NGA)	-1.2702	0.1424
Ruwotokech (RUW)	-127.38	0.0798
Lolupe (LOP)	-2E+08	0.1289
Kopedur (KOP)	-2E+09	0.0832
Lotemwoyes (LOT)	-219.78	0.1336
Low altitude populations	-7.729	0.0965
High altitude populations	-3.3483	0.3178
Overall population structure	-4.7058	0.2617

The population structure of *O. lanceolata* in Lotemwoyes (LOT) was an inverse-J-shaped curve, having more individuals in the smaller size classes and no individuals in the mature size classes (Fig. 5.2).

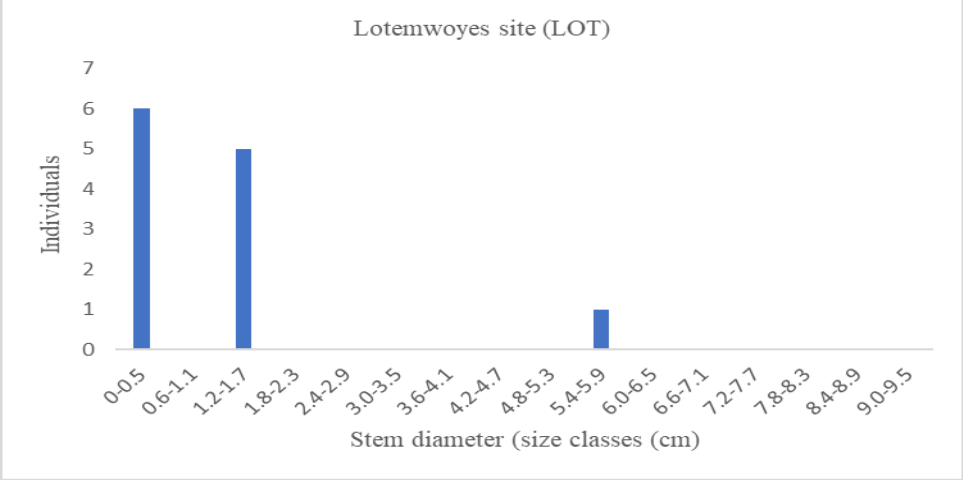


Figure 5.2: Population structure of O. lanceolata in Lotemwoyes (LOT)

Across the low altitudes, the populations exhibited a general irregular population structure with missing individuals in specific size classes. The populations in high altitudes exhibited a relatively bell-shaped population structure, but with missing individuals in the adult size classes (>10cm) (Figure 5.3).

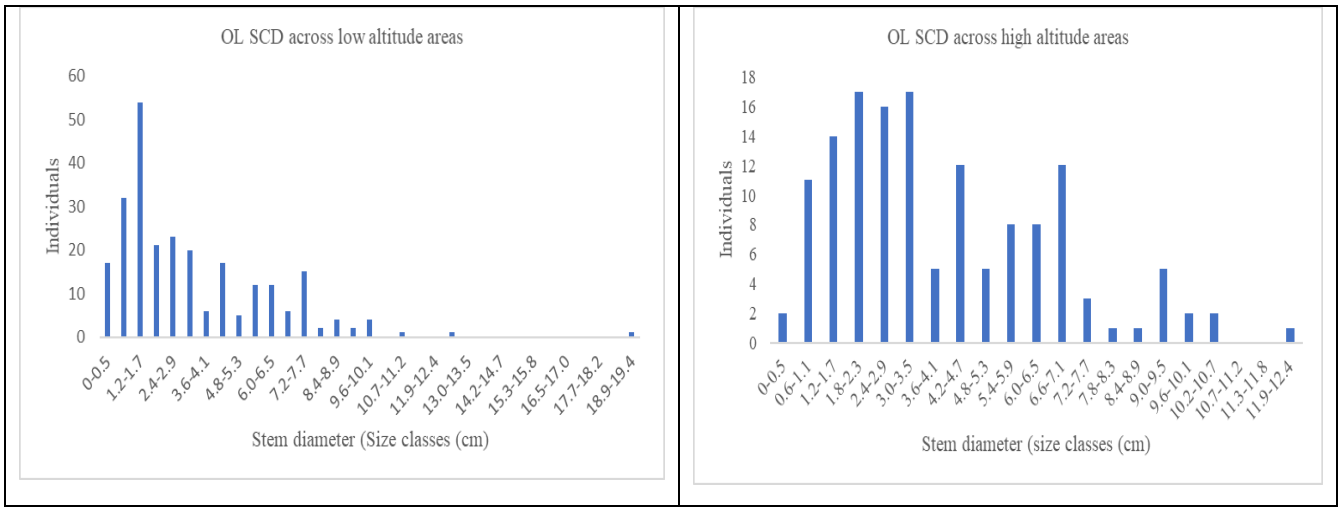


Figure 5.3 Osyris lanceolata stem diameter classes (SCD) across altitudes

Populations in Ngaram (NGA), Kangisa (KAN), Karengepoche (KAR), and Lonyilik (LON) revealed a bell-shaped population structure with more individuals in the middle size classes compared to the smaller and larger size classes (Figure 5.4)

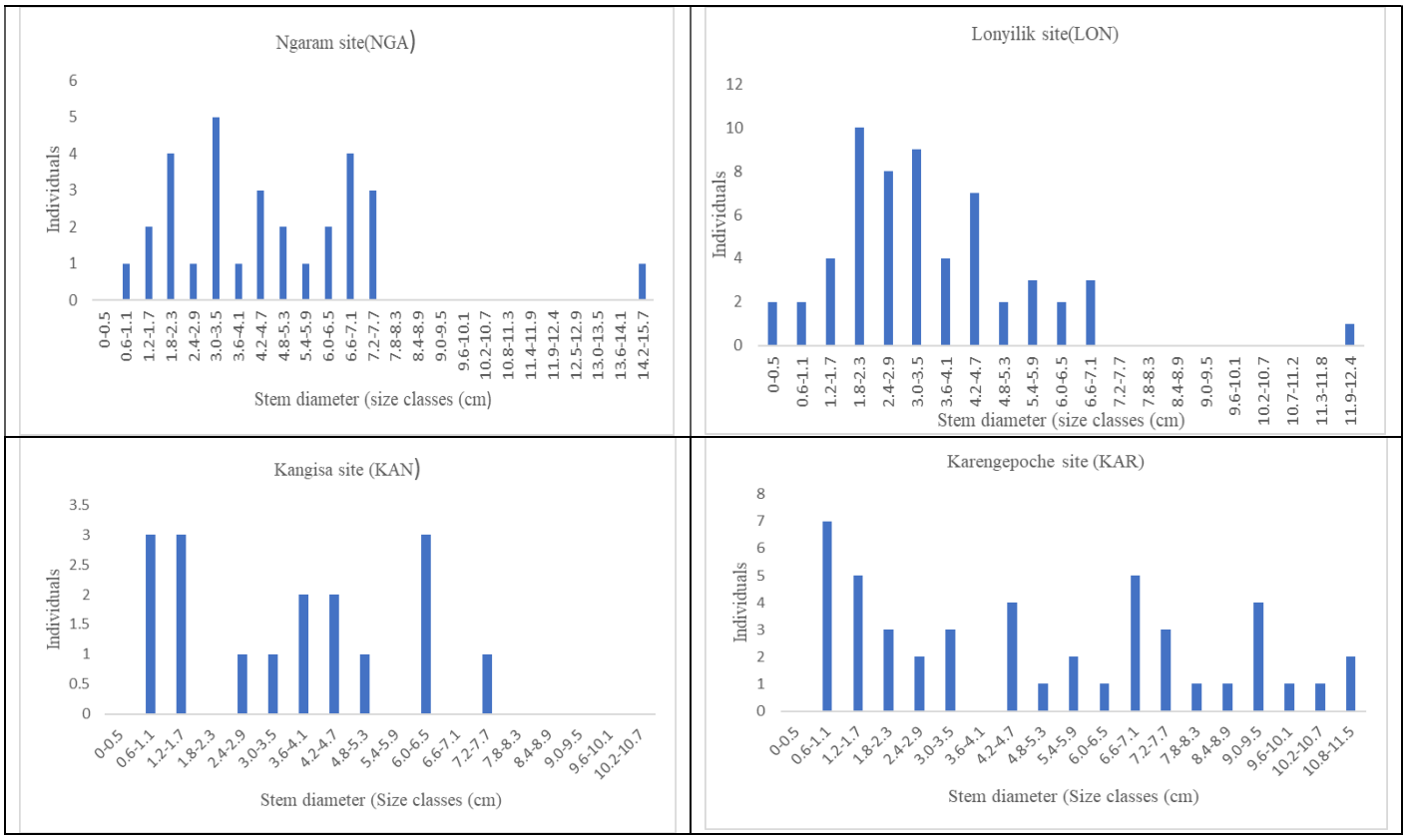


Figure 5.4: Population structure of O. lanceolata in Ngaram (NGA), Lonyilik (LON), Kangisa (KAN), and Karengepoche (KAR).

The populations in Lolupe (LOP), Cheporon (CHP), Korenyang (KOR), Kopedur (KOP), Akariwon (AKA) and Ruwotokech (RUW) exhibited irregular population structures with missing individuals in particular size classes (Figure 5.5).

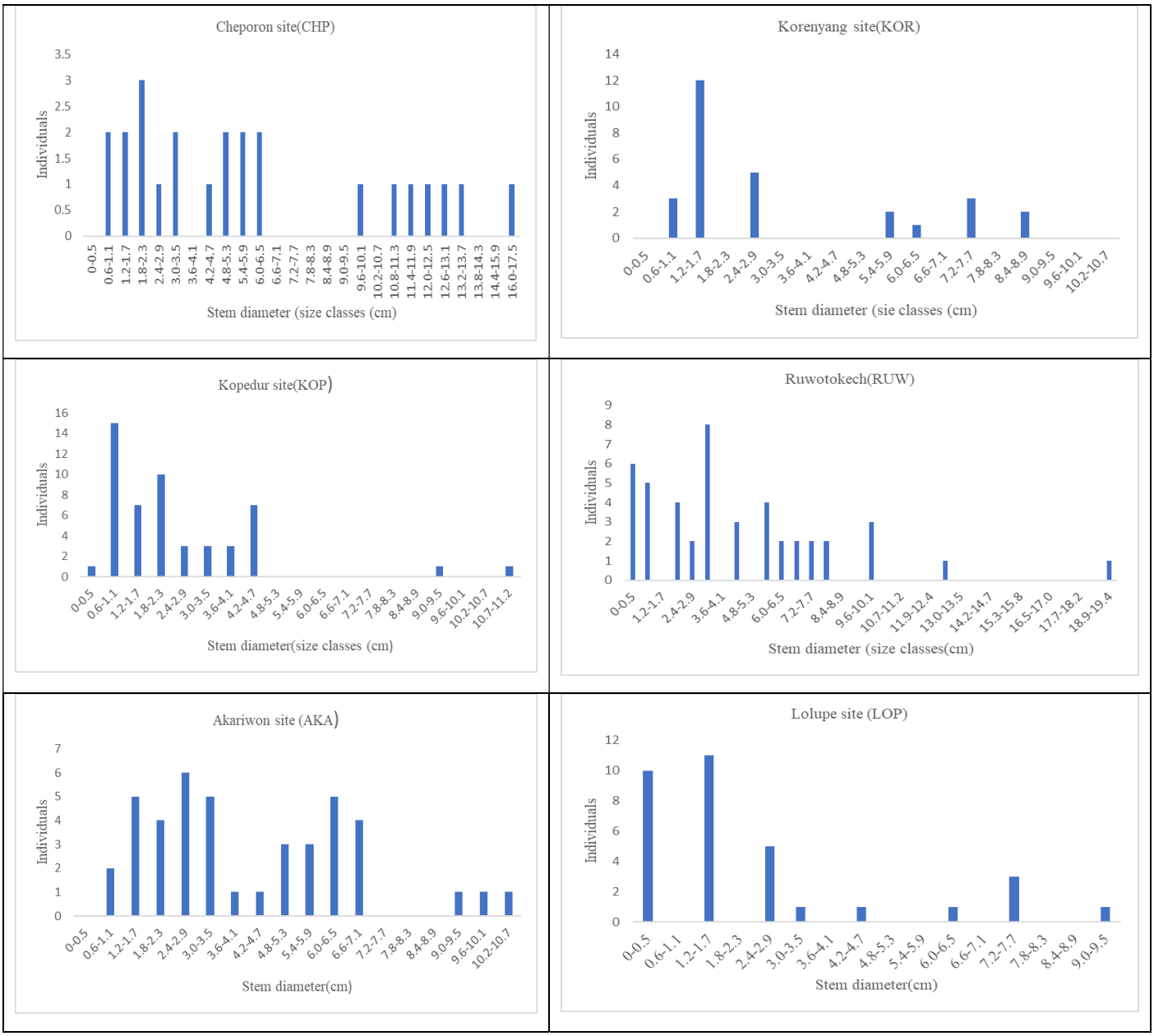


Figure 5.5: Populations structure of O. lanceolata in Cheporon (CHP), Korenyang (KOR), Kopedur (KOP), Ruwotokech (RUW), Akariwon (AKA) and Lolupe (LOP).

5.3.3 Population density and distribution pattern of *O. lanceolata*

The species distribution was patchy and scattered around rocks, deep galleys, streams and even on flat grounds. There was insignificant correlation between *O. lanceolata* density and altitude (cor. coefficient: 0.4174028; t = 1.378, df = 9, and p = 0.2015). Within higher altitudes, the

density of *O. lanceolata* stood at 1.94 trees ha^{-1} and 0.08 trees ha^{-1} in the lower altitudes (Table 5.2)

Table. 5.2 Density of O. lanceolata across different altitudes in Uganda

Altitude Zone	Area sampled (ha)	Abundance (n)	Density (ha^{-1})
Lower altitude	50.878	216	0.0834
Higher altitude	24.114	145	1.0399
Value range	26.764	71	0.9565
Standard Deviation	18.9250	50.204	0.676348
Variance	358.1558	2520.5	0.457446

The species was found to be spread within the altitude range of 1200-1800masl. This range slightly varies from earlier studies that found the species to occur within the altitude range of 1300-1760m.a.s. l. Among the populations, the species densities varied significantly (F= 174.77, $p<0.001$) with the lowest density in Cheporon and the highest in Akariwon (Table 5.3).

Table 5.3: Osyris lanceolata density per sampled population in Uganda

Population	Area Sampled (ha)	Abundance (a)	Density (h^{-1})	Mean deviation (SD)
High altitude (Akariwon)	0.45	42	90.51	0.83
Low altitude (Kopedur)	1.16	51	43.29	0.52
Low altitude (Lolupe)	1.06	33	31.04	0.76
Low altitude (Ruwotokech)	4.57	45	10.73	0.96
Low altitude (Kangisa)	1.78	17	9.55	0.74
Low altitude (Lotemwoyes)	3.29	12	7.59	0.52
Low altitude (Lonyilik)	8.04	57	6.09	0.73
Low altitude (Ngaram)	7.50	30	3.99	0.63
High altitude (Karengepoche)	15.63	46	3.00	0.61
Low altitude (Korenyang)	15.44	28	1.81	0.59
Low altitude (Cheporon)	16.08	24	1.49	0.79

5.3.4 Regeneration patterns of *O. lanceolata*

The observed sources for regeneration of *O. lanceolata* included coppicing (SPRT) and seedlings (germination) detected through independent growth (INDT). However, natural regeneration was rare and irregular and constituted an abnormally low percentage (4%) of seedlings (Figure 5.6)

and 25% of saplings (Figure 4) in all populations. Regeneration was more vegetative through coppicing than seedlings. The coppiced individuals constituted 56.60%, with 35.02% as saplings and 21.60% as adults. The non-coppiced individuals constituted 43.40%, with a population structure of 4% seedlings, 11.7% saplings, and 28.20% adults.

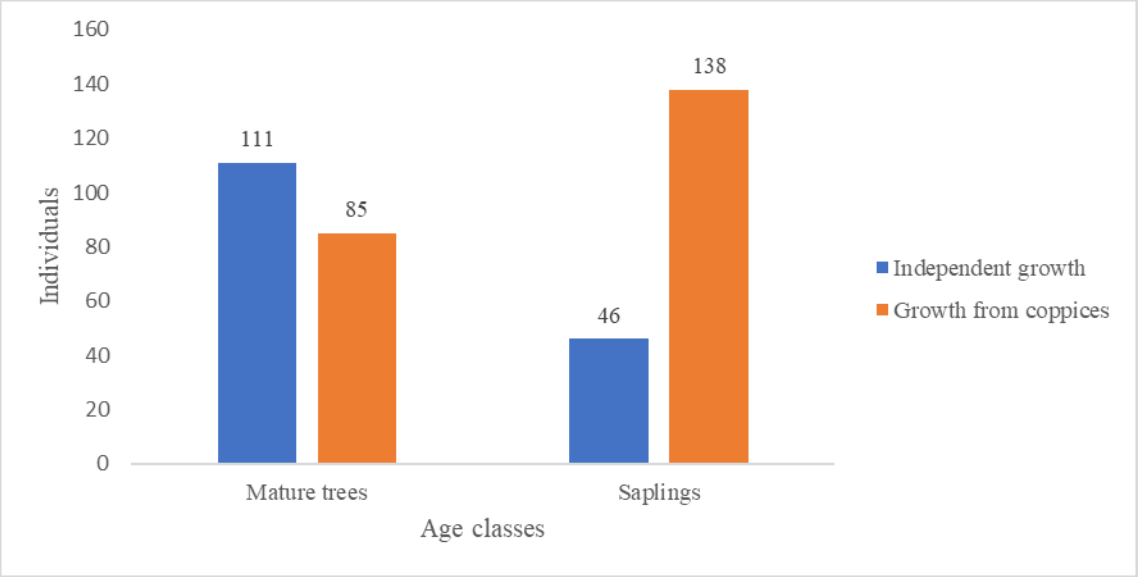


Figure 5.6: Osyris lanceolata age classes under different regeneration strategies in Karamoja

5.4 Discussion

5.4.1 *Osyris lanceolata* distribution and density in Karamoja

O. lanceolata had a patchy distribution in altitude areas ranging between 1200M-1800M.asl. The species patchy distribution may reflect the patchy distribution of survival resources and hence linked to the species hemi parasitic nature, spatial availability of edaphic variables, and patterns of gene flow (Watson et al., 2007, Watson, 2009; Sexton et al., 2016). These factors determine the pattern of species distribution in fragmented habitats (Chen et al., 2014). The species density was generally lower across populations and did correlate with altitude gradient. These findings

resonate with earlier reports that found the species population to be extensively degraded through illegal harvesting across the entire region hence reducing its abundance and density in Karamoja (Tajuba, 2015; FSSD, 2021). Additionally, ecophysiological factors, and environmental isolation also may limit *O. lanceolata* distribution and density (Sexton et al., 2016). The low species regeneration potential due to high seedling mortality also contribute to the lower densities of *O. lanceolata*. The seemingly higher species densities in Akariwon, Lolupe and Kopedur were also characterised with irregularities in availability of individuals in adult and seedling classes which reflects of poor recruitment.

5.4.2 Population structure and regeneration strategy of *Osyris lanceolata*

Osyris lanceolata populations in Uganda exhibited three distinct population structures: reversed-J-shaped, irregular, and bell-shaped structure. The reversed J-shaped structure was characteristic to Lotemwoyes (LOT) (Nakapiripirit) population, with more individuals in the lower size classes and few in mature classes. The irregular structure was observed in Cheporon (CHP), Korenyang (KOR), Kopedur (KOP), Ruwotokech (RUW), Lolupe (LOP) and Akariwon (AKA). The bell-shaped structure was characteristic to Ngaram, Lonyilik, Kangisa and Karengepoche populations. All populations showed consistent lack of seedlings (0 - 1.1cm) and adult classes (7cm-18cm).

Although LOT population showed a reverse- J-shaped structure, its size class distribution did not reflect stability as described by Hall and Bawa, 1993; Lykke, 1998; and Obiri et al., 2002. The population lacked individuals in the sapling size classes (1.7cm-5.3cm), adults (6cm - 18cm) and seedlings. Most juveniles in LOT population were coppiced as opposed to seedlings. Also, the

regression analysis for the population size class distribution revealed a more negative slope (-219.78, r^2 = 0.1336) indicating a weaker, poorly recruiting and unstable population structure (Obiri et al., 2002, Tabuti and Mugula, 2007). These findings provide further insight about the unreliability of using a reverse J-shaped population structure to infer population stability of specific tree species (Cousins et al., 2014). For *O. lanceolata* population structure, the highest number of juveniles largely grows from coppices rather than seedlings.

The bell-shaped pattern of population structure in NGA, LON, KAN and KAR, showed more individuals in the middle size classes but few individuals in the lower and higher size classes. This pattern could be attributed to occurrence of episodic recruitment regimes where certain size classes in some years were not recruited into subsequent classes (Gurmessa et al., 2012). Episodic recruitment may result from environmental bottlenecks that lead to germination failure, or seedling mortality and hence their failure to be recruited to higher size classes. The high seedling mortality could be a result of frequent browsing and grazing of goats and cattle in *O. lanceolata* habitats. Also, damage of *O. lanceolata* seeds and fruits by caterpillars or insect pests (*Dismeqistus sp*) might lead to seed germination failure and hence low abundance of the seedlings (*pers. field observations*). The scarcity of seedlings is an indicator of weak, irregular, and unstable plant populations (Lykke, 1998; Obiri et al., 2002; Kohira and Ninomiya, 2003; Tabuti and Mugula, 2007) with poor regeneration potential (Kohira and Ninomiya, 2003; Bin et al., 2012; Cousins and Witkowski, 2014).

Overall, the study revealed a weakened, irregular, and unstable population structure of *O. lanceolata* with poor regeneration and recruitment. This observation is consistent with earlier

claims on the declining trends in *O. lanceolata* populations in East Africa (CITES, 2013; Muhoozi, 2015; Gathara et al., 2014; Tajuba, 2015; FSSD, 2021). The irregular population structure implies that regeneration of *O. lanceolata* in most populations does not balance with mortality and disturbances which leads to elimination of potential diameter classes that could be recruited into subsequent size classes (Mekonen et al., 2015). The irregularity in size classes also occurs when saplings and adults are harvested before production of seeds to maintain recruitment (Mekonen et al., 2015). Therefore, illegal and unsustainable harvesting of *O. lanceolata* resources could have contributed to the irregular population structure characterised with low densities, poor regeneration and recruitment.

Across populations most individuals (58.68%) regenerated through coppicing compared to natural regeneration (34.47%). Majority of the coppicing individuals were juveniles or saplings (75%) and only 43.3% were mature individuals which indicate coppicing to be a recent activity and a characteristic to the existing species generation. This implies a gradual population shift to a predominantly coppicing population in the future. Besides, the proportion of coppiced *O. lanceolata* individuals (58.68%) also suggests a higher intensity of *O. lanceolata* exploitation over the years resulting into unsustainable population structure. The overexploitation of a species directly impacts on the strength of its population (Osman and Idris, 2012). For instance, the species low densities alter the mating patterns of the species which increase inbreeding and reduce heterozygosity within the populations (Mehes et al., 2009).

These findings raise some questions regarding the strength and stability of the present and future population status of *O. lanceolata*, given the lower number of seedlings. This is because, the

species' reliance on vegetative (coppicing) regeneration strategies may result in dire effects on its genetic diversity (Kohira and Ninomiya, 2003). Much as regeneration through coppicing is believed to enhance the species continuity through regular regrowth of new individuals, it may expose subsequent populations to a higher risk of further degeneration in genetic adaptation potential, due to continued inbreeding (Kohira and Ninomiya, 2003). Previous studies have indicated that weak and irregular population size structure of plant species may predict compromised genetic potential (Mehes et al., 2009; Andiego et al., 2019 and Cueva-Agila et al., 2021). Further investigations should focus on understanding such effects. Thus, assessment of genetic diversity in line with the current population structures helps to understand the impact of species exploitation on genetic diversity and structure, to develop conservation programs such as plant breeding, propagation and domestication (Cueva-Agila et al., 2021; Gathara et al., 2022).

5.5 Conclusion

The findings revealed a coppiced, unstable and irregular population structure of *O. lanceolata* in Karamoja. This population pattern could be a result of seedling mortality, lack of natural seed banks, germination failure and the illegal harvesting. Also, the species' reliance on more vegetative regeneration strategies than seedlings may predict an existing population with compromised genetic potential. The lack of effective population recruitment may degenerate into further decrease in *O. lanceolata* density and genetic diversity. To avert the further decline of *O. lanceolata* populations, in-situ and ex-situ conservation strategies should be applied to restore degraded populations in Moroto, Nakapiripirit and Amudat districts. This will secure the species' germplasm and enhance sustainable commercial production in the future. The *O. lanceolata* provenances for ex-situ conservation should be urgently identified bearing in mind desirable

biochemical properties and survival conditions. However, this requires better understanding of the species site-specific hosts, habitat characteristics and edaphic variables as potential drivers to the species' distribution and survival. Further, understanding the species genetic diversity and structure also provides an insight on suitable provenances for consideration into conservation programs, and development of appropriate strategies for conservation.

CHAPTER SIX

HOST COMPOSITION AND MORPHOLOGY OF *OSYRIS LANCEOLATA* ACROSS ENVIRONMENTAL GRADIENTS IN KARAMOJA REGION, UGANDA

6.1 Introduction

Osyris lanceolata is a highly valuable species because of its essential oils used in pharmaceutical and cosmetic industries (Njoroge and Bussmann, 2006; Muhoozi, 2015; Orwa et al., 2009; Masevhe et al., 2015). However, the species host composition and morphological response to exploitation and altitude gradient are little studied (Mugula et al., 2021). The lack of such information hinders the species' conservation efforts and sustainable use. Earlier studies in Uganda focused on the species' utilisation and habitat threats (FSSD, 2021) with less attention on the host composition, morphological variations and the effect of overexploitation on the morphology of the species. Yet, a better understanding of the species' host composition, and its morphological response to environmental gradients is critical in planning for sustainable management of *O. lanceolata*. This is because, such information, provides insight on suitable conditions of the species (Lomolino, 2001). Unfortunately, much of the research work on root parasites have also focused more on their physiology, anatomy and hosts (Tennakoon et al., 1997) with limited attention to their host diversity and morphological traits. Since hemi parasites differ geographically (Gathara et al., 2014) it is necessary to explore their composition across a continuum of environmental variables in different ecosystems.

Without hosts, the growth of hemiparasites deteriorates rapidly, especially in their advanced stages due to limited capacity to acquire nutrients such as Ca^{2+}, K^{+}, P and Mg^{2+} (Irving and Cameron, 2009; Westwood et al., 2010). For instance, the germination of *O. lanceolata* seeds does not require host influence in the initial stages (Kuijt, 1969; Yoder, 1999) but further development of seedlings require hosts (Westwood et al., 2010). Some hemiparasites co-exist with different hosts (Watson, 2009) while others are extremely host specific (Kuijt, 1969; Irving and Cameroon, 2009). Host specificity is not stationary but keeps on changing with levels of plant diversity in an ecosystem (Furuhashi et al., 2011). Thus, even hemiparasites that appear generalists across the whole habitat range can be specific to particularly abundant hosts in another habitat (Irving and Cameroon, 2009).

A study conducted by Mwang'ingo et al., (2005) revealed common hosts for *O. lanceolata* in controlled environments to include: *Rhus natalensis*, *Dodonaea viscosa* Jacq., *Tecomaria capensis* (Thunb.), *Catha edulis* (Vahl) Forssk. Ex Endl., *Apodytes dimidiata* E. Mey. ex Arn., *Brachystegia spiciformis* Benth., *Maytenus acuminata* var. acuminata and *Aphloia theiformis* (Vahl) Benn. Gathara et al., (2014) also reported the most preferred hosts that predict *O. lanceolata* site suitability to include; *Rhus natalensis* and *Hypoestes forskahlii* in the high and low altitude areas, respectively. Whether similar hosts exhibit consistent pattern of preference in semi-arid habitats is yet to be understood.

The semi-arid environments are characterised with conditions of water stress which largely influence the morphology of endemic species. These species tend to adapt to these habitats by developing morphological modifications in some of their structures. However, such

modifications can be induced by environmental gradients and anthropogenic activities. Similarly, the role of host composition on the survival of *O. lanceolata* can be expressed in the species pattern of distribution and survival strategies (Mathiasen et al., 2008).

Although the ecological significance of hosts at different life stages of hemiparasites and their interaction with hosts is well documented (Matthies,1999; Tennakoon and Cameroon, 2006; Mathiasen et al., 2008), the influence of environmental gradients on the host composition and their proximity to *O. lanceolata* is barely understood (Mugula et al., 2021). This study sought to: (i) establish the host composition and characteristics of *O. lanceolata* and how it affects the species distribution in the semi-arid ecosystem, and (ii) investigate the effect of altitude and compared the morphology of coppiced for exploitation and non-coppiced for no exploitation on the species' morphological traits. In line with these objectives, the study tested the following hypothesis: first; the species distribution is not affected by host preference. Thus, there are no preferred hosts or specific species that characterise the distribution of *O. lanceolata* in the semi-arid ecosystems. Secondly, the habitat altitude and past destructive disturbances have no effect on the species morphology.

Osyris lanceolata has very interesting morphology characterised with multiple stems, and highly branched. The species has variable height ranging between 2-5m, with multiple variations in leaf size. The leaf size variation has been locally attributed to sex identify in East Africa but no empirical data to support such claims (Mugula et al., 2021). It is usually referred to as an evergreen small tree or shrub having alternate and coriaceous leaves that measure about 13mm-50mm in length, sharp pointed with light blue-green color (Vald´es et al., 1987). The study

hypotheses are; first; there are no specific hosts and habitat qualities influencing the distribution of *O. lanceolata* in Karamoja. Secondly, the altitude gradient and exploitation have no effect on the species morphology. Although the morphology of *O. lanceolata* is described in previous studies, there remains a gap to understand the effect of environmental gradients on the species morphology.

6.2 Materials and Methods

The study areas and the criteria for selection of study populations is already described in chapter three of this thesis.

6.2.1 Sampling

To establish the effect of altitude gradient on the morphology of *O. lanceolata*, the number of stems, height, crown cover, and mode of regeneration (coppiced or non-coppiced) were recorded in every sampling point. The altitude for each sampling point was recorded by a GPS (Garmin 64s). The number of stems was used to assess the extent of branching within the species and examine any patterns across altitudes. The height was measured by estimating the total height of the tree from the ground to the last tip of the shoot using the halving method. Height, provides an indicator of the species' stress due to water and nutrient deficiency in the habitats (Wonn, 1998). Also, height helps to assess the ratio of diameter to height which is used to predict the species' stability to wind damage in the habitats (Wonn, 1998). The leaf length and width were measured to examine the extent of variation in leaf size across the altitude gradient and explore their implications for the species' survival. The species crown cover was obtained after measuring the crown diameter in two cardinal directions of the crown.

To assess the host composition, host species were identified around *O. lanceolata* in a radius of 0.5-5meters (m) in each population. The host identity, abundance, frequency, preference, and proximity to *O. lanceolata* individuals were recorded. The host proximity (closeness) to O. lanceolata was assessed by measuring the distance between hosts and *O. lanceolata*. The voucher specimens of un identified hosts were wrapped in newspapers, well pressed and taken to Makerere University Herbarium in Kampala for proper identification and further reference. The *O. lanceolata* hosts are usually clustered with the species and survive at appropriate distances, being not too close and not far away from the *O. lanceolata* (Dueholm et al., 2017). The host plants mainly nourish the growth of *Osyris lanceolata* through a parasitic relationship that involve the exchange of carbon materials, nutrients and water (Tenakoon et al., 1997;). This host-parasitic relationship takes place through a haustorium system established between the host and *O. lanceolata*. (Tenakoon et al., 1997; Okubamichael et al., 2016). Apart from hosts the survival of *O. lanceolata* is also supported by associated plant species popularly known as associates, and such species mainly occur in areas that are suitable for the survival of *O. lanceolata*. There is no direct benefit between the associate species and *O. lanceolata*, and thus their association could be a result of local adaptation through formation of unique spatial patches for their survival (Saiz et al., 2018).

6.2.2 Data analysis

The host plant composition of *O. lanceolata* was assessed in terms of host species identity, families, growth form, abundance, frequency of clustering with *O. lanceolata*, and proximity (closeness) to *O. lanceolata* trees. The host proximity levels to *O. lanceolata* were assessed by

measuring the distance between the host and *O. lanceolata*. The data on host abundancies was used to compute the Sorenson index (*SI*) and illustrate host similarity/dissimilarity among populations as indicated in chapter three:

Sorensen index (S_s) = $^{2a}/$ (2b+c+d); Where, b refers to the number of host species occurring in sites B and C; c refers to the number of host species that occur in site C only or each study site; and d refers to the number of host species that occur in only site B or each site.

The indexes between sites that are < 0.5, indicates dissimilar sites in host species composition and hence *O. lanceolata* have no host preference. In addition, the host proximity to *O. lanceolata* was also determined by estimating the closest and widest distance between *O. lanceolata* and hosts and also identifying specific host species which are closer and those associating at wider distances. In making host site comparisons, the percentage of shared species between sites was calculated using the formula below to establish the level of similarity and dissimilarity in host composition between sites;

SSp (%) = $^{NSSp}/_{TNSp}$ x 100; where,

SSp = percentage of common species between sites A and B sites;

NSSp = number of hosts common between A and B, (A∩B);

TNSp = total number of hosts in site A and B (A∪B).

Canonical correspondence analysis (CCA) was applied to explain the relationships between the species morphological traits: height, leaf length, leaf width, number of stems, stem diameter, crown cover, and natural spacing and the altitude gradient. The data on species morphology was

condensed and transformed by detrended correspondence analysis (DCA) before applying CCA to explain the relationship between the species transformed variables and environmental variables (Carleton, 1984; Anderson and Willis, 2003). The length of the DCA gradients was 4. ANOVA and regression analysis (R) were used to determine the effect of exploitation (coppicing) on the species growth morphology.

The phenotypic plasticity indexes (*PI*) were calculated for each morphological trait to assess whether any variations in structural morphology of *O. lanceolata* was due to coping up with environmental changes (phenotypic plasticity) along or not across the altitude gradient. To do this, *O. lanceolata* individuals were clustered under two altitude zones: lower altitude and higher altitudes. The plasticity indexes (*PI*) were then calculated for each morphological trait as:

$PI = {}^{Lvt\text{-}Stv}/_{Ltv}$; where, *PI*, is the plasticity index, *Lvt* = largest trait value, Stv = smallest trait value.

To assess the effect of exploitation on the species morphology, or growth patterns, *O. lanceolata* individuals arising from coppiced stamps were recorded as evidence of exploitation in the population. The non-coppiced individuals represented unexploited members of the species. ANOVA was used to analyse any differences in morphological traits among the coppiced (exploited) and non-coppiced(un-disturbed) individuals. Altitude was the dependent variable, and morphological traits as independent variables.

6.3 Results

6.3.1 Host composition and proximity to *Osyris lanceolata*

A total of 34 species from 12 families were identified in 322 sample plots. Seven species and mainly trees (44.11%) were hosts for *O. lanceolata,* while others were species considered associates to *O. lanceolata.* Anacardiaceae members were the majority hosts (11.7%), followed by Ebenaceae (8.8%) - Oleaceae (8.8%), Sapindaceae (8.8%), and Malvaceae (8.8%). Among the hosts, five species were highly clustered with *O. lanceolata*, such as; *Euclea racemosa* murray (50.3%), *Rhus natalensis* krauss (65.5%), *Maytenus senegalensis* (47.5%), *Ozorea insignis* (34.5%), and *Terminalia browni* (27.3%) (Table 6.1). The most clustered hosts had minimal variations in proximity to *O. lanceolata*: *Rhus natalensis* (109.31cm), *Euclea racemose* (109.9cm), *Maytenus senegalensis*(119.9cm), *Ozorea insignis*(108.9cm) and *Terminalia brownii* (95.9cm). Most associates included shrubs or small trees (29.41%), typical shrubs (14.7%), succulents (5.88%) and climbers (2.94%) in the Celestraceae family (5.8%), Combretaceae (5.8%), Mimosaceae (5.8%), Fabaceae (2.9%), Burseraceae (2.9%), Asphodelaceae (2.9%), Papilionaceae (2.9%), Convolvulaceae (2.9%), Loganiaceae (2.9%), Rutaceae (2.9%), Rubiaceae (2.9%), Euphorbiaceae (2.9%), Cactaceae (2.9%) and Caeslpiniaceae (2.9%) (Table 6.1).

Table 6.1 Hosts, associates and their proximity to O. lanceolata in Karamoja

Host composition	Family	Growth Form	Frequency (%) in habitats (n=322)	Proximity to *O. lanceolata* (cm)	Ecological role in habitat
Rhus natalensis krauss	Anacardiaceae	S/ST	65.5%	109.31	Ca2+ extraction
Euclea racemose	Ebenaceae	Tree	50.3%	109.97	Calcareous valley clays
Maytenus senegalensis	Celastraceae	Sh-T	47.5%	119.97	Well drained soils
Ozorea insignis	Anacardiaceae	Shrub/small tree	34.5%	108.95	Ca2+ extraction
Terminalia brownii	Combretaceae	Tree	27.3%	95.16	Sandy soils
Acacia mearnsii	Mimosaceae	Tree	19.6%	103.43	N-Fixing
Jasminium dichotomum	Oleaceae	Tree	18.01%	100.77	
Lannea barteri	Anacardiaceae	Tree (fire)	17.7%	114.46	Ca2+ extraction
Diospyros mespiliformis	Ebenaceae	Tree	17.1%	109.82	Calcareous valley clays
Pleurostylis africana	Celestraceae	Shrub/small tree	15.5%	87.28	
Albizia amara	Fabaceae	Tree	13.4%	145.33	N-fixing
Pappea capensis	Sapindaceae	Tree	10.9%	114.47	
Combretum molle	Combretaceae	Tree	6.52%	112.62	
Commiphora africana	Burseraceae	Tree	8.39%	115.04	
Aloevera sp	Asphodelaceae	Succulent plant	6.21%	105.11	
Allophylus rubrifolius	Sapindaceae	Shrub/small tree	0.31%	80.00	
Indigofera sp.	Papilionaceae	Shrub	3.73%	51.17	
Ipomoea hildebrandtii	Convolvulaceae	Shrub	5.59%	80.33	
Strychnos innocua	Loganiaceae	Tree	2.17%	118.33	
Sclerocarya birrea	*Anacardiaceae*	Tree	0.31%	16.00	Ca2+ extraction
Jasminium eminii	Olacaceae	Shrub	1.55%	95.00	
Vepris nobilis	Rutaceae	Shrub/tree	1.55%	75.00	
Pseudocedrella kotschyi	Meliaceae	Tree	1.55%	105	
Diospyros scabra	Ebenaceae	Tree/shrub	1.24%	70	Calcareous valley clays
Dalbergia melanoxylum	Papilionaceae	Tree	1.55%	83.80	
Acacia amythethophylla	Mimosaceae	Tree	0.93%	120	
Piliostigma thoningii	Fabaceae	Tree	0.31%	110	Legume-N-fixing
Gardenis ternifolia	Rubiaceae	Shrub/tree	0.31%	66	
Acalypha fruticosa	Euphorbiaceae	Shrub	0.62%	21.50	
Grewia bicolor	Malvaceae	Shrub/small tree	2,79%	64.44	
Grewia similis	Malvaceae	Shrub/small tree	0.93%	90	
Cactus sp	Cactaceae	Succulents	4.34%	105.71	
Senna sp	Caeslpiniaceae	Shrub	0.62%	55.33	
Ximenia americana	Olacaceae	Shrub/small tree	0.31%	80	

6.3.2 Hosts in low altitude populations

In low altitude areas *O. lanceolata* hosts varied significantly in composition than higher altitudes. *Terminalia brownii* maintained lower frequency throughout the low altitude populations. However, *Rhus natalensis*, *M. senegalensis* and *E. racemosa* had relatively close frequencies in most populations (Figure 6.1).

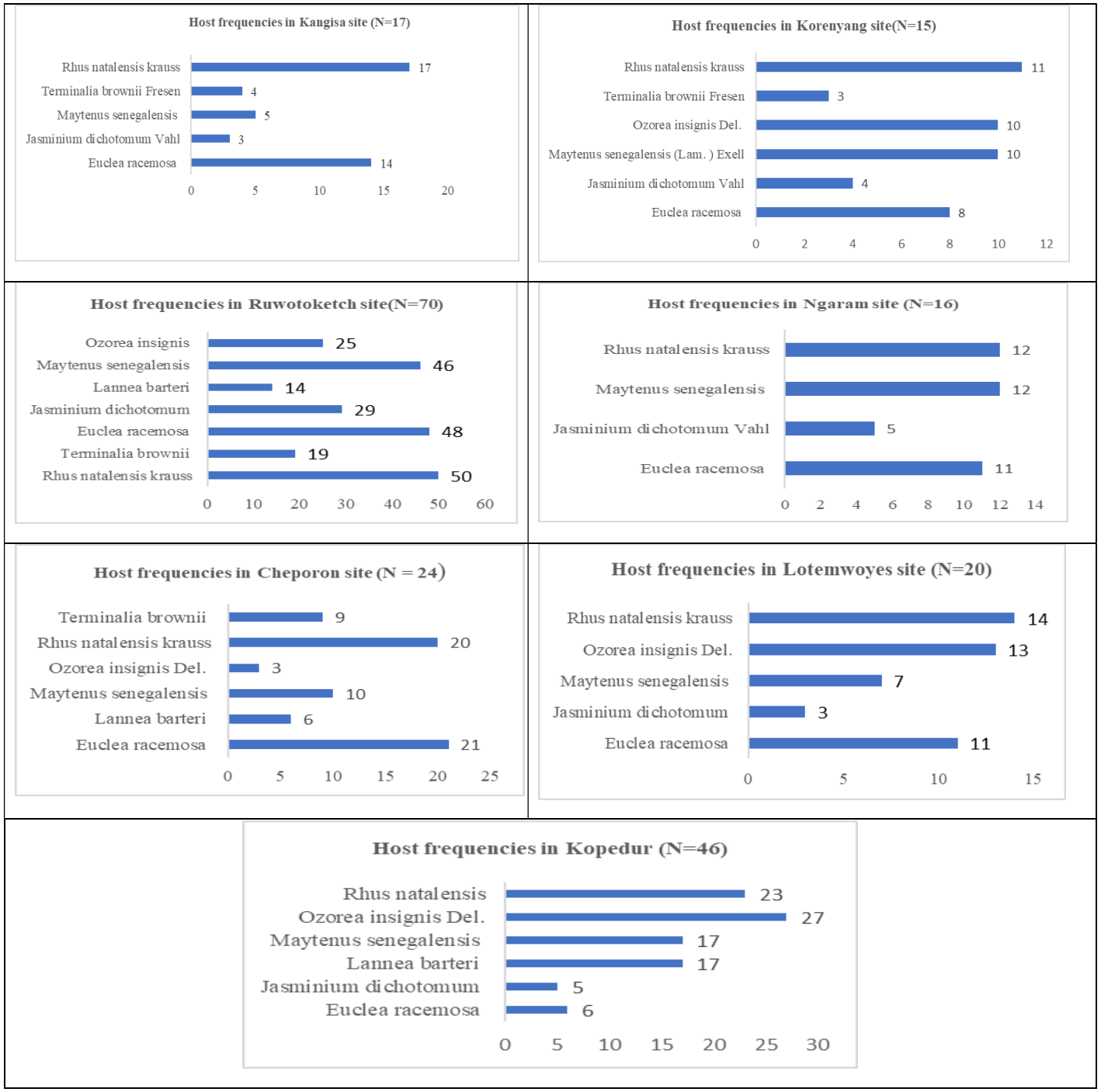


Figure 6.1: Osyris lanceolata hosts in low altitude areas of Karamoja sub-region

Terminalia brownii and *Jasminium dichotomum* were the least common hosts in the Karengepoche site. In Lolupe *Euclea racemosa* was almost absent but dominant in Karengepoche site (Figure 6.2).

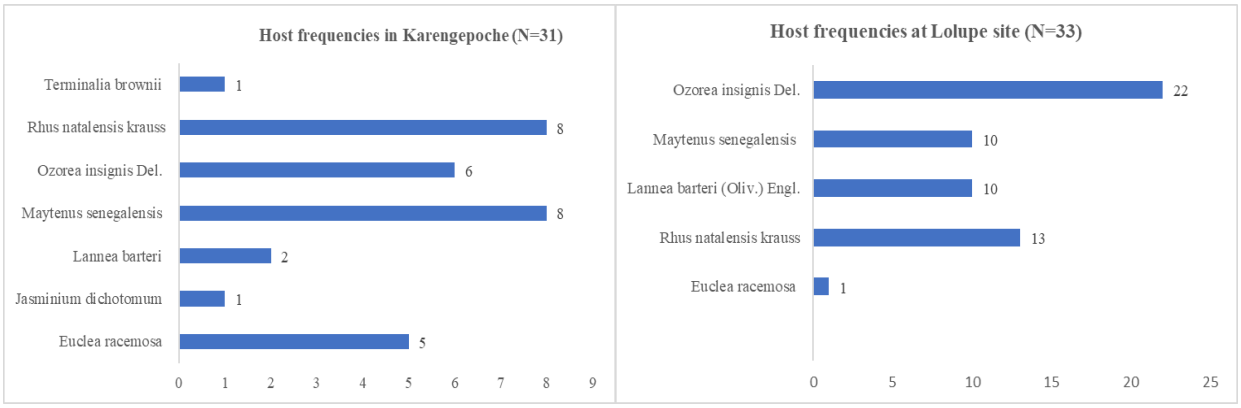


Figure 6.2 Host species in Karengepoche (Amudat) and Lolupe (Nakapiripirit) sites

6.3.3 Host species in high altitude areas of Karamoja

Specific hosts were highly clustered with *O. lanceolata;* in high altitudes except in Karengepoche population, and these included; *Terminalia brownii*; *R. natalensis*, *M. senegalensis*, and *E. racemosa.* However, *T. brownii* maintained a lower frequency in most populations, including Karengepoche which had a higher altitude range with Lonyilik and Akariwon populations (Figure 6.3).

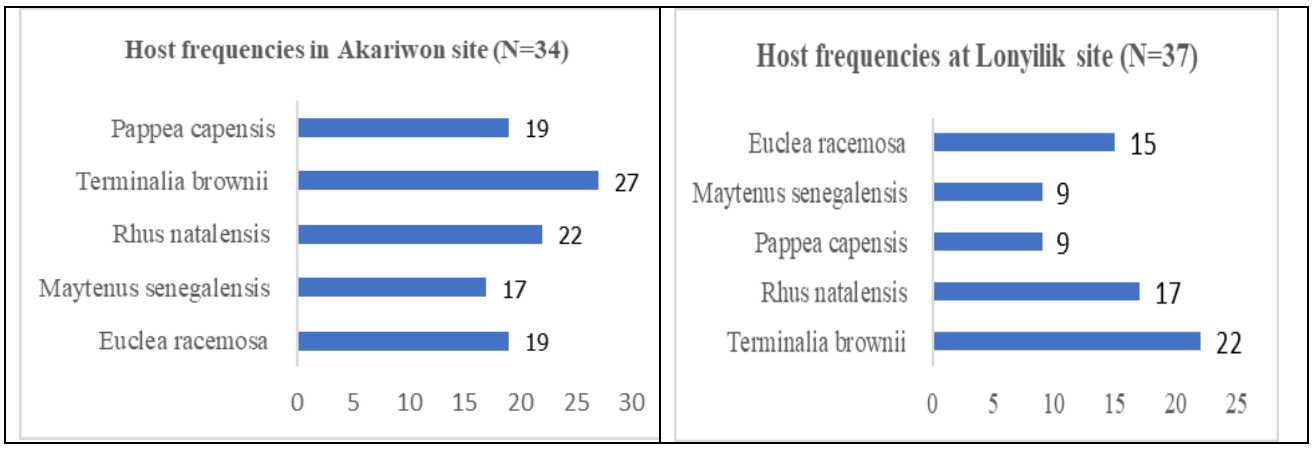


Figure 6.3: Host frequencies in higher altitude areas (Moroto populations)

6.3.4 Host similarity/dissimilarity among populations

The calculated Sorensen indexes (*SI*) between populations were less than <0.5 for both hosts and associates and >0.5 for hosts. This indicates sites dissimilarity in hosts and associates and similarity in hosts across sites respectively (Table 6.2).

Table 6.2 Sorensen indexes of host composition among populations in Karamoja

Sites (populations)	Hosts and Associates (n)	Compared Populations	Sorensen Indexes (*SI*) (Hosts)	Sorensen indexes (*SI*) (Hosts + Associates)
Akariwon (AKA)	12	AKA: LON	1.00	0.33
Lonyilik (LON)	16	CHP: KAR	0.92	0.36
Karengepoche (KAR)	12	LOP: KOP	0.91	0.47
Lolupe (LOP)	12	CHP: NGA	0.60	0.33
Kopedur (KOP)	12	RUW: KOR	0.92	0.45
Lotemwoyes (LOT)	09	KOR: AKA	0.73	0.28
Cheporon (CHP)	16	KOR: LON	0.73	0.35
Ngaram (NGA)	17	KAN: AKA	0.80	0.27
Ruwotokech (RUW)	18	KAN: LON	0.80	0.29
Korenyang (KOR)	15	KAN: RUW	0.83	0.45
Kangisa (KAN)	19	KAN: KOP	0.73	0.42

6.3.5 Morphological attributes of *O. lanceolata* in Karamoja

Regression analysis showed varying degrees of relationship between altitude and growth morphological traits: height ($r^2 = 0.6882$ $p<0.001$), number of stems ($r^2 = 0.0189$, $p = 0.026$), leaf length ($r^2 = 0.4983$, $p<0.001$), leaf width ($r^2 = 0.7727$, $p<0.001$), leaf area ($r^2 = 0.8414$, $p<0.001$), stem diameter ($r^2 = 0.8658$, $p<0.001$), crown size ($r^2 = 0.6425$, $p<0.001$) and natural spacing between *O. lanceolata* individuals ($r^2 = 0.306$, $p<0.001$). The individuals with short stems were found in Lotemwoyes (mean=1.32m, SD = ± - 0.522) and those with tall stems in Ruwotokech (mean = 2.65m, SD = ± 0.9583). The number of stems ranged between 3-6 stems and few individuals had > 6 stems. The individuals with smaller stem diameter were found in Lotemwoyes population (mean =1.54 cm; SD = ±1.7169) while Cheporon population had more

larger individuals in stem diameter (mean = 4.93 cm; SD = ± 4.355). Overall, lower altitudes had more individuals with larger stems than higher altitudes.

The space interval between *O. lanceolata* individuals ranged between 13-91m across populations. The crown size reduced with increasing altitude and increased with decreasing altitude. Despite altitude having no significant effect on the species stem diameter, more trees with larger stems were found in lower altitude than higher altitudes (Figure 6.4).

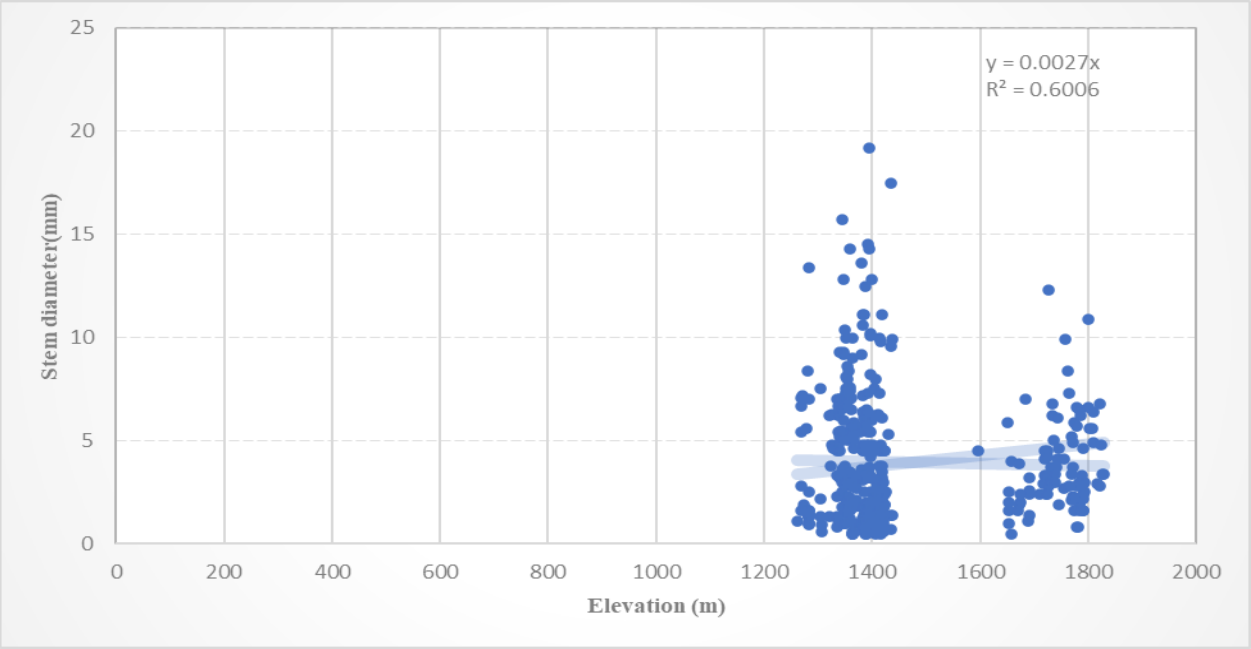


Figure 6.4 Variation of O. lanceolata stem diameter with altitude in Karamoja

6.3.6 Variations in *O. lanceolata* leaf sizes across altitudes in Karamoja

The species leaf sizes varied significantly across altitudes ($p = 0.05$). Most individuals had their leaf sizes between 10 - 35 cm^2. *O. lanceolata* leaf length ranged between 4-8cm; and width 2-4cm**.** Although the species had multiple leaf sizes, two distinct patterns of leaf length and width (4-8cm(length); and 2-4cm (width) were frequently recorded across populations (Table 6.3).

Table 6.3 Osyris lanceolata leaf size frequencies across sites

Population sites	**Altitude** (m)	**Leaf length**				**Leaf width**		
		4cm %Freq	6cm %Freq	7cm %Freq	8cm %Freq	2cm %Freq	3cm %Freq	4cm %Freq
KOP (n = 50)	1416	40	16	16	26	60	12	26
Ngaram (n = 30)	1435	33	10	16	17	57	10	33
Lotemwoyes (n = 25)	1382	60	0	16	24	72	8	20
Lolupe (n = 33)	1407	54	0	9	36	65	0	37
Kangisa (n = 17)	1327	35	0	17	47	53	0	47
Cheporon (n = 24)	1392	33	0	12	54	46	0	55
Korenyang (n = 28)	1275	50	3.6	14	32	57	10	32
Lonyilik (n = 49)	1721	53	4	14	29	74	4	22
Akariwon (n = 41)	1787	29	12	14	36	39	19	42
Ruwotokech (n = 49)	1381	43	6	16	32	69	0	31
Karengepoche (n = 47)	1794	27	2.1	21	49	51	0	50

6.3.7 Morphological plasticity of *O. lanceolata*

The species morphological plasticity varied across altitudes. All traits showed higher variations in plasticity across altitudes (Table 6.4).

Table 6.4 Plasticity indexes (PI) of O. lanceolata morphological traits in Uganda

Altitude zones	PI (Height)	PI (No. of stems)	PI (Leaf size)	PI (Plant spacing)	PI (Stem size)	PI (Crown cover)
Higher altitude(1500-1800m)	0.21	0.25	0.28	1.27	0.264	0.18
Lower altitude (1300-1400m)	0.501	0.5	0.27	0.813	0.688	0.51

6.3.8 The morphology of coppiced and non-coppiced *O. lanceolata*

The stem diameter of *O. lanceolata* significantly varied among coppiced and non-coppiced individuals ($p<0.001$, $df = 1$) across altitudes (Table 6.5).

Table: 6.5 Stem size and height comparison among coppiced and non-coppiced O. lanceolata

Site category	Altitude (m)	Coppiced (dm)	non-coppiced (dm)	Ht-coppiced (m)	Ht-non coppiced (m)
High altitude (Akariwon)	1787	5.6	5.3	2.4	2.7
High altitude (Lonyilik)	1721	3.9	5.2	2.5	2.7
High altitude (Karengepoche)	1794	5.9	7.4	2.9	3.0
Lower altitude (Lolupe)	1407	4.6	7.6	2.7	3.0
Lower altitude (Kopedur)	1416	3.2	6.1	2.5	2.1
Low altitude (Lotemwoyes)	1382	4.2	5.4	1.2	2.5
Low altitude (Cheporon)	1392	3.3	5.7	7.1	2.6
Low altitude (Ngaram)	1345	6.7	6.2	2.7	2.9
Low altitude (Korenyang)	1275	4.7	7.1	2.6	2.5
Low altitude (Kangisa)	1327	2.8	5.3	2.8	2.6
Low altitude (Ruwotokech)	1381	5.2	9.5	3.2	3.4
Standard Deviation:		1.24	1.36	2.03	1.71
Standard Error of Mean:		0.37	0.41	0.61	0.51

DM=diameter; copp=coppiced; non-copp=not coppiced; Ht=height

The past exploitation of *O. lanceolata* as evidenced in coppiced individuals also significantly affected the species height (p-value = <0.001, df = 1). In higher altitudes, the coppiced *O. lanceolata* had bigger stems (mean = 5.11cm) than non-coppiced members (mean = 4.487cm). However, the non-coppiced individuals had bigger stems (mean = 6.72cm) than coppiced individuals (mean = 4.56cm) in lower altitudes. Remarkably, non-coppiced *O. lanceolata* had significantly bigger stems (mean = 5.828cm, p-value = 0.001951, df = 1) than coppiced individuals (4.728cm) across altitudes. In regard to the species height, coppiced individuals were significantly taller (p-value = 0.016744, df = 1) than non-coppiced members across altitudes.

6.3.9 Variation in stem size and height of *O. lanceolata*

The findings indicated a significant positive relationship between the species height and stem diameter (p - <0.001, df = 1) among coppiced *O. lanceolata*. The species height had a linear relationship with stem diameter. Further analysis revealed a significant interaction effect of coppicing (df = 1, F = 7.0938, p < 0.0116) and height (df = 1, F = 27.4656, p < 0.001) on the stem diameter. The combined interaction effect of the two variables (coppicing and height) on stem diameter was also significant (df = 1, F = 22.5890, p < 0.001). Further investigation of the

main simple effects per category (coppiced and non-coppiced *O. lanceolata*) revealed no significant impact of height on stem diameter among coppiced individuals (df=1, F = 4.1954, p = 0.05461), but, a significant effect of height on the species' stem diameter was present among non-coppiced individuals (df = 1, F = 65.248, p< 0.001) (Appendix 9).

Regression analysis of *O. lanceolata* morphological traits revealed a positive coefficient for stem diameter (R = 0.2770) on height; and a negative coefficient (R = - 0.5062) for coppicing on the species' height. The species showed a strong significant relationship between height and stem diameter (R^2 = 0.7939, y = 0.4185x, p< 0.001). Most individuals had their height between 0.5 - 3.5m and the stem diameter was 1 - 9cm (Appendix 8). However, the regression analysis between height and stem diameter of coppiced stems of *O. lanceolata* revealed a weak negative relationship (R^2 = 0.0705, y = -0.2194x) as indicted in Figure 6.5.

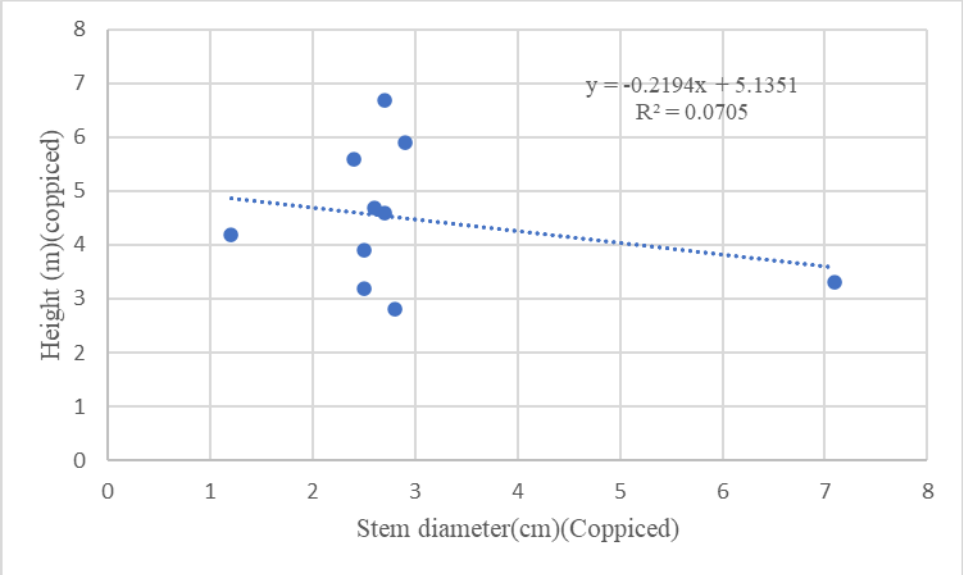


Figure 6.5 Relationship between height of coppiced stems and stem diameter of O. lanceolata

The regression analysis between stem diameter and height of non-coppiced individuals revealed a weak and negligible relationship (Figure 6.6).

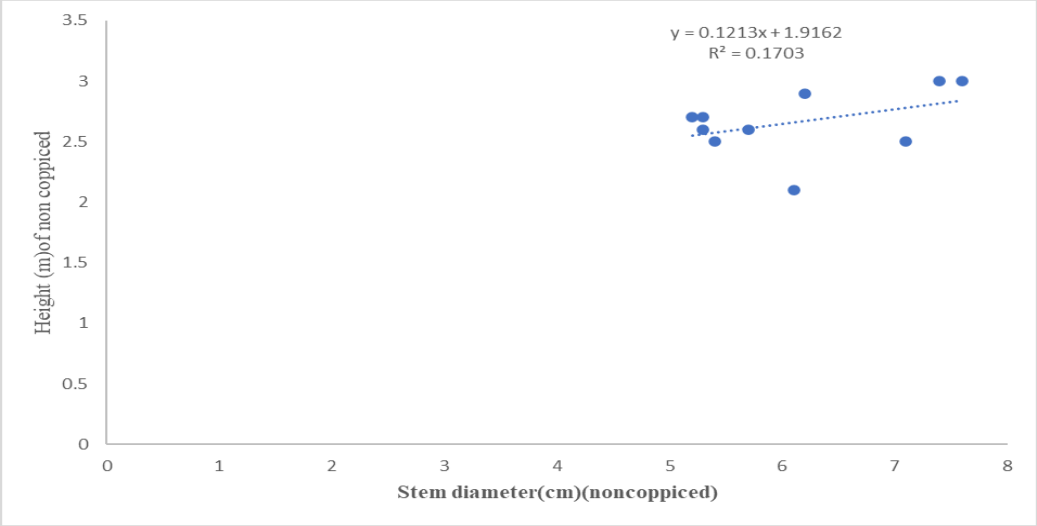


Figure 6.6 Relationship between stem diameter and height of non-coppiced stems of O. lanceolata

The CCA results indicate that the species crown cover, height, life stages and stem diameter are closely associated. Also, natural spacing increases with decreasing elevation and the number of stems is associated with high altitudes. Further, elevation favors increase in the number of stems and a decrease in the species natural spacing, crown size and stem diameter (Fig. 6.7).

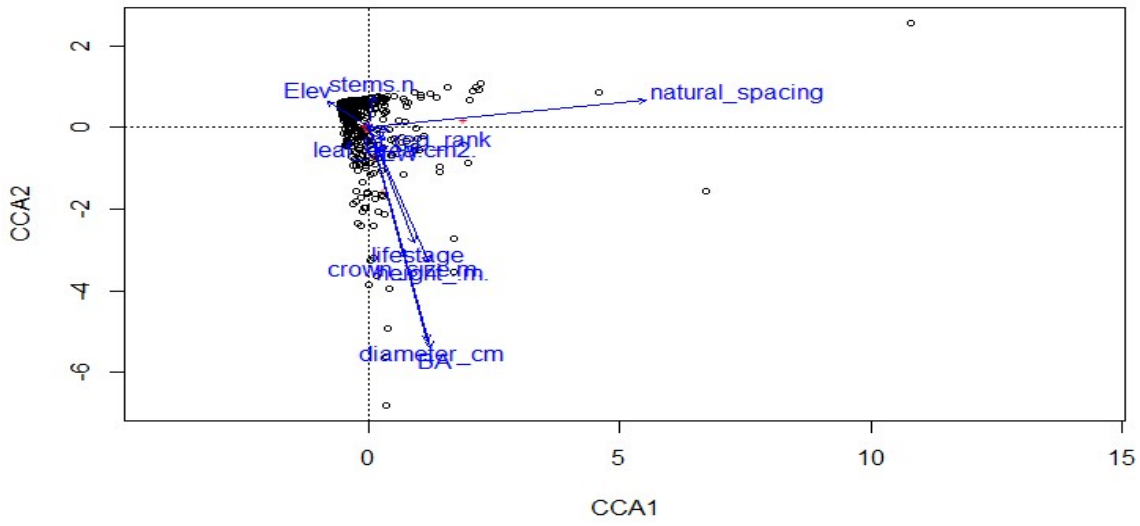


Figure 6.7: CCA results for O. lanceolata morphology across altitudes. (Dots are sampling points).

6.4 Discussion

6.4.1 Host composition

Osyris lanceolata has a diverse composition of hosts, and associated plant species that include trees, shrubs-small trees, typical shrubs, succulents and climbers. The highly clustered hosts belong to family Anacardiaceae, Ebenaceae, Celestraceae, Combretaceae, and Mimosaceae (Table 6.1). These hosts occur in well drained calcareous soils within close proximity to *O. lanceolata* (90 - 105cm) and are capable of extracting calcium from the soil. The hosts' affinity to soil calcium reinforces its role in supporting the species' survival and hence influencing its distribution. The presence of highly clustered and associated species to *O. lanceolata* indicates the species' preference to particular hosts for survival (host preference) and more species associated with the distribution of *O. lanceolata*. Such preferred hosts include; *Euclea racemosa*,

Rhus natalensis krauss *Maytenus senegalensis*, *Ozorea insignis* and *Terminalia browni* (Figure 6.1).

These findings concur with observations made by Gathara et al., (2014) which indicated *Rhus natalensis* to exhibit higher predictive potential for *O. lanceolata* in highland and lowland forests. In contrast, the findings demonstrate that *Maytenus senegalensis*, *Euclea racemosa*, *Terminalia brownii* and *Ozorea insignis* also have capacity to predict *O. lanceolata* availability in addition to *Rhus natalensis* in the semi-arid ecosystems. The findings also confirm earlier claims that most hemiparasites are generalists, though they survive better in presence of specific hosts than others (Sandner et al., 2022). Thus *O. lanceolata* would have specific preference to some hosts amidst a wide range of host species (Sandner et al., 2022). In this case the specific species are the highly clustered hosts. However, these findings contradict the claims of Mwangi et al., (2023) who indicated that *O. lanceolata* frequently parasitises members of family Fabaceae. However, the Fabaceae members were not highly clustered to *O. lanceolata* and exhibited lower frequencies throughout the sampled populations.

The Sorensen indexes revealed a high level of dissimilarity in host and associate composition across *O. lanceolata* sites. This implies that the species has a diversity of associate species that characterise its distribution. The Sorenson index for hosts revealed high similarities across the sites and this characteristic to host specificity of *O. lanceolata*. This further indicates the high adaptive potential of *O. lanceolata* to different ecosystems which indicates the species having an extensive geographical distribution and survival across ecosystems in Africa, Asia, Europe and Socotra Islands (Mugula et al., 2021). Most hosts had strategic contrasting physiognomy to that

of *O. lanceolata* suggesting the quest for similar survival nutrients and conditions between *O. lanceolata* and its host species.

6.4.2 Host proximity to *O. lanceolata* in Karamoja region

The findings indicate that most clustered hosts had similar patterns in proximity to *O. lanceolata*. For instance, *Rhus natalensis* was clustered to *O. lanceolata* at 109.31cm, *Euclea racemosa* (109.9cm), *Maytenus senegalensis* (119.9cm), *Ozorea insignis* (108.9cm) and *Terminalia brownii* (95.9cm). However, the less clustered hosts had wide variations in proximity levels to *O. lanceolata* (16cm-145cm) than clustered host species. The level of proximity between plant-plant intervals is a survival strategy that has important ecological role of ensuring easy access and exchange of nutrients between interacting plants. Host plant species support hemi parasitic plants through exchange of nutrients especially between typical tree hosts and *O. lanceolata*, this survival connection may appear different with climbers, and succulent hosts. The analysis of variations in distance intervals between host species and parasitic plants reveals interesting patterns especially for the highly clustered host species with *O. lanceolata*, and those that are not closely clustered.

Some studies suggest that plant-plant functional distances within forest ecosystems are driven more by environmental factors than genetic relatedness (Villellas et al., 2020). In another study by Trautz et al., 2017, plant performance in terms of growth, fecundity and survival is linked to access for space and resources. The same performance at community level is controlled by limiting resources, and thus plant resource availability is based on how plants are patterned and this is mediated by competitive plant-plant interactions. Also, spacing has the ability to modify the strength of the above and below-ground plant -plant interactions (Trautz et al., 2017).

6.4.3 Effect of altitude on *O. lanceolata* morphology in Uganda

The study findings indicated significant variations in morphological structures (height, leaf size, crown size, number of stems, stem diameter, and natural spacing) across altitudes. This implies that altitude has a significant influence on the species' morphology. The earlier studies also found different altitudes to provide a wide range of conditions that influence the growth patterns and survival of plant species (Chen et al., 2014; Cirimwami et al., 2019; Anic et al., 2010; Cousins et al., 2013; Amundrud, 2020). At lower altitudes most trees have higher stem heights because they tend to grow vertically to capture more light, while at higher altitudes tree height will reduce to allow more horizontal or radial growth or secondary growth which leads to increase in girth and subsequent formation of more wood (Peters et al., 2021; Negi et al., 2024). However, this study revealed specific morphological structures of *O. lanceolata* that are influenced by altitude. The findings also confirm *O. lanceolata* to exhibit phenotypic plasticity along an altitude gradient. This is shown in form of leaf size, stem height, stem diameter, and crown cover. For instance, lower altitudes favored bigger crown covers of *O. lanceolata* than higher altitudes that had smaller crown covers. Similarly, the natural spacing between individuals of *O. lanceolata* reduced with increasing altitude. Other morphological structures that decreased with altitude include height, number of stems, and stem diameter.

This study also found evidence of plasticity in morphological traits in response to altitude gradients. Plant species can adapt to climate change through phenotypic plasticity or local adaptation in their natural range or habitats (Geange et al., 2017). A previous study by Andiego et al., (2022) found *O. lanceolata* to exhibit morphological variations in different populations in

Kenya. Also, the existence of two distinct and most frequent patterns in leaf length and width of *O. lanceolata* across altitude gradients suggests the existence of more species or synonyms of *O. lanceolata* in Karamoja. Secondly, *O. lanceolata* could be exhibiting environmentally induced leaf plasticity (Fritz et al., 2018) as an ecological and evolutionary strategy for adaptation in the semi-arid environment due to global climatic changes (Guerin et al., 2012). In addition, variations in leaf length and width of *O. lanceolata* is also locally attributed to variation in the sex identity (Fay et al., 2010) of the species.

Locally, the longer leaf length of 8cm and width of 4cm (Table 5) of *O. lanceolata* is attributed to the male plant while the smaller leaf length of 4cm and width of 2cm is attributed to the female plant in Karamoja (*OJ. Oziri, pers. comm*) and future studies can help to ascertain such claims to enhance sex identification of the species in the field. Understanding the effect of altitude on morphological structures is necessary to identify suitable environmental gradients that enhance species survival and this is useful in planning suitable sites for in-situ and ex-situ conservation approaches (Nkulu et al., 2022). The significant variations between altitude and *O. lanceolata* morphology shows the role of elevation in shaping the modification of plant structures for adaptation to environmental gradients. It is thus an indicator of plant morphological plasticity to survive under environmental gradients.

6.4.4 The effect of coppicing on height and stem diameter of *O. lanceolata*

The findings suggested coppicing and altitude to have a positive combined effect on the stem diameter of *O. lanceolata*. This is because coppicing only affected the size of *O. lanceolata* at higher altitudes but had no effect at lower altitudes. Thus, coppicing and altitude have a significant combined interaction effect on the stem size of *O. lanceolata*. Further, this implies that the species exploitation through coppicing affects its increase in secondary growth at higher altitudes. The findings build on earlier growth theories that show altitude to have a positive effect on secondary growth as opposed to primary growth of plant species (Bakhtiari et al., 2019). The study findings provide further insights about the effect of environmental gradients and coppicing having significant combined interaction effects on the stem size of *O. lanceolata.* This is a new insight which has never been investigated on the same species. Thus, the study opens new frontiers in understanding the combined effects between environmental gradients, and species management practices on species biology to enhance appropriate strategies for conservation.

6.5 Conclusion

Within the semi-arid ecosystems of Karamoja region in Uganda, *O. lanceolata* was highly clustered with *Euclea racemosa* murray, *Rhus natalensis* krauss, *Maytenus senegalensis*, *Ozorea insignis*, *Terminalia browni, Jasminium dichotomum*, and *Lannea barteri* which could be more preferred for its distribution and survival. The species exhibits morphological plasticity which might be a strategy for adaptation in the semi-arid environments. Further studies should establish the correlation between the species' leaf morphology and genetic identity, sex identity, or ecological adaptation. Also, further investigation of other combined interaction effects of environmental gradients and anthropogenic disturbances on the species morphology, and genetic adaptation potential will unravel new management approaches to responsible conservation of

useful species in the semi-arid ecosystems. Further investigations to establish the driving force behind variations in host proximity levels to *O. lanceolata* should be undertaken. Further studies should establish how coppicing affects the growth morphology of the species in a wider range of habitats.

CHAPTER SEVEN

DISTRIBUTION DRIVERS AND HABITAT CHARACTERISTICS OF *OSYRIS LANCEOLATA* IN KARAMOJA SUB-REGION, UGANDA

7.1 Introduction

Osyris lanceolata (Santalaceae) survives in a range of habitats including unique arid and semi-arid habitats, particularly on rocky and stony soils (Mwangi'ngo et al., 2005; Kamondo et al., 2007; Kokwaro, 2009; CITES, 2013; Teixeira, 2016). It grows naturally in clumped communities along water galleys and is closely associated with *Rhus natalensis* as a suitable host (Mwangi'ngo et al., 2005; Gathara et al., 2014; Mugula et al., press). Since *O. lanceolata* is a hemi parasitic plant, its edaphic requirements and those of the hosts are related (Mwangi'ngo et al., 2005). The understanding of distribution drivers and habitat characteristics for the survival of *O. lanceolata* is a continuous debate among researchers to-date. Previous theories to explain the factors that influence the distribution of parasitic plants have put more emphasis on mistletoes (Aerial hemiparasites) than root hemiparasites (Dean et al., 1994; Watson et al., 2007; Jiang et al., 2008; Watson, 2009; Těšitel et al., 2010; Scalon and Wright, 2015; Dueholm et al., 2017).

In the past studies, mistletoes distribution was said to be influenced by nitrogen levels of an ecosystem (Dean et al., (1994). Rodrigues et al., (2019) also indicated that soil properties especially texture and macro nutrients also regulate the distribution of plant species in tropical habitats. Other studies use the host quality hypothesis (HQH) and the abundant center hypothesis (ACH) to explain the distribution of hemi parasites (Fox, 1997; Pfenninger et al., 2011). HQH highlights water availability and edaphic variables as key factors in driving the spatial

distribution of parasitic plants. For instance, *O. lanceolata* can only grow in habitats with suitable survival conditions for its hosts (Watson et al., 2007; Irving and Cameron, 2009). Again, the ACH suggests that *O. lanceolata* abundance is positively linked to suitable conditions for reproduction and population growth (Watson, 2009) within the hemi-parasitic association framework. Thus, the distribution of *O. lanceolata* seem to be controlled by parasitism, altitude, water and edaphic variables. These factors are further shaped by genetic and anthropogenic factors (Jensen and Mellby, 2012; Hahn et al., (2017). The in-depth understanding of the distribution drivers and suitable habitat characteristics helps to identify better conditions for the species survival within the habitat in order to plan better strategies for the species management including establishment of plantations/woodlots for commercial purposes.

Edaphic variables are measurable soil parameters, that determine the nature and function of a particular type of soil. The converging conclusion from several studies tend to emphasise the fact that a combination of soil variables usually influences the growth and distribution of a particular species (Rodrigues et al., 2019; Chitiki, 2020). In some studies, *O. lanceolata* was observed to have an inconsistent relationship with edaphic variables especially in humid highland and dry lowland forest ecosystems (Gathara et al., 2014). However, the species had a significant correlation with soil nitrogen in the humid highland ecosystems (Gathara et al., 2014) which underpin the role of nitrogen in driving *O. lanceolata* distribution.

To understand how edaphic drivers influence the distribution of a species, it is necessary to consider the species distribution and abundance as a linear function of soil nutrients and habitat characteristics interacting with biotic and abiotic factors to support the species survival in the

habitat (Scalon and Wright, 2015; Rodrigues et al., 2019). Using this approach, the analysis of plant-soil relationship, has been enhanced by the application of ordination techniques (Oksanen, et al., 2016; Flesch, 2017). Such techniques have the ability to detect variation patterns in species data as explained by environmental variables (ter Braak and Verdonschot, 1995).

Therefore, with reference to *O. lanceolata* distribution, suitable sites that support the species survival should have key nutrients to facilitate plant growth. Secondly, because edaphic variables are unequally distributed within the habitats, some variables may exert more influence on the species' survival than other variables (Zhang et al., 2018). To demonstrate this observation, analysis of soil nutrient levels between areas where *O. lanceolata* survives and those where *O. lanceolata* is absent can help to reveal key nutrients influencing the species distribution. Away from hemiparasites, soil nutrients also influence the distribution of non-hemiparasites (Pfenninger et al., 2011; Chen et al., 2014; Cirimwami et al., 2019; Anic et al., 2010; Cousins et al., 2014; Rodrigues et al., 2019; Amundrud, 2020). For the root hemi parasites, some studies have linked their distribution to host quality and water availability (Fox, 1997; Watson et al., 2007; Irving and Cameron 2009; Watson, 2009). This implies that host survival requirements strongly influence the survival of root hemiparasites. Thus, the distribution of *O. lanceolata* and its hosts can be considered as a function of edaphic variables interacting with biotic and abiotic factors in the species microhabitat (Scalon and Wright, 2015; Rodrigues et al., 2019). In this context, the species micro-habitat will exhibit different those areas where the species is not surviving (Zhang et al., 2018). Hence, analysis of soil variables within the species micro-habitat and those distant away from the microhabitat provides insight into specific variables that facilitate the species distribution and survival.

Therefore, further studies are necessary to identify edaphic variables, if any, that influence the species survival in dry savanna habitats as a key step towards long-term conservation strategies (Lomolino, 2001; Winterbottom and Eilu, 2006; UIA, 2016; FSSD, 2021, Mugula et al., 2021). This study builds further on a similar approach to identify edaphic variables that influence the distribution of *O. lanceolata* and its hosts and how the species habitat characteristics vary across an altitude gradient in the semi-arid savanna ecosystem. A better understanding of the habitat characteristics where *O. lanceolata* occurs requires a deliberate assessment of physical habitat qualities in relation to the species distribution and abundance. The key features in this assessment include; average ground habitat conditions, extent of habitat-illumination, organic matter content, and ground vegetation cover. Therefore, the key habitat attributes and edaphic variables that influence the distribution of *O. lanceolata* and its hosts requires further investigations. This helps to test whether; soil variables have no influence on the distribution of *O. lanceolata*, or the soil macro nutrients between Osyris and the non-Osyris microhabitats have no differences. To establish whether habitat qualities vary across altitudes, required testing the null (H_o) hypothesis; "No significant differences in habitat qualities between low and high-altitude populations of *O. lanceolata*". The alternative hypothesis (H_A) would be; "Significant differences in the habitat qualities exist between low and high-altitude populations of *O. lanceolata* in Karamoja"

7.2 Materials and Methods

7.2.1 Study area (s)

The study was conducted in eleven populations of *O. lanceolata* in Karamoja sub-region of Uganda. The selected study sites have been described in chapter three.

7.2.2 Sampling

A total of 388 habitats were sampled and the characteristics for each micro-habitat were recorded. The recorded parameters used to describe *O. lanceolata* habitats included: habitat type categorised into three basing on levels of illumination; full light habitats (FLT), medium light (MLT), and dense habitat (DSD). The habitat characteristics were described using the following parameters: litter cover on ground (LCG), exposed soil (ES), animal trails seen on ground (AT), rock at soil surface (RSS), gulley on ground (GLG), dry water courses, water courses on ground (WCG), mammal droppings (MDP), fallen fruits decomposing on ground (FFD), termite mounds (TMD), open grass understory (OGU), open shrub understory (OSU), and dense impenetrable climbers (DIC). The sampled habitats and their categories are represented in table 7.1

Table 7.1 sampled sites and habitat categories in Karamoja sub-region

Sites (Populations)	Number of habitats	Habitat Category MSD	FLT	DSD
Akariwon (AKA)	42	35	7	0
Lonyilik (LON)	49	36	13	0
Karengepoche (KAR)	25	23	2	0
Lolupe (LOP)	33	19	14	0
Kopedur (KOP)	52	18	32	2
Lotemwoyes (LOT)	20	8	12	0
Cheporon (CHP)	24	21	3	0
Ngaram (NGA)	19	18	1	0
Ruwotokech (RUW)	71	45	26	0
Korenyang (KOR)	28	28	0	0
Kangisa (KAN)	17	15	2	0

MSD = Moderate illuminated habitat; FLT = Full light Habitat; DSD = Dense shed habitat

7.2.3 Edaphic variables

One hundred twelve (112) soil samples were randomly collected from eleven sites in Moroto, Nakapiripirit and Amudat districts of Karamoja, sub-region (Table 7.2). Two locations were considered for sampling at each sampling point: one location within the 5m radius of *O. lanceolata* (*Osyris* samples) and another location at 10m away from *O. lanceolata* (control samples or non-*Osyris* samples). Using a soil auger, the samples were collected at two depths to understand how nutrients vary with depths between *Osyris* and non-*Osyris* samples. Topsoil at 0 - 20 cm, while bottom soil at 20 - 40cm (Gathara et al., 2014). All samples were packed in tight black polythene bags and labeled using non-erasable ink on a masking tape to indicate the study site, sampling point, and sample category. The labeled samples were temporarily stored under room temperature at NARO-Nabuin Zonal Agricultural Research and Development Institute (ZARDI) in Karamoja and later transferred to Makerere University for analysis. Before analysis, all samples were air-dried at room temperature, ground, and sieved through a 2mm sieve. Samples were analyzed for soil pH, measured on a 1:2.5 soil in distilled water suspension using a pH meter. Soil texture was assessed by the bouyoucos or hydrometer method. Exchangeable cations (K^+ Na^+ and Ca^{2+}) were extracted with neutral ammonium acetate solution and then determined directly from emissions measured by a flame photometer. The nitrogen content (N) was determined by the Kjeldahl method, and phosphorus (P) was measured by Bray 1 method and determined using a spectrophotometer (JENWAR 6405UV/vis) (Olsen et al., 1982; Okalebo et al., 2002). Organic matter was determined by the oxidation method (Olsen and Summers, 1982).

Table 7.2 sampled sites in Karamoja sub-region Uganda and number of soil samples collected

Sites (Populations)	District	Number of samples
Akariwon (AKA)	Moroto	18
Lonyilik (LON)	Moroto	08
Karengepoche (KAR)	Amudat	04
Lolupe (LOP)	Nakapiripirit	02
Kopedur (KOP)	Nakapiripirit	12
Lotemwoyes (LOT)	Nakapiripirit	09
Cheporon (CHP)	Amudat	05
Ngaram (NGA)	Amudat	12
Ruwotokech (RUW)	Amudat	17
Korenyang (KOR)	Amudat	08
Kangisa (KAN)	Amudat	17

7.2.4 Data analysis

The non-multidimensional scaling analysis (NMDS) was used to illustrate the difference between soil samples within the species microhabitats and those further away from the microhabitats. A plot of NMDS values of the soil was used to classify the edaphic variables according to their relationship with the distribution of *O. lanceolata* and its hosts. Further, the test for significance in differences between *Osyris* samples and non-*Osyris* samples was done by ANOVA. The canonical correspondence analysis (CCA) was applied at 999 permutations to explain the relationships between the species presence and edaphic variables. All edaphic data was condensed and transformed by detrended correspondence analysis (DCA) before the application of the CCA on species-transformed edaphic variables to explain the relationships between the species distribution, edaphic variables, and altitude (ter Braak and Verdonschot, 1995; Carleton, 1984; Anderson and Willis, 2003). The CCA was implemented by the vegan package in R version.4.1.2 using recommended steps (ter Braak and Verdonschot, 1995).

The multivariate regression modeling (MRM) was used to predict and explain how *O. lanceolata* distribution and abundance are related and edaphic variables influencing the relationships. In this model, the response or predictor variable was *O. lanceolata* density (Osd) per site/population

against edaphic variables (pH, electroconductivity (EC), Na^+, Ca^{2+}, K^+, N, P, and organic matter), as independent variables. Further, this model was expected to be significant because the spatial distribution of hemiparasites is influenced by the nutrient status of the soils (Dean et al., 1994).

To understand habitat conditions for *O. lanceolata*, the relative frequencies of habitat characteristics were assessed to identify the most habitat conditions associated to *O. lanceolata* distribution and survival in the habitats. The categorical data on habitat characteristics was analysed using the chi-square test, performed in R, with the command function "chisq.test ()" at 0.05 level of significance. This was done to establish whether habitat characteristics differ significantly between sampled sites across altitudes. The null (H_o) hypothesis to be rejected was; No significant difference exists in habitat qualities between low and high-altitude populations of *O. lanceolata*. The alternative hypothesis (H_A) was: There are significant differences in the habitat qualities between low and high-altitude populations in Karamoja. Therefore, the chi-square was calculated using the formular:

$X^2 = \sum(O_i - E_i)^2/E_i$, where, O_i=observed value (actual value) and E_i=expected value.

Rejection of the null hypothesis and upholding of the alternative hypothesis (Ho) was done when the calculated probability (p-value) was less than the set level of significance (≤ 0.05). The alternative hypothesis was rejected if the calculated p-value (probability) exceeded the set level of significance (≥ 0.05). This principle was applied to the interpretation of all chi-square tests in this study.

7.3 Results

7.3.1 Edaphic distribution drivers of *O. lanceolata*

Some soil macro nutrient levels varied significantly at 0.005 level of significance across the sampled populations and treatments (Table 7.3)

Table 7.3: Mean nutrient levels within sample treatments in Karamoja

Edaphic variables	Non-*Osyris* samples (>10m away)	*Osyris* samples (within 5m radius)	r^2	*p values*
Organic matter content (OM) (%)	0.38 - 4.68	0.493 - 8.621	0.0078	0.842
Nitrogen content(N) (%)	0.02 - 0.35	0.023 - 0.56	0.2723	0.005**
Sodium (Na^+) (cmols/kg)	0.04 - 1.33	0.048 - 0.909	0.7976	0.001***
Phosphorus (PO_4^{-3}) (ppm)	0.26 - 234.86	0.426 - 255.75	0.6920	0.001***
Calcium (Ca^{2+}) (cmols/kg)	2.64 - 34.80	4.560 - 43.92	0.5223	0.001***
Potassium(K^+) (cmols/kg)	0.17 - 6.12	0.141 - 2.12	0.1012	0.141
pH	5.30 - 7.48	2.960 - 7.82	0.0609	0.310
Salinity (EC) (S m^{-1})	0.39 – 42	0.204 – 461	0.1216	0.101

Significance. codes: *0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

The soil variables that were significantly different between *Osyris* and non-*Osyris* microhabitats include; nitrogen (N), Sodium (Na^+), Phosphorus (PO_4^{-3}) and calcium (Ca^{2+}). The ordination analysis of soil variables (NMDS1) against the category of samples (Osyris and non-Osyris samples) (NMDS2) indicated a significant difference in variables between the Osyris and non-Osyris microhabitats ($p<0.001$). The *Osyris* samples (1 & 2), had distinct nutrient levels from non-Osyris samples. All significant variables, (N, Ca^{2+}, PO_4^{-3} and Na^+) were clustered towards *Osyris* microhabitats while non-significantly different variables (K^+, pH, salinity and organic matter) aggregated towards non-Osyris microhabitats (Figure 7.1).

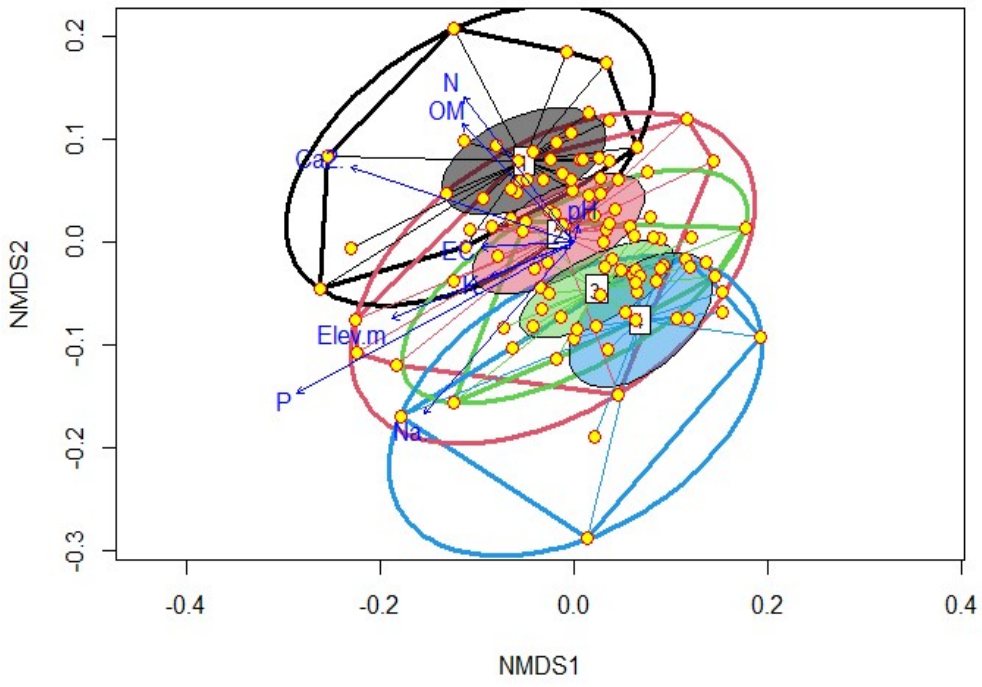


Figure 7.1: NMDS plot results indicating the relationship between Osyris and non-Osyris microhabitats: The sample categories are represented by figures in polygons as follows: 1= Osyris- microhabitat at a depth of 0-20cm; 2 = Osyris-microhabitat at 20-40cm depth; 3 = non-Osyris microhabitat at a depth of 0-20cm and 4 = non-Osyris microhabitat at a depth of 20-40cm). Colored dots represent the sampled sites

Also, the constrained ordination undertaken under the reduced model with 999 permutations to test the goodness of fit for the model investigating differences between all edaphic variables was significant (df = 9, Chi-square = 0.048717, F = 35.639, $p<0.001$). There was a clear indication of a relationship between *O. lanceolata* presence and edaphic variables. The four significantly different variables (N, Ca^{2+}, PO_4^{-3} and Na^{+}) were clustered in component 2 which shows their contribution and influence to the presence/distribution of *O. lanceolata* (Figure 7.1). The potassium (K^{+}) and organic matter (OM) were closely related together than other variables. The significant variables correlated with altitude while Nitrogen (N) correlated with salinity and pH levels of the soils (Figure 7.2).

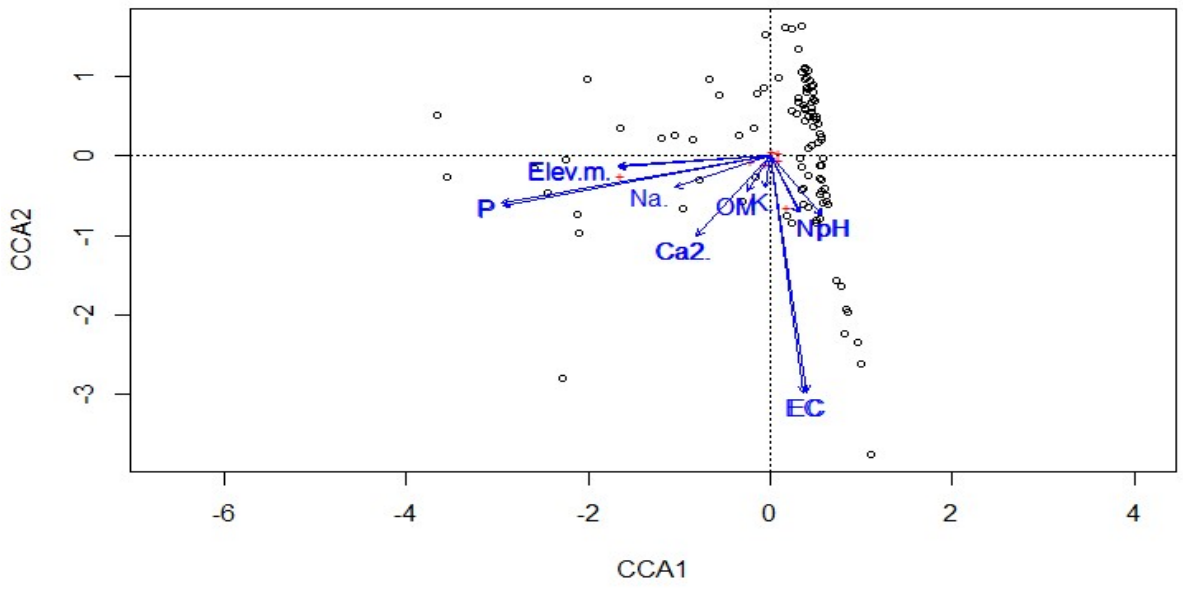


Figure 7.2: CCA Ordination results indicating a relationship between O. lanceolata distribution and edaphic variables. The round dots represent the sampled points; Ca = Calcium; Na = Sodium = Phosphorus; OM = Organic matter; Elev.m = Elevation/altitude in meters; N = Nitrogen; pH = potential of hydrogen ions; EC = electroconductivity/salinity; and K = potassium). The applied model was significant (Permutation level at 999; F-statistic: 41.051 *on 9 and 30 DF, p-value <0.001).*

The multiple regression interaction model (MRIM) revealed that *O. lanceolata* abundance was significantly influenced by; nitrogen(N) (t = 2.083, p = 0.04907), phosphorus (t = 2.262, p=0.034), potassium(K^+) (t = -3.475, p = 0.00215), and sodium (Na^+) (t = 3.679, p = 0.00131).

Regardless of the differences in edaphic variables between microhabitats, nutrient levels indicated a non-uniform pattern in variation with soil depths across sites (Appendix 4). Some nutrients increased with soil depths while others decreased. The amount of nitrogen and phosphorus generally deteriorated with the soil depth whereas an opposite trend was observed for potassium (K^+), sodium (Na^+), and calcium (Ca^{2+}). Nutrient levels for calcium and sodium

consistently increased with an increase in soil depth in high-altitude zones but decreased in low altitudes (Table 7.4)

Table 7.4: Nutrient levels with soil depths across samples in Osyris sites

Osyris sites &depths	Altitude (m)	OM (%)	N (%)	K^+ (cmols/kg)	P (ppm)	Na^+ (cmols/kg)	Ca^{2+} (cmols/kg)	pH	EC S m^{-1}
KAR (top soil)	1794	2.53	0.21	0.94	97.741[	0.13	11.88	4.87	1.72
KAR (bottom)		1.99	0.21	0.94	10.12	0.18	16.88	6.33	139.83
AKA (top)	1787	4.16	0.19	0.90	217.60	0.66	28.08	6.49	136.8
AKA (bottom)		1.42	0.03	0.94	191.48	0.51	30.72	6.51	110.25
LON (top)	1721	2.55	0.17	1.33	27.84	0.22	20.56	6.86	86.63
LON (bottom)		3.47	0.13	1.24	7.43	0.24	26.30	6.63	146.30
KOP (top)	1416	1.58	0.18	0.67	2.64	0.24	17.46	6.57	134.80
KOP (bottom)		1.51	0.13	0.78	2.64	0.08	17.04	6.59	134.28
LOP (top)	1407	1.25	0.14	0.58	78.77	0.13	21.6	6.36	70.30
LOP (bottom)		6.84	0.07	0.74	3.58	0.05	4.56	6.11	47.40
CHP (top)	1392	0.49	0.19	0.94	15.69	0.05	13.44	6.57	41.20
CHP (bottom)		2.53	0.12	0.14	27.42	0.19	13.68	6.18	51.60
LOT (top)	1382	1.41	0.11	0.93	7.71	0.42	8.52	6.29	67.61
LOT (bottom)		2.09	0.13	0.94	3.28	0.08	14.04	6.72	59.60
RUW (top)	1381	2.41	0.13	0.93	4.16	0.09	13.08	6.59	107.78
RUW (bottom)		1.87	0.16	0.82	20.25	0.32	15.04	6.52	139.25
NGA (top)	1435	2.17	0.11	1.00	34.74	0.11	14.7	6.31	102.73
NGA (bottom)		1.76	0.15	0.94	3.88	0.23	12.96	7.07	172.60
KAN (top)	1327	3.97	0.19	0.92	21.53	0.11	17.52	7.14	201.63
KAN (bottom)		1.51	0.11	0.75	24.66	0.08	9.875	6.54	140.05
KOR (top)	1275	1.10	0.19	0.94	11.13	0.07	10.44	6.29	122.60
KOR (bottom)		1.20	0.18	0.94	5.16	0.06	16.08	6.46	130.25

Note: Populations: Karengepoche (KAR); Akariwon (AKA); Lonyilik (LON); Kopedur (KOP); Lolupe (LOP); Cheporon (CHP); Lotemwoyes (LOT); Ruwotokech (RUW); Ngaram (NGA); Kangisa (KAN), and Korenyang (KOR).

7.3.2 Habitat characteristics of *O. lanceolata* in Uganda

A total of 388 habitats were surveyed across different altitudes. The chi-square test to compare significance of differences in habitat characteristics between low and high-altitude sites revealed no significant differences in habitat characteristics (p-value = 0.1991, df = 4 and $\chi 2 = 6$). There were no significant differences in habitat characteristics between low and high altitudes (p-value = 0.1991, df = 4 and $\chi 2 = 6$) and the most distinct habitat attributes across altitudes were: rocky surfaces (RSS) (79.8%), animal trails (AT) (75.2%), moderate illumination (MSD) (70%), gullies (66.8%) and mammal dropping (62.1%). High altitude habitats were not characterised by any of the attributes used to describe the habitats.

The low altitude habitats were characterised by dense impenetrable climbers (DCU), open shrub understory (OSU), termite mounds (MD), fallen wood branches (FWB), water courses (WCW), fallen decomposed fruits (FFD), full light illumination (FLT), litter cover on ground (LCG) and dry water courses (DWC). The dense shed or limited illumination (DSD) attribute was non-existent in high altitudes and only observed in 2.2% of the low altitude habitats. The more moderately illuminated habitats were common in low altitudes (70%) than high altitude areas (25%). The full light habitats were more observed in low altitudes (46%) than high altitudes (5%). Other distinct habitat characteristics observed include: rocky surfaces (77.6%) in low altitudes and less rocky surfaces (37.3%) in higher altitudes. More termite mounds (15.53%) in low altitudes than in the high-altitude areas (3%) (Figure 7.3).

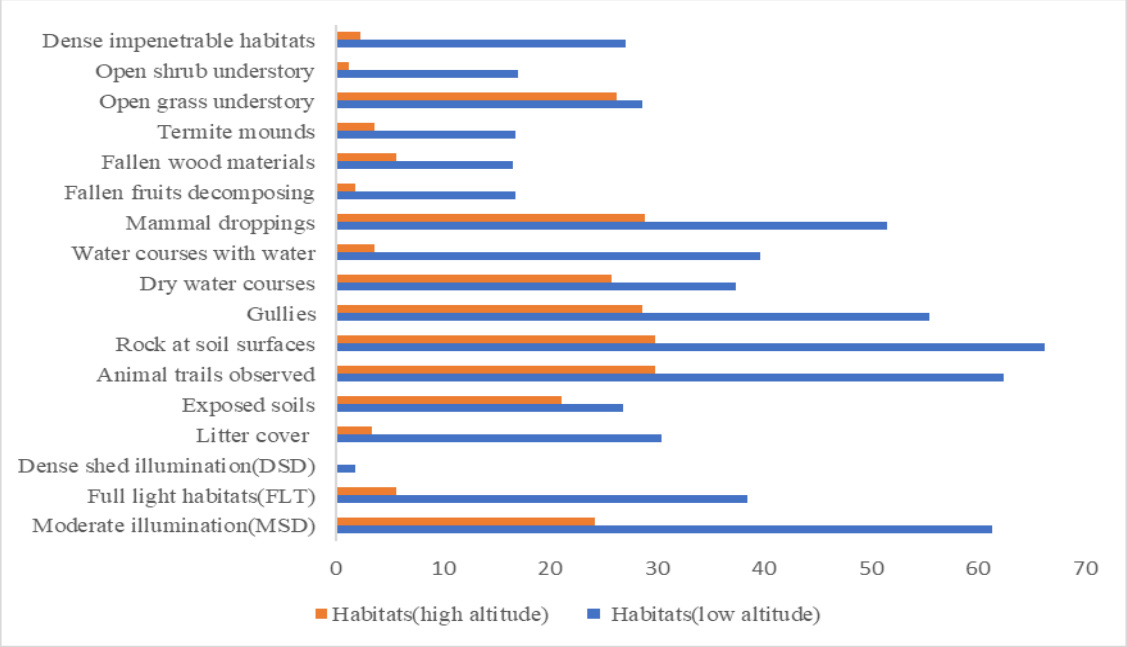


Figure 7.3: Characteristics of habitats for O. lanceolata in Karamoja

7.4 Discussion

7.4.1 Distribution drivers of *O. lanceolata* in Uganda

The findings revealed that calcium, sodium, phosphorus, and potassium were significantly different between Osyris and non-Osyris microhabitats. The multivariate regression modelling predicted similar edaphic variables to significantly influence the species distribution and density. Thus, these results suggest that the distribution and abundance of *O. lanceolata* is potentially driven by a combination of macro nutrients in the soils. The study agrees with the findings of Dean et al., 1994; Těšitel et al., 2010 which emphasised similar nutrients to provide suitable conditions for the survival of *O. lanceolata* in natural habitats. The occurrence of *O. lanceolata* to hosts that survive near hard rocky habitats suggests the need for Ca^{2+} to enhance the species' survival through the acquisition of desired nutrients (Dean et al., 1994; Watson et al., 2007; Těšitel et al., 2010; Dueholm et al., 2017) in line with the study findings. This is because calcium was significantly different between Osyris and non-Osyris sites, which validates its influence in driving the species distribution. Similarly, sodium (Na^{+}), phosphorus, calcium (Ca^{2+}) and nitrogen influenced the distribution of the species as explained by the CCA ordination results. The results concur with other studies that found soil nitrogen, and phosphorus to be critical variables in determining spatial floral composition (Kirkpatrick and Bridle, 1998; Cavieres et al., 2000; Gu¨sewell, 2004; Anic et al., 2010). However, these variables act in concert with environmental gradients, climate, gene flow, and host quality to drive the distribution of root hemiparasites (Watson et al., 2007; Watson, 2009).

7.4.2 Habitat qualities influencing distribution of *O. lanceolata* in Karamoja

The study findings indicate that *O. lanceolata* is a moderately light loving, and drought adapted species with affinity for macro soil nutrients particularly calcium, phosphorus, nitrogen and sodium. The lack of significant differences in habitat characteristics across altitudes reflects the high potential of *O. lanceolata* to survive in a wide range of habitats as a generalist species. The absence of *O. lanceolata* individuals in dense shed or poorly illuminated habitats across altitudes suggest the species' preference to well illuminated habitats. The presence of most distinct habitat attributes provides an insight on desirable habitat conditions for the survival of *O. lanceolata*. The most preferred habitat characteristics for *O. lanceolata* survival were moderate illumination (MSD), high organic matter content (AT), rocky surfaces (RSS), gullies and mammal dropping (MDP). The presence of gullies, water courses, rocky surfaces are in line with the habitat quality hypothesis (HQH) which links the distribution of root hemi-parasites to host quality in terms of nutrients, and water availability (Fox, 1997; Watson et al., 2007; Irving and Cameron, 2009; Watson, 2009). The presence of rocky surfaces, mammals' droppings, fallen decomposed fruit (organic matter) (FFD), litter cover (LCG), wood branches (FWB), and termite mounds (MD) also correlates with the influence of organic matter in the distribution and survival of *O. lanceolata*, while availability of organic matter in the habitats (animal droppings) points to its role in enhancing the species survival in the semi-arid ecosystem. These findings are in line with the work of Dean et al., (1994), that established the abundance of parasitic plants to be significantly linked to levels of nitrogen requirements for the plants and the total nutrient status within an ecosystem.

The presence of open grass understory and shrub understory habitats underpin the role of moderate habitat illumination in influencing the survival of *O. lanceolata*. However, this observation contradicts earlier studies that reported only bushlands (shrub understory habitats) as the most preferred habitats for *O. lanceolata* (Nyingi et al., (2020). In other findings, Nyingi et al., (2020) reported the least preferred habitat conditions for *O. lanceolata* to include to be rocky surfaces and hilly areas especially the steep slopes, gentle slopes and flat slopes. Another study by Bhandari and Zhang (2019) reported altitude to influence plant species richness and biomass than soil nutrients. The findings reveal different insights on the role of habitat characteristics in determining the species distribution and survival that require immediate attention while designing *O. lanceolata* conservation programs. It is therefore important to understand the influence of similar habitat characteristics on the distribution and survival of *O. lanceolata* in other ecosystems across the species' range.

7.5 Conclusion

This study investigated the distribution drivers and habitat characteristics of *O. lanceolata* in a semi-arid ecosystem. These findings suggest that *O. lanceolata* is a moderate light loving, and drought adapted species with affinity for calcium, phosphorus, water, nitrogen, sodium and potassium nutrients. Also, a combination of soil macro nutrients (Ca^{2+}, N, P and Na^{+}) significantly influence the species distribution irrespective of the altitude levels. Restoration, domestication and other conservation efforts should consider moderately illuminated habitats as suitable for the species survival across altitudes. Therefore, ex-situ and in-situ conservation programs for *O. lanceolata* should consider habitat characteristics and soil nutrients as key benchmarks in identifying suitable sites for restoration, and commercial propagation of *O.*

lanceolata across altitudes. Further investigations should focus on analysis of the species biotic conditions for survival and also explore the role of micro-nutrient variables on the species distribution, genetic and biochemical composition such as oil yield and quality to inform appropriate strategies for restoration, breeding, propagation and domestication.

CHAPTER EIGHT

GENETIC DIVERSITY AND STRUCTURE OF *OSYRIS LANCEOLATA* IN UGANDA AND KENYA USING MICROSATELLITES

8.1 Introduction

The East African sandalwood (*Osyris lanceolata*) is a valuable commercial species because of its aromatic wood and essential oils used in perfumery and pharmaceutical industries (CITES, 2013; Texeira et al., 2016). The species occurs across different habitats in Africa, Asia, Europe and Socotra Islands with unknown origin (Palmer and Pitman, 1972; Teklehaimanot et al., 2004; Texeira et al., 2016). It is locally used as medicine for candidiasis, malaria, diarrhea, chest pain and fever in Africa (Njoroge and Bussmann, 2006; Ochanda, 2009; Orwa et al., 2009; Masevhe et al., 2015). The oil has chemo-preventive properties for managing eruptive skin, inflammatory diseases, and urinary infections. The species bark and root have potential in phytoremediation (Xiaohai et al., 2008), and they provide a red dye for skin tanning.

The use of *O. lanceolata* oils in the perfumery industries increased after a decline in the Indian (*Santalum alba*) and Australian (*Santalum spicutum*) sandalwood populations in the 1990's (Mbuya et al., 1994) which shifted pressure to *O. lanceolata* populations in East Africa leading to over-exploitation of the species (CITES, 2013). Several reports of illegal trade and destructive harvesting (whole plant uprooting) of the species have been reported in African countries with no success in conservation strategies to-date (CITES, 2013; Muhoozi, 2013; Tajuba, 2015; USAID, 2015; Otieno et al., 2016; Bunei, 2017).

Osyris lanceolata populations were already being affected by multiple stressors such as habitat loss through urbanization and deforestation, and uncontrolled harvesting for multiple purposes like charcoal production and construction material (Mugula et al., press). Thus, the increasing demand for essential oils added extra pressure to *O. lanceolata* populations in Africa threatening its survival (Page et al., 2012; CITES, 2013). Besides, certain aspects of *O. lanceolata* biology may contribute to a faster decline of this species. For instance, seed germination success is low in East Africa (Mwangi'ngo et al., 2007; Mugula et al., press). Moreover, *O. lanceolata* is a hemi parasitic plant, therefore the survival of their populations is also affected by its host where conservation status is threatened by similar stressors such as non-regulated wood harvesting (Mwaura et al., 2020; Mugula et al., 2021). The species conservation efforts have not been successful to-date because of limited planting materials (seedlings), ineffective propagation techniques, limited provenances, seedbanks and poor understanding of the species ecology and genetics. In fact, *O. lanceolata* is one of the least genetically and ecologically studied species amongst African trees. Even with the current rapid advancement in genetic technology (Kahilainen et al., 2014; Szczeci et al., 2016; Otieno et al., 2016, *O. lanceolata* has limited genetic studies particularly in East Africa (Mugula et al., 2021).

The destructive harvesting of *O. lanceolata* increases the risk of extreme variation in the species sex - ratios, reduction in population size, alteration in dispersion, density, and distribution patterns. These can ultimately contribute to the reduction of populations genetic diversity which has long-term consequences on species survival and resilience to changing environmental conditions (Jensen and Mellby, 2012; Curto et al., 2015; Ellegren & Galtier, 2016). The diversity of alleles and genotypes provide a basis for species survival, evolution and genetic adaptive

capacity to change, hence making populations more resilient to environmental changes (Govindaraj et al., 2015; Ellegren & Galtier, 2016; Fuentes-pardo et al., 2017). Therefore, decline in species genetic diversity weakens the species evolutionary potential, resilience, and adaptation hence pre-disposing the species to higher risk of extinction (Farwig et al., 2008; Alfaro et al., 2014; Graudal et al., 2014). This loss in genetic diversity needs to be quantified to develop efficient conservation measures yet the genetic characterization of *O. lanceolata* populations continue to be lacking in Uganda and Kenya to-date.

High-throughput sequencing technologies enhanced the development of molecular markers like microsatellites that are extremely informative in the assessment of population genetic parameters and therefore evaluate the impact of human activities on evolutionary processes (Castoe et al., 2012; de Barba et al., 2017; Vartia et al., 2016; Fuentes-Pado et al., 2017; Neophytou et al., 2018; Tibihika et al., 2018). Due to their high mutation rate and mostly neutral nature, microsatellite markers are especially informative in retrieving genetic variation patterns within and among populations of the same species and consequently how these are affected by anthropogenic impacts (Curto et al., 2015). The unraveling of the species genetic diversity and structure explains population dynamics and trends in evolutionary processes as a basis for sound conservation strategies (Zong et al., 2015). For instance, analysis of spatial genetic structure detects the distance of gene dispersal and influence of ecosystem disturbances on the non-random distribution of genes leading to inbreeding and loss of genetic diversity in populations (Volis et al., 2016).

It is important to understand the effect of anthropogenic bottlenecks and ecological gradients on the species genetic diversity and structure to enhance conservation strategies. Therefore, genetic data and information is needed to adequately understand what drives the species genetic adaptation potential and develop informed conservation strategies. This study sought to determine whether geographical isolation and altitude gradient influenced the patterns in genetic diversity and structure of *O. lanceolata* between Uganda and Kenya. In order to strengthen the species conservation efforts in Uganda and Kenya, two specific objectives were considered: (i) to characterise the genetic diversity and structure of *O. lanceolata* in Uganda and Kenya, and (ii) to determine whether geographical isolation influence the species genetic structuring between the two countries. This is the first comparative analysis of genetic diversity and structure for *O. lanceolata* across its range in Uganda and Kenya.

8.2 Materials and Methods

8.2.1 Sampling

A total of seven populations, three from Uganda (Karamoja sub-region) and four from Kenya in the rift-valley region were sampled for genetic analysis. The Karamoja populations were; Amudat, Nakapiripirit and Moroto. The Kenyan populations included; Mt. Elgon, Baringo, Laikipia and Mau. Leaf samples were collected from fresh leaves of *O. lanceolata* adult trees, dried on silica gel in paper bags, and stored briefly under room temperature until extraction of genomic DNA. The location of sampled populations of *O. lanceolata* with low and high-altitude clusters in Uganda and Kenya is illustrated in Chapter three, Figure 3.2. The sampling information is also given in Table 8.1.

Table 8.1 Sampling information on the populations of O. lanceolata in Uganda and Kenya

Population	Sample size	Longitude	Latitude	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)
Mt. Elgon (KE)	28	34.81058	1.152700	2007	1280.0	18.50
Baringo (KE)	30	35.78866	0.396067	2040	635.00	25.00
Mau (KE)	30	36.08889	0.607300	2288	1025.0	21.00
Laikipia (KE)	26	36.37124	0.117420	2055	207.27	15.70
Amudat (UG)	60	34.80616	1.484444	1434	154.45	24.99
Moroto (UG)	20	37.71965	2.452242	1754	800.00	20.60
Nakapiripirit (UG)	16	34.71965	1.869604	1401	156.53	25.33

KE= Kenya; UG=Uganda

8.2.2 DNA Isolation, quantification and Fragment analysis

The detailed protocols for extracting genomic DNA, DNA quantification, and multiplexing PCR reactions and DNA fragment analysis are described in chapter three, section 3.3.1-3.3.4 of this thesis.

8.2.3 PCR analysis

Ten primer pairs developed by Otieno et al. (2014) were used to characterise the populations of *O. lanceolata* (Table 8.2). PCR reactions were conducted in a final volume of 5 µL using Qiagen multiplex PCR kit (*Invitrogen, country*), following the manufacturer's guidelines in Veriti^{TM} thermal cycler (Applied Biosystems). The detailed protocol is described in chapter three, section 3.3.5. The primers and their sequences are given (Table 8.2).

Table 8.2: Osyris lanceolata SSR primers and the number of amplified fragments

Primer code	Direction	Primer sequence (5'to 3')	Primer mix	Allele sizes(bp)
KFOL2	F R	AGAATGTCATTTGAAGGCTCGA CCTTTCCTCCGTTCTCCTG	1	178-194
KFOL13	F R	TCCGAGGAACAGGGACTCTT AGCGAAGAACTCATGAGCGAA	1	139-165
KFOL17	F R	CATTGACGAATTGCATCCCGT CGTGAAGTTCAGTGCAAACC	1	178-220
KFOL24	F R	CAACTCGATCGTGCATTGGC TCCGCATATCCATTTGGCCG	2	219-263
KFOL28	F R	ATAAAGGCCCACGAGCTCAG AACATCGCCATGCAGAACAG	2	245-255
KFOL30	F R	CTAAACTGTCAGGGCTTGCT ATACCTTAGCTCCCGTTGCG	2	270-306
KFOL37	F R	TTTCTAGAGCTAACATACCTCTGAA ATGACCTGGGTGCTTTGCTG	3	300-340
KFOL42	F R	AGGTCCTCCTGAGAAT CATAGGGCTGTGATGCGTCA	3	315-337
KFOL47	F R	TTTGATCGTAAATTATAGATGTCCACA CCCTTGCTTGATCTCCAGGTA	3	353-387
KFOL48	F R	GAGTGCATGGAATTATGTGTGCGT TCGCCATGAGAAGGGTTACT	3	369-393

A touchdown thermocycling program used by Otieno et al. (2016), was followed in PCR reactions. This was programmed as follows: initial denaturation at 95^0C for 15 minutes, then 10 cycles at 94^0C for 30 seconds, 57^0C for 90 seconds, and 72^0C for 60 seconds. Annealing starts at 57^0C and decreases by 1^0C for each cycle and elongation at 72^0C for 1 minute for 35 cycles. This was followed by 22 cycles at 94^0C for 30 seconds, 55^0C for 90 seconds. A final extension stage at 60^0C for 30 minutes was implemented. PCR was conducted in a Verit 96 Well Thermocycler (Applied Biosystems, Germany). The amplified PCR products were briefly stored under -20^0C before the DNA fragment analysis.

8.2.4 DNA fragment analysis

The Capillary electrophoresis (CE) was scored against 600 Liz internal size standard using a genetic analyzer (3500, Applied Biosystems, HITACHI, Japan) and used to analyse DNA

fragments. The resulting chromatograms were used to generate allele score bases on amplicon size reported by Otieno et al. (2014), using the expected allele size ranges as reference for quality control (Table 8.2). The SSR primer pairs generating clear peaks in the expected amplification range were used for *O. lanceolata* population analysis and the Gene Mapper 5.0 software (Applied Biosystems, California, USA) captured the genotypic data. Detailed description is provided in general methods, chapter three section 3.3.5

8.2.5 Data analyses

The GeneAlex software, version 6.51b2 (Peakall and Smouse, 2006) was used to assess within and among population genetic diversity parameters including percentage of polymorphic loci; alleles for each polymorphic locus (Na), observed heterozygosity (Ho), expected heterozygosity (He), Fixation index (F) and genetic differentiation. The Gene pop software version 4.7.5 was also used to test for deviations from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium among loci (Maudet et al., 2002; Peakall and Smouse, 2012). Significance of deviations were computed using the Markov chain method (1000 iterations, Mehes et al., 2009). The population structural patterns were modeled using STRUCTURE software, version 4.5.7 (Muriira et al., 2018). The division between one to seven hypothetical populations (K=1 to K=7) were tested and the best K value evaluated by using the deltaK criteria as executed in Structure harvester (Earl and vonHoldt, 2012). The 100,000 generations were run by STRUCTURE after a burning period of 10,000 generations using the default settings. A total of 10 replicates per K value were computed. Results per K values were summarized across replicates using CLUMPAK server (Kopelman et al., 2015).

Measures of genetic differentiation used include; F_{ST}, R_{ST}, Nei's genetic distance, (D), and Nei's genetic identity (I) (Ma et al., 2015). The partitioning of the genetic variation among populations and regions was analysed using analysis of molecular variance (AMOVA) in GeneAlex ver.5.1b2 (Excoffier et al., 1992; Peakall and Smouse, 2012). The null hypothesis was; Ho = No genetic difference among the populations ($F_{ST} = 0$ or $R_{ST} = 0$). The statistical testing for significance of AMOVA was determined by random permutations or shuffling of data at 999 times. The correlation between geographical distance of the population and genetic distance was used to test for the presence of isolation by distance by Pearson's correlation coefficient 'r^2.' The four hundred and sixty-two (462) tests of linkage disequilibrium between pairs of loci in each population were done to detect the existence of any population bottlenecks between Ugandan and Kenyan populations (Appendix 5: Table 13). In addition to Nei's (D) and Nei's (I), the genetic relatedness and structure patterns between the seven populations were visualized using Principal Coordinate Analysis (PCoA).

8.3 Results

8.3.1 SSR markers, allele frequencies and Hardy-Weinberg Equilibrium (HWE) tests

The markers produced 127 alleles with 29.729 (SE = 1.582) as the average number of alleles for each locus. The KFOL17 and KFOL30 primers produced the highest number (22) of alleles while KFOL2 produced 5 alleles. The private alleles constituted about 22% (28 alleles) of the total alleles observed. All populations were highly polymorphic ranging from 80 -100% polymorphic.

Out of the 462 tests for deviation from HWE at each locus in each population, significant deviations were detected in 10 of the tests. Genetic marker KFOL47 showed the highest number of populations (85.71%) with significant deviations from HWE. Marker KFOL37 showed the least number of populations with significant deviations from HWE (28.57%). HWE deviations per marker across all populations may indicate genotyping errors due to null alleles and marker duplication, which was not the case for any of the markers used. In Kenya, Baringo showed 70% of the markers with significant deviations from HWE, followed by Mau and Laikipia with a proportion (40%) of markers showing deviations from HWE. Mt. Elgon had most stable allele frequency with only 10% alleles showing deviations from HWE. Among Ugandan populations, Moroto had (80%) of the markers with significant deviations from HWE, while Nakapiripirit had the least number of markers (30%) with significant deviations (Appendix 1). Generally, the Ugandan populations showed more markers deviating from HWE than Kenyan ones.

8.3.2 Genetic diversity patterns

There were distinct patterns in genetic diversity and structure between populations across environmental gradients in Uganda and Kenya. The patterns in genetic diversity and structure were generally clustered in geographic relation to altitude levels and rainfall. Kenya populations were distributed in higher altitude habitats than Ugandan populations. The average number of effective alleles (Ne) was 3.985 and ranged from 1.455 to 8.954 across the 10 loci among populations in the two countries. The mean fixation index was 0.187 with a range of 0.048 to 0.398. The mean expected heterozygosity was 0.618 while the Shannon information index (I) ranged from 0.478 to 2.340 with a mean of 1.359. The fixation index was fairly high (F = 0.187) (Table 8.5).

The Kenya populations exhibited relatively higher levels of genetic diversity than Ugandan populations for all measures (Table 8.3). The mean number of alleles (Na) for each population in Kenya was 7.471 and ranged from 5.1 to 9.8 alleles across the seven populations. The mean number of alleles for Ugandan populations was 6.7 and ranged from 5.7 to 7.7. The mean effective number of alleles was higher for Kenyan populations (Ne = 4.032) compared to Ugandan populations (Ne = 3.92). The mean genetic diversity (He) of the seven populations was 0.618 and ranged from 0.493 (Amudat) to 0.681(Baringo). In Uganda, Moroto population had higher genetic diversity (He = 0.677) and Amudat with a lower level of genetic diversity (He = 0.493). In Kenya, Baringo had a higher genetic diversity (He = 0.681) and Laikipia with the lowest (He = 0.587). The average fixation index (F) values for Uganda populations were higher (F = 0.2656) than those for Kenyan populations (F = 0.123). The average levels of observed

heterozygosity (Ho) for the seven *O. lanceolata* populations were 0.511, and 0.57 for Kenya and 0.431 for Uganda populations (Table 8.3).

Table 8.3: Mean genetic diversity indices over all loci across populations of O. lanceolata in Kenya and Uganda

Population	Country (Origin)	N	Na	Ne	I	Ho	He	F
Mt. Elgon	Kenya	27.700	8.600	4.256	1.488	0.626	0.663	0.026
Baringo	Kenya	29.800	9.800	4.391	1.600	0.539	0.681	0.207
Mau	Kenya	29.700	8.200	4.671	1.527	0.635	0.670	0.050
Laikipia	Kenya	25.800	5.100	2.808	1.132	0.480	0.587	0.209
Amudat	Uganda	59.600	7.700	3.232	1.118	0.386	0.493	0.264
Moroto	Uganda	19.700	7.200	4.644	1.474	0.417	0.677	0.437
Nakapiripirit	Uganda	15.800	5.700	3.892	1.171	0.491	0.558	0.096
Mean		29.729	7.471	3.985	1.359	0.511	0.618	0.184

N: mean sample Size, Na: mean number of alleles, Ne: mean number of effective alleles, I: Information Index, Ho: Observed Heterozygosity, He: Expected Heterozygosity, and F: Fixation Index

8.3.3 Genetic structure and differentiation

The AMOVA tests showed 91% of the genetic divergence within individuals of *O. lanceolata* and 1% was among the populations (Table 8.4).

Table 8.4: AMOVA results for genetic differentiation among O. lanceolata populations in Kenya and Uganda

Source	Df	SS	MS	Est.var	(%)
Among regions (UG/KE)	1	79834.756	79834.756	281.235	3%
Among populations	5	98506.345	19701.269	77.535	1%
Among individuals	203	3136390.271	15450.198	7552.799	91%
Within individuals	210	72366.000	344.600	344.600	4%
Total	419	3387097.371		8256.168	100%

Df = degree of freedom; SS = sum of squares; MS = mean squares; Est.var; estimated variance component;

However, populations also showed little but significant genetic differentiation (R_{ST} = 0.043, p = 0.001) (Appendix 3). Pairwise comparisons revealed the higher genetic differentiation in allele frequencies between Mt. Elgon and Laikipia (R_{ST} = 0.288), Mt. Elgon and Moroto (R_{ST} = 0.248), and Mt. Elgon and Nakapiripirit (R_{ST} = 0.344). The rest of the population pairs showed little to moderate genetic differentiation (Table 8.5).

Table 8.5: Pairwise populations R_{ST} values for populations in Uganda and Kenya

Populations	Mt. Elgon	Baringo	Mau	Laikipia	Amudat	Moroto	Nakapiripirit
Mt.Elgon	0.000	0.043	0.006	0.288	0.001	0.248	0.344
Baringo	0.029	0.000	0.111	0.358	0.020	0.025	0.021
Mau	0.043	0.013	0.000	0.394	0.001	0.004	0.001
Laikipia	0.009	0.000	0.005	0.000	0.015	0.079	0.044
Amudat	0.078	0.025	0.079	0.044	0.000	0.128	0.051
Moroto	0.007	0.023	0.031	0.018	0.009	0.000	0.396
Nakapiripirit	0.000	0.041	0.077	0.036	0.026	0.000	0.000

Based on F_{ST} values, most populations showed little to moderate but significant genetic differentiation. The greatest inter-population differentiation occurred between Mt. Elgon and Amudat (F_{ST} = 0.260). Among the Kenyan populations, great differentiation existed between Baringo and Laikipia and the least genetic differentiation between Mt. Elgon and Baringo. Amudat and Moroto showed the greatest differentiation in Uganda while Amudat and Nakapiripirit had the lowest differentiation.

The principal component analysis (PCoA) results showed two main clusters among the seven populations. The 66.24% of the total observed variation is explained by the first two coordinates, hence suggesting existence of distinct population structure among the populations (Fig.8.2). The first coordinate separated mostly individuals from different regions/countries being the main exception Mt. Elgon and Moroto where different individuals cluster with populations of both countries showed distinct patterns of clustering compared to other populations. The second cluster separates the Ugandan population of Amudat from other Ugandan populations, although Nakapiripirit individuals are found in both of the resulting clusters (Figure 8.2).

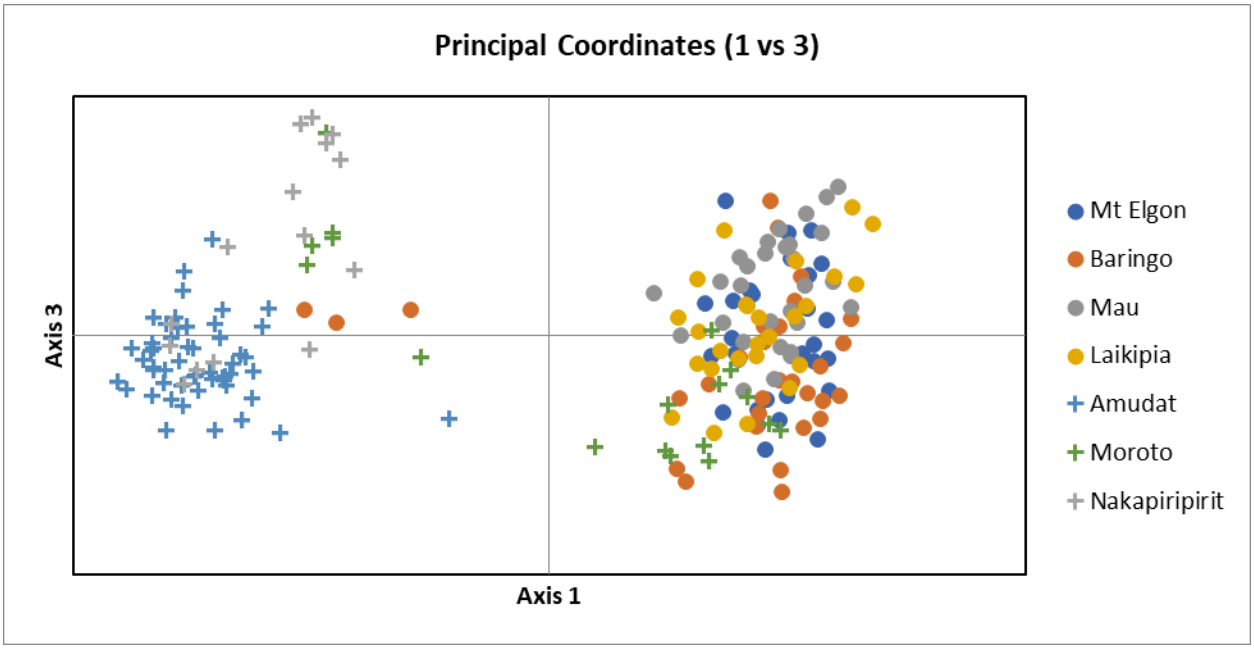


Figure 8.2 Principal Coordinate Analysis (PCoA) based on Nei's genetic distances for seven O. lanceolata populations: The first two coordinates explain 66.24% of the variation among populations

Based on Nei's Genetic Identity (D), Moroto population was genetically closer to all Kenyan populations than Nakapiripirit and Amudat populations (Table 8.6). Among the Kenyan populations, the highest genetic identity existed between Mt. Elgon and Baringo (D = 0. 925) and the least genetic identity occurred between Baringo and Laikipia. In Uganda, Amudat and Nakapiripirit populations had the highest genetic identity while the least genetic identity existed between Amudat and Moroto.

Table 8.6: Pairwise population matrix of Nei's Genetic Identity (D)

Population	Baringo	Mt. Elgon	Mau	Laikipia	Amudat	Moroto	Nakapiripirit
Baringo	1.000						
Mt. Elgon	0.925	1.000					
Mau	0.892	0.851	1.000				
Laikipia	0.776	0.759	0.803	1.000			
Amudat	0.167	0.271	0.173	0.238	1.000		
Moroto	0.664	0.704	0.631	0.561	0.455	1.000	
Nakapiripirit	0.196	0.285	0.208	0.186	0.843	0.504	1.000

Table 8.7: Pairwise population F_{ST} values among the seven populations

Population	Baringo	Mt. Elgon	Mau	Laikipia	Amudat	Moroto	Nakapiripirit
Baringo	0.000						
Mt. Elgon	0.018	0.000					
Mau	0.027	0.036	0.000				
Laikipia	0.064	0.068	0.060	0.000			
Amudat	0.260	0.231	0.267	0.263	0.000		
Moroto	0.087	0.071	0.097	0.129	0.188	0.000	
Nakapiripirit	0.235	0.214	0.241	0.263	0.062	0.159	0.000

Based on delta K variation the best K value for the STRUCTURE analysis was two, clustering together the populations from the same country. The main exception was Moroto that showed assignment to both clusters with some degree of admixture. Although, at lower extent some admixture was also observed in other populations, such as Amudat and Moroto. At higher K values some additional biological meaningful clustering is found, but the greatest structuring occurred at K2 (Figure 8.3). At K3, Laikipia separates from the rest of the Kenyan populations and it shares a cluster with some individuals from Moroto (Figure 8.4). For K=4 these individuals from Moroto are assigned to an independent cluster. At K=5, Mau is separated from Mt. Elgon and Baringo (Fig. 8.5) while at K=6 some individuals from Amudat and Nakapiripirit are grouped into a new cluster. The STRUCTURE analysis results are also consistent with results from the PCoA (Fig. 8.2).

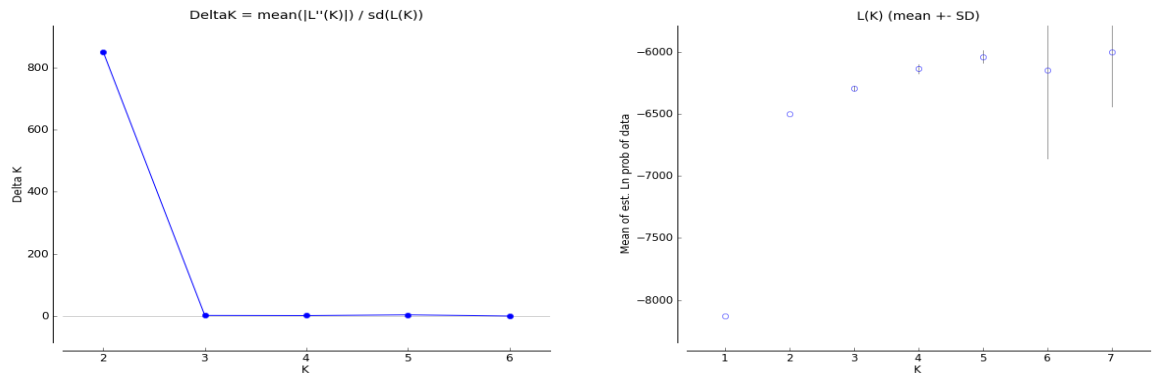


Figure 8.3: Values of ΔK, with its modal value detecting a true K of the two groups (K = 2), ***(b)*** *Log likelihood of the data (n = 210), L (K), as a function of K (the number of groups used to stratify the sample). For each K value, 20 independent runs were considered and data were averaged over the replicates.*

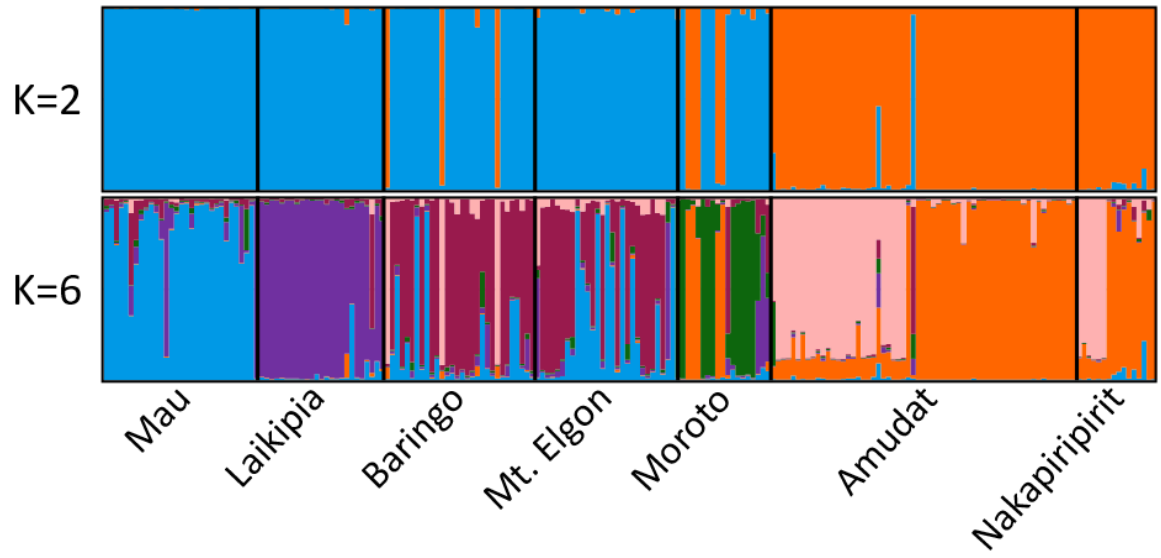


Figure 8.4 Bar plots of proportional group membership for the 210 trees genotyped at nuclear microsatellite loci for K=2 (optimum) and 6 (the highest showing biological meaningful results). Each bar represents a single tree, with color representing the proportion of ancestry derived from each group. Black lines indicate the division between populations.

There was no evidence of isolation by distance was found since there was no correlation between the pairwise R_{ST} matrix and the geographical distance for the pairwise comparisons among the 7 populations (Fig. 8.5, $R^2 = 0.004$, $P = 0.368$).

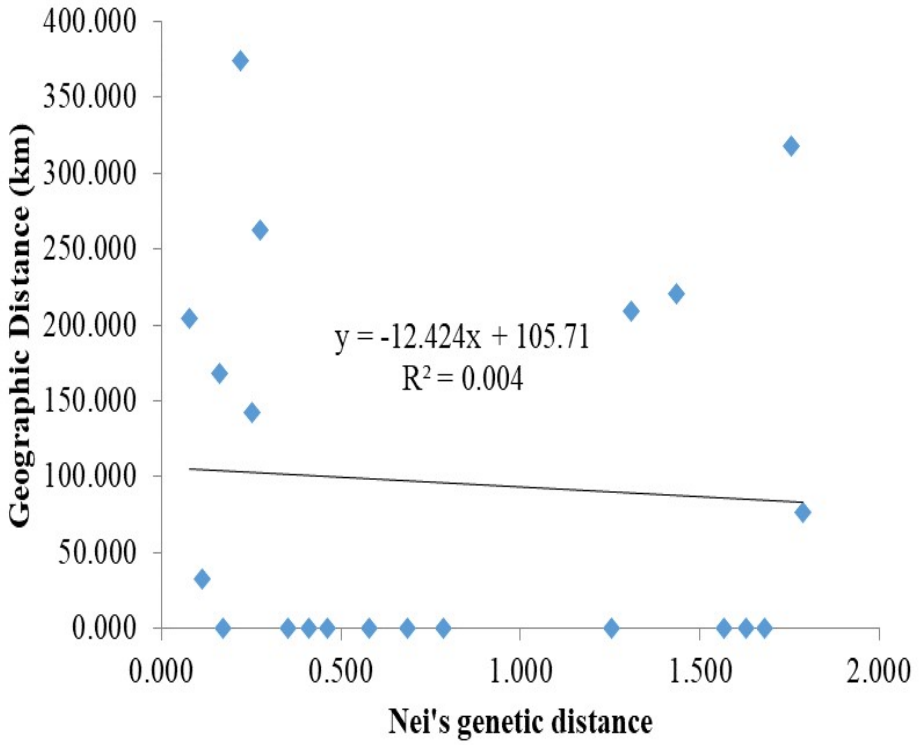


Figure 8.5: The relationship between pairwise geographic distance and Nei's genetic distance across populations in Uganda and Kenya

8.4 Discussion

8.4.1 Population genetic diversity of *O. lanceolata* in Uganda and Kenya

This study analysed the genetic diversity and structure of *O. lanceolata* in Uganda and Kenya taking advantage of the microsatellite markers (SSR) developed by Otieno et al., (2014). These markers were shown to be highly polymorphic and therefore informative to study population genetic patterns of *O. lanceolata.* Based on the findings, the sampled populations showed a high level of genetic diversity (He = 0.49 - 0.68), which indicates genetic diversity preservation in spite of the historical exploitation of these populations (Leberg, 1992; Foulley and Ollivier, 2006). Similar patterns were revealed in another study by Vandepitte et al. (2010), that analysed

the patterns of sex ratio variation and genetic diversity in dioecious species of *Mercurialis perennis*. The study detected a distinct genetic structure and moderately higher levels of genotypic diversity in the species influenced by stochastic events and sex ratios within the populations. A recent study by Soorni et al., (2017), on genetic diversity and genetic structure of Cannabis germplasm (a dioecious species) also revealed moderate levels of genetic diversity, and distinct genetic structure. Although the present study did not evaluate the impact of sex ratios on genetic diversity of *O. lanceolata*, and the fact that the species is a dioecious plant (Mwang'ngo et al., 2007), the sex ration distribution could be an important factor in the influencing variations in genetic diversities among and within populations.

Genetic diversity varied across populations with an overall pattern of higher diversity in Kenya than Uganda populations. These findings are consistent with earlier results obtained by Otieno et al., (2014) and Andiego et al., (2019). Otieno et al., (2014) used SSR markers and obtained higher genetic diversity (He) for Mt. Elgon (He = 0.043 - 0. 902). Andiego et al., (2019), used ISSR markers and also revealed higher genetic diversity for Mt. Elgon and Baringo populations. Interestingly, most populations that showed higher genetic diversity (Baringo, Moroto, Mau and Mt. Elgon) were under protected status as government conservation areas (Petursson et al., 2013; Koech, 2024). The higher degree of genetic diversity in these populations could be attributed to the effectiveness of conservation areas in preserving the germplasm of endangered species than communal and private habitats. Control measures, such as restricted resource harvesting and monitoring resource use, infer higher comparative advantages to species protection than communal populations that are easily accessed by members of the local communities.

Since the SSR-markers of Otieno et al., 2014 were developed for Kenyan *O. lanceolata* populations and hence their application to other populations which could be genetically isolated may lead to bias in genetic diversity estimates because the markers would preferably amplify Kenyan alleles (Heslot et al., 2013; Curto et al., 2019). The presence of genetic admixture between Baringo and Moroto could suggest a possibility of long-distance gene flow as recently observed in *Acacia senegal* populations (Omondi et al., 2023). In addition, other factors such as population disturbance, genetic drift, mutations, also significantly influence genetic variation and diversity (Soorni et al., 2017). Field observations revealed that people in Kenya are embracing the planting of *O. lanceolata* in their farms. Thus, it is also possible that long-distance gene flow could result from deliberate transfer of *O. lanceolata* planting materials such as seeds and seedlings from Uganda to Kenya and Kenya to Uganda hence causing admixture of genes from different countries within a single population through genetic translocations. Moreover, the natural long-distance dispersal is possible through seed dispersal agents such as frugivorous birds, and mammals (Gebirehiwot et al., 2023; Mwangi et al., 2023).

In the past ten years *O. lanceolata* populations in Uganda and Kenya have experienced higher exploitation levels that led to significant decline in the species abundance (Tajuba, 2015; Bunei, 2017; Mugula et al., 2021) and change in allele frequencies. Such disturbances are expected to cause decline in genetic diversity levels among individuals in the disturbed populations. However, populations showed fairly high levels of genetic diversity despite the past disturbance regimes. Most Ugandan populations showed significant deviations from Hardy-Weinberg Equilibrium than Kenyan populations. The positive fixation index for all populations, could have been caused by a lack of observed heterozygosity. Moroto and Amudat are the populations

showing the highest number of markers deviating from HWE and also the highest F values. Given the fact that they show substructure in both PCOA and STRUCTURE analysis this is likely caused by Wahlund effect. For the remaining populations we cannot exclude the possibility that their deviations from HWE are a consequence of bottlenecks caused by over-exploitation, which might be reflected in the difference in deviations between countries. It is possible that *O. lanceolata* populations in Uganda that experienced continuous over-exploitation for a longer period without application of resource control measures could be losing genetic diversity faster than the Kenyan populations resulting in the observed pattern.

Over-exploitation of *O. lanceolata* was detected earlier in Kenya and measures were established in 2007 to protect the resource base through a presidential decree that banned trade in *O. lanceolata* resources (CITES, 2013). The strict control in *O. lanceolata* resource utilisation in Kenya, shifted pressure to Ugandan populations to meet the illegal market demand for the species resources (Tajuba, 2015; Bunei, 2017). Unfortunately, the Uganda government did not establish quick deterrent measures to curb the destructive harvesting of the species which could have severely led to significant variation in genetic diversity through loss of different alleles from the populations leading to genetic differentiation. The destructive harvesting of the species (uprooting of whole plant) for a longer period of time could have played an important role in changing the genetic structure in terms of allele frequencies among the Uganda populations hence weakening the species genetic diversity. The level of genetic drift could have been higher in community habitats compared to the species habitats found in protected areas such as Mt. Elgon in Kenya, Mt. Moroto and Pian-upe areas and this could also explain the differing patterns in genetic deviations from HWE.

8.4.2 Genetic structure patterns of *O. lanceolata* across populations in Uganda and Kenya

The seven populations were mainly structured into two major genetic clusters (K = 2) between Uganda and Kenya populations. This was further supported by other analysis: the PCoA clustering pattern along the first coordinate, the high F_{ST} values and low genetic identity between some populations of different countries, and the fact that AMOVA showed that groupings based on country of origin explained a higher proportion of the variation than by locality. The genetic structuring between Kenya and Uganda *O. lanceolata* populations could be attributed to ecological, evolutionary, and anthropological factors that have been shaping the populations of the species in the two countries.

Geographically, the populations could be spatially isolated to the extent that dispersal mechanisms cannot facilitate free interbreeding between *O. lanceolata* individuals in the two regions. Secondly, the *O. lanceolata* habitats have been highly fragmented, and isolated geographically leading to continued inbreeding that was reflected in the high levels of fixation indexes among the Kenyan and Ugandan populations. Additionally, the high level of anthropogenic disturbances exposed to the species habitats including destructive harvesting between Uganda and Kenya populations have also gradually contributed to significant variations in allele frequencies and genetic variations among populations hence leading to genetic structuring. An earlier study by Ratnaningrum et al., (2015) to understand the evidence of geneflow and selection in *Santalum alba* in different population structures observed that natural barriers influence fragmentation of habitats which eventually disrupt gene flow among populations. Since Ugandan populations are believed to undergo a greater level of exploitation

than the Kenya populations, these could have increased the genetic differentiation between them through genetic drift leading to structuring into two genetic clusters.

Despite geographical isolation seeming to play a crucial role in shaping genetic structure between countries, there was no evidence of isolation by distance meaning that other factors are shaping gene-flow between populations. Similar findings were obtained by Ratnaningrum et al., 2015), where another dioecious Indian sandalwood species (*Santalum alba*) was structured based on similarity in genetic structures rather than geographical locations, perhaps due to long-distance gene flow and translocation of genes into geographically distant populations. Although populations that were geographically close show low levels of genetic differentiation (for instance; Amudat and Nakapiripirit), and some not geographically isolated populations show high levels of differentiation (e.g., Moroto from the remaining Ugandan populations). This isolation within Uganda could have been promoted by the fact that Moroto shows evidence of having divergent gene pools sharing variation with both countries with high degrees of admixture.

The dispersal of *O. lanceolata* seeds by birds as well as human mediated translocations may increase chances of long-distance gene flow hence narrowing the chances of population isolation by distance. Natural, admixture can be beneficial since it increases standing variation and forms new genotypes that can be useful in genetic improvement programs (Muriiru et al., 2018). On the other hand, artificial admixture has been shown to break local adaptation and contribute to 'outbreeding depression (Barker et al., 2019; Tibihika et al., 2020; and Lanner et al., 2021). It is important to further explore the processes contributing to the admixture found in some of these

populations to better understand the role they can play in the conservation of genetic resources of *O. lanceolata*.

8.4.3 Implications for *O. lanceolata* conservation in Uganda and Kenya

The study highlights areas with implications for conservation of genetic resources for *O. lanceolata* in Uganda and Kenya. Genetic variation is clearly organized in two genetic units that should be conserved from extreme population disturbances. The presence of significant levels of differentiation and structuring among populations in Kenya and Uganda require strategic interventions to prevent adverse effects from continuous population differentiation such as loss of genetic diversity, reduction in population size, isolation and loss of the species ability to adapt to climatic change and survive. *Osyris lanceolata* presents special adaptation mechanisms even in harsh environments that are highly fragile. It is important to identify such useful genetic traits and incorporate them in the species tree breeding programs.

The absence of a significant correlation between the species geographical location and genetic distance points to certain factors; genetic, ecological, and anthropogenic that drive the species spatial distribution and these require thorough investigation. The recent attempt to characterise ecological distribution drivers of *O. lanceolata* (Mugula et al., press) is not adequate to understand spatial distribution drivers of the species. It is thus necessary to investigate population genetic factors that drives the species distribution. Although Ugandan populations showed slightly lower genetic diversity than most Kenyan populations, there are still populations with higher genetic diversity that could serve as suitable provenances in boosting in-situ and ex-situ conservation. However, a better criterion for identifying suitable provenances should take

into consideration, morphological traits, ecological, genetic and biochemical properties of the target populations to inform robust management decisions that will generate and promote conservation of desirable traits of the species. To achieve this, it is necessary to characterise the biochemical properties of all potential populations for in-situ and ex-situ conservation programs.

Since the seven populations were structured/clustered along genetic similarities, it is important to explore whether such clustering patterns extend beyond genetic factors such as genetic diversity and geneflow, to biochemical properties like oil yield, quality and composition of active ingredients among the populations in the two countries. By identifying genotypes that are more fit to be cultivated, one can more efficiently pick germplasm for selective breeding. This would release the pressure from harvesting natural populations and hence guarantee long-term conservation and sustainable use of the species. Finally, the sampled populations exhibited potential qualities for in-situ and ex-situ conservation programs due to their considerable levels of genetic diversity, distinctive clustering in some populations, genetic admixture among populations, presence of private alleles and the ability to adapt and survive in harsh and diverse environmental conditions. Finally, the findings revealed anthropogenic and ecophysiological impacts on population genetic diversity and structure patterns of *Osyris lanceolata* in Uganda and Kenya. The high genetic divergence between Uganda and Kenya populations should also be considered an opportunity for conserving a more genetically diverse *O. lanceolata* with a higher genetic potential for adaptation in a wide range of semi-arid habitats in East Africa and beyond.

8.5 Conclusions

Ugandan populations were more threatened than Kenyan populations due to genetic diversity loss. Population bottlenecks and environmental gradients might be key drivers to the species genetic structuring between Uganda and Kenya than isolation by geographical distance. The big genetic divergence between *O. lanceolata* populations in both countries should present an opportunity for conservation of a wide range of the species gene pool. Finally, to save the populations from extreme loss of genetic diversity, restoration (in-situ) and domestication (ex-situ) programs should be implemented to conserve the species germplasm across its natural range for future genetic improvement programs. Finally, sampling of *O. lanceolata* populations should be extended to other regions and beyond Africa to explore evolutionary processes influencing species genetic diversity and structure and also compare biochemical and morphological patterns of *O. lanceolata* populations across East Africa and beyond to scientifically identify populations with more superior attributes for conservation and genetic improvement.

CHAPTER NINE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1 DISCUSSION

The study provided insight into the population structure, distribution drivers and genetic diversity and structure patterns of *Osyris lanceolata* in Uganda and Kenya. However, the findings should not be generalized to all populations in Uganda and Kenya, because the study focused on specific populations in the two countries. Nevertheless, the findings improve our understanding of the current ecological trends and evolutionary processes shaping the structure and status of existing populations of *O. lanceolata* as a basis for development of strategies to conserve and add value to the species resources. The limitations and potential consequences of the design are discussed as well as implications for the results and the key recommendations for future research.

9.1.1 Population structure and regeneration strategy of *O. lanceolata*

Overall, *Osyris lanceolata* has unstable irregular and poorly regenerating population structure characterised with more coppiced and juvenile individuals. The species density was generally lower in Amudat, Nakapiripirit than in Moroto. Although these populations were clustered into lower and higher elevations, altitude did not influence the species density and abundance, but influenced its morphology. The difference in density of *O. lanceolata* across populations also suggest varying levels of species exploitation in the Karamoja sub-region. For instance, Moroto populations lie within Mt. Moroto conservation area which may provide reasonable control and

protection to *O. lanceolata* resources than communal lands in Nakapiripirit and Amudat districts with less restrictions to resource harvesting.

The irregular population structure and availability of more coppiced individuals reflect past harvesting of the species which contributed to the unstable population structure in Karamoja sub-region. Other factors that are responsible for unstable species population structure include poor regeneration and destruction of the species hosts for timber, construction poles, local fencing materials and fuelwood.

The lower rates of natural regeneration through seedlings for *O. lanceolata* was also underscored by past studies in Kenya (Gathara et al., 2014). The factors limiting natural regenerations of *O. lanceolata* include high seedling mortality attributed to absence of appropriate hosts, browsing and grazing pressure. Also, the high seed germination failure limits the species capacity for natural regeneration and this is attributed to destruction of the *O. lanceolata* fruits and seeds by pests such as *Dismeqistus sp* (Mugula et al., press). The destructive effect of *Dismeqistus sp* on fruit and seed viability and regeneration requires further investigations as it was not covered by this study.

Contrary to the hypothesis of this study, that *O. lanceolata* population structure is stable and sufficiently regenerating in Karamoja, the findings reveal the population structure to be unstable, irregular, and hence poorly regenerating. The results also support earlier claims that *O. lanceolata* populations are declining in Karamoja due to overexploitation (Tajuba, 2016; CITES et al., 2013; FSSD et al., 2021; Mugula et al., press) but provides insight into understanding the

current patterns of species densities, size class distributions, regeneration strategies, and the combined interaction effect between coppicing and altitude on the species stem diameter which have been lacking in earlier studies for Ugandan populations. Hence the findings are useful in providing an insight and guidance on which populations require urgent priority for restoration and domestication. Additionally, the ability of *O. lanceolata* to regenerate vegetatively as indicated in this study presents an opportunity for the local communities to harvest the species resources sustainably as opposed to uprooting the whole plant for extraction of essential oils. However, further studies should establish the effect of coppicing on the species genetic diversity, biochemical composition including oil yield and quality.

9.1.2 Distribution drivers of *O. lanceolata*

The species populations showed a highly patchy distribution across the altitude gradient (1200m asl – 1800m asl). The species habitats were largely characterised with moderate illumination, rocky surfaces, water gullies, mammal droppings and rich soils in calcium, nitrogen, phosphorus and sodium. The findings support the alternative hypothesis that *O. lanceolata* distribution is a function of specific edaphic variables acting in concert with other environmental variables to influence its abundance. This is also emphasised by earlier studies that established the spatial distribution of hemiparasites to be influenced by the nutrient status of the soils (Dean et al., 1994). However, there was no significant variation between habitat qualities across the populations. These findings support earlier studies that categorized *O. lanceolata* to be a light loving species. Secondly, the availability of water gullies and rocky surfaces in most habitats also reflect the species strategy to adapt to water stress and the need for specific soil nutrients to facilitate growth (Fox, 1997; Watson, 2009). In line with the habitat quality hypothesis (HQH), the presence of gullies, water courses, and rocky surfaces enhances the species exposure to

suitable nutrients, water availability, and hosts (Dean et al., 1994; Watson et al., 2007; Irving and Cameron, 2009).

The distribution of *O. lanceolata* was characterised with a wide range of hosts in form of trees, shrub-trees, shrubs, succulent plants and climbers. Of these hosts, *Rhus natalensis* (Anacardiaceae), was most clustered to *O. lanceolata* distribution, followed by *Euclea racemosa* (Ebenaceae), *Maytenus senegalensis* (Celastraceae), *Ozorea insignis* (Anacardiaceae), and *Terminalia browni* (Combretaceae). Earlier studies showed *O. lanceolata* survive through interaction with a range of hosts (Nilsson and Svensson 1997), and sometimes being host specific according to local site diversity (Matthies and Egli, 1999; Okubamichael et al., 2016; Marvier, 2014). In Kenya and Tanzania, *O. lanceolata* was found to be highly clustered with *Rhus natalensis* and *Maytenus sp* (Mwang'Ingo et al., 2004; Gathara). However, these results did not concur with the claims of Mwangi et al., (2023) who indicated that *O. lanceolata* frequently parasitises members of family Fabaceae. Although Fabaceae hosts were identified, they were among the least clustered hosts to *O. lanceolata* exhibiting lower frequencies across the sampled populations and hence categorised as least clustered or parasitized by *O. lanceolata* in this study.

The findings revealed a wide range of other species associated to *O. lanceolata* distribution in the semi-arid habitats. Thus, the present study together with earlier studies on *O. lanceolata* hosts in East Africa supports two predictions: (i) *Rhus natalensis* and *Maytennus sp* could be the most highly clustered and universally preferred hosts for *O. lanceolata* across natural habitats, (ii) *Rhus natalensis* and *Maytennus sp* could be the most influential host drivers of *O. lanceolata* distribution across natural habitats. However, further studies should clarify which of the two

hosts; *Maytennus senegalensis* and *Maytennus acuminata* var. *acuminata* (Mwang'Ingo et al., (2004) is highly clustered to *O. lanceolata* in natural habitats. Which among the two is highly clustered around *O. lanceolata*.

Nevertheless, the findings provide interesting insights about members in family Anacardiaceae and Celastraceae having unique features that influence *O. lanceolata* distribution and survival across habitats in Kenya and Uganda. Unravelling such special parasitic associations between *O. lanceolata* and members in these families should be a concern for further studies to understand the key survival strategies of *O. lanceolata*. The findings further support the results of earlier studies that indicated that hosts of hemiparasites differ geographically (Qasem, 2006; Marvier, 1996). Further studies should focus on understanding whether site specific host preference of *O. lanceolata* is due to its adaptation to local flora or because the species has special genetic attributes to survive as a generalist plant.

Since *O. lanceolata* is a hemi-parasitic plant, its distribution drivers also influence the distribution of hosts especially those highly clustered hosts. Given that the abundance and availability of host species highly varied from place or habitat to habitat, it becomes logical to relate *O. lanceolata* distribution drivers to the distribution of key hosts. Although the occurrence of key hosts to *O. lanceolata* was almost similar to the distribution of *O. lanceolata* these findings cannot infer the distribution drivers of the *O. lanceolata* hosts. This requires further studies to establish whether both *O. lanceolata* and their hosts have similar edaphic variables influencing their distribution.

Finally, the generalization from this study is limited to identification of soil variables, host compositions and habitat qualities (above the ground) as key drivers to *O. lanceolata* distribution and survival.

9.1.3 Effect of altitude and coppicing on *O. lanceolata* morphology

The findings revealed higher variations in morphological plasticity of *O. lanceolata*. Altitude and coppicing to had a positive combined interaction effect that favors increase in the stem size of *O. lanceolata*. Higher altitudes facilitated increase in stem size of coppiced individuals than lower altitudes which negatively affected stem size of coppiced individuals. The stem size of non-coppiced individuals was favored in lower altitudes. The findings provide a better understanding of management strategies that can be taken to facilitate quantitative growth of *O. lanceolata* populations in different altitudes. This implies that the increased size in stem diameter of *O. lanceolata* can be attained by managing populations in high altitude habitats through coppicing. In higher altitude, the increase in secondary growth is attained due to more vertical growth than horizontal growth where light is abundant for growth (Peters et al., 2021; Negi et al., 2024). However, coppicing, cannot lead to the increase in stem size for *O. lanceolata* in the lower altitude areas due to limited light intensities in lower altitudes which encourages more horizontal growth in search for higher light intensities for photosynthesis than vertical growth (Peters et al., 2021; Negi et al., 2024).

These findings support the growth theories that emphasise the role of altitude in promoting vertical growth in plants (Negi et al., 2024). However, it is necessary to explore whether altitude and coppicing exerts similar patterns in in other tree species.

The manifestation of higher plasticity levels indicates that *O. lanceolata* uses morphological adaptations to survive in the different environmental gradients. The species leaf size, length and width also varied widely across altitudes. Variation in the species' leaf size may be correlated to genetic identity, sex, or morphological plasticity, but this requires further investigations. These insights provide a basis for designing a species management strategy that facilitates quantitative growth in *O. lanceolata* within the right altitudes. In regard to the species height, coppicing had a general negative effect on the height of *O. lanceolata* irrespective of the altitude level.

9.1.4 Genetic diversity and structure patterns of *O. lanceolata* in Uganda and Kenya

The findings showed relatively lower genetic diversity levels among *O. lanceolata* populations in Uganda than Kenyan populations. However, the results demonstrate evidence of population deviations from Hardy-Weinberg Equilibrium, genetic differentiation and structuring between Kenya and Uganda populations. The populations were genetically clustered into two clusters according to altitude gradients and environmental isolation. The two distinct genetic clusters reveal interesting ecological and evolutionary trends within *O. lanceolata* taxon. These trends could be influenced by genetic drifts, restricted gene flow, and spatial variation in natural selection (Frankham et al., 2002). These results are in support of earlier studies that indicated *O. lanceolata* to be highly threatened in Uganda and Kenya due to over exploitation (CITES, 2013, Otieno et al., 2016, Andiego et al., 2019).

Overall, the study provides clear evidence in contrast to earlier hypothesised scenario in this thesis that no distinct patterns in genetic diversity and structure exist among Uganda and Kenyan populations of *O. lanceolata*. However, due to lack of baseline genetic data on *O. lanceolata* populations in Uganda, the study can only confirm the species to have a weak genetic structure

with evidence of genetic loss, but cannot estimate the extent of genetic diversity loss impacted on the present populations by past disturbances.

Although these findings are limited to the sampled populations, the study highlights emerging ecological and evolutionary trends within the species populations in Uganda and Kenya which has not been reported. The presence of genetic admixture between Uganda and Kenya populations and clustering of populations along altitude gradient is an important genetic pattern that require further investigations. Present findings are consistent with earlier results that revealed higher genetic diversity of *O. lanceolata* in some Kenyan populations (Otieno et al., 2014; Andiego et al., 2019). On the other hand, the study does not support the hypothesis of earlier studies that indicated strong correlations between genetic distance and geographic distance of dioecious species. However, the study revealed new insights into the least and more genetically diverse populations, highly disturbed populations and the degree of differentiation, and evidence of genetic admixture within *O. lanceolata* populations in Kenya and Uganda.

9.2 CONCLUSIONS AND RECOMMENDATIONS

9.2.1 Conclusions

A weak and destabilised population structure of *O. lanceolata*, characterised with highly coppiced stems and poor regeneration points to a greater threat to the species in Uganda. The low numbers of the species seedlings might be a consequence of many factors: first; the past intensive exploitation of mature trees capable of producing seeds, and secondly; the seed germination failure due to poor seed viability and damage by caterpillars (*Dismeqistus sp*) could greatly hinder natural regeneration and recruitment. The predominantly coppiced stems indicate

a higher rate of species exploitation through harvesting of the mother trees which could negatively affect the overall genetic potential of the species in subsequent generations. Based on the study the findings enough evidence is available to support the alternative hypothesis, that "population structure of *Osyris lanceolata* is weak, unstable and poorly recruiting in Karamoja sub-region.

The distribution of *Osyris lanceolata* was influenced by moderate light, water, rocky surfaces, highly clustered hosts namely; *Euclea racemosa*, *Rhus natalensis*, *Maytenus senegalensis*, *Ozorea insignis*, and *Terminalia browni*, and rich soils in calcium, nitrogen, phosphorus, and sodium. Based on the findings, the null hypothesis that "edaphic variables have no influence on the distribution and density of *O. lanceolata*" is rejected. Thus, distribution and density of *O. lanceolata* is influenced by key edaphic variables and specific hosts and habitat qualities.

The most preferred and universally clustered hosts to *O. lanceolata* included; *Rhus natalensis* and *Maytennus sp*. However, altitude influenced the morphology of coppiced *O. lanceolata* individuals. The species morphological plasticity may indicate its adaptation strategy to environmental gradients, taxonomical identity, or sex identity in the semi-arid ecosystems. Based on the findings, there was evidence to reject the null hypothesis that "morphological traits of *O. lanceolata* are not influenced by environmental gradients and species exploitation".

The divergence and trends in genetic diversity and structure patterns of *O. lanceolata* across Uganda and Kenyan populations indicated a higher level of threat to populations in Uganda than in Kenya. The patterns in genetic structuring reveals emerging evolutionary processes across *O.*

lanceolata populations in Uganda and Kenya which could be influenced by elevation gradients, restricted geneflow, genetic drifts and spatial variation in natural selection.

The existence of genetic admixture and genetic structuring may have further evolutionary consequences that could lead to full allopatric speciation within the *O. lanceolata* taxon and thus complicate future management strategies such as identification of suitable provenances when the species taxonomic units are not clearly defined. The study findings provided adequate evidence to reject the null hypothesis in support of the alternative hypothesis that; "distinct patterns in genetic diversity and structure exist between the *Osyris lanceolata* populations in Uganda and Kenya".

9.2.2 Recommendations

To avert the further decline in *O. lanceolata* populations, restoration of degraded populations in Uganda and Kenya through in-situ programs and ex-situ strategies such as gene bank establishments, species restoration, domestication and commercial propagation should be prioritised to conserve the species germplasm.

The species conservation programs should target habitat sites with desirable edaphic variables, universally preferred hosts and habitat qualities to enhance their success in ensuring species survival and productivity. Thus, suitable habitat characteristics and soil nutrients should be key benchmarks in identifying suitable sites for restoration and propagation of *O. lanceolata* across altitudes.

9.2.3 Areas for further research

Further investigations should focus on analysis of the species biotic survival conditions and also explore the role of micro-nutrient variables on the species distribution, genetic and biochemical composition such as oil yield and quality to develop appropriate strategies for restoration, breeding, propagation and domestication. Also, the role of hosts and habitat qualities on the species distribution, genetic adaptation potential, morphology and biochemical properties such as oil yield and quality of *O. lanceolata* should be investigated to enhance conservation programs.

There should be deliberate effort to unravel the implications of morphological plasticity on the species genetic and biochemical compositions. The effect of exploitation on the biochemical properties such as oil yield, composition and quality of *O. lanceolata* should be investigated further to enhance selection of better germplasm for propagation. The *O. lanceolata* leaf morphology should be investigated to establish whether plasticity reflects the species' emerging and unknown taxonomic units, evolutionary and adaptive response to environmental gradients, or the species' sex identity.

Finally, further studies should establish whether the present *O. lanceolata* genetic clusters between populations in Uganda and Kenya represent emergence of yet unknown taxonomic units and explore the genetic relationships within and between populations in the two countries and beyond.

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APPENDICES

Appendix 1 Table 8.3: Probability values for Hardy Weinberg Equilibrium (HWE) tests

Locus	Mt Elgon	Baringo	Mau	Laikipia	Amudat	Moroto	Nakapiripirit
KFOL2	0.128	0.996	0.495	0.126	**0.013**	**0.042**	0.648
KFOL13	0.996	**0.014**	**0.000**	**0.000**	0.500	**0.000**	**0.013**
KFOL17	0.092	0.009	0.899	0.171	**0.000**	0.229	0.074
KFOL24	0.315	0.185	0.216	**0.032**	**0.000**	**0.007**	0.345
KFOL28	0.068	**0.001**	**0.029**	0.563	**0.000**	**0.004**	**0.004**
KFOL30	0.958	**0.002**	0.696	0.761	**0.000**	0.248	**0.001**
KFOL37	0.543	0.273	0.245	0.269	0.074	**0.003**	0.473
KFOL42	1.000	**0.000**	0.998	**0.000**	**0.000**	**0.000**	**0.000**
KFOL47	**0.000**	**0.000**	**0.029**	**0.001**	**0.000**	**0.000**	0.657
KFOL48	0.946	**0.011**	**0.010**	0.325	**0.000**	**0.000**	**0.030**

Appendix 2: Table 8.5: Genetic diversity indices for the 10 loci over the seven studied populations

Loci	Na	Ne	I	Ho	He	F	FST	Nm
KFOL2	4.000	2.104	0.895	0.453	0.508	0.125	0.166	1.252
KFOL13	5.714	2.316	1.020	0.340	0.526	0.345	0.317	0.538
KFOL17	14.000	8.954	2.340	0.814	0.897	0.075	0.056	4.215
KFOL24	10.000	5.854	1.833	0.711	0.780	0.072	0.155	1.359
KFOL28	4.714	2.759	1.012	0.528	0.518	0.048	0.339	0.488
KFOL30	9.571	4.057	1.642	0.620	0.737	0.145	0.124	1.774
KFOL37	9.429	5.064	1.621	0.626	0.678	0.083	0.225	0.860
KFOL42	3.286	1.455	0.478	0.123	0.245	0.398	0.570	0.188
KFOL47	9.143	5.001	1.777	0.523	0.792	0.334	0.099	2.272
KFOL48	4.857	2.284	0.970	0.368	0.503	0.254	0.252	0.741
Mean	7.471	3.985	1.359	0.511	0.618	0.187	0.230	1.369

Appendix 3: Table 8.8 R statistics results

Source	R-statistics	P(rand>=data)
Among regions (UG/KE)	Rrt = 0.034	0.001
Among populations	Rsr = 0.010	0.053
Among individuals	Rst = 0.043	0.001
Within individuals	Ris = 0.956	0.001
Total	Rit = 0.958	0.001
	Nm = 5.500	

Appendix 4: Table 12 Delta K values for the seven K populations proposed

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	20	-8128.8	0.818857	—	—	—
2	**20**	**-6500.18**	**1.676965**	**1628.62**	**1424.84**	**849.654152**
3	20	-6296.4	20.276977	203.78	44.59	2.199046
4	20	-6137.21	36.628834	159.19	62.22	1.698662
5	20	-6040.24	50.157545	96.97	206.82	4.123408
6	20	-6150.09	712.551523	-109.85	257.86	0.361883
7	20	-6002.08	441.960909	148.01	—	—

Appendix 5: Table 13: P-values for Genotypic linkage disequilibrium of each pair of loci across all populations

Population	Locus#1	Locus#2	P-Value
Mt Elgon	KFOL2	KFOL13	0.121
Mt Elgon	KFOL2	KFOL17	0.595
Mt Elgon	KFOL13	KFOL17	0.891
Mt Elgon	KFOL2	KFOL24	1.000
Mt Elgon	KFOL13	KFOL24	1.000
Mt Elgon	KFOL17	KFOL24	1.000
Mt Elgon	KFOL2	KFOL28	0.548
Mt Elgon	KFOL13	KFOL28	0.541
Mt Elgon	KFOL17	KFOL28	0.896
Mt Elgon	KFOL24	KFOL28	1.000
Mt Elgon	KFOL2	KFOL30	0.984
Mt Elgon	KFOL13	KFOL30	0.593
Mt Elgon	KFOL17	KFOL30	0.382
Mt Elgon	KFOL24	KFOL30	0.324
Mt Elgon	KFOL28	KFOL30	0.312
Mt Elgon	KFOL2	KFOL37	0.661
Mt Elgon	KFOL13	KFOL37	0.309
Mt Elgon	KFOL17	KFOL37	1.000
Mt Elgon	KFOL24	KFOL37	1.000
Mt Elgon	KFOL28	KFOL37	0.514
Mt Elgon	KFOL30	KFOL37	0.769
Mt Elgon	KFOL2	KFOL42	0.746
Mt Elgon	KFOL13	KFOL42	0.591
Mt Elgon	KFOL17	KFOL42	0.712
Mt Elgon	KFOL24	KFOL42	0.855
Mt Elgon	KFOL28	KFOL42	0.048
Mt Elgon	KFOL30	KFOL42	0.261
Mt Elgon	KFOL37	KFOL42	0.811
Mt Elgon	KFOL2	KFOL47	0.901
Mt Elgon	KFOL13	KFOL47	0.402
Mt Elgon	KFOL17	KFOL47	0.168
Mt Elgon	KFOL24	KFOL47	0.293
Mt Elgon	KFOL28	KFOL47	0.134
Mt Elgon	KFOL30	KFOL47	0.583
Mt Elgon	KFOL37	KFOL47	0.896
Mt Elgon	KFOL42	KFOL47	0.406
Mt Elgon	KFOL2	KFOL48	0.429
Mt Elgon	KFOL13	KFOL48	0.343
Mt Elgon	KFOL17	KFOL48	0.942
Mt Elgon	KFOL24	KFOL48	0.044
Mt Elgon	KFOL28	KFOL48	0.934
Mt Elgon	KFOL30	KFOL48	0.172
Mt Elgon	KFOL37	KFOL48	0.623
Mt Elgon	KFOL42	KFOL48	0.768
Mt Elgon	KFOL47	KFOL48	0.718
Baringo	KFOL2	KFOL13	0.380
Baringo	KFOL2	KFOL17	0.641
Baringo	KFOL13	KFOL17	0.553
Baringo	KFOL2	KFOL24	0.985
Baringo	KFOL13	KFOL24	0.843
Baringo	KFOL17	KFOL24	0.098
Baringo	KFOL2	KFOL28	0.092
Baringo	KFOL13	KFOL28	0.415
Baringo	KFOL17	KFOL28	0.116
Baringo	KFOL24	KFOL28	0.156
Baringo	KFOL2	KFOL30	0.392
Baringo	KFOL13	KFOL30	0.805
Baringo	KFOL17	KFOL30	0.331
Baringo	KFOL24	KFOL30	0.010
Baringo	KFOL28	KFOL30	0.054
Baringo	KFOL2	KFOL37	0.076
Baringo	KFOL13	KFOL37	0.850
Baringo	KFOL17	KFOL37	1.000
Baringo	KFOL24	KFOL37	0.483
Baringo	KFOL28	KFOL37	0.481
Baringo	KFOL30	KFOL37	0.756

Baringo	KFOL2	KFOL42	0.006
Baringo	KFOL13	KFOL42	0.602
Baringo	KFOL17	KFOL42	0.228
Baringo	KFOL24	KFOL42	0.005
Baringo	KFOL28	KFOL42	0.029
Baringo	KFOL30	KFOL42	0.063
Baringo	KFOL37	KFOL42	0.124
Baringo	KFOL2	KFOL47	0.573
Baringo	KFOL13	KFOL47	0.207
Baringo	KFOL17	KFOL47	0.363
Baringo	KFOL24	KFOL47	0.000
Baringo	KFOL28	KFOL47	0.485
Baringo	KFOL30	KFOL47	0.663
Baringo	KFOL37	KFOL47	1.000
Baringo	KFOL42	KFOL47	0.067
Baringo	KFOL2	KFOL48	0.076
Baringo	KFOL13	KFOL48	0.013
Baringo	KFOL17	KFOL48	0.456
Baringo	KFOL24	KFOL48	0.265
Baringo	KFOL28	KFOL48	0.006
Baringo	KFOL30	KFOL48	0.012
Baringo	KFOL37	KFOL48	0.293
Baringo	KFOL42	KFOL48	0.013
Baringo	KFOL47	KFOL48	0.926
Mau	KFOL2	KFOL13	0.385
Mau	KFOL2	KFOL17	0.639
Mau	KFOL13	KFOL17	0.263
Mau	KFOL2	KFOL24	0.909
Mau	KFOL13	KFOL24	0.792
Mau	KFOL17	KFOL24	1.000
Mau	KFOL2	KFOL28	0.531
Mau	KFOL13	KFOL28	0.021
Mau	KFOL17	KFOL28	1.000
Mau	KFOL24	KFOL28	0.175
Mau	KFOL2	KFOL30	0.276
Mau	KFOL13	KFOL30	0.186
Mau	KFOL17	KFOL30	0.000
Mau	KFOL24	KFOL30	0.518
Mau	KFOL28	KFOL30	0.904
Mau	KFOL2	KFOL37	0.061
Mau	KFOL13	KFOL37	0.846
Mau	KFOL17	KFOL37	0.060
Mau	KFOL24	KFOL37	0.423
Mau	KFOL28	KFOL37	0.489
Mau	KFOL30	KFOL37	0.293
Mau	KFOL2	KFOL42	0.576
Mau	KFOL13	KFOL42	1.000
Mau	KFOL17	KFOL42	0.889
Mau	KFOL24	KFOL42	0.574
Mau	KFOL28	KFOL42	0.883
Mau	KFOL30	KFOL42	1.000
Mau	KFOL37	KFOL42	0.554
Mau	KFOL2	KFOL47	0.016
Mau	KFOL13	KFOL47	0.143
Mau	KFOL17	KFOL47	1.000
Mau	KFOL24	KFOL47	0.719
Mau	KFOL28	KFOL47	0.017
Mau	KFOL30	KFOL47	0.609
Mau	KFOL37	KFOL47	0.616
Mau	KFOL42	KFOL47	0.680
Mau	KFOL2	KFOL48	0.211
Mau	KFOL13	KFOL48	0.008
Mau	KFOL17	KFOL48	0.445
Mau	KFOL24	KFOL48	0.612
Mau	KFOL28	KFOL48	0.358
Mau	KFOL30	KFOL48	0.161
Mau	KFOL37	KFOL48	0.189
Mau	KFOL42	KFOL48	1.000
Mau	KFOL47	KFOL48	0.004

Laikipia	KFOL2	KFOL13	0.039
Laikipia	KFOL2	KFOL17	0.237
Laikipia	KFOL13	KFOL17	0.108
Laikipia	KFOL2	KFOL24	0.683
Laikipia	KFOL13	KFOL24	0.269
Laikipia	KFOL17	KFOL24	0.878
Laikipia	KFOL2	KFOL28	0.162
Laikipia	KFOL13	KFOL28	0.433
Laikipia	KFOL17	KFOL28	0.715
Laikipia	KFOL24	KFOL28	0.030
Laikipia	KFOL2	KFOL30	0.466
Laikipia	KFOL13	KFOL30	0.447
Laikipia	KFOL17	KFOL30	0.015
Laikipia	KFOL24	KFOL30	0.646
Laikipia	KFOL28	KFOL30	0.360
Laikipia	KFOL2	KFOL37	0.244
Laikipia	KFOL13	KFOL37	0.076
Laikipia	KFOL17	KFOL37	0.607
Laikipia	KFOL24	KFOL37	0.963
Laikipia	KFOL28	KFOL37	0.269
Laikipia	KFOL30	KFOL37	0.489
Laikipia	KFOL2	KFOL42	0.480
Laikipia	KFOL13	KFOL42	0.278
Laikipia	KFOL17	KFOL42	0.318
Laikipia	KFOL24	KFOL42	0.690
Laikipia	KFOL28	KFOL42	0.185
Laikipia	KFOL30	KFOL42	0.778
Laikipia	KFOL37	KFOL42	0.963
Laikipia	KFOL2	KFOL47	0.386
Laikipia	KFOL13	KFOL47	0.107
Laikipia	KFOL17	KFOL47	0.321
Laikipia	KFOL24	KFOL47	0.306
Laikipia	KFOL28	KFOL47	0.919
Laikipia	KFOL30	KFOL47	0.219
Laikipia	KFOL37	KFOL47	0.930
Laikipia	KFOL42	KFOL47	0.452
Laikipia	KFOL2	KFOL48	0.415
Laikipia	KFOL13	KFOL48	0.523
Laikipia	KFOL17	KFOL48	0.759
Laikipia	KFOL24	KFOL48	0.645
Laikipia	KFOL28	KFOL48	0.985
Laikipia	KFOL30	KFOL48	0.094
Laikipia	KFOL37	KFOL48	0.026
Laikipia	KFOL42	KFOL48	1.000
Laikipia	KFOL47	KFOL48	0.018
Amudat	KFOL2	KFOL13	0.791
Amudat	KFOL2	KFOL17	0.000
Amudat	KFOL13	KFOL17	0.111
Amudat	KFOL2	KFOL24	0.626
Amudat	KFOL13	KFOL24	0.013
Amudat	KFOL17	KFOL24	0.008
Amudat	KFOL2	KFOL28	1.000
Amudat	KFOL13	KFOL28	1.000
Amudat	KFOL17	KFOL28	0.475
Amudat	KFOL24	KFOL28	0.109
Amudat	KFOL2	KFOL30	0.106
Amudat	KFOL13	KFOL30	0.363
Amudat	KFOL17	KFOL30	0.024
Amudat	KFOL24	KFOL30	0.000
Amudat	KFOL28	KFOL30	0.167
Amudat	KFOL2	KFOL37	0.254
Amudat	KFOL13	KFOL37	0.759
Amudat	KFOL17	KFOL37	0.017
Amudat	KFOL24	KFOL37	0.167
Amudat	KFOL28	KFOL37	-
Amudat	KFOL30	KFOL37	0.129
Amudat	KFOL2	KFOL42	0.621
Amudat	KFOL13	KFOL42	1.000
Amudat	KFOL17	KFOL42	0.008

Amudat	KFOL24	KFOL42	0.315
Amudat	KFOL28	KFOL42	0.083
Amudat	KFOL30	KFOL42	0.007
Amudat	KFOL37	KFOL42	0.371
Amudat	KFOL2	KFOL47	0.020
Amudat	KFOL13	KFOL47	0.430
Amudat	KFOL17	KFOL47	0.000
Amudat	KFOL24	KFOL47	0.000
Amudat	KFOL28	KFOL47	0.239
Amudat	KFOL30	KFOL47	0.000
Amudat	KFOL37	KFOL47	0.011
Amudat	KFOL42	KFOL47	0.081
Amudat	KFOL2	KFOL48	0.196
Amudat	KFOL13	KFOL48	0.109
Amudat	KFOL17	KFOL48	0.000
Amudat	KFOL24	KFOL48	0.004
Amudat	KFOL28	KFOL48	0.065
Amudat	KFOL30	KFOL48	0.000
Amudat	KFOL37	KFOL48	0.062
Amudat	KFOL42	KFOL48	0.054
Amudat	KFOL47	KFOL48	0.218
Moroto	KFOL2	KFOL13	0.231
Moroto	KFOL2	KFOL17	0.093
Moroto	KFOL13	KFOL17	0.031
Moroto	KFOL2	KFOL24	0.475
Moroto	KFOL13	KFOL24	0.192
Moroto	KFOL17	KFOL24	0.035
Moroto	KFOL2	KFOL28	0.184
Moroto	KFOL13	KFOL28	0.111
Moroto	KFOL17	KFOL28	0.197
Moroto	KFOL24	KFOL28	0.029
Moroto	KFOL2	KFOL30	1.000
Moroto	KFOL13	KFOL30	0.622
Moroto	KFOL17	KFOL30	0.011
Moroto	KFOL24	KFOL30	0.012
Moroto	KFOL28	KFOL30	0.089
Moroto	KFOL2	KFOL37	0.509
Moroto	KFOL13	KFOL37	0.214
Moroto	KFOL17	KFOL37	0.022
Moroto	KFOL24	KFOL37	0.002
Moroto	KFOL28	KFOL37	0.002
Moroto	KFOL30	KFOL37	0.000
Moroto	KFOL2	KFOL42	0.360
Moroto	KFOL13	KFOL42	0.320
Moroto	KFOL17	KFOL42	0.566
Moroto	KFOL24	KFOL42	0.014
Moroto	KFOL28	KFOL42	0.001
Moroto	KFOL30	KFOL42	0.002
Moroto	KFOL37	KFOL42	0.001
Moroto	KFOL2	KFOL47	0.712
Moroto	KFOL13	KFOL47	0.511
Moroto	KFOL17	KFOL47	0.239
Moroto	KFOL24	KFOL47	0.032
Moroto	KFOL28	KFOL47	0.000
Moroto	KFOL30	KFOL47	0.000
Moroto	KFOL37	KFOL47	0.113
Moroto	KFOL42	KFOL47	0.001
Moroto	KFOL2	KFOL48	0.752
Moroto	KFOL13	KFOL48	0.045
Moroto	KFOL17	KFOL48	0.717
Moroto	KFOL24	KFOL48	0.274
Moroto	KFOL28	KFOL48	0.029
Moroto	KFOL30	KFOL48	0.000
Moroto	KFOL37	KFOL48	0.059
Moroto	KFOL42	KFOL48	0.000
Moroto	KFOL47	KFOL48	0.000
Nakapiripirit	KFOL2	KFOL13	0.717
Nakapiripirit	KFOL2	KFOL17	0.570
Nakapiripirit	KFOL13	KFOL17	0.393

Nakapiripirit	KFOL2	KFOL24	0.429
Nakapiripirit	KFOL13	KFOL24	0.270
Nakapiripirit	KFOL17	KFOL24	0.257
Nakapiripirit	KFOL2	KFOL28	-
Nakapiripirit	KFOL13	KFOL28	-
Nakapiripirit	KFOL17	KFOL28	-
Nakapiripirit	KFOL24	KFOL28	-
Nakapiripirit	KFOL2	KFOL30	0.447
Nakapiripirit	KFOL13	KFOL30	0.553
Nakapiripirit	KFOL17	KFOL30	0.125
Nakapiripirit	KFOL24	KFOL30	0.486
Nakapiripirit	KFOL28	KFOL30	-
Nakapiripirit	KFOL2	KFOL37	0.520
Nakapiripirit	KFOL13	KFOL37	0.068
Nakapiripirit	KFOL17	KFOL37	0.789
Nakapiripirit	KFOL24	KFOL37	0.493
Nakapiripirit	KFOL28	KFOL37	-
Nakapiripirit	KFOL30	KFOL37	0.116
Nakapiripirit	KFOL2	KFOL42	-
Nakapiripirit	KFOL13	KFOL42	-
Nakapiripirit	KFOL17	KFOL42	-
Nakapiripirit	KFOL24	KFOL42	-
Nakapiripirit	KFOL28	KFOL42	-
Nakapiripirit	KFOL30	KFOL42	-
Nakapiripirit	KFOL37	KFOL42	-
Nakapiripirit	KFOL2	KFOL47	0.814
Nakapiripirit	KFOL13	KFOL47	0.254
Nakapiripirit	KFOL17	KFOL47	0.106
Nakapiripirit	KFOL24	KFOL47	0.009
Nakapiripirit	KFOL28	KFOL47	-
Nakapiripirit	KFOL30	KFOL47	0.337
Nakapiripirit	KFOL37	KFOL47	0.991
Nakapiripirit	KFOL42	KFOL47	-
Nakapiripirit	KFOL2	KFOL48	0.702
Nakapiripirit	KFOL13	KFOL48	0.141
Nakapiripirit	KFOL17	KFOL48	0.331
Nakapiripirit	KFOL24	KFOL48	0.000
Nakapiripirit	KFOL28	KFOL48	-
Nakapiripirit	KFOL30	KFOL48	0.200
Nakapiripirit	KFOL37	KFOL48	0.683
Nakapiripirit	KFOL42	KFOL48	-
Nakapiripirit	KFOL47	KFOL48	0.052

Appendix 6 Table 14 Chi-Square Tests for Hardy-Weinberg Equilibrium per loci per population

Pop	Locus	DF	Chi-Square	Probability	Significance
Mt Elgon	KFOL2	6	9.930	0.128	ns
Mt Elgon	KFOL13	15	4.457	0.996	ns
Mt Elgon	KFOL17	91	109.382	0.092	ns
Mt Elgon	KFOL24	91	96.966	0.315	ns
Mt Elgon	KFOL28	15	23.824	0.068	ns
Mt Elgon	KFOL30	36	22.782	0.958	ns
Mt Elgon	KFOL37	105	102.765	0.543	ns
Mt Elgon	KFOL42	10	0.860	1.000	ns
Mt Elgon	KFOL47	28	70.581	0.000	***
Mt Elgon	KFOL48	10	4.024	0.946	ns
Baringo	KFOL2	10	2.081	0.996	ns
Baringo	KFOL13	36	57.235	0.014	*
Baringo	KFOL17	78	110.628	0.009	**
Baringo	KFOL24	78	88.994	0.185	ns
Baringo	KFOL28	28	56.292	0.001	**
Baringo	KFOL30	78	119.290	0.002	**
Baringo	KFOL37	120	128.879	0.273	ns
Baringo	KFOL42	6	30.104	0.000	***
Baringo	KFOL47	66	221.895	0.000	***
Baringo	KFOL48	10	22.891	0.011	*
Mau	KFOL2	10	9.392	0.495	ns
Mau	KFOL13	36	74.537	0.000	***
Mau	KFOL17	91	74.271	0.899	ns
Mau	KFOL24	45	52.151	0.216	ns
Mau	KFOL28	10	20.078	0.029	*
Mau	KFOL30	66	59.636	0.696	ns
Mau	KFOL37	66	73.538	0.245	ns
Mau	KFOL42	3	0.036	0.998	ns
Mau	KFOL47	36	53.777	0.029	*
Mau	KFOL48	3	11.302	0.010	*
Laikipia	KFOL2	3	5.723	0.126	ns
Laikipia	KFOL13	6	26.954	0.000	***
Laikipia	KFOL17	21	26.985	0.171	ns
Laikipia	KFOL24	28	43.396	0.032	*
Laikipia	KFOL28	10	8.682	0.563	ns
Laikipia	KFOL30	36	29.717	0.761	ns
Laikipia	KFOL37	10	12.238	0.269	ns
Laikipia	KFOL42	3	26.059	0.000	***
Laikipia	KFOL47	10	31.414	0.001	***
Laikipia	KFOL48	1	0.968	0.325	ns
Amudat	KFOL2	10	22.506	0.013	*
Amudat	KFOL13	3	2.367	0.500	ns
Amudat	KFOL17	190	275.102	0.000	***
Amudat	KFOL24	21	122.691	0.000	***
Amudat	KFOL28	3	120.000	0.000	***
Amudat	KFOL30	36	103.897	0.000	***
Amudat	KFOL37	10	17.028	0.074	ns
Amudat	KFOL42	3	37.593	0.000	***
Amudat	KFOL47	91	182.581	0.000	***
Amudat	KFOL48	28	178.359	0.000	***
Moroto	KFOL2	3	8.210	0.042	*
Moroto	KFOL13	3	40.000	0.000	***
Moroto	KFOL17	91	100.694	0.229	ns
Moroto	KFOL24	78	112.041	0.007	**
Moroto	KFOL28	10	25.841	0.004	**
Moroto	KFOL30	21	24.980	0.248	ns
Moroto	KFOL37	55	88.582	0.003	**
Moroto	KFOL42	6	33.645	0.000	***
Moroto	KFOL47	21	66.176	0.000	***
Moroto	KFOL48	10	40.274	0.000	***
Nakapiripirit	KFOL2	3	1.650	0.648	ns
Nakapiripirit	KFOL13	15	29.728	0.013	*
Nakapiripirit	KFOL17	120	143.111	0.074	ns
Nakapiripirit	KFOL24	10	11.159	0.345	ns
Nakapiripirit	KFOL28	Monomorphic			
Nakapiripirit	KFOL30	28	57.371	0.001	***

Nakapiripirit	KFOL37	1	0.515	0.473	ns
Nakapiripirit	KFOL42		Monomorphic		
Nakapiripirit	KFOL47	36	32.049	0.657	ns
Nakapiripirit	KFOL48	15	26.822	0.030	*

Appendix 7 Table 6.3 *O. lanceolata* morphology across sites in Karamoja

Sampled sites	Mean height (m)	Mean number Stems (n)	OL: OL Spacing (m)	Stem Diameter (cm)	Crown Cover(m)
Akariwon (AKA)(HA)	1.99	4	13.76	3.99	1.48
Lonyilik (LON)(HA)	2.17	3	60.94	3.61	1.61
Karengepoche (KAR)(HA)	2.53	4	91.19	4.91	1.81
Lolupe (LOP)(LA)	1.65	4	25.62	2.50	1.18
Kopedur (KOP)(LA)	1.51	3	86.64	2.61	1.07
Lotemwoyes (LOT)(LA)	1.32	3	16.21	1.54	0.92
Cheporon (CHP)(LA)	2.1	5	68.97	4.93	1.60
Ngaram (NGA)(LA)	2.5	3	50.51	4.79	1.80
Ruwotokech (RUW)(LA)	2.65	6	38.72	4.89	1.86
Korenyang (KOR)(LA)	1.89	4	56.71	3.52	1.48
Kangisa (KAN)(LA)	2.01	3	70.53	3.55	1.62

Akariwon (AKA); Cheporon (CHP); Ngaram (NGA); Lolupe (LOP); Lonyilik (LON); Ruwotokech (RUW); Karengepoche (KAR); Lotemwoyes (LOT); Kangisa (KANG); Korenyang (KOR); Osyris lanceolata (OL) and Kopedur (KOP).LA: Low altitude; HA: high altitude

Appendix 8: Table: 6.7 Regression between height and stem diameter of coppiced *O. lanceolata* stems

	Min	1Q	Median	3Q	Max
Coefficients	1.78906	-0.73944	-0.01015	0.81094	2.08985
	Estimate Std.	Error	t value	Pr(>|t|)	
Intercept	5.1797	0.8856	5.849	0.000244 ***	
Height.coppiced.OL.	-0.2109	0.2705	-0.780	0.455459	
Signif. codes:	0 '***'	0.001 '**'	0.01 '	*' 0.05 '.	0.1 1
Residual standard error:	*1.2 on 9 degrees of freedom*				
Multiple R-squared:	*0.06331,*	*Adjusted R-squared:*	*-0.04077*		
F-statistic:	*0.6083 on 1 and 9 DF, p-value:*		*0.4555*		

Appendix 9: Table: 6.8 Regression results on stem diameter and height of non-coppiced stems

	Min	1Q	Median	3Q	Max
	-1.1613	-0.7489	-0.3859	0.8120	1.3908
Coefficients:					
	Estimate Std.	Error	t value	Pr(>|t|)	
Intercept	-1.0732	2.5758	-0.417	0.6867	
Ht_noncopp_OL.m.	2.7535	0.9378	2.936	0.0166 *	
Signif. codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '	0.1 '
Residual standard error:	1.011 on 9 degrees of freedom				
Multiple R-squared:	0.4892,	Adjusted R-squared: 0.4325			
F-statistic:	8.62 on 1 and 9 DF, p-value: 0.01659				