CHARACTERIZATION OF SANDFLY POTENTIAL VECTORS AND RESERVOIR HOSTS OF CUTANEOUS LEISHMANIASIS AND THEIR HABITATS IN BUNGOMA COUNTY, KENYA

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has never been presented for the award of an academic degree in any other university and should not be copied, or reproduced in any format without written authority from the author and/or University of Eldoret.

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DEDICATION

I dedicate this work to my lovely aunt Leah Namalwa for the financial support and encouragement, and to my loving wife, Clare, and my daughters, Princess and Celine for enduring my long absence from home, your prayers, goodwill, encouragement and all the sacrifices you made that enabled me ample time to focus on my postgraduate studies. I wish you God's blessings.

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ABSTRACT

Cutaneous leishmaniasis (CL), a neglected tropical disease caused by *Leishmania* parasites and transmitted by sandfly vectors, is a significant health concern in Bungoma County, and other Kenyan leishmaniasis-endemic counties. The present study aimed to characterize the distribution and ecological attributes of habitats of sandfly vectors and reservoir hosts of CL. The research objectives included: determining sandfly species diversity and abundance in identified sandfly habitats, evaluating the effects of temperature, relative humidity, and altitude on sandfly occurrence, analyzing soil characteristics in sandfly habitats for their physical and chemical properties, determining the association of sandfly populations and plant species populations in the study area, and evaluating the relationship between potential vertebrate reservoir hosts populations and sandfly populations in the study area. Sandflies were collected from their habitats using CDC light traps, dissected and identified morphologically between January 2021 and December 2022. Species diversity and evenness was analyzed using the Shannon-Weinner's diversity index. A total of 6,156 sandflies were captured, with *Phlebotomus pedifer* accounting for 94%, *P. elgonensis* 0.5%, and *Sergentomyia* species 5.5%of the collections. Environmental factors, including temperature (21–29°C), relative humidity (75-90%) and altitude (1,506–3,100 masl), were significant ($P < 0.05$) determinants of sandfly distribution. The correlation between soil properties and sandfly abundance was non-significant. Plant species diversity index (3.14 *H'*) and vertebrate diversity index (2.06 *H'*) indicated strong association with sandfly abundance, highlighting ecological drivers of the vector populations. The study concluded that *Phlebotomus pedifer* is the dominant sandfly vector species of CL in Bungoma County; while it is the people who go to the vector habitats (caves) who get bitten and become infected with CL. These findings underscore the need for habitat-specific interventions, such as environmental management to control sandfly populations in caves to reduce the transmission of CL in the study area and other similar regions.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

AAS -Atomic absorption spectrophotometer

ANOVA - Analysis of variance

C - Carbon

Ca - Calcium

CDC -Center for Disease Control and Prevention

CDC-LT - Center for Disease Control and Prevention Light Trap

CL - Cutaneous leishmaniasis

Cu - Copper

df – Degrees of freedom

e - Shanon Weiner's diversity evenness

Fe - Iron

H' - Shanon Weiner's diversity index

KEMRI - Kenya Medical Research Institute

LSD - Least significance difference

M^2 – Meters square

MASL - Meters above the sea level

MCL - Mucocutaneous leishmaniasis

Mg - Magnesium

Mn - Manganese

MoH - Ministry of Health

MSC - Master of Science

N - Nitrogen

n - total number counts

NACOSTI - National Commission of Science, Technology and Innovation

P - Phosphorus

P -Phlebotomus

PBS - Phosphate buffered saline

r - Correlation value

S – Sergentomyia

Spp -Species

TEA -Trimethylamine

VL - Visceral leishmaniasis

WHO - World Health Organization

χ^2 - Chi square

Zn – Zinc

() – Parenthesis

% - Percentage

± - Plus or minus sign

* - Values that are significantly different at $p < 0.05$

** - Values that are significantly different at $p < 0.01$

CHAPTER ONE

INTRODUCTION

1.1 Background and Scope of Study

Leishmaniasis is a vector-borne protozoan disease caused by obligate intra-macrophagic parasite species of genus *Leishmania* (family *Trypanosomatidae*) (Akhoundi *et al.,* 2016). Leishmaniasis manifests in three clinical forms which include the most common cutaneous leishmaniasis (CL) which causes skin lesions mainly ulcerous on exposed parts of the body, the most serious is visceral leishmaniasis (VL) which is also known as kala-azar that affects the liver, spleen and the bone-marrow, and the muco-cutaneous leishmaniasis (MCL) that partially or totally destroys the mucus membranes of the nose, throat or mouth (WHO, 2023). The clinical disease forms that is manifested is dependent on the species of the infecting parasite and the respective vector species as well as the availability of suitable reservoir vertebrate hosts endemic to a given localized region at any specific time (CDC, 2023).

According to Jones and Welburn, (2021), the prevalence of VL in Kenya is so high and is characterized by epidemic episodes across the arid and semi-arid counties including Baringo, West Pokot, Turkana, Isiolo, Samburu, Garissa, Marsabit, Kitui, Machakos, Meru, Wajir and Mandera. Kenya is amongst the top ten countries with the highest prevalence records of VL that is ever increasing since 2018 that recorded 607 reported cases, 2019 had 1463 cases, while 2021 reported 1746 cases. The incidence rate for Kenya currently stands at 2.96/10000 persons in highly endemic counties, with over 5 m people being at risk (Grifferty *et al*., 2023). The prevalence of CL in Kenya is patchy due to insufficient documentation of research activities around this form of disease that

is endemic in some counties of Kenya including Bungoma, Trans Nzoia, Baringo, Nakuru and Laikipia (Ngere *et al*., 2020).

The disease has complex and diverse transmission modes comprising of over 20 species of different *Leishmania* parasites and over 90 sandfly vectors of the genera *Lutzomyia* and *Phlebotomus* that are known to affect humans in the New World and Old World respectively (WHO, 2023). Leishmaniasis affects large areas in the tropical, sub-tropical and the Mediterranean regions where most of the poorest and most vulnerable communities live and it has been described as a neglected tropical disease. Humans together with some other vertebrates serve as reservoir hosts in the transmission of leishmaniasis (Alten *et al*., 2016). The reservoir hosts get infected when infected female phlebotomine sandflies bite the hosts for blood meals, which are essential for development of the vectors' reproductive cycle (Moncaz *et al*., 2012). The disease is passed to humans as an anthroponosis and/or a zoonosis (Alten *et al*., 2016).

In Kenya, sandfly vectors which transmit visceral leishmaniasis have been commonly associated with lowlands including the Rift Valley basin, the Eastern region and the North-Eastern region; whereas cutaneous leishmaniasis has been confirmed to occur in the highland regions like along the slopes of Mt. Elgon, the Aberdares range and at the Rift Valley plateaus (Anjili *et al*., 2011; Dulacha *et al*., 2019). Some of the specific habitats for vector sandflies in Kenya have been identified as rock crevices, mountain caves, under the dense forest covers, tree holes, decomposing waste debris, in corners of domestic animal shelters, inside animal burrows or nests, termite mounds, and even in the open crevices of human shelter walls as well as at the roofs of grass-thatched houses (Ngere *et al*., 2020, Mukhwana *et al*., 2018).

Successful establishment of leishmaniasis endemicity at any given place requires widely varied vector habitats and factors which are determined by favorable climatic conditions, availability of suitable reservoir vertebrate host, the vector sandfly species, as well as the infective *Leishmania* parasites (Oryan and Akbari, 2016). Favorable habitats for *Leishmania* vectors are therefore diverse as they have been confirmed to range between the warm and humid tropical climates, dry semi-arid places, vegetative and dry arid climates, to a few species of vectors and parasites which survive in the temperate ecological zones (Akhoundi *et al*., 2016; Koch *et al*., 2017).

Sandflies do not only have rural and peri-urban preferences for habitats, but they also colonize urbanized places due to massive movement of people who readily provide blood meal sources for these vectors (Mukhwana, 2018; Ngere *et al*., 2020; Santamaría *et al*., 2020; WHO, 2023). The rural and peri-urban habitation of sandflies also ensures ease of access to reservoir hosts which include wild and domestic animals as well as humans by the female vectors for easily accessible blood meals (Munoz *et al*, 2018). Sandflies' eggs need humid microclimate to develop to larvae which in turn require cool, moist habitats that is rich in decaying matter to be able to develop to adult forms (Feliciangeli, 2004). The adult sandflies often inhabit caves, rock crevices, rodent burrows, tree holes and termite mounds (Moncaz *et al*., 2012; Munstermann 2019; Wijerathna and Gunathilaka 2020). Adult sandflies that inhabit domestic and peri-urban settings shelter in the cool, dark and humid corners of animal shelters and human dwellings respectively (Koch *et al*, 2017).

1.2 Statement of the Problem

According to the World Health Organization, over 350 million people are at risk of acquiring leishmaniasis, with estimated annual new cases of about 700,000 to 1 million being actively recorded worldwide (WHO, 2023). In Kenya, leishmaniasis endemicity

is estimated to affect over 30 counties (Anjili *et al*., 2011). This is indicated by active human cases being reported every year and the presence of different species of sandfly vectors and reservoir hosts but the distribution of their known and unknown habitats has not been characterized (Njau, 2010; Anjili *et al*., 2011; Dulacha *et al*., 2018). Therefore, proper understanding of the epidemiological determinants of this disease, the distribution of the suspected vector sandflies, the potential reservoir hosts and characterization of their habitats will help to generate helpful policies aimed at formulating effective intervention measures towards leishmaniasis disease prevention, control and management.

Previously, research studies on leishmaniasis transmission in the western Kenya region only focused on sandfly vectors found in a few volcanic caves in Mt. Elgon Sub-County. There has been no documentation of evidence of previous research activities on leishmaniasis reported in the other Sub-Counties of Bungoma County, despite the availability of wide habitat ecology suitable for sandfly habitation with features similar to those found in the Mt. Elgon Sub-County. This study therefore aimed at confirming and characterizing suspected leishmaniasis vectors and reservoir hosts habitats in more sub-counties in Bungoma County, which included suspected habitats in Bungoma West (Sirisia constituency), Bungoma Central (Kabuchai constituency), Bungoma South (Kanduyi constituency) and Webuye East constituency. These sub-counties have not been assessed for existence of habitats of sandflies and reservoir hosts of leishmaniasis before.

In Kenya, Baringo County in the Rift valley, is the only known leishmaniasis focus where both VL and CL form of the disease co-occur (Tonui, 2006). However, Kenya's Ministry of Health guidelines to prevention, diagnosis and treatment of visceral leishmaniasis (MoH, 2017), reports that there are many cases of visceral leishmaniasis

around the western Kenya region, but logistical and expansive problems have been a major impediment to carrying out effective epidemiological research to ascertain this. The geographical location of Bungoma County and other counties (including Busia, Trans Nzoia, West Pokot, Turkana), which are along the Kenya–Uganda border, calls for further research investigations to characterize sandfly and reservoir species and their habitats to better understand the extent of potential transmission of CL and VL leishmaniasis in the region. The expansiveness of Bungoma County, existence of different physical features therein and varied climatic conditions (from cool and wet highlands in the slopes of Mt. Elgon to relatively warm and dry low lands of Bumula and Kanduyi Sub-Counties) also presents the opportunity for potential habitats for vector and reservoir host species of leishmaniasis in the wider Bungoma County which needed to be investigated.

Sandflies oviposit in the crevices and holes in the ground, inside houses, in caves, in animal burrows and in tree holes since the eggs require highly moist micro-climate to hatch (Nguyen *et al.,* 2021). Larvae too, need moist, cool microclimate rich in decomposing organic matter such as fungi, animal feces, decaying leaves and arthropods to develop and grow (Peterkova-Koci *et al.,* 2012 and Marayati *et al.,* 2015). Therefore, the biological, physical and chemical constituents of the soils in suspected sandfly habitats needed to be analyzed to determine the soil characteristics that are suitable for sandfly development.

Over the years, the understanding of the sandfly vector-parasite-host interactions has considerably improved. Therefore, there is need to understand the sandfly vector and reservoir host habitat characteristics in the leishmaniasis foci. The mean temperature and relative humidity of habitats should also be assessed to determine the suitable niche conditions for the sandflies. Adult male and female sandflies feed on plant sugars

(Abbasi *et al*., 2018) although the females additionally require vertebrate blood meal for successful reproduction. Therefore, the plants associated with confirmed sandfly habitats should be investigated to determine which ones provide the sugar meals for the vector (Hassaballa *et al*., 2021a). Furthermore, phytochemicals from the plants are considered attractants or repellents to the vectors (Lorenz *et al*., 2013). These habitat characteristics can be used to predict potential sites for targeting in leishmaniasis vectors and reservoir hosts' control.

1.3 Objectives

1.3.1 Broad Objective

To investigate the distribution and characteristics of habitats of sandfly vectors and reservoir hosts of leishmaniasis in Bungoma County, Kenya

1.3.2 Specific Objectives

i To determine sandfly species diversity, their spatial and temporal distribution in their habitats in Bungoma County

ii To assess the impact of temperature, relative humidity and altitude on the occurrence and abundance of sandflies in the identified habitats in Bungoma County

iii To determine the physical and chemical characteristics of soil in the habitats of sandflies and reservoir hosts of leishmaniasis in Bungoma County

iv To assess the sandfly–plant species association in habitats of sandflies in Bungoma County

v To evaluate the relationship between the occurrence of vertebrate hosts in proximity of sandfly habitats, and the occurrence and abundance of the sandflies in the habitats in Bungoma County

1.4 Research Alternate Hypotheses

Ha1 Sandfly species inhabit locations with diverse characteristics in Bungoma County

Ha2 Temperature, relative humidity and altitude influence the occurrence and abundance of sandflies in different habitats in Bungoma County

Ha3 Suspected sandfly vectors and vertebrate potential reservoir hosts of leishmaniasis are found in habitats with different physical and chemical characteristics of the soil in Bungoma County

Ha4 Plant species distribution and abundance influence sandfly species distribution and abundance in Bungoma County

Ha5 Sandfly populations are influenced by the presence of different types of vertebrates found in the sandfly habitats

1.5 Justification for the Study

Cases of CL have been reported in the Mt. Elgon region (Kung'u *et al*., 1972; Sang *et al*., 1993; Mukhwana *et al.*, 2018; Ombaka *et al*., 2023). Sandfly vectors and leishmaniasis reservoir hosts have also been identified in Mt. Elgon region, albeit the previous studies being restricted to a few characteristic caves in Mt. Elgon Sub-County (Mutinga 1975; Makwali, 2021; Ombaka *et al*., 2023). However, caves with similar features exist in other Sub-Counties in Bungoma County but there is no documented data to show that these caves have sandfly vectors and reservoir hosts habitation. During preliminary visits to these other sites, individuals presenting with chronic skin lesions, ulcerative wounds and diffuse face rashes similar to the symptoms of CL described by Mukhwana *et al*., 2018 and Ngere *et al*., 2020, were observed.

Despite leishmaniasis affecting millions of people worldwide; causing significant morbidity and mortality, the ecology that includes vector habitat characteristics, and

behavior of its vectors and reservoir hosts are not fully understood. There is therefore need to further conduct research in this area (Killick-Kendrick & Ward, 1981; Lessa *et al*., 2007; Aoun & Bouratbine, 2014; Bailey *et al*., 2017). Additionally, the current effect of climate change is expected to impact on the distribution and abundance of not only sandfly species and reservoir hosts, but also associated infective pathogens that include *Leishmania* parasites (Githeko *et al*., 2000; Gage *et al*., 2008; Almeida *et al*., 2015; Daoudi *et al*., 2022). The chemical and physical characteristics of soil have also been confirmed in other places that they significantly influence the vector and reservoir hosts populations (Basimike, 1988; Abdullah *et al*., 2017). For these reasons, understanding the ecology of these organisms is akin to predicting future leishmaniasis transmission dynamics, and thus will inform planning and implementation of effective control measures. There is urgent need of coming up with a comprehensive approach to controlling zoonotic diseases including CL by incorporating the diverse determinant risk factors ranging from human, other animals and the environmental variables so as to easily identify obvious/ specific risk factors for transmission of such diseases (Hong *et al*., 2020; de Thoisy *et al*., 2021; Valero *et al*., 2021).

To determine the trend that would help in understanding the actual spatial distribution of the vectors, this research was conducted to establish the relationship between the size of the cave habitats and the sandfly numbers captured in each. The presence of caves that serve as habitats for sandflies was used to predict potential sandfly existence in the study area. This needed to be confirmed by extensive field surveys.

Adult male and female sandflies feed on plant sugars although the females require vertebrate blood meal for successful reproduction. Therefore, there was need to assess the plant species that are associated with sandfly habitats to also assist in prediction of priority areas to be targeted for sandfly vector control.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nature and Global Burden of Leishmaniasis

Leishmaniasis is a protozoan disease that is caused by single-celled obligate intracellular parasites belonging to the genus *Leishmania* (Order: Kinetoplastida) transmitted via bites of infected female sandflies of the genus *Phlebotomus* and *Lutzomyia* in the Old World and New World respectively (WHO 2023).

The disease is caused by infection with over twenty species of the *Leishmania* parasites (Colmenares *et al.,* 2002; Peacock *et al.,* 2007 and Akhoundi *et al.,* 2016). It has been confirmed that over ninety sandfly species are the vectors responsible for the transmission of *Leishmania* parasites causing visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and muco-cutaneous leishmaniasis (MCL) (Killick-Kendrick *et al.,* 1999; Warburg *et al.,* 2011; Bates *et al.,* 2015; and Almeida *et al.,* 2015). Visceral leishmaniasis is also known as kala-azar and is the most fatal form of the disease since about 95% of the cases normally die if they are left untreated (CDC, 2020). VL is characterized by enlarged spleen and liver, anemia, loss of weight and irregular bouts of fever (Sakkas *et al.,* 2016; Goto *et al.,* 2017; CDC, 2020). It is estimated that only less than 45% of VL cases comprising of about 50,000 to 90,000 new cases every year are reported to the WHO globally (Alvar *et al*., 2012; Torres-Guerrero *et al.,* 2017; WHO 2023). VL is one of the top parasitic disease forms with the highest potential of outbreaks and mortality (Desjeux, 2004; des Boer *et al.,* 2009). Most VL cases are usually reported in Brazil, East Africa and India. WHO (2023) reported that 90% of new cases in the year 2020 alone were reported in Brazil, China, Ethiopia, Somalia, South Sudan, Sudan and Yemen. In Africa and South Asia, *L. donovani* is the only

etiological agent for VL whereas *L. infantum* is responsible for VL in India, Middle East, the Mediterranean, Latin America and some parts of Asia (WHO 2023).

Cutaneous leishmaniasis (CL) is described as the most common form of the disease characterized by skin lesions and/or ulcers on the exposed part(s) of the body that leaves permanent scars which may lead to serious disability or stigma (Ngure *et al.,* 2009; Honorio *et al.,* 2016).It is estimated that between 700,000 to 1.2 million new cases of CL are reported worldwide every year with about 95% of the cases occurring in the Americas, the Mediterranean basin, the Middle East and Central Asia (Alvar *et al*., 2012; Ramirez *et al.,* 2016).

Muco-cutaneous leishmaniasis (MCL) is another form of leishmaniasis which is so disfiguring in that it may lead to partial or total destruction of mucus membranes of the nose, mouth and throat (Bittencourt & Barral 1991; David and Craft, 2009; Goto and Lindoso, 2010). It is estimated that over 90% of the MCL cases occur in Bolivia, Brazil, Ethiopia and Peru (Alvar *et al*., 2012). There is also another form of leishmaniasis that is called post kala-azar dermal leishmaniasis (PKDL) which normally presents with general skin rash and it comes as a sequel of most recent infection and recovery from VL (Desjeux *et al.,* 2013 and Zijlstra 2016).

2.2 Infection and Transmission of Leishmaniasis

Infected female sandflies transmit *Leishmania* parasites that cause leishmaniasis to humans as they bite humans for blood meals that are required for eggs development (Williams and Coelho 1978; Bates, 2007). The infective stage of the *Leishmania* parasites called promastigotes are injected from the infected sandflies' proboscis during blood meal feeding into the host's skin (Teixeira *et al.,* 2013). These promastigotes then get phagocytosed by the host's macrophages and other types of mononuclear

phagocytic cells (Veras and Bezerra, 2016). The promastigotes then transform inside these cells into the tissue stage amastigotes forms which multiply by simple division and infect other mononuclear phagocytic cells (Beattie and Kaye, 2011; Giraud *et al.,* 2019).

The form of disease (CL, VL or MCL) which will result from a successful infection, will be determined by the type of parasite, host, vector, and other factors and most of such infections are asymptomatic, while others become symptomatic (Alemayehu & Alemayehu 2017; Bates 2018; Serafim *et al.,* 2021).

Sandflies become infected by ingesting bloodmeal(s) from infected host(s) and inside the sandflies' midgut, the parasites transform from amastigotes into promastigotes which develop in the gut and migrate to the proboscis ready to infect a subsequent host when the sandfly bites for another blood meal (Nzelu *et al.,* 2014; Sant'Anna *et al.,* 2014 and Dantas *et al.,* 2014).

The most common sandfly species in Kenya are *Phlebotomus martini* and *Phlebotomus orientalis* which transmit visceral leishmaniasis while *Phlebotomus pedifer*, *Phlebotomus elgonensis, Phlebotomus duboscqi* and *Phlebotomus guggisbergi* transmit cutaneous leishmaniasis (Ngumbi *et al.,* 2010, Anjili *et al*., 2011 and Gitari *et al.,* 2018). Previously, it was believed that *P. orientalis* did not occur in large numbers to warrant VL outbreaks in Kenya because *P. martini* was the only sandfly species that occurred in large numbers and was always strongly associated with VL outbreaks in the Kenya's kala-azar endemic regions (Young 1982; Mutinga *et al.,* 1989-v, Ngumbi *et al.,* 1992 and Britch *et al.,* 2011) However, Ngumbi *et., al.,* (2010) confirmed that *P. orientalis* was the only sandfly species that was found to transmit *L. donovani* in the VL outbreaks that were reported in Wajir and Isiolo Counties in the years 2000, 2003 and 2006. It

was suspected that *P. orientalis* which has been associated with acacia and balanite tree species and responsible for VL epidemics in Sudan had managed to move southwards into the Kenya's neighboring counties. *Phlebotomus pedifer* and *P. elgonensis* have not been associated with any plant species so far in Kenya. This needs to be investigated.

In-depth knowledge of the sandfly's breeding and resting sites to inform effective vector control and thus management of leishmaniasis has been a hard nut to crack world over. A lot of research activities have been conducted around this subject with the aim of understanding the sandfly habitats so as to develop novel techniques or products that can effectively keep in check the devastating effects brought about by leishmaniasis infection to humans. Up to date, very little has been achieved in attempts to deeply understand, not only the characteristics of sandflies' breeding sites, but also their resting sites too (Moncaz *et al*., 2012). However, it is known that sandfly larvae develop in moist and dark micro-habitats that are rich in organic matter with stable climatic conditions. Srinivasan *et al*., (2015) reported that the fluvial landforms, which are non-porous with high water retention capacity, with high alkaline pH level which supports rice cultivation and luxuriant vegetation cover, had increased sandfly densities than porous and acidic landforms in Southern India. Since sandflies are sensitive to dehydration, fluvial landforms are suitable for their survival and development. Very scanty information exists in Kenya on research focusing on soil characteristics and sandfly habitats (Mutinga and Kamau 1986; Basimike *et al.,* 1992; Elnaiem 2011). It is important to investigate the suitable soil characteristics to enable prediction of locations with soil that are favorable for sandfly development. This knowledge will inform on appropriate sites for targeted vector control.

There are different types of micro-ecological niches incriminated to be suitable for sandfly habitation across varied ecological zones in the areas found to be endemic to

leishmaniasis infections (Ghazanifar & Malik 2016). Some of the most commonly reported locations and sites include caves, rock crevices, termite mounds, animal burrows, cracks in soil, cracked human housing walls, domestic animal shelters, tree holes, bird nests and on organic litter (Moncaz *et al*., 2012).

Research carried out by Pareyn *et al*., (2019) on ecology and seasonality of sandfly in Southern Ethiopia revealed caves as the only habitats harboring sandfly species of *Phlebotomus pedifer* which transmit *Leishmania ethiopica* to cave hyraxes and rodents. Previous studies in Mt. Elgon focus of CL in Western Kenya also found *P. pedifer* in cave habitats and confirmed the species as the vector of *L. ethiopica* (Mutinga 1975, Sang & Chance, 1993 and Mukhwana *et al*., 2018).

Further studies have revealed that some sandfly species are associated with certain tree species, particularly the sandfly vector species, *Phlebotomus orientalis* that inhabit the arid and semi-arid areas (Elnaim, 2011) which are associated with desert tree species (associated with black cotton soils) such as the *Balanite aegyptica* and *Acacia seyal. Phlebotomus martini* and *Phlebotomus celiae* have been associated with termite mounds. These sandfly species are vectors of VL in Eastern Africa (Gebre-Michael & Lane., 1996).

In Kenya, most of the leishmaniasis foci have been found to be highly classified where only a single form of the disease transmission occurs (Tonui, 2006). Mukhwana *et al*., (2018) also reported a single role of *L. ethiopica* parasite species CL transmission focus in the Mt. Elgon focus in Western Kenya. However, the Baringo leishmaniasis transmission focus is so far the only location where transmission of mixed forms of both CL and VL occur (Anjili *et al*., 2011).

Cutaneous leishmaniasis in Kenya occurs in a broad range of environmental settings ranging from river valleys, semi-arid and arid lowlands and also in the heights of highland plateaus and mountains (Feliciangeli 2004; Elnaiem 2011; Anjili *et al*., 2011). CL is caused by *Leishmania major* in Baringo, *L. tropica* in Nyandarua, Kitui, Nakuru, Laikipia, Samburu and Isiolo while in Mt. Elgon focus, CL is caused by *L. ethiopica* (Mutinga *et al.,* 1986-iii; Anjili *et al.,* 2011 and Gitari *et al.,* 2018). Sandfly species responsible for active transmission of cutaneous leishmaniasis in Kenya include *P. duboscqi* and *P. guggisbergi* which transmits *L. major* and *L. tropica* respectively, while *P. pedifer, P. elgonensis* and *P. longipes* have been implicated in the transmission of *L. ethiopica* (Mahamat & Hassanali 1998; Tonui, 2006 and Anjili *et al*., 2011).

Cutaneous leishmaniasis transmission has been shown to be both anthroponotic and zoophilic (Ghazanifar and Malik 2016). This is where sandfly vectors pass the parasites from infected humans to other uninfected humans or from infected animal reservoir hosts to humans respectively.

Visceral leishmaniasis (kala-azar) which is the most severe form of the disease comes with high morbidity and absolute fatality of cases if left untreated, is caused by *Leshmania donovani* in Kenya and other parts of Africa and it is an anthroponosis (Ghazanifar and Malik 2016; WHO 2023). This means that infected humans provide blood meal sources to the vectors which in turn lead to anthroponotic transmission of *Leishmania donovani* parasites responsible for the etiology of VL, transmitted by specific sandfly species from infected humans to uninfected humans via blood meal bites. The vector species for VL in Eastern Africa are *Phlebotomus martini* (the only confirmed vector of VL in Kenya) and possibly *Phlebotomus orientalis* (Anjili *et al*., 2011; Rohousova *et al*., 2015). However, a relatively recent study done by Yared *et al*., (2019) on feeding behavior of sandflies in North-Western Ethiopia revealed that the

blood meals in the guts of *P. orientalis* sandfly species confirmed a consistent association of both human and animal (wild and domestic) blood suggesting that VL could also be transmitted as a zoonosis.

In Kenya, the only confirmed vector of visceral leishmaniasis is *P. martini*; which their implicated habitats include termite mounds, animal burrows, tree holes, and house walls. Diagnosed VL cases have been endemic in the low-lying river valleys, arid low-lands and semi-arid plateaus found in the Rift Valley region including Baringo, West Pokot and Turkana, Eastern region of Machakos, Kitui, Meru, Isiolo and Samburu and North-Eastern region of Mandera, Wajir and Garissa (Basimike & Mutinga, 1997; Elnaiem, 2011; Anjili *et al*., 2011). Research done by Kaburi *et al*., (2019) in the rice growing zones of Mwea Irrigation Schemes which borders Mt. Kenya to the East at an altitude of between 1100- 1300 meters above sea level, has also confirmed the presence of *P. martini* and *P. rhodhuini* which suggested that VL vectors could also occur in the non-arid and/or semi-arid urbanized areas in Kenya as opposed to the earlier documentations.

The transmission settings of leishmaniasis have been found to be around domestic, rural and peri-urban locations because female sandfly vectors are highly opportunistic blood feeders having been observed to feed on large variety of vertebrate animals (Widaa *et al*., 2012; Yared *et al*., 2019). This is further strengthened by the report reviewed by Tonui, (2006) implicating domestic dogs as the only confirmed reservoir host for VL in East Africa; whereas the tree hyrax (*Dendrohyrax arboreus*) and rock hyrax (*Procavia capensis*) as well as the giant rat (*Cricetomys* spp) are confirmed reservoirs of *L. ethiopica* (Mutinga 1975). Other rodent species harbor *L. major* and *L. tropica* which are both etiological pathogens of cutaneous leishmaniasis in Kenya (Owino *et al*., 2019).

2.3 Risk Factors for the Transmission of Leishmaniasis

According to the World Health Organization (WHO 2023) report on leishmaniasis, the major human risk factors for transmission of leishmaniasis particularly in the developing world include poor socio-economic conditions, malnutrition and population mobility. Environmental changes and climate change are the other risk factors.

It has been observed that leishmaniasis is associated with poor housing, poor domestic sanitation, lack of balanced diets, migration of non-immune populations to leishmaniasis endemic zones, occupational exposures, massive deforestation, effects of global warming and human behavior which favor increased cases of leishmaniasis in some parts of the world (Tonui, 2006; Mutungi *et al*., 2019 and Abu-Hurub and Okbah, 2022).

The research done in Ethiopia by Yared *et al*., (2014) on the risk factors for VL also revealed that goat ownership, living in the house with cracked walls, increased family size and the number of days spent in the farm are strongly associated with epidemiology of VL in the particular leishmaniasis foci.

In Kenya, Ngere *et al*., (2020) conducted a study on the burden and risk factors of CL in Gilgil and found out that gender also played a role in the infection prevalence since there were more females (56%) than males with a median of 7-year age persons that were diagnosed with CL. Other risk factors included staying outside after sunset, visiting forests, sharing residence, residing in thatched houses, living in houses with cracked walls, sighting rock hyraxes near residence, residing near a forest and having a close neighbor with CL. However, most of the previous studies done around gender have demonstrated consistent records of males being most vulnerable compared to females (Tonui, 2006; Mutungi *et al*., 2019 and Abu-Hurub and Okbah, 2022). This

difference in gender prevalence may be due to the difference in exposure opportunities between male and female occasioned by their differential socio-economic roles.

2.4 Classification of Sandfly Species Vectors of Leishmaniasis and their habitats in Kenya

A total of 48 sandfly species, belonging to two genera; *Phlebotomus* and *Sergentomyia* are so far known to occur in Kenya (Anjili *et al.,* 2011). The species of sandflies in the genus *Sergentomyia* are only biting nuisants because they do not transmit leishmaniasis, but most of the species of sandflies in the genus *Phlebotomus* are confirmed vectors (Minter 1963; Anjili *et al.,* 2011 and Britch *et al.,* 2011). *Phlebotomus* species belong to 5 further sub-genera: *Phlebotomus, Larroussius, Synphlebotomus, Paraphlebotomus* and *Anaphlebotomus* (Young 1982 and Killick-Kendrick, 1999). The sub-genera *Phlebotomus (Phlebotomus*) has only one species, that is, *Phlebotomus* (*Phlebotomus*) *duboscqui* which transmits *L. major* that causes CL in a small focus in Baringo County due to this species' limited distribution. *P*. (*Phlebotomus*) *duboscqui* is exophagic and a perennial breeding species that is known to inhabit inside rodent burrows surrounded by plenty of vegetation cover. Rodents such as *Aethomys kaiseri, Taterillus emini, Arvicanthis niloticus, Tatera robusta* and *Mastomys natalensis* are known reservoir hosts even though charcoal burners are also major blood meal sources for these vectors (Sang *et al.,* 1994). Humans who live in close proximity to these rodent burrows surrounded by some vegetation get infected when bitten by the vectors (Mebrahtu *et al.,* 1992 and Johnston *et al.,* 1993). These vector species are closely related with *Phlebotomus papatasi* which is found in the neighboring country, Sudan, even though *Phlebotomus* (*Phlebotomus*) *duboscqui* is an effective vector due to its gonotrophic discordance (Sang *et al.,* 1994 and Anjili *et al.,* 2011).

The sub-genus *Larroussius* consist of *P. pedifer*, *P. longipes* and *P. elgonensis* species that are found in the caves of Mt Elgon in Bungoma County where they transmit *L. aethiopica* which causes CL (Sang *et al.,* 1993). Other sandfly species also belonging to the sub-genus *Larroussius* include *P. guggisbergi* and *P. aculeatus* found inside caves in Laikipia and Nakuru Counties respectively where they transmit *L. tropica* that also causes CL (Sang *et al.,* 1994 and Anjili *et al.,* 2011). *P. guggisbergi* has also been found in Karura forest in Nairobi City County (Killick-Kendrick *et al.,* 1994). *P. orientalis* which is associated with tree species such as *Acacia seyal, Balanite aegypti* and *Prosopsis juliflora* which grow in black cotton soils of semi-arid North Eastern regions of Kenya transmits *L. donovani* that causes VL in the region (Ngumbi *et al.,* 2010). The leishmaniasis reservoir hosts associated with sandfly species of sub-genus *Larroussius* include tree hyrax (*Procavia capensis*), rock hyrax (*Dendrohyyrax arboreus*) and giant rats (*Cricetomys gambianus*) which are reservoirs for *L. aethiopica* in Mt Elgon focus; while both *P. johnstoni* and *P. capensis* rock hyrax species are known *L. tropica* reservoirs in Nakuru and Laikipia Counties whereas humans are reservoirs for *L. donovani* in Counties within North Eastern Kenya (Sang *et al.,* 1994; Ngumbi *et al.,* 2010 and Anjili *et al.,* 2011).

The sub-genus *Synphlebotomus* is made up of three species; *P. martini, P. celiae* and *P. vansomerenae* that are all associated with transmission of *L. donovani* which causes VL (Heisch *et al.,* 1962; Minter 1964 and Anjili *et al.,* 2011;).The highly anthropophilic (which is also endophilic) *P. martini* has a wide distribution in the semi-arid regions of Rift-Valley including Baringo, West Pokot and Turkana Counties as well as in the Eastern Kenya's Machakos, Kajiado, Kitui and Meru Counties (Perkins *et al.,* 1988 and Gebre-Michael *et al.,* 2004). *P. celiae* and *P. vansomerenae* have always been caught in small numbers and whose bionomic information is not clearly known as no parasites

have been isolated from them yet (Minter 1963; Marlet *et al.,* 2003 and Gebre-Michael *et al.,* 2013). Analysis of the *P. martini* blood meals have revealed that human, cattle, dog (*Canis familiaris*), wild rabbits (*O. cunculus*) and in more so goats (*Capra hercus*) are this vector's blood meal sources (Ngumbi *et al.,* 1992 and Gebresilassie *et al.,* 2015).

Sub-genus *Paraphlebotomus* constitutes two species of *Phlebotomus saevus* and *P. mirellae* which transmits *L. tropica* and *L. major* which causes CL (Johnston *et al.,* 1993 and Killick-Kendrick *et al.,* 1997). These vector species have been found in Utut, Gilgil in Nakuru County even though transmission history of these species in relation to Kenya is unknown (Anjili *et al.,* 2011).

The sub-genus *Anaphlebotomus* is composed of one species *Phlebotomus rodhaini* which was caught in Machakos County. The species' transmission role of *Leishmania* parasites is still unknown. Members of this species have high preferences for bissel-type termite mound habitats (Anjili *et al.,* 2011).

Thorough literature review has revealed that there is no documented information on the characteristics and distribution of habitats of sandfly vectors of leishmaniasis and the socio-economic risk factors for the disease in Bungoma County. The current study, therefore, aimed at investigating the distribution and characterization of habitats of sandfly vectors and reservoir hosts of leishmaniasis in the region.

CHAPTER THREE

METHODOLOGY

3.1 Study Area and the Study Sites

This research study covered five Sub-Counties in Bungoma County. The physical features which prompted the selection of the Sub-Counties included presence of numerous caves, rock crevices, quarries and other physical features that are suitable as habitats for sandflies and reservoir hosts of leishmaniasis as suggested in published literature (Basimike *et al*., 1992, Feliciangeli 2004 and Ozbel *et al*., 2011). The study sites were also selected based on reported suspected human cases (MoH, personal Communication) with CL like clinical symptoms in the local health facilities. The selected study sampling sites are shown in Figure 3.1.

The selected study areas comprised of sites in Mt. Elgon (Mt Elgon Constituency), Bungoma Central (Kabuchai Constituency), Bungoma South (Kanduyi Constituency), Bungoma East (Webuye East Constituency) and Kimilili (Kimilili Constutuency) Sub-Counties.

The specific sandfly sampling sites in Mt Elgon Sub-County (Mt Elgon Constituency) included; Labot caves in Chepkitale (1°1'2.0435″N and 35°1'36.5261″E), Kaborom caves in Kaptama (0°51'19.506″N and 34°47'12.786″E), Chebwek caves in Chaptais (0°49'41.778″N and 34°28'5.22″E), Chepkutuny (0°49'52.896″N and 34°42'59.52″E), Chepkarai (0°49'48.846″N and 34°42'59.52″E) caves in Kapsokwony, and Kaptanai area (0°47'31.776″N and 34°32'41.562″E) around Namwela-Sirisia-Chwele T-junction.

Kabuchai Constituency is located in Bungoma Central Sub-County adjacent to Mt. Elgon Sub-County while Kanduyi Constituency is in Bungoma South Sub-County bordering Kabuchai to the South. These two Constituencies have extensive rocky hills

with many small caves, rocky crevices and other fascinating physical features. The historical Kabuchai Hills extending from Kisiwa, through Lorio all the way to Sikata is found in Bungoma Central Sub-County. The specific sandfly sampling sites in Bungoma Central Sub-County were Makhonge caves in Chwele (0°45'20.73″N and 34°35'20.364″E), Chebin cave in Kapkateny (0°45'0.924″N and 34°36'43.302″E), and Lorio caves in Sikata (0°37'3.354″N and 34°33'14.772″E).

In Bungoma South Sub-County, Kanduyi Constituency, we sampled the rocky Sang'alo hills (0°31'15.63″N and 34°36'25.908″E) that are unique for Christian religious groups who congregate there all year round for night/day prayers and fasting. The 'Magical' Samulia Hills are located about 3km to the South of Sang'alo Hills bordering Kakamega County. Samulia has magical history in the sense that human footprints, beddings, animal drawings and set of ancient armories were discovered on a hard rock in some of the caves therein. Collection of sandflies and characterization of the sandfly habitats was also carried out in Sang'alo Hills.

Webuye East Constituency has historic Chetambe War Hills where Bukusu people are said to have built communal homesteads where they fought and defeated the Germans, the Wanga and Waganda invaders who had ganged up to forcefully rule over them and take up their land. Chetambe hills also houses historical war cemeteries for Bukusu warriors. This location is characterized with scenic waterfalls along River Nzoia with extensive rocks along the river bank that form sheltered features similar to small caves. Overlapping rocks also form spaces that have over time formed small cave-like features alongside the riverbank. Vector collection and habitat characterization was conducted at Nabuyole cave (0°36'0.282″N and 34°48'3.198″E).

The last sub-county that was sampled was Kimilili Sub-County. This is one of the northern Sub-Counties, including Tongaren where they both border the neighboring Trans Nzoia County. This Sub-County is represented by Kimilili Constituency and the exact sandfly collection point and characterization of habitats was conducted in Tamoi caves in Kipchiria (0°45'54.504N and 34°44'37.5″E).

Figure 3.1 A map of Bungoma County showing the selected study sites (Sampling points). (Source: Author, 2022).

3.2 Research Design and Study Procedures

A cross-sectional field-based study was conducted in different villages within the five selected Sub-Counties in Bungoma County based on existence of suspected sandfly and reservoir host habitats, especially the caves. Data was collected once monthly in each of the twelve (12) study sites for two years (24 months). Sandfly collections and habitat characterization covered the period from January, 2021 to December, 2022.

3.2.1 Sandfly Collection

Center for Disease Control and Prevention light traps, CDC-LT (Model 512; John W. Hock Company, Gainesville, FL) were set inside and/or around the potential sandfly habitats (appendix 4); including caves, rock crevices, termite mounds, waste dumpsites, rodent burrows, human dwellings, stone quarries, trees holes, animal sheds, under dense vegetation covers, around soil cracks and also around natural and artificial water points as previously described (Alexander, 2000 and Alten *et al*., 2015). The CDC light traps were set late in the evenings at around 6 - 7pm and collected early mornings at 6 - 7am; considering the nocturnal activity of the sandflies (Dinesh *et al.,* 2001, Sawalha *et al*., 2017). Every unit of the CDC-LT was powered by a 6-volt battery or 4 new dry cells (1.5 volts each) assembled with power terminals from the source to the trap which is fitted with a trap net with motor connected appropriately so as to produce a “sucking” torque inside the trap. The trap was then suspended onto a support stick or rock surface at a height of about 2 feet above the ground or adjacent to the walls of the habitat where collection was carried out. Trap positions were also marked, described and recorded in terms of distance from the entrance, through the center to the further ends of the cave sites and the collected sandfly abundance per trap position recorded the next day as total counts of sandflies per trap per night. In the case of cave habitats, the sizes of the

caves were also estimated and recorded in length, height and width in meters by use of a tape measure during the stage of trap setting.

The collected sandfly catches were packed in labeled locally assembled field cages and carefully transported to a makeshift field laboratory after tying the net openings to keep the flies inside the cages. In the laboratory, the nets with fly catches were then put inside an air-tight plastic bag into which a cotton wool soaked in trimethylamine (TEA) solution was also put and the bag tied (Chen & Hillyer, 2013). This was done to knock down the insects catches before they were poured onto an open white tray where preliminary identification and sorting of the sandfly catches was done based on the sandflies' published morphological characteristics (Pareyn *et al*., 2020). The rest of the net catch contents of no particular interest to the objectives of the study were discarded.

3.2.2 Sandfly Cleaning, Preservation, Dissection and Identification

The collected sandflies were counted per trap and put into the first glass bowl containing 2% liquid soap detergent solution for washing. A camel brush was used to gently stir and transfer the suspended sandflies to the second glass bowl which contained 10% phosphate buffered saline (PBS) solution for rinsing. A camel brush was again used to transfer the rinsed sandflies into labeled Eppendorf tubes containing 70% ethanol for preservation. These Eppendorf tubes were then transported to the University of Eldoret for refrigeration at -4°C and safe storage, and later transported to the Kenya Medical Research Institute (KEMRI) Nairobi laboratories for dissection and species identification.

Procedures described by Theodor (1932), Killick-Kendrick *et al.,* (1994) with improvements of Brisola-Marcondes *et al*., (1998) were adopted in dissection and identification of the preserved sandflies. Individual flies were picked by use of a camel

brush and each put on a clearly labeled slide and placed under power 10x magnification of a dissection microscope. A drop of water was added to the specimen for easy turning to the right dissecting position. The head was cut at the neck and the 3 terminal segments of the abdomen were also cut using fly dissection pins. The rest of the body parts were returned inside respective labeled individual Eppendorf tubes for storage to be used for other tests. The separated individual heads on the slide were turned to face the upward position where mouth parts would be easily and clearly seen. The 3 terminal segments of the abdomen were similarly mounted. A drop of chloral hydrate gum was then added to the specimens and a cover slip gently placed on top of these specimens. The slides and their contents were later placed on a flat bench in the laboratory to dry overnight at room temperature. These permanent vector slides were later examined using a compound microscope for species identification using published morphological characteristics (Pareyn *et al*., 2020).

The female spermathecae, male genitalia, the teeth, and the pharynx were used as morphological features for species identification (Abonenc and Minter, 1965). Males were identified morphologically by use of aedeagus or the number and position of the hairs on the inner surface of the coxite (Killick-Kendrick *et al.,* 1994), as shown in plates 4.1, 4.2, and 4.3. The total numbers of male and female sandflies were recorded per site and their date of collection (appendix 3).

Males of *P. elgonensis* were identified based on their genitalia (terminalia) which had five terminal spines (two at the tip and three at the sub-terminal) that were less clustered, elongated and slender parameres with few or no setae, slender and pointed penis (aedegus), shorter coxites, and thin and weak surstyles (Killick-Kendrick, 1990). Their females had shorter ducted spermathecae with numerous broad cylindrical rings and regular well-patterned teeth on their cibaria (Aklilu *et al*., 2023). In contrast, the males

of *P. pedifer* species had five spines (two at the tip and 3 sub-terminal) which were more clustered, elongated and strong parameres with more setae, large penis with hooked tip, large elongated coxite with more setae, and strong and large surstyle (Young *et al*., 1977). Their female counterparts were identified by elongate and thin ducted cylindrical-ringed spermathecae, and fewer and dispersed teeth on their cibaria (Lane 1993).

The morphological identification of *Sergentomyia* species proceeded the microscopic observation of the male genitalia, female spermathecae, the number of teeth, pharynx and sometimes their antennae appearance as just like the preceding *Phlebotomus* species (Lewis 1973). *S. africana* was distinguished by their males having five spines (2 terminal and 3 sub-terminal), thin paramere, and styles longer than their coxites; while females had 4-6 teeth that were prominent with weak pigmented patches on the cibarium, weak and short pharyngeal armature, and short (5-7 segmented) spermathecae ducts (Abonenc and Minter, 1965). *S. alderi* males had 4-spined terminalia, short parameres with no setae, and a firm coxite; females had well-formed (7-10) teeth on the cibaria, strong pharynx having conspicuous denticles, and short (4-6 segment) and large spermathecae ducts (Abonenc and Minter, 1965). *S. antennatus* males were 4-6-spined with one at the base, elongated parameres, and large and elongated coxites; females had a conspicuous dark-colored patch with no teeth on their cibaria, thin and long pharyngeal armatures, and more elongated, slender, multi-segmented spermathecal ducts (Lane 1993). *S, bedfordi* males had short 4-spined styles, thin paremeres with minimal setae, and equal-sized coxite and style; while females had psuedoteeth with colored patches on their cibaria, thin and faintly-granulated pharynix, and long segmented (8-10) spermathecal ducts (Young *et al*., 1977). *S. ingrami* was identified by males having 4-spined unevenly spaced styles, fairly setated coxites and

paremeres that are curved; females had multiple granulated patches with weak teeth on the cibaria, very strong pharynx, and very elongated spermathecae containing multiple segments that tapered towards the end (Killick-Kendrick, 1990). Finally, *S. schwetzi* was identified by males possessing 4-spined and regularly spaced styles, densely setated and curved parameres, and slightly long coxites; while females had large pigmented patches with pseudo-teeth on the cibarium, strong pharyngeal armature, and elongated and curved spermathecae that had more than 12 segmental ducts (Abonenc and Minter, 1965).

3.2.3 Association of Climatic Factors and Sandfly Relative Abundance

Ambient cave temperatures were recorded by use of a thermometer. A modified hygrometer made up of wet and dry bulb thermometers was used to determine the relative humidity inside the caves and the other habitats by observing the readings of the dry bulb thermometer and the difference between the wet and dry bulb thermometers readings (Griffiths, 1921). Approximated relative humidity was then determined by comparing the results in the standard relative humidity interpretation scale. The altitudes for all the sites where data was collected were recorded accordingly in units of meters above the sea level by the use of a digital altimeter software application installed on an android system smart phone. The Geographical Positioning Systems (GPS) application installed on android smart phone was also used to determine the longitudinal and latitudinal co-ordinates for every study site.

3.2.4 Soil Samples Collection and Analyses

Random soil samples were collected from the study sites by use of a shovel to scoop the surface soil. The scooped soil from all the sampled spots were first poured inside a bucket, mixed thoroughly and only 250g of composited soil per site was taken for laboratory analysis. At least three spots within each site were sampled depending on

the size of the study site. Soil temperatures were recorded by driving a soil thermometer 5 cm into the soil at each of the spots where soil samples were collected. The mean ambient temperature for the whole habitat was recorded accordingly by summing up the temperatures for all the sampled spots and dividing the total by the number of spots sampled. Collected soil samples were packaged inside multiple airtight well labeled polythene bags and transported carefully to the University of Eldoret soil laboratories for further analysis. The soil physical parameters such as soil type and soil texture were analyzed using hydrometer method, soil moisture content (was analyzed by weighing the crucible before adding 2 g of soil and putting the crucible in an oven at 105^0C overnight and reweighing), humus content (by dry combustion method at 450^0C for 8 hours and reweighing), and soil pH (by use of pH meter). The chemical aspects of soil that were analyzed included carbon (titrimetric analysis), phosphorus and nitrogen (both analyzed by UV -vis spectrophotometric method), calcium, magnesium, iron, manganese, copper and zinc (all analyzed by atomic absorption spectrophotometer - AAS), and sodium and potassium (analyzed by flame photometer).

3.2.5 Plants Species Diversity Associated with Sandfly Species Populations

Material specimens of the plants which were found within a distance of 10 m from the entrance of the cave or inside each of the sandfly habitat sites were noted and collected (as shown on appendices 16, 24, 26,32 and 35). The plant specimens were properly preserved under dry magazine papers and transported to the University of Eldoret's herbarium for taxonomic identification by an expert plant taxonomist. Short plant parts comprising of at least the stem, branch, three leaves, floral parts and the apical end were carefully cut using a pair of scissors or a knife. Accompanying clear photographs of the whole plants were also taken. Proper labels of the plants, sites where they were collected, date of collection and common names used locally (where available) were

written on the tag attached on every plant specimen. The most common plant species and their densities were also recorded for all the sites. The occurrence and abundance of the sandflies was later correlated with the diversities of the plants using the Shannon Weiner diversity test in each habitat site to investigate whether there were any significant associations between the plant species and sandflies occurrence in the sampled habitats.

The Shannon-Weiner diversity index, *H*' indicates how diverse (the number of species present) while the evenness, *E* indicates how species are relatively distributed in a given ecosystem. High diversity and evenness will indicate that there is stability and heterogeneity in that given habitats that supports many species. The implication of a highly diverse vector and reservoir ecology will suggest increased risk of sustaining the pathogens which may eventually lead to increased transmission of *Leishmania* parasites to humans. Low *H*' and *E* means unstable ecosystem or one species is dominating over other species. High diversity index ranges from H' > 3.0, moderate H' = 1.5 -3.0, and low H' < 1.5. Similarly, high evenness is indicated by E = 0.68 -1.0, moderate E = 0.34 -0.67, and low E = 0.00 -0.33.

The Shannon-Weiner index is calculated by the formula, $H' = -\sum (pi \ln pi)$, while evenness is calculated using the formula, $E=H'/\ln S$; where, *pi* represents the proportion of numbers of species *i* of the total number of individuals recorded, and *S* being the total number of species.

3.2.6 Potential Vertebrate Reservoir Hosts Data

During the setting of the traps, attention was given to the numbers and types of all vertebrates that were sighted inside and around the vicinity of the sandfly collection sites in the study areas. Every vertebrate seen was assumed to be a potential reservoir

host of leishmaniasis and/ or a source of bloodmeal for the sandflies. These included domestic and wild animals, large and small animals, ranging from mammals, reptiles and birds. Rodents, donkeys, cows, sheep, goats, dogs, rock hyraxes, giant rats, bats and also people who were sighted, counted and recorded accordingly. Presence of animal footprints, presence of stool, discarded package(s) of used candies and drinks, as well as packages of used condoms were also recorded as indicators of the presence and activities of vertebrate animals (animals and humans) in the respective cave sites. The mean numbers per month of the sighted animals were determined and recorded for each site. This information on numbers and type of vertebrates was later used to correlate with the corresponding densities of sandflies caught per month in every site from where they were collected.

3.3 Data Analyses

The number and species of sandflies, suspected animal reservoir host types and plants species recorded in all the habitat sites were obtained by discrete counts. Mean counts and their associated errors (S.E.) were also calculated for sandflies, animal reservoir hosts and plants, while their respective attributes such as habitat location, altitude, soil type, species, sex, among others were recorded. Sex ratio between male and female sandflies was determined. Taxonomic quantification was done for all collected sandfly species and expressed as percentage proportion of the total collections. Specific quantifications of all recorded parameters across all the sites were also done. The resultant computations were subjected to chi-square test, t-test and ANOVA at 95% confidence level ($p \leq 0.05$) to compare their significance differences. The Shannon Weiner test was used to determine the species diversities (*H'*) and evenness (*e*) in the distribution of sandflies, animal reservoir hosts and plants in all the sampled habitats. High diversity index means there are large species communities that are evenly

distributed, which indicates a fairly stable, heterogenous and healthy ecosystem, and vice-versa is true for low diversity index.

Effects of climatic factors such as ambient temperatures, soil temperatures, relative humidity and altitude on the occurrence and distribution of sandflies were analyzed by conducting correlation tests at a significance level of $p \leq 0.05$. The mean numbers of periodic abundance of sandflies between wet and dry periods of the year were analyzed by use of ANOVA (at $p \leq 0.05$). The significant differences in means were separated using Fisher's least significant difference (LSD). Impacts of soil physical and chemical properties on the distribution of population of sandflies in all the study sites were tabulated and subjected to correlation tests. The mean differences between soil constituents at $p < 0.05$ was considered significant.

Relative abundance and type of vertebrate potential and confirmed reservoir hosts of leishmaniasis in all respective sites were recorded and chi-square test was done at a significance level of $p < 0.05$. Correlation test at $p < 0.05$ was also used to establish the relationship in means between numbers of sandflies and the mean number of each type of the potential animal reservoir host sighted per month. Mean numbers of sandflies collected and vertebrate potential reservoir hosts sighted were used to determine the diversities and evenness in their distribution by the Shannon Weiner test. The same test at $p < 0.05$ was applied to determine the plants' diversity and distribution relationship with sandflies in the sampled habitats in Bungoma County.

CHAPTER FOUR

RESULTS

4.1 Sandfly Species Diversities and Characteristics of Their Habitats in Bungoma County

4.1.1 The Abundance of Sandfly Species and their Morphological Identification Plates in Sampled Habitats in Bungoma County

Sandflies in Bungoma County were found only in cave habitats. The other sampled habitats (that included termite mounds, tree holes, domestic animal sheds, human dwellings, under vegetation, and rock crevices), did not yield any sandfly catches during the study period. A total of 6156 sandflies were collected from the sampled cave habitats in Bungoma County during the study period. Sandfly collections comprised of 1828 male and 4328 female (n = 6156, male/female ratio was: 1828/4328 = 1:2.4). Taxonomic identification categorized the sandfly collection into two (2) genera; *Phlebotomus* and *Sergentomyia* and 8 species namely *Phlebotomus elgonensis*, *P. pedifer, Sergentomyia adleri, S. africana, S. antenatus, S. bedfordi, S. ingrami* and *S. schwetzi.* The most abundant species of sandfly found in the majority of the habitats in the study area was *P. pedifer* which contributed 5789 (94.04%) of the total sandfly collection, while the least abundant species were *S. adleri*, S. *bedfordi* and *P. elgonensis* whose counts were 12 (0.19%), 26 (0.42%) and 29 (0.47%) respectively as shown in table 4.1. The difference in sandfly species mean abundance across the habitats was significant ($p < 0.0001$).

Table 4.1 Comparison of Mean Frequency of Sandfly Species Collected from Cave Habitats in Bungoma County During January, 2021 to December, 2022 Period (p < 05)

Species	Frequency	Percent	Chi square (χ^2); p value
P. elgonensis	29	0.50	χ^2= 606.91
P. pedifer	5789	94.00	df=7
S. adleri	12	0.20	p < 0.0001
S. africana	148	2.40	
S. antenatus	53	0.90	
S. bedfordi	26	0.40	
S. ingrami	65	1.10	
S. schwetzi	34	0.60	
Total	6,156	100.00	

Some of the photographic plates of the different species that were used as morphological taxonomic guidelines are also attached as plates 4.1, 4.2, and 4.3 below. They were the distinctive features that were heavily relied on in the species identification sandflies under compound microscopes after dissection.

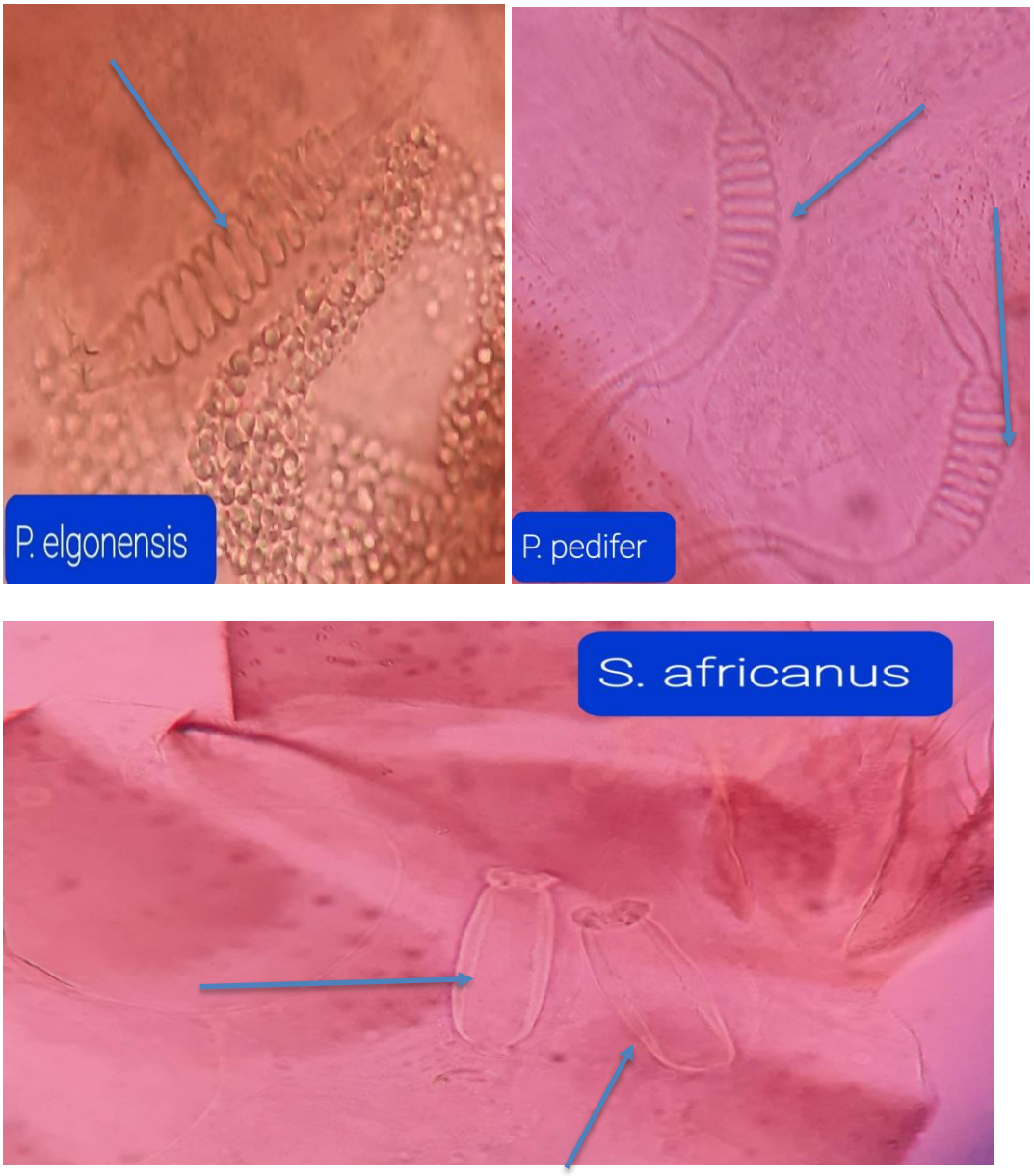
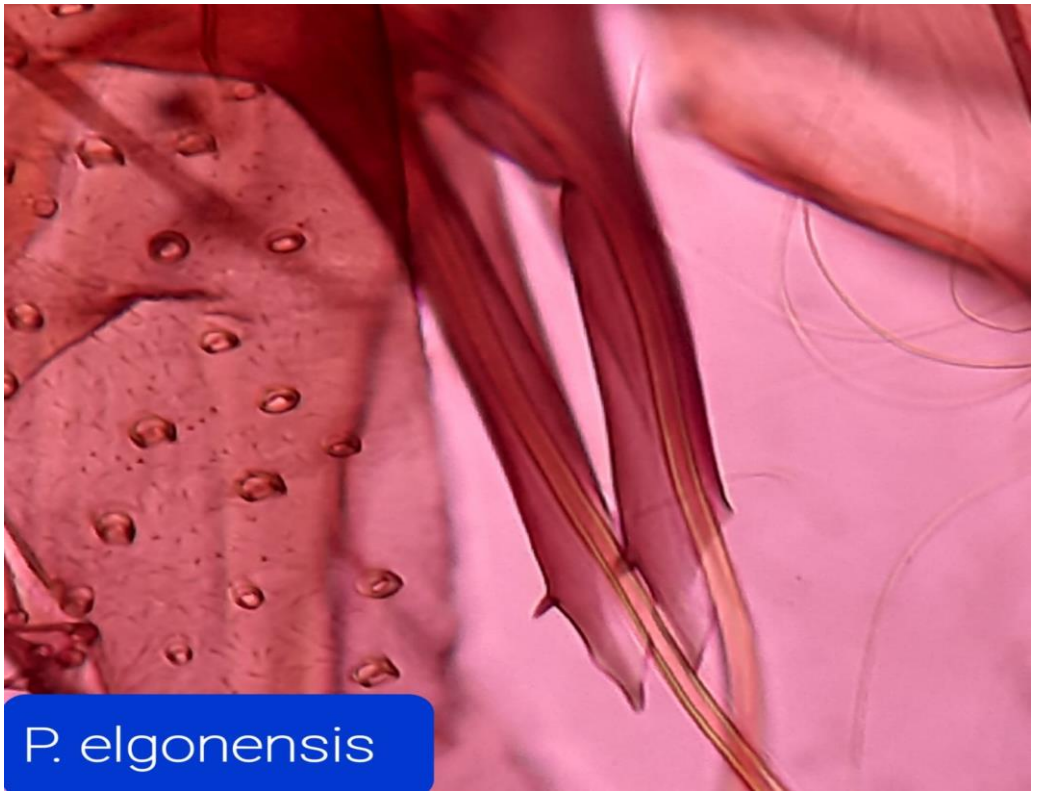


Plate 4.1 Comparative Appearance of Images of Spermathecae of *P. elgonensis*, *P. pedifer* and *S. africana* as Seen Under a Compound Microscope (X400 Magnification): Plates Captured by Self Using an Android Phone Camera 11 June, 2022. (Source: Author, 2022)



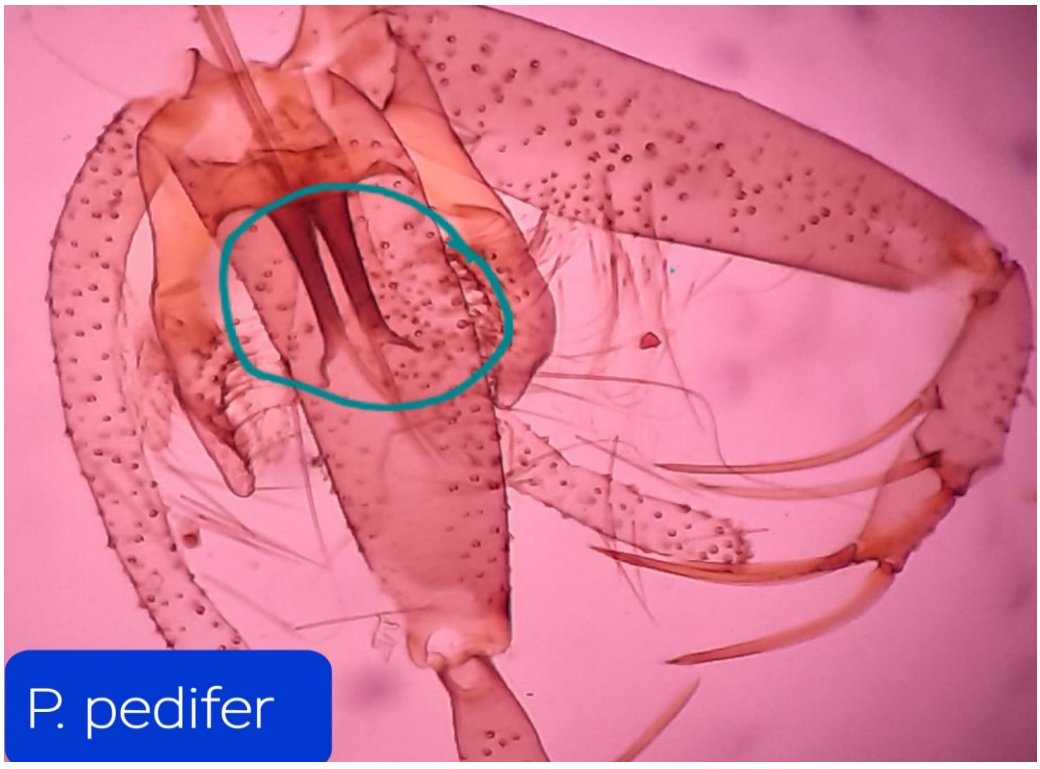


Plate 4.2 Comparative Morphological Distinction of Male Genitalia (Styles) for *P. elgonensis* and *P. pedifer* Species as Seen Under a Compound Microscope (X400 Magnification): Plates Captured by Self Using an Android Phone Camera 11 June, 2022. (Source: Author, 2022

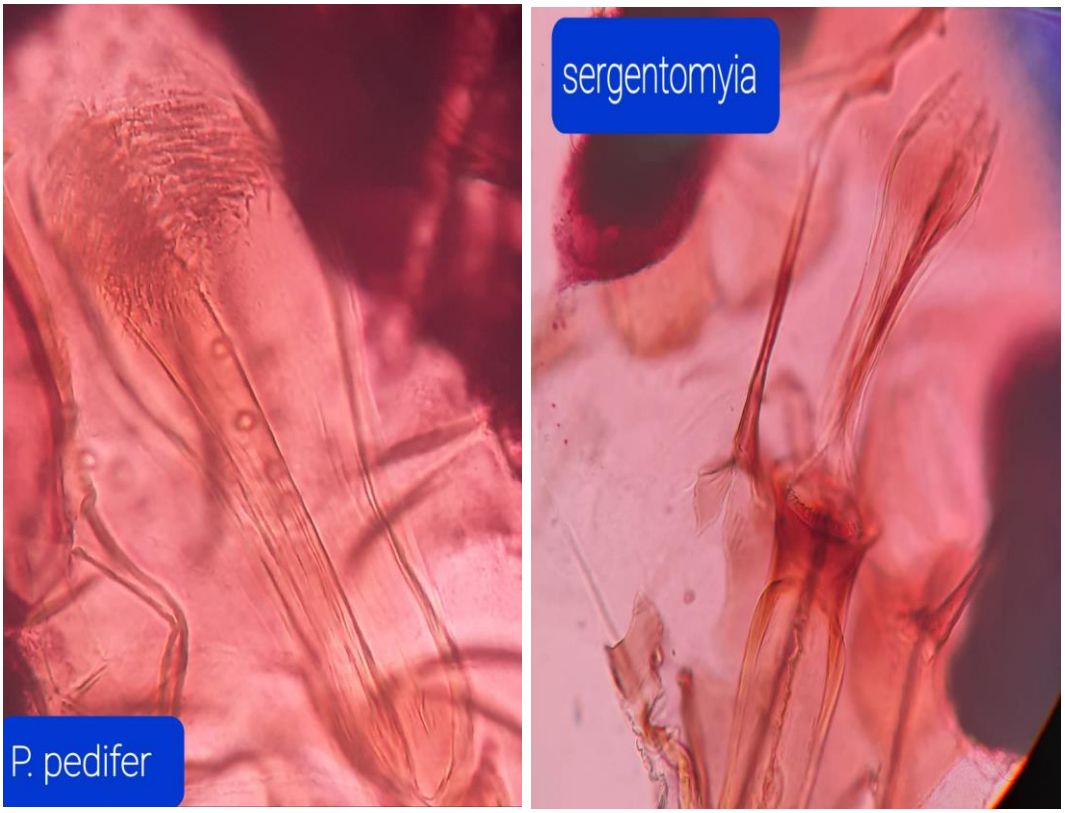


Plate 4.3 Cibarial Plates for *P. pedifer* and *Sergentomyia* species (Source: Author, 2022)

4.1.2 Distribution and Mean abundance of Sandfly Collections in Sampled Cave Sites in Bungoma County

The sandflies were distributed in the 12 sampled sites as shown in Table 4.2. The site where the highest population of sandflies were collected was Chepkarai with a population of 2191 (35.59%) sandflies, followed by Chepkutuny with a population of 1112 (18.06%) sandflies, while Nabuyole with a population of 13 (0.21%) sandflies had the least number of sandflies that were collected. The difference between the numbers of sandfly species collections across the sampled sites was significant (χ^2=135.68, df. =11, $p < 0.0001$). All the *P. elgonensis* (29 individuals = 100.00%) were found only in Labot cave (1°1'2.0435″N and 35°1'36.5261″E) located at the highest

sampled altitude (3100 masl). This species was not found in any other sampled site in the study area. Although a large number 2,191 (35.59%) of *P. pedifer* was found in Chepkarai cave site, none of this species was recorded in Labot cave site.

S. adleri, S. bedfordi, and *S. ingrami* were recorded in Chepkutuny and Sang'alo as shown in Table 4.2. A large proportion of *S. africana* were recorded in Chepkutuny 128 (86.67%) while the rest of this species were recorded in Kaptanai 12 (8.11%) and Chebwek 8 (5.41%) sites.

S. antenatus were collected from two cave sites namely; Chepkutuny (47/53 = 88.68%) and Kaptanai (6/53 = 11.32%). *S. schwetzi* were collected from five cave sites namely; Chepkutuny (12/34 = 35.29%), Lorio (8/34 = 23.53%), Kaptanai (6/34 = 17.65%), Nabuyole (6/34 = 17.65%) and Chebwek (2/34 = 5.88%).

Table 4.2 Distribution of Sandflies in Sampled Sites in Bungoma County During January 2021 to December 2022 Period

Sites	Chebin	Chebwek	Chepkarai	Chepkutuny	Koborom	Kaptanai	Labot	Lorio	Makhonge	Sang'alo	Tamoi	Nabuyole	Total
Species													
P. elgonensis	0	0	0	0	0	0	29	0	0	0	0	0	29
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)
P. pedifer	651	82	2191	887	399	355	0	215	534	56	412	7	5789
	(11.25)	(1.42)	(37.85)	(15.32)	(6.89)	(6.13)	(0.00)	(3.71)	(9.22)	(0.97)	(7.12)	(0.12)	(100.00)
S. adlerai	0	0	0	12	0	0	0	0	0	0	0	0	12
	(0.00)	(0.00)	(0.00)	(100.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)
S. africana	0	8	0	128	0	12	0	0	0	0	0	0	148
	(0.00)	(5.41)	(0.00)	(86.49)	(0.00)	(8.11)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)
S. antenatus	0	0	0	47	0	6	0	0	0	0	0	0	53
	(0.00)	(0.00)	(0.00)	(88.68)	(0.00)	(11.32)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)
S. bedfordi	0	0	0	26	0	0	0	0	0	0	0	0	26
	(0.00)	(0.00)	(0.00)	(100.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)
S. ingrami	0	0	0	0	0	0	0	0	0	65	0	0	65
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(100.00)	(0.00)	(0.00)	(100.00)
S. schwetzi	0	2	0	12	0	6	0	8	0	0	0	6	34
	(0.00)	(5.88)	(0.00)	(35.29)	(0.00)	(17.65)	(0.00)	(23.53)	(0.00)	(0.00)	(0.00)	(17.65)	(100.00)
Total	651	92	2191	1112	399	379	29	223	534	121	412	13	6156
	(10.58)	(1.49)	(35.59)	(18.06)	(6.48)	(6.16)	(0.47)	(3.62)	(8.67)	(1.97)	(6.69)	(0.21)	(100.00)

(Numbers in parenthesis are percentage proportions of the total collections per sandfly species per site)

4.1.3 Sandfly Species Diversities and Distribution

There was a very low overall Shannon Weiner diversity index and evenness for sandfly species, 0.3256 *H'* and 0.1731 respectively, which suggested unstable habitat ecology with one species dominating the rest of the species that were collected. Analysis of sampled sites showed that Chepkutuny (0.75 *H'),* Sang'alo 0.69 *H'* and Nabuyole (0.69 *H')* sites had the highest Shannon Weiner diversity indices while Chebin, Chepkarai, Kaborom, Labot, Makhonge and Tamoi sites had the lowest indices (*H'*< 0.01 each). The overall difference between the sandfly Shannon Weiner diversity indices among the sites was significant ($p < 0.05$) across all sampled sites except for Sang'alo and Nabuyole sites (Table 4.3).

Table 4.3 A Table Showing of the diversity of Sandfly Species and How They are Distributed Across the Sampled Sites

Species Distribution Per Site

Sites **Species**	**Chebin**	**Chebwek**	**Chepkarai**	**Chepkutuny**	**Kaborom**	**Kaptanai**	**Labot**	**Lorio**	**Makhonge**	**Sang'alo**	**Tamoi**	**Nabuyole**	**Total**
P. elgonensis	_	_	_	_	_	_	29	_	_	_	_	_	29
P. pedifer	651	82	2191	887	399	355	_	215	534	56	412	7	5789
S. adleri	_	_	_	12	_	_	_	_	_	_	_	_	12
S. africana	_	8	_	128	_	12	_	_	_	_	_	_	148
S. antenatus	_	_	_	47	_	6	_	_	_	_	_	_	53
S. bedfordi	_	_	_	26	_	_	_	_	_	_	_	_	26
S. ingrami	_	_	_	_	_	_	_	_	_	65	_	_	65
S. schwetzi	_	2	_	12	_	6		8	_	_	_	6	34
Total	651	92	2191	1112	399	379	29	223	534	121	412	13	6156

4.2 Impact of Temperature, Relative Humidity and Altitude on the Occurrence and Abundance of Sandflies in Sampled Sites in Bungoma County

The assessed altitudes ranged from 1506 to 3100 meters above sea level (masl). *P. elgonensis* was the only species of sandfly that was found in a cave site at highest sampled altitude of 3100 masl while *P. pedifer* was found in moderately high altitudes ranging from 1506 to 2147 masl with a high abundance (2191 collected sandflies) being recorded in a cave site at an altitude of 1775 masl. A large proportion of *Sergentomyia* species (225 of 338 sandflies) were found in a cave site at 1740 masl. *S. adleri* and *S. bedfordi* were also found in the cave site at 1740 masl, *S. africana, S. antenatus, and S. schwetzi* were found in cave sites at altitudes ranging from 1506 -2147 masl, while *S. ingrami* was found in a cave site at altitude of 1520 masl. The overall sandfly species diversity distribution differences between different altitudes were significant ($p < 0.05$).

The assessed cave sites had ambient temperatures ranging from 15 to 29 ^{0}C. *P. elgonensis* was only found at a cave site with mean ambient temperature of 15.1 ^{0}C. while *P. pedifer* was found in several cave sites with ambient temperatures ranging from 21 to 29 ^{0}C with the largest proportion being found at cave sites with ambient temperature of above 26 ^{0}C. *Sergentomyia* species were also found inhabiting cave with different ambient temperatures ranges but the majority were found in cave sites with 28^{0}C and above. *S. adleri* and *S. bedfordi* were found only in sites with 28^{0}C, while *S. ingrami* were at cave sites with 27^{0}C. *S. schwetzi, S. africana* and *S. antenatus* were found in cave sites with a range of temperatures (21 - 28^{0}C). The overall differences in sandfly abundance distribution by mean ambient temperature in the caves was significant ($p < 0.0001$).

Soil temperature also influenced distribution of sandflies with all the *P. elgonensis* being recorded in a cave site with soil having mean temperatures of 12.5 ^{0}C. All the

other recorded sandfly species were found in caves with different soil temperatures with significant differences in sandfly abundances in caves with soil having different mean temperatures ($p < 0.0001$).

Size of the caves influenced distribution of the sandfly species with a large proportion of *P. elgonensis* being recorded in a significantly larger cave of more than 1508 m^2 compared to smaller sized caves of <1508 m^2 ($p < 0.0001$).

A large proportion of *P. pedifer* were found in caves with flowing or stagnant water while the rest of the other species were mainly found in caves without water ($p < 0.0001$).

Soil compactness on cave habitat floor influenced sandflies distribution with a large proportion of sandfly species being significantly found in caves with floors characterized by dry loose soil.

There was a negative relationship between altitude and ambient temperature. Increase in altitude resulted in significant decrease in ambient temperature ($r (204) = -0.27$, $p<0.0001$) and soil temperature ($r (204) = -0.27$, $p<0.0001$) respectively. However, increase in altitude led to significant increase in relative humidity ($r (60) = 0.46$, $p<0.0001$). There was a significant positive correlation between soil temperature and ambient temperature ($r (6) = 0.86$, $p<0.0001$). There was a negative correlation between relative humidity and ambient temperature ($r (15) = -0.72$, $p<0.0001$) as shown in Table 4.3.

Sandflies abundance was positively correlated with soil moisture ($r (1080) = 0.118$, $p<0.0001$), ambient temperature of the cave ($r (1000) = 0.125$, $p<0.0001$), soil temperature ($r (13) = 0.83$, $p<0.0001$), water inside the cave ($r (208) = 0.265$, $p<0.0001$), floor type ($r (265) = 0.236$, $p<0.0001$) and relative humidity ($r (7) = 0.95$,

$p < 0.0001$). However, sandfly abundance was negatively correlated with cave size ($r(2300) = -0.083$, $p<0.0001$) as shown in Table 4.3.

Table 4.4 Correlation of Factors Associated with Sandflies Species Distribution in Bungoma County

	Soil moisture	Altitude	Sandfly abundance	Ambient temp	Soil temp	Cave size	Water inside	Cave Floor type	Relative humidity
Soil moisture	1	**.118***	**.118***	0.01	**.119***	0.02	**.149***	0.01	**-.254***
	(1)	(0.00)	(0.00)	(0.26)	(0.00)	(0.09)	(0.00)	(0.35)	(0.00)
Altitude	**.118***	1	0.01	**-.270***	**-.125***	**.126***	**.339***	**-.184***	**.458***
	(0.00)	(1)	0.47	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)
Sandfly abundance	**.118***	0.01	1	**.125***	**.083***	**-.083***	**.265***	**.236***	**0.95***
	(0.00)	0.47	(1)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Ambient temp	0.01	**-.270***	**.125***	1	**.860***	-0.01	0.03	**.512***	**-.072***
	(0.26)	(0.00)	(0.00)	(1)	(0.00)	(0.47)	(0.05)	(0.00)	(0.00)
Soil temp	**.119***	**-.125***	**.083***	**.860***	1	**.046***	**.066***	**.316***	**.208***
	(0.00)	(0.00)	(0.00)	(0.00)	(1)	(0.00)	(0.00)	(0.00)	(0.00)
Cave size	0.02	**.126***	**-.083***	-0.01	**.046***	1	**-.132***	**-.179***	**.068***
	(0.09)	(0.00)	(0.00)	(0.47)	(0.00)	(1)	(0.00)	(0.00)	(0.00)
Water inside	**.149***	**.339***	**.265***	0.03	**.066***	**-.132***	1	**.350***	**.304***
	(0.00)	(0.00)	(0.00)	(0.05)	(0.00)	(0.00)	(1)	(0.00)	(0.00)
Cave Floor type	0.01	**-.184***	**.236***	**.512***	**.316***	**-.179***	**.350***	1	**.085***
	(0.35)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(1)	(0.00)
Relative humidity	**-.254***	**.458***	**0.95***	**-.072***	**.208***	**.068***	**.304***	**.085***	1
	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(1)

*** (Bold font) indicates that correlation is significant at the 0.05 level (2-tailed). (Numbers in parenthesis are p- values).**

The mean abundance of sandflies varied non-significantly (F=1.838, df=4.817, p=0.2611) with the long dry season (January to March) collections of 95.23 ± 24.24, long rainy season (April to June) recorded 67.25 ± 41.90 sandflies, short dry season (July to September) recorded 59.00 ± 52.00 sandflies and the short rainy season (October to December) recorded 266.47 ± 69.20, the highest sandfly collection as illustrated in Figure 4.2.

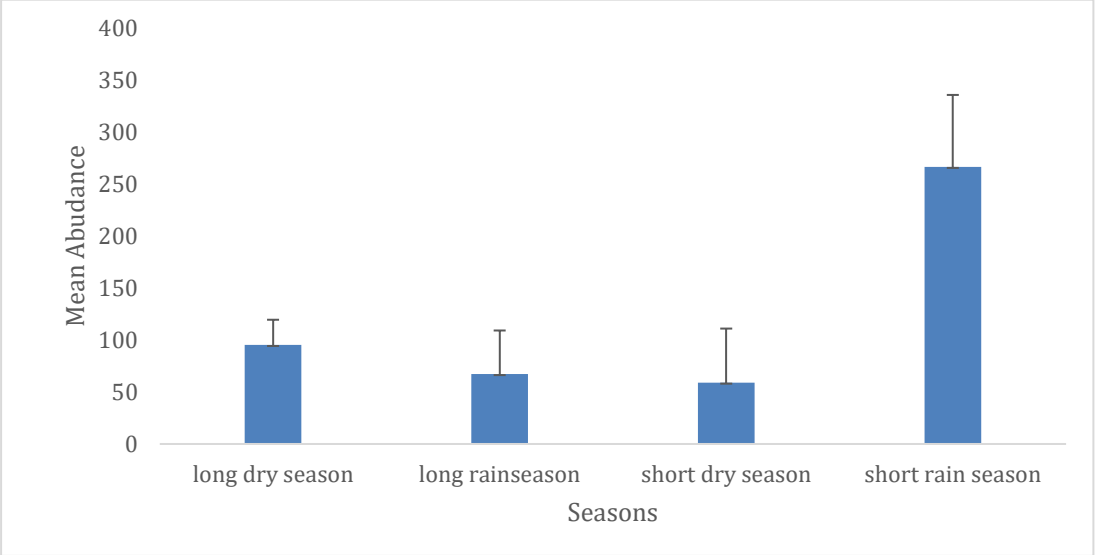


Figure 4.2 Seasonal Distribution of Sandfly Abundance Collected in Caves in Bungoma County

4.3 The Impact of Soil Physical and Chemical Characteristics on the Population of Sandflies in Cave Sites in Bungoma County

4.3.1 Soil Physical and Chemical Characteristics

The assessed soil physical and chemical properties included soil texture, soil pH, soil moisture, soil nitrogen, soil phosphorus, soil carbon, soil iron, soil zinc, soil copper, soil manganese, soil calcium and soil magnesium contents.

All the cave sites contained sandy soils with the majority of the sites having sandy-loam soils. Chepkarai site was the only cave with sandy-clay soil, while Chepkutuny, Chebwek and Lorio caves had porous sandy soils. Correlation analysis between the soil type and sandfly densities revealed that the sandfly density differences were found to be non-significantly associated with soil texture but were positively associated with caves that had dry loose soil floors ($r(9) = 0.3814$, $p = 0.2471$).

The average soil moisture content was 0.28 ± 0.1024. Chepkutuny cave had the highest soil moisture content (0.5160 ± 0.2122) while Makhonge had the least moisture content (0.1010 ± 0.0246). When correlated with the mean number of sandflies caught at each site, there was a positive but non-significant relationship suggesting that sandflies prefer habitats with moist soil ($r(9) = 0.0964$, $P = 0.778$).

The average soil pH was 7.25 ± 1.40 with neutral and alkaline soils ($pH > 6$) being recorded in Chepkutuny, Chebin, Kaptanai, Nabuyole, Tamoi, Makhonge, Kaborom, Sang'alo, Chepkarai and Chebwek caves. Only Lorio cave had acidic soil ($pH = 4.55 \pm 1.24$). Higher densities of sandflies were found inhabiting caves with non-acidic soil.

The average nitrogen content of soil in all the study sites was $0.63 \pm 0.27\%$ and was significantly ($p < 0.05$) high in Chepkutuny ($1.14\% \pm 0.11$), Kaptanai ($0.24\% \pm 0.11$) and Nabuyole ($0.26\% \pm 0.12$) compared to the other study sites.

Soil phosphorus contents varied across the study sites with Chebin cave ($0.33\% \pm 0.09$), Chepkarai cave ($0.33\% \pm 0.12$), Lorio cave ($0.32\% \pm 0.14$), Tamoi cave ($0.30\% \pm 0.19$), Sang'alo caves ($0.30\% \pm 0.08$) and Kaborom cave ($0.30\% \pm 0.21$) having higher percentage phosphorus contents while Nabuyole ($0.06\% \pm 0.01$) had significantly lower phosphorus percentage ($p < 0.05$).

Chepkutuny cave soil recorded the highest carbon content (19.80% ± 2.63) compared to lower carbon contents in soil from the other sites. However, Kaptanai cave soil (0.96% ± 0.38) recorded significantly ($p < 0.05$) the lowest carbon content.

Iron content in soil was significantly high ($p<0.0001$) in Chepkutuny cave (34.93 mg/kg ± 9.2), Chebwek cave (32.64 mg/kg ± 4.27) and Tamoi cave (29.94 mg/kg ± 3.38) and low in Makhonge cave (9.92 mg/kg ± 2.41), Nabuyole cave (9.75 mg/kg ± 3.1), Kaborom cave (9.46 mg/kg ± 2.9), Chepkarai cave (8.15 mg/kg ± 1.32) and Sang'alo caves (7.48 mg/kg ± 2.36) compared to the other study sites.

Zinc content in soil was significantly ($p < 0.0001$) high in Chebwek cave (0.25 mg/kg ± 0.12) compared to the low content in soil in Nabuyole cave (0.11 mg/kg ± 0.09) while the other study sites recorded intermediate levels with insignificant differences ($p < 0.05$). Similar observations were made for copper content in soil. Manganese content in soil was significantly ($p < 0.0001$) high in Chebwek cave (1.79 mg/kg ± 1.28) and lowest in Nabuyole cave (0.05 mg/kg).

Soils in Sang'alo caves had the highest calcium content of 9.02 mg/kg ± 3.02 while soils in Nabuyole (0.06 mg/kg ± 0.03) had the lowest, the difference in calcium content between the sites was highly significant ($p < 0.0001$). Soils in Sang'alo caves had the highest magnesium content content of 9.02 mg/kg ± 2.31 while soils in Nabuyole (0.06 mg/kg ± 0.02) had the lowest, the difference in magnesium content between the sites was highly significant ($p < 0.0001$).

4.3.2 Correlation between Soil Physical and Chemical Characteristics and Sandfly Abundance in Cave Sites in Bungoma County

There was a positive correlation between nitrogen and carbon ($r(9) = 0.9081$, $p = 0.0001$), phosphorus and zinc ($r(9) = 0.6478$, $p = 0.0311$), iron and copper ($r(9) =$

0.8019, p = 0.0030), iron and manganese (r (9) = 0.7942, p = 0.0035), copper and manganese (r (9) =0.8824, p=0.0003), calcium and magnesium (r (9) = 0.7024, p = 0.0160) and between calcium and soil texture (r (9) = 0.7323, p = 0.0104) as shown in Table 4.4. Sandfly abundance was positively but non-significantly ($p > 0.05$) correlated with soil pH, soil moisture, soil nitrogen, soil phosphorus, soil copper, soil zinc, soil calcium, soil carbon contents and the soil texture.

Table 4.5 Correlation Between Sandfly Abundance and Soil Physical and Chemical Characteristics

	Sandfly abundance	Soil moisture	pH	N	% P	C	Fe	Zn	Cu	Mn	Ca	Mg	Soil texture
Sandfly	1	0.0964	0.0177	0.3105	0.2491	0.3739	-0.1004	0.0524	0.0534	-0.1473	0.203	-0.0245	0.3814
abundance	(1)	(0.778)	(0.9587)	(0.3527)	(0.4601)	(0.2572)	(0.7689)	(0.8785)	(0.876)	(0.6656)	(0.5493)	(0.943)	(0.2471)
Soil	0.0964	1	-0.3699	0.4193	0.0402	0.6184	0.4561	0.0257	0.2896	0.4464	0.0363	0.0881	-0.4021
moisture	(0.778)	(1)	(0.2629)	(0.1992)	(0.9065)	**(0.0426)***	(0.1586)	(0.9401)	(0.3876)	(0.1687)	(0.9156)	(0.7967)	(0.2203)
pH	0.0177	-0.3699	1	-0.3742	-0.1801	-0.4527	0.1832	-0.4986	0.11	-0.1288	-0.0861	-0.0185	0.0639
	(0.9587)	(0.2629)	(1)	(0.2569)	(0.5961)	(0.1621)	(0.5897)	(0.1185)	(0.7474)	(0.7059)	(0.8012)	(0.9569)	(0.852)
N	0.3105	0.4193	-0.3742	1	0.2775	0.9081	0.5417	0.2577	0.5044	0.5336	-0.19	-0.0417	-0.207
	(0.3527)	(0.1992)	(0.2569)	(1)	(0.4088)	**(0.0001)***	(0.0852)	(0.4442)	(0.1136)	(0.0909)	(0.5757)	(0.903)	(0.5415)
P	0.2491	0.0402	-0.1801	0.2775	1	0.1248	-0.0159	0.6478	0.2038	0.2899	0.5427	0.299	0.2603
	(0.4601)	(0.9065)	(0.5961)	(0.4088)	(1)	(0.7146)	(0.963)	**(0.0311)***	(0.5477)	(0.3872)	(0.0845)	(0.3718)	(0.4395)
C	0.3739	0.6184	-0.4527	0.9081	0.1248	1	0.5193	0.1782	0.3952	0.4549	-0.2866	-0.0985	-0.4169
	(0.2572)	**(0.0426)***	(0.1621)	**(0.0001)***	(0.7146)	(1)	(0.1017)	(0.6)	(0.2290)	(0.1598)	(0.3928)	(0.7732)	(0.2021)
Fe	-0.1004	0.4561	0.1832	0.5417	-0.0159	0.5193	1	0.0898	0.8019	0.7942	-0.4440	-0.2843	-0.538
	(0.7689)	(0.1586)	(0.5897)	(0.0852)	(0.963)	(0.1017)	(1)	(0.7929)	**(0.0030)***	**(0.0035)***	(0.1713)	(0.3968)	(0.0878)
Zn	0.0524	0.0257	-0.4986	0.2577	0.6478	0.1782	0.0898	1	0.4209	0.5938	0.2334	-0.0268	0.0806
	(0.8785)	(0.9401)	(0.1185)	(0.4442)	**(0.0311)***	(0.6)	(0.7929)	(1)	(0.1974)	(0.0541)	(0.4898)	(0.9376)	(0.8138)
Cu	0.0534	0.2896	0.11	0.5044	0.2038	0.3952	0.8019	0.4209	1	0.8824	-0.0642	-0.2353	-0.0933
	(0.876)	(0.3876)	(0.7474)	(0.1136)	(0.5477)	(0.2290)	**(0.0030)***	(0.1974)	(1)	**(0.0003)***	(0.8513)	(0.4862)	(0.7849)
Mn	-0.1473	0.4464	-0.1288	0.5336	0.2899	0.4549	0.7942	0.5938	0.8824	1	-0.0964	-0.0961	-0.325
	(0.6656)	(0.1687)	(0.7059)	(0.0909)	(0.3872)	(0.1598)	**(0.0035)***	(0.0541)	**(0.0003)***	(1)	(0.778)	(0.7785)	(0.3295)
Ca	0.203	0.0363	-0.0861	-0.19	0.5427	-0.2866	-0.4440	0.2334	-0.0642	-0.0964	1	0.7024	0.7323
	(0.5493)	(0.9156)	(0.8012)	(0.5757)	(0.0845)	(0.3928)	(0.1713)	(0.4898)	(0.8513)	(0.778)	(1)	**(0.0160)***	**(0.0104)***
Mg	-0.0245	0.0881	-0.0185	-0.0417	0.299	-0.0985	-0.2843	-0.0268	-0.2353	-0.0961	0.7024	1	0.2935
	(0.943)	(0.7967)	(0.9569)	(0.903)	(0.3718)	(0.7732)	(0.3968)	(0.9376)	(0.4862)	(0.7785)	**(0.0160)***	(1)	(0.381)
Soil texture	0.3814	-0.4021	0.0639	-0.207	0.2603	-0.4169	-0.538	0.0806	-0.0933	-0.325	0.7323	0.2935	1
	(0.2471)	(0.2203)	(0.852)	(0.5415)	(0.4395)	(0.2021)	(0.0878)	(0.8138)	(0.7849)	(0.3295)	**(0.0104)***	(0.381)	(1)

Correlation (p-value) * with a significant difference at $p \leq 0.05$

4.4 The Correlation Between Plant Diversities/Densities and Sandfly Abundance /Distribution in Bungoma County

4.4.1 Plant Richness, Checklist, Diversity and Distribution in sampled sites in Bungoma County

Twenty-eight plant species belonging to 25 genera were found to have been most commonly associated with caves where sandflies were present. There was a high overall Shannon Weiner diversity index of the plants was 3.14 *H'.* The location/ area with the highest number of observed plant species was Makhonge (16) followed by Nabuyole (15) whereas Chebin, Chepkutuny, Chepkarai, Chebwek, Kaborom, Kaptanai each had 14 plant species that were recorded. The most common plant species were *Achyranthes aspera* J.Jacq*, Clematis vitalba* Buch.-Ham. ex Steud.*, Pavonia burchelii* (DC.) R. A. Dyer and *Solanecio manii* (Hook. F.) C. Jeffrey being found in more than eight study sites out of the twelve sampled study sites. *Urtica massaica* Mildbr was found in all the sampled sites except Nabuyole site. However, *Ocimum grantissimum L., Hisbicus fuscus* Garcke*, Pavonia urens var. irakuensis* (Ulbr.) Verdc. and *Sida ovata* Forssk. were only found in the Nabuyole site (appendix 13). A list of all plant species names is provided in appendix 12, while photographs of all plant species recorded and identified are attached in the appendices 14 – 38).

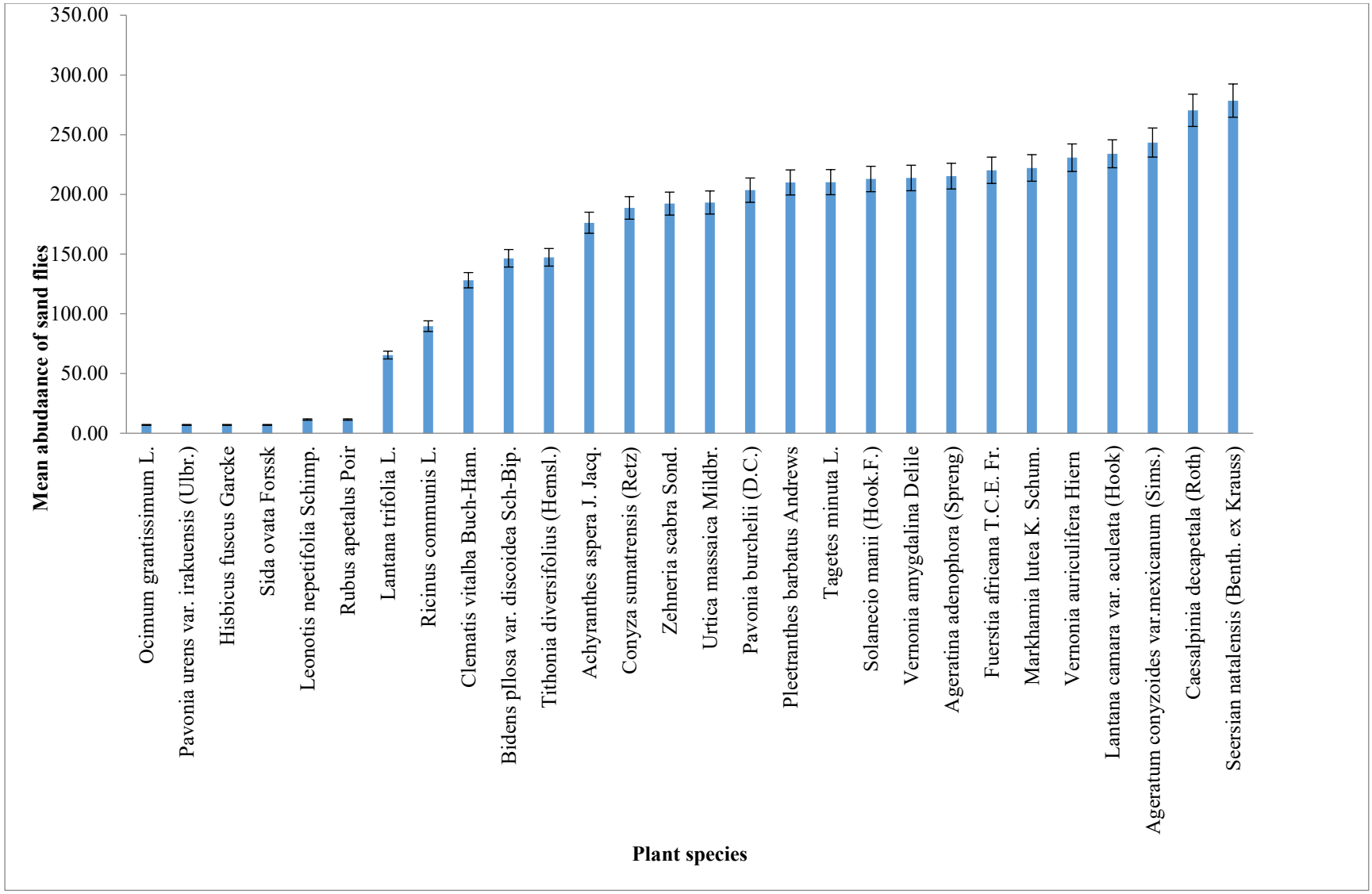


Figure 4.3 Mean Number of Sandflies Correlated with the Abundance of Plant Species in the Study Sites in Bungoma County

4.4.2 Correlation of Mean Number of Sandfly Species with Mean Number of Associated Plant Species

The overall mean number of sandflies recorded per correlated plant species was 155.13±6.34, $r(26) = 0.8256$, $p < 0.05$. The plant species that were found to have the highest mean abundance of associated sandflies was *Searsia natalensis* (Bernh. ex Krauss) F. A. Barkley (278.50±12.65) followed by *Caesalpinia decapetala* (Roth) Alston (270.41±13.97), *Ageratum conyzoides var. mexicanum* (Sims) DC. (243.42±158.83), *Lantana camara var. aculeata* (Hook.) L.H.Bailey (234.00±18.32), *Vernonia auriculifera* Hiern (230.71±16.72) and *Markhamia lutea* K.Schum. (222.14±17.92), whereas *Ocimum grantissimum* L., *Pavonia urens var. irakuensis* (Ulbr.) Verdc., *Hisbicus fuscus* Garcke and *Sida ovata* Forssk. were significantly ($p < 0.05$) associated with the lowest mean abundance of sandflies as shown in Figure 4.3. Figure 4.3 also shows that there were significant ($p < 0.05$) differences in mean number of sandflies collected and abundance of some associated plant species.

4.5 Potential Vertebrate Reservoir Hosts of Leishmaniasis in Bungoma County

4.5.1 Relative Abundance of Potential Vertebrate Reservoir Hosts of Leishmaniasis in Bungoma County

This study observed thirteen different types of vertebrates that were suspected to be potential reservoir hosts of leishmaniasis and/ or providing sources of bloodmeals in the sandfly habitats as shown in Figure 4.4. These vertebrates were sighted in or near caves were counted and recorded at the time of trap setting and traps collection. The numbers of specific type / species recorded were later quanified to obtain their means and their proportions of the total vertebrates sighted in the entire field research period. The vertebrates were bats (29.61%), goats (14.08%), people (13.52%), cows (10.39%),

rock hyraxes (9.27%), birds (7.26%), sheep (7.15%), monkeys (4.58%), dogs (2.68%), giant rats (1.01%), gazelles (0.22%), mice (0.11%) and rabbits (0.11%) with a significant difference in frequency of being sighted ($\chi^2 = 108.79$, d.f.=12, $p<0.0001$).

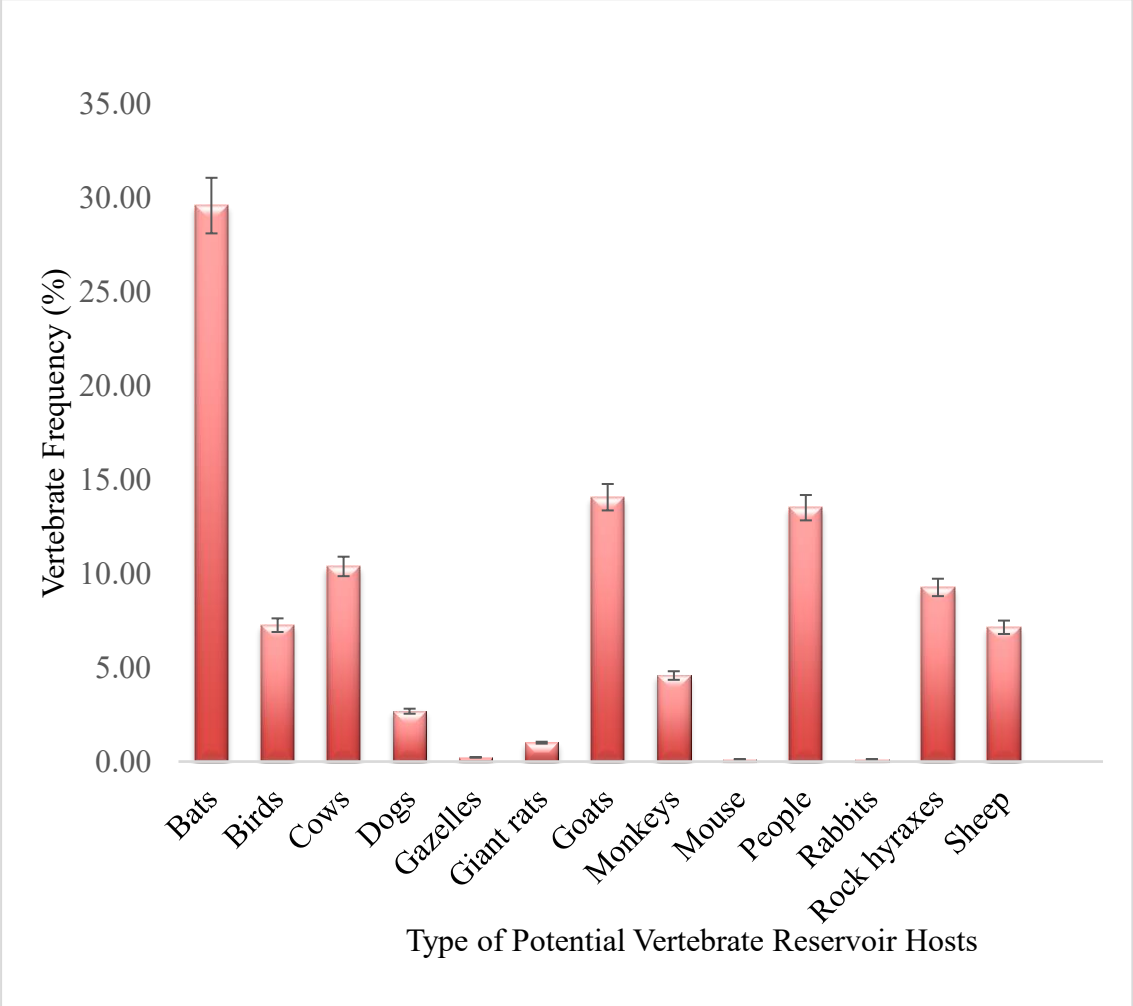


Figure 4.4 Mean Frequency of Potential Vertebrate Reservoir Hosts and Sandfly Habitats in Bungoma County

4.5.2 Correlation between Vertebrate Potential Reservoir Hosts and Sandfly Vector Abundance

The mean estimated number of all of the vertebrate potential reservoir hosts was 68.84±7.78 while the mean collection of the vector was 314.15±37.59 as shown in figure 4.5. There was a non-significant correlation between mean number of sighting the vertebrate potential reservoir hosts and the mean vector number ($r(11) = 0.4802$, $p = 0.0967$).

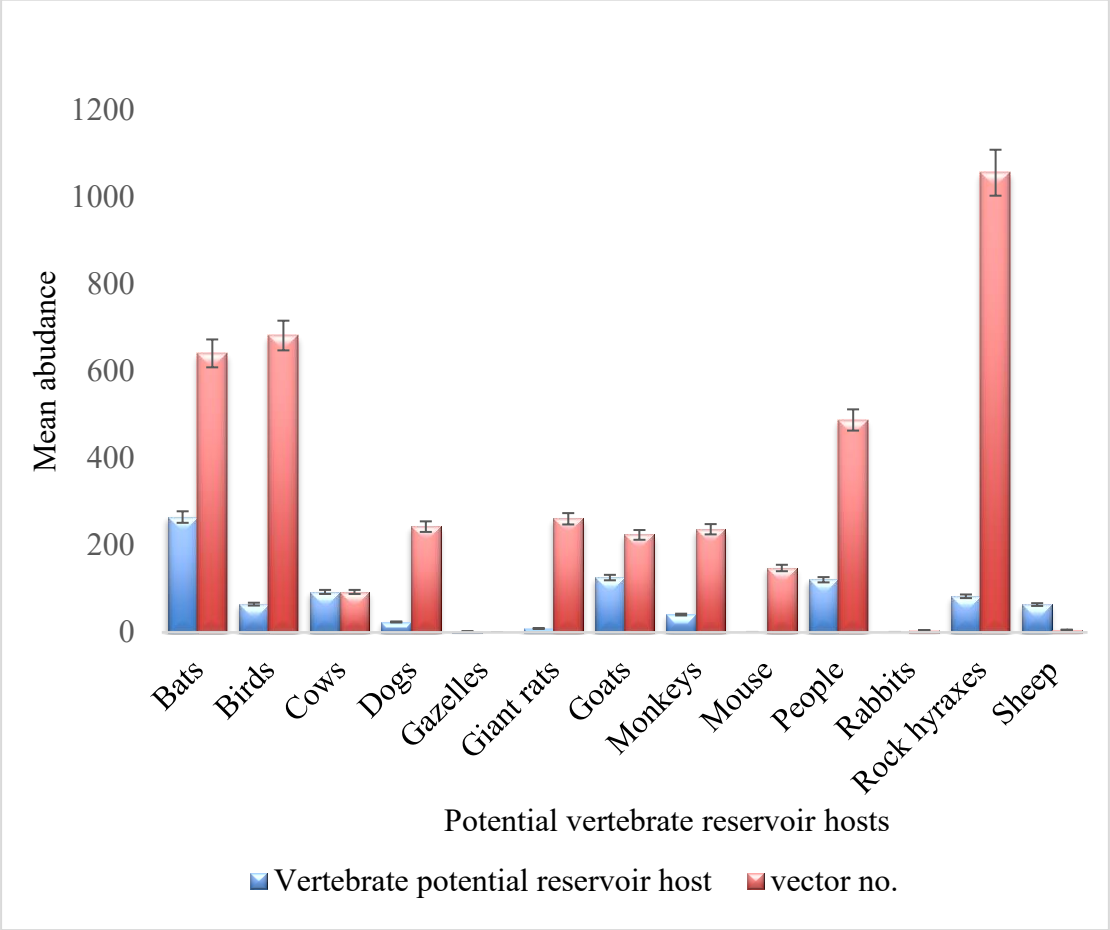


Figure 4.5 Correlation Between Mean Number of Sighting the Potential Vertebrate Reservoir Hosts and Sandfly Vector Mean Abundance

4.5.3 Diversity Index of the Potential Vertebrate Reservoir Hosts and Vector Diversity

Shannon Weiner diversity index for the potential vertebrate reservoir hosts was 2.06 *H'* with evenness of 0.66 e^H/S while the vectors' Shannon Weiner diversity index was 2.09 H' and evenness of 0.67 e^H/S as illustrated in Figure 4.6. This this suggests a moderately high and stable reservoir and vector ecosystems, which may translate to diverse vertebrate species that can support diverse sandfly species increasing chances of transmission of Leishmania parasites to humans in Bungoma county, Kenya.

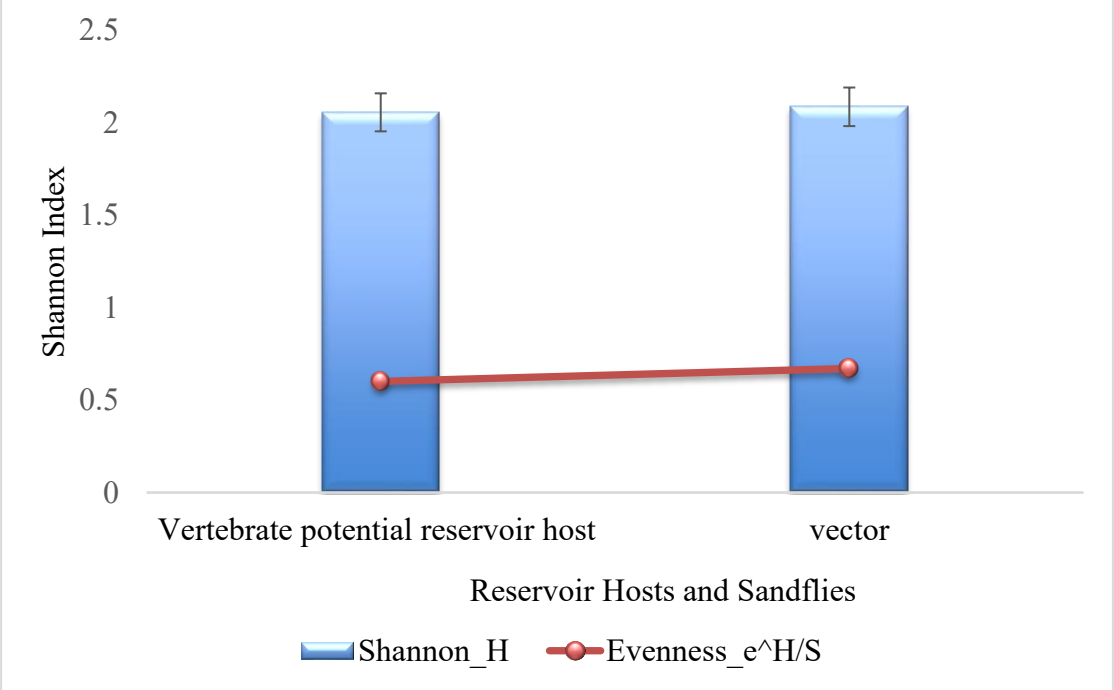


Figure 4.6 Diversity Index of the Potential Vertebrate Reservoir Hosts and Sandfly Vectors Diversity

CHAPTER FIVE

DISCUSSION

5.1 Sandflies Species and Habitat Characteristics in Bungoma County

A total of 6,156 sandflies were collected in caves during the study period which lasted for 24 months, underscoring the high density of sandfly populations in the cave habitats in Bungoma County. If the sandflies were evenly distributed in the twelve sampled caves, 513 sandflies would have been collected in each cave during the study period. Similarly, if the sandflies were evenly distributed during the study period of twenty-four months, about 257 sandflies would have been collected each month. The results of this study show that sandflies are not evenly distributed in spatial and temporal dimensions in the study area because different ecological conditions support sandfly populations differently.

Notably, sandflies were not found in human residences, suggesting that CL transmission is associated with exposure to sandflies in the cave environments. This is because studies in Mt Elgon have revealed that there is CL cases that is caused by *Leishmania aethiopica* and transmitted by *P. pedifer* and *P. elgonensis* (Mutinga *et al*., 1976). Presence of people sighted within the caves, their footprints, discarded package(s) of used items such as soft and hard drink bottles and other candies, as well as used condoms all pointed out to evidence of regular human activities in the caves. Some human bedding materials and firewood cooking points were also discovered in the caves. Upon further oral interrogations, the local communities confided that they regularly go to the caves to collect crystalized salt for their livestock, and to collect bat guano used as organic fertilizer in crop farming. Other human activities reported to

occur in the caves include; prayers and fasting, cultural rituals, touring and leisure, consumption of outlawed local alcoholic brews and other collective and personal reasons. Previous unrests amongst communities living around Mt Elgon region rendered some families homeless and many people settled for caves as their homes as well as safe hiding places from attritions instigated by criminal gangs. Even the most recent uprisings that were prompted by the outlawed Sabaot Land Defense Force (SLDF) drove so many families away from their homes, and most interesting is that the criminals in turn used caves as their operations bases as they fled from security operations.

The current economic pressure has pushed many people who live near the caves to also engage in quarrying activities around the caves, and some have even established harvesting of building stones sourced from inside these caves. The forests that previously sheltered the cave habitats have been cleared for charcoal and firewood, and areas opened up for agricultural activities that are extending up to the entrances of these caves which may have prompted further exposures to vectors-borne infections as people shelter inside the caves while tending to their farms and looking after their livestock during adverse weather conditions.

Out of the 6,156 sandflies collected, female flies outnumbered the male resulting in a male-to-female ratio of 1:2.4. This finding aligns with earlier studies by Mukhwana *et al*. (2018) and Owino *et al.* (2019) that attribute the observed sex ratio differences amongst the collected sandflies to the attraction of more female sandflies to the male-secreted pheromones, which attracted more females that males to the presence of the light traps inside the caves. Dye *et al.,* (1991) used laboratory experiments in Brazil to show that adult male *Lutzomyia longipalpis* sandflies possess sex pheromones which

aid in communication and attracts more female than it does to other male flies. The authors further stated that natural disparities between sites in the sex ratio depends on the number of blood source hosts that are available for the female flies. Because of fast responsiveness to abundance of hosts by male than female (Dye *et al.,* 1991), more females were therefore attracted towards the location of male, thus justifying why more female are caught in the light traps than male sandflies.

Collected sandflies were observed to belong to two genera and eight species, namely *Phlebotomus elgonensis, P. pedifer, Sergentomyia alderi, S. africana, S. antenatus, S. bedfordi, S. ingrami,* and *S. schwetzi.* The most abundant species of sandfly in the study area was *P. pedifer*. This dominance highlights its role as the primary vector of CL in the region (Mutinga,1775). On the other hand, the least abundant species was *S. adleri,* Other previous studies done in the Mt Elgon Sub- County also recorded relatively high abundance of *Phlebotomus* species (Sang and Chance, 1993; Sang *et al.,* 1993; Mukhwana *et al.,* 2018, and Ombaka *et al.,* 2022).

Of all the sandfly species collected, *P. pedifer* and *P. elgonensis* are confirmed vectors of CL. This indicates the actual risk of potential infection threat to people through infective bites with *Leishmania* parasites. This had been confirmed by the previous research studies conducted in Mt Elgon Sub-County implicating the duo as vectors that actively transmit *Leishmania aethiopica* that causes CL to the people living around this area (Mutinga *et al.,* 1986-iii; Sang *et al.,* 1993; Mukhwana*,* 2018; Mukhwana *et al.,* 2018; Ombaka *et al.,* 2022).

Sergentomyia species have for long been referred to as biting nuisance flies. However, some recent studies have incriminated some species in this genus as leishmaniasis vectors. Nzelu *et al*., (2014) and de Souza *et al.,* (2023) reported that *S. ingrami, S.*

mahoni and *S. africana africana* were detected with *L. tropica, L. major* and also *Trypanosoma* species DNA in Ghana, where they were suspected to transmit CL to people. *S. ingrami* were also reported by Mutinga *et al*., (1986 - iv) to be responsible for the transmission of *L. major* parasites to the susceptible rodents inside burrows in Baringo County in Kenya. However, Maia and Depaquits, (2016) and Sadlova *et al.,* (2013) reported that *S. schwetzi, S. alderi, S. bedfordi* and *S. antenatus* were not competent vectors of *L. donovani* and other *Leishmania* species that are pathogenic to humans, and therefore fail to meet the threshold to be incriminated as successful vectors of human CL.

With regards to the areas sampled, Chepkarai had the highest number of sandflies collected followed by Chepkutuny while Nabuyole had the least number of sandflies collected. Sang and Chance (1993) reported that *P. pedifer* and *P. elgonensis* sandflies were collected in 229 out of 237 sampled caves distributed at different altitudes of Mt. Elgon Sub-County. However, they did not list the names/locations of the specific caves that the sandflies were collected from. This current research has listed particular names of caves and GPS coordinates where the sandflies were collected from, not only in extended divisions of Mt. Elgon Sub-County but also across the other Sub-counties in the lower altitudes in Bungoma County that are being reported for the first time. Variations in distribution of numbers and species of sandflies at different sites can be attributed to various ecological characteristics.

Interestingly, all *P. elgonensis* were found in very high altitude habitats (Chepkitale region including Labot caves), while *P. pedifer* were distributed across most of the lower altitude sites. The high altitude of the Labot cave, the cool ambient and soil temperatures, high relative humidity, existence of diverse species of dense natural vegetation cover and very minimal human activities could explain why *P. elgonensis*

uniquely preferred this site. This observation agrees with similar finding by Sang and Chance, (1993) who found that *P. elgonensis* predominated high altitudes (2300-2600 masl) while *P. pedifer* were more at lower altitudes. It also appears that *P. pedifer* and *S. schwetzi* are very well adapted to the local cave conditions of Bungoma County as indicated by their wide distribution across different cave sites except the high-altitude conditions.

However, sandflies in the genus *Sergentomyia* are not yet confirmed vectors of leishmaniasis in Kenya which is still subject to further investigations in the roles they play in co-existence with the confirmed vector sandfly species. The results of the current study highlight the variation in sandfly species distribution across the different sampled areas, which is important in designing targeted control measures for sandfly-borne diseases in the region.

There was a significant difference ($p < 0.05$) in the diversity of sandfly species among the different sampled areas. Chepkutuny, Sang'alo, and Nabuyole caves had the highest Shannon-Weiner diversities at 0.75*H*, 0.69*H* and 0.69*H* respectively. This indicated that they had a higher number of different sandfly species and also had a more even distribution sandfly species compared to other areas that had lower diversity indices. Chepkutuny cave for instance, had the most diverse species (comprising of a total of six) and also recorded the second highest number of total sandflies caught. This can be attributed to the nature of this cave which has a very small almost enclosed entrances, thus it is insulated inside with higher temperatures that favor the sandflies' biological activities such as reproduction and development. There is also limited human activity inside this cave due to its low height and darkness. The conditions of this cave forms good habitat for rock hyraxes, bats, and other vertebrates that probably serve as reservoir hosts that not only provide blood meals for the female adult sandflies but also

provides tons of manure from their fecal matter on which juveniles of sandflies thrive which has not been documented before. Ombaka *et al*, (2022) also confirmed this while working in a similar cave, as well as Mutinga and Odhiambo, (1986 - iii) as they worked on breeding and resting sites of sandflies in unnamed caves of Mt Elgon where they found that *P. elgonensis* and *P. pedifer* breed and rest mainly in the poorly lighted areas of the caves, on the roof, the floor of the caves under objects, and in the cracks and crevices inside the caves; and also were abundant in cracks and crevices outside the caves where hyraxes were found to rest.

Sang'alo hills had several small sheltered caves in which hundreds of Christians of varied religions converged every day and night for prayers and fasting. These congregations may have provided readily needed sources of blood meals that favored several species of sandfly populations. Similarly, Nabuyole caves that had attractive sceneries along River Nzoia bordering historic Chetambe Hills where the Bukusu people fought colonial invaders. People visited these sites for tours and leisure and they may be getting exposed to vector sandfly bites in these sheltered caves. On the other hand, Chebin, Chepkarai, Koborom, Labot, Makhonge, and Tamoi had the least sandfly diversity, with Shannon Weiner index (*H'*) values below 0.01, which suggests that there was only one sandfly species that was found inhabiting these caves. This may be explained by factors, such as climatic and environmental conditions, host availability, and the presence of predators or competitors. However, further research studies are needed to determine the specific factors contributing to the observed differences in sandfly diversity amongst the sampled areas.

5.2 Impact of Temperature, Relative Humidity and Altitude on the Occurrence and Abundance of Sandflies in Bungoma County

P. elgonensis was the only species of sandfly that was found in high altitude, probably because of the cool temperature and high relative humidity that uniquely favored its survival. This sandfly species had unique reddish colour on its external cuticles. This is the first study to document this observation. Previous studies in Mt Elgon have not documented the sandfly colour. The high altitude, low temperatures, and other environmental and climatic conditions are suspected to be the most likely cause of this unique observation. The types of plants from which the sandflies source their sugar meals could be another possible reason for the red coloration.

P. pedifer was found in altitude ranging from 1506 to 2147 masl with a high abundance recorded in altitude of 1775 masl. The results agree with those of Sang and Chance (1993) who reported that *P. pedifer* and *P. elgonensis* were found distributed at different altitudes. *P. pedifer* was found to occur in lower altitudes (1750-1900 m) while *P. elgonensis* predominated the higher altitudes (2300-2600 m), and high occurrence of promastigotes in the guts of phlebotomine sandflies found in lower altitudes below 1900 m (areas associated with *P. pedifer*). They concluded that *P. pedifer* was the major vector of CL in Mt Elgon. Sang, (2011) also indicated that *P. elgonensis* were mainly found in the intermediate higher altitudes and were found associated with river valleys and major cliffs that overlook river valleys. Mukhwana *et al*., (2018) found allopatric distribution of leishmaniasis transmission risks with *P. pedifer* as the only vector species found in both Bungoma and Trans-Nzoia Counties along the slopes of Mt Elgon. The ecology of *P. elgonensis* seems to shift to higher altitudes over time than has previously been recorded. Leishmaniasis in the intermediate and lower altitudes soon may solely be the role of *P. pedifer* that has swept over all the habitat ecosystems

across these altitudes. Additionally, recent publications have not recorded *P. longipes* species that were reported previously in the Mt Elgon region; suggesting a need for further research on this sandfly.

A significant proportion of *Sergentomyia* species, such as *S. adleri* and *S. bedfordi*, were observed in mid-altitude regions, while *S. africana, S. antenatus*, and *S. schwetzi* were identified across a range of altitudes spanning low to high elevations. Interestingly, *S. ingrami* was exclusively found in lower-altitude sites. Although *Sergentomyia* species are traditionally regarded as non-vectors of leishmaniasis, their extensive geographical and environmental distribution calls for further investigation. Relatively recent studies reporting the isolation of *Leishmania* parasites from their digestive systems (Sadlova *et al.,* 2013; Kanjanopas *et al.,* 2013; Maia & Depaquit, 2016) challenge this assumption, suggesting a potential, albeit underexplored, role in disease transmission.

The environmental conditions of the study sites demonstrated considerable variation, influencing sandfly distribution and habitat preferences. For instance, *Phlebotomus elgonensis* was exclusively found in high-altitude regions characterized by cooler temperatures and distinct soil conditions. These challenging environmental conditions likely limit the survival of other sandfly species, and highlighting the unique adaptations of *P. elgonensis* to such niches. Additionally, higher altitudes were associated with increased humidity, which could further explain the habitat specificity observed among sandflies.

The distribution of sandfly species in the study area revealed varying adaptations of the sandflies to environmental conditions. *Phlebotomus pedifer* was predominantly found in warmer sites with ambient temperatures above 26°C, suggesting the preference for

higher temperature ranges. Similarly, *Sergentomyia* species occupied a range of ambient temperature conditions, with the majority being recorded at 28°C. Notably, *S. adleri* and *S. bedfordi* were exclusively found at this temperature, while *S. ingrami* preferred slightly cooler conditions. Other species such as *S. schwetzi, S. africana,* and *S. antenatus* exhibited a broader range of temperature tolerance, with significant differences observed across their habitats.

The study also highlighted the influence of soil temperature on sandfly distribution. While all sandfly species except *Phlebotomus elgonensis* that were found in caves with varying soil temperatures, significant differences were noted in the soil temperature ranges across these sites. This aligns with findings by Basimike and Mutinga, (1990), who identified soil temperature as a stable and critical parameter influencing the distribution of phlebotomine sandflies in termite mounds and animal burrows in Kenya. Such stability in soil temperature could offer a microclimatic refuge for sandflies, enabling their survival and reproduction under otherwise variable ambient conditions.

Interestingly, the current study recorded high sandfly populations during the short rainy season, which is warmer compared to the long rainy season. However, earlier research findings associated sandfly abundance with the hot dry season (Mukhwana, 2018; Ombaka *et al.,* 2022). This observation suggests that sandfly behavior and population dynamics might be influenced not only by temperature but also by seasonal factors such as relative humidity and vegetation cover, which tend to change during the rainy periods. Size of the caves non-significantly influenced distribution of the sandflies with only *P. elgonensis* being recorded in a significantly larger cave of more than 1508 m^2 ($p<0.0001$), but not for other species. This means that the sizes of the caves did not necessarily influence the distribution of sandfly population densities, while some other environmental characteristics could be responsible for this observation. It was noted

that a very large population of bats also inhabited this cave and there was evidence of frequent visits by big mammals (elephant and buffalo excreta on the floor of the cave) for rock mineral licks. The role of this activity in favoring *P. elgonensis* existence in such caves needs further investigation.

The relative humidity of the habitats significantly influenced the population of the vectors. The presence of water also influenced distribution of sandflies with a large proportion of *P. pedifer* being found in caves with flowing or stagnant water while the rest of the other species were found in caves without water. Water within caves contributes to sufficient humidity which has been shown to have significantly influenced the population densities of sandflies (Sang 2011; Mukhwana *et al*., 2018; Pareyn *et al*., 2019). Perhaps the current findings could be indicating that moderately high relative humidity promotes sandflies activity but excessively high relative humidity may be promoting fungal and bacterial activities that tend to check the sandfly population in such habitats. Further research should be conducted to fully understand the role of presence of water points inside the cave habitats.

Sandfly abundance was positively correlated with soil moisture. This agrees with previous studies which found that the development and survival of juvenile sandfly stages highly rely on appropriate soil moisture contents (Bettini & Melis, 1988; Basimike *et al*., 1992; Paul *et al*., 2006; Bhunia *et al*., 2020;). Preference of highly moist soils means that there is sufficient hydration for successful development of juvenile stages to the adult stage.

5.3 The impact of Soil Physical and Chemical Characteristics to the Population of Sandflies in Bungoma County

Correlation tests amongst different soil physical and chemical properties suggested that there were some correlations. These included soil texture, pH, percentage moisture, nitrogen, phosphorus, carbon, iron, zinc, copper, manganese, calcium and magnesium. However, none of these properties appeared to significantly correlate with the sandfly occurrence and abundance. This meant that these elements were not sufficient enough to determine how the distribution of vector populations in different habitats. However, they may have the potential to significantly affect the sandfly population characteristic when combined with other unknown habitat factors.

To start with, the general neutral to alkaline soil pH (7.25 ± 1.40) found in 10 sites was good for sandfly development and colonization of the habitats. Vivero *et al.,* (2015) while working on the natural breeding sites of sandflies in Columbia found that neutral to slightly alkaline ecosystems indicated stable characteristic niches that favor breeding sites in terms of homeostasis, digestion of nutrients, and ionic exchange. Alencar *et al*., (2011), reported that assessment of breeding sites for sandflies revealed slightly acidic to neutral soils and did not show any significant correlations with the number of immature sandflies collected. Srinivasan *et al*., 2013 also reported that sandfly density and diversity were influenced by rural locations on fluvial landforms with alkaline soil surface pH that supported rice cultivation and luxuriant vegetation. These observations on suitability of soil pH (7.25 ± 1.40) are in agreement with the current findings.

This study observed that soil texture also affected the mean collections of sandflies. All the habitats which were positive for sandflies had loam sandy soils, with very high organic matter. This indicated increased capacity of high carbon and nitrogen contents that were found to have significant correlations among the soils collected from all sites.

Decomposed vegetation, dead animals, animals' fecal matter, and other organic compounds all contribute to high carbon and nitrogen content in the soil. Vivero *et al*., (2015) suggested that sand and loam soils enhance sandfly breeding that ensured good drainage and air circulation thus facilitating larval survival.

Similarly, soil compactness on habitat floor influenced sandflies distribution with a large proportion of sandfly species being found on floors with dry loose soil. It has been indicated that the soil forms breeding surface where oviposition and development of juvenile stages of sandflies take place (Bettini, 1989, Singh *et al.,* 2008 and Ranganathan and Swaminathan, 2015and). Dry loose soils provide the best aeration for incubation, easy movement and feeding of the juvenile stages of sandflies by providing sufficient aeration for incubation and easy movement of the larvae within the soil as they feed, that is why many adult sandflies were collected from caves with such soil characteristics. In contrast, Elnaiem, (2011) found many *P. orientalis* sandflies species that transmit *L. donovani* that causes VL distinctively in close association with compact black cotton soil and *Acacia seyal-Balanite aegyptica* vegetation in East Africa. This suggests that *P. orientalis* has adapted to survive in this soil type probably due to its ability to retain moisture in the semi- arid conditions of the sayel climate.

Phosphorus, iron, copper, zinc and calcium were also found to have a positive influence on sandfly population. In contrast, magnesium and manganese were found to have a negative influence on sandfly population. All these elements were present in high concentration which is due to the continuous weathering processes of volcanic rocks found in the cave habitats. A previous study by Khan *et al.,* (2022) in Pakistan reported that the high concentrations of magnesium, potassium, calcium and zinc moderately correlated with immature and adult sandfly collections. A study in Baringo County in Kenya done by Basimike *et al*., (1992) did not find correlation between sandflies of

genera *Phlebotomus/Sergentomyia* and soil chemical-physical factors. However, *Sergentomyia* species were negatively correlated with soil pH and phosphorus. Rahman *et al*., (2018) concluded that physical and chemical characteristics of soil in area of leishmaniasis endemicity have been postulated to contribute towards affording suitable environments for sandfly breeding. Environmental intervention measures towards control of breeding sites could be harnessed in designing further policies to impede breeding of these public health important vectors.

5.4 Sandfly-Plant Relationships

The population dynamics of sandflies across diverse habitats is determined by several factors including their interaction with certain plant species (Torto *et al*., 2022). Understanding of plant-feeding behavior of sandfly vectors of leishmaniasis could provide vital clues on how chemical leads that direct this behavior can be used in sandfly management. Schlein *et al*., (2001) observed that feeding adult sandflies on noxious plants such as *Solanum jasminoides, Ricinus communis* or *Bougainvillea glabra* branches for one night drastically shortened the life span of sandflies.

The overall Shannon Weiner diversity index (3.14 *H'*), for the plants assessed in Bungoma County, which is classified as a high diversity index value scale indicated richness in species diversity and evenness in distribution across the sandflies' varied habitat locations (Spellerberg & Fedor (2003; Strong 2016). Therefore, correlated results for diversity and number of sandflies signaled a general increasing trend in means of sandfly numbers with increase in plant diversity. Of all the plant communities assessed, some have direct influence towards increased sandfly population while some presented deterrent effects that repel or suppress sandfly populations (Schlein & Jacobson 1999; Jacobson & Schlein 1999; Dinesh *et al*., 2014; Hassaballa *et al.,* 2021 and Torto *et al*., 2022).

The most common plant species (frequency) that were found to have been in consistent association with sandfly populations in the study sites included *Achyranthes aspera, Urtica massaica, Zehneria scabra, Tithonia diversifolius, Pavonia burchelii, Solanecio manii* and *Clematis vitalba)* as they were found in more than eight areas out of twelve areas that were assessed. *Ocimum grantissimum, Hisbicus fuscus, Pavonia urens, Sida ovata, Lantana trifolia, Leonotis nepetifolia*, *Rubus apetalus* and *Searsia natalensis* had the lowest frequency score out of the 12 sampled sites. These plant species with the highest frequencies in this research area generally indicated a sure signal for the presence of sandflies in a particular habitat. However, it might not be a true indicator of the sandflies' abundance and diversity.

Ricinus communis and *Tagetes minuta* have shown repellent or insecticidal effects against sandflies (Dinesh *et al*., 2014). *Tithonia diversifolius* has been suggested to be investigated for antileishmanicidal effects (Tagne *et al*., 2018), while *Lantana camara* has also been associated with antileishmanicidal effect (Pohlit *et al*., 2011). Although this study found significant positive correlation between these plant species (*Tithonia diversifolias* and *Lantana camara*) and sandfly density, we suggest that the sandflies which feed on sugars of these plants be investigated for the likelihood of being free from *Leishmania* parasites. Perhaps these plant species have anti-leishmanicidal activity that is why they are fondly found associated with vectors, and thus they enhance the quality of their lives hence prolong these insects' lifespan besides providing useful nutrients that enhances constantly high vector densities in the locations they were found. However, this needs to be investigated and if found to be true, then communities living in leishmaniasis endemic areas should be encouraged to cultivate these plants around the vector habitats as one of the eco-friendly control measures against

leishmaniasis. This will mean that despite the vector bites there will be no effective transmission of the parasites as sandflies will be free from the infective parasites.

Sandflies more often feed on plant-derived sugars such as nectar, honeydew and phloem sap as source of energy (Moore *et al*., 1987; Wallbanks *et al*., 1991; Bonfim-Melo *et al*., 2018). The plant sugar dependence explains the reason why the structure of plant communities can influence the spatial-temporal distribution of communities of sandflies and respective leishmaniasis infection cases in a given ecosystem (Bonfim-Melo *et al*., 2018).

Lantana camara leaves have been studied as biofumigant and in management of stored grain insect pests (Rajashekar *et al*., 2013 and Rajashekar *et al*., 2014), *Seersia natalensis* has also been assessed by Ashoori *et al.,* (2020) and was found to contain antileishmanicidal and antibacterial potential. *Vernonia auriculifera* has pest larvicidal activity and adult insect anti-feedant (Mitiku 2011 and Githua 2013). *Pavonia urens* has been shown to have insecticidal, larvicidal and insect repellency in Ethiopia (Degu *et al*., 2020), while *Hisbicus fuscus* was used for external treatment of insect bites and wounds in Uganda (Bussmann *et al*., 2020). These phytochemical compounds that act as insecticidal and counter-pathogenicidal activities may account for the least mean counts in numbers of sandflies associated with these plant species.

The significant differences in the mean number of sandflies per plant species ($p < 0.05$) could mean existence of special relationships with different plant species which need to be investigated further. This is supported by Schlein & Jacobson, (1994) and Schlein *et al*., (2001) findings which showed that certain plant species which sandflies feed on can inhibit their capacity to grow, develop and reproduce and also interfere with their vectorial capacity to transmit the *Leishmania* parasites. Abbasi *et al*., (2018) also noted

special preference in sandflies' choice for *Canabis sativa* plants compared to other plants. Hassaballa *et al*., (2021) while working in dry ecological VL endemic Baringo County (Kenya) reported that combination of biochemical, molecular and chemical approaches to determine plant-feeding association in sandflies collected from the area indicated similar recent fructose positivity rates for both male and female sandflies. The study also indicated that *Acacia* plant species (family: *Fabaceae*) were the most preferred by both *Phlebotomus* and *Sergentomyia* species; with *Vachellia. tortilis* species being the most preferred as compared to *Senegalia. laeta, Vachellia. nilotica, Faidherbia albida* and invasive *Prosopis juliflora*. Similar findings were reported by Lima *et al*., (2016). This selectivity in the preference of plant species by sandflies could be exploited in designing useful strategies that can be used to control sandfly vectors in such habitats, particularly the extracts of phytochemicals of plant species that attracted the lowest number or no sandflies as insect repellents, toxins and for their antimicrobial activities.

5.5 Vertebrates Abundance- Sandfly Population Relationship

5.5.1 Relative Abundance of Vertebrate Potential Reservoir Hosts of Leishmaniasis in Bungoma County

The diversity of potential vertebrate reservoir hosts of leishmaniasis in the study area was assessed, underscoring the broad range of diverse species that could play role in the transmission of the CL disease. A total of thirteen different vertebrate species were identified as potential reservoir hosts, with varying contributions to the overall reservoir pool. The species included bats, birds, cows, dogs, gazelles, giant rats, goats, monkeys, mice, humans, rabbits, rock hyraxes, and sheep (Some are shown in appendices 5, 6,7, 8, 9, 10, and 11). Some of these like bats, rock hyraxes and giant rats that have been

confirmed as successful reservoirs were very abundant within the habitats, while others like pets, livestock and humans come and leave.

These findings align with earlier studies, such as Mutinga's (1975) work on the reservoir hosts of leishmaniasis in Mt. Elgon, where he recorded the presence of hyraxes (*Dendrohyrax arboreus* and *Procavia capensis*) and giant rats (*Cricetomys* spp.). The current study also found hyraxes and giant rats with dermal lesions (as shown in appendix 7) containing amastigotes of *Leishmania* parasites. This provides a direct link between these animals and their potential to act as reservoir hosts of CL reported in human cases in the study area. Similarly, blood meal analysis from *P. pedifer* sandflies in the area revealed the presence of *Leishmania* parasites, further confirming the transmission cycle involving these vertebrate hosts (as shown in appendix 11). Other studies by Makwali, (2021) and Ombaka *et al.,* (2022) also identified rock hyraxes, giant rats, bats, and cats as important reservoir hosts in the Mt. Elgon cutaneous leishmaniasis (CL) focus.

While some of the identified species may not serve as direct reservoir hosts of leishmaniasis, they can still play a critical role in sustaining sandfly populations. For example, animals such as dogs, goats, and cattle may provide blood meals for sandflies, ensuring the continuation of vector populations in the absence of preferable reservoir hosts. This highlights the complexity of the transmission cycle, where even non-reservoir hosts can support the vector population until they encounter a suitable reservoir host to complete the transmission cycle. The role of these non-reservoir hosts underscores the importance of considering all potential ecological factors, including alternative blood sources, when assessing leishmaniasis transmission risks.

5.5.2 Correlation between Vertebrate Potential Reservoir Hosts and Vector Numbers

The study observed a positive but non-significant correlation between the abundance of vertebrate potential reservoir hosts and sandfly vector numbers ($r(9) = 0.4802$, $p = 0.0967$). This positive correlation suggests that, as the number of vertebrate potential reservoir hosts increases, there is a tendency for sandfly populations to increase as well.

Several factors could contribute to this outcome. The absence of a significant relationship may reflect that the density of sandflies is not solely determined by the number of potential reservoir hosts present. Other ecological variables, such as the type of host, microhabitat conditions, or environmental factors (e.g., temperature, humidity), may also play critical roles in determining sandfly abundance. For instance, some species of reservoir hosts may be more attractive to sandflies or may sustain vector populations in ways that others do not. Furthermore, the presence of non-reservoir hosts could also influence sandfly numbers by providing alternative blood meals, thus sustaining vector populations even when reservoir hosts are less abundant or unavailable.

5.5.3 Diversity Index of the Vertebrate Potential Reservoir Host Type and Vector Diversity

Shannon Weiner diversity index for the vertebrate potential reservoir host type and sandfly vectors species indicated that it was moderately high (2.06 *H'* and 2.09 *H*' respectively). Both groups had high evenness (0.66 e^H/S and 0.67 e^H/S respectively) which indicated species richness and more even distribution across the habitats. This can also be interpreted that reservoir hosts are spread across several vertebrate host types with a generalized vector feeding behavior, possibly biting multiple host species. Rock hyraxes, birds, bats, people, giant rats and dogs were associated with the highest

mean densities of the collected sandflies. These findings can be related with previous findings in other publications that confirmed *Leishmania* parasites in the above listed hosts (Makwali*,* 2021, Ombaka *et al.,* 2022, Mukhwana *et al*., 2018 and Mutinga, 1975). This observation suggests that different species of sandflies prefer specific vertebrate host(s) for blood meal source. Knowledge on preferred blood meal source can be exploited in targeted control of leishmaniasis focused on the actual vertebrate reservoir hosts. However, one should have in mind that control strategies targeting only one vertebrate host species may not be effective because other vertebrates may still maintain the transmission cycle.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study revealed a relatively wide sandfly species diversity (two genera and eight species) namely; *Phlebotomus pedifer, Phlebotomus elgonensis, Sergentomyia adleri, Sergentomyia africana, Sergentomyia antenatus, Sergentomyia bedfordi, Sergentomyia ingrami,* and *Sergentomyia schwetzi* in Bungoma County.
2. CL vectors are widely distributed than previously reported in Bungoma County, and *P. pedifer* is the main vector of CL.
3. Altitude and temperature are key climatic factors that determine the distribution of sandfly vectors in cave habitats of Bungoma County.
4. Both soil physical and chemical factors on their own seem not to determine the abundance and distribution of sandflies in their natural habitats in Bungoma County.
5. *Urtica masaica* Mildbr was found across all the habitats except one, *Searsia natalensis* (Bernh. ex Krauss) F.A.Barkley was highly associated with sandfly abundance compared to other plant species, while *Osimum grantissimum* L. was least associated with sandfly abundance in the cave environment.
6. The abundance of sandflies in a given habitat is not only determined by the number of vertebrate reservoirs but also the host type of the vertebrates, as sandflies express biased preference for specific bloodmeal source animal types than others

6.2 Recommendations

1. Since diverse *Sergentomyia* species were found in the study area alongside the confirmed vector species (*Phlebotomus pedifer* and *Phlebotomus elgonensis*), they should be investigated for their potential in transmission of CL in Bungoma County.

2. Molecular assessment of the sandfly species found in the Bungoma County habitats should be conducted to precisely confirm the specific species taxonomic identities.

3. Policies should be formulated to restrict or minimize human activities inside the caves so as to reduce on the sandflies' bites that could potentially spread leishmaniasis to people who frequent the caves.

4. Extensive and intensive research should be carried out to locate and characterize the exact oviposition and larvae development points in the natural ecosystems for future targeted control interventions so as to interrupt the lifecycle of the sandfly vectors before they become infective adults.

5. *Searsia natalensis,* (Bernh. ex Krauss) F.A.Barkley, *Urtica masaica* Mildbr and *Caesalpinia decapetala* (Roth) Alston should be investigated for their potential as vector attractants; *Ocimum grantissimum* L.*, Pavonia urens* (Albr.) Verdic.*, Fuscus hibiscus* Garcke, and *Sida ovata* Forsk should be investigated for their repellency properties, and all these plants should further be investigated for insecticidal, larvicidal and leishmanicidal properties.

6. Molecular assessment should also be done to characterize the specific plant sugar sources unique to these habitats that could be exploited for effective control of the sandfly vectors of CL in Bungoma County.
7. Further research activities are also recommended to determine and document all the species or types of vertebrate reservoir hosts in Bungoma County that provide blood meal sources and successfully perpetuate the lifecycle of *Leishmania* parasite so that specific control measures are redesigned to limit CL infection cases.

REFERENCES

Abbasi, I., Trancoso Lopo de Queiroz, A., Kirstein, O. D., Nasereddin, A., Horwitz, B. Z., Hailu, A., ... & Warburg, A. (2018). Plant-feeding phlebotomine sand flies, vectors of leishmaniasis, prefer *Cannabis sativa*. *Proceedings of the National Academy of Sciences*, *115*(46), 11790-11795.

Abdullah, A. Y. M., Dewan, A., Shogib, M. R. I., Rahman, M. M., & Hossain, M. F. (2017). Environmental factors associated with the distribution of visceral leishmaniasis in endemic areas of Bangladesh: modeling the ecological niche. *Tropical Medicine and Health*, *45*, 1-15.

Abonnenc, E. & Minter, D.M. (1965). Bilingual keys for the identification of the sandflies of the Ethiopian Region (in Fr. and Eng.). *Cahiers Orstom. Entomologie Médicale 5*:1-63

Abu-Hurub, A. S., Okbah, A. A., Al-Dweelah, H. M. A., Al-Moyed, K. A., Al-Kholani, A. I. M., Al-Najhi, M. M. A., & Al-Shamahy, H. A. (2022). Prevalence of visceral leishmaniasis among adults in Sana'a City-Yemen. *Universal Journal of Pharmaceutical Research*, *7*(2), 21-26.

Akhoundi, M., Kuhls, K., Cannet, A., Votýpka, J., Marty, P., Delaunay, P., & Sereno, D. (2016). A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Neglected Tropical Diseases*, *10*(3), e0004349.

Aklilu, E., Yared, S., Gebresilassie, A., Legesse, B., & Hailu, A. (2023). Phlebotomine sandflies (Diptera: Psychodidae) of Ethiopia. *Heliyon*, *9*(3).

Alemayehu, B., & Alemayehu, M. (2017). Leishmaniasis: a review on parasite, vector and reservoir host. *Health Science Journal*, *11*(4), 1.

Alencar, R. B., De Queiroz, R. G., & Barrett, T. V. (2011). Breeding sites of phlebotomine sand flies (Diptera: Psychodidae) and efficiency of extraction techniques for immature stages in Terra-Firme Forest in Amazonas State, Brazil. *Acta Tropica*, *118*(3), 204-208.

Alexander, B. (2000). Sampling methods for phlebotomine sandflies. *Medical and Veterinary Entomology, 14*(2), 109-122.

Almeida, P. S. D., Andrade, A. J. D., Sciamarelli, A., Raizer, J., Menegatti, J. A., Hermes, S. C. N. M., ... & Gurgel-Gonçalves, R. (2015). Geographic distribution of phlebotomine sandfly species (Diptera: Psychodidae) in Central-West Brazil. *Memórias do Instituto Oswaldo Cruz*, *110*, 551-559.

Alten, B., Maia, C., Afonso, M. O., Campino, L., Jiménez, M., González, E., ... & Gradoni, L. (2016). Seasonal dynamics of phlebotomine sand fly species proven vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*. *PLoS Neglected Tropical Diseases*, *10*(2), e0004458.

Alten, B., Ozbel, Y., Erygunay, K., Kasap, O. E., Cull, B., Antoniou, M., … & Medlock, J. M. (2015). Sampling strategies for phlebotomine sandflies (Diptera: Psychodidae) in Europe. *Bulletin of Entomological Research, 105*(6), 664-678.

Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., ... & WHO Leishmaniasis Control Team. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PloS One*, *7*(5), e35671.

Anjili, C. O., Ngumbi, P. M., Kaburi, J. C., & Irungu, L. W. (2011). The phlebotomine sandfly fauna (Diptera: Psychodidae) of Kenya. *Journal of vector borne diseases*, *48*(4), 183.

Aoun, K., & Bouratbine, A. (2014). Cutaneous leishmaniasis in North Africa: a review. *Parasite*, *21*.

Ashoori, F., Fakdar, M., Goli, H. R., Mirzaee, F., Faridnia, R., Kalani, H., & Shahani, S. (2020). Antileishmanial and antibacterial activities of the hydroalcoholic extract of *Rhus coriaria* L. *Annals of Parasitology*, *66*(2).

Bailey, F., Mondragon-Shem, K., Hotez, P., Ruiz-Postigo, J. A., Al-Salem, W., Acosta-Serrano, A., & Molyneux, D. H. (2017). A new perspective on cutaneous leishmaniasis—Implications for global prevalence and burden of disease estimates. *PLoS Neglected Tropical Diseases*, *11*(8), e0005739.

Basimike, M. (1988). *Studies on Factors Affecting the Distribution and Abundance of Phlebotomine Sandflies in a Leishmaniasis Endemic Focus in Baringo District, Kenya* (Doctoral dissertation, Rivers State University of Science and Technology, Nkpolu, Port-Harcourt).

Basimike, M., & Mutinga, M. J. (1990). Temperature and moisture content of soils of termite mounds and animal burrows in relation to relative abundance of adult phlebotomine sandflies (Diptera: Psychodidae) in Marigat semiarid area, Baringo District, Kenya. *Environmental Entomology*, *19*(3), 486-489.

Basimike, M., & Mutinga, M. J. (1997). Studies on the vectors of leishmaniasis in Kenya: phlebotomine sandflies of Sandai location, Baringo district. *East African Medical Journal*, *74*(9), 582-585.

Basimike, M., Mutinga, M. J., Kumar, R., & Munyinyi, D. (1992). Relative abundance of adult phlebotomine sand flies (Diptera: Psychodidae) in relation to soil characteristics of their breeding sites in Baringo District, Kenya. *Environmental Entomology*, *21*(5), 1114-1120.

Bates, P. A. (2007). Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. *International Journal for parasitology*, *37*(10), 1097-1106.

Bates, P. A. (2018). Revising *Leishmania*'s life cycle. *Nature Microbiology*, *3*(5), 529-530.

Bates, P. A., Depaquit, J., Galati, E. A., Kamhawi, S., Maroli, M., McDowell, M. A., ... & Warburg, A. (2015). Recent advances in phlebotomine sand fly research related to leishmaniasis control. *Parasites & Vectors*, *8*(1), 1-8.

Beattie, L., & Kaye, P. M. (2011). *Leishmania*–host interactions: what has imaging taught us?. *Cellular Microbiology*, *13*(11), 1659-1667.

Bettini, S. (1989). Sandfly breeding sites. *Leishmaniasis: The Current Status and New Strategies for Control*, 179-188.

Bhunia, G. S., Shit, P. K., Bhunia, G. S., & Shit, P. K. (2020). Sandfly ecology of kala-azar transmission. *Spatial Mapping and Modelling for Kala-Azar Disease*, 77-89.

Bittencourt, A. L., & Barral, A. (1991). Evaluation of the histopathological classifications of American cutaneous and mucocutaneous leishmaniasis. *Memórias do Instituto Oswaldo Cruz*, *86*, 51-56.

Bonfim-Melo, A., Ferreira, E. R., Florentino, P. T., & Mortara, R. A. (2018). Amastigote synapse: the tricks of *Trypanosoma cruzi* extracellular amastigotes. *Frontiers in Microbiology*, *9*, 1341.

Brisola Marcondes, C., Leuch Lozovei, A., Galati, E. A., & Taniguchi, H. H. (1998). The usefulness of Bergmann's rule for the distinction of members of *Lutzomyia intermedia* species complex (Diptera, Psychodidae, Phlebotominae). *Memórias do Instituto Oswaldo Cruz*, *93*, 363-364.

Britch, S. C., Linthicum, K. J., Walker, T. W., Farooq, M., Gordon, S. W., Clark, J. W., ... & Chepchieng, C. (2011). Evaluation of ULV applications against Old World sand fly (Diptera: Psychodidae) species in equatorial Kenya. *Journal of Medical Entomology*, *48*(6), 1145-1159.

Bussmann, R. W., Paniagua-Zambrana, N. Y., & Njoroge, G. N. (2020). *Abutilon* sp. *Abutilon angulatum* (Guill. & Perr.) Mast. *Abutilon grandiflorum* (Will.) Sweet. *Abutilon longicuspe* Hochst. ex A. Rich. *Abutilon mauritianum* (Jacq.) Medik. *Hibiscus* sp. *Hibiscus calyphyllus* Cav. *Hibiscus vitifolius* L. Malvaceae. *Ethnobotany of the Mountain Regions of Africa*, 1-12.

Center for Disease Control and Prevention (2020). Leishmaniasis. https://www.cdc.gov/parasites/leishmaniasis/index.html

Center for Disease Control and Prevention (2023). https://www.cdc.gov/

Chen, W., & Hillyer, J. F. (2013). FlyNap (triethylamine) increases the heart rate of mosquitoes and eliminates the cardioacceleratory effect of the neuropeptide CCAP. *PLoS One*, *8*(7), e70414.

Colmenares, M., Kar, S., Goldsmith-Pestana, K., & McMahon-Pratt, D. (2002). Mechanisms of pathogenesis: differences amongst *Leishmania* species. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96, S3-S7.

Dantas, M. L., Oliveira, J. M. G. C. D., Carvalho, L., Passos, S. T., Queiroz, A., Guimarães, L. H., ... & Arruda, S. (2014). Comparative analysis of the tissue inflammatory response in human cutaneous and disseminated leishmaniasis. *Memórias do Instituto Oswaldo Cruz*, *109*, 202-209.

Daoudi, M., Outammassine, A., Amane, M., Hafidi, M., Boussaa, S., & Boumezzough, A. (2022). Climate change influences on the potential distribution of the sand fly *Phlebotomus sergenti*, vector of *Leishmania tropica* in Morocco. *Acta Parasitologica*, *67*(2), 858-866.

David, C. V., & Craft, N. (2009). Cutaneous and mucocutaneous leishmaniasis. *Dermatologic Therapy*, *22*(6), 491-502.

de Souza, D. K., Addo, S. O., Desewu, K., Nzelu, C. O., Mosore, M. T., Nimo-Paintsil, S., ... & Boakye, D. A. (2023). Investigating potential sand fly vectors after the first reported outbreak of cutaneous leishmaniasis in Ghana. *Parasites & Vectors*, *16*(1), 154.

de Souza, D. K., Desewu, K., Nzelu, C. O., Addo, S. O., Mosore, M. T., Odoom, S., ... & Boakye, D. A. (2023). Identifying cutaneous leishmaniasis vectors after an outbreak: anthropophily of *Sergentomyia* species and detection of *Leishmania major* DNA within *Sergentomyia ingrami*, in Ghana.

De Thoisy, B., Duron, O., Epelboin, L., Musset, L., Quénel, P., Roche, B., ... & Guégan, J. F. (2021). Ecology, evolution, and epidemiology of zoonotic and vector-borne infectious diseases in French Guiana: Transdisciplinarity does matter to tackle new emerging threats. *Infection, Genetics and Evolution*, *93*, 104916.

Degu, S., Berihun, A., Muluye, R., Gemeda, H., Debebe, E., Amano, A., ... & Tadele, A. (2020). Medicinal plants that used as repellent, insecticide and larvicide in Ethiopia. *Pharmacy & Pharmacology International Journal*, *8*(5), 274-283.

den Boer, M. L., Alvar, J., Davidson, R. N., Ritmeijer, K., & Balasegaram, M. (2009). Developments in the treatment of visceral leishmaniasis. *Expert Opinion on Emerging Drugs*, *14*(3), 395-410.

Desjeux, P. (2004). Leishmaniasis: current situation and new perspectives. *Comparative immunology, microbiology and infectious diseases*, *27*(5), 305-318.

Desjeux, P., Ghosh, R. S., Dhalaria, P., Strub-Wourgaft, N., & Zijlstra, E. E. (2013). Report of the post kala-azar dermal leishmaniasis (PKDL) consortium meeting, New Delhi, India, 27–29 June 2012. *Parasites & vectors*, *6*(1), 196.

Dinesh, D. S., Ranjan, A., Palit, A., Kishore, K., & Kar, S. K. (2001). Seasonal and nocturnal landing/biting behaviour of *Phlebotomus argentipes* (Diptera: Psychodidae). *Annals of Tropical Medicine & Parasitology*, *95*(2), 197-202.

Dinesh, N., Kaur, P. K., Swamy, K. K., & Singh, S. (2014). Mianserin, an antidepressant kills *Leishmania donovani* by depleting ergosterol levels. *Experimental parasitology*, *144*, 84-90.

Dulacha, D., Mwatha, S., Lomurukai, P., Owiny, M. O., Matini, W., Irura, Z., ... & Network, A. F. E. (2019). Epidemiological characteristics and factors associated with visceral leishmaniasis in Marsabit County, Northern Kenya. *J Interv Epidemiol Public Heal*, *2*(1), 3.

Dye, C., Davies, C. R., & Lainson, R. (1991). Communication among phlebotomine sandflies: a field study of domesticated *Lutzomyia longipalpis* populations in Amazonian Brazil. *Animal Behaviour*, *42*(2), 183-192.

Elnaiem, D. E. A. (2011). Ecology and control of the sand fly vectors of *Leishmania donovani* in East Africa, with special emphasis on *Phlebotomus orientalis*. *Journal of Vector Ecology*, *36*, S23-S31.

Feliciangeli, M. D. (2004). Natural breeding places of phlebotomine sandflies. *Medical and Veterinary Entomology*, *18*(1), 71-80.

Gage, K. L., Burkot, T. R., Eisen, R. J., & Hayes, E. B. (2008). Climate and vectorborne diseases. *American Journal of Preventive Medicine*, *35*(5), 436-450.

Gebre-Michael, T., & Lane, R. P. (1996). The roles of *Phlebotomus martini* and *P. celiae* (Diptera: Phlebotominae) as vectors of visceral leishmaniasis in the Aba Roba focus, southern Ethiopia. *Medical and Veterinary Entomology*, *10*(1), 53-62.

Gebre-Michael, T., Balkew, M., Yimer, M., & Hailu, A. (2013). First record of *Phlebotomus (Synphlebotomus) vansomerenae* (Diptera: psychodidae) in Ethiopia. *Journal of Medical Entomology*, *50*(3), 659-663.

Gebre-Michael, T., Malone, J. B., Balkew, M., Ali, A., Berhe, N., Hailu, A., & Herzi, A. A. (2004). Mapping the potential distribution of *Phlebotomus martini* and *P. orientalis* (Diptera: Psychodidae), vectors of kala-azar in East Africa by use of geographic information systems. *Acta Tropica*, *90*(1), 73-86.

Gebresilassie, A., Yared, S., Aklilu, E., Kirstein, O. D., Moncaz, A., Tekie, H., ... & Gebre-Michael, T. (2015). Host choice of *Phlebotomus orientalis* (Diptera: Psychodidae) in animal baited experiments: a field study in Tahtay Adiyabo district, northern Ethiopia. *Parasites & Vectors*, *8*(1), 1-10.

Ghazanfar, M., & Malik, M. F. (2016). Sandfly and leishmaniasis: A Review. *J Ecosys Ecograph*, *6*(3), 1-5.

Giraud, E., Martin, O., Yakob, L., & Rogers, M. (2019). Quantifying *Leishmania* metacyclic promastigotes from individual sandfly bites reveals the efficiency of vector transmission. *Communications Biology*, *2*(1), 84.

Grifferty, G., Shirley, H., O'Brien, K., Hirsch, J. L., Orriols, A. M., Amechi, K. L., ... & Wamai, R. (2023). The leishmaniases in Kenya: A scoping review. *PLOS Neglected Tropical Diseases*, *17*(6), e0011358.

Gitari, J. W., Nzou, S. M., Wamunyokoli, F., Kinyeru, E., Fujii, Y., Kaneko, S., & Mwau, M. (2018). Leishmaniasis recidivans by *Leishmania tropica* in central rift valley region in Kenya. *International Journal of Infectious Diseases*, *74*, 109-116.

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., & Patz, J. A. (2000). Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organization*, *78*(9), 1136-1147.

Goto, H., & Lindoso, J. A. L. (2010). Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert Review of Anti-Infective Therapy*, *8*(4), 419-433.

Goto, Y., Cheng, J., Omachi, S., & Morimoto, A. (2017). Prevalence, severity, and pathogeneses of anemia in visceral leishmaniasis. *Parasitology Research*, *116*, 457-464.

Griffiths, E. (1921). Some modified forms of hygrometers. *Proceedings of the Physical Society of London*, *34*(1), viii.

Hassaballa, I. B., Sole, C. L., Cheseto, X., Torto, B., & Tchouassi, D. P. (2021). Afrotropical sand fly-host plant relationships in a leishmaniasis endemic area, Kenya. *PLoS Neglected Tropical Diseases*, *15*(2), e0009041.

Hassaballa, I. B., Sole, C. L., Cheseto, X., Torto, B., & Tchouassi, D. P. (2021b). Afrotropical sand fly-host plant relationships in a leishmaniasis endemic area, Kenya. *PLoS Neglected Tropical Diseases*, *15*(2), e0009041.

Hassaballa, I. B., Torto, B., Sole, C. L., & Tchouassi, D. P. (2021a). Exploring the influence of different habitats and their volatile chemistry in modulating sand fly population structure in a leishmaniasis endemic foci, Kenya. *PLoS Neglected Tropical Diseases*, *15*(2), e0009062.

Heisch, R. B., Wijers, D. J. B., & Minter, D. M. (1962). In pursuit of the vector of kala-azar in Kenya. *British medical journal*, *1*(5290), 1456.

Hong, A., Zampieri, R. A., Shaw, J. J., Floeter-Winter, L. M., & Laranjeira-Silva, M. F. (2020). One health approach to leishmaniases: understanding the disease dynamics through diagnostic tools. *Pathogens*, *9*(10), 809.

Honório, I. D. M. H., Cossul, M. U., da Silva Bampi, L. N., & Baraldi, S. (2016). Quality of life in people with cutaneous leishmaniasis. *Revista Brasileira em Promocao da Saude*, *29*(3), 342.

Jacobson, R. L., & Schlein, Y. (1999). Lectins and toxins in the plant diet of *Phlebotomus papatasi* (Diptera: Psychodidae) can kill *Leishmania major* promastigotes in the sandfly and in culture. *Annals of Tropical Medicine & Parasitology*, *93*(4), 351-356.

Johnson, R. N., Ngumbi, P. M., Robert, L. L., Anjili, C. O., Killick-Kendrick, R., & Meredith, S. E. O. (1993). Phlebotomine sandflies of Kenya (Diptera: Psychodidae). II. *Phlebotomus aculeatus* as a probable vector of *Leishmania tropica* sl. *Annals of Tropical Medicine & Parasitology*, *87*(5), 541-543.

Jones, C. M., & Welburn, S. C. (2021). Leishmaniasis beyond east Africa. *Frontiers in Veterinary Science*, *8*, 618766.

Kaburi, J. C. (2019). *Evaluation Of Lambda-Cyhalothrin (Icon®) Incorporated Into 1, 4-Dichlorobenzene (Pcb®) For the Control of Leishmaniases and Malaria Vectors* (Doctoral dissertation, University of Nairobi).

Kanjanopas, K., Siripattanapipong, S., Ninsaeng, U., Hitakarun, A., Jitkaew, S., Kaewtaphaya, P., ... & Leelayoova, S. (2013). *Sergentomyia* (*Neophlebotomus*) *gemmea*, a potential vector of *Leishmania siamensis* in southern Thailand. *BMC Infectious Diseases*, *13*, 1-4.

Khan, K., Khan, N. H., Anwar, F., Ullah, I., Badshah, N., Irfan, I., ... & Shah, S. U. (2022). Characterization of sand fly breeding sites in district Malakand, Khyber Pakhtunkhwa, Pakistan, and evaluation of risk factors for cutaneous leishmaniasis in the region. *Zoonoses and Public Health*, *69*(1), 33-45.

Killick-Kendrick, R. (1990). Phlebotomine vectors of the leishmaniases: a review. *Medical and veterinary entomology*, *4*(1), 1-24.

Killick-Kendrick, R. (1999). The biology and control of phlebotomine sand flies. *Clinics in Dermatology*, *17*(3), 279-289.

Killick-Kendrick, R., & Ward, R. D. (1981). Ecology of *Leishmania*. *Parasitology*, *82*(4), 143-152.

Killick-Kendrick, R., Tang, Y., Killick-Kendrick, M., Johnson, R. N., Ngumbi, P. M., Sang, D. K., & Lawyer, P. G. (1994). Phlebotomine sandflies of Kenya (Diptera: Psychodidae). III. The identification and distribution of species of the subgenus *Larroussius*. *Annals of Tropical Medicine & Parasitology*, *88*(2), 183-196.

Killick-Kendrick, R., Tang, Y., Johnson, R. N., Ngumbi, P. M., & Robert, L. L. (1997). Phlebotomine sandflies of Kenya (Diptera: Psychodidae). V. Phlebotomus (Paraphlebotomus) mireillae n. sp. *Annals of Tropical Medicine & Parasitology*, *91*(4), 417-428.

Koch, L. K., Kochmann, J., Klimpel, S., & Cunze, S. (2017). Modeling the climatic suitability of leishmaniasis vector species in Europe. *Scientific Reports*, *7*(1), 1-10.

Kung'u, A., Mutinga, M. J., & Ngoka, J. M. (1972). Cutaneous leishmaniasis in Kenya. *East African Medical Journal*, *49*(6), 458-465.

Lane, R. P. (1993). Introduction to the arthropods. In *Medical Insects and Arachnids* (pp. 30-47). Dordrecht: Springer Netherlands.

Lessa, M. M., Lessa, H. A., Castro, T. W., Oliveira, A., Scherifer, A., Machado, P., & Carvalho, E. M. (2007). Mucosal leishmaniasis: epidemiological and clinical aspects. *Revista Brasileira de Otorrinolaringologia*, *73*, 843-847.

Lewis, D. J. (1973). The Simuliidae (Diptera) of Pakistan. *Bulletin of Entomological Research*, *62*(3), 453-470.

Lima, L. H. D. M., Mesquita, M. R., Skrip, L., de Souza Freitas, M. T., Silva, V. C., Kirstein, O. D., ... & Costa, C. H. (2016). DNA barcode for the identification of the sand fly *Lutzomyia longipalpis* plant feeding preferences in a tropical urban environment. *Scientific Reports*, *6*(1), 1-6.

Lorenz, L., Maia, M., & Moore, S.J. (2013). The use of plants in vector control. *Biological and Environmental of Disease vectors*, 42-66.

Mahamat, H., & Hassanali, A. (1998). Cuticular hydrocarbon composition analysis for taxonomic differentiation of phlebotomine sandfly species (Diptera: Psychodidae) in Kenya. *Journal of Medical Entomology*, *35*(5), 778-781.

Maia, C., & Depaquit, J. (2016). Can *Sergentomyia* (Diptera, Psychodidae) play a role in the transmission of mammal-infecting *Leishmania*? *Parasite*, *23*.

Makwali, J. (2021). *Leishmania* Reservoirs and Sandfly Vectors: Dangers in Caves.

Marayati, B. F., Schal, C., Ponnusamy, L., Apperson, C. S., Rowland, T. E., & Wasserberg, G. (2015). Attraction and oviposition preferences of *Phlebotomus papatasi* (Diptera: Psychodidae), vector of Old-World cutaneous leishmaniasis, to larval rearing media. *Parasites & Vectors*, *8*, 1-9.

Marlet, M. V. L., Sang, D. K., Ritmeijer, K., Muga, R. O., Onsongo, J., & Davidson, R. N. (2003). Emergence or re-emergence of visceral leishmaniasis in areas of Somalia, North-Eastern Kenya, and South-Eastern Ethiopia in 2000–2001. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *97*(5), 515-518.

Mebrahtu, Y. B., Lawyer, P. G., Ngumbi, P. M., Kirigi, G., Mbugua, J., Gachihi, G., ... & Roberts, C. R. (1992). A new rural focus of cutaneous leishmaniasis caused by *Leishmania tropica* in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *86*(4), 381-387.

Ministry of Health (2017). Prevention, Diagnosis and Treatment of Visceral Leishmaniasis (Kala-Azar) in Kenya: National Guidelines for Health Workers.

Minter, D. M. (1963). Three new sandflies (Diptera, Psychodidae) from East Africa, with notes on other species. *Bulletin of Entomological Research*, *54*(3), 483-495.

Minter, D. M. (1964). The distribution of sandflies (Diptera, Psychodidae) in Kenya. *Bulletin of Entomological Research*, *55*(2), 205-217.

Mitiku, M. (2011). *Phytochemical Investigation on the Root Extract of Vernonia auriculifera Hiern* (Doctoral dissertation).

Moncaz, A., Faiman, R., Kirstein, O., & Warburg, A. (2012). Breeding sites of *Phlebotomus sergenti,* the sand fly vector of cutaneous leishmaniasis in the Judean Desert. *PLoS Neglected Tropical Diseases*, *6*(7), e1725.

Moore, J. S., Kelly, T. B., Killick-Kendrick, R., Killick-Kendrick, M., Wallbanks, K. R., & Molyneux, D. H. (1987). Honeydew sugars in wild-caught *Phlebotomus ariasi* detected by high performance liquid chromatography (HPLC) and gas chromatography (GC). *Medical and Veterinary Entomology*, *1*(4), 427-434.

Mukhwana, D. (2018). Peri-urban cutaneous leishmaniasis transmission dynamics with regard to associated risk factors in Mt. Elgon Focus, Kenya.

Mukhwana, D. W., Makwali, J., Ngeiywa, M. M., Anjili, C. A., & Oyoo-Okoth, E. (2018). Spatial-temporal distribution and risk factors associated with phlebotomine sand flies in Mt. Elgon region, Western Kenya. *Journal of Entomology and Zoology Studies* 6: 1 – 7 (E – ISSN: 2320 – 7078; P – ISSN: 2349 – 6800).

Mukhwana, W.D., Makwali, A. J., Ngeiywa, M. and Anjili, C. A. (2018b) Peri-urban cutaneous leishmaniasis transmission dynamics with regard to associated risk factors in mt. elgon focus, kenya. *South Asian Journal of Parasitology 1*(2): 1-9; Article no. SAJP.44077

Muñoz, C., Risueño, J., Yilmaz, A., Pérez-Cutillas, P., Goyena, E., Ortuño, M., ... & Berriatua, E. (2018). Investigations of *Phlebotomus perniciosus* sand flies in rural Spain reveal strongly aggregated and gender-specific spatial distributions and advocate use of light-attraction traps. *Medical and Veterinary Entomology*, *32*(2), 186-196.

Munstermann, L. E. (2019). Phlebotomine sand flies and moth flies (Psychodidae). In *Medical and Veterinary Entomology* (pp. 191-211). Academic Press.

Mutinga, M. J. (1975). The animal reservoir of cutaneous leishmaniasis on Mount Elgon, Kenya. *East African Medical Journal*, *52*(3), 142-151.

Mutinga, M. J., & Kamau, C. C. (1986). Investigations of the epidemiology of leishmaniases in Kenya—II: The breeding sites of phlebotomine sandflies in Marigat, Baringo District, Kenya. *International Journal of Tropical Insect Science*, *7*(1), 37-44.

Mutinga, M. J., & Odhiambo, T. R. (1986). Cutaneous leishmaniasis in Kenya—III. The breeding and resting sites of *Phlebotomus pedifer* (Diptera: Phlebotomidae) in Mt Elgon focus, Kenya. *International Journal of Tropical Insect Science*, *7*(2), 175-180.

Mutinga, M. J., Kamau, C. C., & Kyai, F. M. (1986). Investigations on the epidemiology of leishmaniases in Kenya—IV: Breeding habitat of *Phlebotomus duboscqi* (Diptera: Psychodidae), a vector of *Leishmania major* in Marigat, Baringo District, Kenya. *International Journal of Tropical Insect Science*, *7*(6), 727-729.

Mutinga, M. J., Kamau, C. C., Kyai, F. M., & Omogo, D. M. (1986). Epidemiology of leishmaniases in Kenya: V. Wider search for breeding habitats of phlebotomine sandflies in three kala-azar endemic foci. *East African Medical Journal*, *66*(3), 173-182.

Mutinga, M. J., Kyai, F. M., Kamau, C., & Omogo, D. M. (1986). Epidemiology of leishmaniasis in Kenya—III: Host preference studies using various types of animal baits at animal burrows in Marigat, Baringo District. *International Journal of Tropical Insect Science*, *7*, 191-197.

Mutungi, A. K., Kariuki, C. H., Kandie, G. J., Njoroge, A. W., Anampiu, J. M., & Kibebe, H. (2019). Prevalence of Parasitic Infections amongst Children in Primary Schools in Tharaka South Sub County, Kenya.

Ngere, I., Gufu Boru, W., Isack, A., Muiruri, J., Obonyo, M., Matendechero, S., & Gura, Z. (2020). Burden and risk factors of cutaneous leishmaniasis in a peri-urban settlement in Kenya, 2016. *PloS one*, *15*(1), e0227697.

Ngumbi, M. P., Kaburia, C. J., Anjili, O. C., & Haas, F. (2010). *Phlebotomus (Larroussius) orientalis* (Diptera: Psychodidae) as a probable secondary vector of visceral leishmaniasis in Kenya.

Ngumbi, P. M., Lawyer, P. G., Johnson, R. N., Kiilu, G., & Asiago, C. (1992). Identification of phlebotomine sandfly bloodmeals from Baringo district, Kenya, by direct enzyme-linked immunosorbent assay (ELISA). *Medical and Veterinary Entomology*, *6*(4), 385-388.

Ngure, P. K., Kimutai, A., Rukunga, G., & Tonui, W. K. (2009). A review of leishmaniasis in Eastern Africa. *Journal of Nanjing Medical University*, *23*(2), 79-86.

Nguyen, H. M., Kowacich, D. J., & Wasserberg, G. (2021). Temporal bet-hedging in sand fly oviposition: Pharate *Phlebotomus papatasi* sand fly neonates regulate hatching time in response to organic matter and proximity to conspecific eggs. *Vector-Borne and Zoonotic Diseases*, *21*(4), 275-279.

Njau, J. (2010, April). Leishmaniasis control in Kenya: Current challenges and future strategies. In *Geneva Health Forum* (pp. 19-21).

Nzelu, C. O., Kato, H., Puplampu, N., Desewu, K., Odoom, S., Wilson, M. D., ... & Boakye, D. A. (2014). First detection of *Leishmania tropica* DNA and *Trypanosoma* Species in *Sergentomyia* Sandflies (Diptera: Psychodidae) From an Outbreak Area of Cutaneous Leishmaniasis in Ghana. *PLoS Neglected Tropical Diseases*, *8*(2), e2630.

Ombaka, K. O., Makwali, J., & Ngeiywa, M. (2022). *Leishmania* parasite infections and blood meal source apportionment in sand fly vector species in Mt. Elgon habitats (Kenya).

Ombaka, K. O., Ngeiywa, M. M., & Makwali, J. (2023). Natural infection of sandfly vector and reservoir hosts species with *Leishmania* parasites in Mt. Elgon cave habitats in Kenya. *International Journal of Tropical Disease & Health*, *44*(3), 17-25.

Oryan, A., & Akbari, M. (2016). Worldwide risk factors in leishmaniasis. *Asian Pacific Journal of Tropical Medicine*, *9*(10), 925-932.

Owino, B. O., Matoke-Muhia, D., Alraey, Y., Mwangi, J. M., Ingonga, J. M., Ngumbi, P. M., ... & Masiga, D. K. (2019). Association of *Phlebotomus guggisbergi* with *Leishmania major* and *Leishmania tropica* in a complex transmission setting for cutaneous leishmaniasis in Gilgil, Nakuru County, Kenya. *PLoS Neglected Tropical Diseases*, *13*(10), e0007712.

Ozbel, Y., Sanjoba, C., Alten, B., Asada, M., Depaquit, J., Matsumoto, Y., …& Matsumoto, Y. (2011). Distribution and ecological aspects of sand fly (Diptera: Psychodidae) species in Sri Lanka. *Journal of Vector Ecology, 36, S77-S86.*

Pareyn, M., Dvorak, V., Halada, P., Van Houtte, N., Girma, N., De Kesel, W., ... & Volf, P. (2020). An integrative approach to identify sand fly vectors of leishmaniasis in Ethiopia by morphological and molecular techniques. *Parasites & Vectors*, *13*, 1-13.

Pareyn, M., Van den Bosch, E., Girma, N., van Houtte, N., Van Dongen, S., Van der Auwera, G., ... & Leirs, H. (2019). Ecology and seasonality of sandflies and potential reservoirs of cutaneous leishmaniasis in Ochollo, a hotspot in southern Ethiopia. *PLoS Neglected Tropical Diseases*, *13*(8), e0007667.

Paul, S. K., Jeyaram, A., Kishore, K., Ranjan, A., Palit, A., Kesari, S., & Jayaraman, V. (2006). Application of remote sensing and GIS in identifying and mapping sandfly distribution in endemic and non-endemic kala-azar foci in Bihar and Jharkhand. In *57th International Astronautical Congress* (pp. B1-P).

Peacock, C. S., Seeger, K., Harris, D., Murphy, L., Ruiz, J. C., Quail, M. A., ... & Berriman, M. (2007). Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nature Genetics*, *39*(7), 839-847.

Perkins, P. V., Githure, J. I., Mebrahtu, Y., Kiilu, G., Anjili, C., Ngumbi, P. S., ... & Koech, D. K. (1988). Isolation of *Leishmania donovani* from *Phlebotomus martini* in Baringo district, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *82*(5), 695-700.

Peterkova-Koci, K., Robles-Murguia, M., Ramalho-Ortigao, M., & Zurek, L. (2012). Significance of bacteria in oviposition and larval development of the sand fly *Lutzomyia longipalpis*. *Parasites & Vectors*, *5*(1), 1-8.

Pohlit, A. M., Rezende, A. R., Baldin, E. L. L., Lopes, N. P., & de Andrade Neto, V. F. (2011). Plant extracts, isolated phytochemicals, and plant-derived agents which are lethal to arthropod vectors of human tropical diseases–a review. *Planta Medica*, *77*(06), 618-630.

Rahman, M. F., Banu, S., Alam, M. J., Uddin, M. J., Sarker, M. A. S., Sanjoba, C., ... & Rahman, M. B. (2018). Effects of climate on sandflies and leishmaniasis in endemic areas of Bangladesh. *Journal of Armed Forces Medical College, Bangladesh*, *14*(1), 10-14.

Rajashekar, Y., Kumar, H. V., Ravindra, K. V., & Bakthavatsalam, N. (2013). Isolation and characterization of biofumigant from leaves of *Lantana camara* for control of stored grain insect pests. *Industrial Crops and Products*, *51*, 224-228.

Rajashekar, Y., Ravindra, K. V., & Bakthavatsalam, N. (2014). Leaves of *Lantana camara* Linn. (Verbenaceae) as a potential insecticide for the management of three species of stored grain insect pests. *Journal of food science and technology*, *51*(11), 3494-3499.

Ramírez, J. D., Hernández, C., León, C. M., Ayala, M. S., Flórez, C., & González, C. (2016). Taxonomy, diversity, temporal and geographical distribution of cutaneous leishmaniasis in Colombia: A retrospective study. *Scientific Reports*, *6*(1), 1-10.

Ranganathan, S., & Swaminathan, S. (2015). Sandfly species diversity in association with human activities in the Kani tribe settlements of the Western Ghats, Thiruvananthapuram, Kerala, India. *Memórias do Instituto Oswaldo Cruz*, *110*, 174-180.

Rohousova, I., Talmi-Frank, D., Kostalova, T., Polanska, N., Lestinova, T., Kassahun, A., ... & Baneth, G. (2015). Exposure to *Leishmania* spp. and sand flies in domestic animals in northwestern Ethiopia. *Parasites & Vectors*, *8*, 1-10.

Sadlova, J., Dvorak, V., Seblova, V., Warburg, A., Votypka, J., & Volf, P. (2013). *Sergentomyia schwetzi* is not a competent vector for *Leishmania donovani* and other *Leishmania* species pathogenic to humans. *Parasites & Vectors*, *6*(1), 1-10.

Sakkas, H., Gartzonika, C., & Levidiotou, S. (2016). Laboratory diagnosis of human visceral leishmaniasis. *Journal of Vector Borne Diseases*, *53*(1), 8.

Sang, D. K. (2011). The caves of Mount Elgon and their sandfly fauna David K.

Sang, D. K., & Chance, M. L. (1993). Studies on the *Phlebotomus* fauna of Mount Elgon, Kenya. *Annals of Tropical Medicine & Parasitology*, *87*(5), 509-515.

Sang, D. K., Njeru, W. K., & Ashford, R. W. (1994). A zoonotic focus of cutaneous leishmaniasis due to *Leishmania tropica* of Utut, Rift Valley Province, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *88*(1), 35-37.

Sang, D. K., Okelo, G. B. A., & Chance, M. L. (1993). Cutaneous leishmaniasis due to *Leishmania aethiopica*, on Mount Elgon, Kenya. *Annals of Tropical Medicine & Parasitology*, *87*(4), 349-357.

Sant'Anna, M. R., Diaz-Albiter, H., Aguiar-Martins, K., Al Salem, W. S., Cavalcante, R. R., Dillon, V. M., ... & Dillon, R. J. (2014). Colonisation resistance in the sand fly gut: *Leishmania* protects *Lutzomyia longipalpis* from bacterial infection. *Parasites & Vectors*, *7*, 1-10.

Santamaría, E., Cabrera, O. L., Marceló, C., Goenaga-Olaya, S., & Maestre-Serrano, R. (2020). Behavioral aspects of phlebotomine sand flies associated with a case of cutaneous leishmaniasis in Atlántico, Northern Colombia. *The American Journal of Tropical Medicine and Hygiene*, *102*(4), 768.

Sawalha, S. S., Ramlawi, A., Sansur, R. M., Salem, I. M., & Amr, Z. S. (2017). Diversity, ecology, and seasonality of sand flies (Diptera: Psychodidae) of the Jenin District (Palestinian Territories). *Journal of Vector Ecology*, *42*(1), 120-129.

Schlein, Y., & Jacobson, R. L. (1994). Mortality of *Leishmania major* in *Phlebotomus papatasi* caused by plant feeding of the sand flies. *The American Journal of Tropical Medicine and Hygiene*, *50*(1), 20-27.

Schlein, Y., & Jacobson, R. L. (1999). Sugar meals and longevity of the sandfly *Phlebotomus papatasi* in an arid focus of *Leishmania major* in the Jordan Valley. *Medical and Veterinary Entomology*, *13*(1), 65-71.

Schlein, Y., Jacobson, R. L., & MÃ¼ller, G. C. (2001). Sand fly feeding on noxious plants: a potential method for the control of leishmaniasis. *The American Journal of Tropical Medicine and Hygiene*, *65*(4), 300-303.

Serafim, T. D., Coutinho-Abreu, I. V., Dey, R., Kissinger, R., Valenzuela, J. G., Oliveira, F., & Kamhawi, S. (2021). Leishmaniasis: the act of transmission. *Trends in Parasitology*, *37*(11), 976-987.

Singh, R., Lal, S., & Saxena, V. K. (2008). Breeding ecology of visceral leishmaniasis vector sandfly in Bihar state of India. *Acta Tropica*, *107*(2), 117-120.

Spellerberg, I. F., & Fedor, P. J. (2003). A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener'Index. *Global Ecology and Biogeography*, *12*(3), 177-179.

Srinivasan, R., Jambulingam, P., & Vanamail, P. (2013). Sand fly (Diptera: Psychodidae) abundance and species diversity in relation to environmental factors in parts of coastal plains of southern India. *Journal of Medical Entomology*, *50*(4), 758-763.

Srinivasan, R., Jambulingam, P., Kumar, N. P., Selvakumar, M., Edwin, B., & Kumar, T. D. (2015). Temporal distribution and behaviour of sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus of the Kani Tribe settlements in the Western Ghats, India. *Acta Tropica*, *148*, 147-155.

Strong, W. L. (2016). Biased richness and evenness relationships within Shannon–Wiener index values. *Ecological Indicators*, *67*, 703-713.

Tagne, A. M., Marino, F., & Cosentino, M. (2018). *Tithonia diversifolia* (Hemsl.) A. Gray as a medicinal plant: A comprehensive review of its ethnopharmacology, phytochemistry, pharmacotoxicology and clinical relevance. *Journal of Ethnopharmacology*, *220*, 94-116.

Teixeira, D. E., Benchimol, M., Rodrigues, J. C., Crepaldi, P. H., Pimenta, P. F., & de Souza, W. (2013). The cell biology of *Leishmania*: how to teach using animations. *PLoS Pathogens*, *9*(10), e1003594.

Theodor, O. (1932). On the structure of the buccal cavity, pharynx and spermatheca in South American *Phlebotomus. Bulletin of Entomological Research*, *23*(1), 17-23.

Tonui, W. K., & Titus, R. G. (2006). *Leishmania major* soluble exo-antigens (Lm SEAgs) protect neonatal BALB/c mice from a subsequent challenge with *L. major* and stimulate cytokine production by *Leishmania*-naïve human peripheral blood mononuclear cells. *Journal of Parasitology*, *92*(5), 971-976.

Torres-Guerrero, E., Quintanilla-Cedillo, M. R., Ruiz-Esmenjaud, J., & Arenas, R. (2017). Leishmaniasis: a review. *F1000Research*, *6*.

Torto, B., Hassaballa, I. B., & Tchouassi, D. P. (2022). Chapter 8: Chemical ecology of sand fly plant-feeding behaviour. In *Sensory Ecology of Disease Vectors* (pp. 235-252). Wageningen Academic Publishers.

Valero, N. N. H., Prist, P., & Uriarte, M. (2021). Environmental and socioeconomic risk factors for visceral and cutaneous leishmaniasis in São Paulo, Brazil. *Science of the Total Environment*, *797*, 148960.

Veras, P. S. T., & Bezerra de Menezes, J. P. (2016). Using proteomics to understand how *Leishmania* parasites survive inside the host and establish infection. *International Journal of Molecular Sciences*, *17*(8), 1270.

Vivero, R. J., Torres-Gutierrez, C., Bejarano, E. E., Peña, H. C., Estrada, L. G., Florez, F., ... & Muskus, C. E. (2015). Study on natural breeding sites of sand flies (Diptera: Phlebotominae) in areas of *Leishmania* transmission in Colombia. *Parasites & Vectors*, *8*, 1-14.

Wallbanks, K. R., Moore, J. S., Bennett, L. R., Soren, R., Molyneux, D. H., Carlin, J. M., & Perez, J. E. (1991). Aphid derived sugars in the neotropical sandfly--*Lutzomyia peruensis*. *Tropical Medicine and Parasitology: Official Organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ)*, *42*(1), 60-62.

Warburg, A., & Faiman, R. (2011). Research priorities for the control of phlebotomine sand flies. *Journal of Vector Ecology*, *36*, S10-S16.

Widaa, A., Claro, T., Foster, T. J., O'Brien, F. J., & Kerrigan, S. W. (2012). *Staphylococcus aureus* protein A plays a critical role in mediating bone destruction and bone loss in osteomyelitis. *PloS one*, *7*(7), e40586.

Wijerathna, T., & Gunathilaka, N. (2020). Diurnal adult resting sites and breeding habitats of phlebotomine sand flies in cutaneous leishmaniasis endemic areas of Kurunegala District, Sri Lanka. *Parasites & Vectors*, *13*(1), 1-10.

Williams, P., & Coelho, M. D. V. (1978). Taxonomy and transmission of *Leishmania*. *Advances in Parasitology*, *16*, 1-42.

World Health Organization (2023). Leishmaniasis. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis

Yared, S., Deribe, K., Gebreselassie, A., Lemma, W., Akililu, E., Kirstein, O. D., ... & Hailu, A. (2014). Risk factors of visceral leishmaniasis: a case control study in north-western Ethiopia. *Parasites & Vectors*, *7*(1), 1-11.

Yared, S., Gebresilassie, A., Abbasi, I., Aklilu, E., Kirstein, O. D., Balkew, M., ... & Gebre-Michael, T. (2019). A molecular analysis of sand fly blood meals in a visceral leishmaniasis endemic region of North-Western Ethiopia reveals a complex host-vector system. *Heliyon*, *5*(7), e02132.

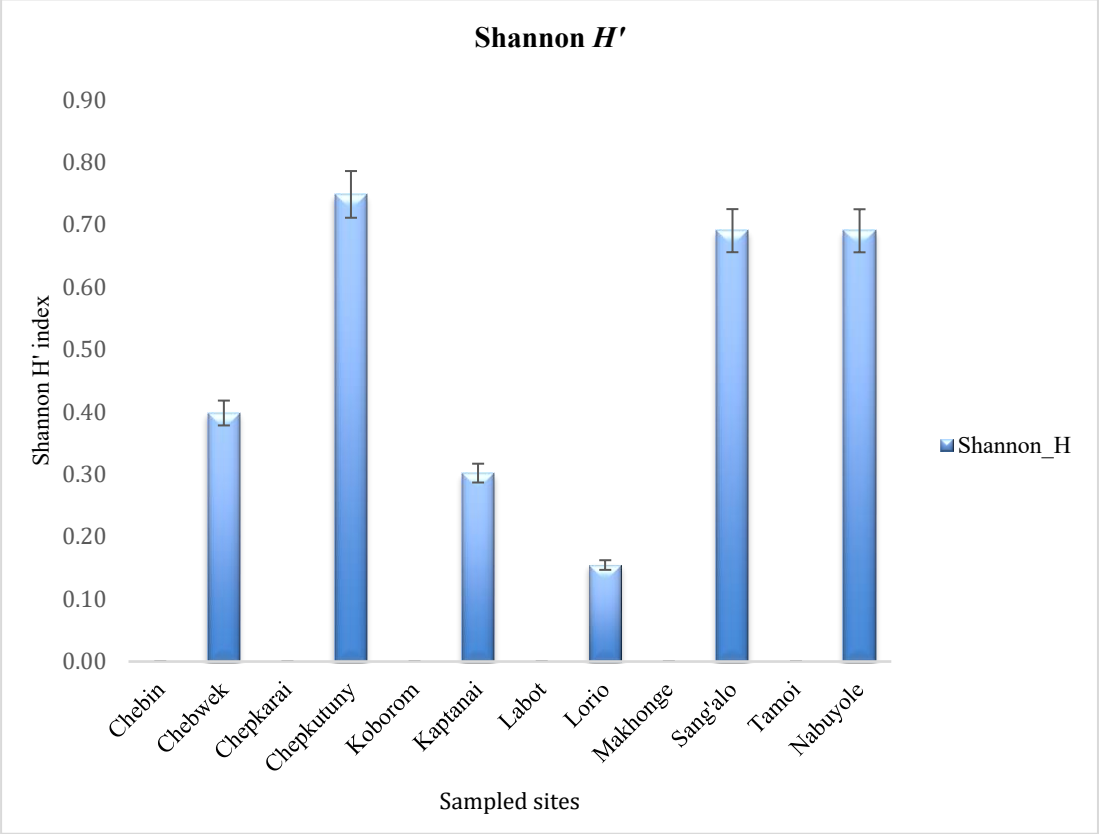
Young, D. G. (1982). *Studies in Phlebotomine Sand Flies*. Florida Univ Gainesville Dept of Entomology and Nematology.

Young, D. G., & Arias, J. R. (1977). Lutzomyia sand flies in the subgenus Evandromyia Mangabeira with descriptions of a new species from Brazil (Diptera: Psychodidae). *Acta Amazonica*, *7*(1), 59-70.

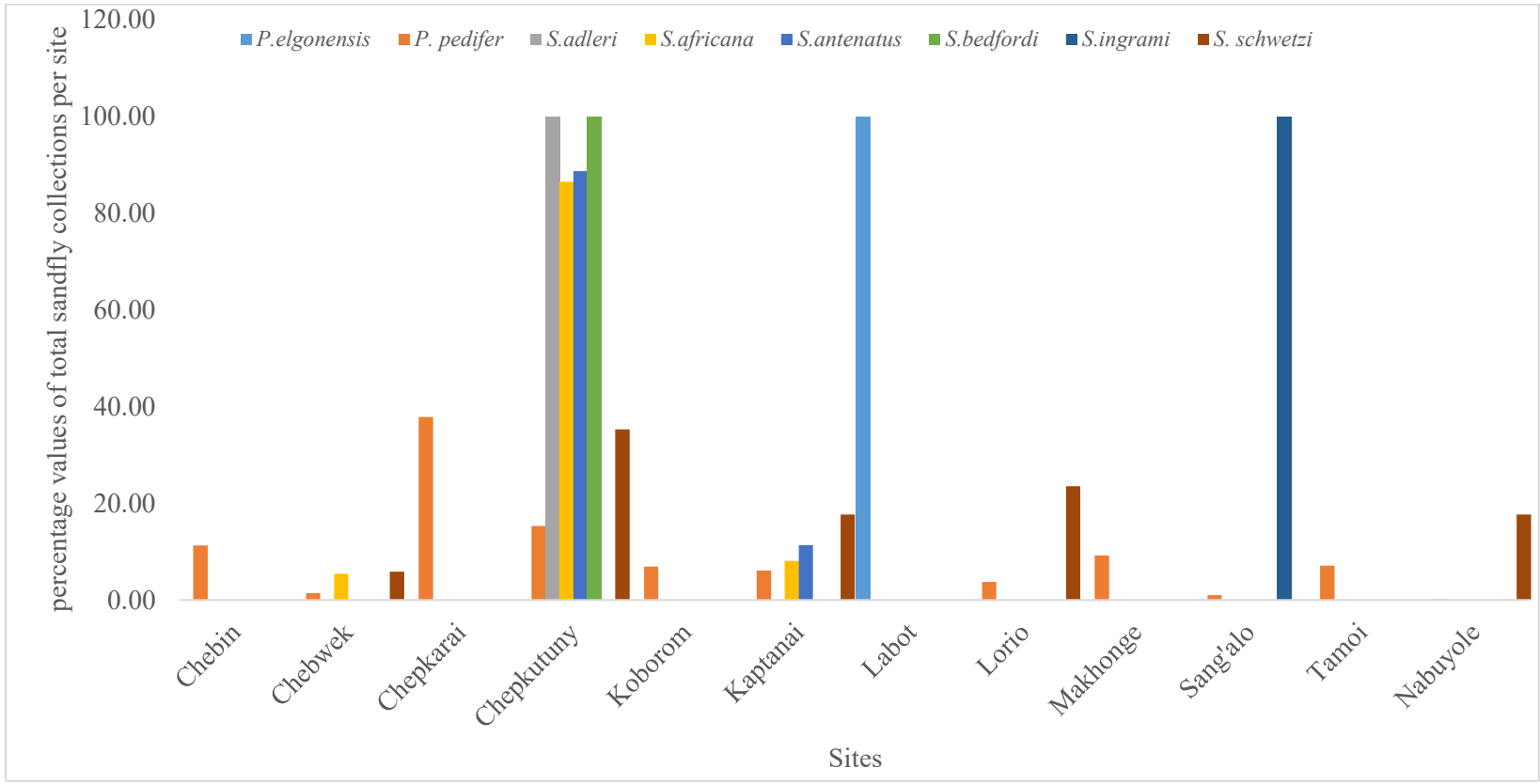
Zijlstra, E. E. (2016). The immunology of post-kala-azar dermal leishmaniasis (PKDL). *Parasites & vectors*, *9*(1), 1-9.

APPENDICES

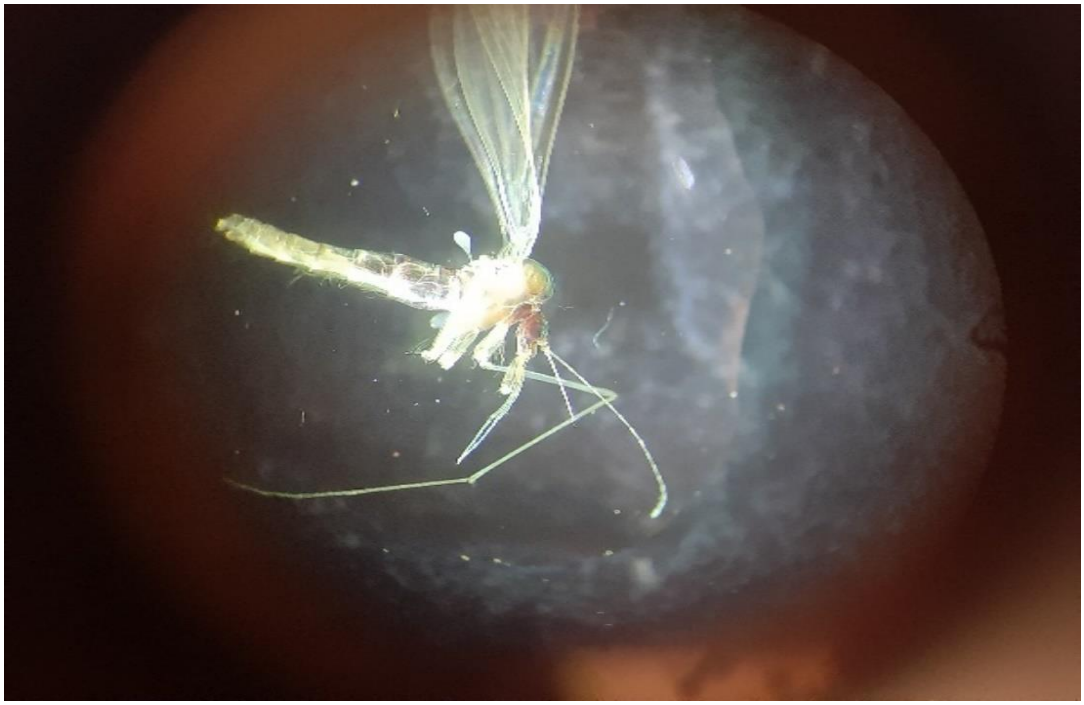
Appendix I Comparison of Shannon Weiner Diversity Indices Between Sandfly Species Collected from Cave Sites in Bungoma County



Appendix II A Figure Showing Percentage of the Mean Numbers of Sandfly Species Collected in Sampled Sites in Bungoma County during November, 2021 to December, 2022 Period



Appendix III A Female *Phlebotomus pedifer* Sandfly under a Microscope. (Source: Author, 2022)



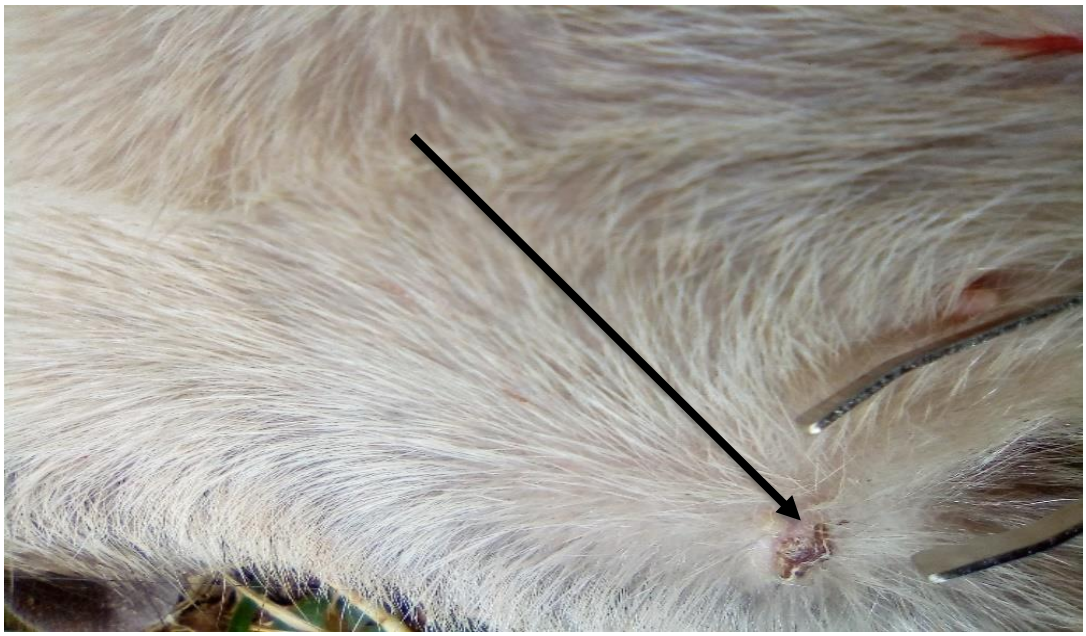
Appendix IV A Set CDC Light Trap in Action inside a Cave. (Source: Author, 2022)

Appendix V People Sleeping in Sang'alo Cave During Prayer and Fasting Camps.

(Source: Author, 2022)

Appendix VI Giant Rats. (. (Source : Author, 2022

Appendix VII A Giant Rat with a Leishmanial Lesion. (Source: Author, 2022)



Appendix VIII A Giant Rat with Ticks on the Ear. (Source: Author, 2022)

Appendix IX A Young Rock Hyrax in the Laboratory. (Source: Author, 2022)

Appendix X One of my Supervisors, a Team from KEMRI and I at Tamoi Cave. (Source: Author, 2022)

Appendix XI Some Plates of Typical Clinical Cases of CL in Bungoma County

(Source: Author, 2022)

Apendix XII Reference List of Plant Species Collected from Bungoma County and Their University of Eldoret's Herbarium Voucher Numbers

Achyranthes aspera Jacq., Ecl. Pl. Rar. 109. t. 74. (UOE/ACAS/JMW/22)

Ageratina adenophora (Spreng.) R. M. King & H. Rob., Phytologia 19: 211 (1970). (UOE/AGAD/JMW/22)

Ageratum conyzoides var. mexicanum (Sims) DC. Prodr. [A. P. de Candolle] 5: 108 (1836). (UOE/AGCO/JMW/22)

Bidens pilosa var. discoidea Sch. Bip. (UOE/BIPI/JMW/22)

Caesalpinia decapetala (Roth) Alston, Handb. Fl. Ceylon vi. Suppl., 89 (1931). (UOE/CADE/JMW/22)

Clematis vitalba Buch. -Ham. ex Steud., Nomencl. Bot. [Steudel], ed. 2. i. 379, 380. (UOE/CLVI/JMW/22)

Conyza sumatrensis (Retz.) E. Walker, J. Jap. Bot. 46(3): 72 (1971). (UOE/COSU/JMW/22)

Fuerstia africana T.C.E.Fr., Acta Univ. Lund. xxv. No. 17, 3 (1929). (UOE/FUAF/JMW/22)

Hisbicus fuscus Garcke (UOE/HIFU/JMW/22)

Lantana camara var. aculeata (Hook.) L. H. Bailey (UOE/LACA/JMW/22)

Lantana trifolia L., Sp. Pl. 2: 626 (1753). (UOE/LATR/JMW/22)

Leonotis nepetifolia Schimp. ex Benth. Prodr. [A. P. de Candolle] 12: 535 (1848), pro syn (UOE/LENE/JMW/22)

Markhamia lutea K. Schum., Nat. Pflanzenfam. [Engler & Prantl] iv. 3b (1895) 242. (UOE/MALU/JMW/22)

Pavonia burchellii (DC.) R. A. Dyer, Bull. Misc. Inform. Kew 1932(3): 152 (1932). BHL (UOE/PABU/JMW/22)

Pavonia urens var. irakuensis (Ulbr.) Verdc., Fl. Trop. E. Africa, Malv. 12 (2009). (UOE/PAUR/JMW/22)

Plectranthus barbatus Andrews, Bot. Repos. 9(pts. 121-126): t. 594 (1810). (UOE/PLBA/JMW/22)

Ricinus communis L., Sp. Pl. 2: 1007 (1753). (UOE/RICO/JMW/22)

Rubus apetalus Poir., Encycl. [J. Lamarck & al.] 6(1): 242 (1804). (UOE/RUAP/JMW/22)

Sida ovata Forssk., Fl. Aegypt. -Arab. 124. (1775). (UOE/SIOV/JMW/22)

Tagetes minuta L., Sp. Pl. 2: 887 (1753). BHL (UOE/TAMI/JMW/22)

Tithonia diversifolia (Hemsl.) A. Gray, Proc. Amer. Acad. Arts xix. 5 (1883). (UOE/TIDI/JMW/22)

Urtica massaica Mildbr., Notizbl. Bot. Gart. Berlin-Dahlem viii. 275 (1923). (UOE/URMA/JMW/22)

Vernonia amygdalina Delile, Cent. PL Meroe 41. (1826). (UOE/VEAM/JMW/22)

Vernonia auriculifera Hiern, Cat. Afr. Pl. (Hiern) 1(3): 539 (1898). (UOE/VEAU/JMW/22)

Zehneria scabra Sond., Fl. Cap. (Harvey) 2: 486 (1862). BHL (UOE/ZESC/JMW/22)

Appendix XIII A Table of Plant Richness, Checklist, Diversity and Distribution in Bungoma County

Plant species	Chebin	Chepkutuny	Chepkarai	Cheptais	Kaborom	Kaptanai	Kipchiria	Loriro	Makhonge	Nabuyole	Sang'alo	Total
Achyranthes aspera J. Jacq.	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	10
Ageratina adenophora (Spreng.) R. M. King & H. Rob.	✓	✓	✓	✓		✓	✓		✓			7
Ageratum conyzoides var. *mexicanum* (Sims) DC.	✓	✓	✓				✓		✓	✓	✓	7
Bidens pilosa var. discoidea Sch. Bip.	✓		✓	✓	✓					✓	✓	6
Caesalpinia decapetala (Roth) Alston		✓	✓		✓	✓			✓			5
Clematis vitalba Buch. -Ham. ex Steud.		✓		✓	✓		✓	✓	✓	✓	✓	8
Conyza sumatrensis (Retz.) E. Walker					✓				✓		✓	3
Fuerstia africana T.C.E.Fr.		✓	✓	✓					✓	✓		5
Hisbicus fuscus Garcke										✓		1
Lantana camara var. aculeata (Hook.) L. H. Bailey		✓	✓		✓				✓	✓		5
Lantana trifolia L				✓	✓							2
Leonotis nepetifolia Schimp. ex Benth.										✓	✓	2
Markhamia lutea K. Schum.	✓	✓	✓					✓	✓	✓	✓	7
Ocimum grantissimum L.										✓		1
Pavonia burchelii (DC.) R. A. Dyer	✓	✓	✓	✓		✓	✓	✓	✓		✓	9

Pavonia urens var. Irakuensis (Ulbr.) Verdc.										✓		1
Plectranthus barbatus Andrews	✓			✓	✓			✓	✓			5
Ricinus communis L.						✓	✓				✓	3
Rubus apetalus Poir.										✓	✓	2
Searsian natalensis (Bernh. ex Krauss) F. A. Barkley			✓			✓						2
Sida ovata Forssk.										✓		1
Solanecio manii (Hook. F.) C. Jeffrey	✓	✓	✓	✓	✓	✓	✓	✓	✓			9
Tagetes minuta L.	✓			✓	✓						✓	4
Tithonia diversifolia (Hemsl.) A. Gray	✓			✓	✓	✓	✓	✓	✓	✓	✓	9
Urtica massaica Mildbr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	11
Vernonia amygdalina Delile	✓	✓				✓	✓					4
Vernonia auriculifera Hiern	✓	✓	✓	✓	✓		✓		✓			7
Zehneria scabra Sond.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		10

Total Shannon Weiner diversity Index =3.14 *H'*

Photograph Plates of Common Plant Species in Bungoma County Cave Habitats

Appendix XIV *Achyranthes aspera* J. Jacq. (Source: Author, 2022) - UOE/ACAS/JMW

Appendix XV *Ageratina adenophora* (Spreng.) R. M. King & H. Rob. (Source: Author, 2022) -UOE/AGAD/JMW/22

Appendix XVI *Ageratum conyzoides* var. mexicanum (Sims) DC. (Source: Author, 2022) -UOE/AGCO/JMW/22

Appendix XVII *Bidens pilosa var. discoidea* Sch. Bip. (Source: Author, 2022)

UOE/BIPI/JMW/22

Appendix XVIII: *Caesalpinia decapetala* (Roth) Alston. (Source: Author, 2022) - UOE/CADE/JMW/22

Appendix XIX *Clematis vitalba* Buch. -Ham. ex Steud. (Source: Author, 2022) - UOE/CLVI/JMW/22

Appendix XX *Conyza sumatrensis* (Retz.) E. Walker. (Source: Author, 2022) - UOE/COSU/JMW/22

Appendix XXI *Fuerstia africana* T. C. E. Fr. (Source: Author, 2022) - UOE/FUAF/JMW/22

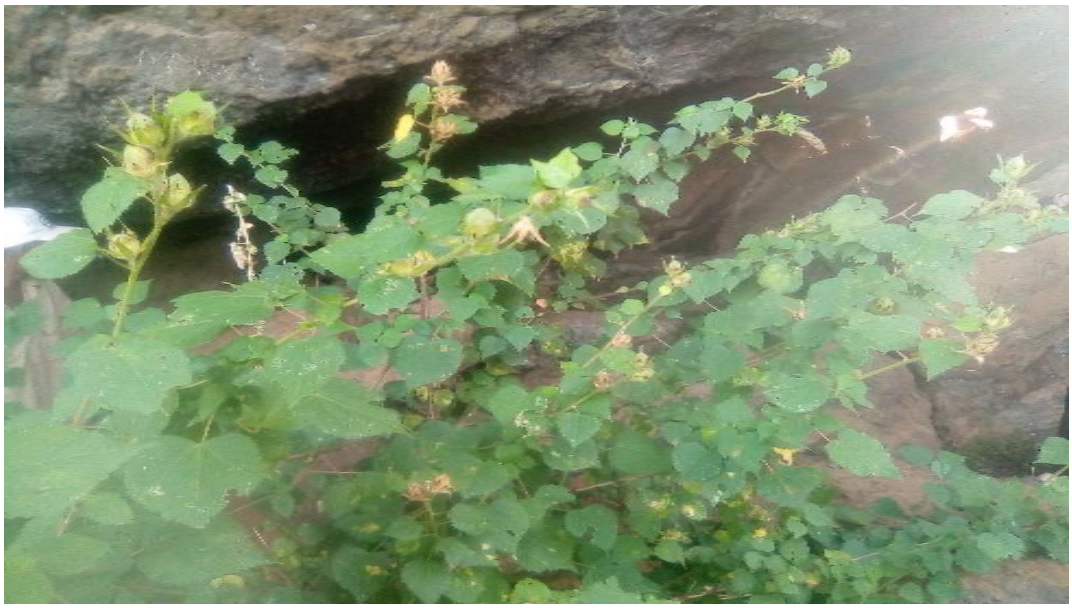
Appendix XXII *Lantana camara var. aculeata* (Hook.) L. H. Bailey. (Source: Author, 2022 -UOE/LACA/JMW/22

Appendix XXIII *Lantana trifolia* L. (Source: Author, 2022) -UOE/LATR/JMW/22

Appendix XXIV *Markhamia lutea* K.Schum. (Source: Author, 2022) - UOE/MALU/JMW/22

Appendix XXV *Ocimum grantissimum* L. (Source: Author, 2022) UOE/OCGR/JMW/22

Appendix XXVI *Pavonia burchellii* (DC.) R. A. Dyer. (Source: Author, 2022) - UOE/PABU/JMW/22



Appendix XXVII *Plectranthus barbatus* Andrews. (Source: Author, 2022) UOE/PLBA/JMW/22

Appendix XXVIII *Ricinus communis* L. (Source: Author, 2022) UOE/RICO/JMW/22

Appendix XXIX *Rubus apetalus* Poir. (Source: Author, 2022) UOE/RUAP/JMW/22

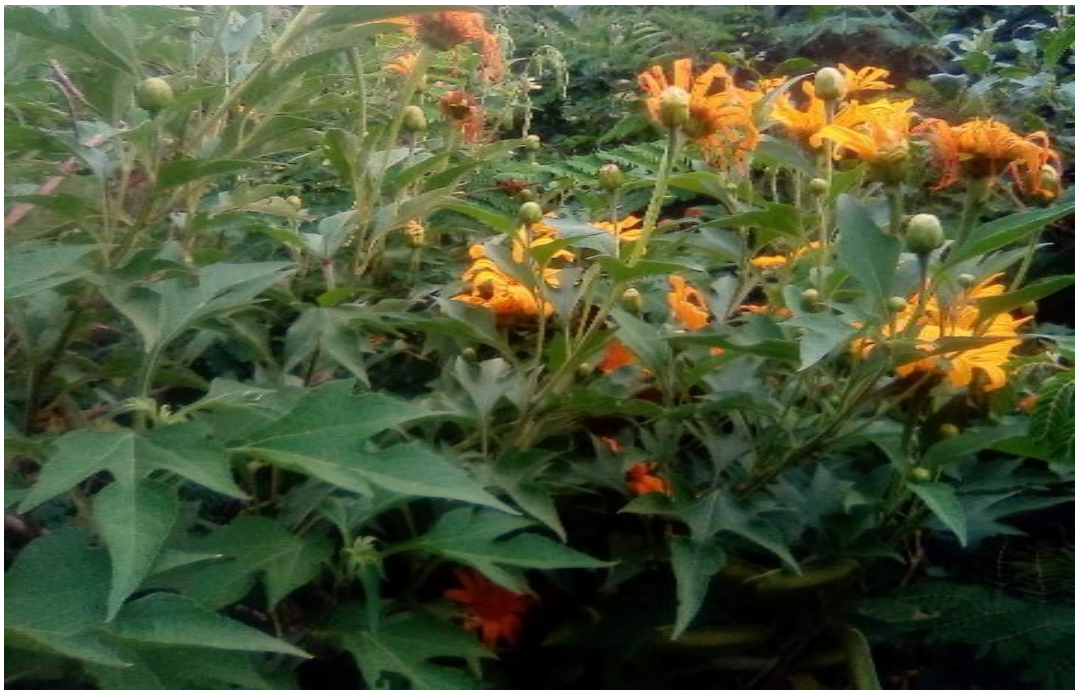
Appendix XXX *Searsia natalensis* (Bernh. ex Krauss) F. A. Barkley. (Source: Author, 2022) UOE/SENA/JMW/22

Appendix XXXI *Sida ovata* Forssk. (Source: Author, 2022) UOE/SIOV/JMW/22

Appendix XXXII *Solanecio manii* (Hook. F.) C. Jeffrey. (Source: Author, 2022) - UOE/SOMA/JMW/22

Appendix XXXIII *Tagetes minuta* L. (Source: Author, 2022) - UOE/TAMI/JMW/22

Appendix XXXIV *Tithonia diversifolia* (Hemsl.) A. Gray. (Source: Author, 2022) -UOE/TIDI/JMW/22



Appendix XXXV *Urtica massaica* Mildbr. (Source: Author, 2022) - UOE/URMA/JMW/22

Appendix XXXVI *Vernonia amygdalina* Delile. (Source: Author, 2022) - UOE/VEAM/JMW/22

Appendix XXXVII *Vernonia auriculifera* Hiern. (Source: Author, 2022) - UOE/VEAU/JMW/22

Appendix XXXVIII *Zehneria scabra* Sond. (Source: Author, 2022) UOE/ZESC/JMW/22

Appendix XXIX NACOSTI Research Permit

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