MOLECULAR SCREENING OF POTENTIAL BAT-BORNE HANTAVIRUSES AND DETERMINATION OF SPECIES RICHNESS AND COMPOSITION OF BATS IN SELECTED HABITATS WITHIN THE WOODLAND AND SAVANNA BIOMES IN NAMIBIA

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ABSTRACT

Bats are present and they play unique ecological roles in various habitats in Namibia. Yet the patterns of species richness and composition of bats associated with these habitats within different biomes in Namibia still remain unknown. Bats are also important carrier hosts and transmitters of zoonotic viruses of public health significance including old and emerging hantaviruses. The burden of hantavirus disease is globally well-documented but this remains unknown and unrecognised in many African countries including in Namibia. This study investigated the patterns of species richness and composition of bats as well as the potential prevalence of bat-borne hantaviruses at selected habitats in the broad-leaved tree and shrub savanna, and the Acacia tree and shrub savanna biomes in Namibia. Live bats were sampled using Dinier mist nets, biometrically processed and the species identifications were morphologically and molecularly determined. Blood and organ tissue samples were collected from live and sacrificed bats respectively, and they were molecularly screened for hantavirus using the two-step Pan-Hanta RT- PCR technique. A total of 219 bat captures were recorded from 17 selected habitats in both the broad-leaved tree (n = 7) and Acacia tree (n = 10) savanna biomes. Only 103 bat lung and blood samples were collected and screened for hantavirus. No hantavirus however was detected. A combined total of 11 species of bats were recorded from selected habitats in both biomes, and these included the following four families of the order Chiroptera: 1) Pteropodidae (1 species); 2) Molossidae (3 species); 3) Vespertilionidae (5 species); and 4) Rhinolophidae (2 species). Seven bat species were observed in selected habitats in the broad-leaved and eight in the Acacia tree savanna biomes, while 9 and 12 species,

respectively, were predicted. Species richness of bats did not differ significantly across the biomes. In addition, there was no significant difference in species composition between selected habitats in the two biomes. Thus the influence of the biomes on the species richness and composition of bats appears to be minimal at a broad spatial scale. The results indicate a compositional overlap in species of bats in selected habitats across biomes which suggest that the two biomes broadly harbour similar species. Habitats characterised by riparian vegetation, non-perennial riverine vegetation, and semi-urban environments were up to four times more species rich than other habitats. These habitats may represent important areas that support high species richness of bats characterised by variable patterns of species composition, based on species habitat requirements. In addition, four highway bridges at selected habitats in the Acacia tree and shrub savanna biomes showed high abundance of Robert's flat-headed bat (*Sauromys petrophilus*), indicating the importance of bridges to the roosting ecology and hence conservation of the species. Since no hantavirus could be detected, future detection of hantavirus and to gain more insights into our understanding of *in situ* patterns of bat species richness and composition may require more intensive sampling efforts and screening of bats in various habitats, and using a range of active (e.g., mist nets and harp traps) and passive (e.g., acoustic recording devices such as an Anabat) efforts.

Key words: hantavirus, bats, species richness and composition, molecular techniques, cytochrome b, Namibia

DEDICATION

This thesis is dedicated to the honour and memory of my late parents Kefas and Lahja who encouraged, insisted and sacrificed all the little they had to get me educated. May they both continue to Rest in Eternal Peace!

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DECLARATION

I, Augustinus T. Mbangu, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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Name of Student	Signature	Date

LIST OFACRONYMS

ANDV	Andes virus
ANOSIM	Analysis of Similarity
ATSS	Acacia tree and shrub savanna (biome)
BLAST	Basic Local Alignment Search Tool
BLTSS	Broad leaved tree and shrub savanna (biome)
BOWV	Bowie virus
DNA	Deoxyribonucleic acid
DOBV	Dobrava-Belgrade Virus
EHF	Epidemic hemorrhagic fever
ELISA	Enzyme linked immunosorbent assay
GPS	Global Positioning System
HCA	Hierarchical cluster analysis
HCPS	Hantavirus cardiopulmonary syndrome
HFRS	Hemorrhagic fever with renal syndrome
HPS	Hantavirus pulmonary syndrome
HTNV	Haantan virus
IFA	Indirect fluorescent assay
IFAT	Immunofluorescent antibody test
IgM	Immunoglobulin M
KHF	Korean hemorrhagic fever
M	Medium
MGBV	Magboi Virus
N	Nucleocapsid protein
NCBI	National center for Biotechnology information
NE	Nephropathia Epidemica

NSs	Non structural proteins
OKH	Okahandja
OPW	Opuwo
PCR	Polymerase chain reaction
PUUV	Pumaala Virus
PRNT	Plague reduction neutralization test
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SARS-CoV	Severe Acute Respiratory Syndrome-Coronavirus
SEOV	Seoul virus
SNV	Sin Nombre virus
TANGV	Tanganya virus
TULV	Tula virus
UNAM	University of Namibia
USA	United States of America
WHK	Windhoek
WHO	World health organisation

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CHAPTER 1

INTRODUCTION

1.1 General introduction

Bats are a unique group of small mammals to have evolved wings with a well-developed ability for powered flight. They exhibit evolutionary characteristics that to a large extent are considered socially, physiologically and ecologically unique and peculiar (Calisher *et al*., 2006). Bats are classified under the Order Chiroptera which was previously divided into the suborders Megachiroptera and Microchiroptera, generally comprising large bodied frugivorous bat species called mega bats and small-bodied bat species of largely insectivorous bats which consisted of micro bats respectively (Calisher *et al*., 2006). Latest taxonomic reconfigurations have however reclassified the Order Chiroptera into the suborder Pteropodiformes, comprising the families; 1) Pteropodidae; 2) Hipposideridae; 3) Rhinolophidae and; 4) Megadermatidae (Monadjem, Taylor, Cotterill, & Schoeman, 2010). The remainder of the bat families which include among others; Emballonuridae, Miniopteriadae, Molossidae and Vespertilionidae are classified under the suborder Vespertiliniformes (Monadjem *et al*., 2010). Bats are the most abundant group of mammals in terms of population numbers with a great diversity of over 1000 extant species that is second only to those of the Order Rodentia (Kunz *et al*., 2011; Kasso & Balakrishnan, 2013; Shapiro & Monadjem, 2016). Their wings enable bats to fly and cover long distances (Kunz, 1982). As such, bats have exploited this attribute to explore a wide variety of ecosystems during feeding and migratory activities (Kasso & Balakrishnan, 2013). This accounts for their wide

geographic distribution and high ecological diversity, occurring over all major continents except Antarctica and some polar regions (Calisher *et al*., 2008; Kasso & Balakrishnan, 2013).

In addition to flight ability, most bats can also echolocate, show roosting behaviour, enter torpor and hibernate in winter and have unusually long life spans for such smaller-sized mammals (Calisher *et al*., 2008; Kunz *et al*., 2011). These well-developed and unique socio-physiological adaptations may be enabling factors that help many bat species to thrive and survive. Bats are generally active at night and show different feeding habits, emerging from their roosting sites after sunset to forage and feed on a wide variety of dietary resources including insects, flower nectar, fruits, seeds, fish, frogs, small mammals as well as blood (Calisher *et al*., 2006; Kasso & Balakrishnan, 2013). Bats are characterised by a relatively small body size whose body weights varies between 2 grams, such as in the Bumblebee bat (*Craseonycteris thonglongyai*) and the Golden capped fruit bat (*Acerodon jubatus*), the largest species of bats with a body mass of up to 1.2 kilograms (Safi, Meiri, & Jones, 2013). The wing spans of bats generally ranges from a mere 8 centimetres to 1 meter in length (Calisher *et al*., 2006). They show roosting behaviour that enables them to live in a wide variety of natural structures including caves, trees, tree hollows, the under bark of trees as well as man-made structures such as house roofs, abandoned mines and bridges (Kunz, 1982; Knight & Jones, 2009).

In southern Africa, extensive efforts have been made in examining various ecological aspects, reproductive strategies, distribution and diet composition of some bat species especially in South Africa (Gelderblom, Bronner, Lombard, & Taylor, 1995; Van Der Merwe & Stirnemann, 2007; Monadjem *et al*., 2010; Bohmann *et al*., 2011; Schoeman, Cotterill, Taylor, & Monadjem, 2013;

Cooper-Bohannon *et al*., 2016). In Namibia however, published ecological studies on bats are almost non-existent. In fact, much of what is currently known about the occurrence and distribution of bat species is largely based on records of museum specimens that were collected as far back as the late 1960s (Monadjem *et al*., 2010), which may not necessarily represent the current patterns and status of bat distribution and occurrence. The biology and ecology of many bat species in Namibia remains unexplored, let alone bat community-based studies focusing on species richness and composition at localised habitats in the different biomes. Species richness generally refers to a count of the number of species in a given area or habitat and it is used as a basic measure for quantifying biological diversity through assessment and records of the presence or absence of species within given geographic areas (Chao & Chiu, 2016; Gotelli & Chao, 2013). Species composition entails the identities of species forming part of an assemblage and the associations of such species to particular habitats (Dorji, Moe, Klein, & Totland, 2014). Bats are sensitive to changing environments and they are considered suitable biological indicators of environmental quality, serving as agents for monitoring changing ecosystems (Jones *et al*., 2009). Lima, Varzinczak, & Passos, (2016) reported that the interactions of factors associated with habitat structure and seasonality influence the composition of local bat assemblages. In essence, bat communities at local scales are influenced by interacting factors, where the availability of suitable roosts and easy access to foraging habitats with abundant food resources takes precedence (Falcão, Esprito-Santo, Fernandes, & Paglia, 2018).

The determination of patterns of species richness and composition in local habitats therefore, help to characterise important conservation areas of high species richness and the associated species that such habitats may support. The effective conservation of biodiversity and particularly bats

may also hinge on our understanding of changing environments and how these changes affect species richness and composition of bats particularly in localised habitats. Characterising patterns of species richness and composition of local habitats within these biomes would represent a starting point and a positive step towards understanding the biology, ecology, patterns of species occurrence and distribution of some bat species in Namibia.

In addition, bats have recently come under increasing attention with regards to their roles as hosts for a large number of both old and emerging zoonotic viruses that have the potential to spill over and infect humans and other animals with significant economic and public health implications (Newman, Field, de Jong, & Epstein 2011). Calisher *et al*. (2006) and Smith and Wang (2013) reported that as many as 66 types of viruses including Ebola virus, Hendra virus, Nipah virus and Severe acute respiratory syndrome coronavirus (SARS-CoV) have been reported to be carried by bats without showing any pathogenic effects or any symptom of disease. Although bats carry this important status of being hosts for viruses with significant public health impact, they are equally suitable carriers of new and emerging viruses, including the potential emergence of hantaviruses in Africa (Calisher *et al*., 2006; Witkowski et al., 2014). Accordingly, the detection of the first novel African bat-borne Magboi hantavirus (MGBV) confirmed the status of bats as suitable hosts for hantaviruses and this has drawn attention to these viruses and their potential public health significance on the African continent (Weiss *et al*., 2012; Witkowski *et al*., 2014).

Hantaviruses are a group of emerging viruses sharing similar antigenic and genetic properties (Spickler, 2009). Hantaviruses are classified under the genus *Orthohantavirus*, of the family Hantaviridae and the Order Bunyavirales, and comprises of different hantavirus genotypes that

are traditionally named after the place where each virus was first detected from their carrier hosts (Bi, Formenty, & Roth, 2008; Adams *et al*., 2017). These viruses inhabit rodent, shrew and bat hosts that are dispersed and adapted to different environments globally (Bi *et al*., 2008; Reusken & Heyman, 2013). The hosts excrete the hantavirus in saliva, urine and faeces while infections may happen through inhalation of excreted virus containing aerosols, contact with contaminated excreta, contaminated food or by bites from particularly carrier rodents that may occur (Calisher *et al*., 2006; Spickler, 2009; Reusken & Heyman, 2013). Human infections by the rodent-borne Hantaan virus (HTNV) and the Seoul hantavirus (SEOV), as well as Puumala and Dobrava-Belgrade hantaviruses cause Hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, while the Sin Nombre (SNV) and Andes (ANDV) hantaviruses cause hantavirus pulmonary syndrome (HPS) in the Americas (Botten *et al*., 2003; Guo *et al*., 2013).

In Africa, there is a conspicuous absence of recorded cases of HFRS. The extent and impact of hantavirus disease has largely remained unknown, with only one isolated case of HFRS reported in the Central African Republic in 1987 (Bi *et al*., 2008). Although pathogenic hantaviruses are traditionally known to be carried by rodents, there is emerging evidence that shows hantavirus infections of humans emanating from non-rodent (shrew) carrier hosts particularly in west Africa (Heinemann *et al*., 2016). The need to screen for possible prevalence of hantaviruses especially in suspect bats can contribute towards ascertaining the status of hantavirus prevalence in Namibia.

1.2 Statement of the problem

Although bats are highly diverse and abundant in Africa, the ecology and distribution of many bat species remains largely unknown in many countries, particularly in southern Africa including in

Namibia (Shapiro & Monadjem, 2016). For example, the extent to which insectivorous bats may be assisting with rendering crop pest suppression services through their feeding habits in communal subsistence crop farming set-ups of rural communities in Namibia and Africa in general, if at all, remains to be determined. The potential beneficial roles of frugivorous and nectar-feeding bats in the pollination and seed dispersal of important local wild fruits and economically beneficial plant species that support the livelihoods of many rural communities in Namibia and Africa in general is also not yet known.

Even if it is known that bats occur in Namibia (Monadjem *et al*., 2010), the most basic data about species occurrence, distribution and ecology of bats in local habitats is absent. There is no reliable and updated national inventory of bat species that are recorded in different habitats within various biomes in Namibia. In fact, the current information on the occurrence and distribution of bat species in Namibia are largely based on outdated, old and arbitrary museum specimen collection records which does very little to reveal the patterns of bat species richness and composition at broader and local scale habitats in Namibia. In addition, associated patterns of bat species richness and composition in various habitats within different biomes in Namibia have remained largely undetermined and the conservation status of many bat species in Namibian habitats, is seemingly unknown, particularly in non-protected areas. Consequently, habitats that potentially support high bat species richness and the identities of associated bat species that such habitats may support have not yet been characterised. Hence the conservation and protection of bat species as well as management and protection of bat species-rich habitats especially within non-protected areas in Namibia may be hampered.

Furthermore, hantaviruses have over the years become the focus of global attention not only due to increasing incidents of New and Old World hantavirus disease but also the emergence of novel hantaviruses in new geographic regions of the world, including in new carrier host species (Witkowski *et al*., 2014). In Africa however, evidence for these viruses has largely and fairly recently been reported in West Africa, Gabon, Kenya and Ethiopia with an on-going search in South Africa and Namibia (Klempa *et al*., 2006; Meheretu *et al*., 2012; Witkowski *et al*., 2014, 2016; Tesicova *et al*., 2017). Whilst there is some evidence of hantavirus infection of humans demonstrated through sero-prevalence surveys in some parts of southern Africa (Ithete *et al*., 2014; Chau *et al*., 2017), hantaviruses have not been reported despite their potential prevalence in southern Africa. In addition, the extended incidence of hantavirus prevalence, especially in bat host species and human populations at local and continental scales, has not been fully explored. As an emerging virus, sufficient public and scientific hantavirus awareness is markedly lacking in many African countries (Chau *et al*., 2017). As such, hantavirus disease is not recognised in many African health systems, and can thus neither be reliably diagnosed nor treated.

The purposes of this study were therefore; 1) to determine the species richness and composition of bats; and 2) to investigate the potential prevalence of hantaviruses in bats using molecular techniques. These were based on bats sampled from selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia as a case study.

1.3 Aims and objectives

The aims of the study were to determine species richness and composition of bats and to investigate the potential prevalence of bat-borne hantaviruses in selected habitats in the broad-

leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia as a case study.

The specific objectives of the study were to:

(i) Determine and compare bat species richness in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia;

(ii) Determine and compare bat species composition in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia; and

(iii) Determine potential hantavirus prevalence through molecular screening of bats sampled from selected habitats within broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

1.4 Research questions

The study attempted to address the following research questions:

1.4.1 (a) What are the patterns of bat species richness that are associated with selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia?

(b) Is there a significant difference in bat species richness between selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia?

1.4.2 (a) What are the patterns of bat species composition associated with selected habitats in relation to the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia?

(b) Is there a significant difference in bat species composition between selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia?

1.4.3 Are there any individuals or species of bats in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia that harbour any hantavirus?

1.5 Research hypotheses

From the research questions above, the current study tested the following research hypotheses:

1.5.1. Ho: There is no significant difference in the species richness of bats in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

Ha: There is a significant difference in the species richness of bats in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

1.5.2. Ho: There is no significant difference in the species composition of bats between selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

Ha: There is a significant difference in the species composition of bats between selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

1.5.3. Ho: There is no individual or species of bats sampled in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia that harbours any hantavirus.

Ha: Some individual or species of bats sampled in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia do harbour some hantaviruses.

1.6 Significance of the study

Ecosystems represent units within which complex ecological interactions amongst living organisms and the non-living environmental component that support life occur. These natural ecosystems include but are not limited to forests, grasslands and deserts (Kunz *et al*., 2011). Bats constitute a significant proportion of the mammalian fauna, are highly diverse and form an integral part of the food web dynamics that characterise ecosystems; thereby regulate important ecological processes (Aguirre, 2002). Bats are highly adaptable animals with well-developed life history traits that inhabit the earth, exploiting a wide variety of these ecosystems (Ducummon,

2000; Kunz *et al*., 2011). Through their feeding habits, bats help with the suppression of pests, pollinate flowers, recycle energy and nutrients as well as help re-seed forests through dispersal of plant seed (Estrada & Coates-Estrada, 2001; Boyles, Cryan, Mccracken, & Kunz, 2011; Mccracken *et al*., 2012; Kasso & Balakrishnan, 2013).

Obtaining knowledge about the number of species in particular areas can therefore reveal associated patterns of species richness, identify threats to species and characterise endemic areas which are important parameters for assessing and managing biodiversity (Stoffberg, Schoeman, & Matthee, 2012). Research-based understanding of the biology and ecology of insectivorous bat species may hold the future potential for attracting insectivorous bats and relying on their feeding behaviour as biological control agents in rural subsistence farming set-ups in Africa. Elsewhere, bats have been explored for their role as biological control agents of crop pests in commercial agriculture (Boyles *et al*., 2011). Experimental attempts have been made to build bat friendly boxes near commercial crop plantations in order to attract bats on to crop fields to kill and reduce insect crop pests (Riccucci & Lanza, 2014). The reduction in crop pests not only serve to increase crop yields but also contribute towards a reduction in the costs and quantities of pesticide outputs into the environment (Riccucci & Lanza, 2014).

The future possibility of this in Namibia and Africa in general may present an opportunity through which local communities can be sensitised regarding the beneficial value of bats in order to help improve subsistence farming practices. This might at the same time also help to ameliorate negative perceptions and myths that are generally associated with bats, thereby contributing positively to the promotion of community based bat conservation strategies.

Namibia is generally categorised as an arid landscape with certain parts largely characterised by communal land tenure systems underpinned by subsistence farming (Ministry of Environment and Tourism, 2005). These, coupled with a growing population, lead to over-exploitation of natural resources resulting in deforestation, wild fires and soil erosion, thereby intensifying the problem of desertification (Jensen, Gaseb, & Nawaseb, 2002; Ministry of Environment and Tourism, 2005; Rosengren, 2011; Coetzee *et al*., 2014). In addition, human activities relating to agriculture, wood logging, and animal farming are reported to transform the vegetation structure of natural habitats and consequently affect natural ecological processes (Avila-Cabadilla *et al*., 2012). Therefore, land use practices that result into anthropogenic transformation of habitats compounded by the impact of climate change is likely to negatively affect biodiversity and by extension, impact on patterns of species richness and composition of bat assemblages in local habitats (Linden, Gaigher, Weterings, & Taylor, 2014). Data on the presence of species, the number of species and the association of particular bat species to certain habitats indicate habitat use by bats and may reveal compositional structures of bat assemblages in local habitats (Lourenco *et al*., 2014). The effective protection of bats may therefore depend on the knowledge regarding the habitats in which they occur and the prevailing factors that influence their distribution and association to specific habitats. Investigating the spatial patterns of bat species richness and composition associated with local habitats may therefore help to highlight important habitats of high bat species richness and may indicate intricate species-habitat associations that may be pertinent for the management of habitats and effective conservation of bat species in Namibia.

Given the above, the starting point was therefore to investigate the patterns of bat species richness and composition associated with some local habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia. This may help to characterize important habitats that contain high species richness and to understand the overall patterns of bat species-habitat relationships. These aspects may significantly contribute to the effective conservation and management of bat species and the habitats that support the existence of such species. The absence of basic knowledge on bat species richness and composition may impair our understanding of the role of bats in local ecosystems. Since bats are also considered to be effective bio-indicators of habitat quality, knowledge of the presence and absence of species in habitats may potentially assist in long terms efforts to monitor the impact of climate change and the effect of anthropogenic land-use pressures on the environment.

Bats are also implicated as carriers of hantaviruses which are classified as emerging viruses globally (Weiss *et al*., 2012). Although no bat-borne hantaviruses are known to cause disease in humans thus far, hantavirus infections in general are responsible for a number of human illnesses globally. Up to 150,000 cases of HFRS are recorded each year in Asia and Europe while an average of 200 HPS cases are recorded annually in the Americas (Bi *et al*., 2008), signifying their public health importance. In addition, given the lack of knowledge and public awareness on African hantaviruses and their potential public health impact, it may be possible that undiscovered hantaviruses may continue to infect and inflict illnesses in humans un-noticed, considering that HFRS may be mistaken for other illnesses such as acute manifestations of pneumonia and malaria which showcases similar clinical signs (Bi *et al*., 2008).

CHAPTER 2

LITERATURE REVIEW

2.1 The taxonomy, distribution and ecology of bats in Southern Africa

The Order Chiroptera comprises two bat suborders that occur in southern Africa (Monadjem *et al*., 2010). The suborder Pteropodiformes contains the families Pteropodidae, Hipposideridae, Rhinolophidae and Megadermatidae. The suborder Vespertilioniformes contains the families Molossidae, Emballonuridae, Miniopteridae and the Nycteridae. Both suborders and their associated families account for a combined total of 116 species of bats that occur in the southern African sub-region (Monadjem *et al*., 2010). In addition, it is possible that over 30 species of bats representing all eight southern African families occur in Namibia (Monadjem *et al*., 2010). The inclination of bat species to some habitats are influenced by a host of environmental factors largely associated with the availability of food and roosting space (Aguirre, Lens, & Matthysen, 2003; Ferrara & Leberge, 2005; Kaňuch & Krištín, 2005; Falcão *et al*., 2018). Factors such as variation in elevation, vegetation structure, primary productivity, localised climate, higher plant species richness and overall annual precipitation have been identified as constituting important factors influencing the richness and distribution of bats in southern Africa (Schoeman *et al*., 2013; Cooper-Bohannon *et al*., 2016). Among other factors, Linden *et al*. (2014) investigated changes in bat species richness across an altitudinal gradient and recorded the highest richness of bats at low altitudes and the lowest richness at much higher altitudes.

The southern African sub-continent is broadly classified into the following distinct biotic regions: 1) the Indian ocean coastal forest mosaic; 2) the Afromontane; 3) the south-west arid; 3) dry savanna; 4) the Highveld; 5) the moist savanna; and 5) the south Western Cape fynbos (Monadjem *et al*., 2010; Happold & Lock, 2015; Figure 1). These ecological regions are without doubt resident to a diverse number of bat species that are distributed based on for example, roosting, dietary, climatic and precipitation requirements (Schoeman *et al*., 2013).

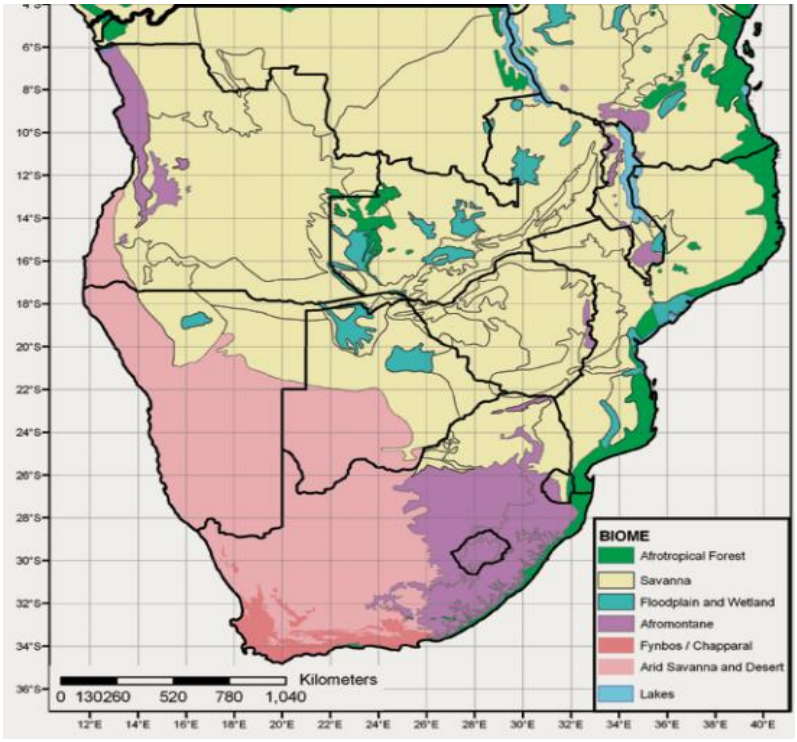

Figure 1 The six major biomes in southern Africa, characterised mainly on the basis of vegetation structure. The distribution of bat species is based on dominant vegetation structure, climate and precipitation within these Biomes. *Source:* Monadjem *et al*. (2010).

In order to understand the broader spatial patterns of bat species richness, there is a need to consider the predicted overall regional patterns of bat species richness in southern Africa. There is consensus that the distribution of mammal species including bats in southern Africa generally follows a positively correlated trend with plant species richness, with an associated pattern of a west to eastward increase in average annual rainfall (Qian, Kissling, Wang, & Andrews, 2009; Schoeman *et al*., 2013; Cooper-Bohannon *et al*., 2016; Monadjem, Conenna, Taylor, & Schoeman, 2017; Schoeman & Monadjem, 2018). In this regard, Andrews and O'Brien (2000) alludes to the spatial variations of climatic conditions especially relating to its effect in availing precipitation and energy dynamics, coupled with habitat productivity. A combination of these environmental dynamics over broader spatial scales may produce variable levels of primary productivity that influence patterns of plant diversity and mammal species richness within southern Africa (Qian *et al.*, 2009). Schoeman and Monadjem (2018) noted that the interaction between high rainfall and high plant diversity may result in high primary productivity, which produces abundant food resources in the form of fruits and insects, hence a positive correlation between high rainfall, plant species diversity and the associated high bat species richness.

In addition, notable progress has been made in developing tools of analysis for estimating the distribution patterns of species based on a set of dominant ecological and environmental factors that influence the spatial distribution of taxa in different environments (Schoeman *et al*., 2013). Species distribution models (SDM) use bioclimatic characteristics and species occurrence records to predict and estimate the suitability of species to some habitats in order to extrapolate the distribution of species (Phillips, Dudik, & Schapire, 2004; Schoeman *et al*., 2013). In this regard,

Maximum Entropy (Maxent) represents a widely used algorithm to estimate the distribution of species according to the suitability of specific habitats for some species (Phillips *et al*., 2004).

Schoeman *et al*. (2013) and Cooper-Bohannon *et al*. (2016) reported that SDM was applied in southern Africa in order to estimate the dispersal patterns of 64 and 58 bat species, respectively. They found that bat species richness was generally predicted to be highest in the eastern half of the southern African sub-region (Schoeman *et al*., 2013; Cooper-Bohannon *et al*., 2016). Habitats associated with higher bat species richness in southern Africa are those that form part of the Afromontane region in north-eastern South Africa and Zimbabwe, including in southern parts of Mozambique, Malawi and Zambia which may be associated with higher levels of primary productivity (Schoeman *et al*., 2013).

Specifically, the Afro-montane and the Indian Ocean forest coastal mosaic biomes were recognised to be regions showing the highest patterns of bat diversity followed by the dry and then moist savanna biomes (Schoeman *et al.*, 2013). Similarly, the north-western parts of South Africa that border with the southwestern arid region of Namibia and the savanna woodland showed high predicted bat species richness. The abundant river catchment water of the Okavango delta in the northern parts of Botswana was highlighted to be a key factor influencing the high species richness of bats in the area (Cooper-Bohannon *et al*., 2016).

2.2 Terrestrial biomes and sampled habitats in Namibia

The broad category of the savanna biome extends over many parts of Africa (Rutherford *et al.*, 2006). The savanna biome covers > 50% of the land surface area in southern Africa, highlighting

the variable patterns of vegetation and a diversity of plant life (Rutherford *et al*., 2006; O'Brien, 1998). The broad-leaved and fine-leaved categories of the savanna biome are mainly recognised in southern Africa (Schoeman & Monadjem, 2018). In Namibia, two broad categories of broad-leaved woodland and thorn-bush categories of the savanna biomes are recognised amongst others (Mendelsohn, Jarvis, Roberts, & Robertson, 2002). The broad-leaved woodland savanna, the thorn-bush savanna, the Karoo and desert biomes in Namibia are generally distinct geographic areas characterised by dominant vegetation types that are influenced by unique climatic and geological conditions prevailing in the areas categorised as biomes (Mendelsohn *et al*., 2002; Irish, 2008). The entire expanse of land is constituted by small distinct clusters of vegetation which are influenced by local soil types, geology and rainfall patterns. Generally, the vegetation in the central north and north-eastern parts of the country is constituted by taller and larger broad-leaved trees that are generally ascribed to the woodland biome where the annual rainfall is the highest and exceed 500 mm (Mendelsohn *et al*., 2002). The Woodland biome is thus constituted by the eastern Zambezi floodplains, Mopane woodland of eastern Zambezi region, the Kavango valley, the Omatako drainage, the Kalahari woodland and northern Kalahari regions (Christelis & Struckmeier, 2001; Mendelsohn *et al*., 2002; Mendelsohn & El Obied, 2005). The woodland biome is also referred to as the "broad-leaved tree and shrub savannah biome" (Barnard, Bethune, & Kolberg, 1998). The size and height of trees are taller in the broad-leaved tree and shrub savanna biome but progressively reduces into smaller and shorter trees and shrubs towards the southern and extreme western parts of the country (Mendelsohn *et al.*, 2002). Consequently, the thorn-bush savanna or "Acacia tree and shrub savannah biome" (Figure 2) is constituted by the Cuvelai drainage, southern Kalahari, the north western Mopane shrub land, the

western highlands, western Kalahari and the thorn bush shrub land regions (Mendelsohn *et al*., 2002; Mendelsohn & El Obied, 2005).

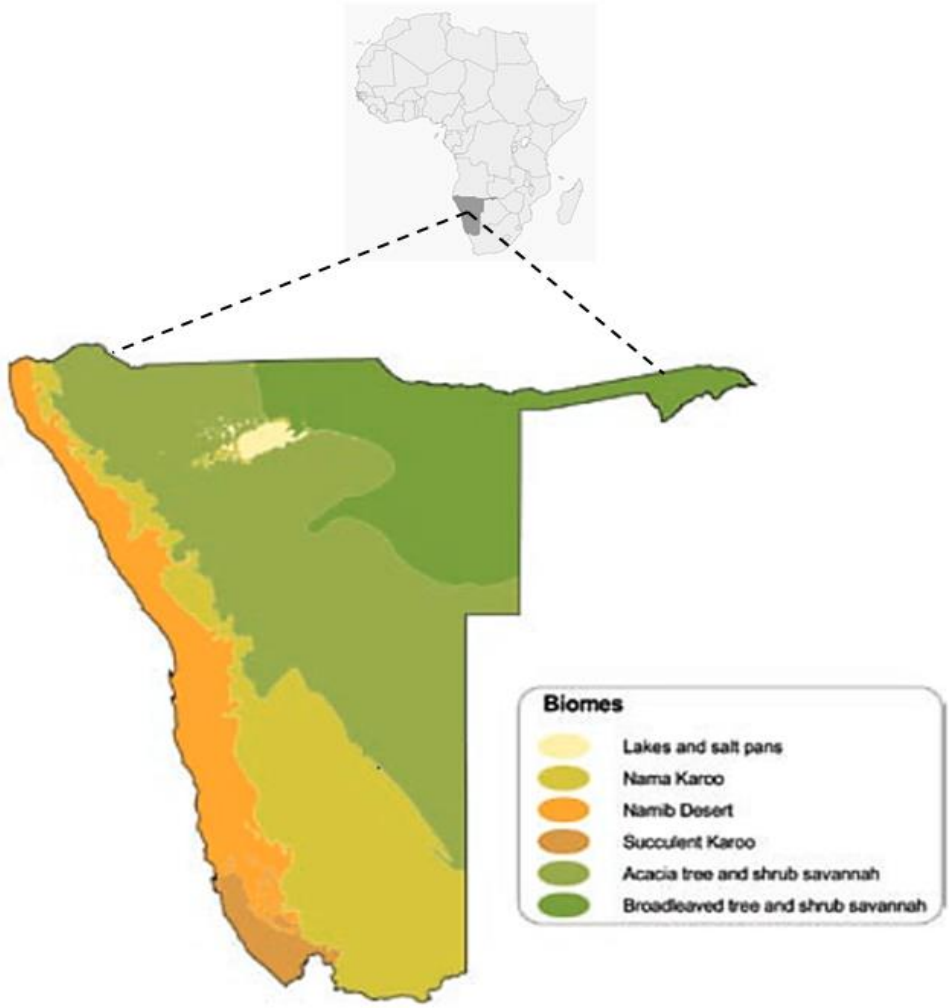

Figure 2 A map of major biomes of Namibia (with an insert map of Africa). The Tree and Shrub savanna is divided into the broad-leaved tree and shrub savanna biome as well as the Acacia tree and shrub savanna biomes. The Nama Karoo, the Succulent Karoo and the Namib Desert biomes are also shown. *Source*: Map of Namibian biomes: Namibian Ministry of Environment and Tourism (2010); insert map of Africa: https://www.info-namibia.com/info/namibias-geography

The desert vegetation zone (Figure 2) is spread along the western edge beyond the mountain escarpment towards the Atlantic Ocean and comprises the northern, central and southern Namib, which mainly consist of small succulent plants, supported by limited amounts of rainfall (Mendelsohn & El Obied, 2005). The Nama and Succulent Karoo biomes (Figure 2) are located in the central south and south-western edge of the country, respectively (Barnard *et al*., 1998).

For the current study, bats were sampled only in selected habitats in the broad-leaved tree and shrub savanna biome in the Zambezi region and those in selected habitats of the scrubland in the central and north-western areas of the Acacia tree and shrub savannah biome (Figure 2). The savanna biome is generally associated with high levels of biodiversity such as plants and vertebrates including bats which are influenced by varying effects of climate, energy and habitat quality dynamics at different spatial scales (O'Brien, 1998; Qian *et al*., 2009; Schoeman & Monadjem, 2018). The savanna biome represents an important area with good conservation value for biodiversity. The contrasting nature of the vegetation structures (Figure 2), represented by varying levels of annual rainfall and climate within the broad-leaved and Acacia tree savanna biomes in Namibia indicated the possibility of variable habitats and hence variable habitat structure with associated patterns of bat species richness and composition at a broader scale. The variability in terms of habitat vegetation structure and contrasting levels of land use patterns within the broader categories of the two biomes also suggested the potential variability in associated patterns of bat species richness and composition. Hence the current study focused on sampling bats in selected habitats within the two biomes as a case study, in order assess and

compare associated patterns of species richness and composition of bats and to molecularly screen bats for potential prevalence of any bat-borne hantaviruses.

2.3 Ecological and economic importance of bats

Bats are highly diverse and exhibit complex social, physiological and feeding habits that contribute positively to the well-being of ecosystems rendering services that are considered both ecologically and economically beneficial to humans and the environment (Kasso & Balakrishnan, 2013). Bats produce large amounts of guano in caves, which has a long history of being used as a natural fertilizer for agricultural crops as a rich source of nitrogen and phosphorus, potassium as well as trace elements that are crucial for stimulating growth, enhancing root development, and strengthening stems of plants (Kasso & Balakrishnan, 2013; Shetty & Sreepada, 2013; Bharambe, 2016). As a result, this does not only provide monetary benefits but also stimulates and supports the growth of important commercial cash crops such as cotton and maize (Ducummon, 2000; Ghanem & Voigt, 2012; Kasso & Balakrishnan, 2013). Similarly, bat guano has been reported to support several cave ecosystems as a primary source of energy for cave-dwelling micro-organisms that include bacteria, fungi, nematodes, and arthropods (Emerson & Roark, 2007; Shetty & Sreepada, 2013). Although not much emphasis is placed on the role of bats as prey for other animals, reports indicate that they constitute some proportion of the diets of birds, reptiles and some carnivorous mammals (Vargas, Landaeta, & Simonetti, 2002; Esbérard & Vrcibradic, 2007; Kasso & Balakrishnan, 2013; Mikula et al., 2016). While owls account for the largest threat of predation on bats, snakes and birds of prey such as hawks and falcons as well as small carnivores such as raccoons and feral cats have also

been reported to feed on bats (Motta JR & Taddei, 1992; Esbérard & Vrcibradic, 2007; Kasso & Balakrishnan, 2013; Mikula *et al*., 2016). Although predation on bats is still considered incidental and does not constitute a major threat to bat populations, it nevertheless still represents an important ecological contributor to the food chain dynamics that assist in maintaining the functionality of ecosystems (Mikula *et al*., 2016).

Conversely however, insectivorous bats through their diverse feeding patterns are known for being effective predators of a wide variety of insects including common pests of agricultural crops and forest ecosystems (Boyles *et al*., 2011; Mccracken *et al*., 2012). Some attempts have been made to identify insect prey by either microscopy or molecular analysis of insect fragments recovered from faecal pellets and stomach contents of bats including those that are associated with agricultural ecosystems (Ducummon, 2000; Feldhamer, Carter, & Whitaker Jr, 2009; Bohmann *et al*., 2011; Rolfe, Kurta, & Clemans, 2014). Common pests of economically-important forestry plants and agricultural crops such as beetles, corn ear worms, cucumber beetles, leaf hoppers have been identified from the faeces of many insectivorous bats that are associated with agricultural ecosystems (Whitaker Jr, Shalmon, & Kunz, 1994; Feldhamer et al., 2009; Mccracken *et al*., 2012; Rolfe *et al.*, 2014). Given their roosting behaviour, some species of insectivorous bats such as the bracken cave dwelling Brazilian free-tailed bats (*Tadarida brasiliensis*) are known to form large aggregations of up to 2 million individual bats in communal roosting caves of northern Mexico (Ghanem & Voigt, 2012). These bats descend on agricultural crop fields in large numbers and consume large quantities of these insect crop pests per night which substantially reduces the numbers of crop pests and thus save farmers on financial costs for pesticides which may ultimately result in increased crop yields due to reduced crop pests by bats

(Boyles *et al*., 2011; Kunz *et al*., 2011). In addition, insectivorous bats contribute to a reduction in the output quantities of chemical pesticides that have the potential to cause negative environmental effects (Ghanem & Voigt, 2012).

Pollination is a process which results in the transfer of pollen grains between male and female parts of the flower or between plants; and this facilitates the reproductive success of many plants, achieved by mutual interactive relationships with their pollinators (Rymer *et al*., 2005). A legitimate pollinator is regarded as one that regularly visits a plant and effectively causes the transfer of pollen grains between flowers or plants, resulting in pollination success (Ratto, Simmons, Spake, & Zamora-gutierrez, 2018). Although bat-driven pollination of plants is considered to be uncommon, over 500 species of plants in the tropics have been reported to depend on bats for their pollination (Acharya *et al*., 2015). The phenomenon of co-evolution has resulted in mutualistic relationships between bats and their associated plants creating inter-dependencies between bats and flowers or fruits, including the pollination of some economically-important plants (Aziz *et al*., 2017).

Nectar-feeding bats are important in the pollination of plants and have been reported to sustain the reproductive success of plants of tropical regions and wild islands (Kasso & Balakrishnan, 2013; Aziz *et al*., 2017). Consequently, a few case studies highlighting the mutualistic interdependent "pollination syndrome" between some nectar-feeding bats and their associated economically-important plants emphasize on the economic value of bats.

The African baobab (*Adansonia digitata*) for example, grows into a giant tree that forms part of the woodland savanna landscapes in sub-Saharan Africa and has important economic and social

roles in the livelihoods of many African communities including Namibia (Kamatou, Vermaak, & Viljoen, 2011; Kehlenbeck, Padulosi, & Alercia, 2015). Buchmann, Prehsler, Hartl, & Vogl, (2010) and Kehlenbeck *et al*. (2015) reported on the many traditional uses of the tree and its commercial potential in the nutrition, medicinal and cosmetic industries. Most notably, the flowers of the African baobab have been shown to rely mainly on nectar-feeding species of bats such as *Eidolon helvum*, *Epomophorus gambianus* and *Rousettus aegyptiacus* which have been identified as primary pollinators and seed-dispersers of these economically important plants (Marshall, 1983; Start, 2008). The white flowers of the African baobab are specially adapted to night opening and show outgrowths of staminal tubes that emit a scent which attracts nectar-feeding bats such as has been observed in the African straw coloured fruit bat (*Eidolon helvum*) and black flying foxes (*Pteropus alecto*) of Australia which have recorded to feed on anthers of baobab flowers and thereby facilitating pollination (Kamatou *et al*., 2011; Groffen, Rethus, & Pettigrew, 2016).

In addition, the evolutionary convergent mutualism between nectarivorous bats, especially the dawn bat (*Eonycteris speleae*) and Durian plants (*Durio zibenthinus*) in east Asia is well-documented (Bumrungsri, Sripaoraya, Chongsiri, Sridith, & Racey, 2009; Acharya, Racey, Sotthibandhu, & Bumrungsri, 2015; Aziz *et al*., 2017;). Durian fruits are common and in high demand in the east and south Asian economies due to their taste, flavour and enriched protein, fats, carbohydrate contents, as well as their associated cultural and economic benefits (Lim & Lauders, 1997; Bumrungsri *et al*., 2009). Over 200 cultivated varieties of Durian plants have been domesticated and are cultivated as cash crops for commercial exports and domestic trade, generating millions of dollars for the east Asian economy (Lim & Lauders, 1997; Aziz *et al*.,

2017). Flowers of these Durian plants rely on the nectar-feeding dawn bat (*Eonycteris spelaea*) for their pollination to produce these fruits (Bumrungsri *et al.*, 2009; Acharya *et al*., 2015). These plants have adaptive strategies such as flowering at night, coupled with the emission of a strong smell and an outgrowth of unusually high numbers of nectar and pollen that appear and smell attractively for easy access by these nectar-feeding bats (Acharya *et al*., 2015). Furthermore, fruit-eating bats are suitable seed dispersal agents that assist to re-seed and rejuvenate forests thereby contributing positively to the sustenance of economic benefits derived thereof (Ducummon, 2000; Ghanem & Voigt, 2012).

Bats also form part of the bush meat trade in many countries, and mainly large-bodied species are hunted in Africa and south east Asia as a source of food and commercial trade (Harrison *et al*., 2011; Kamins *et al*., 2011). Bat meat is considered to be a rich source of protein and some vitamins, and has formed a part of the bush meat trade in Ghana and Indonesia, being sold at prices ranging from $5 US to 10 US$ per bat, respectively (Harrison *et al*., 2011; Kamins *et al*., 2011; Kasso & Balakrishnan, 2013). Bat species such as *Eidolon helvum, Pteropus rufus, Rousettus madagascariensis* have been reported to be hunted for bush meat and commercial trade in Ghana and Madagascar, while the *Pteropus tonganus* and *Pteropus vampyrus* have been reported to be harvested in large numbers in Indonesia and the broader south east Asian region (Jenkins & Racey, 2008; Harrison *et al*., 2011; Kamins *et al*., 2011).

Bats also represent an invaluable and significant source of knowledge and they are considered suitable candidates possessing some life history traits that can be used for scientific modelling (Kasso & Balakrishnan, 2013). For example, new advances in medicinal research in ultrasound,

wireless systems, and ship navigation technology as well as sonar systems are reported to be based and modelled on principles of bat echolocation and movement (Gudra, Furmankiewicz, & Herman, 2011; ; Kunz *et al*., 2011; Kasso & Balakrishnan, 2013).

In addition, wildlife watching is an activity that involves the appreciation of nature, its associated wildlife and beauty. Pennisi, Holland, and Stein (2004) reported that successful wildlife watching is influenced by the predictability of occurrence of target animals in fairly small areas. The collective and predictable pattern of emergence of a large number of bats from caves, bridges and tunnel roosts at dusk for foraging activities not only create an impressive spectacle that attracts appreciation from visiting spectators but it also generates excitement and adventure for recreational tourists (Bagstad & Wiederholt, 2013). Thus, bat-watching is appreciated as a form of recreational tourist activity with the potential to generate income for local economies through entrance charges, guided tours and accommodation fees (Pennisi *et al.*, 2004; Bagstad & Wiederholt, 2013; Kasso & Balakrishnan, 2013). For instance, the congress avenue bridge in Texas, USA is a roost site for over 1.5 million Mexican free-tailed bats (*Tadarida brasiliensis*) which attracts over a 100,000 visitors annually, generates an estimated income of > $10 million US annually, illustrating the potential economic benefit of bats through recreational tourism (Pennisi *et al*., 2004). Although its economic value has not yet been quantified, the Arnhem cave in Namibia which consists of six species of bats has become a centre of attraction for recreational tourists and generates income through guided tours and accommodation fees for surrounding lodges.

2.4 General global threats to bats

There are several factors that cause a decline to bat populations globally. For example, the growing problem of climate change has highlighted the necessity for scaling-down on the global use of non-renewable energy sources which has been identified to cause negative environmental impacts (Arnett *et al*., 2016). Wind energy generation has become a suitable substitute to non-renewable energy sources, although with negative impacts on fauna (Arnett *et al*., 2016). Bats are clear casualties of wind turbines which strike and cause unprecedented mortalities of large numbers of bats (Arnett *et al*., 2016). Wind turbines have thus globally been recognised to pose a threat to declining bat populations (Boyles *et al*., 2011; Arnett *et al*., 2016).

In addition, bats are faced with negative public perceptions, myths and the general fear of acquiring diseases (Kunz *et al*., 2011). The tendency of bats to be active at night has rendered them susceptible to negative traditional beliefs that perceive bats to be sources of evil or as agents used by witches to cast negative spells on people in some parts of Africa (Cumes, 2013; Musila, Prokop, & Gichuki, 2018). Bat carcasses are sometimes used as part of traditional portions by *Sangomas* (traditional healers) when performing healing and spell casting rituals (Nzouankeu, 2010). At times, these negative perceptions have led to instances where house-roosts and other roosting areas of bats have become targets of destruction (Musila *et al*., 2018). Furthermore, frugivorous bats that have fed on ripe fruits in commercial plantations have attracted negative reactions from farmers, leading to large-scale culling and eradication efforts (Mickleburgh *et al.*, 2002; Aziz *et al.*, 2016). A fatal fungal infection by the fungus *Geomyces destructans*, which has killed a large number of bats in North America is also recognised as a growing threat to bat populations (Boyles *et al*., 2011). Moreover, the general threat of overhunting of bush meat

particularly of large-bodied species of bats still persist in some parts of the world and has been flagged as global factors that contribute to the decline in bat populations (Mickleburgh *et al*., 2002; Calisher *et al*., 2006; Kunz *et al*., 2011).

2.5 Global history and aetiology of hantavirus disease

From the mid-1930s, outbreaks of diseases characterised by a combination of abdominal pain, kidney dysfunction, haemorrhages and feverish symptoms have plagued some parts of the world. The causative agent associated with these diseases was unknown in the earlier manifestations of hantavirus disease. Thus, the description of Nephropathia Epidemica (NE) in the Scandinavian and Russian regions in the early 1930s and the outbreak of the Korean haemorrhagic fever (KHF) among army troops in the 1950s in Asia show that HFRS was previously classified under different names (Mccaughey & Hart, 2000; Bi *et al*., 2008; Spickler, 2009). The incidents of Nephropathia Epidemica (NE) and Korean hemorrhagic fever (KHF) were recognised earlier. The viral etiologic agent, the Hantaan virus (HNTV) causing the Asian forms of hantavirus disease was however, only isolated from the striped field mouse (*Apodemus agrarius*) in 1978 in South Korea (Mccaughey & Hart, 2000). Subsequently, the identification of the Seoul hantavirus (SEOV) in Asia as well as the Puumala (PUUV) and Dobrava-Belgrade (DOBV) hantaviruses in Europe, which were found to be related to the Hantaan Virus (HTNV) led the World Health Organisation (WHO) to collectively classify their related forms of disease as Haemorrhagic Fever with Renal Syndrome (Bi *et al*., 2008; Schmaljohn, 2009; Spickler, 2009). While many species of Old and New World hantaviruses have been isolated from rodents, shrews (Radosa *et al.*, 2013), and bats (Sumibcay *et al*., 2012; Weiss *et al.*, 2012), only the rodent-borne HNTV and SEOV are

known to cause HFRS in the east Asian region, while the PUUV and DOBV infections cause disease in Europe (Botten *et al*., 2003; Schmaljohn, 2009; Guo *et al*., 2013).

The outbreak in 1993 of a related form of hantavirus disease known as hantavirus pulmonary syndrome (HPS) was found to be caused by the rodent borne Sin Nombre hantavirus (SNV) isolated from the deer mouse (*Peromyscus maniculatus*) in the United States of America (Bi *et al*., 2008; Witkowski *et al*., 2014). The Sin Nombre hantavirus (SNV), together with the Andes hantavirus (ANDV) cause HPS which is prevalent in the greater American region (Plyusnin *et al*., 1996; Johansson *et al*., 2010; Guo *et al*., 2013).

2.6 The status of hantavirus research in Africa

Whilst it is clear that hantaviruses have extensively been studied in other parts of the world, it is noteworthy that the first African hantavirus was only molecularly detected from the African wood mouse (*Hylomyscus simus*) sampled from Guinea in 2006 (Klempa *et al*., 2006). The discovery of this first novel African hantavirus in West Africa has resulted in intensive hantavirus search efforts in the sub-region, leading to the discovery of the shrew-borne Tanganya hantavirus in Guinea (Klempa *et al*., 2007), Magboi hantavirus in Sierra Leone, Azagny hantavirus in Cote d'Ivoire, and Bowe hantavirus in Guinea (Kang *et al*., 2011; Weiss *et al*., 2012; Gu *et al*., 2013). In addition, the bat-borne Mouyassué hantavirus (MOUV) has also been detected in West Africa. Hantaviruses have also been reported in Central and East Africa, including the Mouyassué-related hantaviruses detected in bat and rodent hosts from Ethiopia and Kenya, respectively (Meheretu *et al*., 2012; Witkowski *et al*., 2016; Tesicova *et al*., 2017). The above African scenario shows that what is currently known about the existence of African hantaviruses is

largely concentrated in the west, central and eastern tropical forest region of Africa. Recent efforts to search for hantaviruses in South Africa and Namibia, which focused on rodent and shrew hosts, has so far not yielded any positive results (Witkowski *et al.*, 2014). Knowledge of the prevalence of hantavirus disease (HFRS) in many African regions remains unknown. Hantavirus sero-prevalence surveys in human populations in Ivory coast, Guinea, South Africa and Mozambique however, have demonstrated the presence of antibodies against hantavirus, indicating possible evidence of human infections by hantaviruses, including those from non-rodent hosts (Klempa *et al*., 2010; Ithete *et al.*, 2014; Heinemann *et al*., 2016; Chau *et al.*, 2017).

2.7 Genomic organization of hantaviruses

Hantaviruses are negative sense, single stranded RNA viruses (Jonsson *et al*., 2010). The Hantavirus genome is divided into three major segments with a simplified coding strategy where: 1) the large (L) segment encodes for the viral RNA dependent RNA polymerase; 2) the Medium (M) segment encodes for surface glycoprotein (Gn and Gc); and 3) the Small (S) segment encodes for the Nucleocapsid (N) proteins (Plyusnin *et al*., 1996; Mccaughey & Hart, 2000; Klempa *et al*., 2006; Bi *et al*., 2008; Reusken & Heyman, 2013). Although some hantaviruses were suggested to contain non-structural (NSs) proteins in their genomes, these have not been shown in viral-infected cells in the laboratory (Plyusnin *et al*., 1996; Mccaughey & Hart, 2000).

2.8 The classification and taxonomy of hantaviruses

Hantaviruses represent a group of old and emerging viruses sharing similar antigenic and genetic properties (Spickler, 2009). Previously, hantaviruses (Genus: *Hantavirus*) were classified under the family Bunyaviridae, which comprised the following five genera: 1) *Tospovirus* (which

contained plant viruses); 2) *Orthobunyavirus*; 3) *Hantavirus*; 4) *Nairovirus*; and *Phlebovirus* (Weidmann *et al*., 2003). The International Committee on the Taxonomy of Viruses (ICTV) however, has recently taxonomically treated the Family Bunyaviridae as a separate Order *Bunyavirales* (Adams *et al*., 2017). The genus previously designated as *Hantavirus* is currently taxonomically elevated as the Hantaviridae, consisting a single genus *Orthohantavirus*, under which 41 hantavirus species (renamed Orthohantaviruses) are taxonomically allocated (Briese *et al*., 2016; Adams *et al*., 2017).

2.9 Hantavirus disease, prevention, vaccine development and treatment

HFRS may occur in mild, moderate to severe forms characterised by fever, haemorrhages and kidney dysfunction with up to 15% case fatality rate while, hantavirus pulmonary syndrome (HPS) is marked by pulmonary and cardiovascular dysfunctions with recorded case fatality rates reaching 50% (Bi *et al*., 2008; Guo *et al.*, 2013). Since there are currently no approved drugs available, the prevention of hantavirus infection has been highlighted as the method of disease surveillance which is achieved by minimising human proximity to rodents and their contaminated excreta (Bi *et al*., 2008). The prevention of exposure to rodents is achieved through effective monitoring of rodent population dynamics, adoption of measures to make homes inaccessible to rodents and undertaking effective public education programmes to sensitise communities on how best to minimise contact with rodents (Bi *et al*., 2008; Schmaljohn, 2009). In addition, developing effective food storage measures that are resistant to rodent infestation, adoption of policies and measures for overall rodent management and scientific monitoring of patterns of hantavirus out-breaks in rodent populations have assisted in reducing the number of HFRS cases in China (Bi *et al*., 2008; Ullman, Souza & Langoni, 2008; Schmaljohn, 2009).

Other than adopting preventive measures, vaccination against hantavirus infection would be an effective preventative approach (Schmaljohn, 2009). Ideally, a vaccine would initiate the production of protective antibodies that work against hantavirus, which can prevent hantavirus infections in immunised individuals (Schmaljohn, 2009). There are currently no approved or licensed vaccines; however, for prevention of hantavirus infections (Maes et al., 2004; Schmaljohn, 2009, 2012).

Currently, there are also no drugs that are available for the treatment of both HFRS and HPS, and the only way to administer relief to patients with hantavirus disease is supportive treatment covering different stages of the progression of the disease (Bi *et al*., 2008; Mahmud-al-rafat & Taylor-robinson, 2014). Consequently, tranquilisation, oxygenation, and the continuous supply of body fluids are highlighted as critical intervention mechanisms during the feverish phase of HFRS (Bi *et al*., 2008; Ullman *et al*., 2008). During the kidney failure stage, it is also important to monitor and maintain blood pressure for patients with hypertension and a strict body fluid balance assisted by haemodialysis (Ullman *et al*., 2008). In HPS patients, the lungs should be ventilated during the initial stages together with careful monitoring of body fluid and electrolyte balance (Ullman *et al*., 2008).

Efforts at developing drugs for the treatment of hantavirus disease are still being undertaken at the experimental level, including Ribavirin which bears potential as a broad spectrum antiviral experimental drug that has been shown to cause antiviral effect against some hantaviruses in experimental animals (Mahmud-al-rafat & Taylor-robinson, 2014; Pettersson, 2015). Most mice that were infected with HTNV and subjected to treatment with Ribavirin survived compared to mice that were treated with experimental negative control substances (Maes *et al*., 2004;

Pettersson, 2015). HFRS patients in China and Korea subjected to Ribavirin treatment showed a significant reduction in rates of disease prevalence compared to patients treated with control substances (Maes *et al*., 2004).

2.10 Techniques used for detection of hantaviruses

Although epidemiological information and clinical data on hantavirus disease in humans are important, the accurate diagnosis of hantavirus infections is however dependent on the use of laboratory tests in order to screen samples of patients showcasing clinical signs and symptoms (Mattar, Guzman, & Figueiredo, 2015). Suitable laboratory diagnostic approaches have used principles of serologic detection of hantavirus antibodies, neutralisation tests, molecular approaches and even cell culture based techniques (Mattar *et al*., 2015). The specific tests include: 1) indirect fluorescent assay (IFA); 2) immunofluorescent antibody test (IFAT); 3) agglutination reactions; 4) western blotting; 5) plague reduction neutralisation tests (PRNT); and 6) reverse transcriptase polymerase chain reaction (RT-PCR) (Bi *et al*., 2008; Ullman *et al*., 2008; Witkowski *et al*., 2014; Mattar *et al*., 2015). The primary diagnosis of a hantavirus infection can be located in the serologic application of the Enzyme linked Immunosorbent Assay (ELISA) which has wide application (Bi *et al*., 2008; Witkowski *et al*., 2014). The early stages of hantavirus disease induces increased levels of hantavirus-specific IgG and IgM antibodies, thus the ELISA technique has the sensitivity to detecting these antibodies (Bi *et al*., 2008; Ullman *et al*., 2008). In addition, the PRNT has been used as a gold standard for the identification of a specific hantavirus strain responsible for an infection, given its ability to detect and measure neutralising antibody titers (Mattar *et al*., 2015). More importantly, following its first application

in the 1990s, the polymerase chain reaction (PCR) technique is now globally considered a very useful tool for the detection of hantaviruses (Mccaughey & Hart, 2000). Although largely used as a research tool, two versions of the two-step Pan-Hanta reverse transcription polymerase chain reaction (RT-PCR) test are mainly used to molecularly detect hantavirus from clinical samples (Mattar *et al*., 2015; Vial *et al*., 2016). A quantitative real time reverse transcriptase polymerase chain reaction (qRT-RT-PCR) method is considered gold standard and is used for fast and early detection and diagnosis of hantavirus infections in patients (Kramski *et al*., 2007; Mattar *et al*., 2015; Vial *et al*., 2016). The conventional two step Pan Hanta RT-PCR method is largely used as a non-clinical research tool and has been designed to detect hantaviruses using Hantaviridae family or genotype-specific primers (Klempa *et al.*, 2006; Bi *et al*., 2008; Jonsson *et al*., 2010; Witkowski *et al*., 2014). The latter molecular approach has especially been used in research to successfully detect and demonstrate hantavirus using dried blood spots, frozen blood samples, frozen lung, liver and kidney tissues as well as from ethanol fixed liver tissues sampled from wild rodent, shrew and bat carrier hosts (Klempa *et al*., 2006; Meheretu *et al*., 2012; Sumibcay *et al*., 2012; Weiss *et al*., 2012; Kang *et al*., 2014; Witkowski *et al.*, 2016; Tesikova *et al*., 2017). The success of the above hantavirus detection efforts were largely based on the two-step Pan-Hanta RT-PCR assay designed by Klempa *et al.* (2006), which uses two sets of degenerate Oligonucleotide primers in a nested RT-PCR assay. The Oligonucleotide primers target a conserved region of the Large (L) hantavirus genomic segment which encodes for the RNA dependent RNA polymerase (RdRp), and are capable of detecting both old and new hantaviruses (Klempa *et al*., 2006). The successful RT-PCR amplification of target hantavirus genome, especially from non-clinical wild small mammal research samples appears to be anchored on the

use of a plasmid-derived positive control hantavirus cDNA, which is used for validating the PCR assay (e.g., see Iithete, 2013). In venturing into investigating the potential prevalence of bat-borne hantaviruses from selected habitats in Namibia, the current study adopted the same molecular approach as above, which has conventionally been demonstrated to detect even novel hantaviruses.

2.11 Molecular identification of bat species

The identification of species based on morphological characteristics of living organisms have traditionally been used widely to taxonomically describe over 1.5 million species (Mayer, Dietz, & Kiefer, 2007). The deficiencies associated with morphology-based identifications led to the development of new technologies in order to enhance the ability to identify and describe species (Nadin-davis, Guerrero, Knowles, & Feng, 2012). With the advent of molecular techniques and their application in ecology, the identification of species and the reliable classification of organisms have been made more compelling (Irwin, Kocher, & Wilson, 1991; Meganathan *et al*., 2012). Mitochondrial DNA (mtDNA) sequences have become useful for molecular identification of species and for inferring phylogenetic relationships between species because of its presence in multiple copies within the cells of organisms and its high rate of mutation compared to nuclear DNA (Meganathan *et al*., 2012). Sequences of target genes can be obtained and be used as a basis for molecular identification of species and in phylogenetic analyses, in order to compare and elucidate evolutionary relationships between species of interest (Nadin-davis *et al*., 2012). Phylogenetic analysis can be performed using computer software such as MEGA (Tamura *et al*., 2011). These analysis are based on statistical methods for calculating genetic distances between

DNA sequences, which among others may include Maximum Likelihood (ML) and Neighbour joining (NJ), in order to construct phylogenetic trees (Mayer *et al*., 2007; Nadin-davis *et al*., 2012; Lei & Dong, 2016; Demos *et al*., 2019). It is on the basis of these phylogenetic analyses that evolutionary relationships between species can be inferred, although based on genes (Mayer et al., 2007; Demos *et al*., 2019). Consequently, a number of bat species have been identified and their phylogeny determined using mtDNA sequence data (Clare *et al*., 2007; Lamb et *al*., 2008).

2.12 Data analyses

2.12.1 Species accumulation curves and species richness estimators

Species accumulation curves represent a cumulative plot of the number of species against some level of sampling effort which may be measured in relation to the number of individuals, samples, unit area or time (Colwell, Mao, & Chang, 2004; Dorazio, Royle, Orazio, & Oyle, 2005). The species accumulation curve can then be smoothed by applying different randomised re-samplings of the data, in order to produce an average smoothed-out curve from the different randomized permutations of the curve using a statistical software such as Paleontological Statistics (PAST) software for windows (Hammer, Harper, & Ryan, 2001). The appropriate use of species accumulation curves and predictions of species richness is predicated on sampled data that satisfies the following three basic assumptions that: 1) a record of observed species be compiled; 2) communities being surveyed and compared must come from different geographic locations; and 3) some measure of randomness but replicated and consistent sampling effort from each geographic location has been undertaken (Dorazio *et al*., 2005). Stevens, Willig, & De Fox, (2004) and Dorazio et al. (2005) proffered that sample-based species accumulation curves

provide a better representation of communities and should take precedence over individual-based ones, as they tend to cater for different levels of variability in species based on each sample.

Species richness is generally defined as a count of the number of species over an area and it is used as a basic measure for quantifying biological diversity through assessment and records of the presence or absence of species within given geographic areas (Gotelli & Chao, 2013; Chao & Chiu, 2016). The successful assessment of total species richness relies on a good level of sampling success and a comprehensive coverage of survey areas (Dorazio *et al*., 2005; Hortal, Borges, & Gaspar, 2006). The collation and collection of species richness data therefore requires more elaborate efforts. Survey methods however are constrained by the inherent shortcomings of not being able to detect all species within habitats or communities of interest (Chao & Chiu, 2016; Colwell, 2009; Colwell *et al*., 2004; Dorazio *et al*., 2005). This is because the natural complexities of communities may be different and the behavioural traits as well as relative abundances of species may affect the levels of detectability of some species (Colwell, 2009). Since the true species richness of a given area is the sum of the detected species and the undetected ones, therefore a mere survey and count of species observed in a sample leads to negatively understating true species richness of any area (Chao & Chiu, 2016).

Consequently, the acceptable approach is dependent on the collation of sample data from communities or areas of interest from which the highest number of species can be approximated. Species richness estimation is therefore, an important analytical method for evaluating the degree of sampling completeness and is considered suitable for partially sampled habitats when gauging overall richness and the determination of taxa of given areas by using sample data (Colwell, 2009; Gotelli & Chao, 2013; Chao & Chiu, 2016). Brose and Martinez (2004), Hortal *et al*.

(2006), and Gotelli and Colwell (2011) reported that there are parametric and non-parametric statistical methods for estimating species richness and quantification of biological diversity. The use of non-parametric approaches which relies on statistical extrapolation principles calculate the asymptote of the species accumulation curve from where the total number of species are extrapolated has proven more reliable and are thus preferred than parametric methods and they are widely used (Brose & Martinez, 2004; Gotelli & Colwell, 2011; Gotelli & Chao, 2013).

2.12.2 Determination of bat species composition

A measure of properties of any given biological community requires the use of an appropriate statistical framework in order to gain insights into trends within it. One common and applicable ecological procedure is the assessment of taxonomic similarities or differences in plant or animal community structures that are influenced by ecological dynamics that characterise those communities (Clarke, Somerfield, & Chapman, 2006). The Bray-Curtis similarity index is used widely to assess community structure as it can calculate the similarity or dissimilarity coefficients which are used to determine the compositional similarities or differences between different sampling areas using a simplified measure expressed as a percentage (Clarke, 1993; Clarke *et al*., 2006).

Comparisons of the degree of distinctiveness in species composition between groups can be achieved by application of the analysis of similarity (ANOSIM, Clarke, 1993). ANOSIM is appropriate for assessing levels similarity or difference in composition of species within and between groups (Chapman & Underwood, 1999). Consequently, it has been used to assess and compare differences in plant species composition across different categories of plant communities

(Ross, 2014; Sandor & Chazdon, 2014), to evaluate compositional differences in marine and benthic communities (Heaven & Scrosati, 2008), and to compare differences in compositional structure of bat communities across variable categories of habitats (Heer *et al*., 2015; Lourenço *et al*., 2014; Pereira *et al*., 2009). ANOSIM is a distribution-free multivariate test which compares average ranked distances of [dis]similarity measures based on multiple species and their abundances within and between groups in order to generate a value of the *R* test statistic which indicates the magnitude of separation between the groups and this value ranges between 0 and 1 (Chapman & Underwood, 1999; Ramette, 2007). The null hypothesis of the ANOSIM test assumes no significant difference between groups when the *R* test statistic is scaled to equal 0 while an *R* value of 1 represent the highest degree of separation between groups (Chapman & Underwood, 1999). The statistical significance between groups represented by the observed sample-based *R* test statistic is determined by the *P*-value resulting from different permutations of simulated re-arrangements of sample group memberships (Clarke & Warwick, 2001). The significance level at which to reject the null hypothesis is determined by the chosen total number of randomised permutations on which the test is undertaken. For instance, Clarke (1993), and Clarke and Gorley (2006) noted that when running the ANOSIM test using 1000 permutations, the significance level for rejecting the null hypothesis is 1 in a 1000. So the *P*-value must be less than 0.01% (i.e. $P < 0.001$) for the null hypothesis to be rejected, provided that the test was conducted using 1000 permutations.

In light of all the above, this study therefore sought to collect data in order assess patterns of bat species richness and composition in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia. The gaps in knowledge

relating to these aspects have remained unexplored and there are currently no efforts to characterise potential areas of high bat species richness and establish habitats which are associated with particular species of bats, especially in non-protected areas, where there are no specifically targeted conservation efforts. Given the current land use practices in non-protected areas in Namibia which may likely transform habitats and result in elevated threats to biodiversity, the effective conservation of bats, particularly in light of the growing phenomenon of climate change and the human land use impacts on potential habitats will be made difficult without basic data on the species richness, distribution, ecology and species-habitat relationships (i.e. species composition). The broad-leaved and Acacia tree and shrub savanna biomes constitute potential habitats within which rich biodiversity may co-exist. Bats are significant indicators of habitat quality and the assessment and characterisation of richness and composition patterns associated with various habitats, particularly over broad spatial scales such as in different biomes may help improve our understanding of changing environments and efforts to characterize species rich habitats.

In addition, the study also sought to screen bats for the potential prevalence of bat-borne hantaviruses. The reported expanded host range of hantaviruses which include shrews and bats may potentially signify a more broader global biogeographic spread and hence increased global risk of hantavirus disease. Despite this possibility, there appears to be very little to no knowledge and recognition of hantavirus disease in many African countries including in Namibia. Hence very little or no diagnostic and surveillance mechanisms have been put in place for the effective collection of epidemiological data on hantavirus disease. The potential prevalence of hantavirus in human or non-human small mammal wild hosts has not been determined and thus, the

possibility of undetected hantavirus infections of humans cannot be discounted. Bats are implicated as carriers of significant viral zoonosis including the emergence hantaviruses especially in Africa (Weiss *et al*., 2012; Sumibcay *et al*., 2012; Tesicova *et al*., 2017). Bats are known to form large high density social groups at roost sites, they live unusually long lifespans and many species hibernate in winter; adaptations that are suggested to facilitate viral maintenance and spread (Calisher *et al*., 2006). In addition, Witkowski *et al*. (2016) stressed that bats' high diversity and powered flight facilitate their ability to occupy a wide variety of habitats including roosting in human dwellings, thereby increasing the potential to facilitate spill over infections to humans. The need thus arose to survey potential wild bat carriers using the conventional Pan Hanta RT-PCR molecular screening technique as a starting point, in order to assess the potential prevalence of hantaviruses within suspected wild bat hosts in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study areas

3.1.1 Climate

Namibia is generally characterised by spring, summer and winter seasons (Mendelsohn & El Obied, 2005). Hot annual summer rain season occurs between November and April and rainfall figures vary depending on prevailing local climate in different parts of the country. Recorded annual rainfall figures range from less than 50 mm in the Namib Desert to over 600 mm in the extreme north-eastern flood plains of the Zambezi region (Figure 3), supported by vast and contrasting soil types and landscapes which influence a great diversity of plant life (Mendelsohn *et al*., 2002).

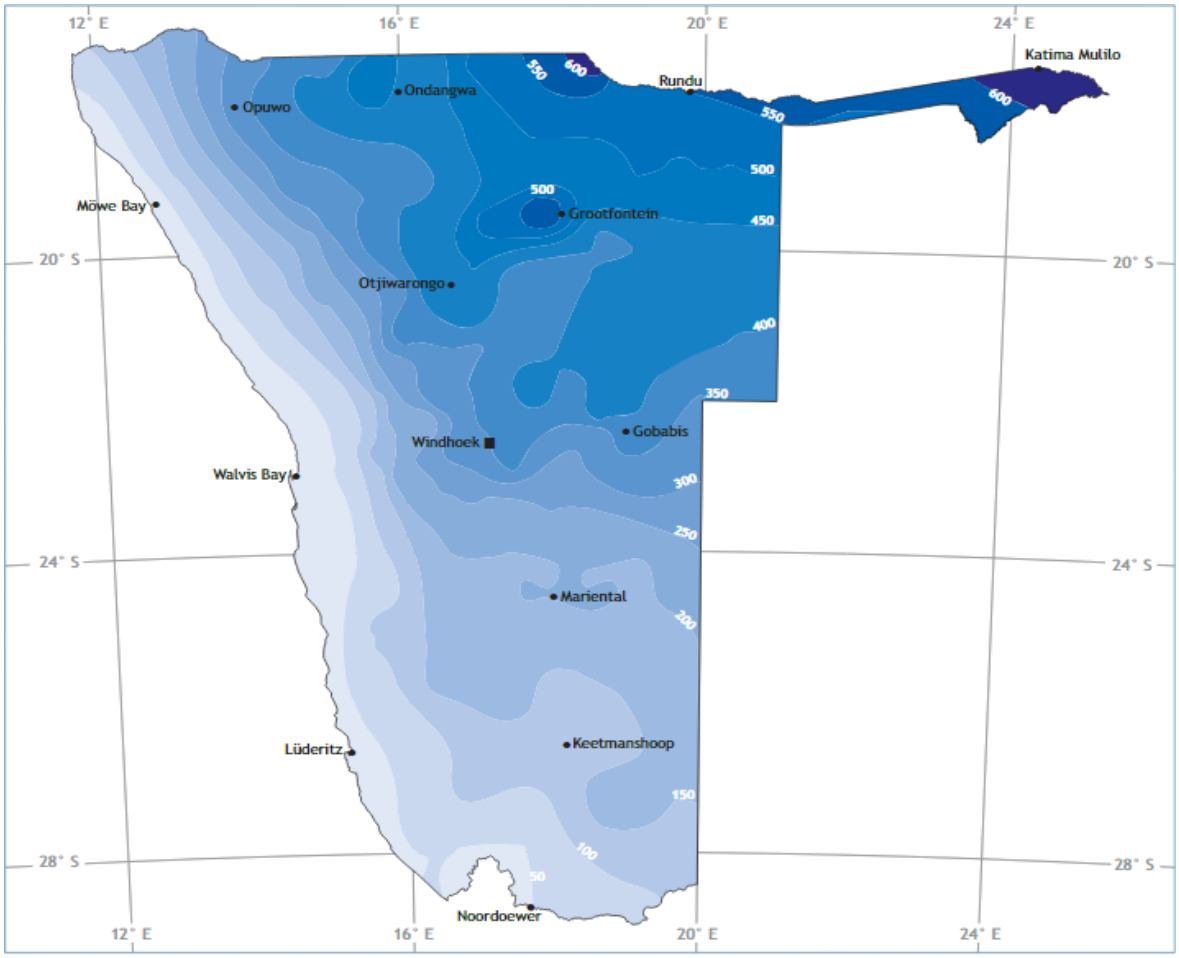

Figure 3 A map of Namibia showing its average annual rainfall (mm) pattern. *Source*: Mendelsohn & El Obied (2005)

From the eastern end of the Zambezi strip, there follows a general south-ward and west-ward reduction in annual rainfall, with 450 mm in the central-east, 150 mm in the south to less than 50 mm in the north-central and southern Namib desert (Mendelsohn & El Obied, 2005). Seasonality is central to recorded annual average temperatures. The average maximum temperatures recorded during summer vary between 30-34^{o} C in the northern and north-eastern parts of Namibia during

September and October. The highest temperatures are recorded in central south Namibia (Mariental) at above 36^{o} C in January while in the Namib Desert, maximum annual temperatures vary between 18-26^{o} C recorded in February (Mendelsohn *et al*., 2002; Mendelsohn & El Obied, 2005). Average minimum temperatures vary between 0-4^{o} C in north, central and south-east Namibia in July, and 8-12^{o} C in north, central and southern Namib in August (Mendelsohn et al., 2002).

3.2. Overall study areas and sampling sites

Under a research and collection permit (number 2089/2015; appendix 1) granted by the Ministry of Environment and Tourism, bats were sampled at the identified sampling sites in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes. The combined sample data was collected in the summer months from December 2015 until the first week of May 2016, taking into consideration sampling time limitations and avoiding the cold winter season within which bat activity is generally reported to reduce substantially for some bat species (Meyer, Schwarz, & Fahr, 2004; Meyer, Senulis, & Reinartz, 2016) in order to avoid potential sampling bias. According to Mendelsohn *et al*. (2002), the general sampling area in the broad-leaved tree and shrub savanna falls within two broad vegetation types. 1) Selected habitats in localities around Kongola (i.e Kamenga and Waldo lodge) and Katima Mulilo (i.e. Wenela, MET, Katima east site 1 and 2) towns are located within the broad-leaved mopane woodland vegetation, where the habitat types are generally characterised by large stemmed and large canopy woody vegetation. 2) The Chinchimane habitat is typically described as falling part of the north-eastern Kalahari woodland with some small alternating transitions between broad-leaved large canopy vegetation and *Senegalia* and *Vachellia*

type of vegetation (Mendelsohn *et al*., 2002). The general sampling area within the Acacia tree and shrub savanna broadly comprises of characteristic thornbush and woody *Senegalia* and *Vachellia* type of vegetation in the central east, central areas and the mopane shrubby vegetation is characteristic in the northwestern areas of Namibia (Mendelsohn et al., 2002). The selected habitat types included: 1) The characteristic *Senegalia* and *Vachellia* type shrub-land and woodland mosaic vegetation of the central east Namibia; 2) the dense thorn-bush *Senegalia* and *Vachellia* scrublands of central Namibia; and 3) the dominant northwestern mopane shrubland vegetation, with some large patches of grasslands.

The current study focused on sampling from the general population of bats from selected sampling sites in selected habitats within the broad-leaved tree and shrub savanna and Acacia tree and shrub savanna terrestrial biomes in Namibia. These included 7 selected habitats within the broad leaved savanna and 10 selected habitats within the Acacia savannah terrestrial biomes based on "Biome" characterisations described by Barnard *et al*. (1998) and also as depicted in figure 2. The selected habitats in each study area within the biomes were broadly assessed for suitability prior to being sampled. The assessments of potential habitats for selection were done based on vegetation structure, suitability as potential sites of bat activity, accessibility and their overall suitability for sampling with mist nets. Some sampled habitats were selected based on information provided by local residents relating to areas where bats have recently been sighted (e.g. Katima east site 1 and 2; Ben hur). Specific sampling sites in each selected habitat were chosen based on observed habitat features that may be associated with bat activity. These included the presence of water such as at a river or water hole, open and channelling spaces within dense vegetation, on the edge of forest vegetation, dry river beds aligned with vegetation

cover, near observed roost sites such as bridges and buildings and any other place suspected to attract bat foraging activity in order to maximise sampling success. Ideally, ten selected habitats ought to have been sampled in each biome. Only seven selected habitats could however be sampled within the broad-leaved tree and shrub savanna, largely due to limitations of time, resources and inaccessibility to some areas. Selected habitats were generally characterised based on recorded observations of characteristics such as dominant vegetation, soil type, grass cover and presence of any notable feature as observed at the sampling site and the surrounding area. Habitats were then generally characterised into categories such as riparian, riverine vegetation, roost sites, water points and semi-urban based on recorded characteristics observed in selected habitats such as described under section 3.2.1 and 3.2.2.

3.2.1 Sampling sites in selected habitats within the Acacia tree and shrub savanna biome (Figure 4).

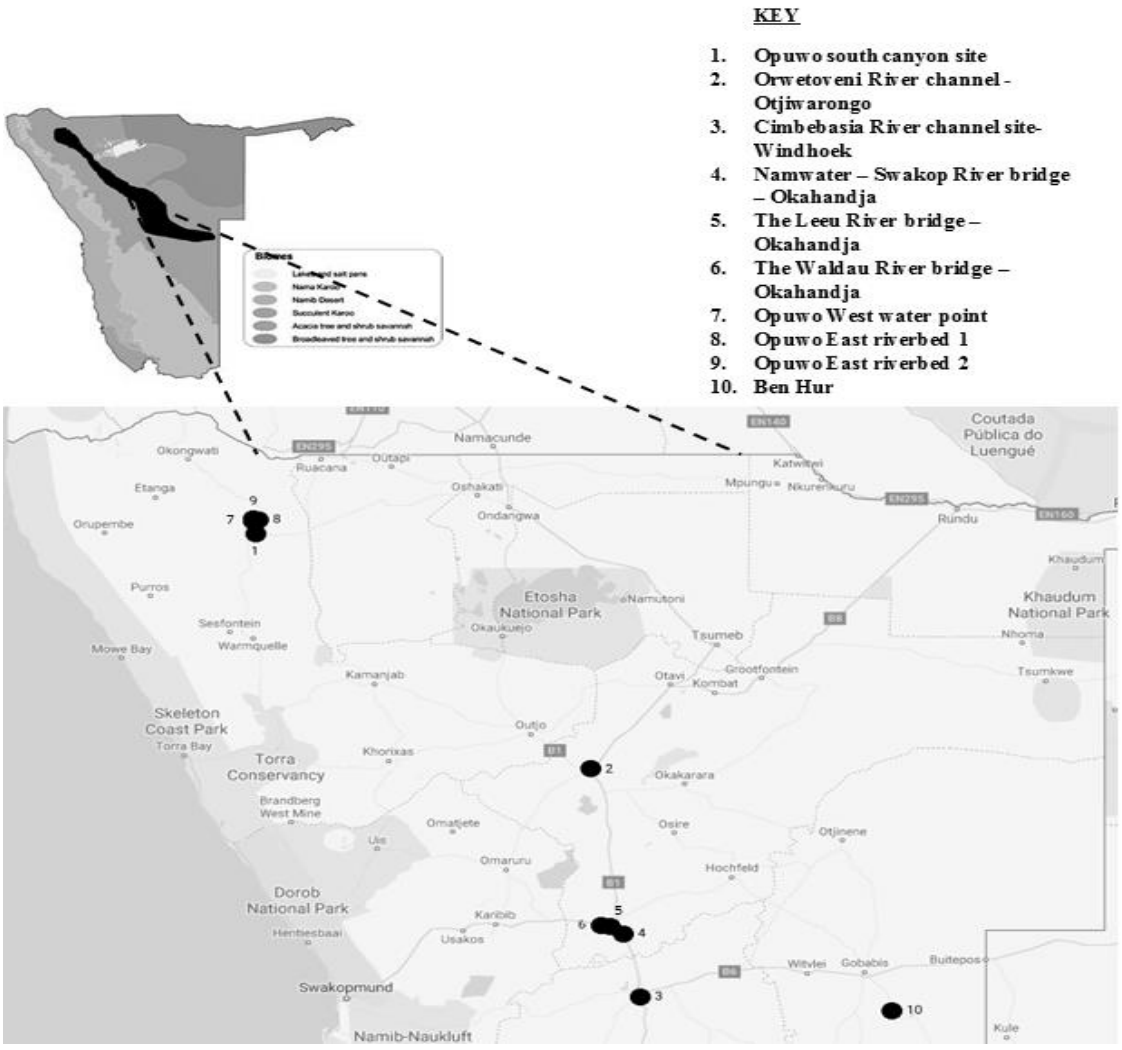

Figure 4 Partial Google earth map of Namibia showing numbered Global Positioning System (GPS) locations (numbered black dots) including a key of the name of each numbered sampling site in selected habitats (with a partially modified insert map of biomes in Namibia showing the location of the overall sampling area (shaded in black)) in the Acacia tree and shrub savanna biome. *Source:* Insert map: Namibian Ministry of Environment and Tourism, (2010).

Bats were sampled at the following sampling sites within selected habitats in the Acacia tree and shrub savanna biome in Namibia.

1) Opuwo South canyon site (18°09'47.0"S,13°50'23.4''E)

This sampling site lies *ca.* 25 km south of the town of Opuwo and is characterised by the presence of a heavy soil erosion by rain water, forming large trenches, gullies and water-flow channels. Bat sampling was undertaken at the base of the deep main river gorge lined with large stemmed trees and dense vegetation comprising *Vachellia erioloba* with some sparse *Termialia prunoides* and *Dichrostachys cinerea* on the banks. The entire surrounding was dominated by small mopane trees (*Colophospermum mopane*) and shrubs. The site was also characterised by red sand.

2) Orwetoveni River channel - Otjiwarongo (20°27'02.9"S, 16°39'52.5"E)

This sampling site is located on a small riverine channel that runs parallel to the northern outskirts of the town of Otjiwarongo near Orwetoveni suburb. The banks of the riverine channel are lined with thick and dense *Vachellia* and *Senegalia* vegetation, *Ziziphus mucronata* and also dominated with large alien *Prosopis* trees. The ground was muddy and well-covered in grass (90%). There were largely open adjacent surroundings and the site was in close proximity to residential buildings and large street lamps.

3) Cimbebasia River channel site (22°37’59.2”S, 17°05’13.7”E)

This sampling site is located on the eastern outskirts of the suburb of Cimbebasia in the southern parts of the city of Windhoek, located near a bridge roosting site on a river bed that undercuts the B1 road. The site is characterised by > 80% grass cover and is dominated by *Ziziphus mucronata* and *Vachellia erioloba* trees lining the banks of the dry river bed covered with rocks with some small patches of sand. Further away from the river channel, the surrounding vegetation was mainly dominated by *Senegalia mellifera* shrubs.

4) Namwater – Swakop River bridge - Okahandja (22°02’01.2”S, 16°56’10.8”E)

This sampling site is adjacent to the Namwater Head Office just outside the town of Okahandja around the Swakop River bridge roost site. The dominant vegetation around the sampling site includes *Senegalia mellifera, Senegalia fleckii, Vachellia erioloba, Dichrostachys cinerea* and *Combretum apiculatum*. Grass grows over and covers (50%) the floor of the rocky Swakop River channel and has characteristic patches of sandy soils.

5) The Leeu River bridge - Okahandja (21°57’40.6”S, 16°50’01.2”E)

The Leeu River lies *ca.* 6 km west of the town of Okahandja on Okahandja-Karibib Road. The river channel is cut across by the Leeu bridge in which numerous bats were observed to roost. Bats were sampled around the bridge roosting site where the surrounding vegetation included the dominant *Vachellia erioloba* with the sparse presence of *Ziziphus mucronata*, *Senegalia mellifera* and some shepherd trees (*Boscia albitrunca*), with a rocky soil surface and low grass cover (20%). The dry river channel floor was open and covered with clear white sand.

6) The Waldau River bridge - Okahandja (21°57'01.2"S, 16°45'28.8"E)

This bridge sampling site lies further from the town of Okahandja on the Okahandja-Karibib road and is cut across by the Waldau bridge which hosts numerous bridge-dwelling bats. The site is characterised by a conspicuous sandy river bed channel with dominant *Vachellia hebeclada* trees. It also has *Vachellia erioloba* trees and *Boscia albitrunca* shrubs with very low grass cover (20%).

7) Opuwo West water point (18°02'45.2"S, 13°49'25.7"E)

This sampling site represents a small river channel with standing water on the western outskirts of the town of Opuwo. Large numbers of sparsely positioned mopane trees (*Colophospermum mopane*) surround the water hole and the bare and open ground is mainly covered with white rocks with no grass cover. This sampling site is characterised by the constant presence of cattle and humans.

***8) Opuwo East riverbed* 1** (18°01'48.1"S, 13°51'35.8"E)

This sampling site has a small and sandy riverine channel and lies *ca.* 5 km to the east of the town of Opuwo, a few hundred meters from a national gravel road that connects Opuwo to nearby areas. It is characterised by open vegetation comprising mainly of small to medium-sized mopane trees (*Colophospermum mopane*). This sampling site is also characterised by *Terminalia prunoides* and *Burkea africana*, with bare ground surfaces covered in gravel stones in some parts. The river bed was bare and covered in sand with signs of human activities associated cattle grazing and sand mining.

9) Opuwo East riverbed 2 (18°01'01.3"S, 13°49'25.7")

This sampling site falls within the same riverbed described under section (9) above and shares the same characteristics. It is however, located further downstream *ca.* 5 km towards the east from the above described site under section (9).

10) Ben Hur (22°45'42.6"S, 19°12'07.5"E)

Ben-Hur is a small village settlement located *ca.* 55 km south-east of the town of Gobabis in the Omaheke region. The site was also characterised by large and old trees of *Vachellia erioloba*, *Combretum sp*, and *Ziziphus mucronata* and a fairly low grass cover (25%) over the extreme sandy surfaces. It was also in close proximity to old residential and office buildings.

3.2.2 Sampling sites in selected habitats within the broad-leaved tree and shrub savanna biome (Figure 5).

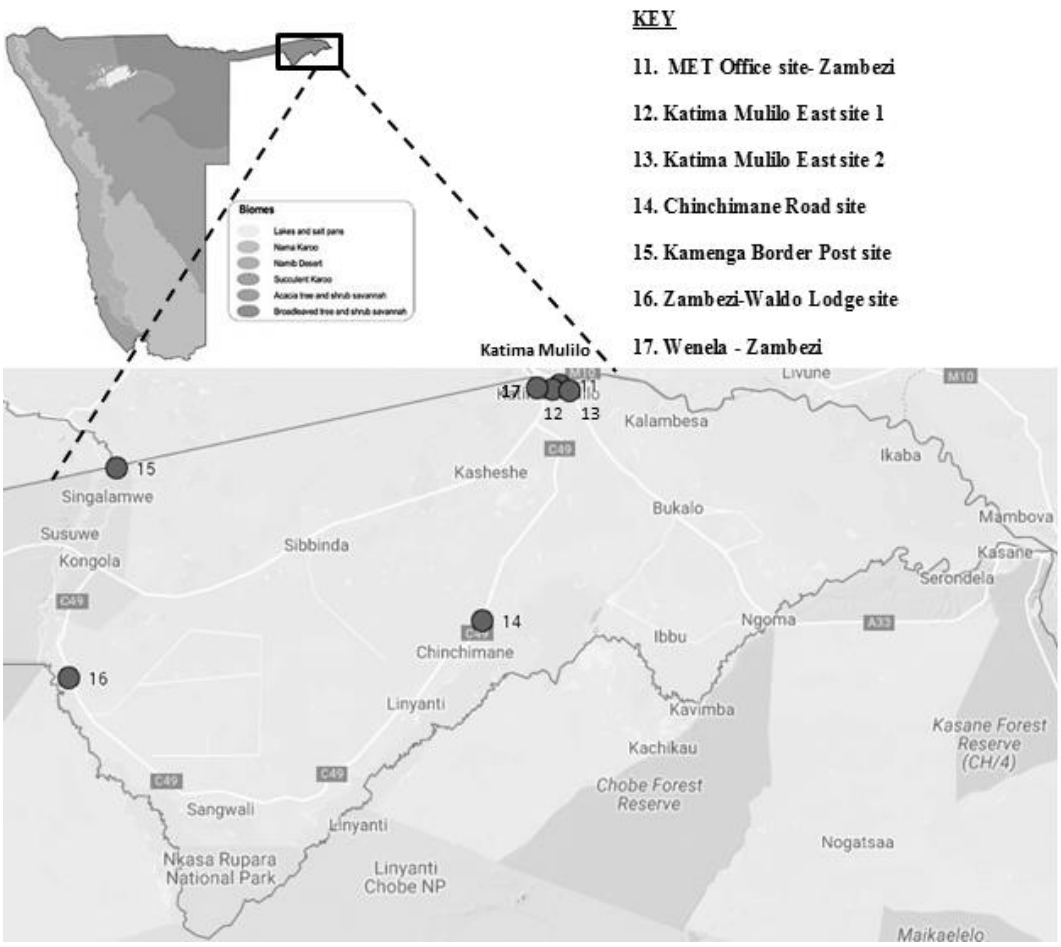

Figure 5 Partial Google earth map of the far north east of Namibia showing numbered GPS locations (numbered dark grey dots) including a key displaying the name of each numbered sampling site in selected habitats (with a partially modified insert map of biomes in Namibia, showing the location of the overall sampling area) in the broad-leaved tree and shrub savanna biome. *Source:* Insert map: Namibian Ministry of Environment and Tourism, (2010).

Bats were sampled at the following sites in the selected habitats:

11) MET Office site- Zambezi (17°29'13.9", 24°18'09.5"E)

This sampling site lies *ca.* 3 km east of Katima Mulilo. It is located on the banks of the Zambezi River, near the Ministry of Environment and Tourism Regional Office. The sampling site was characterised by two large fig trees (*Ficus sp*). The banks of the Zambezi River at this sampling site are dominated by the glossy velvet karree (*Rhus quartinaina*). The sampling site was characterised by the presence of medium and small sized shrubs such as *Peltophorum africanum*, *Grewia flava* in characteristic dense bush. The soil was dark, loamy and fairly covered with grass (50%).

12) Katima Mulilo East site 1(17°29'41.6"S, 24°17'17.8"E)

This sampling site is located *ca.* 2 km east of the centre of Katima Mulilo near a residential area. It also has characteristic African teak (*Baikiaea plurijuga*).

13) Katima Mulilo East site 2 (17°29'53.5"S, 24°19'10.7"E)

This sampling site is located *ca.* 4 km east of Katima Mulilo, near a residential area. Large African teaks (*Baikiaea plurijuga*) were also recorded with over 90% grass cover at the time of sampling in January 2016.

14) Chinchimane Road site (17°55'07.8"S, 24°08'58.3"E)

This sampling site lies along the Chinchimane Road and is located near Masokotoane settlement. It is largely characterised by large and tall African teaks (*Baikiaea plurijuga*). *Burkea africana*

and *Terminalia sericiae* are also present. Shrubby and fruit-producing plants such *Ximenia caffra* and *Grewia flava* are also present. Grass cover was fairly open (30% estimate).

15) Kamenga Border Post site (17°38'18.5"S, 23°20'32.8")

This sampling site is located a few meters from the Kamenga Border Post. It lies *ca.* 20 km north of Kongola town in the Zambezi region on the border between Namibia and Zambia. The sampling site is characterised by red sand covered with thick grass (85%) and a vast range of trees such as *Baikiaea plurijuga* with some isolated *Guibourtia coleosperma* and *Burkea africana*.

16) Zambezi-Waldo Lodge site (18°01'25.6"S, 23°20'32.8"E)

This sampling site is located adjacent to the ground road leading up to the Kwando River Lodge on the banks of the Kwando River flood plains. It is surrounded by tall and large trees dominated mainly by *Combretum colium* (50%) and sparse presence of *Baikiaea phurijuga,* smaller sized *Combretum imberbe* with some isolated *Acacia erioloba* trees and some small unidentified shrubs. The estimated grass cover at the time of sampling in February 2016 was close to 70%.

17) Wenela - Zambezi (17°29'30.3"S, 24°15'27.0"E)

This sampling site is located a few hundred meters east of the Wenela Border Post on the western outskirts of the town of Katima Mulilo. Dominant plant species include *Burkea Africana* (80%) which make up some small dense forest and include some isolated *Acacia sp*. Clear sandy soils with minimal grass cover (20%) and the sampling site is located in close proximity to the Zambezi River.

3.3 Sampled population

Based on previously reported bat sampling procedures (Estrada *et al*., 1993; Aguirre, 2002; Esberard, 2009; Oprea *et al*., 2009; Linden *et al*., 2014), the main focus of the current study was to sample as many bats as possible from the general population of bats in a variety of selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in order determine associated patterns of bat species richness and composition, as well as collect samples for molecular screening of potential bat-borne hantaviruses. Strict conditions imposed on the issuance of the research and collection permit and approval of the research proposal by the Namibian ministry of Environment and tourism: 1) excluded sampling of bats from protected areas; and 2) only permitted the collection of a maximum of ten whole bat specimens of each bat species per sampling site. Consequently, sampling of bats for the current study was only based non-protected areas and only up the first ten individual bats of each species could be consecutively sacrificed and collected at each sampling site were this number could be achieved. The sacrificed specimens were used both as voucher specimens and for extraction of organs for molecular screening of potential bat-borne hantaviruses. Where this could be achieved, blood samples were collected from bats which could not be sacrificed in order to supplement the sample size for hantavirus screening. Taking the two terrestrial biomes into account, a combined total number of 219 bats were sampled from a variety (17) of selected habitats. The data from these sites were used to determine and compare bat species richness and composition in the two biomes. Only a total of 97 organ samples from whole bat specimens and six blood samples from live bats were collected and molecularly screened to determine the potential prevalence of

hantaviruses and for molecular identification of bat species by PCR amplification, sequencing and analysis of the mitochondrial cytochrome b gene.

3.4 Sampling procedures

3.4.1 Field sampling and the identification of bats

This study was conducted as part of a broader international research project (DFG) focusing on molecular screening of bat borne hantaviruses, arenaviruses and coronaviruses. Hence the choice of target hosts for molecular screening of potential hantavirus prevalence was pre-determined. Since the prolonged and repeated bat sampling at the same place has been reported to reduce bat capture rates as bats rely on echolocation, olfactory and visual attributes to develop net avoidance strategies (Esbérard, 2009; Larsen *et al*., 2007; Sedgeley, 2012). Sampling of bats was therefore, undertaken at each sampling site in selected habitats using four Dinier nylon mist nets (Ecotone, Gdynia, POLAND Mesh: 16mm, Shelves: 5, Dimensions: 2.6 M x 6M [2 nets]; 2.6M x 12M [2 nets]) over three consecutive nights. The mist nets were placed at water holes, dry river channels/river beds, near identified bridge roosting sites, along a perennial river and any other sampling sites (as described under sections 3.2.1 and 3.2.2) in selected habitats identified or suspected to be a potential site of bat activity following Meyer *et al*. (2004). At the sampling sites, mist nets were hoisted at heights ranging between 3 to 5 m, in assumed flight paths, corridors and in the under-storey, in order to maximise bat sampling success. The mist nets were consistently opened shortly before sunset, at around 18h00 during each sampling night and were monitored and checked every 10 minutes for an extended duration from sunset until midnight

(Estrada & Coates-Estrada, 2001), at which bat activity would have substantially subsided. All bats sampled in the mist nets were immediately removed and individually processed.

Using appropriate personal protective equipment (PPE), each live animal was removed from the mist net by physical handling and morphologically identified with the aid of a reference field guide for Southern African bats (Monadjem *et al*., 2010). The biometric measurements (forearm length, ear length, tail length, head body and body mass) and a determination of sex and age, as well as any other morphological features necessary for the identification of each sampled animal were taken and recorded on site. For the purpose of collecting organ samples and maximising the possibility of hantavirus detection as well as collection of voucher specimens, a maximum of the first 10 individual bat specimens per species were sacrificed and collected; euthanized using chloroform, where this number could be attained.

3.4.2 Sampling of bat blood and organs

Where feasible, each animal was restrained in one hand with one wing extended outwardly. After rubbing the underwing area of muscle with an ethanol-soaked cotton wool, a small needle (STERIJECT; 0.80 x38 mm) was used to puncture the blood vessel on the rubbed muscle tissue, resulting in the sampling of blood on the surface. A capillary tube (LASEC; 75 mm, 80 µL) or micropipette (Labnet, New Jersey, USA) was used to sample blood (up to 25uL to 200 µL total blood quantity per animal; based on the prescribed ratio of 5µL of blood per gram of animal body weight) from the punctured blood vessel of the bat following the procedures described by Smith *et al*. (2010) and Epstein *et al*. (2013). The sampled blood was placed in Eppendorf tubes and mixed with Phosphate buffered saline (PBS) to a final dilution of 1:10 and stored in a mobile

freezer at -20^{o} C and transported to the laboratory where samples were processed further for hantavirus screening. Due to difficulties of carrying out the blood sampling technique exacerbated mainly by the small body sizes of bats, most attempts to sample blood from bats were either unsuccessful or very small quantities of blood were sampled. As a result, only six sufficient blood samples could be sampled. Thus, 97 bats that were sacrificed and sampled as voucher specimens had to be dissected, in order to extract body organs that included lung, liver, kidney and spleen tissues. The extracted organ tissues were placed in separate 2 mL cryo-vials and appropriately labelled with reference numbers corresponding with that of the voucher specimens and immediately frozen in a -20^{o} C mobile freezer and transported to the laboratory for processing and long-term storage.

3.5 Analysis of bat species richness and composition

3.5.1 Species richness

All species of bats sampled at selected habitats in the broad-leaved tree and shrub savanna and Acacia tree and shrub savanna biomes were counted and recorded based on bat species as identified by a combination of molecular and morphological data. The data was used to determine species richness through the construction of species accumulation curves and the asymptotic estimation of species richness in order to assess the level of sampling success based on sampling effort.

Moreno and Halffter (2000) reported that species accumulation curves are necessary for assessing the effectiveness and completeness of sampling effort within or comparatively across different areas, interpreted based on the patterns of asymptotic levelling of the curves. Consequently, the

mean number of species were then cumulatively generated using 100 randomisations of the Mao Tau algorithm in PAST software (Hammer *et al*., 2001), based on mist-netting sampling data for each biome. Species accumulation curves of the sample (X-axis) plots against the number of species (Y-axis) were then generated using Microsoft Excel (2010) for windows.

In addition, the maximum species richness of bats from the selected habitats in the broad-leaved tree and shrub savanna (n = 7) and the Acacia tree and shrub savanna (n = 10) biomes were separately estimated for several parametric and non-parametric estimators (Chao1, Chao 2, abundance based estimators [ACE], incidence based estimator [ICE], Jackknife1, Jackknife 2, Michaelis-Menten means [MM Means] and the Bootstrap method) using sample data collected from each of the biomes in EstimateS software for windows (Colwell *et al*., 2012). Chao and Chiu (2016) noted that the Jackknife species richness estimators in general are a non-parametric approach designed to lessen the natural bias associated with sample data. The first order Jackknife (Jackknife1) was particularly chosen in the current study by relying on its performance, exhibiting high levels of accuracy and least bias in comparison to Chao1, ACE and the Michaelis-Menten (MM) estimators reported by Brose and Martinez (2004). In addition, Walther and Morand (1998) (cited in Gotelli & Colwell, 2011) found the Jackknife1 together with the incidence-based Chao 2 estimator, to be the best performing richness estimators. The choice of the Jacknife1 was to some extent also influenced by following previous studies on species richness of bats (Monadjem & Reside, 2008; Fahr & Kalko, 2011; Lourenço *et al*., 2014). Sample coverage or completeness was calculated and expressed as a percentage of the observed species richness to that of the estimated number of species.

In order to compare species richness between the two biomes, observed bat species richness (counts) data from the different selected habitats was tested for normality using the Shapiro-Wilk test for normality in the IBM Statistical Package for the Social Sciences (SPSS) statistics for windows, version 25 (IBM Corp., Armonk, New York., USA). The Kruskal-Wallis test for independent samples was used to compare species richness of different selected habitats between the broad-leaved tree and shrub savanna (7 selected habitats) and the Acacia tree and shrub savanna biomes (10 selected habitats) using IBM SPSS statistics for windows, version 25 (IBM Corp., Armonk, New York., USA). The Kruskal-Wallis test is a non-parametric test that represents a suitable statistical test for assessing differences between two or more independent groups where sample data are not normally distributed (Chan, 1997; Van Hecke, 2010).

3.5.2 Species composition

The Bray-Curtis similarity measure was used to compute and compare bat species similarity pairwise distances between pairs of the selected habitats of the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes using Primer Software (Clarke & Gorley, 2006). A triangular matrix containing similarity distances between differently paired permutations of selected habitats for presence/absence data was generated. Using Primer software (Clarke & Gorley, 2006), Hierarchical cluster analysis (HCA) was used in order to generate different clusters of selected habitats based on the Bray-Curtis similarity distance using the group average linkage method (Clarke & Gorley, 2006), which was based on the above-generated triangular matrix. This was necessary in order to assess patterns of species composition in selected habitats of the broad-leaved tree and shrub savanna and the Acacia tree and shrub

savanna biomes. An HCA dendrogram was therefore generated to separate groupings (clusters) of selected habitats based on shared levels of compositional similarities in species of bats.

In order to assess for potential significant differences in species composition between the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes, one-way analysis of similarity (ANOSIM; Clarke, 1993) was undertaken, based on the Bray-Curtis similarity measure. This was done using sample-based abundances of species compared across the two groups of selected habitats representing biomes at the default 1000 permutations using PRIMER software (Clarke & Gorley, 2006).

3.6 RNA extraction, cDNA synthesis and reverse transcriptase PCR (RT-PCR) and Agarose gel electrophoresis for screening of bat-borne hantaviruses

Total RNA was extracted from blood and tissue samples collected as stipulated above, using the QIAGEN RNAESY tissue and blood RNA purification kits (QIAGEN, Hilden, Germany) and ZYMO tissue RNA purification kit (ZYMO RESEARCH Corp, California, USA) following the procedures outlined for use by the manufacturers. The concentrations of extracted RNA were measured using the series 2000 Nanodrop (ThermoFisher Scientific, Massachusetts, USA). Consequently, only RNA samples yielding concentrations measuring at least 200 ng/µl or more at 260/280 ratios of between 1.90 to 2.0 were considered for complimentary DNA (cDNA) synthesis. Repeated RNA extractions for a few samples yielding RNA concentrations below the above stipulated minimum threshold were undertaken to the point of meeting the 200 ng/µl set minimum RNA concentration threshold. From the extracted RNA above, cDNA was synthesized

using the Protoscript II cDNA synthesis kit (New England Biolabs inc., Hitchin town, UK). Tzanetakis *et al*. (2005) reported that the use of the enzyme reverse transcriptase has been exploited positively in synthesizing cDNA templates of RNA molecules through an *in vitro* process of reverse transcription. Consequently, cDNA synthesis was achieved by firstly mixing 1 µg RNA, 2 µl of random primers and topped up with nuclease-free water to a total volume of 8 µL in a 0.2 mL reaction tube. Firstly, this RNA mixture was denatured at 70^{o} C for 5 minutes, centrifuged for 15 seconds and immediately cooled on ice. Secondly, 2 µl M-MuLV enzyme mix and 10 µl of M-MuLV reaction mix were added, making up a total of 20 µl reaction volume. The 20 µl cDNA reaction mixture was then incubated in a thermal cycler (BIORAD, California, USA) at 25^{o} C for 5 minutes, followed by a 1 hour cycle at 42^{o} C, then an enzyme deactivation step at 80^{o} C for 5 minutes and reaction mixture was held at 4^{o} C. This cDNA reaction mix was then topped up with nuclease free water to a final volume of 50 µL and either immediately used for Pan Hanta RT-PCR or stored at -80^{o} C for future use. Subsequently, a two-step Pan Hanta RT-PCR assay was undertaken using degenerate oligonucleotide primers (Table 1) in order to amplify the targeted conserved region of hantavirus genome following the procedures described by Klempa et al. (2006).

Table 1 Names and sequences of degenerate oligonucleotide primers used in a two-step polymerase chain reaction (PCR) assay to screen for the hantavirus (Klempa et al., 2006) in bats from Namibia.

Name of Oligonucleotide primer	**Oligonucleotide primer sequence**
HAN-L-F1	ATGTAYGTBAGTGCWGATGC
HAN-L-R1	AACCADTCWGTYCCRTCATC
HAN-L-F2	TGCWGATGCHACIAARTGGTC
HAN-L-R2	GCRTCRTCWGARTGRTGDGCAA

During the primary step, a 25 µl PCR reaction mixture containing 12.5 µl 2x Mastermix, 0.5 µL of 10 µM of each primer (HAN-L-F1 and HAN-L-R1), 2.5 µL of Template cDNA and 1 µL of PCR enhancer then topped up to a total of 25 µL reaction volume with nuclease-free water was undertaken. The primary PCR was undertaken as based on an initial denaturation step at 95^{o} C for 2 minutes. This was followed by 40 cycles of denaturation at 95^{o} C for 30 seconds, annealing at 53^{o} C for 45 seconds and extension at 72^{o} C for 30 seconds. A final extension step at 72^{o} C for 6 minutes and the reaction was held at 4^{o} C for infinity in a thermal cycler (BIORAD, California, USA).

During the secondary step, a 50 µL nested PCR reaction mixture was undertaken containing 25 µl 2x Mastermix, 1 µL of 10 µM of each primer (HAN-L-F2 and HAN-L-R), 2, 5 µL DNA of the primary PCR product and 1 µL PCR enhancer then topped up to 50 µL reaction volume with

nuclease-free water. In addition to the study samples, a RT-PCR reaction containing a diluted form (1000x) of a plasmid derived hantavirus cDNA was prepared and amplified across the primary and secondary RT-PCR hantavirus screening assay together with the study samples. This was necessary as an internal positive control mechanism, in order to ensure optimization of the RT-PCR cycling conditions and reliability of the results (see section 4.3, hantavirus results).

The secondary RT-PCR mixtures were run in a BIORAD thermal cycler (BIORAD, California, USA) using the following cycling conditions as described by Klempa *et al*. (2006) with some minor modifications. Denaturation was first undertaken at 95^{o} C for 15 minutes and followed by 25 cycles of denaturation at 95^{o} C for 30 seconds. An annealing step was undertaken at 53^{o} C for 45 seconds followed by an extension step for 1 minute at 72^{o} C and a final extension at 72^{o} C for 5 minutes. The secondary PCR reaction was then undertaken at 4^{o} C for infinity. Amplified products were examined by electrophoresis, using a 2% Agarose gel stained with GR green (AMRESCO Inc., Ohio, USA) nucleic acid stain.

3.7 Molecular identification of bat species

Fresh DNA from tissues of bat species was extracted using the ZYMO tissue DNA purification kit (ZYMO RESEARCH Corp, California, USA) following the manufacturer's instructions. The DNA of each individual bat was amplified by PCR using Oligonucleotide primers (Table 2) targeting the cytochrome b gene of bat mitochondrial DNA. A 50 µL reaction mix containing 25 µL of 2x Mastermix, 1 µL of 10 µM of each primer, 5µL of template DNA and topped up to 50 µL reaction volume with nuclease-free water and placed in a thermal cycler and the reactions were performed under the following conditions as described by Bastos *et al*. (2011) with

modifications. An initial denaturation step at 95^{o} C for 2 minutes, followed 35 cycles of 95^{o} C for 60 seconds, annealing at 50^{o} C for 45 seconds, extension at 72^{o} C for 1 minute and a final extension step at 72^{o} C for 5 minutes. The resultant amplicons were examined by electrophoresis in a 1% agarose gel stained GR green nucleic acid. PCR amplified products, and the sequencing was undertaken by INQABA BIOTEC (PTY) LTD in South Africa.

Table 2 Oligonucleotide primers used for amplification of the cytochrome b gene, for molecular identification of bat species from Namibia (Bastos *et al*., 2011; O'Brien *et al*., 2017).

Name of oligonucleotide primer	**Oligonucleotide sequence**
L15171	CATGAGGACAAATATCATTCTGAGG
UMMZ04	TCTTCATTTYWGGTTTACAAGAC
L14724	CGAAGCTTGATATGAAAAACCATCGTTG
H15915	GGAATTCATCTCTCCGGTTTACAAGAC

3.8 Bat DNA sequence editing and searching using basic local alignment search tool (BLAST)

Cytochrome b sequences were manually edited through base calling using Chromaslite 201 for Windows. Pairwise alignments of sequences were undertaken using the BioEdit sequence alignment editor (for Windows) after which consensus sequences were generated where this was required. In order to identify bat species, generated sequence searches were performed in NCBI

basic local alignment search tool (BLAST) in order to search for the best matching DNA sequences on the NCBI sequence database for purposes of identifying bat species. In addition, generated consensus sequences of all bat species were subjected to multiple sequence alignment using Multiple Alignment using Fast Fourier Transform (MAFFT).

For bat species whose DNA sequences could not be used to identify species mainly due to poor quality, morphological identification of species was done; whereby biometric measurements and other morphological features recorded in the field for each bat were considered and used as a basis for the identification of such bat species.

CHAPTER 4

RESULTS

4.1 Bat species richness in selected habitats in the broad-leaved tree and shrub savanna and in the Acacia tree and shrub savanna biomes

After 41 nights of sampling bats in selected habitats using mist nets, a combined total of 219 bat samples belonging to 11 species were recorded. Of these, 42 (~20%) were sampled in selected habitats in the broad-leaved tree and shrub savanna (BLTSS) biome and comprised 7 species belonging to 4 families (Table 3). The remaining 177 (~80%) bats were sampled in selected habitats in the Acacia tree and shrub savanna (ATSS) biome, representing 8 species belonging to four families (Table 3). *Sauromys petrophilus* had the largest proportion (61%) of the total number of individual bats for the combined data (Table 3).

Table 3 The total number of bats captured (abundance) for each species (and family) at different selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

Family and species name	**Common English name**	**Number of bats**		**Proportion (%) of each species to the total number of bats**
		BLTSS biome	**ATSS biome**	
Pteropodidae				
Epomophorus crypturus	Peter's epauletted bat	15	0	6.85
Rhinolophidae				
Rhinolophus d. damarensis	Damara horseshoe bat	0	7	3.20
Rhinolophus fumigatus	Rupell's horseshoe bat	0	8	3.65
Molossidae				
Sauromys petrophilus	Roberts's flat-headed bat	0	133	60.70
Mops midas	Midas free- tailed bat	1	4	2.30
Chaerephon pumilus	Little- free tailed bat	4	0	1.83
Vespertilionidae				
Scotophilus dinganii	Yellow-bellied house bat	8	13	9.59
Scotophilus viridis	Green house bat	6	0	2.74
Scotophilus leucogaster	White-bellied house bat	2	2	1.83
Neoromicia capensis	Cape serotine	0	6	2.74
Neoromicia zuluensis	Zulu serotine	6	4	4.57
Totals		**42**	**177**	**100**
Overall total number of bats		**219**		

The bat species with the highest number of sampled individuals in selected habitats in the broad-leaved tree and shrub savanna biome was *Epomophorus crypturus* with 15 individuals sampled at 4 selected habitats and constituted 6.85% of total captures (Table 3). *Sauromys petrophilus* had the highest number of sampled individuals with 133 (60.70%) individual bats sampled at 5 selected habitats in the Acacia tree and shrub savanna biome (Table 3).

The randomised species accumulation curve for 7 selected habitats in the broad-leaved tree and shrub savanna biome (Figure 6) showed a steady rising of the curve but appears to indicate some asymptotic levelling. The species accumulation curve for 10 selected habitats in the Acacia tree and shrub savanna biome on the other hand (Figure 6), showed a steep rise without any indication of asymptotic levelling. While the observed bat species richness from bat sampling data recorded a total of 7 and 8 species in broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes, respectively (Figure 6), the total estimated (Jackknife1) number of bat species was 9 for selected habitats in the broad-leaved tree and shrub savanna biome and 12 for selected habitats in the Acacia tree and shrub savanna biome. Assuming that the species richness estimator (Jackknife1) is more accurate, given its in-built mechanism that corrects for underestimation, the sample coverage, calculated as a proportion of the observed species richness to the estimated species richness for the sample data in the current study was 80% in the broad-leaved tree and shrub savanna biome and 69% in the Acacia tree and shrub savanna biome.

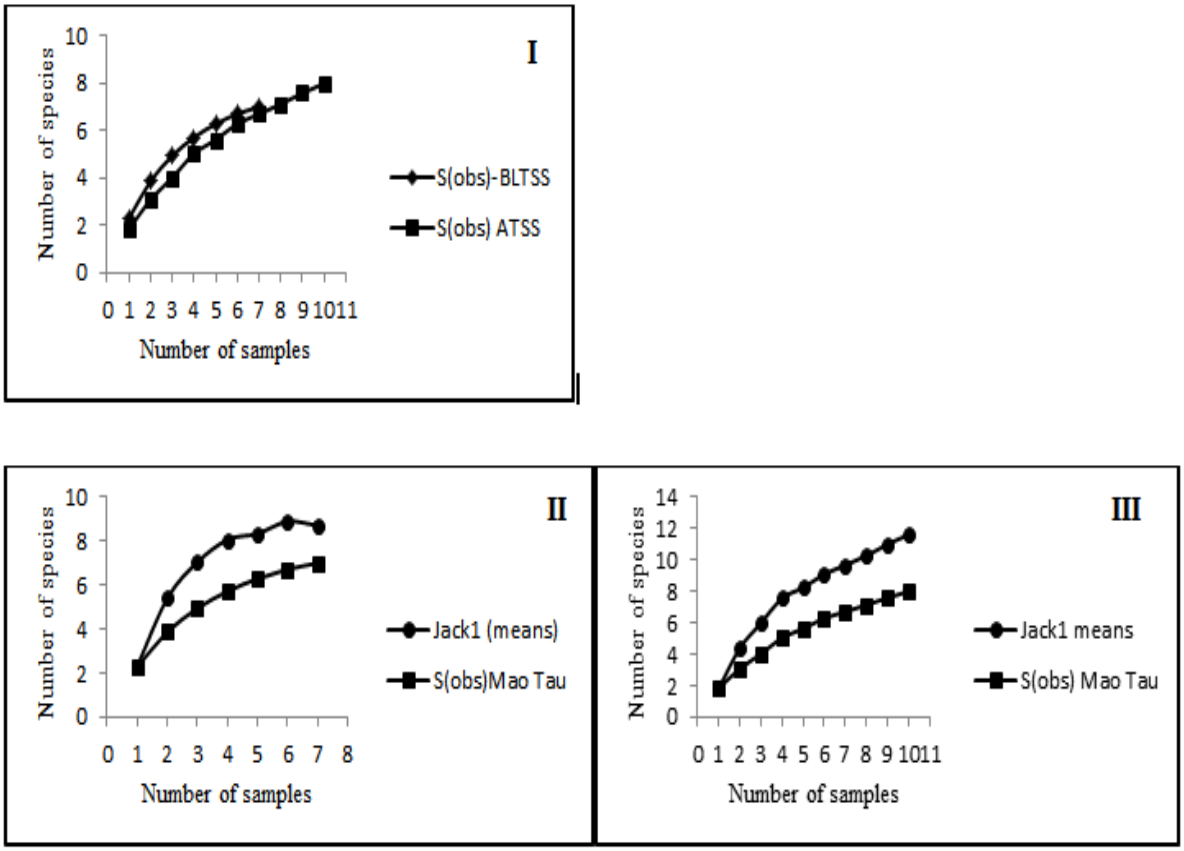

Figure 6 (I) Mao Tau randomized species accumulation curves showing observed species richness [S(obs)] (Hammer *et al*., 2001) ; Acacia tree and shrub savanna biome (in shaded squares) and the broad-leaved tree and shrub savanna biome (in shaded diamonds). Observed bat species [S(obs)] richness (shaded squares) *versus* Jackknife1(Jack1) estimated species richness (shaded circles) for selected habitas sampled in the; (II) broad-leaved tree and shrub savanna biome [total number habitats = 7; each sampled for maximum of 3 nights]; and (III) the Acacia tree and shrub savanna biome [total number habitats = 10; each sampled for maximum of 3 nights] (Colwell *et al*., 2012).

Habitat-specific species richness (total number) of bats in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes is presented in Figure 7. The highest species richness (4 species) of bats was recorded at Wenela-Zambezi and the Opuwo south river canyon falling in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes, respectively. The second highest bat species richness (3 species) was recorded at 2 sites (Kamenga and MET-Zambezi River) within the broad-leaved tree and shrub savanna biome, and one site (Orwetoveni-Otjiwarongo) from the Acacia tree and shrub savanna biome. Two sites (Zambezi-Chinchimane and Zambezi Waldo lodge) and three sites (Ben Hur, Opuwo East riverbed 2 and Opuwo West water point recorded 2 species from the broad-leaved tree and shrub savanna and Acacia tree and shrub savanna biomes, respectively. The rest of the sites from both biomes recorded only one species each.

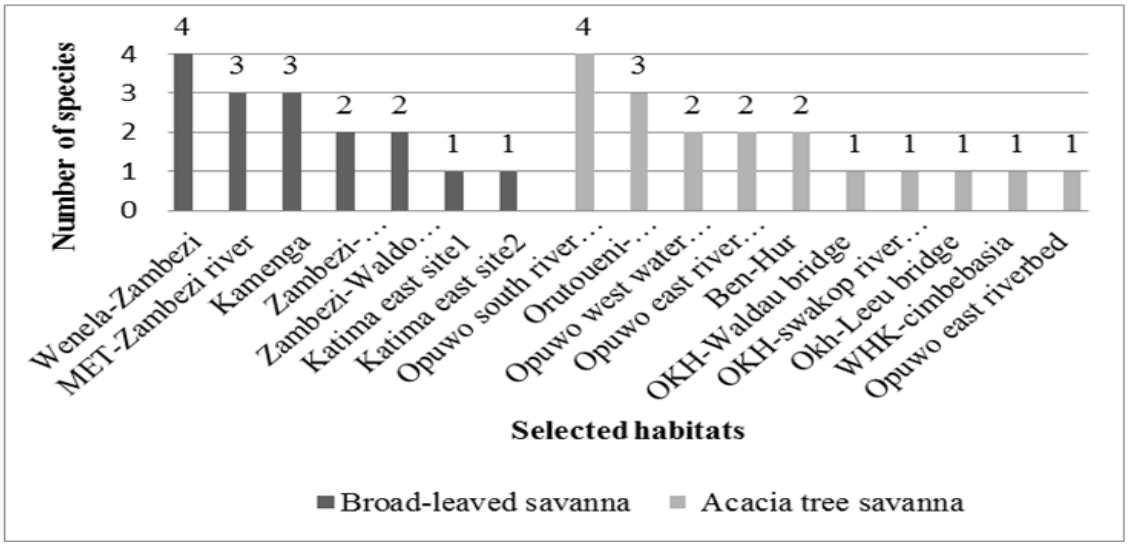

Figure 7 Species richness (total count of species) at different sampling sites in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia.

The Kruskal-Wallis test showed no significant difference ($H_{d.f.}$ = 1; n = 17; P = 0.329) in the median species richness of bats (Figure 8) between selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes.

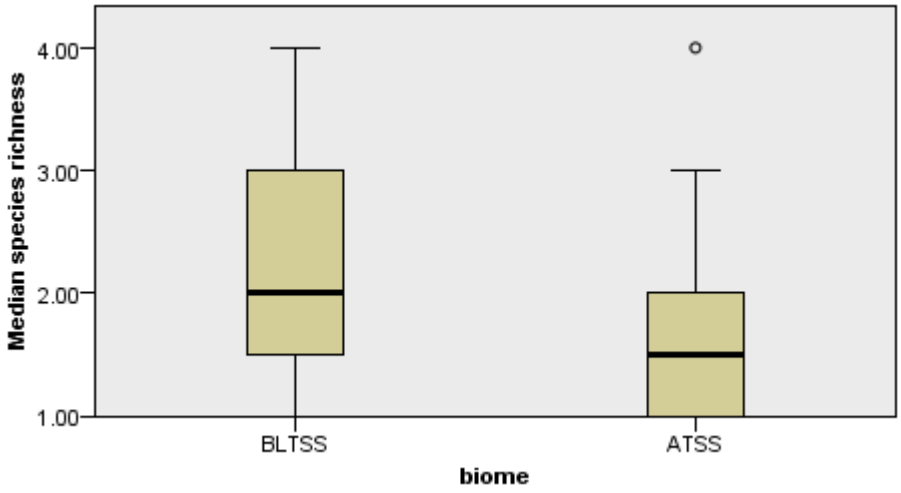

Figure 8 Whisker box plots of the Kruskal-Wallis test showing the distributions of medians of species richness for selected habitats in the broad-leaved tree and shrub savanna (n = 7) and the Acacia tree and shrub savanna (n = 10) biomes in Namibia.

4.2 Bat species composition in selected habitats in the broad-leaved tree and shrub savanna and Acacia tree and shrub savanna biomes

The family Molossidae accounted for the highest proportion of the total number of bat samples with 142 (65%), followed by Vespertilionidae with 47 (21%), Pteropodidae and Rhinolophidae with 15 (7%) each for the combined sample data. The Vespertilionidae accounted for the highest number of species with 5 (45%) species, followed by the Molossidae, Rhinolophidae and Pteropodidae with 3 (27%), 2 (18%) and 1 (9%) species, respectively (Table 3).

There were overlaps of four bat species (*Scotophilus dinganii*, *Scotophilus leucogaster*, *Neoromicia zuluensis* and *Mops midas*) that were sampled in some selected habitats of both the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes. *Scotophilus*

viridis, *Chaerephon pumilus* and *Epomophorus crypturus* however, were only sampled in some selected habitats falling within the broad-leaved tree and shrub savanna biome, while *Neoromicia capensis*, *Sauromys petrophilus*, *Rhinolopus darlingi damarensis* and *Rhinolopus fumigatus* were only sampled at selected habitats within the Acacia tree and shrub savanna biome (Table 3).

The 17 selected habitats representing samples (Figure 9) did not result in exclusive and distinctive groupings of selected habitats according to the BLTSS nor the ATSS biomes except for two groups (Figure 9; HCA Clusters 1 & 2) where only one species was common to each group or cluster. Two selected habitats of the broad-leaved tree and shrub savanna biome where only one species (*Chaerephon pumilus*) was sampled (Figure 9; HCA Cluster 1) and four selected habitats (Figure 9; HCA Cluster 2) in the Acacia tree and shrub savanna biome at which only one species (*Sauromys petroptilus*) was predominantly sampled, were grouped as two separate HCA clusters. The rest of the selected habitats were grouped together (Figure 9; HCA Cluster 3) based on species compositional similarities and did not conform to specific biomes.

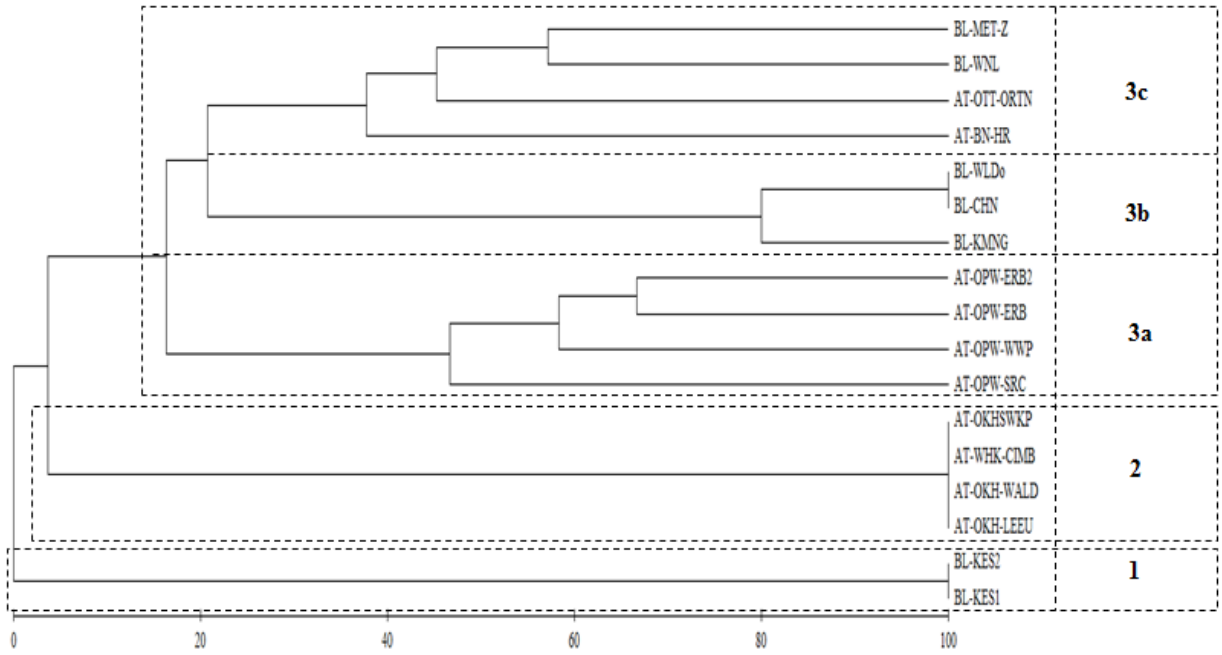

Figure 9 Hierarchical Cluster Analysis (HCA) using group average linkage for presence/absence data showing differences in species composition between selected habitats of the broad-leaved tree and shrub savanna (BL-labelled samples) and those of the Acacia tree and shrub savanna (AT-labelled samples) biomes in Namibia. The horizontal axis displays the Bray-Curtis similarity percentage scale (0-100%) and the vertical axis shows clusters and names of selected habitats representing different samples.

The HCA results (Figure 9) show three distinct broad HCA clusters (1, 2 and 3) based on compositional similarities (< 5%) in species of bats at each selected habitat. Below are descriptions of the relationships between clusters based similarities in species of bats:

Cluster 1: A very small and close association between 2 selected habitats located on the eastern outskirts of Katima Mulilo (Katima East site 1 (BL-KES1) and Katima East site 2 (BL-KES2)). *Chaerephon pumilus* was the only species sampled at both of these sites; hence complete similarity in species composition (100% similarity) was achieved between these selected habitats.

Cluster 2: Contains a close association between 4 selected habitats, all falling within the ATSS biome. These included Swakop River bridge - Okahandja (AT-OKH-SWKP), Waldau bridge - Okahandja (AT-OKH-WALD), Leeu bridge - Okahandja (AT-OKH-LEEU) and Cimbebasia bridge-Windhoek (AT-WHK-CIMB) habitat sites. *Sauromys petrophilus* was common to all the 4 selected habitats under this cluster and was the only species sampled at all trapping sites in these selected habitats, although the species was also sampled at Opuwo South canyon (AT-OPW-SRC).

Cluster 3: A close association between 3 sub-clusters (3a, 3b and 3c) comprising a total of 10 selected habitats. Sub-cluster 3a and 3b show a 10% species compositional similarity and contain a total of 7 selected habitats. Sub-cluster 3a shows *ca*. 15% species compositional similarity to sub-clusters 3b and 3c and comprises Opuwo South river canyon site (AT-OPW-SRC), Opuwo West water point (AT-OPW-WWP), Opuwo East riverbed 1 (AT-OPW-ERB) and Opuwo East riverbed 2 (AT-OPW-ERB2). *Rhinolophus*

darlingi damarensis was common to all these 4 selected habitats. *Rhinolophus fumigatus* and *Neoromicia zuluensis* were common to 2 sites (Opuwo South canyon site (AT-OPW-SRC and Opuwo east riverbed 2 (AT-OPW-ERB2)), while *Scotophilus dinganii* was only found at Opuwo West water point (AT-OPW-WWP). Sub-cluster 3b comprises of Waldo-Zambezi (BL-WLDo), Chinchimane-Zambezi (BL-CHN), Kamenga (BL-KMNG) and MET office site-Zambezi (BL-MET-Z) selected habitats. *Epomophorus crypturus* and *Neoromicia zuluensis* were common to all selected habitats under sub-cluster 3b. Each of these 2 species however, was also sampled at MET office site-Zambezi (BL-MET-Z) and Wenela-Zambezi (BL-WNL), respectively.

Sub-cluster 3c comprises Ben Hur (AT-BN-HR), Orwetoveni-Otjiwarongo (AT-ORTN-OTT), Wenela-Zambezi (BL-WNL) and MET office site-Zambezi (BL-MET-Z) selected habitats. *Scotophilus dinganii* was common to all selected habitats under sub-cluster 3c. In addition, *Scotophilus leucogaster* and *Neoromicia capensis* were also sampled at Orwetoveni-Otjiwarongo (AT-ORTN-OTT), while *Scotophilus viridis* was common to 2 sites, Wenela-Zambezi (BL-WNL) and MET office site-Zambezi (BL-MET-Z). In addition, based on Clarke (1993), and Clarke and Gorley (2006), the species composition of bats in the broad-leaved tree and shrub savanna and Acacia tree and shrub savanna biomes did not differ significantly (ANOSIM; $R = 0.29$; $n = 17$; $P = 0.003$).

4.3 Hantavirus detection

A total of 97 bat lung tissues and 6 bat blood samples (see appendix 2) were screened via the 2-Step Pan-Hanta RT-PCR assay. No hantaviruses however were detected in all the samples. A representative agarose gel image of hantavirus secondary RT-PCR amplification results is presented in Figure 10 where lane 26 of the Agarose gel image shows the 100 base-pairs (bp) DNA molecular weight marker and lane 50 contains the internal hantavirus positive control cDNA (~400 bp). There are no amplicons that are visible on the gel lanes (L to R): 27-49 and thus PCR amplification of hantavirus target cDNA could not be achieved and hence negative results.

Figure 10 A 1% Agarose gel image of hantavirus secondary RT-PCR amplification results.

4.4 BLAST and morphological identification of bat species

Sequences of bat species sampled for the current study were subjected to BLAST searches and the results showcasing the best matching sequences in the GenBank and hence identities of species are presented in Table 4.

Table 4 Search results for identification of bat species based on Genbank matches of bat cytochrome b sequences searched in the NCBI database using BLAST.

Bat field reference	Species	Fraction sequence match	Percentage sequence identity score	GenBank accession of sequence matches
KAM02	*Epomophorus crypturus*	746/757	99%	KX823308.1
KAM03	*Epomophorus crypturus*	754/765	99%	KX823308.1
KAM04	*Epomophorus crypturus*	752/763	99%	KX823308.1
BH0023	*Mops midas*	618/624	99%	EF474031.1
BH0052	*Mops midas*	645/653	99%	EF474031.1
TSE01	*Chaerephon pumilus*	803/806	99%	GQ489140.1
TSE02	*Chaerephon pumilus*	852/864	99%	GQ489140.1
BH0053	*Scotophilus dinganii*	613/630	97%	EU750996.1
WEN01	*Scotophilus dinganii*	733/751	98%	EU750996.1
WEN06	*Scotophilus viridis*	730/750	98%	EU750951.1
MET02	*Scotophilus viridis*	733/750	98	EU750951.1
OPW9	*Rhinolophus d. damarensins*	682/713	96%	KU531289.1
OPW2	*Rhinolophus fumigatus*	660/660	100%	KU531289.1
OPW8	*Rhinolophus fumigatus*	713/714	99%	KU531325.1
OPW7	*Rhinolophus fumigatus*	720/722	99%	KU531325.1
OPW1	*Neoromicia zuluensis*	707/708	99%	KX375187.1
OTT8	*Neoromicia capensis*	705/708	99%	KX375175.1
OTT9	*Neoromicia capensis*	708/709	99%	KX375175.1

Bat species whose DNA sequences were of poor quality and could not be identified using BLAST were morphologically determined and their identities are presented in Table 5.

Table 5 Morphologically identified bat species

Bat field reference number	**Morphologically determined species name**
SWKP 04	*Sauromys petrophilus*
WEN 07	*Scotophilus leucogaster*

CHAPTER 5

DISCUSSION

5.1 Bat species richness in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes

5.1.1 Determination of overall patterns of bat species richness in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia

For the current study, 11 bat species were sampled from selected habitats in the broad-leaved tree and shrub savanna (7 species) and the Acacia tree and shrub savanna (8 species) biomes, with an overlap of 4 species falling in both biomes. The Natural History Museum's bat specimen collection records for Namibia (see Monadjem *et al*., 2010) show that up to 35 species of bats falling into 8 southern African families are recorded to occur in Namibia. The museum records recorded up to 24 bat species from localities within the broad-leaved tree and shrub savanna and 26 species within the Acacia tree and shrub savanna biomes, with some species recorded in both biomes. There are no reported previous studies on bat species richness in Namibian habitats. In southern Africa however, Sirami, Jacobs, & Cumming, (2013) recorded 9 bat species in a range of habitats characterised by shrubby vegetation in the Western Cape Province of South Africa, while Seamark and Brand (2005) also recorded 9 species in habitats around the Cederberg region of the Western Cape Province in South Africa. In addition, acoustic recording surveys conducted by Adams and Kwiecinski (2018) in open Acacia thicket recorded 12 bat species belonging to four families in the north-western parts of South Africa that included a portion of the southern Kalahari desert close to its borders with Namibia and Botswana. Similar to findings in the current study, their study also recorded the following bat species amongst other species:

Rhinolophus fumigatus, Chaerephon pumilus, Sauromys petrophilus, Neoromicia capensis, Scotophilus viridis and *Scotophilus dinganii*.

Based on species accumulation curves, the species sampled in selected habitats of the BLTSS biome appear to have been relatively well-covered in the sample as the curve shows some sign of levelling, albeit an incomplete curve without a clear asymptote in contrast to those of the Acacia tree and shrub savanna biome where the curve shows no sign of levelling (Moreno & Halffter, 2000). This may indicate that some species were not sampled and therefore, not represented in the observed species richness. This was not unusual considering that insectivorous bat species use echolocation to avoid nets, so it is possible that some species may have been missed. Other species such as those considered high fliers, open-air above-canopy foragers such as those of the family Molossidae (Monadjem *et al*., 2017) may have been missed by mist nets whose use was only limited to under-storey sampling and only hoisted to a maximum height of 5 m above ground. Regional predictions of area-specific patterns of bat species richness reported by Cooper-Bohannon *et al.* (2016), for southern African localities indicate that local species richness in sampled areas for the current study in the broad-leaved tree and shrub savanna biome are predicted to contain up to 10 bat species. Thus, the Jackknife1 estimation of 9 species appears reasonable and agrees with the above predicted local species richness for the eastern parts of the Zambezi region. The bats sampled within the Acacia tree and shrub savanna biome, regional predictions of bat species richness outlined for specific areas by Schoeman *et al*. (2013) and Cooper-Bohannon *et al*. (2016) show that the areas covering the selected habitats from which bats were sampled may contain between 10 to 15 species of bats. Similarly, the first order of Jackknife (Jackknife1) estimated a total number of 12 species of bats for the current study. Although it is

possible that the estimated species richness for selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savannah biomes may be understated considering that species richness estimators are generally designed to estimate the lower bound of the maximum possible number of species (Chiu, Wang, Walther, & Chao, 2014), it is still reasonable to argue that the predicted total number of bat species is acceptable and thus falls within the regionally-predicted species richness covering the study areas in both biomes.

When reconciling the specimen record counts of species numbers broadly counted in the 2 biomes to the observed and predicted for species richness of bats in the current study, however, the species richness count recorded and predicted for the current study only accounts for far less than the recorded number of bat species in each biome. This was to be expected, especially when considering that the sample effort did not extend over the entire expanse of each biome but rather, only specifically selected habitats were sampled from each biome. For instance, the 7 selected habitats sampled in the broad-leaved tree and shrub savanna biome all fell to the extreme north-eastern parts of the Zambezi strip, to the east of the Kwando River, consisting of a small part of the entire biome. No other samples in any other parts of the broad-leaved tree and shrub savanna biome were sampled due time limitations and inaccessibility of some areas. The observed and estimated number of bat species in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes however, only represent assemblage sample data reflecting combined local patterns of species richness of bats associated only with selected habitats and may not be attributed to the entire expanse of the biomes.

5.1.2 *Habitat based patterns of bat species richness in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia*

The results of the current study revealed observed habitat specific patterns of bat species richness with variations in the numbers of bat species observed at locally selected habitats. The number of bat species sampled at each selected habitat ranged from one species to a maximum of four species. Selected habitats at which three to four bat species were sampled were those noted to be characterised by riverine vegetation, either located around a perennial river such as at Wenela-Zambezi and MET office-Zambezi sampling sites or at some non-perennial water drainage channels such as at Opuwo south river canyon, and Orwetoveni-Otjiwarongo. It is important to consider the reported potential habitat characteristics that may bear some influence on the roosting and foraging requirements of the co-existing bat species sampled at each of the aforesaid selected habitats. Habitat variations in relation to availability of roosting sites, feeding habits, food availability, ecological interactions, and the potential effect of predation have been reported to influence the presence, absence or co-existence of bat species within habitats (Fenton *et al*., 1998; Monadjem *et al.*, 2017; Schoeman & Monadjem, 2018).

The above observed pattern of habitat specific bat species richness at Zambezi riparian and non-perennial riverine habitats is supported by reported patterns of bat species richness elsewhere (Monadjem & Reside, 2008). Conventionally, riverine vegetation is generally associated with high species richness, diversity and abundance of bats in comparison to the often adjacent inland vegetation (Rautenbach, Fenton, & Whiting, 1996; Monadjem & Reside, 2008; Ober & Hayes, 2008; Lourenço *et al*., 2014; de la Pena-Cuellar *et al*., 2015; Zarazua-Cabajal *et al*., 2018). These

habitats, especially around perennial water streams, are considered important foraging platforms for insectivorous bats, given their associated high abundance of food, availability of water, and an expansive above water foraging space by insectivorous bats for exploitation of insect prey (Hagen & Sabo, 2011).

In addition, non-perennial drainage river streams consist of parallel strips of usually dense, closed canopy vegetation with tall and large diameter trees that align these streams in contrast to surrounding vegetation, providing suitable habitats for a diversity of organisms including bats (de la Pena-Cuellar *et al*., 2015). The riverine water drainage streams and their aligning vegetation may also form important navigational corridors that connect different habitats, facilitate foraging activities and provide suitable roosts for bats (de la Pena-Cuellar *et al*., 2015). These may be related to the high number of bat species (4 and 3 species respectively) sampled in selected habitats located at the Zambezi River in the broad-leaved tree and shrub savanna biome. The bat species *Scotophilus dinganii*, *Scotophilus leucogaster*, *Scotophilus viridis* and *Neoromicia zuluensis* and *Epomophorus crypturus* were sampled around the Zambezian riverine habitats. With the exception of *Epomophorus crypturus*, these are species of the family Vespertilionidae, which are categorised in the clutter edge functional group (Monadjem *et al*., 2017). They are reported to forage on the edge and corridors of forest vegetation (Denzinger & Schnitzler, 2013). The transition between riparian vegetation and the open above water space of the Zambezi river stream can be considered to provide a suitable clutter edge environment consistent with the foraging requirements of the aforementioned species.

In contrast, bat species richness patterns observed at sampling sites in selected habitats located largely in woody non-riparian savanna vegetation within the broad-leaved tree and shrub savanna biome revealed the following: 1) A single species was sampled at both Katima east site 1 and Katima east site 2 sampling sites. This may be attributed the fact that the two sampling sites are located within a residential area near an urban environment were human activity may have displaced other species such as *Epomophorus crypturus* and *Neoromicia zuluensis* which were sampled at other sites within the same biome and are generally associated with riparian and natural woodland savanna vegetation **(**Shackleton, 2005; Salata, 2012; Bonaccorso *et al*., 2014**)**. 2). Two bat species were sampled at the other non-riparian habitats except for the Kamenga border post site at which three bat species similar to one of the riverine sampling sites were sampled in the woodland savanna vegetation within the broad-leaved tree and shrub savanna biome. this may be attributed to the importance of woodland biomes, which are recognised as important habitats that provide roosting space for tree roosting bats (Fenton *et al*., 1998). For instance, in addition to the presence of abundant rock crevices and caves, the high level of bat species richness in Zimbabwe is partly associated with the availability of a high density of large diameter tree stems of the Miombo woodland, which provide large numbers of cavities and tree hollows which are exploited by tree roosting bats (Fenton et al., 1998). *Epomophorus crypturus* and *Neoromicia zuluensis* are associated with woodland biomes and they were sampled in the non-riparian Zambezian habitats in the broad-leaved tree and shrub savanna biome, which is consistent with their roosting and habitat selection requirements such as predicted in part by Salata (2012).

In the Acacia tree and shrub savanna biome, three and four bat species were sampled around non-perennial riverine vegetation from selected habitats at Orwetoveni-Otjiwarongo and Opuwo south river canyon site respectively. 1) The Orwetoveni sampling site falls within a semi-urban environment, a habitat that comprises a non-perennial riverine drainage stream aligned with typical dense *Senegalia* and *Vachellia* type vegetation, including large barky *Vachelia erioloba* and the alien *Prosopis* tree species. *Neoromicia capensis*, *Scotophilus dinganii* and *Scotophilus leucogaster* are the bat species that were sampled at the site and they have been reported in semi-urban habitats in which they adapted to roosting in the building roofs and remnant patches of urban riverine vegetation (Jacobs & Barclay, 2009; Schoeman & Waddington, 2011). The semi-urban habitat where street lights were in close proximity, together with the presence of characteristic thick vegetation around the stream, compared to more open surrounding areas may be associated with the occurrence of the three species in the habitat. The transition between vegetation aligned stream corridor and the more open adjacent areas may provide a clutter edge environment associated with foraging habits of these vespertilionid bats. Street lights have also been reported to constitute foraging spaces of some bat species in some urban environments due to associated high abundance of insects that are attracted to the lights (Avila-flores & Fenton, 2005). The extent to which the illumination provided by street lamps observed within close proximity to the sampling site in this habitat may have been useful as a source of insect prey, which may have served to attract the observed clutter edge foraging species of bats can however only be speculated.

2) The Opuwo south river canyon site consisted mainly of a deep gorged dry river channel characterised by trenches, gullies and long cliffs aligning on each side of the stream. The

vegetation along both sides of the stream consisted of more denser, larger diameter and taller mopane, *Vachellia* and some *Terminalia* trees in comparison to a largely woody mopane shrub-land in the surrounding vicinity. It is suggested that the uncluttered dry riverine stream and its aligning riverine vegetation, coupled with observed presence of trenches, rocks, crevices and large cracks on the walls of the aligning cliffs may have served to provide ample roosting spaces and a suitably unhindered foraging platform that could support the occupancy of bat species in this selected habitat. This to some extent may have facilitated the high number (four species) of sampled co-existing species in this habitat in comparison to; 3) the other 3 selected habitats (Opuwo east river bed 1, Opuwo east river bed 2 and Opuwo west water point) sampled within the same area which consisted much smaller sized streams, with largely open mopane shrubs aligning the streams where local richness of bat species may possibly have been influenced by frequent human related land use activities such as observed at some selected habitats in the Opuwo district. These included sand mining from the sampled river bed, livestock grazing, potential vehicle noise from one nearby national road and constant presence of livestock at one water point which constituted a sampling site. 4) The rest of the selected habitats within the Acacia tree and shrub savanna biome constituted sampling sites located in close to bridge roosts at which only the species *Sauromys petrophilus* was sampled as well a semi- urban residential area based at Ben Hur settlement.

5.1.3 *Comparison of patterns of bat species richness in selected habitats across the broad-leaved tree and Acacia tree and shrub savanna biomes in Namibia*

The results of the current study also showed that there was no significant difference in bat species richness between selected habitats of the broad-leaved tree and shrub savanna and those of the

Acacia tree and shrub savannah biomes, supporting the null hypothesis which predicted no significant difference in bat species richness across the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes. This suggests that the two biomes may largely support indistinctive numbers of bat species at a broader spatial scale. In any case, it may hold true that the two biomes investigated in the current study do not support statistically distinctive numbers of bat species. Given the overall patterns of bat species richness based on natural history records and assuming that such represents a true reflection of the status quo in the biomes in Namibia, it could be a further indication that the 2 biomes may support similar numbers of bat species.

In addition, when juxtaposing the above postulated climate theory to the surveyed biomes, different levels of annual rainfall are noted and vegetation structure constitute the broad-leaved woodland and thorn bush open Acacia savanna in the separate biomes, within which contrasting levels of plant diversity is observed. Ideally, over a broader spatial scale, patterns of bat species richness ought to show some correlated variation across the 2 biomes based on the potential overarching influence of the aforementioned climate, energy and habitat productivity dynamics. The balance of evidence in the current study does not however, corroborate this observed pattern in that no significant difference in species richness could be demonstrated in the surveyed biomes, perhaps owing to the fact that sampling was done at much smaller spatial scales and did not encompass entire biomes. This however, could also be indicative of the low degree of distinctiveness in terms of biome-scale associated patterns of bat species richness. Nevertheless, the overall broader spatial similarity in patterns of species richness observed in selected habitats across the biomes may suggest that the above postulated biome spatial elements of climate and habitat variability may not majorly be influencing the patterns of species richness of bats at local

spatial scales, such as in locally selected habitats. According to Chambers *et al*. (2016), species relate to habitats at different magnitudes of spatial or temporal scales. Species may thus select specific habitats in response to a multiplicity of environmental variables perhaps via some ranked mechanism of characterising scale depended habitat attributes in a certain order of importance (Ober & Hayes, 2008). Bats may respond to dictates of climate elements of rainfall, energy and habitat complexity as broad scale filters that can influence their biogeographic extent of dispersal (Schoeman & Monadjem, 2018). However, alpha diversities or local richness patterns of bats may probably be shaped by local habitat based factors pertaining to vegetation characteristics such as riparian, forest or woodland (Fenton *et al*., 1998; Grindal, Morissette, & Brigham, 1999; Lima *et al*., 2016). Furthermore, associated landscape features relating to close proximity to water, distance to foraging habitats and roost availability have also been highlighted to shape patterns of richness in local habitats including the potential influence of competition and the risk of predation (Swystun, Lane, & Brigham, 2007; Rainho & Palmeirim, 2011). Therefore, the competing habitat elements that support roosting and foraging requirements of bats may feature more prominently for bats at local spatial scales, thus exerting more influence in the selection and exploitation of habitats (Aguirre *et al*., 2003).

5.2 Bat species composition in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes

5.2.1 Determination of overall patterns of bat species composition in selected habitats within the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia

The current study provides an account on investigated patterns of species composition of selected local bat assemblages in the broad-leaved tree and shrub savanna and the Acacia tree and shrub

savanna biomes in Namibia. The essential question was; do bat assemblages of selected habitats differ according to the biomes in which they were sampled or, were bat assemblages sampled in selected habitats in the two biomes similar in their composition of species? Hierarchical Cluster Analysis (HCA) did not result in separate clusters of sampling sites in selected habitats based on each biome, which indicates that the composition of bat species in selected habitats between the broad leaved tree shrub savanna and the Acacia tree shrub savanna biomes was largely similar. HCA results which were based on Bray-Curtis similarity measures produced three main clusters of selected habitats based on bat species compositional similarities.

The first group (Cluster1) is composed of only 2 sampling sites in selected habitats in the broad-leaved tree and shrub savanna biome. The bat species *Chaerephon pumilus* was solely common to both sites. The 2 trapping sites in these selected habitats fall within a semi-urban residential area but largely characterized by presence of large trees. Contrary to the observed pattern of composition implicating the species at only 2 habitats in the broad-leaved tree and shrub savanna biome, Mickleburgh *et al*. (2014) and Monadjem *et al*. (2016) reported that *Chaerephon pumilus* is widespread in Africa where it occurs in a wide variety of habitats including in open arid savannas of the south Western Cape and the eastern coastal forest belt biomes in South Africa. This bat species is considered to be more inclined to forested and woodland habitats outside of urban environments (Mickleburgh *et al*., 2014), where it naturally roosts in tree hollows, cavities and crevices within rocky habitats but prefer to roost in buildings within urbanized environments (Bouchard, 1998; Mickleburgh *et al*., 2014). It feeds by foraging in open air above the tree canopy and trap insects using its wings while in flight and its diet is reported to largely constitute insects of the Order *Hemiptera, Lepidoptera* and *Coleoptera* (Bouchard, 1998; Monadjem *et al*.,

2010). Although, the above described habitat characteristics for the species are to some extent consistent with the 2 habitats in which the species was observed in the present study, the specimen collection records (Monadjem *et al*., 2010) however, appear to indicate that its distribution within Namibia may be limited to the extreme north eastern parts of the broad-leaved savanna woodland biome.

The second group (Cluster 2) is composed of four sampling sites in selected habitats where bats were sampled closer to bridge roosts in the Acacia tree and shrub savanna biome. Only the species *Sauromys petrophilus* was sampled at the four selected habitats and hence the close association although in addition, some specimens of the species were also sampled at Opuwo south river canyon site. The species dominantly constituted the largest proportion (n = 133; 61%) of the total number of bats sampled for the combined data.

The high abundance and numerical dominance of *Sauromys petrophilus* in the combined sample data is not surprising and can be attributed to many possible contributing factors. The four sampling sites in selected habitats where the species was sampled were all located near bridge roost sites where large numbers of these bats were observed to be roosting in the narrow gaps and connecting spaces in the highway bridges. As bats flew out of the bridge after dusk and foraged in the adjoining riverbeds, the likelihood of capture by mist nets that were set up around these bridges was high and hence the high numbers of the species recorded, compared to sampling sites in selected habitats that were not located closer to roosts.

This may indicate the importance of bridges as manmade structures to the roosting ecology, management and conservation of these bats. Roosting structures are considered to be an essential

element that influences the survival and reproductive success of bats, although tied to other ecological factors (Kaňuch & Krištín, 2005). Bat species composition in microhabitats and particularly at roost sites may be influenced by factors pertaining to the roosting requirements of specific species of bats concerned. Primarily, roost sites provide shelter and security from predators, protection from harsh and undesirable weather and easy access to feeding areas (Ferrara & Leberge, 2005). In addition, bridge roosts are sites at which bats aggregate to realize life history characteristics such as conservation of energy, metabolism, hibernation, reproductive and maternal activities as well social interactions (Adam & Hayes, 2000; Ferrara & Leberge, 2005). Keeley and Tuttle (1999) also emphasised that concrete bridges produce warm temperatures which are suitable for maternity and lactating colonies of bats that take advantage of the constant warmth in the crevices to stimulate the growth and development of the young. In addition, the narrow vertical and connecting crevices of the bridge provide suitable narrow and dark spaces for day roosting bats to ensure safety from predation. Most notably, the bridge dwelling Brazilian-free tailed bat (*Tadarida brasiliensis)* has been reported to reproduce and fulfil maternal activities by roosting in bridges in the USA (Allen, Richardson, Mccracken, & Kunz, 2010; Keeley & Keeley, 2004). Bat populations numbering up to 3000 bats per single bridge have been reported to occupy different bridges in the USA (Keeley & Tuttle,1999). In addition, 24 different species of bats, including *Tadarida brasiliensis*, *Epesticus fiscus* and *Myotis lucifugus,* have been noted to partially or permanently exploit bridge roosts (Adam & Hayes, 2000; Keeley & Keeley, 2004; Keeley & Tuttle, 1999).

Furthermore, this species was only sampled in selected habitats within the Acacia tree and shrub savanna biome. In southern Africa, *Sauromys petrophilus* is reported to prefer rocky savanna

habitats as important roosting areas (Monadjem et al., 2010). This species was observed to be roosting in groups of up to four individuals in small cracks, crevices and spaces under the rocks in South Africa (Jacobs & Fenton, 2002). It is considered that *Sauromys petrophilus* is well associated with Acacia woody vegetation in areas generally characterised by rocky outcrops and hills (Jacobs et al., 2016). Interestingly, the study by Salata (2012) in South Africa strongly associated the distribution of *Sauromys petrophilus* closely with the bushveld, the arid savanna and Nama Karoo biomes. This may indicate why this species was only captured in selected habitats falling solely within the arid Acacia tree and shrub savanna biome in Namibia.

The total abundance of Molossidae bats inclusive of *Sauromys petrophilus*, comprised the largest proportion (*n* =142; 65%) of all bats sampled for the combined sample data. This family represented the second largest number of species in the combined sample data, consisting 3 species (*Mops midas, Sauromys petrophilus* and *Chaerephon pumilus*) which accounted for 27% of all species recorded for the current study. Only one and five bats of the species *Mops midas* and *Chaerephon pumilus* respectively, were however sampled in the broad-leaved tree and shrub savanna while the rest of the captures (*n*=136) under the family Molossidae were largely recorded in the Acacia tree and shrub savanna biome. This may indicate the importance of savanna biomes particularly the Acacia tree and shrub savanna to Molossid bats. Molossids are agile animals that hunt insect prey in flight and are thus suggested to prefer foraging in open spaces or above the tree canopies (Cotterill & Ferguson, 1993; Monadjem *et al*., 2017). They have adaptive narrow and long wings which enables rapid flight with reduced ability to navigate in dense vegetation (Jung & Kalko, 2010; Monadjem *et al*., 2017). The open savanna habitats are ideal foraging platforms for these insectivorous bats given their characteristic sparse vegetation

for exploitation of abundant insects as food resources (Cotterill & Ferguson, 1993; Aguirre et al., 2003). This is important, especially when it is considered that the seasonal patterns of insect abundance have been reported to peak during the wet seasons (Cumming & Bernard, 1997). Denlinger (1980) described a positive association in the high abundance of insects to high rainfall in a study conducted in the Nairobi National park in Kenya. In the same way, Pinheiro, Diniz, Coelho, and Bandeira (2002) also showed a positive association in the recorded higher concentrations of *Coleoptera*, *Hemiptera* and *Isoptera* orders of insects to the rainy season in Brazil compared to dry seasons. Studies by Bernard (2002) in the Amazon forests of Brazil and Cumming and Bernard (1997) have reported a positive relationship between rainfall, insect abundance and increased reproductive activities of both fruit and insectivorous bats. In fact, Cumming and Bernard (1997) stated that mono-estrous bats in the paleo-tropics time their reproductive activities to coincide with rainy seasons in which insect and fruit resources become abundant.

The above stated conventional aspect of insect abundance in the wet season was not investigated in the present study. However, assuming that it holds true, it is suggested that this may also be attributed to the high observed abundance of Molossid bats, particularly *Sauromys petrophilus* at sampling sites in some selected habitats in the open Acacia tree and shrub savanna biome in Namibia, since sampling was done during the rainy season.

The third group (Cluster 3) is a collection of various (10) sampling sites in selected habitats that accounted for 9 other bat species reported for the current study. Three sub-clusters of sampling sites in selected habitats were formed based on bat species compositional similarity. An interplay

of factors and ecological processes at different spatial or temporal scales may interact to influence the composition of bat species in natural habitats (Bellamy, Scott, & Altringham, 2013; Ober & Hayes, 2008). It is reported that roosting requirements and food availability are considered to be important among other factors that influence habitat selection by bats (Aguirre *et al*., 2003). The observed association of cluster 3 selected habitats under different sub-groups may be related to small variations or similarities in habitat characteristics in relation to the roosting and foraging ecology of shared implicated species of bats.

The first sub-group (3a) is an association of selected habitats in localities around the town of Opuwo, aggregated based on the commonly shared species such as *Rhinolophus darlingi damarensis*, *Rhinolophus fumigatus* and *Neoromicia zuluensis,* while *Scotophilus dinganii* was also incidentally sampled at one selected habitat. The association of these habitats with *Neoromicia zuluensis* and partly *Scotophilus dinganii* was expected. The two species are largely regarded to be widespread in southern Africa and have been noted to be habitat generalists that occur over a wide variety of habitats including in urban environments (Jacobs & Barclay, 2009; Schoeman & Waddington, 2011).

However, the species in the genus *Rhinolophus* were only sampled in some selected habitats particularly in the woody mopane shrub-land vegetation around the town of Opuwo in the Acacia tree and shrub savanna biome. The observed habitat characteristics at selected habitats where Rhinolophoid bats were sampled included riverine large stemmed and dense vegetation, associated landscape features such as rocks, crevices, cracks and presence of water at one of the selected habitats at which these two species, *Rhinolophus fumigatus* and *Rhinolophus darlingi*

damarensis were sampled may support the roosting and feeding habits of these species. These Rhinolophoid species together constituted only 7% (n =15) of total captures and were only sampled in the northwestern woody mopane shrub-land vegetation in the current study. This observation is similar to reported trends of habitat preferences for Rhinolophoid bats in general. In particular, *Rhonolophus fumigatus* has been reported to roost in high humidity caves (Churchill, Draper, & Marais, 1997), mines (Maree & Grant, 1997) and culverts (Monadjem *et al.*, 2010). However, Maree and Grant (1997) largely associated this species with typical rocky savanna habitats in which it uses tree roosts. In contrast, *Rhinolophus darlingi damarensis* is reported to largely be restricted along the western half of the southern African subregion, in habitats largely characterised by aridity and also based on Museum specimen collection records (Monadjem *et al*., 2010; Monadjem *et al*., 2017). Jacobs *et al*. (2013) reported the species to occur in rocky and arid savanna habitats including in the north-western shrub-lands in Namibia, were the species may use tree hollows and rock crevices as potential roost sites. These habitats are consistent with features observed in selected habitats at which the two Rhinolophoid species were sampled for the current study. Monadjem *et al*. (2017) highlighted that Rhinolophidae are generally regarded as forest foraging bats and were reported to associate more with habitats characterized by woody savanna vegetation. They are classified as clutter foragers and thereby hunt insect prey closer to the ground in vegetated environments, where they may catch prey on the wing or by gleaning (Jones & Rayner, 1989; Pavey & Burwell, 2004). Mtsetfwa, Mccleery, and Monadjem (2018) stressed that Rhinolophoid bats being highly manoeuvrable slow flyers are more inclined to foraging in the midst of vegetation clutter and may prefer natural woody savanna vegetation as opposed to open and transformed agricultural plantations. This may be due to the

fact that Rhinolophoid bats feed partly by gleaning insects directly from tree branches and ground or rocky surfaces. Rautenbach *et al*. (1996) also reported the species *Rhinolophus fumigatus* to be more associated with dense and more cluttered riverine vegetation in the Kruger national park, indicating the preference these bats for vegetated habitats. These observations are similar to other studies done and reported elsewhere for species in the genus *Rhinolophus*. In Australia, the species *Rhinolophus megaphyllus* reportedly foraged by flying within vegetation gaps and mainly kept within woodland vegetation and open canopy forest habitats and while avoiding the adjacent and more open areas (Pavey & Burwell, 2004). This was further corroborated by similar studies done in Europe, where Rhinolophoid bat species such as *R. mehelyi* and *R.euryale* exhibited selective association to woodlands, riverine vegetation, forested habitats and forestry plantations, were they foraged on the edge of vegetation but completely avoided open areas (Russo *et al*., 2005; Salsamendi *et al*., 2012). This may suggest the potential association of Rhinolophoid bats to vegetated habitats which may hold significance to their conservation and management.

The composition of species in some of these local habitats may in part also be shaped by the potential influence of interactive behavioural dynamics of niche separation by co-existing species. The role of ecological morphology in influencing habitat selection is necessary in understanding the potential influence of interactive behaviour on the species composition dynamics in local habitats. The results of the current study showed four morphologically variable species of insectivorous bats belonging to 3 different functional groups that were sampled at Opuwo south river canyon. These were *Sauromys petrophilus* (open air forager), *Neoromicia zuluensis (*clutter edge forager), *Rhinolophus fumigatus* and *Rhinolophus darlingi damarensis* (clutter foragers). In these habitats the co-existence of bat species and assemblage composition

may to a certain extent be influenced by morphology or competition driven niche dynamics. Bergeson *et al*. (2013), stated that the connection between animal behaviour, body morphology and ecology is considered to influence community structure and composition of species in habitats. Physical morphology is associated with the ecological performance of species. Therefore, the species' morphological differences may connote distinctions in levels of ecological performance which can give rise to intra species variations in diets and foraging behaviour (Bergeson et al., 2013). In functioning ecosystems, the co-existence of species in a habitat may be predicated on the assumption of niche differentiation, attained through the usage and segregation of resources by the co-existing species (Reside & Lumsden, 2011).

In relation to bats, differences in wing surface area (wing size) in relative to body weight (wing loading capacity) is associated with flight performance in terms of speed and ability to navigate in cluttered environments which influence the choice of foraging habitats by bats (Aldridge & Rautenbach, 1987; Bergeson *et al*., 2013). In addition, morphology is linked to a bat species' associated echolocation call parameters where the frequency and duration of echolocation calls affect foraging success in terms of the ability of bats to detect and differentiate background obstacles from prey items which influences choice of foraging habitats(Aldridge & Rautenbach, 1987; Schnitzler & Kalko, 2001).

Although these aspects were not investigated in the current study, it is suggested that the observed co-existence (composition) of four species belonging to 3 functional groups at one selected habitat in the Opuwo district may in part have been characterised by the dynamics of resource partitioning mediated ecological interactions. For instance, *Sauromys petrophilus* is a

Molossid bat that forages in open air, while *Rhinolophus fumigatus* and *Rhinolophus darlingi damarensis* are clutter foraging bats that hunt insect in vegetated environments and may co-exist with other species (Jones & Rayner, 1989; Monadjem *et al*., 2017). The species *Neoromicia zuluensis* is a clutter edge forager that hunts insect prey on the edge and within gaps of vegetated habitats where it is reported to mainly feed on *Coleoptera* and *Lepidoptera* insect prey (Monadjem *et al*., 2010). *Rhinolophus fumigatus* reportedly feeds largely on *Diptera, Hemiptera* and *Coleoptera* insects and the diet of *Rhinolopus darlingi damarensis* is mainly composed of *Lepidoptera* insects (Monadjem *et al*., 2010).

Although it may be inferred that there are diet overlaps between the above co-existing species, the differences in foraging habits of the observed species of bats such as at Opuwo south river canyon may indicate that these species could exploit different sections of that specific habitat, suggesting some form of niche separation. In fact, variable feeding patterns of bats are reported to influence co-existence of species in habitats, in that feeding behaviour facilitate occupation of distinct trophic levels by the co-existing species of bats, even with some degree of overlap in diets of these co-existing species (Varzinczak *et al*., 2015; Lima *et al*., 2016). This may also indicate the link between functional traits and niche differentiation between these species.

The second sub-cluster (3b) under this main group consisted of 3 selected habitats which were all located in the broad-leaved tree and shrub savanna biome in the Zambezi strip. The sub-grouping of these woodland habitats were based on bat species compositional similarity based on the species *Epomophorus crypturus* and *Neoromicia zuluensis* although in addition, the Kamenga sampling site included one specimen of the bat species *Mops midas* sampled in addition to the

aforementioned species. Of the 42 bats sampled in selected habitats in the broad-leaved tree and shrub savanna biome biome, *Epomophorus crypturus* made up the largest proportion (*n* =15, 36%), sampled at four different selected habitats. The high abundance of *Epomophorus crypturus* in the broad-leaved tree and shrub savanna sample data may not necessarily indicate its numerical dominance in the habitats but may be attributed to the fact that this frugivorous species does not possess echolocation ability. This may have rendered the species more vulnerable to capture by mist nets in comparison with insectivorous bats that uses echolocation to sense, detect and avoid obstacles including mist nets. The distribution of *Epomophorus crypturus* in Namibia seems to mainly be concentrated to the extreme north eastern edges of the country within the broad-leaved tree and shrub savanna biome, at least based on existing specimen collection records (Monadjem *et al*., 2010). While Salata (2012) predicted the distribution of this species to respond favourably to warm mopane woodlands and the savanna biome in general, Bonaccorso *et al*. (2014) associated this species with riparian vegetation, primarily due to the species' close association with fig trees that are noted to grow mainly in riverine habitats. Interestingly, in the current study, 53% (*n= 8)* of the total number of samples (*N* =15*)* taken for this species were at a single sampling site in a selected habitat (MET office-Zambezi) located on the banks of the Zambezi river at which large fig trees were recorded (see description of study areas, section 3.2.1). Furthermore, *Epomophorus crypturus* appears to be associated with riverine savanna habitats and they have been recorded to frequent Sycamore fig trees (*Ficus sycamorus*) which they are reported to be pollinators and seed disperser (Adams & Snode, 2015). The fruits of *Ficus sycamorus* are considered to constitute their preferred source of food as observed in the Kruger

National Park, including displaying mating behaviour when visiting these trees (Bonaccorso *et al*., 2014; Adams & Snode, 2015).

This observation may not be unusual, given that riparian habitats are generally regarded as important areas for conservation due to their associated high diversity and richness of species (Zarazua-Cabajal *et al.*, 2018). High bat species richness at riverine habitats is associated with high abundance of insects, fruits, water availability and dense vegetation (Zarazua-Cabajal *et al*., 2018). Assessments of bat communities around riverine habitats seem to indicate a pattern of association of fruit bats to these habitats (Zarazua-Cabajal *et al*., 2018). For example, reported large proportions of total bats sampled at riverine habitats constituted 53% and 77% fruit bats. (see Monadjem & Reside, 2008 and Zarazua-Cabajal *et al*., 2018).

The third sub-cluster (3c) is a mixed aggregation of two selected habitats in the Acacia tree and shrub savanna and two selected habitats from the broad-leaved tree and shrub savanna biome. The sub-grouping of the habitats was based on the main shared species *Scotophilus dinganii* and *Scotophilus leucogaster*. The other bat species *Scotophilus viridis*, *Neoromicia zuluensis*, *Neoromicia capensis*, *Epomophorus crypturus* and *Mops midas* were also affiliated to one or two of the four habitats under this sub-cluster. The observed composition of bat species in the Zambezi riverine habitats at Wenela-Zambezi and MET office-Zambezi sampling sites, mainly implicated clutter-edge foragers of the family Vespertilionidae. These are insectivorous bats that hunt insects on the edges of vegetation, near water surfaces and in gaps within vegetated habitats (Schnitzler & Kalko, 2001). The bat species *Scotophilus dinganii*, *Scotophilus leugogaster*, *Scotophilus viridis* and *Neoromicia zuluensis* were mainly sampled at the aforesaid riverine

habitats in addition to *Epomophorus crypturus* which is frugivorous (discussed elsewhere). The observed association of *Scotophilus* bats to riverine savanna woodland habitats is supported by other studies (Fenton, 1983; Barclay, 1985). The roosting and foraging habits of bat species in the genus *Scotophilus*, and particularly *Scotophilus dinganii*, *Scotophilus viridis* and *Scotophilus leucogaster* have also been studied in natural woodland savanna habitats where these bats largely occupied smaller and narrow hollows of *Colophospermum mopane* (Fenton, 1983; Barclay, 1985; Fenton, Brigham, Mills, & Rautenbach, 1985) and *Brachystegia* trees (Fenton *et al*., 1998).

Radio tracked *Scotophilus leucogaster* and *Scotophilus viridis* bats were also reported to exploit riparian woodland habitats in Zimbabwe and South Africa (Barclay, 1985; Fenton *et al*., 1985). Upon emergence from tree roosts, *Scotophilus leucogaster* bats foraged individually and quickly flew to the nearby swamps to drink water of the Sagwe river in Zimbabwe, foraging by hunting insects above water and kept transcending between the swamps and adjacent woodland habitats where they fed on *Coleoptera* and *Hemiptera* insect prey (Barclay, 1985). In addition, radio tracked *Scotophilus viridis* bats were reported to undertake feeding bouts to the Livhuvhu River in the Kruger National Park, in which they foraged along the edge of riparian mopane woodland vegetation (Fenton *et al*., 1985). The observed composition of 3 *Scotophilus* species bats at selected habitats along the Zambezi River may be due to above similar observed foraging activities of the same species in Zimbabwe and South Africa, which may have influenced the species composition.

Riparian habitats may be significant as they contain characteristic large diameter stemmed trees which provide cavities that are exploited by bats as roosting spaces (Swystun *et al*., 2007).

Vespertillionid bats such as those of the genus *Scotophilus* may also take advantage of the edge effect of riverine habitats and the water stream to forage and feed on usually high associated abundance of insect prey (Grindal *et al*., 1999; Swystun *et al*., 2007). Furthermore, the expansive and acoustically simple above water space of river streams and non-perennial riverine channels aligned with vegetation may be used by bats as navigational corridors or flight paths to reach isolated patches of foraging habitats and return to roosting sites (Sera-Cobo, López-roig, Marques-Bonnet, & Lahuerta, 2000; Yovel & Ulanvosky, 2017). These open water spaces and riverine channels also serve as areas where bats locate mates or use for long distance migratory flights. A studied migratory species of *Miniopterus* bat when released, tended to fly to the river first and then used the stream as a navigational corridor to locate roost sites even within familiar roosting habitats (Sera-Cobo *et al*., 2000; Yovel & Ulanvosky, 2017).

In addition, some of the Vespertilionid bat species observed at the Zambezi riverine habitats, especially those of the genus *Scotophilus* have also been noted to co-exist in sympatric fashion which may likely have some influence on patterns of observed species composition. In these habitats, *Scotophilus* bats may select and exploit similar roosting and foraging resources. The extent to which these sympatric interactions may facilitate eco-morphology or competition driven partitioning of resources between the co-existing species in the habitats is unclear. For instance, Jacobs and Barclay (2009) and Monadjem, Raabe, Dickerson, Silvy, and Mccleery (2010) conducted niche differentiation studies on the sympatric bat species *Scotophilus dinganii* and *Scotophilus viridis* and could not establish any morphology or echolocation mediated differences in foraging behaviour nor habitat selection between the two species. In terms of roost use, however, Monadjem *et al*. (2010) could also not establish any evidence of niche separation in

relation to roost use between the two species in a natural savanna habitat in which both species shared the same habitats and roosted in hollows within large *Combretum imberbe* trees. Jacobs and Barclay (2009), however, reported a difference in roost use as the morphologically larger *Scotophilus dinganii* roosted in buildings while the smaller *Scotophilus viridis* only roosted in trees but only in respect of an urban environment, indicating the potential influence of anthropogenic structures on the roosting ecology of these bats. In terms of foraging behaviour, Jacobs and Barclay (2009) found no significant difference in both diet composition and size of prey insect captured by the two sympatric species. This observation was also corroborated by Reside and Lumsden (2011) who did not find evidence to suggest eco-morphology and echolocation mediated partitioning of resources, when investigating niche separation aspects of two sympatric *Mormopterus* species of bats in Australia. It was concluded that since the two Australian species used the same habitats and consumed similar prey, the observed variations in body size and wing morphology only resulted in inconsequential differences in flight and foraging behaviour. Despite observed differences in wing aspect ratio, skull and body size in the above studies, none but one urban influence aspect of resource partitioning could be demonstrated, which may suggest that the composition of the morphologically similar vespertilionid bats in the Zambezi riverine habitats may not be dictated nor shaped by any variation in patterns of foraging or roosting behaviour, but perhaps by other factors unrelated to niche dynamics.

The Acacia tree and shrub savanna habitats under sub-cluster (3c) were those located at semi-urban environments on the periphery of the Orwetoveni suburb in Otjiwarongo and a sampling site located in the midst of large *Vachellia erioloba* trees adjacent to multiple buildings at the Ben

Hur settlement in the Omaheke region. The similarity in these habitats in relation to vegetation and close proximity to urban or semi-urban residential areas is evident. The implicated species of bats at both habitats are *Scotophilus dinganii, Scotophilus leucogaster*, *Neoromicia capensis* and *Mops midas*, which are associated with roosting in both tree hollows and buildings (Monadjem *et al.*, 2010). In relation to the Ben Hur settlement habitat, it is most likely that the sampled yellow African bats and the Midas free tailed bats may have been roosting in the buildings. These bats were observed flying at external lights of the residential buildings probably targeting insects.

The riverine closed canopy vegetation and the possible influence of nearby residences and street lighting at the Otjiwarongo habitat site may be attributed to the sampled composition of clutter edge vespertilionid species of bats. In essence, the effects of urban and semi-urban environments on the foraging and roosting activities of bats may be dependent on landscape attributes and the intensity of human activities that may support or deject the occurrence of bat species (Avila-flores & Fenton, 2005). Distance to urbanised environments, assisted by the presence of street light illuminated areas at night have been reported to influence selection of foraging habitats by some species of bats, due to high abundances of insects at street lights (Rainho & Palmeirim, 2011). In addition, urban and semi urban habitats may offer anthropogenic infrastructure in the form of bridges, buildings and patches of remnant vegetation which may serve as roosting and foraging spaces (Avila-flores & Fenton, 2005; Krauel & Lebuhn, 2016). It is well understood that urbanisation is generally associated with species decline (Avila-flores & Fenton, 2005). Opportunistic and habitat generalist species of bats that are adaptable to the effect of urban settlement may however, take advantage of urban features to hunt large volumes of insect prey that usually aggregate at street light and roosting in buildings, thereby thrive and increase their

population abundances in urban environments (Gaisler, Zukal, Rehak, & Homolka, 1998; Avila-flores & Fenton, 2005; Jacobs & Barclay, 2009).

Clutter edge generalist species such as *Scotophilus dinganii*, *Scotophilus leucogaster* and *Neoromicia capensis*, which were partly implicated at the two semi-urban habitats for the current study, have been reported to exploit remnant patches of the urban riparian habitats within the Durban metropolitan city in South Africa (Schoeman & Waddington, 2011). *Scotophilus dinganii* and *Scotophilus viridis* have also been shown to exploit urban environments in which these bat species roosted in buildings and trees respectively (Jacobs & Barclay, 2009), which shows the association of these bats to urban habitats.

Furthermore, the species *Scotophilus dinganii*, *Neoromicia zuluensis* also constituted the most frequently sampled species in selected habitats. These together with *Scotophilus leucogaster* and *Mops midas* formed a group of species that overlapped in selected habitats across both biomes. The overlap of the species across the two biomes may be related to the general observation that species in the genera *Scotophilus* and *Neoromicia,* particularly those recorded in the current study are largely considered widespread habitat generalists that have been reported in a wide variety of habitats including in urban environments in which they are adapted to roosting in buildings (Fenton *et al*., 1998; Shackleton, 2005; Schoeman & Waddington, 2011). The species *Neoromicia zuluensis* and *Neoromicia capensis* association with woody savanna vegetation may be related to their dependence on under bark of trees as roosting spaces (Shackleton, 2005). The selected habitats at which *Scotophilus* and *Neoromicia* bat species were sampled are consistent

with a presence of large woody vegetation, dense riverine vegetation as well as semi-urban environments.

5.2.2 Comparison of patterns of bat species composition in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes in Namibia

Analysis of similarity (ANOSIM) revealed that there was no significant difference in the species composition of bats between selected habitats across the two biomes. This may be attributed to the aforementioned patterns of overlap of four species of bats in selected habitats transcending across the two biomes. In addition, two of the overlapping species constituted the most frequently captured species in the combined sample data. The scale and magnitude of the observed *R* statistic ($R = 0.29$) suggests a high level of bat species compositional similarities in selected habitats between the two biomes. This may also indicate that compositional resemblances of bat species are lesser within than they are between (across) the biomes (groups) owing to the possible influence of the intra biome species overlap, including the habitat generalists species with the highest frequencies (not abundances) observed between the biomes. This observation appears to be corroborated. For instance, Monadjem and Reside (2008) when comparing species composition of bats between riverine and inland habitats obtained a similar value of the *R* test statistic. Since the inland bat community in that study mainly constituted a subgroup of the riverine community (i.e. overlap of mainly same species across both habitats), it was concluded that the observed significant difference was not as a result of differences in the composition of species (given low *R* statistic) between the two habitat types but rather due to differences in the overall contributing strengths of the species across the two habitats.

Nevertheless, the magnitude of the observed compositional similarity in species of bats in the current study indicates some small degree of correlation (influence) of the biomes to the composition of bat species. The relationship is however not strong, which may suggest that biome scale spatial or temporal environmental correlates that may influence the dispersal of bat species appear to minimally affect patterns of species composition in selected habitats. This may further indicate that the two biomes may largely harbour a composite of similar species of bats at a broader spatial scale, although with some small variations at least on the balance of evidence of the current study. The results of the current study support the initial hypothesis of no comparable significant difference in bat species composition between the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes. Consequently, the current study accepts the null hypothesis and rejects the hypothesis in the alternative.

5.2.3 General observations on patterns of bat species composition in selected habitats in the broad-leaved tree and shrub savanna and the Acacia tree shrub savanna biomes in Namibia

Since the was no significant difference in bat species composition across the two biomes, the observed variations in patterns of species composition in the current study which was based on specific clusters and sub-clusters of selected habitats (Figure 9, HCA clusters; 1, 2 and sub-cluster 3a, 3b, 3c) may perhaps be attributed to the similarities and influence of local scale, habitat attributes and landscape features that may influence local composition of species in the selected habitats. The biogeographic dispersal of many bat species may extend over large areas and they may respond to broad spatial scale factors such as the category of “woodland” biome which encompasses its distributional range (Ober & Hayes, 2008). Habitat selection decisions of

bats may however be largely influenced by small scale, spatial or temporal habitat attributes that support their foraging and roosting habits (Wordley, Sankaran, Mudappa, & Altringham, 2015). These may include the presence of caves for roosting, a body of water at which it can drink or catch insect prey or human land use activities like agricultural plantations and forested as well as riparian vegetation (Fenton *et al*., 1998; Ober & Hayes, 2008; Wordley *et al*., 2015; Mtsetfwa *et al*., 2018). Bellamy *et al*. (2013) suggested that bats may often undertake small scale feeding leaps to exploit resources in separated patches of local habitats stretched over short distances away from roosts, showing that bats may exploit habitats in response to local environmental and biotic attributes that are pertinent to their roosting and foraging requirements.

For example, multi-scale habitat suitability modelling studies done by Wordley *et al*. (2015) and Chambers *et al.* (2016) demonstrated the importance of forested habitats, forest fragments, forestry plantations and riparian habitats to be significant local spatial correlates that support the occurrence of bat species. Wordley *et al*. (2015) particularly suggested a possible dependence on forested habitats by Rhinolophoid and Hipposideros bats by demonstrating a positive association between increasing distance away from forest and woody vegetated habitat patches with declining activity and capture rates of these bats at locally finer distance scales.

In addition, a local spatial scale study on activity patterns of bats in agricultural plantations in comparison to natural savanna vegetation in southern Africa reported a strong affiliation of Rhinolophoid bats to natural savanna vegetation in comparison to open agricultural areas, while activities of Molossid bats were strongly associated with open agricultural plantations (Mtsetfwa *et al*., 2018). Ober and Hayes (2008) also demonstrated the importance of local spatial scale

habitat attributes of vegetation relating to canopy structure in riverine habitats at local finer scales which influenced high activity patterns of bats, suggesting that habitat selection and foraging activities by insectivorous bats may be decided based on local spatial and temporal scale habitat attributes.

The roosting and foraging ecology influences a bat's decision to select specific habitats; species composition of bats at localised habitats may not necessarily be influenced by broad-based spatial or temporal scale factors pertaining to the effect of climate and habitat complexity or a category of biome. Instead, local spatial and temporal scale habitat specific attributes relating to availability of roosts, feeding habits, and the potential influence of predation as well as interactive behaviour may be deterministic of species compositional structure of local bat assemblages (Kunz, 1982; Grindal *et al*., 1999; Kühnert *et al*., 2016; Schoeman & Monadjem, 2018).

The balance of evidence adduced in the current study appears to corroborate the above observation in that no significant differences in the patterns of species composition of bats could be established across the two biomes when considered at a broader (biome) scale. Instead, observed variations in patterns of richness and composition of bats can be attributed to specific selected habitats or categories of selected habitats, which may be a result of the deterministic influence of local scale habitat attributes that may support the occurrence and habitat requirements of the sampled species of bats. In particular, habitats generally characterised by riparian vegetation and the non-perennial water drainage riverine streams with their associated dense aligning vegetation and habitat features such as water, proximity to urban and semi-urban areas and highway bridges may be important potential influencers of observed patterns of species composition of bats at selected habitats in the current study.

5.3 Hantavirus detection

The results of the current study revealed that no hantavirus was detected from a combined total of 97 lung tissues and 6 blood samples of bat species sampled from selected habitats in Namibia. These results are similar to previous studies done on rodents and shrews in Namibia and South Africa (Witkowski *et al.*, 2014). Some evidence of hantavirus sero-prevalence in humans has however, been demonstrated in South Africa (Ithete *et al*., 2014) and more recently in Mozambique (Chau *et al*., 2017), and may direct future hantavirus detection efforts in southern Africa. The study samples were screened based on the two-step Pan Hanta RT-PCR assay using degenerate Hantaviridae specific Oligonucleotide primers that were designed to detect a wide variety of both known and novel hantaviruses (Klempa *et al*., 2006). This PCR assay has been used successfully in other studies and have demonstrably detected various novel hantaviruses from a diverse number of host species especially in Africa (Meheretu *et al*., 2012; Weiss *et al.*, 2012; Kang *et al*., 2014; Witkowski *et al*., 2016; Tesikova *et al*., 2017). The success of this method was dependent on the use of a plasmid derived hantavirus positive control cDNA, in order to optimize and validate the PCR assay which can amplify the targeted hantavirus genome in the samples (Iithete, 2013; S.Weiss, personal communication, 2019). As apparent from the results, an internal hantavirus positive control cDNA sample was included in the PCR assay together with the study samples and was successfully amplified, indicating that both the Oligonucleotide primers and the PCR cycling conditions were optimised and hence capable of amplifying target hantavirus genome that could have been present in the samples. The samples that were analysed in the current study were frozen at -20 ^{o}C in a mobile freezer at the point of sampling and the cold chain was maintained throughout, including during sample transportation,

storage (-80 ^{o}C), and processing in the laboratory. In addition, the current study samples were also sent to the hantavirus reference laboratory at the institute of Virology of Charite University in Berlin, Germany for confirmatory testing. The negative hantavirus results of the current study were reproduced for all the samples. Therefore, to the extent that the failure to detect hantavirus may be attributed to a failure in sample integrity or a laboratory detection methodological failure, a negligible possibility of these is countenanced.

The results of the current study therefore support the initial hypothesis that hantavirus was not prevalent in the sample population of bats in selected habitats within the broad-leaved tree and shrub savanna as well as the Acacia tree and shrub savanna biomes in Namibia. Consequently, the study accepts the null hypothesis and rejects the alternative hypothesis in respect of hantavirus prevalence. The hantavirus negative results of the current study may suggest that either there are no hantaviruses in habitats that were sampled in the two biomes within Namibia, or may reflect sampling failure to detect the hantaviruses. Especially when taking into account some possible factors that may have contributed to the inability to detect hantavirus in the current study:

1) It is necessary to stress that the total number of tissue organs and blood samples screened in the current study constituted a small sample size, was more localised and may also have possibly been confined to unaffected specific habitats compared to other studies conducted elsewhere especially in sub-Saharan Africa. The combined sample size (103 samples) that was screened for hantavirus in the current study composed of 11 bat species and the largest proportion (n = 47) consisted of the species *Sauromys petrophilus*. The composition of the

other bat species in the combined sample for hantavirus screening consisted of, *Scotophilus dinganii* (*n* = 17), *Epomophorus crypturus* (*n* = 10), *Chaerephon pumilus* (*n* = 7), *Neoromicia zuluensis* (*n* = 7) and *Mops midas* (*n* = 5). The bat species *Rhinolophus fumigatus, Scotophilus viridis*, *Scotophilus leucogaster*, *Neoromicia capensis* and *Rhinolophus darlingi damarensis* collectively constituted the remainder of the samples (*n* = 10).

It is possible that the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes may collectively contain up to 30 species of bats (Monadjem *et al*., 2010). Thus, the 11 bat species sampled for the current study and the small number of samples screened for each species represents far less than half the total number of bat species and the study sample may constitute a minuscule proportion of the total population of bats in the two biomes, especially when considering the fact that bats are generally highly diverse and constitute some of the largest populations (Calisher *et al*., 2006). In comparison to other studies, it must be noted that only 1 in 612 blood samples belonging to three broad genera of rodents sampled from a variable range of habitats in Guinea, West Africa and molecularly tested to demonstrate the detection of the first African hantavirus showed a positive result (Klempa *et al*., 2006). In addition, only 1 in 525 organ tissue samples belonging to 28 different genera of bat species sampled from variable habitats in West Africa yielded a positive result to reveal the first African bat-borne Magboi hantavirus (Weiss *et al*., 2012). Furthermore, Tesicova *et al*. (2017) also demonstrated only 2 hantavirus-positive results by screening 1866 samples of dried blood spots of rodents, shrews and bats sourced from 5 countries in sub-Saharan Africa. This may indicate that the sample

size for the current study may be insufficient to conclusively confirm and demonstrate the presence of hantaviruses among bats in the selected habitats within the 2 biomes in Namibia. Hence the results of the current study do not rule out the possibility of hantavirus prevalence within the sampled habitats nor in the two biomes or generally in Namibia.

2) The inability to demonstrate the prevalence of bat-borne hantavirus in the sampled habitats could perhaps also be attributed to the likelihood that the sample effort may have missed infected individual bats, particularly those that may belong to implicated carrier species. This may be due to the fact that hantaviruses tend to show host-specificity and are generally inclined to strictly inhabit mainly a single, but at most two specific primary carrier host species (Klingstrom *et al*., 2002; Zeier *et al*., 2005; Sedda & Rogers, 2012; Avsic-Zupanc *et al*., 2019). The peculiarity of hantavirus-host relationships have not yet been fully understood in Africa. Nevertheless, the bat-borne African Mouyassue hantavirus in west Africa and a closely related east African bat-borne hantavirus similarly appear to show a close relationship to the bat species *Neoromicia nanus* and *Neoromicia capensis*, respectively (Sudi *et al*., 2018; Tesicova *et al*., 2017; Weiss *et al*., 2012).
3) It may also be possible that the sampling effort may have been conducted out of potential seasons, thereby side-stepping the important seasonal, environmental and ecological conditions that may facilitate increased population abundances of carrier bats and high frequency of hantavirus occurrence in carrier populations. These seasonal or environmentally induced increases in population densities could have resulted in an increased possibility of hantavirus infected bats in the study sample, thereby facilitating its detection. The exact mechanisms in which individual host infections occur within a given population, the social

and environmental determinants that stimulates the cross transfer of hantavirus across different species or populations of bats have yet to be understood. Host infections by the hantavirus may be influenced by environmental and ecological conditions especially in rodents (Manigold & Vial, 2014). When ambient conditions become favourable so as to facilitate and support population growth of infected carrier rodents, the risk of parallel hantavirus host infection between vulnerable rodents is also heightened and thus result in an increased number of infected carriers (Manigold & Vial, 2014). Many bat species are known to form and live in large non-human social aggregations within small and confined spaces at roost sites (Calisher *et al*., 2008; Ghanem & Voigt, 2012), reaching numbers of up to 300 individual bats per square meter of space, thus creating a crowded condition that is reported to facilitate cross transfer of other viruses across bats (Calisher *et al*., 2008). It may be possible that hantavirus may be cross transferred between individual bats or across species in a similar manner, aided largely by environmental and ecologically induced population dynamics. Hence seasonal, environmental and ecological dynamics that facilitate increase host populations may have been side-stepped by the sampling efforts in the current study.

4) Assuming that hantavirus is prevalent in the areas investigated in the current study, the failure to detect it may also be attributed to the possibility that the sampling effort may have missed possible hantavirus hotspots or micro habitats. This is possible when considering that hantavirus prevalence has been reported to show a tendency to be confined to specific microhabitats or some broadly defined geographic regions, the so called hantavirus hotspots (Price *et al*., 2014). For example, deer mice identified as primary carriers of the Sin Nombre hantavirus are considered to be widely distributed throughout many parts of the USA

including the desert (Witmer & Moulton, 2012). Armién *et al*. (2016) however, reported that during the initial out-break of HPS, only a small portion of the habitat in the south western parts of North America was observed to be suitable for deer mice (*Peromyscus maniculatus*) that were infected with the Sin Nombre hantavirus (SNV). Thus, only a small portion of this environment located in the four corners region is recognised as a hantavirus hotspot within which hantavirus prevalence rates remains consistently high, possibly aided by favourable environmental conditions and vegetation structure that supports high densities of carrier host species (Price *et al*., 2014).

5.4 Potential limitations of the current study

The current study involved sampling bats in selected habitats in the broad-leaved tree and shrub savanna and Acacia tree and shrub savanna biomes in Namibia. The data collection and processing methods may have had some limitations. Mist netting has been reported for its inherent limitations as a tool for assessing bat populations (Larsen *et al*., 2007). Mist netting sampling rates have been noted to under-represent abundances of species in the natural environment (Larsen *et al*., 2007; Sedgeley, 2012). The size, type and placement of mist nets at different heights and positions during bat sampling, the length of time spent sampling bats (i.e., effort), prevailing weather conditions (including lightning) can affect capture rates (Esbérard, 2009). In addition, behavioural attributes such as foraging activity patterns relating to flight height and the reliance on echolocation, olfaction and eyesight by bats to avoid nets have also been highlighted to adversely affect capture rates of mist nets (Larsen *et al.*, 2007; Esbérard, 2009).

It must be stressed that neither environmental nor weather conditions were recorded at all sampling sites within selected habitats, as the current study was not investigating the influence of environmental conditions on the richness and composition of bats. The focus of the current study was to sample bats in order to determine the prevalence of hantaviruses as well as bat species richness and composition in selected habitats in the 2 biomes of interest, regardless of underlying environmental influences. All efforts were made to offset some limitations where possible, including avoiding trapping during winter which is reported to affect bat foraging activity, on

bright moon nights and by changing net positions to minimise net avoidance by bats (Meyer *et al*., 2004).

In addition, the identification of the species *Sauromys petrophilus* and *Scotophilus leucogaster* were only based on traditional taxonomy through the assessment of morphological features and biometric measurements. This is because the DNA sequences obtained for these species were of poor quality and could not be useful for molecular identification. Consequently, it is suggested that these identifications be regarded as preliminary until molecular fingerprints are obtained and the species identities are confirmed. This is important, especially for *Sauromys petrophilus* whose morphological features resemble and have also been reported to share roosts as well as occur within the same broadly rocky habitats with those of *Tadarida aegyptiaca* (Jacobs & Fenton, 2002). Thus, it may be possible that the molecular identity of the species may turn out to be either of the 2 species.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of the current study was to determine species richness and composition of bats, and to investigate the potential prevalence of bat-borne hantaviruses in selected habitats within the woodland and savanna biomes in Namibia. Specific objectives of the study were to: determine the species richness and composition of bats and molecularly screen bats to investigate the prevalence of bat-borne hantaviruses in bats sampled from selected habitats within broad-leaved tree and shrub and the Acacia tree and shrub savanna biomes in Namibia.

Overall, observed patterns of bat species richness did not differ significantly in selected habitats across the two categories of the savanna biome. The observed patterns of species composition in selected habitats across to the broad-leaved tree and shrub savanna and the Acacia tree and shrub savanna biomes showed overall similarity in compositional structure of bat assemblages, albeit with some variations represented by the low value of the *R* statistic, which suggest a high degree of shared species across the biomes. Variations in observed patterns of bat species richness and composition in selected habitats, however, may highlight the importance of localised habitats to the conservation and management of bat species. Observed numbers of bat species in specific selected habitats, particularly in those generally characterised by riverine vegetation as observed and noted in the current study was up to four times than those observed in open savanna habitats

and twice those in inland woodland habitats. The results of the current study may indicate that both perennial and non-perennial riverine habitats may represent important habitats that may support high species richness as well as associated patterns of species composition of bats. The numerical dominance of *Sauromys petrophilus* and observed association of the species to highway bridge roosts may also indicate the importance of the highway bridges to the roosting ecology and conservation of the species as observed in selected habitats in the Acacia tree and shrub savanna biome.

Moreover, the screening of lung and blood samples of bats sampled in selected habitats in broad-leaved tree and shrub savanna and Acacia tree shrub savanna for hantavirus using Pan-Hanta RT-PCR did not yield any positive results. Consequently, the current study did not find hantaviruses.

6.2 Recommendations

The current study recommends that:

1) Sampling diverse habitats and screening large numbers of bats (larger sample size) including screening a variety of other organs such as the kidneys, the spleen and liver will probably increase the likelihood of detecting hantaviruses. Recently, Chau *et al*. (2017) serologically detected and confirmed four cases of hantavirus infections of humans showing febrile symptoms in Mozambique. Interestingly, the four cases were wrongly diagnosed as malaria cases, given the similarity of febrile symptoms. This scenario may characterise many southern African communities including Namibia. It is thus recommended that the future hantavirus searches in southern African may have to adopt a 2-pronged approach that screens animal hosts while at the

same time sourcing human blood samples from local hospitals for patients showing febrile symptoms in order to determine sero-prevalence of hantavirus among humans. This may provide an indication of possible areas of focus in the screening of hosts using patient information;

2) Since the current study was conducted over a relatively short period during the summer months, it is recommended that these results should be considered preliminary until more studies are undertaken for a more comprehensive comparison and conclusive analysis to determine patterns of bat species richness, composition and hantavirus prevalence;

3) Due to inherent deficiencies associated with traditional morphology-based taxonomic identification of species especially in the order Chiroptera, molecular-based approaches have become a reliable and popular tool for identification and classification of taxa (Mayer *et al*., 2007; Nadin-davis *et al*., 2012; Stoffberg *et al*., 2012). The current study has collected occurrence records and voucher specimens for 11 species and DNA fingerprints of at least 9 of the 11 species. It is recommended that the application of molecular tools in the identification of species be adopted for correct and reliable classification of bat species in Namibia; and

4) Future ecological assessments of bats should adopt a dual-sampling approach that includes concurrent use of mist nets and harp traps, including hand nets, assessments in caves, bridges, culverts, tree hollows and house roofs together with acoustic recording devices. These measures will likely increase detection levels of bat species and result in much higher numbers of recorded bat species richness.

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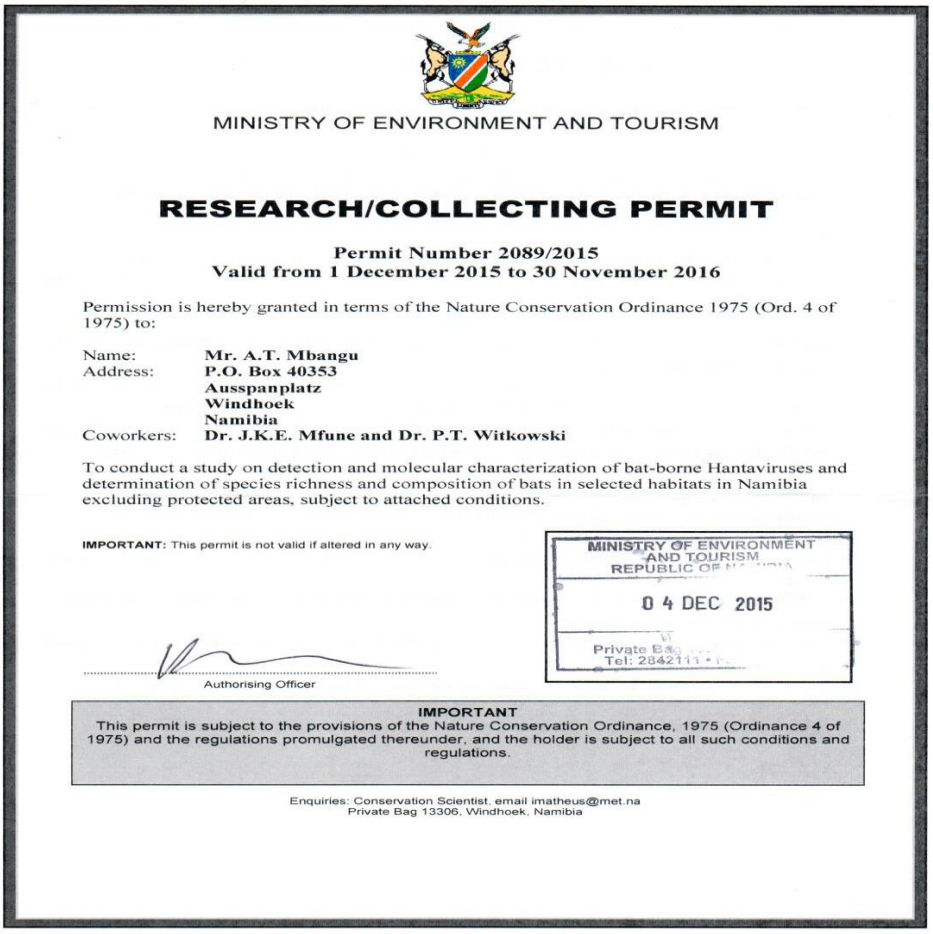
APPENDICES

Appendix 1: Research and collection permit



MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 2089/2015
Valid from 1 December 2015 to 30 November 2016

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: **Mr. A.T. Mbangu**
Address: **P.O. Box 40353**
Ausspanplatz
Windhoek
Namibia
Coworkers: **Dr. J.K.E. Mfune and Dr. P.T. Witkowski**

To conduct a study on detection and molecular characterization of bat-borne Hantaviruses and determination of species richness and composition of bats in selected habitats in Namibia excluding protected areas, subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
04 DEC 2015
Private Bag
Tel: 2842111

Authorising Officer

IMPORTANT
This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Conservation Scientist, email imatheus@met.na
Private Bag 13306, Windhoek, Namibia

Appendix 2: Details of 103 bat lung and blood samples screened for hantavirus using the 2-step Pan-Hanta PCR and the results.

Bat field	Bat species name	Sample type	Pan Hanta

reference			**Result**
KAM 01	*Epomophorus crypturus*	Lung	Negative
KAM 02	*Epomophorus sp*	Lung	Negative
KAM 03	*Epomophorus sp*	Lung	Negative
KAM 04	*Epomophorus sp.*	Lung	Negative
KAM 05	*Mops sp. (midas)*	Lung	Negative
KAM 06	*Neoromicia sp.*	Lung	Negative
OTT8	*Neoromicia capensis*	Lung	Negative
OTT9	*Neoromicia capensis*	Lung	Negative
WAL 01	*Sauromys sp*	Lung	Negative
WAL 02	*Sauromys sp*	Lung	Negative
WAL 03	*Sauromys sp*	Lung	Negative
WAL 04	*Sauromys sp*	Lung	Negative
WAL 05	*Sauromys sp*	blood	Negative
WAL 06	*Sauromys sp*	blood	Negative
WAL 07	*Sauromys sp*	blood	Negative
WAL 08	*Sauromys sp*	Lung	Negative
WAL 09	*Sauromys sp*	Lung	Negative
WAL 10	*Sauromys sp*	Lung	Negative
WAL 11	*Sauromys sp*	Lung	Negative
WAL 12	*Sauromys sp*	Lung	Negative

WAL 13	*Sauromys sp*	Lung	Negative
BH 01	*Scotophilus sp*	Lung	Negative
BH 02	*Scotophilus sp*	Lung	Negative
BH 03	*Scotophilus sp*	Lung	Negative
BH 04	*Mops sp.*	Lung	Negative
BH 0006	*Scotophilus sp*	Lung	Negative
BH 0023	*Mops sp.*	Lung	Negative
BH 0024	*Scotophilus sp*	Lung	Negative
BH 0035	*Mops sp*	Lung	Negative
BH 0052	*Mops sp*	Lung	Negative
BH 0053	*Scotophilus sp*	Lung	Negative
BH 0074	*Scotophilus sp*	Lung	Negative
BH 0081	*Scotophilus sp*	Lung	Negative
WEN 01	*Scotophilus sp.*	Lung	Negative
WEN 02	*Scotophilus sp.*	Lung	Negative
WEN 03	*Scotophilus sp.*	Lung	Negative
WEN 04	*Scotophilus sp.*	Lung	Negative
WEN 05	*Scotophilus sp*	Lung	Negative
WEN 06	*Scotophilus viridis*	Lung	Negative
WEN 07	*Scotophilus sp*	Lung	Negative
WEN 08	*Scotophilus sp*	Lung	Negative

WEN 09	*Neoromicia sp.*	Lung	Negative
LEEU 01	*Sauromys sp*	Lung	Negative
LEEU 02	*Sauromys sp*	Lung	Negative
LEEU 03	*Sauromys sp*	Lung	Negative
LEEU 04	*Sauromys sp*	Lung	Negative
LEEU 05	*Sauromys sp*	Lung	Negative
LEEU 06	*Sauromys sp*	Lung	Negative
LEEU 07	*Sauromys sp*	Lung	Negative
LEEU 08	*Sauromys sp*	Lung	Negative
LEEU 09	*Sauromys sp*	Lung	Negative
LEEU 10	*Sauromys sp*	Lung	Negative
MET 01	*Epomophorus sp.*	Lung	Negative
MET 02	*Scotophilus viridis*	Lung	Negative
MET 03	*Scotophilus sp.*	Lung	Negative
MET 04	*Scotophilus sp.*	Lung	Negative
MET 05	*Scotophilus sp.*	Lung	Negative
MET 06	*Epomophorus sp.*	Lung	Negative
MET07	*Epomophorus sp.*	Lung	Negative
MET 08	*Epomophorus sp.*	Lung	Negative
MET 12	*Epomophorus sp.*	Lung	Negative
TSE 01	*Chaerephon sp.*	Lung	Negative

TSE 02	*Chaerephon sp.*	Lung	Negative
TSE 03	*Chaerephon sp.*	Lung	Negative
ALDO 01	*Neoromicia sp.*	Lung	Negative
ALDO 02	*Epomophorus sp.*	Lung	Negative
ALDO 03	*Neoromicia sp.*	Lung	Negative
MAS 01	*Chaerephon sp.*	Lung	Negative
CIMB 01	*Sauromys sp*	Lung	Negative
CIMB 02	*Sauromys sp*	Lung	Negative
CIMB 03	*Sauromys sp*	Lung	Negative
CIMB 04	*Sauromys sp*	Lung	Negative
CIMB 05	*Sauromys sp*	Lung	Negative
CIMB 06	*Sauromys sp*	Lung	Negative
CIMB 07	*Sauromys sp*	Lung	Negative
CIMB 08	*Sauromys sp*	Lung	Negative
CIMB 09	*Sauromys sp*	Lung	Negative
CIMB 10	*Sauromys sp*	Lung	Negative
SWKP 01	*Sauromys sp*	Lung	Negative
SWKP 02	*Sauromys sp*	Lung	Negative
SWKP 03	*Sauromys sp*	Lung	Negative
SWKP 04	*Sauromys sp*	Lung	Negative
SWKP 05	*Sauromys sp*	blood	Negative

SWKP 06	*Sauromys sp*	blood	Negative
SWKP 07	*Sauromys sp*	blood	Negative
SWKP 08	*Sauromys sp*	Lung	Negative
SWKP 09	*Sauromys sp*	Lung	Negative
SWKP 10	*Sauromys sp*	Lung	Negative
SWKP 11	*Sauromys sp*	Lung	Negative
SWKP 12	*Sauromys sp*	Lung	Negative
SWKP 13	*Sauromys sp*	Lung	Negative
CHIN 01	*Neoromicia sp.*	Lung	Negative
CHIN 02	*Neoromicia sp.*	Lung	Negative
CHIN 03	*Neoromicia sp.*	Lung	Negative
CHIN 04	*Epomophorus sp.*	Lung	Negative
OPUW1	*Neoromicia zuluensis*	Lung	Negative
OPUW2	*Rhinolophus fumigatus*	Lung	Negative
OPUW3	*Sauromys sp*	Lung	Negative
OPUW4	*Sauromys sp*	Lung	Negative
OPUW5	*Sauromys sp*	Lung	Negative
OPUW7	*Rhinolophus fumigatus*	Lung	Negative
OPUW8	*Rhinolophus fumigatus*	Lung	Negative
OPUW9	*Rhinolophus darlingi*	Lung	Negative