MOLECULAR CHARACTERIZATION OF RODENT-AND-SHREW-BORNE VIRUSES

AND THE PREVALENCE AND INTENSITY OF INFESTATION OF SMALL MAMMAL FLEAS, IN NAMIBIA

A thesis submitted in fulfilment of the requirements for the degree of

Master of Science

of

The University of Namibia

By

Julia Hoveka

200515527

April 2015

Main Supervisor: Dr. J. K.E. Mfune

Co-supervisor: Professor P. M. Chimwamurombe

ABSTRACT

Small mammals host diverse communities of both ectoparasites (e.g. fleas and ticks) and endoparasites (e.g. bacteria and viruses). Some parasites are vectors of diseases that infect both humans and wildlife. The main objective of the study was to discover and molecularly characterize novel Hantaviruses and Arenaviruses and to determine the prevalence and intensity of fleas on small mammals in selected areas of Namibia. Small mammals were trapped from six different regions and screened for both Hantavirus and Arenavirus. The regions are, namely, Hardap, Khomas, Kunene, Okavango, Omaheke, and Otjozondjupa. Nucleotide sequence analysis of PCR of the partial large segments of both a Hantavirus and Arenavirus were amplified from RNA extracted from the lungs of small mammal hosts. Hantaviruses were not prevalent in any of the small mammals trapped. However, two new Arenaviruses, not previously recorded in Namibia were both isolated from the Namaqua rock mouse, *Micaelamys namaquensis*, trapped in Okahandja and Mariental. The Arenavirus isolated from rodents trapped in Okahandja and Mariental respectively, is closely related to the Merino Walk virus isolated from the Bush vlei rat, *Myotomys unisulcatus,* captured in South Africa and Luna virus discovered in Zambia in the host, the Natal multimammate mouse, *Mastomys natalensis*.

A generalised linear model (GLM) was used to analyse the significant influence that different factors would present on the prevalence and intensity of flea infestation in three (Hardap, Khomas and Okavango, of the six regions. Factors tested for included host sex, body mass, head-body length and reproductive status. A total of 358, fleas belonging to 9 species (*Chiastopsylla rossi, Xenopsylla cheopis, X. brasiliensis, X.*

hirsuta, X. nubica, X. philoxera, X. piriei, X. versuta, Listropsylla aricinae) were recovered. These fleas were recovered from 5 rodent species, namely *Gerbilliscus leucogaster, Mastomys* sp., *M. namaquensis, Rhabdomys pumilio* and *Saccostomus campestris*. Fleas were prevalent in only 52.3% of the hosts (114 small mammals) examined, of which 54.4% were female and 89.5% were breeding. The GLM analysis showed that host sex, body mass and head body length (representing size of the hosts) and reproductive status of hosts, did not significantly influence the prevalence of fleas on hosts in the three regions ($p = 0.225, 0.053, 0.275, 0.086$, respectively). Male hosts had a significantly high intensity of infestation of fleas compared to females, in Mariental ($p = 0.005$). Host body mass had a significant effect on the intensity of infestation of fleas in Neudamm ($p = 0.003$), whereas host head-body length exerted significantly influence on the intensity of infestation ($p = 0.005$). *Gerbilliscus leucogaster* was the only host species represented, in comparable numbers, in all three regions of study. The results of GLM analysis revealed that there was no significant difference in both the prevalence and intensity of infestation of *G. leucogaster* in the three regions of study.

The use of PCR has revealed the presence of rodent-borne Arenaviruses. Various factors that possibly influence the intensity and prevalence of flea infestation in small mammals have been determined using the GLM analysis.

DEDICATION

This thesis is dedicated the Lord Almighty, who has carried me from the beginning to the end of this program in His strength.

It is also dedicated to my mother, Ms. Luciana Hoveka, who has brought me up to be the person I am today. If it were not for her, I would not have been able to achieve what I have achieved thus far.

ACKNOWLEDGMENTS

I firstly acknowledge the Lord who has been a great source of strength, comfort and wisdom throughout this project.

This project would not have been possible without the funding of Deutsche Forschungsgemeinshaft (DFG) and therefore I thank them wholeheartedly for their financial assistance. I would like to thank the University of Namibia for granting me the opportunity to do this project.

I would like to express my deep appreciation to my supervisor Dr. J. K.E. Mfune and co-supervisor Prof. P. M. Chimwamurombe. Their guidance and inputs into this work has really motivated me to do more and achieve better. I thank my supervisors for taking time to read through drafts and guiding me along the way. I especially thank Dr. Mfune for his assistance during trapping session and for his caring heart, and Prof. Chimwamurombe for always being able to encourage and motivate me with his mere words.

I thank the Ministry of Environment and Tourism for granting me the necessary permission to set traps within the country (permit number 1666/2012). I thank the staff members at Charité in Berlin, Germany and Tygerberg, Cape Town, South Africa, for allowing me to make use of their laboratory facilities to do my work. I thank Ms Ndapewa Ithete at Tygerberg, for her continuous support and help with information and resources that I used for the project. Special thanks to Dr. Peter Witskowski, Charité, who has been a great source of help and encouragement during this project. I greatly thank the staff of the Department of Biological Sciences at the

University of Namibia, for their help and support and for availing their laboratories and facilities for the duration of this project. I would also like to thank the staff at and Central Veterinary Laboratory for allowing me to temporarily use their laboratory facilities.

I would also like to thank Mr Mbangu, Michael and Lego for their willingness and readiness to assist with trapping sessions. I would like to thank the following who allowed us to set traps in their land: farm owners, the municipality of Mariental, Ms Zuraide in Mariental, the management and staff members of the Cheetah Conservation Fund (who also availed space in their laboratories to process and store organ specimens).

Special thanks go out to Professor Kazembe, Mr Klemens Mutorwa, Mrs Mandene Morkel and Mrs Tabitha Dumba and Ms Noa for helping me with data analysis by availing their statistical software and knowledge whenever the need arose. I also extend my appreciation to Mr Dominique Schouw who was always willing to go the extra mile to ensure that all is in order. I would like to extend my deepest appreciation to my husband, Mr Ivan Musuuo who has been a strong pillar of support, motivation and encouragement every step of the way. I thank him for his understanding and his caring heart. I am humbled. I thank my family and all friends for their prayers and continuous support. Without some of them, I would not have had the extra motivation to continue through every moment of weakness. May the Lord bless each one for their support. It is greatly appreciated.

DECLARATION

I, Julia Hoveka, declare hereby that this thesis is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

No part of this thesis may be reproduced, stored in any retrieval system, or transmitted in any form, or by means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author, or The University of Namibia in that behalf.

I, Julia Hoveka, grant The University of Namibia the right to reproduce this thesis in whole or in part, in any manner or format, which the University of Namibia may deem fit, for any person or institution requiring it for study and research; providing that the University of Namibia shall waive this right if the whole thesis has been or is being published in a manner satisfactory to the University.

…… Date……

Julia Hoveka

ACRONYMS AND ABBREVIATIONS

CCF	Cheetah Conservation Fund
CCHFV	Crimean-Congo hemorrhagic fever
cDNA	complementary deoxyribonucleic acid
Cr	*Chiastopsylla rossi* Waterston 1909
CytB-F	Cytochrome B forward
CytB-R	Cytochrome B reverse
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide Triphosphate
DTT	Ditiothreitol
G1/2	Glycoprotein 1/2
GPC	Glycoprotein precursor
GPS	Global Position System
HAN-F	Hantavirus forward primer
HAN-R	Hantavirus reverse primer
HCPS	Hantavirus cardiopulmonary syndrome
HPS	Hantavirus pulmonary syndrome
HFRS	Haemorrhagic fever with renal syndrome

Lar	*Listropsylla aricinae* DeMeillon 1949
LCMV	Lymphocytic choriomeningitis virus
M-MLV RT	Moloney Murine Leukemia Virus Reverse Transcriptase
MWV	Merino Walk virus
N protein	Nucleocapsid protein
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RVFV	Rift Valley Fever Virus
SANGV	Sangassou virus
SDS	Sodium Dodecyl Sulphate
TGNV	Tanganya virus
UNCBD	United Nations Convention on Biological Diversity
Xb	*Xenopsylla brasiliensis* Baker 1904
Xc	*Xenopsylla cheopis* Rothschild 1903
Xh	*Xenopsylla hirsuta* Ingram 1928
Xn	*Xenopsylla nubica* Rothschild 1903
Xph	*Xenopsylla philoxera* Hopkins 1949
Xpi	*Xenopsylla piriei* Ingram 1928

Xv *Xenopsylla versuta* Jordan 1925

TABLES

FIGURES

APPENDICES

TABLE OF CONTENT

1. INTRODUCTION

1.1. Hantavirus

Viruses are extremely small parasites (approximately between 10 and 30 nanometers) that are not visible with an ordinary light microscope (Cann, 1993). They are dependent on host cells for their existence and are therefore not able to live outside of a host's cell for a long period of time (Cann, 1993). Many viruses cause diseases in humans (Murray, Rosenthal, and Pfaller, 2005). Viruses are thought to have the greatest diversity compared to the kingdoms of bacteria, plant and animal put together (Cann, 1993). Viruses are very successful at parasitizing all groups of living organisms (Cann, 1993).

Hantaviruses belong to the family *Bunyaviridae* and genus *Hantavirus* (Groen, Suharti, van Gorp, Sutaryo, and Lundkvist, 2002). They differ from the genus Nairovirus, Orthobunyavirus, Phlebovirus and Tospovirus, of this family in that they are not hosted by arthropods (arboviruses) but by rodents (roboviruses) (Lednicky, 2003) and other small mammals like insectivores and bats (Guo, Lin, Wang, Tian, Cong, Zhang, Wang, Zhou, Wang, Li, Xu, Holmes, and Zhang, 2012). Hantaviruses are negative strand RNA viruses. Their genome consists of large (L), medium (M) and small (S) genomic segments (Figure 2.1) (Khaiboullina, Morzunov and Jeor, 2005; Borges, Campos, Moreli, Souza, Aquino, Saggioro, and Fiueredo, 2006). These segments respectively code for the RNA dependent RNA polymerase, which is the L protein; the glycoprotein precursor of two envelope glycoprotein, namely

glycoprotein 1 (G1) and glycoprotein 2 (G2); and the nucleocapsid protein, known as the N protein (Figure 2.1) (Khaiboullina *et al*., 2005; Borges *et al*., 2006). The large segment of the genome ranges 6530-6550 nt in size, the medium segment 3613-3707 nt and the small segment 1696-2083 nt (Bi, Formenty and Roth, 2008).

Two human zoonoses are caused by Hantaviruses which may result in fatality (Fernandez, 2001; Lundkvist, Lindegren, Sjölander, Mavtchoutko, Vene, Plyusnin, and Kalnina, 2002; Klempa, Fichet-Calvet, Lecompte, Auste, Aniskin, Meisel, Denys, Koivogui, ter Meulen, and Krüger, 2006). The one is known as Hemorrhagic Fever with Renal Syndrome (HFRS) and the second is Hantavirus Pulmonary Syndrome (HPS, also known as Hantavirus cardiopulmonary syndrome (HCPS)) (Fernandez, 2001; Klempa *et al*., 2006). There are about 22 Hantaviruses that cause diseases in humans (Muranyi, Bahr, Zeier and van der Woude, 2005; Bi *et al*., 2008; Krüger, Schönrich and Klempa, 2011) as presented below in Table 1.1:

Table 1.1: Hantaviruses pathogenic to humans.

Hantavirus	**Distribution**	**Disease**
Amur virus (AMRV)	Far East Russia, China, Korea	HFRS
Dobrava virus (DOBV)	Balkans	HFRS
Hantaan virus (HTNV)	China, Korea, Russia	HFRS
Puumala virus (PUUV)	Europe	HFRS
Saaremaa virus (SAAV)	Europe	HFRS
Seoul virus (SEOV)	Worldwide	HFRS

Tula virus (TULV)	Europe	HFRS
Andes virus (ANDV)	Argentina, Chile, Uruguay	HPS
Araraquara virus (ARAV)	Brazil	HPS
Bayou virus (BAYV)	Southeastern USA	HPS
Black Creek Canal virus (BCCV)	USA	HPS
Castelo dos Sonhos virus (CASV)	Brazil	HPS
Choclo virus (CHOV)	Panama	HPS
Hu39694	Argentina	HPS
Juquitiba virus (JUQV)	Brazil	HPS
Laguna Negra virus (LNV)	Paraguay, Bolivia	HPS
Lechiguana virus (LECV)	Argentina	HPS
Monongahela virus (MGLV)	Eastern USA	HPS
New York virus (NYV)	USA	HPS
Orán virus (ORNV)	Northen Argentina	HPS
Rio Mamoré virus (RIOMV)	Bolivia	HPS
Sin Nombre virus (SNV)	USA, Canada	HPS

HFRS mainly occurs in Europe and Asia, whilst HPS occurs in North and South America (Fernandez, 2001; Klempa *et al*., 2006). Zoonotic infections in humans are caused by pathogens that have evolved in wildlife (Begon, Townsend and Haper, 2006; Ulrich, Schmidt-Chanasit, Schlegel, Jacob, Petz, Mertens, Wenk, Büchner, Masur, Sevke, Groschup, Gerstengarbe, Pfeffer, Oehme, Wegener, Bemmann, Ohlmeyer, Wolf, Zoller, Koch, Brockmann, Heckel, and Essbauer, 2008). Biting, scratching, ingestion and inhalation of viral particles are ways in which Hantaviruses

are transmitted from rodent to rodent which seem to suffer no ill effects (Fernandez, 2001). When humans inhale aerosolized excreta, namely urine, faeces and saliva, from rodents persistently infected by the viruses, they become secondary hosts of Hantaviruses (Krüger, Ulrich, and Lundkvist, 2001). Hantaviruses may infect humans through ingestion of food and water that are contaminated by viruses (Krüger *et al*., 2001). Transmission between humans has not yet been widely known except of one case reported among health care personnel in South America (Fernandez, 2001). There has also been mention of a case in which a Hantavirus was transmitted from mother to foetus (Hofmann, Führer, Bolz, Waldshläger-Terpe, Meier, Lüdders, Enders, Oltmann, Meisel, and Krüger, 2012).

The first signs of Hantavirus infections include fever, headache, muscle aches and chills (Krüger *et al*., 2001). The symptoms quickly develop to becoming more extreme with up to 40% of the cases leading to renal or lung failure and shock (Krüger *et al*., 2001). Hantaviruses were thought to only prevail in Europe, Asia, North America and South America but no claims for the virus had been made in the African continent until 2006 (Klempa *et al*., 2006). There is a global increase in the incidences of Hantavirus diseases, being attributed to environmental conditions such as climate that may influence population dynamics of rodents thereby making it easier for transmission of the virus (Klempa, 2009). Agricultural activities may increase the potential of contact between infected rodents and humans especially where these coincide with high population densities of rodents (Nichol, Arikawa, and Kawaoka, 2000). Transmission of Hantavirus to humans may also be influenced by human disturbances such as deforestation, which may affect rodent host behaviour

and hosts physiological responses (Nichol *et al*., 2000; Mills, Ksiazek, Ellis, Rollin, Nichol, Yates, Gannon, Levy, Engelthaler, Davis, Tanda, Frampton, Nichols, Peters, and Childs, 1997). For example, some rodents may seek habitats close to human habitation for shelter and food. Such potential for transmission may increase chances of discovery of Hantaviruses in humans.

The first Hantavirus in Africa, was discovered in 2006 in an African wood mouse *Hylomyscus simus* (Klempa *et al*., 2006). The virus was named Sangassou virus (SANGV), after the village Sangassou in the southeastern regions of Guinea in West Africa (Klempa *et al*., 2006). A second virus, named the Tanganya virus was discovered in Guinea, isolated from the Therese's shrew, *Crocidura theresae* (Klempa *et al*., 2006).

The aim of this research was to investigate, by means of molecular techniques, the prevalence of Hantavirus in small mammals in Namibia. Knowledge of prevalence of Hantavirus would add to the already existing pool of information concerning rodent-borne viruses worldwide, encourage more studies to be undertaken on African rodents and the Hantavirus and characterise African Hantaviruses after their discovery.

1.2. Arenavirus

Arenaviruses belong to the family Arenaviridae and their size range from 50 nm to 300 nm in diameter (LeDuc, 1997). Arenaviruses have single stranded genome that comprises of two segments – a large (7.2 kb) and small (3.4 kb) segments (Cann, 1997; LeDuc, 1997; Sánchez and de la Torre, 2005). The virus is described to have an ambisense genomic organisation because half of the small segment and a short portion of the large segment, both on the 5' ends of the genome, have a positive sense whereas half of the small segment and the longer portion of the large segment are of negative sense (Cann, 1997). The full complement of proteins for the Arenavirus is made up of four proteins: the large segment codes for the viral RNA-dependent RNA polymerase and the protein that binds zinc; and the small segments code for the nucleoprotein and the glycoprotein pre-cursor (Cann, 1997; Botten, Whitton, Barrowman, Sidney, Whitmire, Alexander, Kotturi, Sette, and Buchmeier 2010).

The genus Arenavirus is the only genus in the Family *Arenaviridae* (Cann, 1997; Botten *et al*., 2010). Arenaviruses are classified into two phylogenetic groupings: the Old World Arenaviruses and the New World Arenaviruses (Rowe, Murphy, Bergold, Casals, Hotchin, Johnson, Lehmann-Grube, Mims, Traub, and Webb, 1970). The Lymphocytic-Lassa complex, the Old World serogroup, comprises of the virus lymphocytic choriomeningitis and other African Arenavirus which include Lassa virus (Fulhorst, Charrel, Weaver, Ksiazek, Bradley, Milazzo, Tesh, and Bowen, 2001; Fehling, Lennartz, and Strecker, 2012). All South American Arenaviruses form the New World serogroup, alternatively known as the Tacaribe complex

(Fehling, *et al.*, 2012). According to Botten *et al.*, (2010) approximately eight Arenaviruses cause diseases in humans, as represented in Table 1.2 below:

Table 1.2: Arenaviruses pathogenic to humans (http://www.cfsph.iastate.edu/Factsheets/pdfs/viral_hemorrhagic_fever_arenavirus.pdf).

Arenavirus	**Distribution**	**Disease**
Junin virus (JUNV)	Argentina	Argentine hemorrhagic fever
Machupo virus (MACV)	Bolivia	Bolivian hemorrhagic fever
Guanarito virus (GTOV)	Venezuela	Venezuelan hemorrhagic fever
Sabia virus (SABV)	Brazil	Brazilian hemorrhagic fever
Whitewater Arroyo (WWAV)	Southwesten USA	Whitewater Arroyo hemorrhagic fever
Lassa virus (LASV)	West Africa	Lassa fever
Lujo virus (LUJV)	Zambia, South Africa	Lujo hemorrhagic fever
Lymphocytic choriomeningitis (LCMV)	Worldwide	Lymphocytic choriomeningitis

Lassa virus and Lujo virus from Africa may cause haemorrhagic fevers whilst Lymphocytic choriomeningitis (LCMV) virus, which occurs globally, may cause aseptic meningitis (Botten *et al.*, 2010). LCMV virus is an agent that can cause malformation of an embryo or foetus and is also able to cause high fatalities in patients whose immune systems are suppressed (Botten *et al.*, 2010). There is approximately twenty-two virus species contained in the Arenavirus genus (Charrel,

Coutard, Baronti, Canard, Nougairede, Frangeul, Morin, Jamal, Schmidt, Hilgenfeld, Klempa, and De Lamballerie, 2011).

However, several new viruses were discovered worldwide including: one South American virus with the proposed name Chapare virus (Delgado, Erickson, Agudo, Blair, Vallejo, Albariño, Vergas, Comer, Rollin, Ksiazek, Olson, and Nichol, 2008); four North American Arenaviruses, namely Catarina virus (Fulhorst, Milazzo, Charrel and Bradley, 2002; Cajimat, Milazzo, Bradley and Fulhorst, 2007); Skinner Tank virus (Cajimat, Milazzo, Borcher, Abbott, Bradley, and Fulhorst, 2008), Big Brushy Tank virus (Milazzo, Cajimat, Haynie, Abbott, Bradley, and Charles, 2008), and Tonto Creek virus (Milazzo *et al*., 2008); and three African viruses, namely Kodoko virus (Lecompte, ter Meulen, Emonet, Daffis and Charrel, 2007), Morogoro virus (Günther, Hoofd, Charrel, Röser, Becker-Ziaja, Lloyd, Sabuni, Verhagen, Van der Groen, Kennis, Katakweba, Machang'u, Makundi, and Leirs, 2009), and Lujo virus (Briese, Paweska, Mcmullan, Hutchison, Street, Palacios, Khristova, Weyer, Swanepoel, Egholm, Nichol, and Lipkin, 2009).

Humans mainly become infected with Arenaviruses when they breathe in or eat infected aerosols or if they directly come into contact with infected material through a cut or wound in the skin (Emonet, de la Torre, Domingo, and Sevilla, 2009). Rodents inhabit areas where humans live so they feed on agricultural products and waste substances and make their homes in the buildings of humans (Nursyazana, Mohdzain, and Jeffery, 2013). A case of vertical (human-to-human) transmission has been reported with lymphocytic choriomeningitis virus, in which an organ

transplanted to a immunocompromised recipient resulted in severe disease and inherent infections that led to severe brain and kidney injury that could not be reversed (Cordeya, Sahli, Moraz, Estrade, Morandi, Cherpillod, Charrel, Kunz, and Kaiser, 2011). There are different syndromes related to Arenavirus infections, which in most cases are dependent on the specific virus that causes the infection (Charrel *et al*., 2011). Viruses shown in Table 1.2, except LCMV, are all associated with viral haemorrhagic fever (Charrel *et al*., 2011).

1.3. Parasitism

Parasitism is a relationship in which an organism (the parasite) obtains energy and nutrition from a host organism (Cameron, 1939). The parasite may also have shelter as they live either inside (endoparasite) or on (ectoparasite) the host's body (Price, 1984). Unlike other predators that consume the entire prey, parasites only feed on part of their host (Begon, Townsend and Haper, 2006). This consequently leads only to harmful outcomes but seldom to the death of hosts (Begon *et al*., 2006). Parasites make up a great fraction of diversity of living organisms (Morand and Poulin, 2002), and are thought to be the most common means of obtaining nutrients for living organisms (Laudisoit, Leirs, Makundi and Krasnov, 2009). It is estimated that approximately 50% of all plants and animals are parasitic at some stage during their life cycle (Bush, Fernández, Esch and Seed, 2001). Parasitism is considered to be one of the most successful means of life displayed by living organisms (Poulin and Morand, 2001).

Parasites can be divided into two groups according to size; microparasites and macroparasites (Morand, Krasnov and Poulin, 2006; Jollies, Ezenwa, Etienne, Turner and Olff, 2008) or whether they live in (endoparasites) or on (ectoparasites) their hosts. Microparasites are tiny organisms that live and reproduce within their host, the most common of which are bacteria, viruses, single-celled protozoans and multicellular myxozoans (Begon, *et al*., 2006; Morand *et al*., 2006). Examples of microsparasites are the measles virus or typhoid bacterium that infects animals; the yellow net virus that infect beetroot and tomatoes; protozoa that infect animals; and some fungi infesting plants (Begon, *et al*., 2006). Macroparasites are large enough to be visible without a microscope (Bush *et al*., 2006). They mainly live on the host's body (as ectoparasites, for example fleas, ticks, lice and mites) though in some cases may live within their hosts (as in endoparasites, for example tape worms, roundworms, inworms and ringworms), for instance, the gut of the host (Begon *et al*., 2006; Bush *et* al., 2006; Morand *et al*., 2006). While inside the hosts, macroparasites produce specialised stages of the offspring that go on to infect new hosts (Begon, *et al*., 2006). Parasites are also divided into those directly transmitted from one host to another (for example monogenean worms and lice) or those that require a vector for transmission (malarial parasites and trypanosomes) (Fagir and El-Rayah, 2009). The present study was restricted to the determination of prevalence and intensity of infestation of small mammal fleas in different biomes in Namibia.

1.3.1. Fleas

Fleas belong to Order Siphonaptera and are ectoparasites that suck the blood of warm- blooded animals (Ford, Fagerlund, Duszynski and Polechla, 2004; McCauley, Keesing, Young and Dittmar, 2008). Globally there exists approximately 2,500 species of fleas, belonging to 220 genera and 15 families (Dobler and Pfeffer, 2011), most of which are parasites of mammals and birds (about 25 genera) (Shipley, 1914; Mehlhorn, 2001; Dobler and Pfeffer, 2011). Adult fleas are small in size (1-8 mm in size), orange-brown to dark brown in colour, wingless, laterally compressed, with mouthparts adapted for piercing into the skin and sucking blood and long legs that enable them to jump large distances, (Scholtz, and Holm, 1985; Hall, 1997; Ford *et al*., 2004; Dobler and Pfeffer, 2011). Fleas have periods when they occur on the host's body and periods when they occur in their host's nest (Krasnov, Shenbrot, Khokhlova and Poulin, 2007).

Due to the repetitive sucking action of fleas on different hosts to feed, fleas are able to transmit various pathogens but are mainly vectors of the plague which is caused by the bacterium, *Yersinia pestis* (Keeling and Gilligan, 2000; Mehlhorn 2001). The painful bites of fleas make them pests to humans (Despommier, Gwardz and Hotez, 1982). Fleas are vectors of significant diseases that affect both humans and animals, such as Cat scratch disease and flea-borne spotted fever (Opavsky, 1997; Chae, Yu, Shringi, Klein, Kim, Chong, Lee, and Foley 2008; Rahbari, Nabian, Nourolahi, Arabkhazaeli and Ebrahimzadeh, 2008; Alarcón, Huang, Tsai, Chen, Dubey and Wu, 2011; Dobler and Pfeffer, 2011). Some fleas are vectors of disease-causing agents, carrying them from one host to another while other fleas serve as intermediate hosts

within which parasites grow and multiply. Other than plague, fleas are intermediate hosts of tapeworms, such as *Diplidium caninumi* and *Hymenolepis* spp., which are transmitted to cats and dogs, murine typhus which is transmitted to man and may transmit dermatitis in man and animals (Mehlhorn, 2001; Bitam, Dittmar, Parola, Whiting, and Raoult, 2010 and Alarcón *et al*., 2011) and tularemia (Kundrotaitė and Paulauskas, 2009). Fleas may also irritate both animals and human beings and in turn may cause outbreaks of allergic reactions (Dobler and Pfeffer, 2011)

1.4. Statement of problem

There is a lack of knowledge about the presence or absence of rodent-borne viruses in Namibia despite increasing interest in emerging diseases for which some rodents are vectors and reserviours. Viruses of rodents have not been studied in Namibia despite their medical and veterinary importance. This status quo is probably due to limited resources such as virology laboratories and equipment necessary to undertake such complex studies. It is known from previous studies done in other countries across the world that rodent-borne viruses can be fatal. Studies on rodent-borne viruses fill this knowledge gap and provide sound baseline data and basis to help prepare Namibia to face the potential challenge to deal with fatal diseases spread by rodents in future.

The diversity of living organisms in Namibia is greatly influenced by the diversity of climate, geography, geology and human influences (UNCBD, 2010). Namibia is

characterised by a steep south-west to northeast rainfall gradient, whilst the seasonal and daily temperature variation increase from north and northeast to west and south-west of the country (UNCBD, 2010). The proliferation of emerging diseases such as those caused by Hantaviruses, and their discovery in West Africa, has prompted need to search for these viruses in southern Africa, including Namibia. This present study is part of a larger study on Hantaviruses in southern African.

Namibia has a high diversity of landscapes and associated ecosystems and habitats (Barnard *et al*., 1998), each associated with high diversity of small mammals. Despite this, little has been studied and documented regarding flea prevalence and intensity of infestation in small mammals. It has also been noted that little research has been conducted on the contributions that host sex, body mass, host body length, and host reproductive status, have on parasite prevalence and intensity of small mammals in Namibia.

1.5. Aims and objectives

1.5.1. Aims

The main aim of this study was to investigate the presence of rodent- and shrew-borne viruses and the effect of selected host traits on infestation patterns of flea species on small mammals in selected areas in Namibia.

1.5.2. Objectives

1. To survey, by use of molecular techniques, novel zoonotic viruses in rodent and shrew species in selected areas of Namibia.

2. To determine the prevalence of known zoonotic viruses in rodent and shrew reservoirs in selected areas of Namibia.

3. To determine the effect that host sex, body mass, head-body length and reproductive status have on the prevalence of fleas infesting small mammals from selected geographical regions in Namibia.

4. To determine the effect that host sex, body mass, head-body length and reproductive status have on the intensity of fleas infesting small mammals from selected geographical regions in Namibia.

1.6. Significance of the study

It was important that rodent and shrew species in Namibia be tested for the presence of rodent-borne viruses in order to investigate the possibility to discover new rodent and shrew-borne viruses. Discovery of rodent and shrew-borne viruses would assist epidemiologists correctly diagnose certain diseases to ensure that correct measures are taken to treat infected patients and prevent further spread of diseases (Klempa, Koivogui, Sylla, Koulemou, Auste, Krüger, and ter Meulen, 2010).

There has been a rapid increase in the rate at which new haemorrhagic fever viruses are discovered in recent years (Lednicky, 2003). With the recent discoveries of new viruses, it is likely that many other viruses are yet to be discovered (Henttonen, Buchy, Suputtamongkol, Hapalapong, Herbreteau, Laakkonen, Chaval, Galan, Charbonnel, Michaux, Cosson, Morand and Hugot, 2008; Jonsson, Figueiredo and Vapalahti, 2010). Many newly discovered viruses naturally inhabit one of the most diverse and dynamic group of mammal hosts: the rodents and shrews. The population densities of small mammals vary in response to changes in the environment including climate or agricultural practices (Klempa, 2009). These could be responsible for the emergence of new viral pathogens Advances in technology such as molecular diagnostics have contributed to the detection of new viruses and enabled investigators to isolate and characterize viruses quickly (Jonsson *et al*., 2010).

The differences in topographic diversity along the country could carry with it a vast diversity of small mammal species as well as flea species infesting these small mammals. Apart from environmental influences on the distribution and load of parasite infestations, there are host-specific factors that affect the prevalence and intensity of parasite infestation. Some of these traits include the sex of the host, host body size, and host reproductive status. Studying the prevalence and intensity of flea infestation in relation to the host traits could shed some light as to how parasite infestation is influenced by the host and not merely the environment the host

inhabits. Furthermore, it is important to record the trend of flea prevalence and intensity found in the different biomes of Namibia.

CHAPTER 2 LITERATURE REVIEW

Viruses are parasites that are very small and require a host's cell to live in (Cann, 2001). Viruses are regarded as non-living organisms because they do not possess the seven characteristics common to all living organisms; they are not able to grow or reproduce without invading a cell of a living organism (Cann, 2001). Viruses do not have the necessary genetic information that would enable them to make energy necessary for the chemical reaction; and neither are they able to make proteins, both of these processes are needed for them to maintain life (Cann, 2001). For these reasons, viruses are incapable of surviving outside of a host's cell and are completely reliant on them (Cann, 2001).

According to Morzunov, Rowe, Ksiazek, Peters, Jeor, and Nichol (1998), like most other living organisms, viruses are classified into Families, Subfamilies, Genera, and Species. The parameters used to classify viruses are: firstly the type of nucleic acid and structure of the virion, such as whether the genome is single stranded or double stranded, circular or linear DNA, positive or negative stranded RNA; secondly, the form and structure of the virus such as whether it is enveloped or naked; and thirdly, the strategy which the virus uses for genetic replication and the genetic expression of the virus (Morzunov *et al*., 1998).

Physicians have, for hundreds of years, tried to detect infectious diseases by the medical symptoms they portrayed (Fields, 1985). Those diseases caused by viruses that were distinguished first were ones that showed to have intense and unique

symptoms, these are viruses such as rabies, smallpox and influenza (Fields, 1985). However, it so happened that diseases such as smallpox and measles could not be distinguished initially and for these diseases specific diagnostics and the investigative intelligence of Sudenham were necessary to differentiate between the two diseases (Fields, 1985). With the introduction of the light microscope, *in vitro* immunologic reactions and tissue culture, diagnosis of diseases in laboratories became easier (Fields, 1985). John Brown Buist who was a Scottish pathologist in 1886 was the first to diagnose diseases caused by viruses in a laboratory (Fields, 1985). From the skin wounds of patients who suffered from smallpox, he collected some body fluid from which he detected simple forms, which he believed were responsible for causing the smallpox disease (Fields, 1985). Many other techniques for the diagnosis of viral diseases have been developed after that. These include radio immunoassays, enzyme immunoassays, and immunofluorescence tests, which constitute serological techniques. Others techniques include, light and electron microscopy, which are methods used to enable prompt discovery of viruses (Fields, 1985).

The 1970's brought forth other advances through John Kates who discovered that the mRNA of the *Vaccinia virus* were polyadenylated at their 3' ends (Cann, 2001). In the same year, the enzyme reverse transcriptase was discovered by Howard Temin and David Baltimore, in retrovirus cells (Cann, 2001). With the enabling of purifying enzymes from retrovirus particles, complimentary DNA could was cloned which in turn allowed for RNA viruses to be studied (Cann, 2001). The advancement of

technology caused a shift in the study of nucleic acids instead of proteins and consequently allowed for nucleotides to be sequenced, virus genomes to be influenced and presented advancements in the detection of viruses and their infections (Cann, 2001). The polymerase chain reaction (PCR) is one of the more diagnostic techniques developed *in vitro*, used to amplify nucleic acid and known to be a more sensitive method that enables for the recognition of short fragments of virus nucleic acids (Cann, 2001).

2.1. Hantaviruses

2.1.1. Structure

Hantaviruses are rodent-borne members of the family Bunyaviridae (Martin, Lindsey-Regnery, Sasso, McCormick and Palmer, 1985; Schmaljohn *et al*., 1985). Hantaviruses are enveloped particles that are round in shape and range between 80 to 110 nm in diameter size (Krüger *et al*., 2001). The Hantavirus genome is negative sense and consists of a single RNA strand (Klempa, Stanko, Labuda, Ulrich, Meisel, and Krüger, 2005). Negative sense viruses form part of a group of pathogens capable of causing infectious diseases in both plants and animals (Liljeroos and Butcher, 2013). The Hantavirus genome is divided into three segments, namely small, medium and large segments. (Klempa *et al*., 2005). The small segment codes for the nucleocapsid protein and is approximately 1.6 to 1.8 kb in size. The medium segment is approximately 3.6 to 3.7 kb in size and encodes for the glycoprotein precursor of two viral glycoproteins. The large segment, which is approximately 6.5 to 6.6 kb,

codes for the viral RNA polymerase (Figure 2.1) (Morzunov, *et al*., 1998; Heyman, Vervoort, Escutenairec, Degraved, Konings, Vandenveldee, and Verhagen, 2001; Krüger *et al*., 2001; Hepojoki, Strandin, Lankinen and Vaheri, 2012).

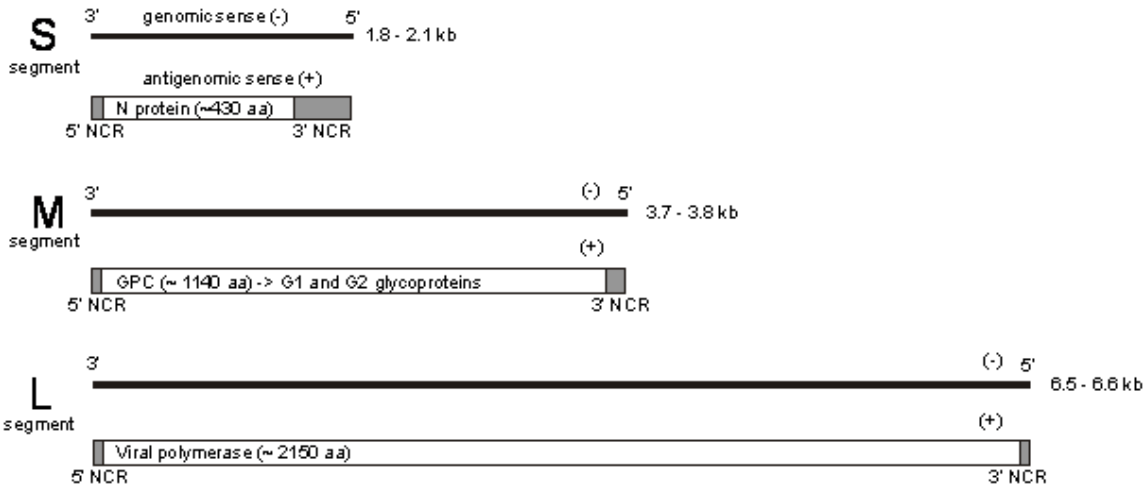

Figure 2.1: Hantavirus (Hantaan virus) genome structure (Klempa, 2004).

Hantaviruses share structural and genetic resemblances with members of the other genera of the Family Bunyaviridae (Schmaljohn and Dalrymple, 1983; Schmaljohn *et al*., 1985). Hantaviruses are transmitted by rodents and not transmitted by arthropods, as is the case for the members of the other genera in the family (Schmaljohn and Dalrymple, 1983). Not only are Hantaviruses harboured by rodents but there have also been many reports of Hantaviruses isolated from shrews (Song, Kang, Song, Truong, Bennett, Arai, Truong, and Yanagihara, 2007; Song, Kang, Song, Truong, Bennett, Arai, Truong, and Yanagihara, 2007). Rodents that serve as vectors of Hantaviruses belong to subfamilies of the Family Muridae including, Arvicolinae, Murinae and Sigmodontinae (Monroe Morzunov, Johnson, Bowen,

Artsob, Yates, Peters, Rollin, Ksiazek, and Nichol 1999). When the nucleotide sequences of Hantaviruses are analysed, it is noted that they group into different genetic lineages (according to subfamilies) which are evidently linked with their principal host species (Morzunov *et al*., 1998; Monroe *et al*., 1999).

2.1.2. Host association

Hantaviruses are divided into three subgroups, namely, *Murinae*-, *Arvicolinae*-, and *Sigmodontinae*- associated Hantaviruses (Schmaljohn and Hjelle, 1997). Examples of *Murinae*-associated Hantaviruses include; Hantaan virus, carried by the striped field mouse (*Apodemus agrarius*), Seoul virus by the rat (*Rattus rattus* and *R. norvegicus*), and Dobrava virus is carried by the yellow-necked field mouse (*Apodemus flavicolis*) and the striped field mouse (*A. agrarius*) (Yamanishi, Dantas, Takahashi, Yamanouch, Domae, Takahashi, and Tanishita, 1984; Scharninghausen, Meyer, Pfeffer, Davis, Honeycutt, 1999; Papa, Mills, Kouidou, Ma, Papadimitriou, and Antoniadis, 2000; Wang, Yashimatsu, Ebihara, Ogino, Araki, Kariwa, Wang, Luo, Li, Hang, and Arikawa, 2000; Cueto, Cavia, Bellomo, Padula and Suárez, 2008). Puumala virus carried by the bank vole (*Myodes* (previously *Clethrionomys*) *glareolus*) (Traavik, Sommer, Mehl, Berdal, Stavem, Hunderi, and Dalrymple, 1984) and Tula virus carried by European common voles (*Microtus arvalis* and *M. levis*) (Plyusnin, Vapalahti, Lankinen, Lehväslaiho, Apekina, Myasnikov, Kallio-Kokko, Henttonen, H., Lundkvist, Brummer-Korvenkontio, Gavrilovskaya, and Baher 1994) are examples of *Arvicolinae*-associated Hantaviruses. Sin Nombre virus carried by the deer mouse (*Peromyscus maniculatus*) (Childs, Ksiazek, Spiropoulou, Krebs,

Morsunov, Maupin, Gage, Rollin, Sarisky, Enscore, Frey, Peters, and Nichol, 1994) is a member of the *Sigmodontinae*-associated Hantaviruses (Peters and Khan, 2002). Various Hantavirus species are strongly associated with their reservoir host species. Hence, Hantavirus species might have coevolved with their host species (Plyusnin *et* al., 1994) and therefore their geographical distribution is dependent, in part, on the distribution of their reservoir hosts (Schmaljohn and Hjelle, 1997). It is believed that Hantaviruses have co-existed with their host and hence are closely adapted for success in their natural hosts. This could explain the host's ability to be infected with the virus and yet remain apparently healthy (Schmaljohn and Hjelle, 1997). They disperse and infect hosts through aerosolized rodent excreta and in the case of their natural hosts, they produce an asymptomatic persistent infection (Tsai, 1987).

2.1.3. Disease in humans

Hantaviruses cause two human zoonoses: haemorrhagic fever with renal syndrome, which is persistent in Asia and Europe (primarily caused by Hantaan virus, Seoul virus, Puumula virus and Dobrava virus) (Park, Kang, Kang, Jung, Jang, and Jung, 2011); and Hantavirus pulmonary syndrome which is persistent in the Americas, primarily caused by Sin Nombre and Andes viruses (Vincent, Quiroz, Gracia, Sanchez, Ksiazek, Kitsutani, Ruedas, Tinnin, Caceres, Gracia, Rollin, Mills, Peters, and Nichol, 2001). The Hantaan virus was the first recognized Hantavirus isolated in Korea in 1978. It was discovered to be the causative agent of the condition initially referred to as Korean hemorrhagic fever, now known as hemorrhagic fever with renal syndrome (HFRS). It affected approximately 3000 United Nations soldiers in Korea

during the time of the Korean War (Lee, Lee, and Johnson, 1978). HFRS affects approximately 200 000 people each year (Muranyi *et al*., 2005) with the rate of fatality ranging from less than 1% to 20% depending on the causative agent (Hjelle, Torrez-Martínez, Koster, Jay, Ascher, Brown, Reynolds, Ettestad, Voorhees, Sarisky, Enscore, Sands, Mosley, Kioski, Bryan, and Sewell, 1996).

Depending on which specific Hantavirus is causing illness, symptoms of HFRS can range from mild, moderate, to severe disease (Schmaljohn and Hjelle, 1997; Meisel, Lundkvist, Gantzer, Bär, Sibold, and Krüger, 1998; Botros *et al*., 2004). Clinical manifestations of HFRS are divided into five phases, namely, febrile, hypotensive, oliguric, polyuric, and convalescent phases (Wichmann, Gröne, Frese, Pavlovic, Anheier, Haller, Klenk, and Feldmann, 2002). The febrile stage is characterized by the inception of fever, chills, malaise, headache, myalgias, back pain, abdominal pain, nausea, vomiting, thirst, photophobia, blurred vision and dizziness. This can be followed by a reduction in the degree of fever and severe shock, which defines the second stage of HFRS (Schmaljohn and Hjelle, 1997; Fernandez, 2001). The third stage is characterized by hypervolemia, which is responsible for approximately half of all deaths (Schmaljohn and Hjelle, 1997; Fernandez, 2001). In the fourth stage, the azotaemia and proteinuria decreases but the fluid and electrolyte imbalances continue and can persist for days to weeks (Fernandez, 2001). The final phase can last weeks to months before recovery is complete (Schmaljohn and Hjelle, 1997) but anemia and hyposthenuria have been reported to occur many months after recovery (Fernandez, 2001).

In May of 1993, the Hantavirus Pulmonary Syndrome (HPS) was first reported after a number of members of the Navajo Nation, situated in New Mexico, who were young and healthy suddenly died after a short period of illness (Schmaljohn *et al*., 1995). At that time, an influenza-like infection broke out in the Four Corner regions of the United States of America, namely, Arizona, Colorado, New Mexico and Utah (Hjelle, Goade, Torrez- Martínez, Lang-Williams, Kim, Harris, and Rawlings, 1996; Fernandez, 2001; Borges *et al*., 2006). The patients had symptoms that included fever, headache, muscle aches, and chills that progressed to severe respiratory disease with respiratory failure, shock, and death occurring in two to ten days after the commencement of illness in approximately 50% of patients. The cause of death was not known at the time and so the health officials termed it as "unexplained adult respiratory distress syndrome". It was later discovered that sera from the ill patients reacted with Hantavirus antigens, which then led to the discovery of a new Hantavirus isolated from tissues of patients and of rodents that were trapped near the homes where the patients lived (Schmaljohn *et al*., 1995).

On average, approximately 200 people are diagnosed with HPS yearly with mortality rates of 40 to 50% regardless of the lower numbers of infection compared to HFRS (Vincent *et al*., 2001; Bi *et al*., 2008). Clinical manifestation of HPS can be divided into three phases, namely the prodromal, cardiopulmonary, and convalescent phases (Fabbri and Maslow, 2001). The prodromal stage, asymptomically involves fever, chills and myalgias and may include abdominal pain, nausea, vomiting, headache,

diarrhoea and dizziness; symptoms that may be misdiagnosed for flu (Fernandez, 2001; Fabbri and Maslow, 2001). The cardiopulmonary phase is characterized by a dry cough, dyspnea, tachycardia, tachypnea, dizziness, diaphoresis and somnolence (Fernandez, 2001). During the cardiopulmonary phase, there is a leaking syndrome that occurs in the lung capillaries, resulting in respiratory failure and cardiogenic shock (Fernandez, 2001; Jonsson *et al*., 2010). In the convalescent phase, patients may experience difficulty breathing because disease affects the lungs (Fernandez, 2001). Although the target organs for HPS are the lungs, some patients with HPS have been reported to have renal sequelae (Pergam, Schmidt, Nofchissey, Hunt, Harford, and Goade, 2009; Rasmuso, Andersson, Norman, Haney, Evander, and Ahlm, 2011).

Rodents, though they seem to be apparently healthy animals, are said to be chronically infected with Hantaviruses, and often shed the virus in urine, faeces and saliva (Hutchinson, Rollin and Peters, 1998; Botros *et al*., 2004). Activities such as farm work, threshing, sleeping on the ground, military exercises, peridomestic cleaning, and agricultural activities increase exposure of people to Hantaviruses (Fink, 1996). People of lower socio-economic standing are believed to be more prone to Hantavirus infections due to poor housing conditions and agricultural activities that expose them to small mammals, hence increasing the risk of exposure to Hantavirus (Lee, 1996). However, people of middle and upper incomes may be exposed to Hantavirus infections during times of outdoor recreational activities such as wilderness camping (Schmaljohn and Hjelle, 1997).

Although Hantaviruses are generally considered to be zoonotic transmission of the viruses between people has been reported for the Andes virus in Argentina (Wells, Estani, Yadon, Enria, Padula, Pini, Mills, Peters, Segura, and the Hantavirus Pulmonary Syndrome Study Group for Patagonia 1997). Wells *et al*., (1997) report 20 cases of patients who contracted HPS after coming into contact with people infected with the disease. One of the cases reported is of a doctor contracted the virus after her husband had died of HPS. The doctor was treated in Buenos Aires where it is suspected that she had infected two of the doctors who had attended to her in hospital.

Another case of man-to-man transmission was reported by Martinez, Bellomo, San Juan, Pinna, Forlenza, Elder, and Padula (2005). A father, known to be a healthy veterinarian worker, had been working on a farm away from home. He became exposed to the HPS virus when he came into contact with pathological particles (Martinez *et al*., 2005). He is believed to have infected his son as they slept on the same bed during the time that the father was showing clinical manifestation of HPS. Another case study reported in the results of Martinez *et al*. (2005), is the case of a man who became infected with Hantavirus during his time on holiday, and was said to have infected one of the passengers next to him who he was travelling with on a bus. It is reported that the said stranger who is believed to have been infected on the bus trip continued to infect one of his friends and someone else he was working with (Martinez *et al*., 2005).

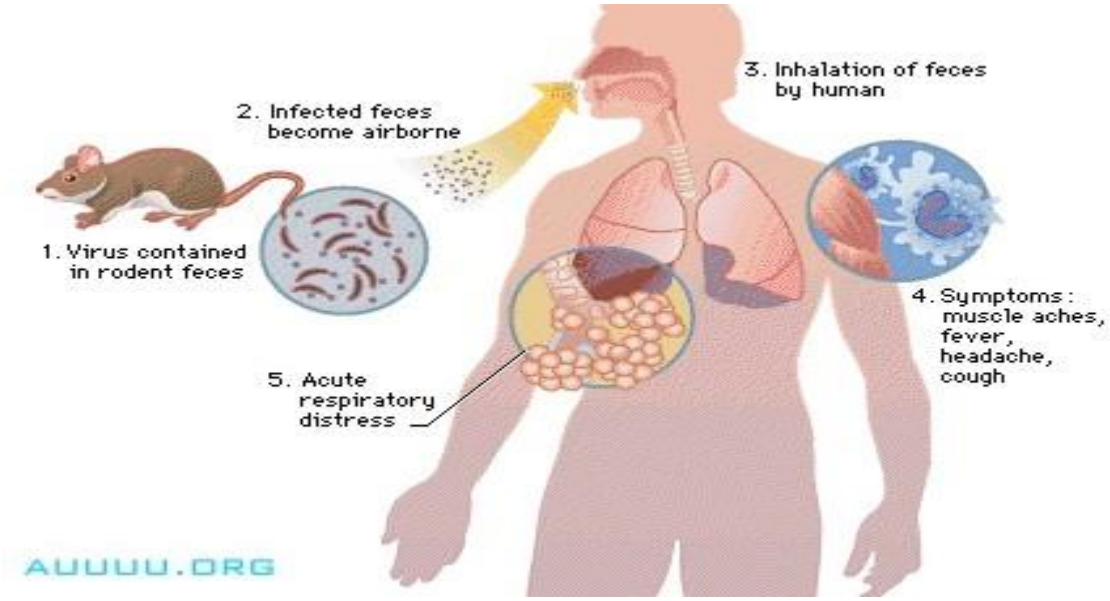

Figure 2.2: Diagram showing the transmission of Hantavirus from small mammal host to human infection and subsequent symptoms of infection. The symptoms described in 4 and 5 are typical for HPS. (http://msbridgetaisch.blogspot.com/2012/09/wider-warning-after-3rd-yosemite.html)

2.1.4. Discoveries of Hantaviruses

For years, Hantaviruses were believed to only cause diseases in Europe, Asia and America. That is, until in 2006 when an indigenous African Hantavirus was detected in an African wood mouse, *Hylomyscus simus*, in Sangassou, Guinea (Klempa *et al*., 2006). In this study, proof was produced of the first Hantavirus genetically detected in Africa and this strain formed part of the *Murinae*- associated Hantaviruses. The virus was named Sangassou virus because the rodent was trapped in a forest habitat near the village of Sangassou, Guinea, West Africa (Klempa *et al*., 2006).

Insectivores have not only been shown to serve as reservoir hosts for Hantaviruses but have also been demonstrated to habour genetically distinct Hantaviruses. The first ever isolated shrew Hantavirus dates back to 1971 when Thottapalayam virus was discovered in the Asian house shrew or brown musk shrew (*Suncus murinus*) in India (Yadav, Vincent and Nichol, 2007). With Hantaviruses only discovered and henceforth considered to infest rodents, Thottapalayam virus was for decades considered to be the only exception of a Hantavirus infesting a non-rodent host reservoir (Yadav *et al*., 2007). There is however, evidence of new and genetically distinct Hantaviruses isolated from non-rodent hosts (presented in Table 2.1).

Table 2.1: Shrew-borne Hantaviruses and their small mammal host species.

Hantavirus	**Host**	**Reference**
Seewis virus	Eurasian common shrew (*Sorex araneus*)	Song *et al*., 2007; Kang *et al*., 2009
Cao Bang virus	Chinese mole shrew (*Anouro-sorex squamipes*)	Song *et al*., 2007
Ramp Ripley virus	Northern short-tailed shrew (*Blarina brevicauda*)	Arai *et al*., 2007
Tanganya virus	Therese's shrew (*Crocidura theresae*)	Klempa *et al*., 2007
Ash River virus	Masked shrew (*Sorex cinereus*)	Arai *et al*., 2008
Jemez Spring virus	Dusky shrew (*Sorex monticolus*)	Arai *et al*., 2008
Asama virus	Japanese shrew mole (*Urotrichus talpoides*)	Arai *et al*., 2008
Oxbow virus	American shrew mole (*Neurotrochus gibbsii*)	Kang *et al*., 2009
Nova virus	European common mole (*Talpa europaea*)	Kang *et al*., 2009
Kenkeme virus	Flat-skulled shrew (*Sorex roboratus*)	Kang *et al*., 2010
Rockport virus	Eastern mole (*Scalopus aquaticus*)	Kang, Bennett, Hope, Cook and Yanagihara, 2011
Azagny virus	African pigmy shrew (*Crocidura obscurior*)	Kang, Kadjo, Dubey, Jacquet, and Yanagihara, 2011
Boginia virus	Eurasian water shrew (*Neomys fodiens*)	Gu *et al*., 2013

Hantaviruses have also been isolated from bats (Order Chiroptera) including the slit-faced bat (*Nycteris hispida)* in Sierra Leone, named Magboi virus (Weiss, Witkowski, Auste, Nowak, Weber, Fahr, Mombouli, Wolfe, Drexler, Drosten, Klempa, Leendertz, and Kruger, 2012*)* and from the banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire named Mouyassué virus (Sumibcay, Kadjo, Gu, Kang, Lim,

Cook, Song, and Yanagihara, 2012). The discovery of Hantaviruses in bats adds on to the existing pool of viruses harboured by bats. Flight in bats and their close social lifestyle enable pathogens to thrive and spread (Calisher, Childs, Field, Holmes, and Schountz, 2006). Other viruses known to be hosted by bats include, Coronoviruses, Hepaciviruses, Marburg viruses, Pegiviruses, and Paramyxoviruses, (Towner, Pourrut, Albariño, Nkogue, Bird, Grard, Ksiazek, Gonzalez, Nichol, and Leroy, 2007; Drexler, Corman, Müller Maganfa, Vallo, Binger, Gloza-Rausch, Rasche, Yordanov, Seebens, Oppong, Sarkodie, Pongombo, Lukashev, Schmidt-Chanasit, Stöcker, Carneiro, Erbar, Maisner, Fronhoffs, Buettner, Kalko, Kruppa, Franke, Kallies, Yandoko, Herrler, Reusken, Hassanin, Krüger, Matthee, Ulrich, Leroy, and Drosten, 2012; Ithete, Stoffberg, Corman, Cottontail, Richards, Schoeman, Drosten, Drexler, and Preiser, 2013; Quan, 2013).

2.2. Arenaviruses

2.2.1. Structure

Arenaviruses are enclosed viruses, with a genome divided into two segments, which is of negative sense (Jahrling, 1997). The virus is round to oval shaped and can have two or more structural shapes, is enclosed by a lipid membrane and measures in a range of 50 to 300 nm in diameter (Murphy, Webb, Johnson, Whitfield and Chappell, 1970; Rowe *et al*., 1970). Each viral particle has a single strand that is divided into two segments; namely the large segment which is approximately 7.3 kb in size and the small segment, which is approximately 3.5 kb in size (Vezza, Gard, Compans and Bishop, 1977; Ghiringhelli, Rivera-Pomer, Lozano, Grau and Romanowski, 1991). The RNA of the small segment encodes for the virus' glycoprotein precursor (GPC)

and the nucleoprotein (NP) (Harnish, Dimock, Bishop and Rawls, 1983) whilst the RNA of the large segment encodes for the virus' RNA dependent RNA polymerase (Salvato, Shimomaye and Oldstone, 1989), also known as the L polymerase, and also encodes for the RING finger protein Z (Salvato and Shimomaye, 1989).

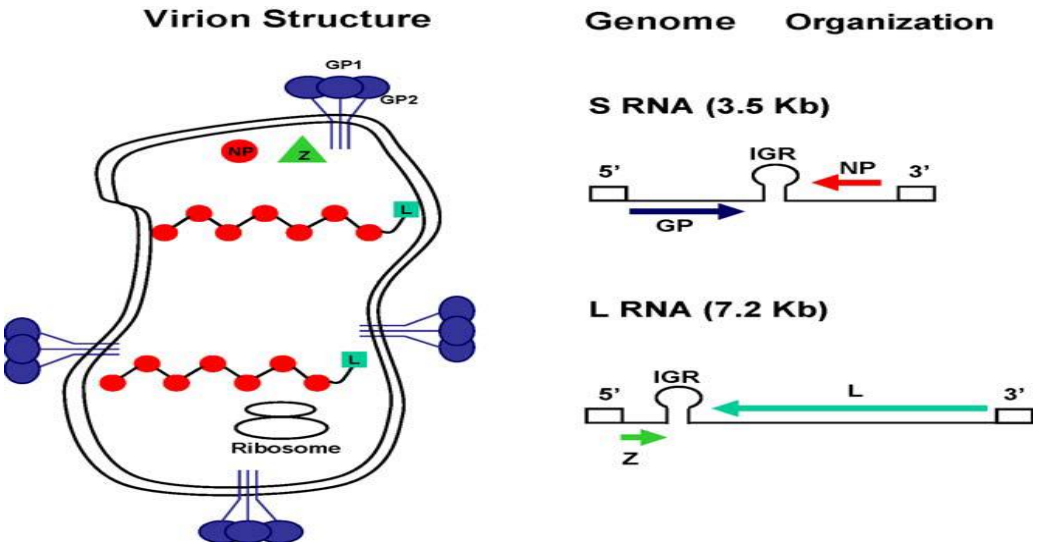

Figure 2.3: Arenavirus virion structure and genome organization (Emonet *et al*, 2009).

2.2.2. Host association

Family *Arenaviridae* comprises of 24 viral species, according to the International Committee on Taxonomy of Viruses (Fehling, *et al.*, 2012). Arenaviruses are divided into two major groups: firstly, there is the Tacaribe serocomplex also referred to as the New World group and secondly the Lassa-Lymphocytic choriomeningitis serocomplex also known as the Old World group (Fulhorst, Bennett, Milazzo, Murray, Webb, Cajimat, and Bradley, 2002). The Lymphocytic choriomeningitis-

Lassa complex consists of Lymphocytic choriomeningitis, Lassa, Mobala, Mopeia and Ippy viruses, which are the African Arenaviruses (Fulhorst *et al*., 2002). The Tacaribe complex comprises of the Whitewater Arroyo virus, Tacaribe virus, Tamiami virus, Bear Canyon virus, Allpahuayo virus, Flexal virus, Parana virus, Pichinde virus, Pirital virus, Amapari virus, Guanarito virus, Junin virus, Machupo virus, Sabia virus, Oliveros virus and the Latino virus which are the South American Arenaviruses (Fuhorst *et al*., 2002; Cajimat *et al*., 2007).

Each Arenavirus is associated with one rodent species or closely related rodent species and circulate in the area in which the host species is endemic (Moncayo, Hice, Watts, de Rosa, Guzman, Russel, Calampa, Gozalo, Popov, Weaver, and Tesh, 2001; Charrel, Lemasson, Garbutt, Khelifa, De Micco, Feldmann and De Lamballerie, 2003). For example, the Natal multimammate mouse (*Mastomys natalensis*) is a principal host of both the Lassa viruses occurring in West Africa (Monath, Newhouse, Kemp, Setzer, and Cacciapuoti, 1974; Lecompte, Fichet-Calvet, Daffis, Koulémou, Sylla, Kourouma, Doré, Soropogui, Aniskin, Allali, Kan, Lalis, Koivogui, Günther, Denys, and ter Meulen, 2006), and Mopeia virus in Mozambique (Wulff, McIntosh, Hamner, and Johnson, 1977). Whitewater Arroyo virus found in the United States is hosted by the woodrats (*Neotoma* species) (Fulhorst *et al*., 1996); the hispid cotton rat (*Sigmodon hispidus*) which is the principal host of Tamiami virus in the United States (Calisher *et al*., 1970). Lymphocytic choriomeningitis virus, however, is an exception to this rule because

the house mouse (*Mus musculus*) that carries this virus is distributed throughout the world (Zapata and Salvato, 2013).

The primary hosts of Arenaviruses are rodents from the family Muridae but Tacaribe virus is an exception because it is not transmitted by rodents but rather by bats, more specifically the Artibeus fruit-eating bats (Price, 1978). The subfamily Murinae hosts all the Old World Arenaviruses whilst the New World Arenaviruses are hosted by rodents from the subfamilies Sigmodontinae and Neotominae (Emonet *et al*., 2009). Arenaviruses from African are predominantly hosted by rodents from family Muridae, of genus *Mastomys*, *Praomys*, or *Arvicanthis* (Zapata and Salvato, 2013). Rodents from the Families *Cricetidae*, of the genera *Oryzomys*, *Sigmodon*, *Neotoma*, *Nephelomys*, *Oecomys*, *Calomys*, *Zygodontomys*, *Neacomys*, or *Akodon* are responsible for carrying Arenaviruses that are persistent in the Americas (Zapata and Salvato, 2013). The Neotominae subfamily primarily habour North American Arenaviruses whereas rodents from Sigmodondinae subfamily habour the South American Arenaviruses (Emonet *et al*., 2009). The idea that Arenaviruses have co-evolved with their particular host species is explained partly by the fact that Arenaviruses infect their rodent hosts persistently for life without causing any ill effects (Emonet *et al*., 2009).

2.2.3. Disease in humans

The risk of humans being infected with Arenaviruses depends of the nature of the rodent species, the factors that contribute to the changes of rodent populations, the behaviour of rodents and human work-related factors that could cause for human to be exposed to rodent excreta (Emonet, Retornaz, Gonzalez, Lamballerie and Charrel, 2007). Rodents infected with the viruses move freely in nature and may move into areas inhabited by humans and this could lead to the exposure of humans to Arenaviruses (Monath, 1975).

Although infections in rodent populations are asymptomatic, a few studies have shown that Arenaviruses are able to cause different symptoms in infected hosts, ranging from changes in weight and fertility, increased mortality and, reduction in size of survivors. These changes are dependent on the viral load, the viral strain and the genetic background of the mice (Webb, Justines and Johnson, 1975; Vitullo and Merani, 1988; Calisher, Nabitym, Root, Fulhorst and Beaty, 2001). Human exposure to Arenviruses could lead to fatal haemorrhagic fevers which include Lassa (caused by Lassa virus; hosted by *Mastomys natalensis*) (Frame, Baldwin, Gocke, and Troup, 1970), Argentine (caused by Junin virus; hosted by *Calomys masculinus*, *Akodon azarae*, and *Bolomys obscurus*) (Maiztegui, 1975), Bolivian (caused by Machupo virus; hosted by *C. callosus*) (Casals, 1975), Venezuelan (caused by Guanarito virus; hosted by *Zygodontomys brevicauda*) (Salas *et al*., 1991), and Brazilian (caused by Sabiá virus) (Lisieux, Coimbra, Nassar, Burrattini, de Souza, Ferreira, Rocco, de Rosa, Vasconcelos, Piheiro, LeDuc, Rico-Hesse, Gonzalez, Jahrling, and Tesh, 1994) haemorrhagic fevers.

Transmission of the virus from rodent host to humans is usually through humans getting into contact with infected rodents and excreta and secreta via inhalation of aerosolised virus (Casals, 1975; Yanagihara, Amyx and Gajdusek, 1985); this may happen when rodents move into human habitation or when rodents move into human dwellings (Charrel *et al*., 2011). Contact between humans and rodents or their excreta and secreta infected with the viruses could most likely lead to transmission of the virus to humans (LeDuc, 1997). However, most cases of arenaviruses transmissions to humans happen after recreational or agricultural activities occurring in the environment and thereby exposing humans to rodent hosts. Scientists working with infected rodents in the laboratories also provide for contact opportunities between rodents and humans and consequently exposure to infectious diseases (Sewell, 1995).

When humans eat mice, they increase the chance of eating virus-infected mice. This is common in those that eat rodents infected with Lassa virus (Ter Meulen, Lukashevich, Sidibe, Inapogui, Marx, Dorlemann, Yansane, Koulemou, Change-Claude, and Schmitz, 1996). Person-to-person transmission may possibly occur from direct contact with blood, tissues, secreta and excreta of infected patients or through sexual contact with infected patients (Fisher-Hoch *et al*., 1995). There has been reports of person-to-person transmission of vertical transmission of LCMV through a case of organs (liver, lungs and kidneys) transplanted from one donor to four recipients. All four organ recipients fell ill three weeks after the organs transplant and LCMV was identified as the cause of the illnesses (Morbidity and Mortality Weekly Report, 2005; Fischer, Graham, Kuehnert, Kotton, Srinivasan, Marty,

Comer, Guarner, Paddock, DeMeo, Shieh, Erickson, Bandy, DeMaria, Davis, Delmonico, Pavlin, Likos, Vincent, Sealy, Godsmith, Jernigan, Rollin, Packard, Patel, Rowland, Helfand, Nichol, Fishman, Ksiazek, Zaki, and the LCMV team, 2006).

The first isolation of the Lymphocytic choriomeningitis virus was in 1933, when human material was acquired from a deadly infection during the serial passage in monkeys in the first epidemic of St. Louis encephalitis (Armstrong and Lillie, 1934). The virus usually begins with the onset of fever, headache, malaise, myalgia, and gastrointestinal tract signs, leukopenia and thrombocytopenia. This might be followed by neurological symptoms such as meningitis or encephalitis and in rare cases hydrocephalus, myelitis, myocarditis, as well as abnormalities in cerebrospinal fluid (Barton and Mets, 2001). Abnormalities in the cerebrospinal fluid include high protein concentration, high white blood cell count and a decrease in glucose concentration (Chesney, Katcher, Nelson and Horowitz, 1979). The case fatality rate of LCMV is approximately less than 1% (Jay, Glaser and Fulhorst, 2005).

Lassa fever is regularly found in Western Africa mainly in Sierra Leone, Guinea, Liberia and Nigeria (Bowen, Rollin, Ksiazek, Hustad, Bausch, Demby. Bajani, Peters, and Nichol 2000). The onset of fevers followed by retrosternal chest pain, pharyngitis, back pain, cough, gastrointestinal tract illness, or hepatitis are clinical signs of Lassa fever. The development of hypotension, peripheral vasoconstriction, decreased urinary output, facial and pulmonary edema, mucosal haemorrhage, severe prostration, or shock may occur as the disease becomes more severe (Woodruff,

Monath, Mahmoud, Pain, and Morris, 1973; Macher, and Wolfe, 2006). Some clinical laboratory findings in Lassa fever patients may include changes in the concentration of leukocytes, thrombocytopenia, albuminuria or proteinuria (Keane and Gilles, 1977). Lassa fever is the cause of death in approximately 30% of women pregnant in their third semester and 75% of infant deaths (Price, Fisher-Hoch, Craven and McCormick, 1988).

The South American hemorrhagic fever viruses cause case-fatalities ranging from 15% to 30% and the different viruses have been found to display different clinical features (Jay *et al*., 2005). Clinical features of Argentine hemorrhagic fevers may include leukopenia, thrombocytopenia, impairment of renal function, hypotension, shock, petechiae, ecchymoses, bleeding and neurologic signs (Maiztegui, 1975). Main clinical features of Venezuelan and Bolivian hemorrhagic fevers include, fever, malaise, headache, neuralgic pain in joints, sore throat, vomiting, abdominal pain, diarrhoea, convulsions, thrombocytopenia, and leukopenia (De Manzione, Salas, Paredes, Godoy, Rojas, Araoz, Fulhorst, Ksiazek, Mills, Ellis, Peters, and Tesh, 1998).

2.2.4. Discoveries of Arenaviruses

An increase in research on viruses of African small mammals has contributed to new discoveries of Arenaviruses (Fulhorst *et al*., 2002; Cajimat *et al*., 2007). Discoveries of Arenaviruses made within the last fifteen years are represented in Table 2.2.

Table 2.2: Newly discovered Arenaviruses and their small mammal host species.

Arenavirus	**Host species**	**Reference**
Bear Canyon virus	California mice (*Peromyscus californicus*)	Fullhorst *et al*., 2002
Catarina virus	Southern plains woodrat (*Neotoma micropus*)	Cajimat *et al*., 2007
Morogoro virus	*Mastomys* species	Vieth *et al*., 2007
Kodoko virus	African pygmy mouse (*Mus* (*nannomys*) *minutoides*)	Lecompte *et al*. 2007
Pinhal virus	Vesper mouse (*Calomys tener*)	Charrel and de Lamballerie, 2010
Oliveros virus	Cark bolo mouse (*Bolomys obscurus*)	Bowen, Peters, Mills and Nichol, 1996; Charrel *et al*., 2008
Skinner Tank virus	Mexican woodrat (*Neotoma mexicana*)	Cajimat *et al*., 2008
Tonto Creek virus	White-throated woodrar (*Neotoma albigula*)	Milazzo *et al*., 2008
Big Brushy Tank virus	White-throated woodrat (*Neotoma albigula*)	Milazzo *et al*., 2008
Real de Catorce virus	White-toothed woodrat (*Neotoma leucodon*)	Inizan *et al*., 2010
Merino Walk virus	Bush vlei rat (*Myotomys unisulcatus*)	Palacios *et al*., 2010
Luna virus	Natal multimammate mouse (*Mastomys natalensis*)	Ishii *et al*., 2011
Menetre virus	*Hylomyscus* species	Coulibaly-N'Golo *et al*., 2011
Gbagroube virus	Peters's mouse (*Mus* (*nannomys*) *setulosus*)	Coulibaly-N'Golo *et al*., 2011
Ocozocoautla de Espinosa virus	Mexican deer mouse (*Peromyscus mexicanus*)	Cajimat, Milazzo, Bradley, and Fulhorst in 2012

2.3. Fleas

2.3.1. General morphology

Fleas have backward pointing bristles, which enable them to move through the hair of their host with ease (Scholtz and Holm, 1985; Bitam *et al*., 2010). Once a flea has attached itself onto a host, the host may not easily remove it because the hairs on the flea's body are used by the flea for grasping the host's hairs and for securing itself into the hairs or the feathers of the host (Braack, 1991). Some adult fleas are characterized by combs or ctenidia that are positioned on their head (Dobler and Pfeffer, 2011). Some species of fleas also have these combs on their bodies (*Macropsylla hercules*) and while other species do not have combs at all (for example the Oriental rat flea, *Xenopsylla cheopis*) (Dobler and Pfeffer, 2011).

Their antennae are triple-segmented, short and positioned in deep hollows found on both sides of their heads (Scholtz and Holm, 1985). The antennae are used solely by male fleas as claspers and provide support for female fleas during times of copulation (Scholtz and Holm, 1985; Bitam *et al*., 2010). Some species of fleas do not have eyes whilst others have shrunken eyes (Dobler and Pfeffer, 2011).

Their mouthparts are situated close to the abdomen and in adults are changed into a tubular feeding organ used for sucking blood of their host (Scholtz and Holm, 1985; Braack, 1991). Their mouthparts are made of three needle-like appendages namely, three stylets; the maxillary and labial palps, maxillary laciniae which are long, cutting blades serrated at the distal end, and the epipharynx which is a long piercing stylet (Scholtz and Holm, 1985; Mehlhorn, 2001).

Hind legs of fleas are well established and bigger than their front legs which enables them to jump great distances (Braack, 1991). They are able to jump a considerable distance of up to 31 cm (in the case of the rat and the human flea) or up to 150 times their own body length, in one single jump, as they travel from one host individual to another (Mehlhorn, 2001; Bitam *et al*., 2010). Their ability to jump such significant distances is enabled by their possession of a resilin pad, described to be a protein that stores energy and releases it similar to elastic (Mehlhorn, 2001).

2.3.2. General life cycle

During development, fleas undergo the egg, larval, pupal and adult stages (Despommier *et al*., 1995). The heat of the host or a blood meal stimulates copulation of fleas and this occurs on the infested host or in the host's nest (Scholtz and Holm, 1985). For the female flea, a blood meal is often vital for the maturing of her ovaries (Scholtz and Holm, 1985). The eggs laid on the host's body or in the host's nest are usually white in colour and have an oval shape, though for some species of fleas the size, shape and the colour of the eggs would be different (Scholtz and Holm, 1985; Bitam *et al*., 2010). Flea eggs are usually not cemented onto the host's body hence they fall off the host and end up on the ground or in the nest of the host as explained by Scholtz and Holm (1985). Eggs dropped off on the host's body are usually smooth whereas those dropped in the host's nest are adhesive and stick to the inside of the nest (Scholtz and Holm, 1985).

The period of gestation of fleas ranges between two and twelve days, but is dependent on the flea species (Scholtz and Holm, 1985). The hatching of the eggs depends on the surrounding temperature and the growth of the embryo requires a

relatively low humidity (Mehlhorn, 2001; Dobler and Pfeffer, 2011). The larvae have no legs or eyes, they are lively, have an off white colour and normally hide in the materials found in the host's nest and therefore are not seen. They feed on waste and organic fragments found in the nest (Despommier *et al*., 1995), whilst some would feed on flea adult faeces that is dried and contains blood, on arthropods or they may feed on other larvae (Dobler and Pfeffer, 2011).

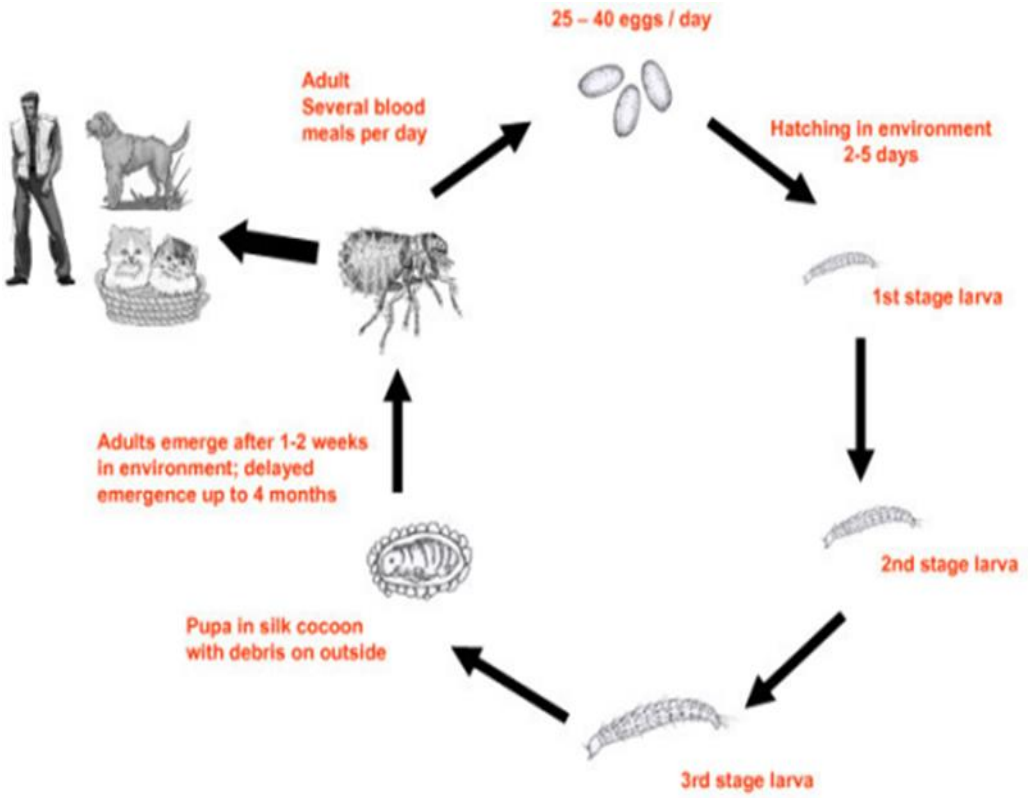

Figure 2.4: Schematic life cycle of *Ctenocephlides felis* (Dobler and Pfeffer, 2011).

2.3.3. Medical importance

Fleas are parasites to small mammals all around the world and harbour many diseases that affect human beings (Murray *et al*., 2005). Some of the diseases carried by fleas include plague (Eskey, 1938; Simond, Godley and Mouriquand, 1998), the

flea borne disease caused by *Rickettsia typhi*, murine typhus and Rocky Mountain fever (Rafinejad, Piazak, Dehghan, Shemshad, and Basseri, 2013). Fleas do not only carry disease that infect humans but they also carry diseases that can be transmitted to animals in the wild and those that are domesticated (Borror, Tripelehorn and Johnson, 1989). The most significant disease spread by fleas is plague, alternatively named, Black Death (Shipley, 1914). Plague is caused by the bacterium, *Yersinia pestis* (Borror & De Long, 1964). In the sixth century A.D., approximately 100 million people were recorded to have died of the plague epidemic – this was the first occurrence of the plague epidemic (Borror, *et al*., 1989). The primary vector of plague known to Southern Africa is the flea species *Xenopsylla brasiliensis* (Zimba, Pfukenyi, Loveridge, and Mukaratirwa, 2011). Along with *X. cheopis*, they are considered to be the most important plague vectors within this genus *Xenopsylla* (Segerman, 1995; Zimba, *et al*, 2011). The bubonic plague is principally carried from rodent to man by the flea species *Xenopsylla cheopis* (Borror *et al*., 1989). *Xenopsylla cheopis* is successful at being a common distributor of human plague because of its cosmopolitan distribution (Shihepo, Eiseb and Cunningham, 2008).

The three ways in which plague can be transmitted is; firstly, by the regurgitation of the plague bacteria at the time the flea is biting its host or feeding, secondly is when faeces infected with the bacterium is scratched into the host's skin; and thirdly, when the host swallows a flea infected with the bacterium (Borror *et al*., 1989).

The sporadic incidences of plague around the world is said to be caused by and associated with an increase in rodent numbers and an increase in other mammals that host vector fleas (Parmenter, Yadav, Parmenter, Ettestad, and Gage, 1999). In New

Mexico, Parmenter *et al*. (1999) showed that an increase in precipitation lead to an increase in rodent population, which subsequently increased the flea population and led to an increase in the occurrence of plague incidences.

2.3.4. Previous studies on fleas

In a study conducted by Laudisoit *et al* (2009) based on the seasonal and habitat dependence of fleas parasitic on small mammals in Tanzania, they investigated host and flea species composition across different habitats during the dry and rainy seasons in the Western Usambara Mountains in Tanzania. In that study they observed that during both the dry and rainy seasons, similarity in flea species composition increased with an increase in the similarity in host species composition. It was also observed that host density was lowest in crop fields and natural forest, higher in commercial plantations and open shrubbery, and highest in closed shrubbery. Host individuals harbored, on average, significantly more flea species during the dry season than during the rainy season (Laudisoit *et al*., 2009).

Krasnov *et al* (2011) tested the hypothesis that host gender affects the expression of infracommunity structures of fleas in South Africa, Tanzania, central Europe (Slovakia) and Western Siberia (Russia). The outcome of the results suggested that different species of fleas randomly infested female hosts more than they did male hosts and that the changing of dynamics in flea communities might be a determined by male host mammals (Krasnov *et al*., 2011). It is known that species of male small mammals move around more frequently than do female small mammals (Krasnov *et al*., 2011) and therefore would explain how male host species get exposed to higher

flea diversities. For instance, male rodents of many species have bigger territories and travel extensive distances compared to female rodents (Krasnov *et al*., 2011). Therefore as male rodents move over longer distances and could have their home territories join; this could result in males being exposed to more flea species (Krasnov *et al*., 2011).

Not many studies have been conducted on ectoparasite abundances in relation to small mammal body size (Morand and Poulin, 2002; Krasnov, 2006). Age dependent patterns on the infestation of fleas on seven species of rodents were studied by Krasnov *et al*. (2006). In this study the age of the host was estimated from the host's body mass and it was expected that hosts of medium size would have a higher number of flea infestation compared to hosts of small and large body sizes (Krasnov *et al*., 2006). The results of the study showed that the abundance of flea infestation increased with an increase in host body size in five out of the seven host species studied (Krasnov *et al*., 2006).

Little is also known about the effects of small mammal reproductive statuses on the prevalence, intensity and abundance of parasite infestation. Viljoen, Bennett, Ueckermann and Lutermann (2011) studied the effects that host sexual reproductive status has on parasite burdens in the Highveld mole-rat (*Cryptomys hottentotus pretoriae*). Their study revealed that reproductive status did not significantly affect the prevalence of *Androlaelaps* species on the rats. Another study by Sundari, Bogdanowics, Varman, Marimuthu and Rajan (2012) revealed that pregnant and

lactating female bats had significantly higher parasite loads than non-breeding female and male hosts.

Zimba *et al* (2011) investigated the abundance and distribution of *X. brasiliensis* on small mammals caught in certain types of habitats adjoining urban areas in Harare, Zimbabwe. They also investigated the cohabitation of other flea species with *X. brasiliensis* (Zimba *et al*., 2011). They found that the rodent species *Mastomys natalensis, Rattus rattus, Gerbilliscus leucogaster*, and *Rhabdomys pumilio* caught in the areas of study were all infested with *X. brasiliensis* (Zimba *et al*., 2011). In the study, flea species *X. brasiliensis* cohabited with other flea species, namely *Dinopsyllus lypusus* and *Ctenophthalmus calceatus* on *M. natalensis*, *R. rattus*, and *T. leucogaster* whereas no flea species was discovered to cohabit with *X. brasiliensis* on the host *R. pumilio* (Zimba et al., 2011).

A study by Amutenya in 2004 found that the prevalence and intensity of small mammal fleas were different between three sites of study (Uusiku, 2007; Shihepo *et al*., 2008). The sites studied were Gross Hertzog farm, the University of Namibia and Gamsberg (Uusiku, 2007; Shihepo *et al*., 2008). It was indicated that the difference in prevalence and intensity was due to the differences in plant cover and the species of plants found in the different sites (Uusiku, 2007; Shihepo *et al*., 2008).

Uusiku (2007) conducted a study on the seasonal occurrence of fleas and other ectoparasites on small mammals at Waterberg Plateau in Namibia. The study revealed that there was a significant difference in the prevalence and intensity of

fleas amongst the five months of the study. The difference in prevalence observed in the study is explained by the differences in temperature, humidity and rainfall observed during the different months of the study.

A study by Shihepo *et al*. (2008) was conducted to investigate the prevalence, the richness of flea species and the intensity of fleas sampled from twelve different areas across the Northern parts of Namibia. The difference in conditions of the environment in different areas could explain why there are different compositions of fleas observed in different locations (Laudisoit *et al*., 2009). Not many studies have been done on the differences of parasite communities in different areas (Laudisoit *et al*., 2009). Reason for this could be because a parasite's environment is effected by the host that it parasitizes and the environment of the host parasitized (Laudisoit *et al*., 2009). Meaning that the habitation of the flea is dependent on the host it infests and the living and non-living environment in which the host is located (Laudisoit *et al*., 2009). Living and non-living components of the host's environment most especially plays an important role in the location of fleas as their distribution is highly influenced by the host's environment (Laudisoit *et al*., 2009).

Mfune, Kangombe and Eiseb (2013) conducted a study on the host specificity, prevalence and intensity of infestation of fleas of small mammals at selected sites in the city of Windhoek, Namibia. In their study they showed that the hosts' body mass had no significant impact on the intensity of flea infestation and also that the intensity of infestation of fleas between host species and host sex were not significantly different.

It is for these reasons that this study on fleas was conducted, to determine the influence of host's traits on the distribution of fleas in selected regions of Namibia.

CHAPTER 3 MATERIALS & METHODS

3.1. Description of the study sites

Small mammals studied in this research were trapped as part of a larger study on Hantaviruses in Southern Africa, including Namibia. Rodent organ samples (kidneys, lungs, and spleen) were collected from six regions in Namibia, which included: Kavango Region (Rundu); Kunene Region (Palmwag); Otjozondjupa Region (Cheetah Conservation Fund (CCF) and Okahandja); Khomas Region (Neudamm Campus); Omaheke Region (Talismanus and Ben-Hur Farmstead) and Hardap Region (Mariental) (Figure 3.2).

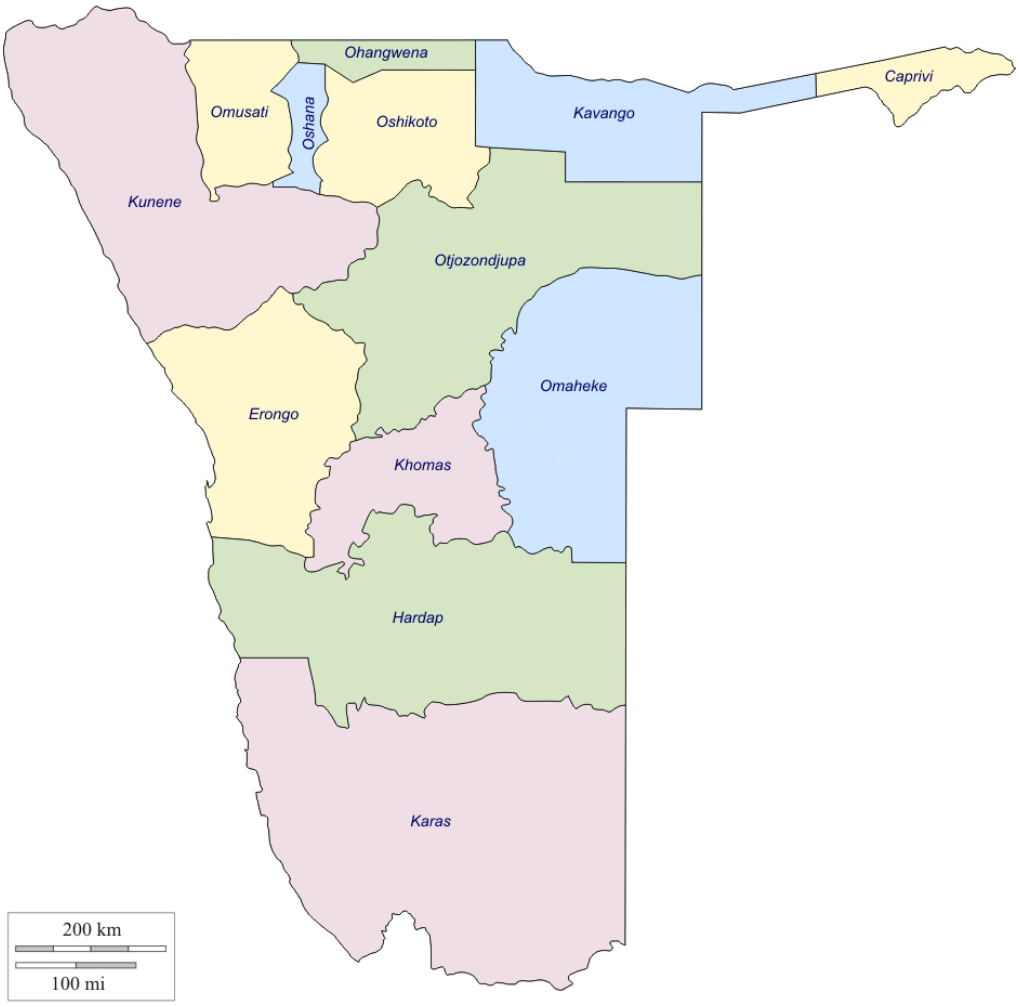

Figure 3.1: A map depicting the regions of Namibia. (Source http://d-maps.com/carte.php?num_car=35459&lang=en).

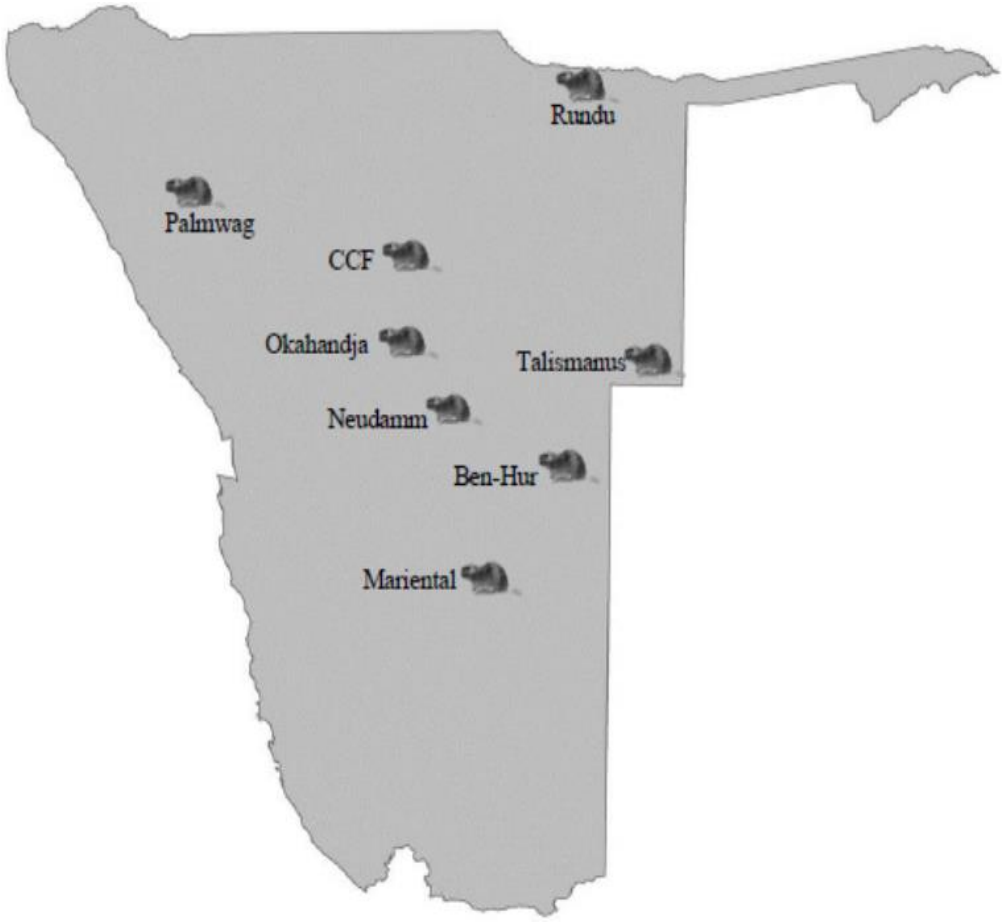

Figure 3.2: Location of the trapping sites for the study in Namibia. Rundu is located in the Okavango Region, Palmwag in Kunene Region, CCF and Okahandja in Otjozondjupa Region, Neudamm in Khomas Region, Talismanus and Ben-Hur in Omaheke Region and Mariental in Hardap Region.

A Global Position System (**GPS**) was used to mark the location of each collection site in each Region. The choice of trapping sites was based on choosing sites that have environmental conditions suitable for the habitation of small mammals. These conditions included suitable soil that enable the small mammals to dig burrows, sufficient grass cover (thick grass cover would restrict movement of small mammals) and shrub cover (Laudisoit *et al*., 2009).

3.1.1 Location and vegetation

In the Kavango Region, trapping for small mammal hosts was carried out at two sites. One site was at the Vungu maize irrigation scheme, located at 17˚53.624’ S and 019˚50.495’ E. Trapping was conducted on a habitat within the scheme which was a furrow area, not planted to maize. The second site was located at 17˚56.645’ S and 020˚05.109’ E area, a mixed woodland habitat located along the roadside between Rundu and Divundu. The vegetation at the trapping sites along Divundu road in the Okavango Region was dominated by *Terminalia sericea, Commiphora* sp., some scattered *Dichrostachys cinerea, Grewia* and *Combretum* species while *Acacia erioloba, Dichrostachys cinerea* and thick grass cover dominated the vegetation at the Vungu Irrigation Scheme.

In the Kunene Region, trapping was conducted at Palmwag (19˚53.23’ S, 013˚56.35’ E) adjacent to an ephemeral Uniab river close to the campsite at Palmwag River Lodge. The lodge is situated on the gravel plains of Damaraland at the foot of the Northwestern plateau. The Euphorbia damarana bushes dominated vegetation at Palmwag.

In the Otjozondjupa Region trapping was conducted at the Cheetah Conservation Fund (CCF) which is located approximately 44km east of Otjiwarongo (16˚39.0’ E, 20˚28.12’ S). CCF was dominated by *Combretum* sp., *Grewia flavescens* and *Boscia albitrunca.* CCF is a research and conservation facility that aims to protect and conserve the existence of cheetahs in Namibia. Trapping in the Otjozondjupa Region

was also conducted in Okahandja (21˚98.33’ S, 016˚91.32’ E). Okahandja is a town situated north of the Namibian capital city, Windhoek and is surrounded by game farms and lodges that serve as tourist attractions and thereby contribute to the economic income of the country. Okahandja is dominated by *A. erioloba*, *A. erubescens* and *A. karoo*. CCF and Okahandja both contain loam soil.

Neudamm is the University of Namibia’s agricultural and fisheries campus, and is located approximately 35km east of Windhoek (22˚49.93’ S, 017˚34.76’ E). Neudamm is a farm situated on an arid land grazed on by livestock that are kept on the campus. Beef, dairy cattle, goats, sheep, pig and local chicken are farmed at the campus. Neudamm was dominated by *Acacia* species.

Talismanus was predominantly encroached with *Terminalia sericea* and other species recorded were *A. erioloba*, and *Grewia* shrubs. In the Omaheke region, trapping was done in Talismanis (21˚84.30’ S, 20˚73.91’ E) and at a farmstead called Ben-Hur (22˚87.26’ S, 019˚21.10’ E). Both these areas are dominated by sandy soil and are inhabited by communal farmers who breed cattle. Ben-Hur farmstead was dominated by *A. erioloba*, *T. sericea* and *Grewia* shrubs.

Dwarf shrubs of A. erioloba dominated Mariental. In the Hardap Region, trapping was conducted in the Mariental district (24˚62.08’ S, 017˚95.93’ E). Mariental, a

small hot and arid small town, lies close to the Hardap Dam, which is one of the water reservoirs in Namibia.

3.1.2 Climate

Rainfall

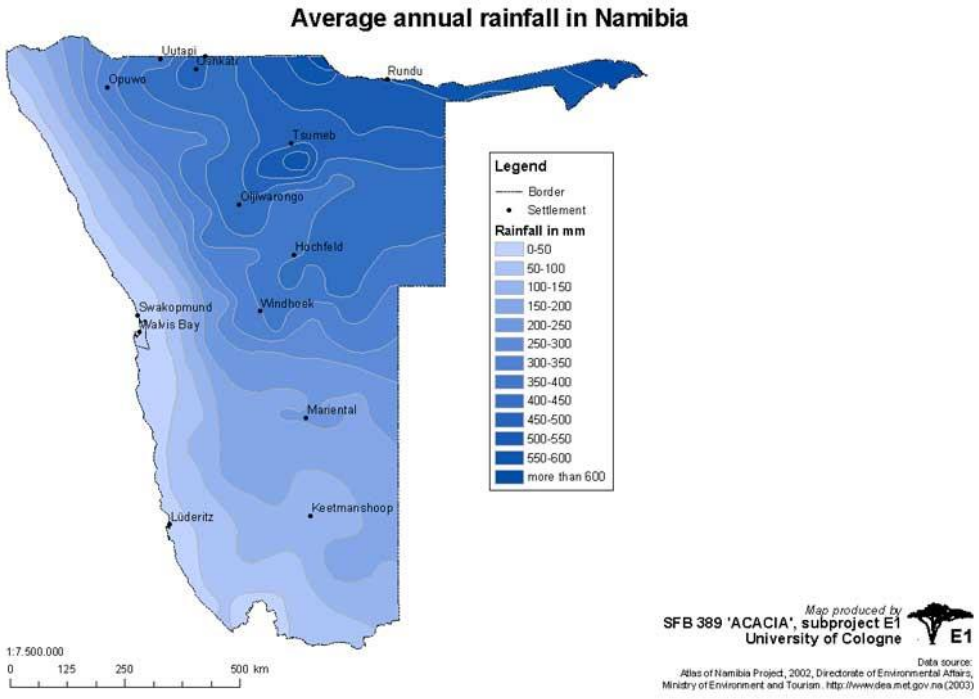

Figure 3.3: A map depicting the annual rainfall across Namibia. (Source: http://www.unikoeln.de/sfb389/e/e1/download/atlas_namibia/e1_download_climate_e.htm).

The annual rainfall pattern across the trapping sites ranges from 0-600mm per year with the highest recorded in the Kavango Region and the lowest rainfall recorded in

the desert regions (along the coast) of Namibia. Kavango Region falls in a range of 400-500mm of rain per year, on average. CCF receives approximately 400-450 mm of year per annum. Okahandja falls within the range of 350-400mm/annum, Neudamm (300-350mm/annum), Talismanis (300-350mm/annum), Ben-Hur (350-400mm/annum), and Mariental (200-250mm/annum) as according to Figure 3.3.

Temperature

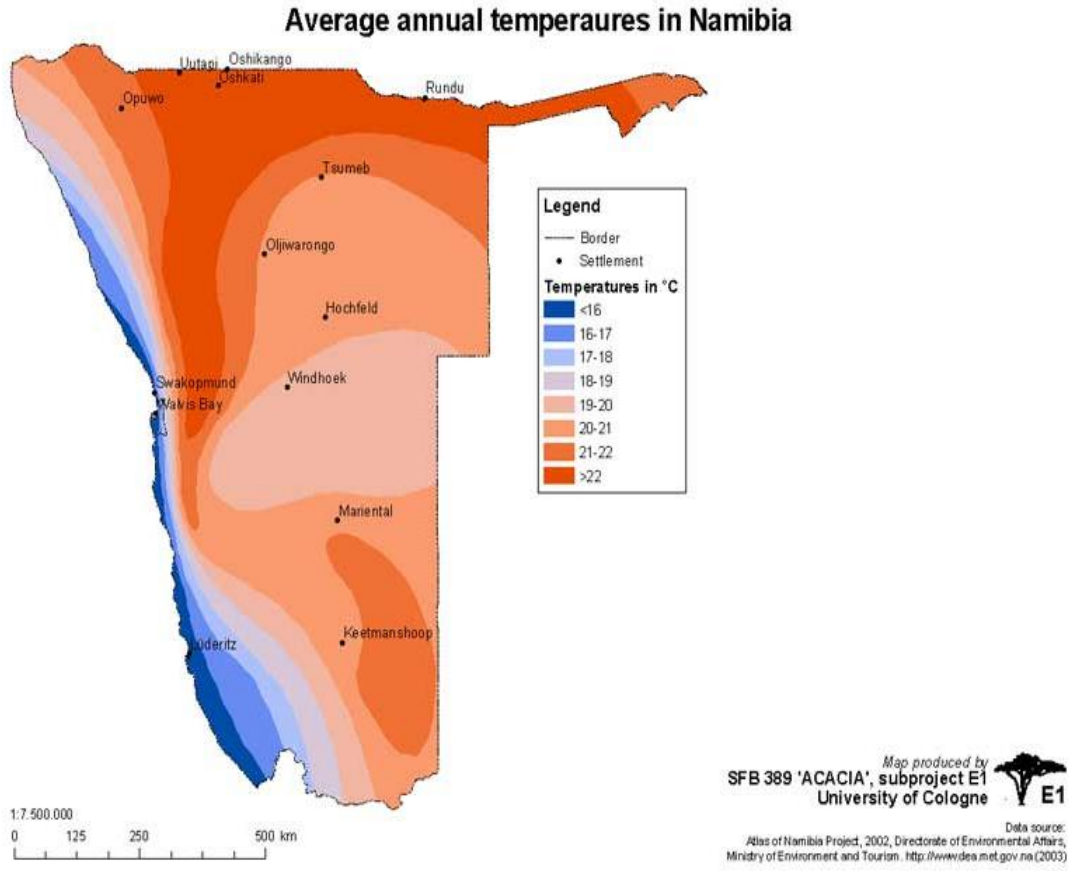

Figure 3.4: A map depicting the annual temperature across Namibia. (Source: http://www.uni-koeln.de/sfb389/e/e1/download/atlas_namibia/pics/climate/temperature-annual.jpg

The annual temperature pattern across the trapping sites ranges from less than 16 °C to more than 22 °C per annum. In the Kavango Region temperatures reach above 22 °C per year, on average. CCF and Okahandja have temperatures ranging 20–21 °C per annum; Neudamm and Ben-Hur farmstead (19–21 °C per annum); Talismanus (20-21 °C per annum); and Mariental (20–21 °C per annum) as according to Figure 3.4.

3.1.3. Biomes

In this study, the investigations on fleas was restricted to fleas collected from small mammal hosts trapped in Rundu (Kavango Region in the North), Neudamm (Khomas Region in the Central) and Mariental (Hardap Region in the South) (UNCBD, 2010).

Table 3.1: Description of biomes, temperature (°**C)**, rainfall (mm/annum) and vegetation type at sites where small mammals were trapped for fleas

Site (Region)	**Biome**	**Vegetation type**	**Temperature ranges (**°C)	**Rainfall (mm/annum)**
Rundu (Kavango)	Broad-leafed Savannah	Deciduous tree species (e.g. Zambezi teak and mopane)	>22	450-700
Neudamm (Khomas)	Acacia savannah	Acacia shrubs	19-20	250-450
Mariental (Hardap)	Nama karoo	known as "Karoo bushes"	20-21	100-250

The three biomes were chosen to investigate how the prevalence and intensity of flea infestation might differ in the highly contrasting habitats.

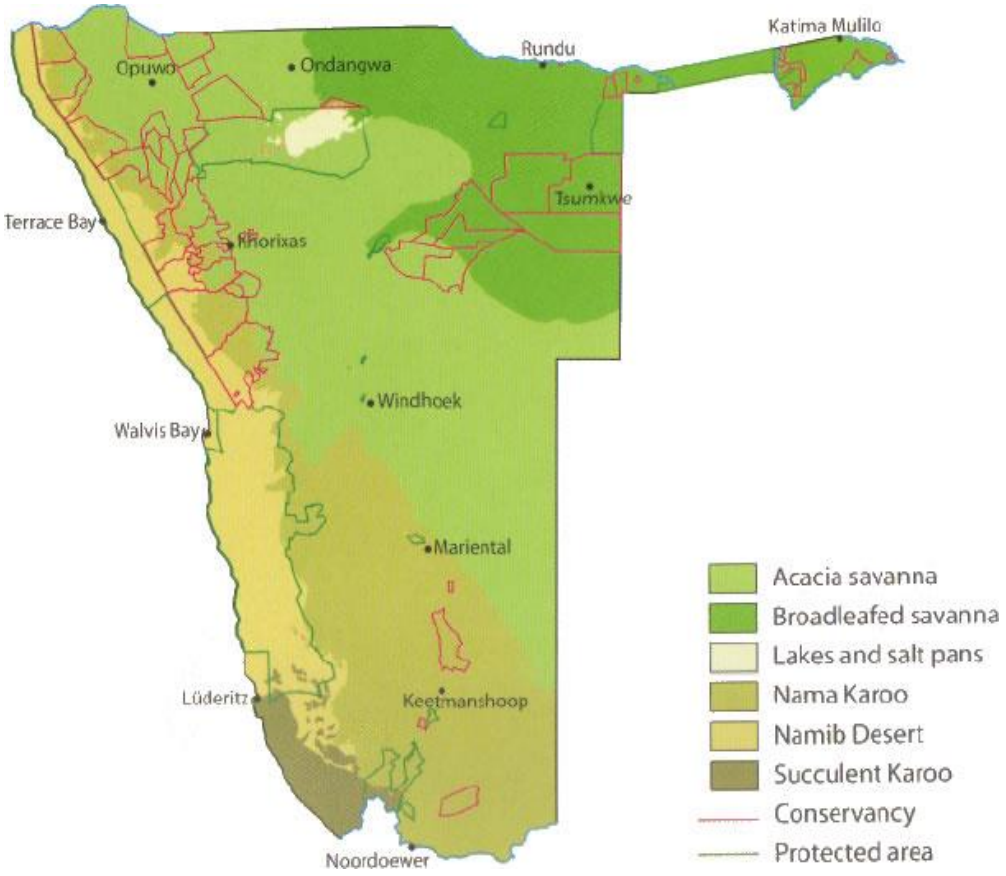

Figure 3.5: Map of Namibia showing divisions of the six major biomes. Retrieved October, 16, 2013 from http://www.nacso.org.no/SOC_2006/chapter2.php

3.2. Field trapping of small mammals

3.2.1. Method of sampling

Trapping sessions were conducted in the following months: Neudamm in September (2010) and January (2012); Rundu in January (2011); CCF in February (2011); Ben-

hur farmstead in November (2011); Talismanus in December (2011); Mariental in April (2012) and Okahandja in June (2012). The decision of where exactly to trap was based on small mammal activities in a specific location and the vegetation structure of the area in question. Active burrows were an indication of small mammal activity and based on how much activity was observed, traps were then set. At each trapping site, a total of 100 Sherman live-traps, baited with mixture of peanut butter and oats, to attract small mammals, were set in line transects that varied depending on the suitable habitats. In some habitats, two line transects, 25 metres were laid down in which 50 traps in each transect were set 10 m apart. Similar line transect trapping layout have been used elsewhere (Caceres, Nápoli and Hannibal, 2011). Traps were set at sunset and inspected an hour after sunrise the next morning, to allow time to capture some diurnal small mammals, for example *R. pumilio*. At each site, traps were set for four consecutive nights.

In the mornings, traps were inspected for the presence of small mammals. Traps that did not capture small mammals were closed and left for next trapping session, or were collected for use at another site if the current site did not produce a sufficient trapping success rate of at least 10%. Traps that captured small mammals were taken to the workstation for processing.

3.2.2. Collection of ectoparasites

Small mammals were carefully removed from individual traps and placed in a zip-lock plastic bag containing cotton wool saturated with chloroform, to euthanize the

host. All small mammal hosts were humanely and ethically euthanized (i.e. the animals were put to death in a way that was efficient, quick and involved minimal pain using chloroform) (Sikes, Gannon, and the animal care and use committee of the American society of mammalogists, 2011). The dead host was carefully transferred from the plastic bag and placed on a white tray where it was thoroughly brushed, using a toothbrush, for the collection of ectoparasites. Ectoparasites were put into vials containing 70% ethanol with the use of fine jeweller's forceps. Each vial was specifically labelled with the following information: trap site, species name, individual reference number and date of collection. The toothbrushes were carefully cleaned with fine tweezers and paper towels before the processing of the next animal.

3.2.3. Processing of the small mammals

Dead host specimens were removed from the tray and placed on a paper towel where standard measurements including the head-body length, tail length, hind foot length, and ear length were taken (in to the nearest millimetre) with a ruler. The body mass (to the nearest g) of the small mammal host was determined using a Pesola spring balance. The host's sex was also determined and recorded. Other host information regarding the small mammal, such as individual reference number (or identification number) assigned to each host, species name and reproductive status were also determined and recorded into data sheets.

3.2.4. Field identification of host species

The Field Guide to Mammals of Southern Africa by Stuart and Stuart (2007) was used to identify small mammal host to species level. Different features of the host were used to compare with features described in the field guide.

3.2.5 Collection of organs for screening of Hantavirus and Arenavirus

Dead host animals were dissected to remove internal organs (lungs, kidneys, liver and spleen) and each respective organ was stored in a separate labelled vial (with the date, reference number assigned to the individual host, and specific organ contained in the vial). Carcasses of small mammals were stored in a field freezer set to –20 °C and later transferred to a freezer set at -80°C. Both organs and carcasses were stored in freezers in the Department of Biological Sciences at the University of Namibia.

3.3. Rodent screening for viruses using Polymerase Chain Reaction

3.3.1. RNA extraction

The protocol from the RNeasy Mini Handbook was used to extract RNA from the small mammal lung tissues. In this study, only lungs were used to screen for both Hantavirus and Arenaviruses. Lungs are considered the best organ from which to screen for Hantavirus and Arenaviruses, not only because they are the first organ to be infected after inhalation of infectious particles (Dr. P. Witkowski, personal communication) but also because other Hantaviruses have been successfully extracted from lungs of host species (Lee, Lee and Johnson, 1978; Klempa *et al*.,

2006). The first viremia takes place exactly in the lungs and can be found in lung tissue even before it is detectable somewhere else (Dr. P. Witkowski personal communication). In the present study, organs of the mammals were screened for the presence of Hantavirus and Arenaviruses using the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method**.** RT-PCR was based on primers in a highly conservative region of the genomic L segment and is able to amplify also novel virus species (Vieth, Drosten, Lenz, Vincent, Omilabu, Hass, Becker-Ziaj, ter Meulen, Nichol, Schmitz, and Günthera, 2007).

Between 20-30 µg of the lung tissue was transferred into a 2 ml sample tube. RLT buffer (including β-mercaptoethanol) (400 µl) was added to the tube, and a steel pellet was used to homogenize the sample in a tissue disruptor. The contents were transferred to a Qiatube shredder and centrifuged at 14000 rpm for 5 minutes. The supernatant was transferred into another sample tube and one volume of 70 % ethanol was added to the sample tube and mixed by pipetting. Into a 2 ml collection tube, 700 µl of the sample was added and centrifuged at 10,000 rpm for 15 s. The flow through was discarded and 700 µl Buffer RW1 was added to the spin column and centrifuged at 10000 rpm for 15 s. The flow through was discarded and 500 µl Buffer RPE was added to the spin column and centrifuged at 10,000 rpm for 15 s. The flow through was discarded and 500µl Buffer RPE was added to the spin column and centrifuged at 10000 rpm for 2 minutes. The spin column was then placed into a new 2 ml collection tube, which was centrifuged at full speed for 1 minute. The spin column was then placed into a new 1.5 ml collection tube; 50 µl nuclease free water

was added directly to the spin column membrane and centrifuged at 10000 rpm for 1 minute to elute the RNA. The RNA was then stored in a -80 ºC freezer.

3.3.2. Reverse Transcription

PCR amplification is readily adapted to RNA templates by first converting them to complementary DNAs using reverse transcriptase (Karp, 2006). From the extracted RNA material that was to be analysed, single-stranded cDNA was synthesized using random hexamer DNA primers and the enzyme reverse transcriptase, which is able to synthesize DNA from RNA templates (Wink, 2006).

A master mix, for 39 samples, was prepared in a sample tube using various reagents and quantities as follows: 5 x First Strand Buffer (150 µl); Random hexamers (76 µl); water (72.2 µl); DTT (3.8 µl); dNTP Mix (114 µl); RNAase inhibitor (19 µl); and M-MLVRT (19.5 µl) to produce a total volume of about 760 µl. Though only 37 lung RNA samples were used for each reaction, a master mix was prepared for 39 samples to make up for the volumes lost during the process of pipetting. From the master mix, 10 µl was transferred into 8-strip PCR tubes (0.2 ml), and 10 µl of the extracted RNA was added into each of the tubes containing the master mix, respectively. The strips were then closed with caps and ran in the PCR machine under the following settings: 25 °C (10 minutes), 42 °C (10 minutes), 96 °C (6 minutes), and a hold at 4 °C.

3.3.3. Hantavirus screening

3.3.3.1. Primary PCR

Primers used for primary PCR are presented in Table 3.2 below. Colleagues working on the Hantavirus project at the Charité in Berlin designed primers used for Hantavirus screening.

Table 3.2: Primers used for the primary PCR screening of Hantaviruses**.**

Name	Sequence	Annealing Temp. (˚C)
HAN-L-F1	5' ATGTAYGTBAGTGCWGATGC '3	53
HAN-L-R1	5' AACCADTCWGTYCCRTCATC '3	53

The master mix for primary PCR was prepared by pipetting the following reagents and quantities into a new sample tube: 5 x Hot Start Mix (500 µl); HAN-F1 (forward primer) (100 µl); HAN-R1 (reverse primer) (100 µl); water (80 µl); and Betaine Enhancer (20 µl) to produce a total volume of 1000 µl. For primary PCR, 20µl of the master mix was transferred into the 8-strip PCR tubes and 5 µl of the cDNA was added into respective tubes containing the master mix. The strips were then closed with caps and placed in the thermocycler on the following amplification settings: 95 °C (15 minutes) for hot start; 95 °C (30 seconds); 53 °C (45 seconds); 72 °C (30 seconds), these were set to run for 40 cycles respectively; 72 °C (6 minutes) and a final hold at 4 °C.

3.3.3.2. Nested PCR

Primers used for nested PCR are presented in Table 3.3 below:

Table 3.3: Primers used for the nested PCR screening of Hantaviruses**.**

Name	Sequence	Annealing Temp. (˚C)
HAN-L-F2	5' TGCWGATGCHACIAARTGGTC '3	53
HAN-L-R2	5' GCRTCRTCWGARTGRTGDGCAA ' 3	53

The master mix for nested PCR was prepared by pipetting the following reagents and quantities into a new sample tube: 2 x Hot Start Mix (1000 µl); HAN-F2 (forward primer) (200 µl); HAN-R2 (reverse primer) (200 µl); water (520 µl); and Betaine Enhancer (40 µl) to produce a total volume of 2000 µl. From the master mix, 49µl was transferred into 8-strip PCR tubes (0.2 ml), and 1 µl of the PCR product from the primary PCR were transferred into each respective tube of the 8-strip PCR tubes.

The strips were then closed with caps and placed in the thermocycler on the following amplification settings: 95 °C (15 minutes) for hot start; 95 °C (30 seconds); 53 °C (45 seconds); 72 °C (30 seconds), these were set to run for 25 cycles respectively; 72 °C (6 minutes) and then an infinite hold at 4 °C. The final PCR products were stored in a -20 °C freezer.

3.3.3.3. Loading of PCR product onto agarose gel

After the preparation of 1% agarose gel, the samples where loaded into the gel once it was set. A 100 bp DNA ladder, mixed with 2 µl of loading dye was loaded into the first well. Individual cDNA samples (5 µl) were also first mixed with 2 µl of the

loading dye and loaded into individual wells onto the gel. The electrophoresis tank was connected to the power supply of 105 V and ran for about 45 minutes. The gel was viewed in a gel documentation system under UV light and pictures were taken. The pictures were labelled and saved on the computer according to the date on which the gels were electrophoresed and observed.

3.3.4. Arenavirus screening

3.3.4.1. Arenavirus PCR

The extracted RNA product was also used for screening of lung sample tissues for the presence of Arenaviruses. Screening was performed by using a pan–Old World Arenavirus reverse transcription–PCR (RT-PCR) specific for the large (L) gene as according to Vieth *et al*. (2007).

The master mix for Arenavirus PCR, per sample, was prepared by pipetting the following reagents and quantities into a new sample tube: 25 µl template mix, 1µl betaine enhancer, 10.5 µl arena primer mix and 8.5 µl water. The primers that were used for the screening of Arenaviruses are presented in Table 3.4.

Table 3.4: Primers used for PCR screening of Arenaviruses. Primers used for Arenavirus screening were obtained from literature (Vieth *et al*., 2007).

Name	Sequence	T(˚C)
LVL3359A+	5' AGAATTAGTGAAAGGGAGAGCAATTC 3'	55
LVL3359D+	5' AGAATCAGTGAAAGGGAAAGCAATTC 3'	55
LVL3359G+	5' AGAATTAGTGAAAGGGAGAGTAACTC 3'	55
LVL3754A-	5' CACATCATTGGTCCCCATTTACTATGATC 3'	55
LVL3754D-	5' CACATCATTGGTCCCCATTTACTGTGATC 3'	55

Unlike Hantavirus screening, screening for Arenavirus only involves a one step process. RNA (5 µl) sample was added to a PCR tube containing 45 µl of Arenavirus master mix. The tubes were then closed and placed in a thermocycler under the following amplification conditions: 95 °C for 2 minutes, 45 cycles at 95 °C for 20 seconds, 55 °C for 45 seconds, 72 °C for 50 seconds, and a final extension at 72 °C for 5 minutes.

The same procedures for gel electrophoresis used for Hantavirus was used for Arenavirus (refer to section 3.3.3.3).

3.3.4.2. Gel extractions

For gel extraction of positive bands, the product was purified by the GeneJet Gel Extraction Kit (Fermentas) following the user's guide.

A gel slice with the desired DNA fragment was cut out and weighed. A 1:1 volume of binding buffer was added to gel slice and the solution was incubated at 50°C to 60°C for 10 minutes. For DNA binding, about 800 µl of the gel solution was added to a spin column and centrifuged for 1 minute, flow through was discarded and spin column put back in the same collection tube.

To wash the residue off the column, wash buffer (700 µl) was added to the spin column and centrifuged for 1 minute, flow through was discarded and the empty column was once again centrifuged for 1 minute. The column was then placed in a fresh 1.5 mL microcentrifuge tube. To elute the DNA, 50 µl of the elution buffer was added to the spin column and centrifuged for 1 minute. The flow through was kept and sequenced using the BigDyeV3.1 Terminator chemistry.

3.3.4.3. DNA sequencing

For sequencing, the protocol in the user's guide was followed using the BigDyeV3.1 Terminator reagent by Applied Biosystems.

A sequencing buffer mix was mixed using the following reagents: ready reaction premix (4 µl), BigDye sequencing buffer (2 µl), primer (3.2 pmol), DNA template (10 ng) and deionized water to have a final volume of 20 µl.

The tubes were placed in a thermal cycler and set to the correct volume. The thermo cycler was ran under the following conditions: 96 ˚C for 1 minute, for 25 cycles, 96 ˚C for 10 seconds, 50 ˚C for 5 seconds and 60 ˚C for 4 minutes. The contents of the tubes were then spun down. The extension products were purified using method stipulated in the BigDyeV3.1 Terminator guide.

To precipitate 20 µl sequencing reaction, 2 µl 2.2 % sodium dodecyl sulphate (SDS) solution was added to a spin column, the tube was sealed and mixed thoroughly and heated to 98 ˚C for 5 minutes. The tubes were allowed to cool to room temperature. The contents in the tubes were spun down briefly. The column was gently tapped to cause the gel material to settle to the bottom of the column, the upper end cap was

removed and 0.8 mL of deionized water was added. The cap was replaced, inverted a few times to mix the water and gel material and the gel was allowed to hydrate at room temperature for at least 2 hours. The upper end cap was first removed, followed by removal of the bottom cap and the column was allowed to drain completely by gravity.

The column was inserted to a provided wash tube, spun for 2 minutes to remove interstitial fluid; the column was removed from the wash tube and inserted into a sample collection tube. The SDS mixture was removed from its tube and loaded onto the centre of the gel material, which was then spun for 2 minutes; the column was discarded as the sample was now in the sample collection tube. The sample was dried in a vacuum centrifuge for 10-15 minutes. Samples were run on the sequencer after which the ABI-files containing the raw sequences were obtained. Raw sequence files were evaluated by BioEdit software for nucleic acid sequences.

The MEGA software was used for multiple sequence alignment and phylogenetic analysis (Tamura, Peterson, Peterson, Stecher, Nei and Kumar, 2011). The nucleic acid sequence of the PCR product was fed into an online BLAST (http://blast.ncbi.nlm.nih.gov/) to look for similar sequences already known in the databases. The generated nucleic acid sequences were translated into corresponding protein sequences. The amino acid sequences of the samples were aligned with those of other Arenaviruses by use of the MUSCLE algorithm from the MEGA package.

The method used for generating a phylogenetic tree was estimated by the MEGA software, which contains a “Model test” which gives a phylogenetic model best suited for the given dataset.

3.3.4.4. Host identification

In order to get the Cytochrome B sequences for host identification, the primers that were used for the PCR are presented in Table 3.5:

Table 3.5: Primers used for the PCR of Cytochrome B sequences for the identification of host species.

Name	Gene	Sequence	T(˚C)
CytB-F	Cytochrome B	5´-CGA AGC TTG ATA TGA AAA ACC ATC GTT G	55°C
CytB-R	Cytochrome B	5´-CTG GTT TAC AAG ACC AGA GTA AT	55°C

The PCR products were cycle sequenced using the primers presented in Table 3.5. The sequences were compared with available data for CytB from small mammals in the GeneBank.

3.4. Fleas

3. 4.1. Processing and identification of fleas

The present study utilised only fleas collected from small mammal hosts trapped at three sites namely Rundu, Neudamm and Mariental. Stored flea specimens were placed in distilled water for one hour to rinse off the alcohol. Specimens were then transferred onto petri dishes with 15 % Potassium hydroxide (KOH) and left at room temperature for 4-6 days to dissolve and clear endodermal and mesodermal tissues, leaving only the exoskeleton which is required for the identification of fleas (Uusiku, 2007). The pH was neutralized for 30 minutes with 10 % acetic acid. The flea specimens were again rinsed with distilled water for one hour. The specimens were

dehydrated using different concentrations of alcohol: 70 %, 80%, and 96 % each for 30 minutes and finally in absolute alcohol for an hour. Specimens were then softened using oil of cloves and mounted onto glass microscope slides in Canada balsam. Slides were labelled according to specimen's identification number and were left to dry by air for 2 weeks.

An identification key for flea species known to occur in the Southern African sub-region, developed by Segerman (1995) was used to identify flea species. Species identifications of specimens of fleas were confirmed at the National Museum of Namibia.

3.5. Data analysis

3.5.1. Screening of viruses

The presence of a band, from the samples being tested, that was in line with the expected band of the positive strand after the PCR samples were run through the electrophoresis revealed that the specific virus tested for is present in the RNA extracted from the animal. The absence of that band showed that the specific virus tested for was not present in the tested animal or that the assay was not able to amplify an unknown virus RNA. Hence, for each host sampled, the presence or absence of Hantavirus and Arenavirus was recorded.

3.5.2. Virus screening

The software that was used for multiple sequence alignment and phylogenetic analysis is called MEGA (version 5.2). PCR products generated in the laboratory were sent for Sanger-sequencing in Germany, after which the sequence data is obtained. The sequencing data is the nucleic acid sequence of the PCR products generated from the screened samples. These nucleic acid sequences are fed into an online BLAST search (http://blast.ncbi.nlm.nih.gov/) to look for similar sequences already known in the databases. After this information was obtained RNA sequences were translated into corresponding protein sequences. This was done because the nucleic acid sequences of Arenaviruses tend to be very diverse and difficult to compare. Protein sequences have more in common and are easier to compare. The amino acid sequences of the screened samples and those of other RNA-viruses already known were aligned by the MUSCLE algorithm from the MEGA package. This alignment was used for generating the tree (also within the MEGA programme). The method used for tree generation was estimated by the MEGA software. There is a "Model test" implemented within the package which gives a phylogenetic model best suited for the given dataset. A total of 518 lung samples were screened for Hantavirus and Arenavirus.

3.5.3. Fleas

The Statistical Package for the Social Sciences (SPSS) version 20 for Windows was used to determine whether flea data was normally distributed. The Kolmogorov-Smirnov normality test was used to determine whether the data (the total number of

individual fleas collected from the regions of study) were normally distributed. Fleas were not normally distributed (K-S=0.256; df=114; p=0.000) and therefore Generalised Linear Models (GLM) were used to determine the effect that selected host specific traits / factors might have on the prevalence and intensity of fleas infesting small mammal hosts.

3.5.3.1. Intensity of infestation of fleas

In the study, we defined flea intensity as the number of parasites per infested host (McCauley *et al*., 2008). Intensity of fleas was calculated as the total number of individual fleas collected divided by the total number of host animals infested by fleas. The median intensity of infestation, which is the middle value of a ranked data set, was used because the data collected for this study was not normally distributed (df=114; p=0.000). For this study, the intensity of infestation was compared between the regions (North, Central and South) for host *G. leucogaster*, host species per region, host sex, body mass, head-body length, and reproductive status.

3.5.3.2. Prevalence of infestation of fleas

Prevalence of infestation of fleas refers to the percentage (or proportion) of host animals that are infested by fleas (McCauley *et al*., 2008). It was calculated as the total number of infested host small mammals (using presence/absence data; not the number of fleas on each host) divided by the total number of host animals examined multiplied by a hundred. For this study, the intensity of infestation was compared

between the regions (North, Central and South) for host *G. leucogaster,* host species per region, host sex, body mass, head-body length, and reproductive status.

3.5.3.3. Jaccard's similarity index

Jaccard similarity index (J) was used to determine the similarity or diversity in species of hosts between the three regions of study (Jaccard, 1912; Mulqueen, Stetz, Beaubien and O'Connell, 2001).

J = a/a + b + c

Where: a = the number of species present in both communities

b = number of species present in community 1 but not in 2

c = number of species present in community 2 but not in 1

For data analysis on fleas, R statistical software (The R foundation for Statistical Computing Version 2.15.0) was used. The Generalised Linear Model (GLM) was used to analyse the effect of host sex, body mass, head-body length and reproductive status, on the prevalence and intensity of flea infestation. The number of individuals host was used as replicates whilst the regions in which the trappings were conducted (in case for analyses for *G. leucogaster*), host sex, body mass, head-body length and reproductive status, were used as factors. The model assumes that data analysed is not normally distributed (Bolker, Brooks, Clark, Geange, Poulsen, Stevens and White, 2008). The binomial model of distribution was used to determine the effects of selected host traits on the prevalence of infestation of fleas on the small mammals. The model assumes that each individual small mammal has the same probability of

infestation and that each outcome is independent from the other (Quinn and Keough, 2002).

The intensity of infestation of fleas was determined by using the Poisson regression model of distribution, which deals with data that are in the form of counts (i.e. the number of fleas infesting individual hosts) (Quinn and Keough, 2002). Poisson is used to measure how many times a certain event occurs in a specific time interval or in a specific length or are (Quinn and Keough, 2002).

A GLM was used to investigate whether the prevalence and the intensity of infestation of fleas on small mammals was influenced by the following parameters of the hosts: - sex, body mass, size (using head body length as an index of body size) and reproductive status. In this GLM analysis, female hosts were the reference category for sex and non-breeding hosts were the reference category for reproductive status. For analysis on the prevalence and intensity of flea infestation on *G. leucogaster* between the regions of study, Mariental was the reference category.

CHAPTER 4 RESULTS

In this study, a total of 518 small mammals representing, 3 orders, 3 families, and 13 genera of 15 species (2 shrews and 13 rodents) were trapped. Organs (lungs) from all 518 small mammals were screened for Hantavirus and Arenaviruses, while only 218 small mammals were used to investigate prevalence and intensity infestation patterns of fleas. The sample sizes of small mammal hosts at different regions are presented in Table 4.1. The species of small mammals represented were as follows: *Aethomys chrysophilus*, *Crocidura fuscomurina*, *Dendromus melanotis*, *Elephantulus intufi*, *Gerbilliscus leucogaster*, *Gerbillurus paeba*, *Gerbillurus setzeri*, *Lemniscomys rosalia*, *Mastomys* sp., *Micaelamys namaquensis*, *Mus indutus*, *Pedromyscus collinus*, *Rhabdomys pumilio*, *Saccostomus campestris*, and *Thallomys paedulcus*.

Table 4.1: The number of small mammal hosts caught from the eight different sites for this study.

Trap site	**Number of hosts caught**
Rundu	45
Palmwag	13
CCF	59
Okahandja	106
Neudamm	153
Ben-hur	47
Talismanus	31
Mariental	64
Total	**518**

4.1. Hantavirus

A total of 518 lung tissue samples were screened for the presence of Hantavirus. There were no Hantavirus specific amplicons detected and therefore no Hantaviruses were discovered in this study. The same cDNA samples used to screen for Hantaviruses were further used to screen for Arenaviruses.

4.2. Arenaviruses

The same RNA extracted from the 51lung tissue samples were used for the screening of Arenaviruses. In this study, four of the samples screened, yielded bands of expected size for Arenaviruses (Figure 4.1).

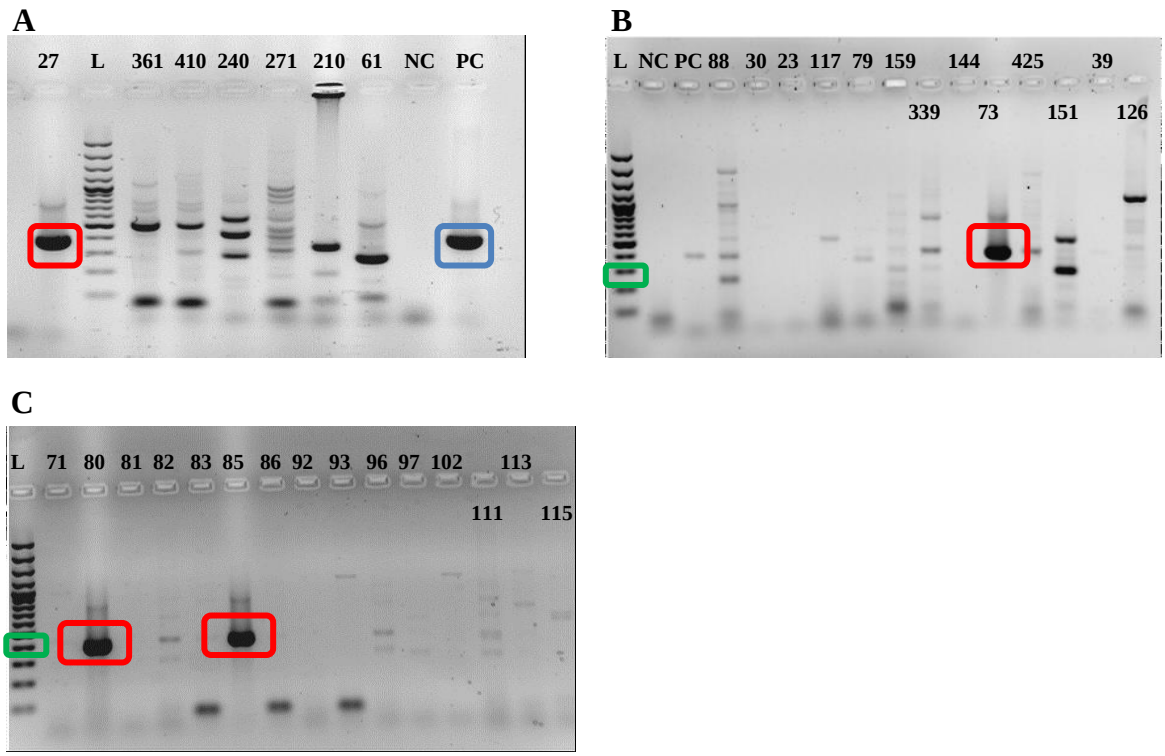

Figure 4.1: Gel showing results of samples ran on gel for Arenavirus screening. N27 (A), N73 (B), N80 and N85 (C) (those enclosed in red squares) were the only samples that were detected to be positive for Arenavirus for this study. Note: L = GeneRuler 100bp Plus DNA Ladder; NC = Negative Control; PC = Positive Control; red boxed = positive results; green boxed = expected band size (400 bp); blue box = positive control; all numbers are reference numbers that were designated to the samples screened for the viruses.

The Arenavirus PCR detected four positive samples, three of which were from the lung tissue of rodents caught in Okahandja (N73, N80, and N85) and one from Mariental (N27). Sequencing and phylogenetic analysis of partial L gene sequence showed that the virus sequences from Okahandja samples are closely related to Merino Walk Arenavirus known from South Africa (host: *Myotomys unisulcatus*), while the Mariental sample material is related to viruses from Lassa virus and Ippy virus clades (Figure 4.1).

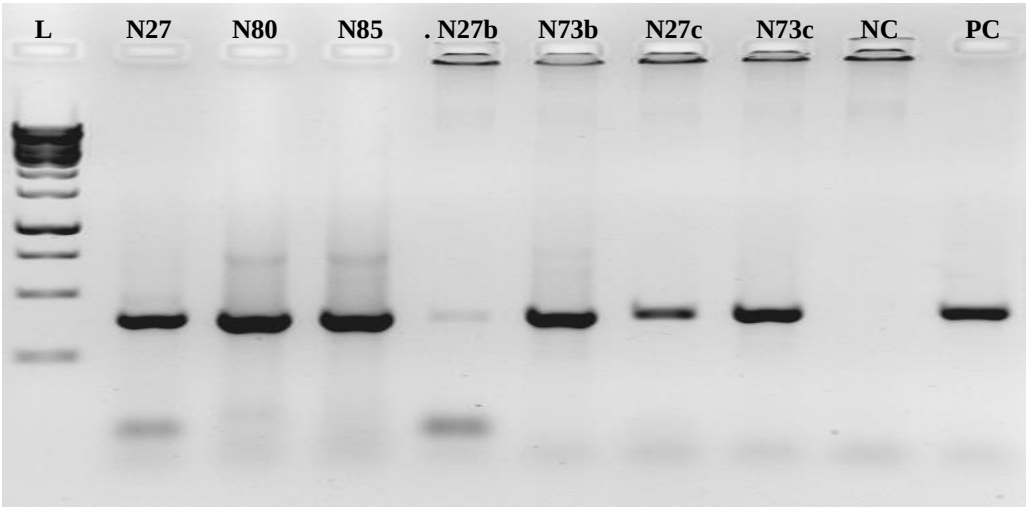

Figure 4.2: DNA products of positive samples ran on gel. Note: L = GeneRuler 1kb DNA Ladder; NC = Negative Control; PC = Positive Control; N27, 73, 80 and 85 were the references designated for the samples tested for Arenaviruses for this study.

Figure 4.2 shows results of confirmation of positive samples that were run on a gel through electrophoresis. The bands had expected size (approximately 400 bp) of the positive control band and therefore were confirmed to be positive for Arenavirus.

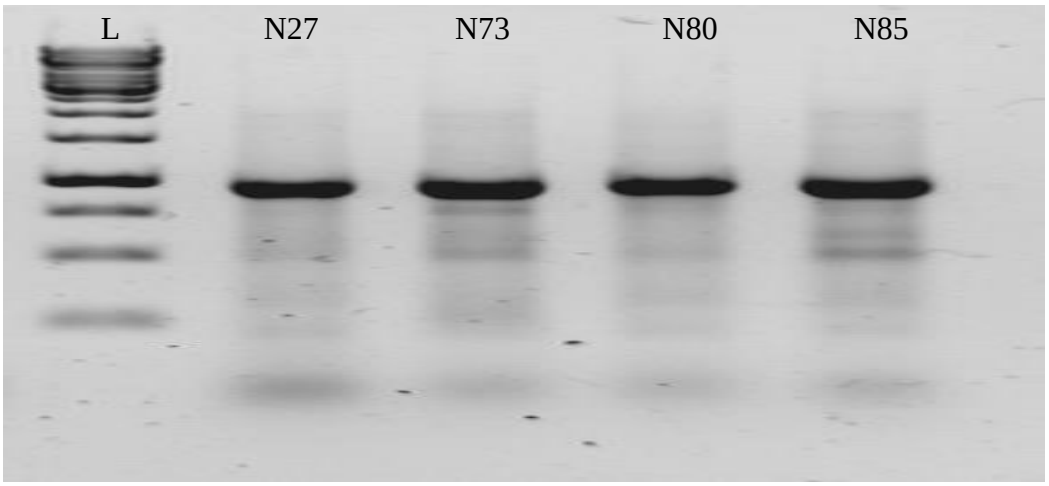

Figure 4.3: DNA products ran on a gel for confirmation of host species (Cytochrome b). Note: L = GeneRuler 100bp Plus DNA Ladder; N73, N80, and N85 were the references designated for the samples tested for Arenaviruses for this study.

Figure 4.3 shows the gel results from the Cytochrome B analysis. The analysis was conducted to confirm the host species of the four samples that tested positive for Arenavirus. The results confirmed that the four host species from which Arenavirus positive samples were obtained, were all Namaqua rock mouse, *Micaelamys namaquensis* belonging to the Family Murinae.

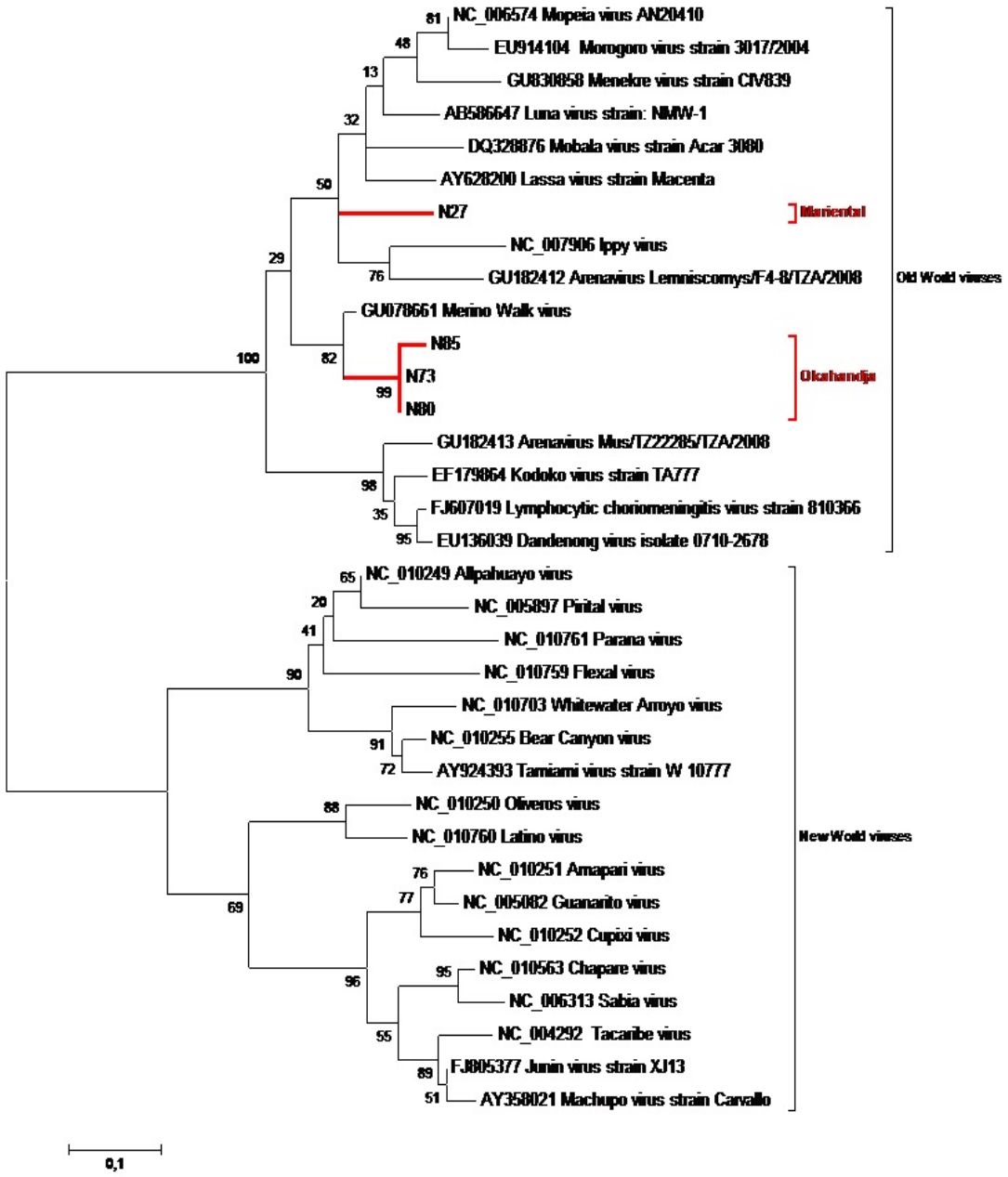

Figure 4.4: Molecular phylogenetic analysis based on partial L gene sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Statistical bootstrap analysis consisted of 500 repetitions.

4.3. Fleas

4.3.1. Occurrence of small mammals and fleas

In order to investigate patterns of infestation of small mammal fleas, only fleas collected from hosts captured in Rundu (Broad-leaved biome, designated North), Neudamm (Acacia savannah biome, designated Central), and Mariental (Nama karoo biome, designated South) were utilised. A total of 218 small mammal hosts, representing 8 rodents, 3 shrew species, and 1 sengi species were examined for the presence of fleas in this study. Of these hosts examined, 114 rodents, representing 5 species, were infested with fleas (Table 4.2). There were no fleas collected from any of the shrew (n=7) and sengis (n=2) hosts collected for the study. Considering the 114 rodent host species infested with fleas, regardless of where they were trapped, a total of 358 fleas, were collected from small mammals and these represented 9 different species of fleas (Table 4.3). Table 4.2 indicates the number of host species captured in each respective region and the number of hosts infested by fleas.

Table 4.2: Number of individual host species captured and infested, per region.

Region	Species	Total	Infested
Rundu	*Gerbilliscus leucogaster*	16	6
	Mastomys sp.	25	9
	Micaelamys namaquensis	2	1
Neudamm	*Gerbilliscus leucogaster*	11	8
	Mastomys sp.	3	2
	Micaelamys namaquensis	65	46
	Rhabdomys pumilio	23	16
	Saccostomus campestris	1	1
Mariental	*Gerbilliscus leucogaster*	22	9
	Mastomys sp.	11	5
	Micaelamys namaquensis	11	6
	Rhabdomys pumilio	9	5

The Namaqua rock mouse, *Micaelamys namaquensis* (A. Smith, 1834) was most prevalent amongst the captured small mammals (46.5%), followed by the bush veld gerbil, *Gerbilliscus leucogaster* (Peters, 1852) (20.2%) whereas, the pouched mouse, *Saccostomus campestris* (Peters, 1846) was the least captured host (0.009%). From Table 4.3, it is evident that the flea species *Xenopsylla cheopis* was prevalent on all host species caught, irrespective of the location while the flea species *Listropsylla aricinae* had the highest number of individuals amongst all the flea species prevalent on the small mammal hosts, with a total number of 230 individual fleas infesting the hosts. The least prevalent flea species was *Chiastopsylla rossi* with only two individuals of the flea species collected from two different host species presented in Table 4.3. Host species *M. namaquensis* was infested with the highest total number

of fleas collected from the different host species (n=210), followed by *Mastomys* sp. (Thomas, 1915) (n=55), the four-striped mouse *Rhabdomys pumilio* (Sparrmann, 1784) (n=49), and then *S. campestris* (n=1) with the least number of flea species collected. The flea species *Xenopsylla cheopis* was prevalent on all five of the host species trapped, regardless of location (Table 4.3). *Listropsylla aricinae*, which had the highest number of fleas collected from all the different host species, was only collected from four out of the five individual host species but with about 76.7% collected from *M. namaquensis*. *Micaelamys namaquensis* was the only host that was infested with all nine of the flea species collected from the hosts, followed by *Mastomys* sp., which was infested with seven flea species, and lastly *S. campestris*, which was infested with only one flea species (Table 4.3).

Table 4.3: Host species caught in relation to the numbers of the different flea species examined on the host, irrespective of the region, irrespective of the region (Rundu, Neudamm and Mariental) of capture of the hosts. n=: refers to the total number of individual host species caught and the number of flea species collected from all the infested hosts.

	Gerbilliscus leucogaster (n=23)	*Mastomys* sp. (n=16)	*Micaelamys namaquensis* (n=53)	*Rhabdomys pumilio* (n=21)	*Saccostomus campestris* (n=1)
Chiastopsylla rossi (n=2)	0	0	2	0	0
Listropsylla aricinae (n=230)	16	29	161	23	1
Xenopsylla brasiliensis (n=14)	4	7	3	0	0
Xenopsylla cheopis (n=41)	16	9	10	6	0
Xenopsylla hirsuta (n=4)	0	3	1	0	0
Xenopsylla nubica (n=14)	0	0	12	2	0
Xenopsylla philoxera (n=13)	0	1	4	8	0
Xenopsylla piriei (n=8)	6	1	1	2	0
Xenopsylla versuta (n=32)	1	5	16	10	0
TOTAL	**43**	**55**	**210**	**49**	**1**
Flea species richness	**5**	**7**	**9**	**5**	**1**

The Jaccard's index of similarity of species of fleas amongst the three regions where small mammal hosts were trapped, revealed that there was high similarity of species of fleas of 60%, 75%, and 80% between the central and north, north and south, and central and south regions, respectively (Table 4.3).

Table 4.4: Jaccard's similarity index (J) comparing species of fleas collected from small mammal hosts captured each region among the three regions of study.

Regions	**North**	**Central**	**South**
North	-	-	-
Central	0.600	-	-
South	0.750	0.800	-

4.3.2. Prevalence of infestation

The prevalence of infestation of fleas on different small mammal hosts and for males and female hosts that were trapped in Mariental (Southern region), are presented in Figure 4.5 and Figure 4.6, respectively.

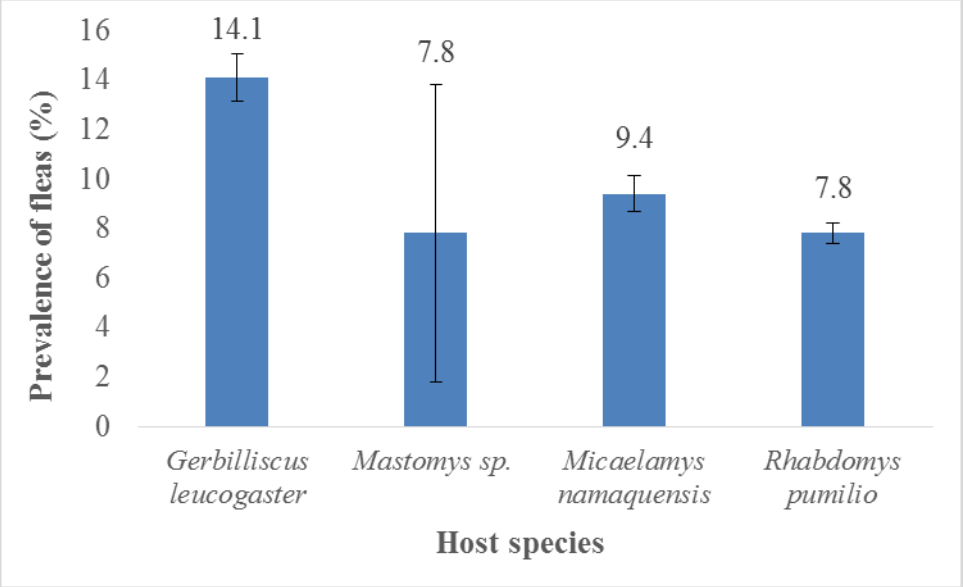

Figure 4.5: Prevalence (%) of infestation of fleas on small mammal host species trapped in the Mariental. Note that the bars represent the standard error of the mean.

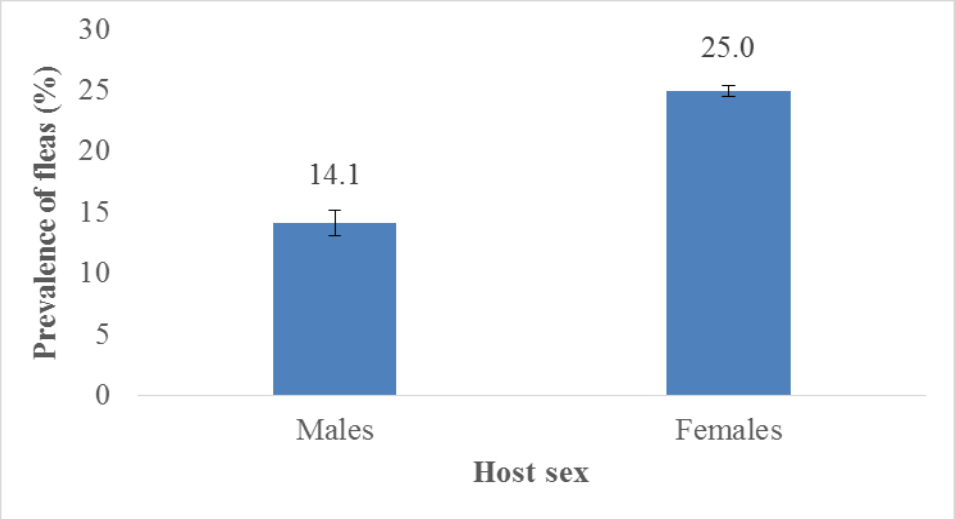

Figure 4.6: Overall prevalence (%) of fleas infesting male and female hosts in Mariental. Note that the bars represent the standard error of the mean. Males hosts (n=9) and female hosts (n=16).

The outcome of the GLM analysis for the prevalence of infestation of hosts trapped in Mariental are presented in Table 4.5.

Table 4.5: Results of Generalised Linear Model evaluating the influence of host sex, body mass (g), head-body length (mm) and reproductive status (breeding or non-breeding) on the prevalence of fleas infesting small mammal hosts in Mariental.

Variable	Estimate	df	P value
Sex (Male)	0.764	63	0.2249
Body mass	0.061		0.0532
Head-body length	-0.023		0.2745
Reproductive status (Breeding)	1.171		0.0864

The results of the GLM analysis revealed that there was no significant influence of sex on the prevalence of fleas on small mammal hosts in Mariental ($p>0.05$, $df=63$) although the positive association suggests that males may be more likely to be infested than females (Table 4.5). The results further show that body mass did not significantly influence the prevalence of infestation of fleas on small mammals ($p>0.05$, $df=63$) (Table 4.5). Similarly, host body size had no significant influence on the prevalence of fleas infesting small mammal hosts in Mariental ($p>0.05$, $df=63$) (Table 4.5). The results from the GLM analysis also revealed that the host's reproductive status did not significantly influence the prevalence of flea infestation ($p>0.05$, $df=63$) (Table 4.5).

The prevalence of infestation of fleas on different small mammal hosts and for males and female hosts that were trapped in Neudamm (Central region) are presented in Figure 4.7 and Figure 4.8, respectively.

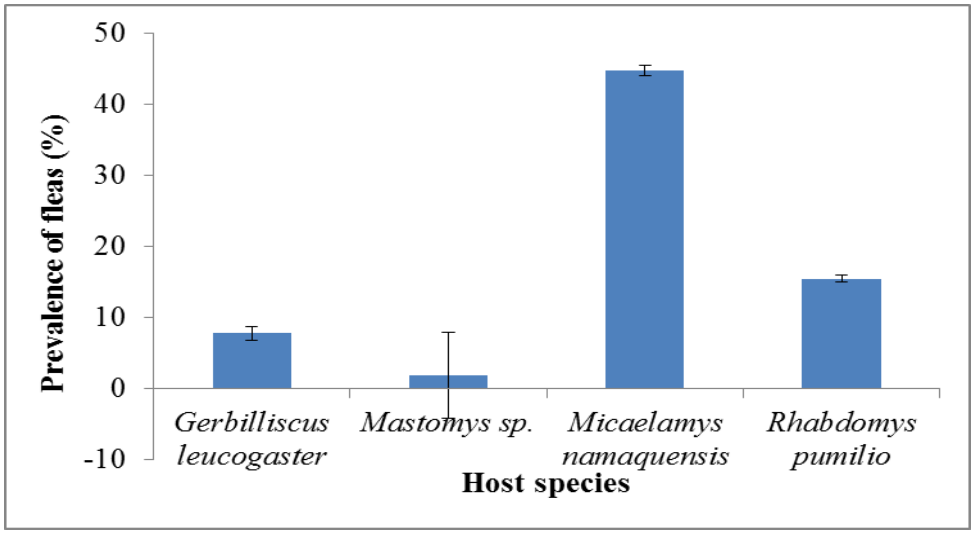

Figure 4.7: Prevalence (%) of infestation of fleas on small mammal host species trapped in the Neudamm. Note that the bars represent the standard error of the mean.

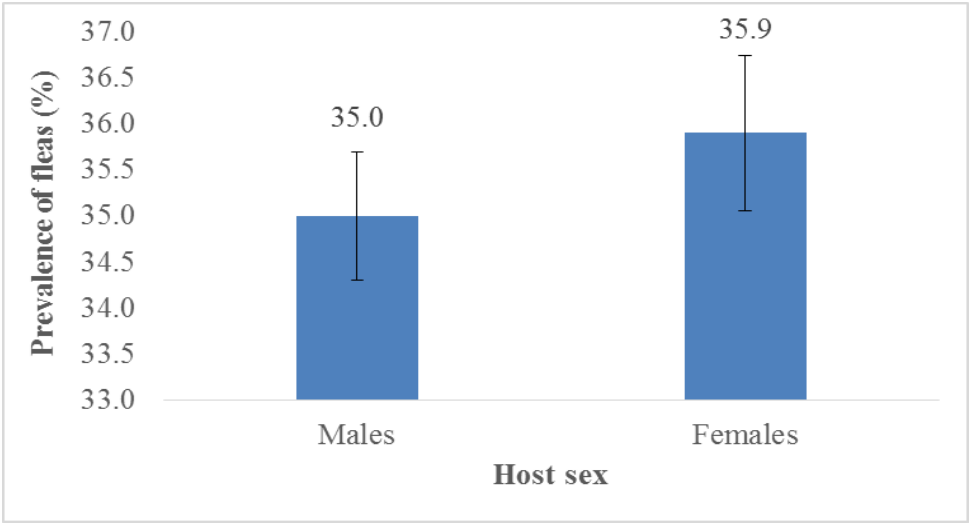

Figure 4.8: Overall prevalence (%) of fleas infesting male and female hosts in Mariental. Note that the bars represent the standard error of the mean. Males hosts (n=36) and female hosts (n=37).

The outcomes of the GLM analysis for the prevalence of infestation of fleas on small mammal hosts trapped in Neudamm are presented in Table 4.6.

Table 4.6: Results of Generalised Linear Model evaluating the influence of host sex, body mass (g), head-body length (mm) and reproductive status (breeding or non-breeding) on the prevalence of fleas infesting small mammal hosts in Neudamm.

Variable	Estimate	df	P value
Sex (Male)	-0.069	108	0.883
Body mass	-0.00014		0.997
Head-body length	0.047		0.233
Reproductive status (Breeding)	0.201		0.792

The GLM analysis revealed that host sex did not significantly influence the prevalence of fleas infesting small mammal hosts in Neudamm ($p>0.05$, df=108) (Table 4.6). In addition, host's body mass was shown to have significantly influenced the prevalence of flea infestation on small mammals hosts ($p>0.05$, df=108), as shown in Table 4.6. In Neudamm, small mammal body sizes also did not exert a significant influence in the prevalence of flea infestation (>0.05, df=108). Similarly, the GLM analysis results showed that the reproductive status of small mammals hosts trapped in Neudamm, did not significantly influence the prevalence of flea infestation ($p>0.05$, df=108) (Table 4.6).

The prevalence of infestation of fleas on different small mammal hosts and for males and female hosts that were trapped in Rundu (Northern region) are presented in Figure 4.9 and Figure 4.10, respectively.

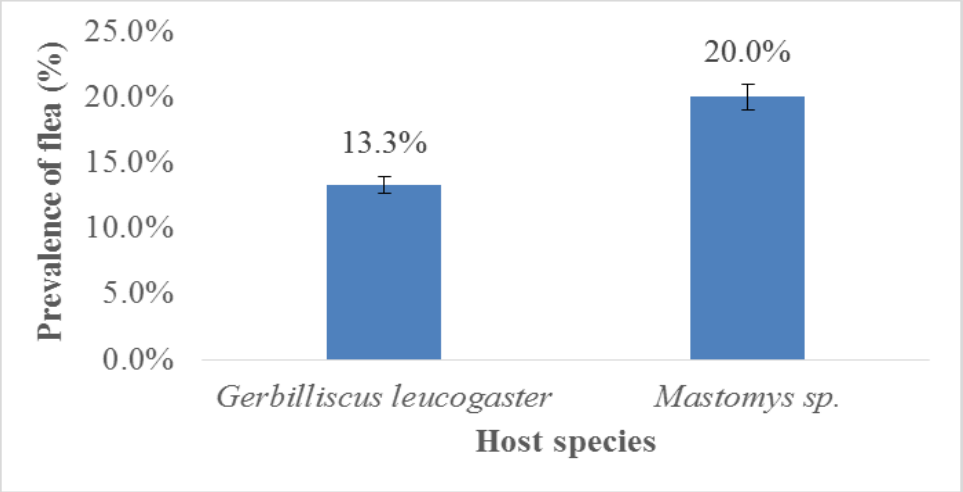

Figure 4.9: Prevalence (%) of infestation of fleas on small mammal host species trapped in the Rundu. Note that the bars represent the standard error of the mean.

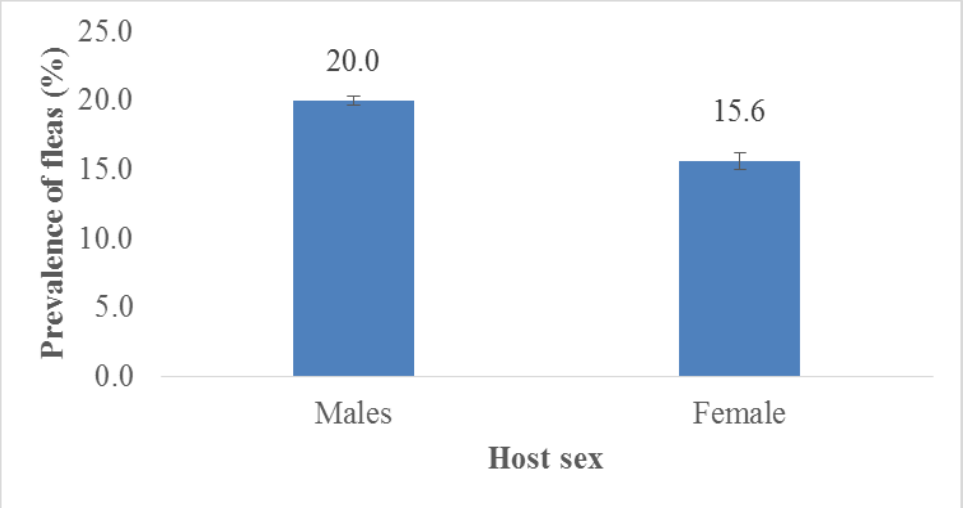

Figure 4.10: Overall prevalence (%) of fleas infesting male and female hosts in Rundu. Note that the bars represent the standard error of the mean. Males hosts (n=7) and female hosts (n=9).

The results of the GLM analysis for the prevalence of infestation of hosts trapped in Rundu are presented in Table 4.7.

Table 4.7: Results of Generalised Linear Model evaluating the influence of host sex, body mass (g), head-body length (mm) and reproductive status (breeding or non-breeding) on the prevalence of fleas infesting small mammal hosts in Rundu.

Variable	Estimate	df	P value
Sex (Male)	0.520	44	0.454
Body mass	0.007		0.720
Head-body length	0.006		0.705
Reproductive status (Breeding)	-0.238		0.784

Results from the GLM analysis revealed that host sex did not significantly affect the prevalence of fleas infesting small mammal hosts in Rundu ($p>0.05$, $df=44$) (Table 4.7). The results further reveal a non-significant influence by host body mass on the prevalence of flea infestation ($p>0.05$, $df=44$) (Table 4.7). Host size did not significantly influence the prevalence of fleas infesting small mammal hosts in Rundu ($p>0.05$, $df=44$) (Table 4.7). Host reproductive status did not influence the prevalence of flea infestation significantly ($p>0.05$, $df=44$) (Table 4.7) on small mammals trapped in Rundu.

The prevalence of infestation of fleas on small mammal host, *G. leucogaster*, trapped in the three regions of study, is presented in Figure 4.11.

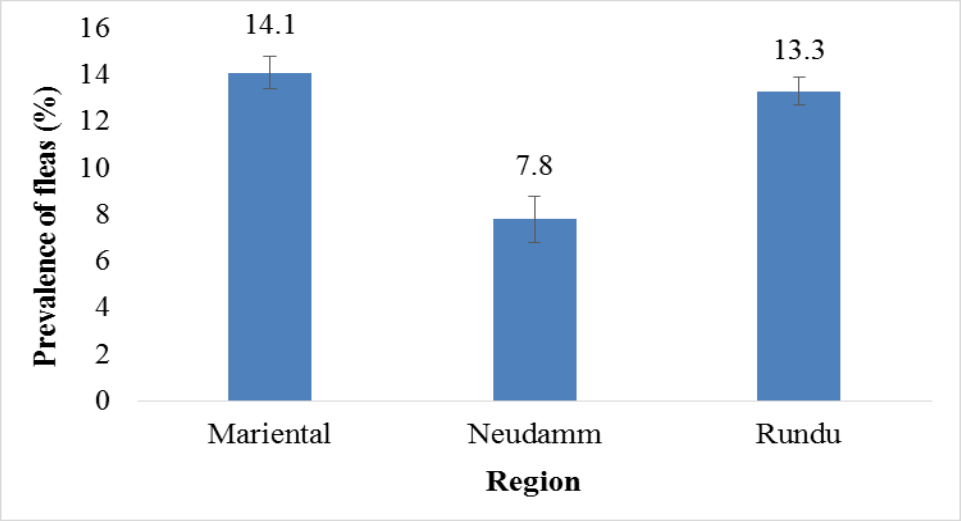

Figure 4.11: Prevalence of *G. leucogaster* in the three regions of study. Note that the bars represent the standard error of the mean. Mariental (n=9), Neudamm (n=8), Rundu (n=6).

The results of the GLM analysis for the prevalence of infestation of fleas infesting *G. leucogaster*, are presented in Table 4.8.

Table 4.8: Results of Generalised Linear Model evaluating the influence of host sex, body mass (g), head-body length (mm) and reproductive status (breeding or non-breeding) on the prevalence of fleas infesting small mammal hosts for *G. leucogaster* in the three sites / Regions

Variable	Estimate	df	P value
Sex (Male)	0.237	48	0.778
Body mass	0.014		0.624
Head-body length	-0.000099		0.995
Reproductive status (Breeding)	0.560		0.513
Neudamm	0.982		0.252
Rundu	-0.446		0.571

Gerbilliscus leucogaster was the only host species represented in all three regions of this study (Figure 4.11). There was no significant influence of host sex on the prevalence of fleas infesting *G. leucogaster* ($p>0.05$, df=48). There was no significant difference in the prevalence of fleas infesting *G. leucogaster* trapped in Mariental and Neudamm ($p>0.05$, df=48) and those trapped in Mariental and Rundu ($p>0.05$) (Table 4.8). There was also no significant difference seen with regard to body mass and its influence on the prevalence of flea infestation ($p>0.05$, df=48) (Table 4.8). Similarly, the body length of hosts did not significantly influence the prevalence of fleas infesting *G. leucogaster* ($p>0.05$, df=48) (Table 4.8). Reproductive state did not significantly influence the prevalence of flea infestation ($p>0.05$, df=48) (Table 4.8). Though the differences were not significant, the prevalence of fleas infesting *G. leucogaster* at Neudamm was higher than the prevalence fleas infesting *G. leucogaster* trapped in Mariental ($p>0.05$, df=48). It was also revealed that *G. leucogaster* trapped at Rundu had a lower prevalence of infestation compared to those captured at Mariental, though not significant ($p>0.05$, df=48) (Table 4.8).

4.3.3. Intensity of infestation

4.3.3.1. Intensity of infestation by region

The intensity of infestation of fleas on different small mammal hosts trapped in Mariental (Southern region) is presented in Figure 4.12.

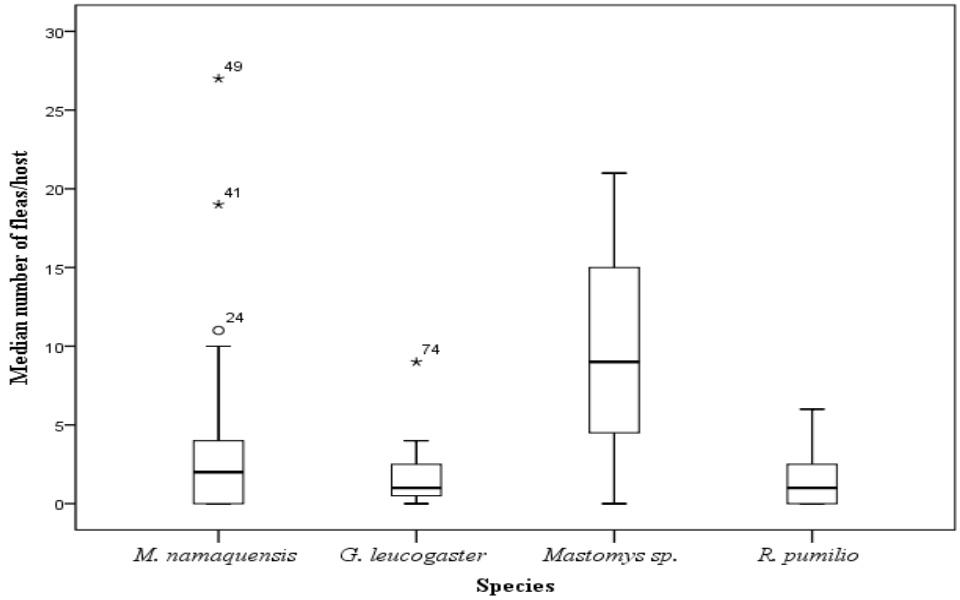

Figure 4.12: Median number of fleas infesting individual rodent species in Mariental. The line in the open box is the median. The top part of each open box represent represents the 75th percentile (upper quartile) while the lower part is the 25th percentile (lower quartile). The end of each line above the open box indicates the largest value that is not an outlier. Asterisk represents extreme values.

The results of the GLM analysis for the intensity of infestation of fleas on small mammal hosts trapped in Mariental are presented in Table 4.9.

Table 4.9: Results of Generalised Linear Model evaluating the influence of host sex, body mass, head-body length and reproductive status on the intensity of flea infestation on small mammals trapped in Mariental. Value with * indicates significant variables.

Variable	Estimate	df	P value
Sex (Male)	0.705	63	0.0048*
Body mass	0.024		0.087
Head-body length	-0.0016		0.873
Reproductive status (Breeding)	0.550		0.110

Host sex revealed a significant positive association with the intensity of infestation of fleas on small mammals in Mariental ($p<0.05$, df=64) (Table 4.9). Male host had higher intensity of flea infestation compared to female hosts. There was no significant influence of host body mass on the intensity of flea infestation ($p>0.05$, df=63). Host body length did no significantly influence the intensity of infestation of fleas on small mammals in Mariental ($p>0.05$, df = 63) (Table 4.9). It was also observed that intensity of infestation was not significantly influenced by host reproductive status ($p>0.005$, df=63) (Table 4.9).

The intensity of infestation of fleas on different small mammal hosts trapped in Mariental (Southern region) is presented in Figure 4.13.

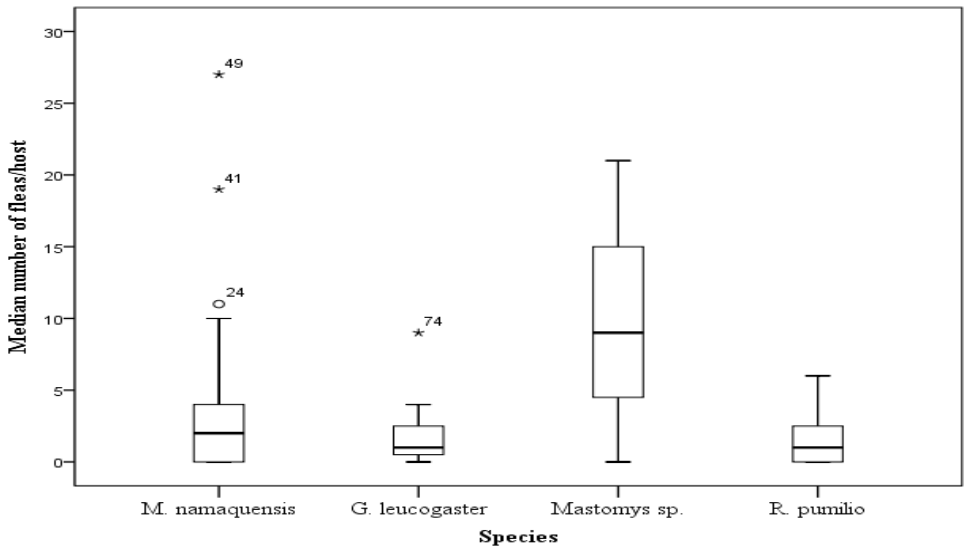

Figure 4.13: Median number of fleas infesting individual rodent species in Neudamm. The line in the open box is the median. The top part of each open box represent represents the 75th percentile (upper quartile) while the lower part is the 25th percentile (lower quartile). The end of each line above the open box indicates the largest value that is not an outlier. Asterisk represents extreme values.

The results of the GLM analysis for the intensity of infestation of fleas on small mammal hosts trapped in Neudamm are presented in Table 4.10.

Table 4.10: Results of Generalised Linear Model evaluating the influence of host sex, body mass, head-body length and reproductive status on the intensity of flea infestation in Neudamm. Values with * indicates significant variables.

Variable	Estimate	df	P value
Sex (Male)	0.179	108	0.153
Body mass	-0.034		0.00300*
Head-body length	0.031		0.00518*
Reproductive status (Breeding)	0.357		0.179

In Neudamm, host sex did not significantly influence the intensity of fleas infesting small mammal hosts ($p>0.05$, df =108). However, it was observed that host body mass significantly influenced the intensity of flea infestation, in Neudamm ($p<0.05$, df=108). It was further revealed that host body size significantly influenced the intensity of fleas infesting small mammal hosts ($p<0.05$, df =108). The GLM analysis showed a non-significant influence of host reproductive state on the intensity of infestation of fleas on small mammal hosts ($p>0.05$, df=108) (Table 4.10).

The intensity of infestation of fleas on different small mammal hosts trapped in Neudamm (Central region), is presented in Figure 4.14.

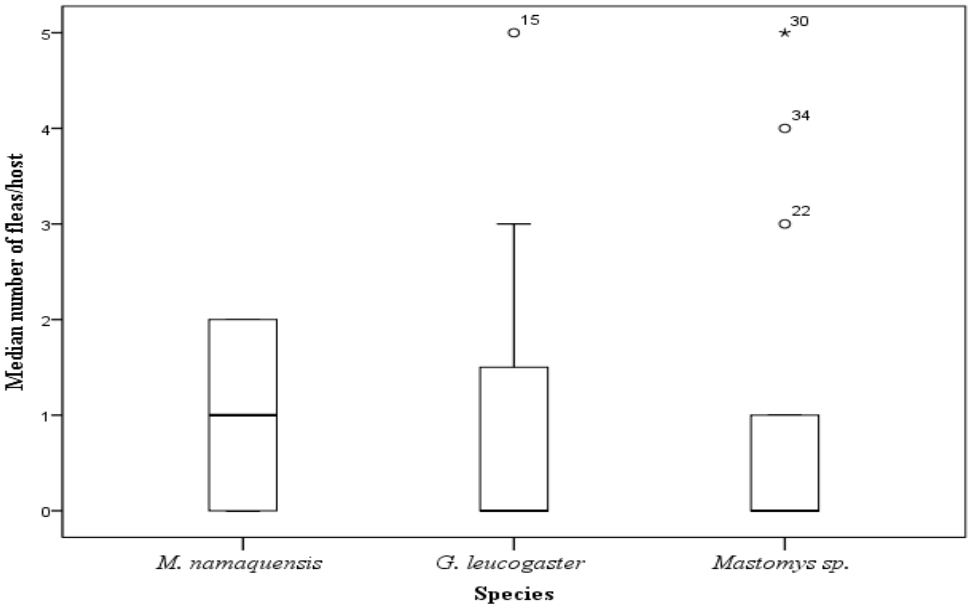

Figure 4.14: Median number of fleas infesting individual rodent species in Rundu. The line in the open box is the median. The top part of each open box represent represents the 75th percentile (upper quartile) while the lower part is the 25th percentile (lower quartile). The end of each line above the open box indicates the largest value that is not an outlier. Asterisk represents extreme values.

The results of the GLM analysis for the intensity of infestation of hosts trapped in Rundu are presented in Table 4.11.

Table 4.11: Results of Generalised Linear Model evaluating the influence of host sex, body mass, head-body length and reproductive status on the intensity of fleas infesting small mammals in Rundu.

Variable	Estimate	df	P value
Sex (Male)	-0.065	48	0.862
Body mass	0.0023		0.863
Head-body length	0.015		0.286
Reproductive status (Breeding)	-0.088		0.857

Results from the GLM analysis show that host sex did not have a significant influence on the intensity of fleas infesting small mammal hosts in Rundu ($p>0.05$, $df=44$) (Table 4.11). The intensity of flea infestation was not significantly influenced by host body size ($p>0.005$, $df=44$) (Table 4.11). Similarly, results showed that host body length did not significantly influence the intensity of flea infestation ($p>0.05$, $df=44$) (Table 4.11). Furthermore, host reproductive state did not show a significant influence on the intensity of fleas infesting small mammal hosts in Rundu ($p>0.05$, $df=44$) (Table 4.11).

The intensity of infestation of fleas on small mammal host, *G. leucogaster*, trapped in the three regions of study, is presented in Figure 4.15.

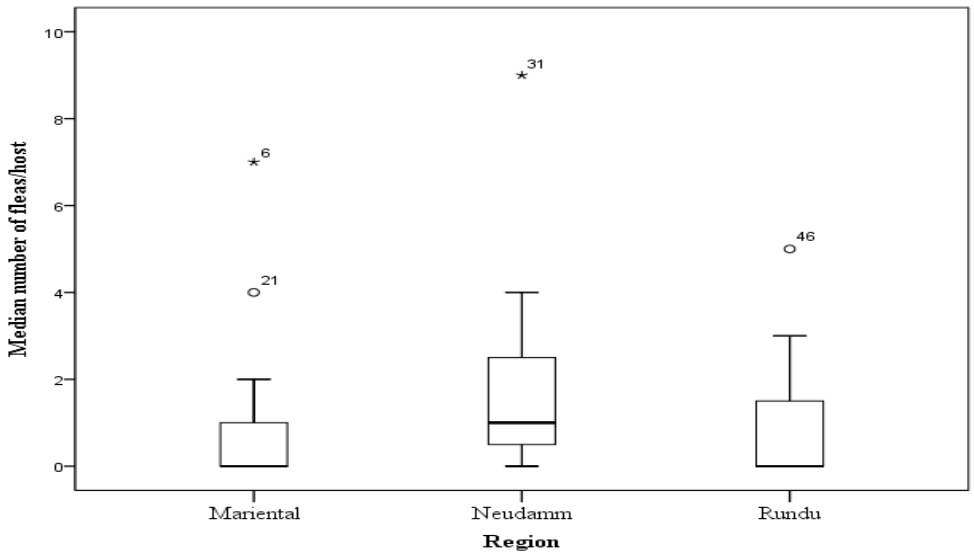

Figure 4.15: Median number of fleas infesting individual rodent species in *G. leucogaster* in the three regions of study. The line in the open box is the median. The top part of each open box represent represents the 75th percentile (upper quartile) while the lower part is the 25th percentile (lower quartile). The end of each line above the open box indicates the largest value that is not an outlier. Asterisk represents extreme values.

The results of the GLM analysis for the intensity of infestation of fleas infesting *G. leucogaster*, in the three selected regions (Mariental, Rundu and Neudamm) are presented in Table 4.12.

Table 4.12: Results of Generalised Linear Model evaluating the influence of host sex, body mass, head-body length and reproductive status on the intensity of flea infestation for *G. leucogaster*.

Variable	Estimate	df	P value
Sex (Male)	-0.395	44	0.324
Body mass	0.013		0.327
Head-body length	0.004		0.621
Reproductive status (Breeding)	- 0.034		0.931
Neudamm	0.658		0.053
Rundu	-0.137		0.727

Results from the GLM analysis show that host sex did not significantly influence the intensity of fleas infesting *G. leucogaster* amongst the three regions of study ($p>0.05$, df=44) (Table 4.12). The mass of *G. leucogaster* did not significantly influence the intensity of flea infestation ($p>0.05$, df=48) (Table 4.12). No significant influence of body length on the intensity of flea infestation was revealed by the GLM analysis ($p>0.05$, df=48) (Table 4.12). Similarly, the reproductive status of host did not have a significant influence on the intensity of flea infestation ($p>0.05$, df=48) (Table 4.12). There was no significant influence of the intensity of flea infestation on small mammals recorded amongst the three regions of study in relation to the intensity of fleas infesting *G. leucogaster* (Figure 4.15). The intensity of infestation of fleas on small mammals trapped in Neudamm were marginally significantly different from those trapped in Mariental ($p>0.05$, df=48) (Table 4.12). However, the intensity of

infestation of fleas on small mammal hosts trapped in Rundu, were not significantly different from hosts captured in Mariental ($p>0.05$, $df=48$) (Table 4.12).

CHAPTER 5 DISCUSSION

The objectives of this study were to survey small mammals for Hantaviruses and Arenaviruses and to determine the prevalence and intensity of fleas infesting small mammals in selected regions of Namibia.

5.1. Hantavirus

In this study, no Hantaviruses were detected in the 518 lung samples analysed. The absence of Hantavirus in the samples screened for this study cannot exclude the possibility for the existence of a Hantaviruses in the small mammals of Namibia. For the initial study of Hantaviruses in Guinea (West Africa), in which they also screened over 2,500 rodent organs, only two PCR products of expected size were obtained. The first virus, named the Sangassou virus (SANGV) (Klempa *et al*., 2006), was found in the African wood mouse, *Hylomyscus simus*. The second virus was named Tanganya virus (TGNV) and was detected from a male Therese shrew *Crocidura theresae* (Klempa, Tkachenko, Dzagurova, Yunicheva, Morozov, Okulova, Slyusareva, Smirnov and Krüger, 2007). Both viruses represent the first recorded indigenous African Hantaviruses and were both respectively named after the villages around which they were trapped. Tanganya viruses represented the second non-rodent borne hantavirus (Therese's shrew) as the first one was isolated from an Asian house shrew (*Suncus murinus*) in India and as afore mentioned is called the Thottapalayam virus (Yadav *et al*., 2007).

Further studies were undertaken to determine the true host reservoir of Sangassou virus. The initial study, as mentioned above, only had one representative of the rodent host species that yielded a positive PCR produce for SANGV. It was unclear whether the *H. simus* was the natural host for SANGV since it was detected in only 1 out of 4 African wood mice and therefore did not provide irrefutable proof that *H. simus* was the reservoir host (Klempa, Witkowski, Popugaeva, Auste, Koivogui, Fichet-Calvet, Strecker, ter Meulen, and Krüger, 2012). In Klempa *et al*. (2012) one additional SANGV sequence was detected in another *H. simus* host. This increased the probability that *H. simus* is the natural reservoir of SANGV. This suggests that even in Namibia, the possibility of Hantavirus in small mammals cannot be ruled out despite failure to detect it in host species in this current study.

It is speculated that the small sample size in the present study might have been a contributing factor to failure to detect Hantavirus in the rodents screened. Another possible explanation is that the virus is quite fragile and storage or handling conditions might not have been optimal. Another explanation is the case of sequence disparity where the PCR assay might have missed detecting distantly related Hantaviruses (Dr. P. Witkowski, personal communication). It should also be noted that the number of non-rodent animals investigated for the presence of Hantaviruses was low and therefore the presence of Hantavirus in non-rodent small mammals in Namibia cannot be ruled out.

Screening for Hantaviruses was also conducted at the University of Stellenbosch where more than 2,317 lung tissue samples were used for the screening between 2009 and 2013. The results for that study also yielded no detection of a new African Hantavirus (Dr. N. Ithete, personal communication). Hantaviruses might not have been detected because it is either not prevalent in Namibia or because it is not prevalent in the areas that were included for the present study. More sampling can be conducted in regions that were excluded from this present study and the collected organs can be screened for the presence of Hantaviruses.

A rapid immunochromatographic test was developed by Sirola, Kallio, Koistinen, Kuronen, Lundkvist, Vaheri, Vapalahti, Henttonen, and Närvänen (2004), to detect anti-PUUv IgG antibodies in *Clethrionomys glareolus*. Tests conducted using fresh and undiluted blood samples resulted in 100% efficacy whilst test with frozen and diluted blood samples resulted in 91% efficacy (Sirola *et al*., 2004).

Amada, Yoshimatsu, Yasuda, Shimizu, Koma, Hayashimoto, Gamage, Nishio, Takakura, and Arikawa (2013) developed an immunochromatographic (ICG) test to detect antibodies that are specific to Hantavirus. They had used sera from *Rattus* species for their study and it was revealed that the ICG was as sensitive as the ELISA test and about 100 times higher than IFA (Amada, *et al*., 2013). They found that the test was able to detect antibodies to SEOV, Hantaan, Dobrava/Belgrade, and Thailand viruses (Amada, *et al*., 2013).

5.2. Arenavirus

In this study, two, possibly new, Arenaviruses were detected in the 518 lung samples analysed. The criteria used for assigning a virus in the genus *Arenavirus* to a new species are: (i) association with a specific host species or group of species; (ii) presence in a defined geographical area; (iii) etiological agent (or not) of disease in humans; (iv) significant differences in antigenic cross-reactivity, including lack of cross-neutralization activity where applicable; and (v) significant divergence from other species in the genus at the amino acid level (Charrel, De Lamballerie, and Emonet, 2008; Emonet *et al*, 2009). A new isolate will be placed into an existing species if it shares with its members 4 or 5 common characters (Emonet *et al*., 2009), whereas a tentative species will be proposed if it presents at least two differences with any other Arenavirus (Charrel *et al*, 2008; Emonet *et al*, 2009).

The present study has isolated two Arenaviruses from the Namaqua Rock Mouse – *M. namaquensis* (Figures 4.2 and 4.3). These two viruses have not been recorded before in the rock mouse and therefore are likely to be new Arenaviruses. This is supported by the fact that they have not been recorded anywhere in Africa or the world except now in Namibia, in the present study and that phylogenetic reconstruction reveals (Figure 4.4) significant difference from other species in the genus.

For now, one can safely assume that the natural host for the two Namibian Arenaviruses is *M. namaquensis* (Family: Muridae) (Figure 4.3). Arenaviruses however have been isolated and discovered from other rodent species, for example in the Natal Multimammate Mouse, *Mastomys natalensis* (Family Muridae), (Ishii, Thomas, Moonga, Nakamura, Ohnuma, Hang'ombe, Takada, Mweene, and Sawa, 2011). As suggested by Ishii *et al*. (2011) rodents of the Family Muridae may be the natural reservoir of Arenavirus in Africa. Discovery of a new Arenavirus in the present study that belongs to the Family Muridae lends support to the suggestion by Ishii *et al*., (2011). More studies that are similar should be carried out in Africa and in rodents of Family Muridae, to discover more Arenaviruses to be added to the list of Arenaviruses prevalent in small mammal hosts. The two Arenaviruses discovered in the present study form part of the Old World Arenaviruses and hence increases the number of recognized species of Old World Arenaviruses (Figure 4.4). Sequencing and phylogenetic analysis of partial L gene sequence showed that the virus sequences from the Okahandja samples are closely related to Merino Walk Arenavirus known from South Africa (host: *Myotomys unisulcatus*), while the Mariental sample material is related to Lassa virus and Ippy virus clades (Figure 4.4).

Until 2007, Lassa virus was the only known Arenavirus to cause haemorrhagic fever in Africa. However, during September–October 2008, 5 haemorrhagic fever cases caused by a novel Arenavirus named Lujo virus occurred in South Africa (Ishii *et al*., 2011). The initial case occurred in Zambia; the patient was transported to South Africa for treatment, where the virus spread to 4 other persons. Four patients died; the source of infection in the index patient was not determined (Ishii *et al*, 2011). In

their study to further the epizootiologic understanding of Arenaviruses, they investigated their prevalence and genetic background among *M. natalensis* rodents in Zambia during May 22–August 28, 2009 (Ishii *et al*, 2011). Their trapping was conducted in Lusaka, Mufwe and Namwala and they used the kidney tissues to screen for the presence of Arenaviruses, using the RT-PCR method. Their results showed positives from four samples collected from Lusaka and one sample collected from Namwala (Ishii *et al*, 2011). The Lusaka and Namwala strains were classified as members of the Old World Arenaviruses; both strains are closely related to the Mobala, Morogoro, and Mopeia viruses. Thus, they concluded that the Zambian strains belong to the same virus species and that the novel Arenavirus differs from other known strains and proposed that these Zambian strains be designated Luna virus (Lusaka-Namwala) (Ishii *et al*, 2011).

Merino Walk virus (MWV), a proposed novel tentative species of the family Arenaviridae, was isolated from the Bush Karoo Rat, *Myotomys* (*Otomys*) *unisulcatus*, collected at Merino Walk, Eastern Cape, South Africa, in 1985. Full-length genomic sequence confirmed MWV as an Arenavirus related distantly related to Mobala, Mopeia and Ippy viruses, all members of the Old World Arenavirus complex. MWV was proposed as a tentative novel species in the Lassa–lymphocytic choriomeningitis virus complex, based on its isolation from a novel rodent species and its genetic and serological characteristics (Palacios, Savji, Hui, da Rosa, Popov, Briese, Tesh and Lipkin, 2010).

In an article written by Charrel *et al* (2008), it was noted that eight new Arenaviruses were discovered, of which two were human pathogens. Charrel *et al* (2008) added that this strongly suggests that many more Arenaviruses may be discovered in the future, and this study confirms, with the discovery of two, possibly new Arenaviruses.

There have been reports of cases of hemorrhagic fever in humans, in Namibia, particularly, Crimean-Congo hemorrhagic fever (CCHF) and Rift Valley fever (RVF) (Joubert, Prozesky, Lourens, van Straten, Theron, Swanevelder, Meenehan, and Van der Merwe, 1985; Kisting, 2010; Monaco, Pinoni, Cossedd, Khaiseb, Calistri, Molini, Bishi, Conte, Scacchia, and Lelli, 2013; Noden and van der Colf, 2013). A more recent report by Kisting (2010) told of a farmer from Aroab in the Karas region (Namibia), who had fallen ill and was diagnosed with Congo fever (Crimean-Congo hemorrhagic fever). Though CCHFV (transmitted by ticks of genus *Hylomma*) and RVFV (transmitted by mosquitoes) are transmitted by arthropods, it is evidence that there have been reports of zoonotic disease outbreaks in Namibia (Simpson, 1978; Chinikar, Mirahmad, Moradi, Ghiasi, and Khakifirouz, 2012; Monaco *et al*., 2013). The likelihood of rodent- or shrew-borne viruses existing in Namibia can be considered and investigated. The pathogenic status of the two discovered Arenaviruses are not known and should be investigated for clarity thereof.

5.3. Fleas

All the small mammals caught for this study have been recorded before in various parts of the country and therefore are not unique to this study. All flea species collected for this study have been recorded in other studies conducted in Namibia (Uusiku. 2007; Mfune *et al*., 2013). Most flea species that infest rodents are not very host-specific and infest more than two host species belonging to the same family, more than two host families or across different rodent orders (Poulin, Krasnov, Georgy and Shenbrot, 2008). This explains why most flea species examined for this study were found to infest more than one host species, except for *C. rossi, which* was found to infest only *M. namaquensis* (Table 4.3). Host specificity cannot be assumed by flea species *C. rossi* as only two fleas were collected for this study.

It is noted that few studies have been undertaken that compare the prevalence, intensity and abundance of flea species on small mammal hosts in different regions, despite the large land surface of Namibia. Land surfaces are well known for their contrasting landscapes and diversity of ecosystems and habitats mainly influenced by contrasting patterns of rainfall and associated vegetation structure. Shihepo *et al*. (2008) carried out a descriptive study of fleas associated with small mammals in selected areas in Northern Namibia. The present study was carried out to determine and compare the prevalence and intensity of infestation of fleas in different regions (representing different biomes) in Namibia.

5.3.1. Prevalence of flea infestation

The present study has shown that host sex, mass, head-body length and reproductive state did not significantly influence the prevalence of fleas infesting small mammal hosts in the three regions of study. Neither was there any significance in prevalence for the host species *G. leucogaster* amongst the three regions of study. Rodents are known for their migratory habits, which affect the spatial distribution of ectoparasites (Nateghpour, Akhavan, Hanafi-Bojd, Telmadarraiy, Ayazian, Hosseini-Vasoukolaei, Motevalli-Haghi, and Akbarzadeh, 2012). The nearly universal distribution of small mammal hosts means that individuals of the same species or those of different species would always come into contact and in turn facilitate the transmission of parasites (Rahbari *et al*., 2008). This is because fleas do not permanently infest hosts, but feed on a host for a short period of time, pass onto another host of the same species or onto a different host species (Rahbari *et al*., 2008).

The Jaccard's index of similarity showed that there was high similarity in the host composition amongst the three regions of study. This could serve as a possible explanation for why the prevalence of flea species infesting host, *G. leucogaster* did not differ significantly between the three regions of study. The availability of a host's nutritious resources could be associated with the host's body condition, which in turn may result in parasites obtaining resources from those individual hosts that are in a good condition (Rueesch, Lemoine and Richner, 2012). Hence, irrespective of the differences in the physical environment of the host species finds itself in, its body is

regarded as a means to resources by fleas (Cameron, 1939; Price, 1984) and so they will infest the host to obtain the needed resources.

Uusiku (2007) reported a significant difference in the prevalence of fleas infesting small mammal hosts between a site that was heavily grazed and one that was not heavily grazed. This was attributed to the cover provided by the dense vegetation, which would accommodate a more diverse habitat for small mammals, greater mobility and therefore an increase in the exposure and exchange of parasites between individual hosts (Uusiku, 2007 cited Eiseb (2000)).

Host sex was did not have a significant influence on the prevalence of fleas infesting small mammals within the three regions of study and between the regions of study with *G. leucogaster*. The results to this study are contrary to those of Krasnov *et al*. (2011) and Mfune *et al*. (2013), who reported significantly higher prevalence of flea infestation in male hosts than female hosts. They attributed this to the higher mobility of male small mammals, which enables frequent encounters of male host individuals with parasites, and thereby consequently lead to a higher prevalence of flea infestation (Krasnov *et al.*, 2011).

This study showed no significant influence of host body mass and length in relation to the prevalence of flea infestation. Hillegrass, Waterman, and Roth (2008) also recorded similar results, confirming that the prevalence of fleas infesting hosts is not

influenced by the body mass of Cape ground squirrel. The study by Lefebvre *et al*. (2002) also indicated a non-significant effect of total body length on the prevalence of parasites infesting eels. Molina, Casanova and Feliu (1999) reported a significantly higher prevalence of the parasite *Graphidium strigosum* in older heavier wild rabbits than in juveniles. It is assumed that heavier hosts are likely to be older animals that may have acquired immunity against parasite infestation (Viljoen, Bennett, Ueckermann, and Lutermann, 2011).

Host reproductive status did not significantly affect the prevalence of fleas infesting hosts for the current study. Results from a study by Viljoen *et al*. (2011) also reported a non-significant effect of host's reproductive status on prevalence of parasites. This was linked to the possible fact that rodent colony members share burrows for extended periods of time, irrespective of sex and reproductive statuses. This enables rodents to become exposed to parasites over time (Viljoen *et al*., 2011). Molina et al., 1999, have reported a higher prevalence of Andrya cuniculi in lactant and pregnant female rabbits compared to non-breeding females.

5.3.2. Intensity

The study showed that there was a significant difference between the sexes of host in Mariental. Males were shown to have a higher intensity of flea intensity ($p=0.005$). This still can be explained by the high mobility of male hosts, which causes for them to frequently be exposed to small mammal host with the likelihood of fleas being

transferred between individual hosts (Krasnov *et al.*, 2011). Perez-Orella and Schult-Hostedde (2005) observed the parasite loads of male and female hosts of the northern flying squirrels and found that male hosts carried more parasite loads than female hosts. Male hosts probably travel greater distances in search for female hosts during the mating season (Perez-Orella and Schult-Hostedde 2005).

Ectoparsites leave the hosts nest whilst on the host animal as the host leaves the nest. This creates opportunities for ectoparasites to be dispersed to other individuals and reduce the chances of inbreeding ectoparasites (Perez-Orella and Schult-Hostedde, 2005). Except for the higher mobility exhibited in male small mammal hosts and the reduced immunocompetence caused by the androgen hormones, male hosts may have a higher intensity of parasite infestation due to their body size (Perez-Orella and Schult-Hostedde, 2005). Males also spend less time on grooming than females as a mechanism of energy conservation for finding and defending mates rather than grooming. This in turn results in male hosts being more parasitized than females (Perez-Orella and Schult-Hostedde, 2005). This is contrary to the studies conducted by Mfune *et al* (2013), Shihepo *et al* (2008), and Uusiku (2007), who all reported to have observed to have no significant difference in the intensity of infestation between male and female hosts.

Host body mass significantly influenced the intensity of flea infestation in Neudamm ($p=0.003$). Intensity increased with a reduction in body mass. This could be due to reduced acquired immunity against parasitic infection by younger host individuals, which are regarded as those hosts with a lighter body mass. On the contrary, in the

study by Krasnov *et al*. (2006) the abundance of fleas increased with an increase in host body size. It assumed that hosts individuals with a larger mass would habour more fleas than those of a smaller body mass (Krasnov *et al*., 2006). This is because larger hosts also provide for an abundant selection of niches that can accompany the infestation of more flea assemblages (Krasnov, Shenbrot, Khokhlova and Degen, 2004). It has also been reported that larger bats have a higher load of ectoparasite infestations (Luz, Costa, Gomes and Esbérard, 2009).

Neudamm also observed a significant effect on host body length and intensity of infestation ($p=0.005$) was also observed. The increase in body length was associated with an increase in intensity. Lefebvre *et al*., (2002) recorded same effect of an increase in parasite intensity with an increase in host size. Hosts with larger body sizes are considered to be older animals. Extended lengths of exposure to parasites and a larger body surface area for parasites to establish themselves (Lefebvre et al. 2002) may cause an increase in parasite intensity infesting larger hosts. The significance in host length and mass observed in Neudamm might also be related to the density of hosts captured in that region of study. High host population densities are said to be associated with high parasite intensity among hosts due to frequent interactions between hosts (Patterson and Ruchstuhl, 2013). Dense vegetation cover characterized the Central region, in which the median intensity of flea infestation was recorded to be highest for this study, the South is characterized by land that is more open and spars shrubbery, whereas the north has less shrubs and therefore less cover (Okitsu, 2010). The closed shrubbery in the region may have influenced the high

intensity in the Central region. The closed shrubbery enables more mobility of hosts and thereby increases in the exposure of fleas on the small mammals (Laudisoit *et al*., 2009).

There was also found to be a non-significant effect of host's reproductive state on the intensity of fleas infesting small mammals. Because most rodent species are social and colonial, it would be easy for fleas to vertically transmit between host individuals, irrespective of reproductive statuses of the host individuals (Viljoen *et al*., 2011). Molina *et al*. (1999) found a significantly higher intensity of *Trichostrongylus retortaeformis* and *Nematodiroides zembrae* in lactant as well as lactant and pregnant female rabbits.

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

6.1. Hantavirus

The objectives of this study was to use molecular techniques to survey the presence of rodent-borne viruses in Namibia, namely Hantaviruses and Arenaviruses. Hantaviruses were not detected during this present study, but it is recommended that the search for Hantaviruses in the small mammal population of Namibia be continued. This should be done by trapping for small mammals in other parts of the country and screening can then be carried out on the harvested lungs of those small mammals. Another recommendation is that other organs harvested also be screened for Hantaviruses. The possibility that they may result in a positive outcome cannot be ruled out and therefore that option can be explored. Another recommendation is that other serological methods, such as enzyme immunoassays or immunofluorescence test, be used to screen for Hantaviruses. The screening of Hantaviruses should not only be limited to the PCR method.

6.2. Arenavirus

The present study detected what might be two possibly novel Arenaviruses that have never been recorded in Namibia before. It is the first time that research of this nature has been conducted in Namibia. Lung organ specimen from four rodents of the species *Micaelamys namaquensis* have been screened for the presence of Arenaviruses; three from rodents caught in Okahandja and one from a rodent caught in Mariental. The virus from Okahandja is closely related to the Merino walk virus that was discovered in South Africa in host species *Myotomys unisulcatus*, whilst the

virus from Mariental is related to Lassa virus and Ippy virus clades. The use of molecular techniques used for this study showed to be successful in the detection of not only rodent-borne viruses but two new Arenaviruses.

It is recommended that more studies be conducted on the surveying of viruses in the small mammal population of Namibia. More small mammals should be trapped and their organs screened for Hantaviruses, Arenaviruses and other viruses that are known to be prevalent in small mammals. This would increase the knowledge on the viruses that are harboured by small mammals across the globe. With the accelerated increase in the discovery of new viruses harboured in small mammals worldwide, Namibia is no exception to the viruses that already exist but are yet to be discovered. Another recommendation is that the pathogenicity of the Arenaviruses from Namibia needs to be determined.

6.3. Fleas

The present study was set out to determine the effect that host factors have on the prevalence and intensity of fleas infesting small mammals in selected regions of Namibia. These factors included host sex, body mass, head-body length and the reproductive status. *Micaelamys namaquensis*, *Gerbilliscus leucogaster*, *Mastomys* species, *Rhabdomys pumilio* and *Saccostomus campestris* were the rodents species found to be infested with fleas. *Chiastopsylla rossi, Listropsylla aricinae, Xenopsylla brasiliensis, X. cheopis, X. hirsute, X. nubica, X. philoxera* and *X. versuta* were the flea species collected from the infested host species.

This study revealed no significant effect of host traits on the prevalence of fleas infesting small mammal host species in the regions (north, central and south) of study. The prevalence of *G. leucogaster* between the three regions of study also showed a non-significant outcome with regard to the influence of host traits on the prevalence of fleas infesting the host species.

Male hosts had higher intensity of fleas infesting small mammal hosts than females in Mariental. Host body mass and head-body length had a significant effect on the intensity of small mammal hosts in Neudamm. None of the host traits were observed to have a significant influence on the intensity of flea infestation in Rundu. Results also revealed a non-significant effect of host traits on the intensity of infestation on *G. leucogaster* amongst the three regions of study.

It is recommended that the flea species that were collected from other regions (Figure 3.1 and 3.2), not included in this study, be identified and analysed for patterns that may arise in the prevalence and intensity of infestation on the small mammal hosts. Trapping should also be conducted in other regions that were not included in this present study. Not many studies have been conducted on fleas infesting small mammal hosts in different geographical regions of Namibia. This would be important for the expansion on the knowledge of biodiversity of not only flea species infesting the small mammals but also the small mammals that inhabit these different regions, which might be influenced by the difference in vegetation and climate that characterizes these regions. It is also recommended that seasonal studies on fleas be conducted to identify any possible seasonal variations in the prevalence and intensity

of infestation of fleas that may occur on the small mammal hosts examined. It was revealed by Uusiku (2007) that the prevalence of infestation of fleas on host species, differed significantly between seasons. It is also generally accepted that the composition of communities change with a change in space and time due to the differences in environmental conditions (Laudisoit *et al*., 2009) therefore it would be of importance to be able to capture the differences that flea communities undergo with a change in environmental conditions.

Another recommendation is that fleas be screened for the presence of bacteria and viruses they are known to be vectors of various diseases. It is known that *Yersinia pestis* is carried by fleas and transmitted to humans to cause plague. Fleas collected from small mammals can thus be screened for the presence of *Y. pestis*. It would be considered a good research area through which the presence or absence of *Y. pestis* in small mammals could be determined.

REFERENCES

1. Alarcón, M. E., Huang, C., Tsai, Y., Chen, W., Dubey, A. K., and Wu, W. (2011). Life cycle and Morphology of *Steinina ctenocephali* (Ross 1909) comb. Nov. (Eugregarinorida: Actinocephalidae), a Gregarine of *Ctenocephalides felis* (Siphonaptera: Pulicidae) in Taiwan. *Zoological Studies*, 50(6), 763-772.
2. Amatre, G., Babi, N., Enscore, R. E., Ogen-Odoi, A., Atiku, L. A., Akol, A., Gage, K. L. and Eisen, R. J. (2009). Flea Diversity and Infestation Prevalence on Rodents in a Plague-Endemic Region of Uganda. *American Journal of Tropical Medicine and Hygiene*, 81, 718–724. doi:10.4269/ajtmh.2009.09-0104.
3. Armstrong, C., and Lillie, R. D. (1934). Experimental Lymphocytic Choriomeningitis of monkeys and mice product by a virus encountered in studies of the 1933 St Louis encephalitis epidemic. *Public Health Report*, 49(34), 1019-1027.
4. Arai, S., Song, J. W., Sumibcay, L., Bennett, S. N., Nerurkar, V. R., Cook, J. A., Yates, T. L. and Yanagihara, R. (2007). Hantavirus in Northern Short-tailed Shrew, United States. *Emerging Infectious Diseases*, 13, 1420-1423. doi: 10.3201/eud1309.070484

5. Arai, S., Bennett, S. N., Sumibcay, L., Cook, J. A., Song, J. W, Hope, A., Parmenter, C., Nerurkar, B. R., Yates, T. L., and Yanagihara, R. (2008). Phylogenetically distinct Hantaviruses in the Masked Shrew (*Sorex cinereus*) and Dusky shrew (*Sorex monticolus*) in the United States. *American Journal of Tropical Medicine and Hygiene*, 78(2), 348-351.

6. Barton, L. L., and Mets, M. B. (2001). Congenital Lymphocytic Choriomeningitis virus infection: Decade of Rediscovery. *Clinical Infectious Diseases*, 33, 370-374. doi: 10.1086/321897.

7. Beevi, M. R., and Radhakrishnan, S. (2012). Community ecology of the metazoan parasites of freshwater fishes of Kerala. *Journal of Parasitic Diseases*, 36, 184-196. doi: 10.1007/s12639-012-0101-8.

8. Begon, M., C., Townsend, C. R. and Harper, J. L. (2006). *Ecology:from Individuals to Ecosystems* (4th Edition). Blackwell Publishing Ltd. United States of America.

9. Bi, Z., Formently, B. H., and Roth, C. E. (2008). Hantavirus Infections: a review and global update. *Journal of Infection in Developing Countries*, 2(1), 3-23.

10. Bitam, I., Dittmar, K., Parola, P., Whiting, M. F., and Raoult, D. (2010). Flea and flea-borne diseases. *International Journal of Infectious Diseases*, 14, 667-676. doi: 10.1016/j.ijid.2009.11.011.

11. Bolder, B. M, Brooks, M. E, Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and White, J. S. (2008).Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, 24, 127-135. doi: 10.1016/j.tree.2008.10.008.

12. Borges, A. A., Campos, G. M., Moreli, M. L., Souza, R. L. M., Aquino, V. H., Saggioro, F. P., and Fiueredo, L. T. M. (2006). Hantavirus cardiopulmonary syndrome: Immune response and pathogenesis. *Microbes and Infections*, 8, 2324-2330. doi:10.1016/j.micinnf.2006.04.019.

13. Borror, Triplehorn and Johnson. (1989). *Introduction to the study of insects* (6th Edition). Holt, Rinehart and Wintson Printing, Inc, United States of America.

14. Botros, B. A., Sobh, M., Wierzba, T., Arthur, R. R., Mohareb, E. W., Frenck, R., El Refaie, A., Mahmoud, I., Chapman, G. D., and Graham, R. R. (2004). Prevalence of Hantavirus antibody in patients with chronic renal disease in Egypt. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98, 331-336. doi:10.1016/S0035-9203(03)00063-4.

15. Botten, J., Whitton, J.L., Barrowman, P., Sidney, J., Whitmire, J. K., Alexander, J., Kotturi, M. F., Sette, A. and Buchmeier, M. J. (2010). A Multivalent Vaccination Strategy for the Prevention of Old World Arenavirus Infection in Humans. *Journal of Virology*, 84, 9947-9956. doi:10.1128/JVI.00672-10.

16. Bowen, M. D., Peters, Cl. J., Mills, J. N., and Nichol, S. T. (1996). Oliveros Virus: A Novel Arenavirus from Argentina. *Virology*, 217(124), 362-366.

17. Bowen, M. D., Rollin, P. E., Ksiazek, T. G., Hustad, H. L., Bausch, D. G., Demby, A. H., Bajani, M. D., Peters, C. J., Nichol, S. T. (2000). Genetic diversity among Lassa virus strains. *Journal of Virology*, 74(15), 6992-7004.

18. Braack, L., E., O. (1991). *Field Guide to Insects of the Kruger National Park.* Struik Publishers. Cape Town, South Africa.

19. Briese, T., Paweska, J. T., McMullan, L. K., Hutchison, S. K., Street, C., Palacios, G., Khristova, M. L., Weyer, J., Swanepoel, R., Egholm, M., Nichol, S. T., and Lipkin, W. I. (2009). Genetic Detection and Characterization of Lujo Virus, a New Hemorrhagic Fever-Associated Arenavirus from Southern Africa. *PLoS Pathogens*, 5, 1-5.

20. Bush, A. O., Fernández, J. C., Esch, G. W., and Seed, J. R. (2001). Parasitism: The diversity and ecology of animal parasites. Cambridge University Press. Cambridge, United Kingdom.

21. Caceres, C. C., Nápoli, R. P., and Hannibal, W. (2011). Differential trapping success for small mammals using pitfall and standard cage traps in a woodland savannah region of southwestern Brazil. *Mammalia*, 75, 45-52. doi: 10.1515/MAMM.2010.069.

22. Cajimat, M. N. B., Milazzo, M. L., Bradely, R. D., and Fuhorst, C. F. (2007). Catarina Virus, an Arenaviral Species Principally Associated with *Neotoma micropus* (Southern Plains Woodrat) in Texas. *The American Journal of Tropical Medicine and Hygiene*, 77(4), 732-736.

23. Cajimat, M. N. B., Milazzo, M. L., Borchert, J. N., Abbott, K. D., Bradley, R. D., and Fulhorst, C. F. (2008). Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the Mexican woodrat (Neotoma Mexicana). *Virus Research*, 133(2), 211-217.

24. Cajimat, N. N. B., Milazzo, M. L., Bradley, R. D., and Fulhorst, C. F. (2012). Ocozocoautla de Espinosa Virus and Hemorrhagic Fever, Mexico. *Emerging Infectious Diseases*, 18, 401-405. doi:http://dx.doi.org/10.3201/eid1803.111602.

25. Calisher, C. H., Nabity, S., Root, J. J., Fulhorst, C. F., and Beaty, B. J. (2001). Transmission of an Arenavirus in White-throated wootrats (*Neotoma albigula*), Southeastern Colorado, 1995-1999. *Emerging Infectious Diseases*, 7(3), 397-402.

26. Calisher C. H., Childs J. E., Field H. E., Holmes K. V., and Schountz T. (2006). Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Review*, 19, 531–545. doi:10.1128/CHM.00017-06.

27. Cameron, T. W. (1939). Parasitism and its importance. *Canadian Journal of Comparative Medicine*, 3(7), 175-181.

28. Cann, A. J. (1993). *Principles of molecular virology*. Academic Press Limited Limited, Great Britan. United Kingdom.

29. Cann, A. J. (1997). *Principles of molecular virology* (2nd Edition). Academic Press Limited, Great Britan. United Kingdom.

30. Cann, A. J. (2001). *Principles of molecular virology* (3rd Edition). Academic Press Limited, Great Britan. United Kingdom.

31. Casals, J. (1975). Arenaviruses. *The Yale Journal of Biology and Medicine*, 48(2), 115-140.

32. Centers for Disease Control and Prevention (CDC). (2005). Lymphocytic choriomeningitis virus infection in organ transplant recipients--Massachusetts, Rhode Island, 2005. *Morbidity and Mortality Weekly Report* 2005, 54(21), 537-539.

33. Charrel, R. N., Lemasson, J. J., Garbutt, M., Khelifa, R., De Micco, P., Feldmann, H., and de Lamballerie, X. (2003). New insights into the evolutionary relationships between arenaviruses provided by comparative analysis of small and large segment sequences. *Virology*, 317, 191-196. doi: 10.1016/j.virol.2003.08.016.

34. Charrel, R. N., De Lamballerie, X., and Emonet, S. (2008). Phylogeny of the Genus Arenavirus. *Current Opinion in Microbiology*, 11, 362-368. doi:10.1016/j.mib.2008.06.001.

35. Charrel, R. N., and De Lamballerie, X. (2010). Zoonotic aspects of arenavirus infections. *Veterinary Microbiology*, 140, 231-220. doi:10.1016/j.vetmic.2009.08.027.

36. Charrel, R.N., Coutard, B., Baronti, C., Canard, B., Nougairede, A., Frangeul, A., Morin, B., Jamal, S., Schmidt, C.L., Hilgenfeld, R., Klempa, B., and de Lamballerie, X. (2011). Arenaviruses and Hantaviruses: From epidemiology and genomics to antivirals. *Antiviral Research*, 90, 102–114. doi:10.1016/j.antiviral.2011.02.009.

37. Chesney, P. J., Katcher, M. L., Nelson, D. B., and Horowitz, S. D. (1979). CSF eosinophilia and chronic lymphocytic choriomeningitis virus meningitis. *Journal of Pediatrics*, 94(5), 750-752.

38. Childs, J. E., Ksiazek, T. G., Spiropoulou, C. F., Krebs, J. W., Morzunov, S., Maupin, G. O., Gage, K. L., Rollin, P. E., Sarisky, J., Escore, R. E., Frey, J. K., Peters, C. J., and Nichol. S. T. (1994). Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the Southwestern United States. *Journal of Infectious Diseases*, 169(6), 1271-1280.

39. Chinikar, S., Mirahmad, R., Moradi, M., Ghiasi, S. M., and Khakifirouz, S. (2012). Crimean-Congo hemorrhagic fever (CCHF). In: Zoonosis, Lorenzo-Morales, J. (Ed). ISBN: 978-953-51-0479-7, In Tech.

40. Cordey, S., Sahli, R., Moraz, M., Estrade, C., Morandi, L., Cherpillod, P., Charrel, R. N., Kunz, S. and Kaiser, L. (2011). Analytical validation of a lymphocytic choriomeningitis virus real-time RT-PCR assay. *Journal of Virological Methods*, 177, 118–122. doi:10.1016/j.viromet.2011.06.018.

41. Coulibaly-N'Golo, D., Allali, B., Kouassi, S. K., Fichet-Calvet, E., Becker-Ziaja, B., Riege, T., Ölschläger, s., Dosso., H., Denys, C., ter Meulen, J., Akoua-Koffi, C., and Günther, S. (2011). Novel Arenavirus sequences in *Hylomyscus* species and *Mus* (*Nannomys*) *setulosus* from Côte d'Ivoire: implication for evolution of Arenaviruses in Africa. *PLoS One*, 6, 1-9.

42. Cueto, G. R., Cavia, R., Bellomo, C., Padula, P. J., and Suárez, O. V. (2008). Prevalence of Aires City, Argentina. *Tropical Medicine and International Health*, 13, 46-51. doi: 10.1111/j.1365-3156.2007.01968.x.

43. Delgado, S., Erickson, B.R., Agudo, R., Blair, P. J., Vallejo, E., Albariño, C. G., Vergas, J., Comer, J. A., Rollin, P. E., Ksiazek, T. G., Olson, J. G., and Nichol, S. T. (2008). Chapare Virus, a newly discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. *PLoS Pathogens*, 4, 1-6. doi:10.1371/journal.ppat.1000047.

44. De Manzione, N., Salas, R. A., Paredes, H., Godoy, O., Rojas, L., Araoz, F., Fulhorst, C. F., Ksiazek, T. G., Mills, J. N., Ellis, B. A., Peters, C. J., and Tesh, R. B. (1998). Venezuelan hemorrhagic fever: Clinical and epidemiological studies of 165 cases. *Clinical Infectious Diseases*, 26(2), 308-313.

45. Despommier, D. D., Gwadz, R.W. and Hotez, P. J. (1982). *Parasitic Diseases* (3rd Edition). Springer-Verlag. New York, Inc. United States of America.

46. Dobler G., and Pfeffer, M. (2011). Fleas as parasites of the family Canidae. *Parasites and Vector*, 4, 1-12. doi: 10.1186/1756-3305-4-139.

47. Drexler, J. F. Corman, V. M., Müller, M. A., Maganga, G. D., Vallo, P., Binger, T., Gloza-Rausch, F., Rasche, A., Yordanov, S., Seebens, A., Oppong, S., Sarkodie, Y. Y., Pongombo, C., Lukashev, A. N., Schmidt-Chanasit, J., Stöcker, A., Carneiro, A. J. B., Erbar, S., Maisner, A., Fronhoffs, F., Buettner, R., Kalko, E. K. V., Kruppa, T., Franke, C. R., Kallies, R., Yandoko, E. R. N., Herrler, G., Reusken, C., Hassanin, A., Krüger, D. H., Matthee, S., Ulrich, R. G., Leroy, E. M., and Drosten, C. (2012). Bats host major mammalian paramyxoviruses. *Nature Communications*, 3, 796. doi: 10.1038/ncomms1796.

48. Emonet, S., Retornaz, K., Gonzalez, J-P., de Lamballerie, X., and Charrel, R. N. (2007).Mouse-to-human transmission of variant Lymphocytic Choriomeningitis virus. *Emerging Infectious Diseases*, 13(3), 472-475.

49. Emonet S. F., de la Torre, J. C., Domingo, E., Sevilla, N. (2009). Arenavirus genetic diversity and its biological implications. *Infection, Genetics and Evolution*, 9, 417–429. doi:10.1016/j.meegid.2009.03.005.

50. Eskey, C. R. (1938). Fleas as vectors of plague. *American Journal of Public* Health, 28(11), 1305-1310.

51. Fabbri, M. and Maslow, M. J. (2001). Hantavirus Pulmonary Syndrome in the United States. *Current Infectious Disease*, 3(3), 258-265.

52. Fehling, S. K., Lennartz, F., and Strecker.T. (2012). Multifunctional Nature of the Arenavirus RING Finger Protein Z. *Viruses*, 4, 2973-3011. doi:10.3390/v4112973.

53. Fernandez, G. E. (2001). Hantavirus. *Elsevier Science*, 8(2), 53-58.

54. Fields, B. N. in Knipe, D. M. (1985). Introduction. In B. N. Fields (Ed), Virology. (pp.1-4). New York: United States of America.

55. Fischer, S. A., Graham, M. B., Kuehnert, M. J., Kotton, C. N., Srinivasan, A., Marty, F. M., Comer, J. A., Guarner, J., Paddock, C. D., DeMeo, D. L., Shieh, W. J., Erickson, B. R., Bandy, U., DeMaria, A., Davis, J. P., Delmonico, F., Pavlin, B., Likos, A., Vincent, M. J., Sealy, T. K., Goldsmith,C. S., Jernigan, D. B., Rollin, P. E., Packard, M. M., Patel, M., Rowland, C., Helfand, R. R., Nichol, S. T., Fishman, J. A., Ksiazek, T., Zaki, S. R., and the LCMV in Transplant Recipients Investigation Team. (2006). Transmission of Lymphocytic Choriomeningitis virus by organ transplantation. *New England Journal of Medicine*, 354(21), 2235-2249.

56. Fisher-Hoch, S. P., Tomori, O., Nasidi, A., Perez-Oronoz, G. I., Fakile, Y., Hutwagner, L., and McCormick, J. B. (1995). Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. *British Medical Journal*, 311(7009), 857-859.

57. Fink, T. M. (1996). Rodents, Human Remains, and North American hantaviruses: Risk Factors and Prevention Measures for Forensic Science Personnel – a Review. *Journal of Forensic Science*, 41(6), 1052-1056.

58. Ford, P. L., Fagerlund, R. A., Duszynski, D. W., and Polechla, P. J. (2004). Fleas and lice of mammals in New Mexico. General Technical Report RMRS-GTR-123. Fort Collins, CO: United States Department of Agriculture, Forest Service, Rocky Mountain Research Station.

59. Frame, J. D., Baldwin, J. M. Jr., Gocke, D. J., and Troup, J. M. (1970). Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings. *American Journal of Tropical Medicine and Hygiene*, 19(4), 670-676.

60. Fulhorst, C. F., Charrel, R. N., Weaver, S. C., Ksiazek, T. G., Bradley, R. D., Milazzo, M. L., Tesh, R. B., and Bowen, M. D. (2001). Geographic Distribution and Genetic Diversity of Whitewater Arroyo Virus in the Southwestern United States. *Emerging Infectious Diseases*, 7(3), 403-407.

61. Fulhorst, C. F., Bennett, S. G., Milazzo, M. L., Murray Jr, H. L., Webb Jr, J. P., Cajimat, M. N. B., and Bradley, R. D. (2002). Bear Canyon Virus: an Arenavirus naturally associated with the California Mouse (*Peromuscus carlifornicus*). *Emerging Infectious Diseases*, 8, 717-721. doi:10.3201/eid0807.010281.

62. Fulhorst, C., Milazzo, M. L., Charrel, R. N., and Bradley, R. D. (2002). Natural host relationship and genetic diversity of Whitewater Arroyo virus in Southern Texas. *American Journal of Tropical Medicine and Hygiene*, 67, 114-118.

63. Ghiringhelli, P. D., Rivera-Pomar, R. B., Lozano, M. E., Grau, O., and Romanowski, V. (1991). Molecular organization of Junin virus S RNA: complete nucleotide sequence, relationship with other members of the Arenaviridae and unusual secondary structures. *Journal of General Virology*, 5(5), 651-652.

64. Groen, J., Suharti, C., van Gorp, E. C. M., Sutaryo, J., and Lundkvist, Å. (2002). Serological Evidence of Human Hantavirus Infections in Indonesia. *Infection*, 30, 326-327. doi:10.1007/s15010-002-2194-y.

65. Gu, S. H., Markowski, J., Kang, H. J., Hejduk, J., Sikorska, B., Liberski, P. P., and Yanagihara, R. (2013). Boginia virus, a newfound Hantavirus harboured by Eurasian water shrew (*Neomys fodiens*) in Poland. *Virology Journal*, 10, 160. doi: 10.1186/1743-422X-10-160

66. Guo, W. B., Lin, X.D., Wang, W., Tian, J. H., Cong, M. L., Zhang, H. L., Wang, M. R., Zhou, R. H., Wang, J. B., Li, M. H., Xu, J., Holmes, E. C., and Zhang, Y. Z. (2012). Phylogeny and origins of Hantaviruses harboured by bats, insectivores and rodents. *PLos One*, 9, e1003159. doi: 10.1371/journal.ppat.1003159.

67. Günther, S., Hoofd, G., Charrel, R., Röser, C., Becker-Ziaja, B., Lloyd, G., Sabuni, C., Verhagen, R., van der Groen, G., Kennis, J., Katakweba, A., Machang'u, R., Makundi, R., and Leirs, H. (2009). Mopeia Virus-related Arenavirus in Natal Multimammate Mice, Morogoro, Tanzania. *Emerging Infectious Diseases*, 15, 2008-2012. doi:10.3201/eid1512.090864.

68. Harnish, D. G., Dimock, K., Bishop, D. H., and Rawls, W. E. (1983). Gene mapping in Pichinde virus: assignment of viral polypeptides to genomic L and S RNAs. *Journal of Virology*, 42(2), 638-641.

69. Henttonen, H., Buchy P., Suputtamongkol, Y. J., Hapalapong, S., Herbreteau, V., Laakkonen, J., Chaval, Y., Galan, M., Charbonnel, N., Michaux, J., Cosson, J. F., Morand, S., and Hugot, J. P. (2008). Recent discoveries of new Hantaviruses widen their range and question their origins. *Annals of the New York Academy of Sciences*, 1149, 84-89. doi: 10.1196.annals.1428.064.

70. Hepojoki, J., Strandin, T., Lankinen, H., and Vaheri, A. (2012). Hantavirus structure – molecular interactions behind the scene. *Journal of General Virology*, 93, 1631-1644. doi:10.1099/vir.0.042218-0.

71. Heyman, P., Vervoort, T., Escutenairec, S., Degraved, E., Konings, J., Vandenveldee, C. and Verhagenf, R. (2001). Incidence of hantavirus infections in Belgium. *Virus Research*, 77, 71–80. doi:10.1007/s10096-004-1196-3.

72. Hillegrass, M. A., Waterman, J. M., and Roth, J. M. (2008). The influence of sex and sociality on parasite loads in an African ground squirrel. *Behavioral Ecology*, 19, 1006-1011. doi:10.1093/beheco/am070.

73. Hjelle, B., Torrez-Martínez N., Koster F. T., Jay, M, Ascher, M. S., Brown, T., Reynolds, P., Ettestad, P., Voorhees, R. E., Sarisky, J., Enscore, R. E., Sands, L., Mosley, D. G., Kioski, C., Bryan, R. T., and Sewell, C. M. (1996) Epidemiologic linkage of rodent and human hantavirus

genomic sequences in case investigations of hantavirus pulmonary syndrome. *Journal of Infectious Diseases*, 173(4), 781-786.

74. Hjelle, B., Goade, D., Torrez-Martinez, N., Lang-Williams, M., Kim, J., Harris, R. L., and Rawlings, J. A. (1996). Hantavirus pulmonary syndrome, renal insufficiency, and myositis associated with infection by Bayou Hantavirus. *Clinical Infectious Diseases*, 23(3), 495-500.

75. Hofmann, J., Führer, A., Bolz, M., Waldschläger-Terpe, J., Meier, M., Lüdders, D., Enders, M., Oltmann, A., Meisel, H., and Krüger, D. H. (2012). Hantavirus infections by Puumala or Dobrava-Belgrade virus in pregnant women. *Journal of Clinical Virology*, 55, 266-269. doi: 10.1016/j.jcv.2012.07.011.

76. Hutchinson, K. L., Rollin, P. E., and Peters, C. J. (1998). Pathogenesis of a North American Hantavirus, Black Creek Canal virus in experimentally infected Sigmodon hispidus. *American Journal of Tropical Medicine and Hygiene*, 59(1), 58-65.

77. Inizan, C. C., Cajimat, M. N. B., Milazzo, M. L., Barragán-Gomez, Bradley, R. D., and Fulhorst, C. F. F. (2010). Genetic Evidence for a Tacaribe Serocomplex Virus, Mexico. *Emerging Infectious Diseases*, 16, 1007-1010. doi:10.3201/eid1606.091648.

78. Ishii, A., Thomas, Y., Moonga, L., Nakamura, I., Ohnuma, A., Hang'ombe, B., Takada, A., Mweene, A. and Sawa, H. (2011). Novel Arenavirus, Zambia. *Emerging Infectious Diseases*, 17, 1921-1924. doi:10.3201ieid1710.10452.

79. Ithete, N. L., Stoffberg, S., Corman, V. M., Cottontail, V. M., Richards, L. R., Schoeman, M. C., Drosten, C., Drexler, J. F., and Preiser, W. (2013). Close relative of human Middle East respiratory syndrome coronavirus in bats, South Africa. *Emerging Infectious Diseases*, 10, 1697-1699. doi: 10.3201.eid1910.130946.

80. Jaccard, P. (1912). The distribution of the flora in the Alpine zone. *New Phytologist*, 11(2), 37-50.

81. Jay, M. T., Glaser, C., and Fulhorst, C. F. (2005). The Arenaviruses. Veterinary Medicine Today: *Zoonosis Update*, 227(6), 904-915.

82. Jollies, A. E., Ezenwa, V. O., Etienne, R. S., Turner, W. C., and Olff, H. (2008). Interactions between macroparasites and microparasites drive infection patterns in free-ranging African Buffalo. *Ecology*, 89(8), 2239-2250.

83. Jonsson, C. B., Figueredo, L. T. M., and Vapalahti, O. (2010). A global perspective on Hantavirus ecology, epidemiology and disease. *Clinical Microbiology Reviews*, 23, 412-441. doi: 10.1128/CMR.00062-09.

84. Joubert, J. J., Prozesky, O. W., Lourens, J. G., van Straten, A. M., Theron, J. W., Swanevelder, C., Meenehan, G. M., and van der Merwe, C. A. (1985). Prevalence of Hepatitis virus and some arbovirus infections in Kavango, Northern SWA/Namibia. *South African Medical Journal*, 67(13), 500-502.

85. Kang, H. J., Arai, S., Hope, A. G., Song, J. W., Cook, J. A., and Yanagihara, R. (2009). Genetic diversity and phylogeography of Seewis virus in the Eurasian common shrew in Finland and Hungary. *Virology Journal*, 6, 208-213. doi: 10.1186/1743-422X-4-114.

86. Kang, H., Bennett, S. N., Dizney, L., Sumibcaya, L., Arai, S., Ruedas, L. A., Song, J. and Yanagihara, R. (2009). Host switch during evolution of a genetically distinct Hantavirus in the American shrew mole (*Neurotrichus gibbsii*). *Virology*, 388, 8–14. doi:10.1016/j.virol.2009.03.019.

87. Kang, H., Bennett, S. N., Sumibcaya, L., Arai, S., Hope, A. G., Mocz, G., Song, J. W., Cook, J. A., and Yanagihara, R. (2009). Evolutionary insights from a genetically divergent Hantavirus harboured by the European common mole (*Talpa europaea*). *PLoS One*, 4, e6149. doi: 10.1371/journal.pone.0006149.

88. Kang, H. J., Arai, S., Hope, A. G., Cook, J. A., and Yanagihara, R. (2010). Novel Hantavirus in the Flat-skulled shrew (*Sorex roboratus*). *Vector-borne and Zoonotic diseases*, 10, 593-598. doi. 10.1089/vbz.2009.0159.

89. Kang H. J, Kadjo B, Dubey S, Jacquet F, and Yanagihara R. (2011). Molecular evolution of Azagny virus, a newfound Hantavirus harbored by the West African pygmy shrew (*Crocidura obscurior*) in Côte d'Ivoire. *Virology Journal*, 8(373), 1-7.

90. Kang, H. J., Kosoy, M. Y., Shrestha, S. K., Shrestha, M. P., Pavlin, J. A., Gibbons, R. V., and Yanagihara, R. (2011). Genetic diversity of Thottapalayam virus, a Hantavirus harboured by the Asian house shrew (*Suncus murinus*) in Nepal. *American Journal of Tropical Medicine and Hygiene*, 83,540-545. doi: 10.4269/ajtmh.2011.11-0034.

91. Karp, G. (2006). *Cell and Molecular Biology: Concepts and Experiments* (5thed.). John Wiley & Sons Inc., Canada.

92. Keane, E., and Gilles, H. M. (1977). Lassa fever in Panguma Hospital, Sierra Leone. *British Medical Journal*, 1(6073), 1399-1402.

93. Keeling M. J. and Gilligan C. A. (2000). Metapopulation dynamics of bubonic plague. *Nature*, 407, 903-906. doi:10.1038/35038073.

94. Khokhlova, I. S., Serobyan, V., Degen, A. A., and Krasnov, B. R. (2010). Host gender and offspring quality in a flea parasitic on a rodent. *Journal of Experimental Biology*, 213, 3299-3204. doi:10.1242/jeb.046565.

95. Kisting, D. (2010). Crimean-Congo hemorrhagic fever - Namibia (Karas). Retrieved from http://www.promedmail.chip.org/pipermail/promed-eafr/2010-August/000439.html

96. Klempa, B. (2004). *Dobrava and Tula hantaviruses from Central Europe: molecular evolution and pathogenic relevance*. Doctoral dissertation. Malacky, Slowakia.

97. Klempa, B., Stanko, M., Labuda, M., Ulrich, R., Meisel, H., Krüger, D. H. (2005). Central European Dobrava Hantavirus Isolate from a Striped Field Mouse (*Apodemus agrarius*). *Journal of Clinical Microbiology*, 43, 2756-2763. doi:10.1128/JCM.43.6.2756-2763.2005.

98. Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Denys, C., Koivogui, L., ter Meulen, J., and Krüger, D. H. (2006). Hantavirus in African Wood Mouse, Guinea. *Emerging Infectious Disease*s, 12(5), 838-840.

99. Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Barrière, P., Koivogui, L., ter Meulen, J., and Krüger, D. H. (2007). Novel Hantavirus Sequences in Shrew, Guinea. *Emerging Infectious Diseases*, 13(3), 520-522.

100. Klempa, B. (2009). Hantaviruses and Climate Change. European Society of *Clinical Microbiology and Infectious Diseases* 15: 518-523.

101. Klempa, B., Koivogui, L., Sylla, O., Koulemou, K., Auste, B., Krüger, D. H., and ter Meulen, J. (2010). Serological evidence of human Hantavirus infections in Guinea, West Africa. *Journal of Infectious Diseases*, 201, 1031-1034. doi: 10.1086/651169.

102. Klempa, B., Witkowski, P. T., Popugaeva, E., Auste, B., Koivoguie, L., Fichet-Calvet, E., Strecker, T., ter Meulen, J., and Krüger, D. H. (2012). Sangassou virus, the first Hantavirus isolate from Africa, displays genetic and functional properties distinct from those of other *Murinae*-associated Hantaviruses. *Journal of Virology*, 86, 3819-3827. doi:10.1128/JVI.05879-11

103. Khaiboullina, S. F., Morsunov S. P., and St Jeor, S. C. (2005). Hantaviruses: Molecular biology, evolution and pathogenesis. *Current Molecular Medicine*, 5(8), 773-790.

104. Krasnov, B. R., Shenbrot, G. I., Khokhlova, I. S., and Degen, A. A. (2004). Flea species richness and parameters of host body, host geography and host 'milieu'. *Journal of Animal Ecology*, 73, 1121-1128. doi:10.1111/j.0021-8790.2004.00883.x.

105. Krasnov, B. R., Stanko, M., Miklisova, D. and Morand, S. (2006). Habitat variation in species composition of flea assemblages on small mammals in central Europe. *Ecological Research*, 21, 460–469. doi:10.1007/s11284-005-0142-x.

106. Krasnov, B. R., Shenbrot, G. I., Khokhlova, I. S., and Poulin, R. (2007). Geographical variation in the 'bottom-up' control of diversity: fleas and their small mammalian hosts. *Global Ecology and Biogeography*, 16, 179-186. doi:10.1111/j.1466-8238.2006.00273.x.

107. Krasnov, B. R, Stanko, M., Matthee, S., Laudisoit, A., Leirs, H., S. Khokhlova, I. S., Korallo-Vinarskaya, N. P., Vinarski, M. V. and Morand, S. (2011). Male hosts drive infracommunity structure of ectoparasites. *Oecologia*, 166, 1099–1110. doi:10.1007/s00442-011-1950-z.

108. Krasnov, B. R., Mouillot, D., Shenbrot, G. I., Khokhlov, I. S. and Poulin, R. (2011). Beta-specificity: The turnover of host species in space and another way to measure host specificity. *International Journal for Parasitology*, 41, 33–41. doi:10.1016/j.ijpara.2010.06.001.

109. Krüger, D. H., Ulrich, R., and Lundkvist, Å. (2001). Hantavirus infections and their prevention. *Microbes and Infection*, 3(13), 1129-1144.

110. Krüger, D. H., Schönrich, G., and Klempa, B. (2011). Human pathogenic Hantaviruses and prevention of infection. *Human vaccines*, 7, 685-693. doi: 10.4161/hv.7.6.15197.

111. Kundrotaitė, I. and Paulauskas, A. (2009). Fleas (*Insecta: Siphonaptera*) on Lithuania small rodents. *Acta Biologica*, 9(1), 7-12.

112. Laudisoit, A., Leirs, H., Makundi, R. and Krasnov, B. R. (2009). Seasonal and habitat dependence of fleas parasitic on small mammals in Tanzania. *Integrative Zoology*, 4, 196–212. doi: 10.1111/j.1749-4877.2009.00150.x.

113. Lecompte, E., Fichet-Calvet, E., Daffis, S., Koulémou, K., Sylla, O., Kourouma, F., Doré, A., Soropogui, B., Aniskin, V., Allali, B., Kan, S. K., Lalis, A., Koivogui, L., Günther, S., Denys, C., and ter Meulen, J. (2006). Mastomys natalensis and Lassa fever, West Africa. *Emerging Infectious Diseases*, 12, 1971-1974. doi: 10.3201/eid1212.060812.

114. Lecompte, E., ter Meulen, J., Emonet, S., Daffis, S., and Charrel, R. N. (2007). Genetic identification of Kodoko virus, a novel Arenavirus of the African pigmy mouse (*Mus Nannomys minutoides*) in West Africa. *Virology*, 364, 178-183. doi:10.1016/j.virol.2007.02.008.

115. Lednicky, J. A. (2003). Hantavirus: A short review. *Archives of Pathology and Laboratory Medicine*, 127(1), 30-35.

116. LeDuc, J. W. (1997). Hantaviruses. In A. S. Evans and R. A. Kaslow (Eds), *Viral Infections of Humans: Epidemiology and Control*. (pp. 345-357). Plenum Publishing Corporation, New York.

117. Lee, H. W., Lee, P, W., and Johnson, K. M. (1978). Isolation of the etiologic agent of Korean hemorrhagic fever. *Journal of Infectious Diseases*, 190(9), 1711-1721.

118. Lee, H. W. (1996). Epidemiology and pathogenesis of hemorrhagic fever with renal syndrome. In: Elliott R.M., (Ed.). *The Bunyaviridae*. Plenum Press. New York: United States of America.

119. Lefebvre, F., Contournet, P., Priour, F., Soulas, O., and Crivelli, A. J. (2002). Spatial and temporal variation in *Anguillicola crassus* counts: results of a 4 year servey of eels in Mediterranean lagoons. *Diseases of Aquatic Organisms*, 50, 181-188.

120. Liljeroos, L., and Butcher, S. J. (2013). Matrix proteins as centralized organizers of negative-sense RNA virions. *Frontiers in Bioscience*, 18, 696-715.

121. Lisieux, T., Coimbra, M, Nassar, E. S., Burrattini, M. N., de Souza, L. T., Ferreira, I., Rocco, I. M., de Rosa, A. P., Vasconcelos, P. F., Piheiro, F. P., Leduc, J., Rico-Hesse, R., Gonzalez, J. P., Jahrling, P. B., and Tesh, R. B. (1994). New Arenaviruses isolated in Brazil. *Lancet*, 343(8894), 391-392.

122. Lundkvist, Å., Lindegren, G., Sjölander, K. B., Mavtchoutko, V., Vene, S., Plyusnin, A., and Kalnina, V. (2002). Hantavirus Infections in Latvia. *European Journal of Clinical Microbiology and Infectious Diseases*, 21, 626-629. doi:10.1007/s10096-002-0784-3.

123. Luz, J. L., Costa, L. M., Gomes, L. A. C, and Esbérard, C. E. L. (2009). The chiggerflea Hectopsylla pulex (Siphonaptera: Tungidae) as an ectoparasite of free-tailed bats (Chiroptera: Molossidae). *Memórias do Instituto Oswaldo Cruz*, 104(4), 567-569.

124. Macher, A. M., and Wolfe, M. S. (2006). *Emerging Infectious Diseases*, 12(5), 835-837.

125. Maiztegui, J. I. (1975). Clinical and epidemiological patterns of Argentine haemorrhagic fever. *Bulletin of the World Health Organization*, 52(4-6), 567-575.

126. Martin, M. L., Lindsey-Regenery, H., Sasso, D. R., McCormick, J. B., and Palmer, E. (1985). Distinction between Bunyaviridae genera by surface structure and comparison with Hantaan virus using negative strain electron microscopy. *Archives of Virology*, 86(1-2), 17-28.

127. Martinez, V. P., Bellomo, C., San Juan, J., Pinna, D., Forlenza, R., Elder, M., and Padula P. J. (2005). Person-to-Person Transmission of Andes Virus. *Emerging Infectious Diseases*, 11(12), 1848-1853.

128. McCauley, D. J., Keesing, F., Young, T. and Dittmar, K. (2008). Effects of the removal of large herbivores on fleas of small mammals. *Journal of Vector Ecology*, 33, 263-268. doi:http://dx.doi.org/10.3376/1081-1710-33.2.263.

129. Mehlhorn, H. (Ed.). (2001). *Encyclopedia reference of Parasitology: Biology, Structure, Function* (2nd Edition). Springer-Verlag. Berlin, Germany.

130. Meisel, H., Lundkvist, Å., Gantzer, K., Bär, W., Sibold, C., and Krüger, D. H. (1998). First Case of Infection with Hantavirus Dobrava in Germany. *European Journal of Clinical Microbiology and Infectious Diseases*, 17(12), 884-885.

131. Mfune, J. K. E., Kangombe, F., and Eiseb, S. (2013). Host specificity, prevalence and intensity of infestation of fleas (Order Siphonaptera) of small mammals at selected sites in the city of Windhoek, Namibia. *International Journal of Science and Technology Namibia*, 1(1), 64-77.

132. Milazzo, M. L., Cajimat, M. N. B., Haynie, M. L., Abbott, K. D., Bradley, R. D., and Charles, F. F. (2008). Diversity among Tacaribe serocomplex viruses (Family Arenaviridae) naturally associated with the White-Throated woodrat (*Neotoma albigula*) in the South western United States. *Vector-borne and zoonotic diseases*, 8, 523-540. doi: 10.1089/vbz.2007.0239.

133. Mills, J. N., Ksiazek, T.G., Ellis, B. A., Rollin, P. E., Nichol, S. T., Yates, T. L., Gannon, W. L., Levy, C. E., Engelthaler, D. M., Davis, T., Tanda, D. T., Frampton, J. W., Nichols, C. R., Peters, C. J., and Childs, J. E. (1997). Patterns of associateion with host and habits: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the South western United States. *American Journal of Tropical Medicine and Hygiene*, 56(3), 273-284.

134. Monaco, F., Pinoni, C., Cossedd, G. M., Khaiseb, S., Calistri, P., Molini, U., Bishi, A., Conte, A., Scacchia, M., and Lelli, R. (2013). Rift Valley fever in Namibia, 2010. *Emerging Infectious Diseases*, 19, 2025-2027. doi: 10.3201/eid1912.130593.

135. Monath, T. P., Newhouse, V. F., Kemp, G. E., Setzer, H. W., and Cacciapuoti, A. (1974). Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. *Science*, 185, 263-265. doi: 10.1126./science.185.4147.263.

136. Moncayo, A. C., Hice, C. L., Watts, D. M., de Rosa, T., Guzman, H., Russel, K. L., Calampa, C., Gozalo, A., Popov, V. L., Weaver, S. C., and Tesh, R. B. (2001). Allpahuayo Virus: A Newly Recognized Arenavirus (Arenaviridae) from Arboreal Rice Rats (*Oecomys bicolor* and *Oecomys paricola*) in Northeastern Peru. *Virology*, 284, 277-286. doi:10.1006/viro.2000.0803.

137. Monroe, M. C., Morzunov, S. P., Johnson, A. M., Bowen, M. D., Artsob, H., Yates, T., Peters, C. J., Rollin, P. E., Ksiazek, T. G., and Nichol, S. T. (1999). Genetic diversity and distribution of *Peromyscus*-borne Hantaviruses in North America. *Emerging Infectious Diseases*, 5(1), 75-86.

138. Morand, S. and Poulin, R. (2002). Body size-density relationship and species diversity in parasitic nematodes: patterns and likely processes. *Evolutionary Ecological Research*, 4, 951-961.

139. Morand, S., Krasnov, B. R., and Poulin, R. (2006). *Micromammals and Macroparasites: From Evolutionary Ecology to Management*. Springer-Verlag. Tokyo, Japan.

140. Morzunov, S. P., Rowe, J. E., Ksiazek, T. G., Peters, C. J., Jeor, S. C., and Nichol, S. T. (1998). Genetic Analysis of the Diversity and Origin of Hantaviruses in *Peromyscus luecopus* mice in North America. *Journal of Virology*, 72(1): 57-64.

141. Mulqueen, C. M., Stetz, T. A., Beaubien, J. M., and O'Connell, B. J. (2001). Developing dynamic work roles using Jaccard similarity indices of employee competency data. *Ergometrika*, 2, 26-37.

142. Murray, P. R., Rosenthal, K. S., and Pfaller, M. A. (2005). *Medical Microbiology* (5th Edition). Elsevier Inc, USA.

143. Muranyi, W., Bahr, U., Zeier, M., and van der Woude, F. J. (2005). Hantavirus Infection. *Journal of the Amercian Society of Nephrology*, 16, 3669-3679. doi: 10.1681/ASN.2005050561.

144. Murphy, F. A., Webb, P. A., Johnson, K. M., Whitefield, S. G., and Chappell, W. A. (1970). Arenoviruses in vero cells: Ultrastructural studies. *Journal of Virology*. 64(4), 507-518.

145. Namibia's Draft Fourth National Report to the United Nations Convention on Biological Diversity (UNCBD). (2010). Compiled by the Namibia Ministry of Environment and Tourism. Retrieved from www.cbd.int/doc/world/na/na-nr-04-en

146. Nateghpour, M., Akhavan, A. A., Hanafi-Bojd, A. A., Telmadarraiy, Z., Ayazian, M. S., Hosseini-Vasoukolaei, N., Motevalli-Haghi, A., and Akbarzadeh, D. (2013). Wild rodents and their ectoparasites in Baluchistan area, Southeast of Iran. *Tropical Biomedicine*, 30(1), 72-77.

147. Nichol, S. T., Arikawa, J., and Kawaoka, Y. (2000). Emerging viral diseases. *Proceedings of the National Academy of Science of the United States of America*, 97(23), 12411-12412.

148. Noden, B. H., and van der Colf, B. E. (2013). Neglected tropical diseases of Unresolved mysteries. *Acta Tropica*, 125, 1-17. doi: 10.1016/j.actatropica.2012.09.007.

149. Nursyazana, M. T., Mohdzain, S. N., and Jeffery, J. (2013). Biodiversity and macroparasitic distribution of the wild rat population of Carey Island, Klang. *Tropical Biomedicine*, 30(2), 199-210.

150. Okitsu, S. (2010). Vegetation structure of the biomes in Southwestern Africa and their precipitation patterns. *African Study Monographs Supplement*, 40, 77-89.

151. Opavsky, M. A. (1997). Cat scratch disease: The story continues. *Canadian Journal of Infectious Diseases*, 8(1), 43-49.

152. Palacios, G., Savji, N., Hui, J., da Rosa, A. T., Popov, V., Briese, T., Tesh, R. and Lipkin, W. I. (2010). Genomic and phylogenetic characterization of Merino Walk virus, a novel Arenavirus isolated in South Africa. *Journal of General Virology*, 91, 1315–1324. doi:10.1099/vir.0.017798-0.

153. Papa, A., Mills, J. N., Kouidou, S., Ma, B., Papadimitriou, and Antoniadis, A. (2000). Preliminary characterization and natural history of Hantaviruses in rodents in Northern Greece. *Emerging Infectious Diseases*, 6(6), 654-655.

154. Park, K. H., Kang, Y. U., Kang, S. J., Jung, Y. S., Jang, H. C., and Jung, S. I. (2011). Experience with extrarenal manifestations of hemorrhagic fevers with renal syndrome in a tertiary care hospital in South Korea. *Americal Journal of Tropical Medicine and Hygiene*, 85, 229-233. doi: 10.4269/ajtmh.2011.10.0024.

155. Parmenter, P. R., Yadav, E. P., Parmenter, C. A., Ettestad, P. and Gage, K. (1999). Incidence of plague associated with increased winter-spring precipitation in New Mexico. *The American Society of Tropical Medicine and Hygiene*, 61(5), 814–821.

156. Patterson, J. E. H. and Ruchstuhl, K. E. (2013). Parasite infection and host group size: a meta-analytical review. *Parasitology*, 140, 803-813. doi:10.1017/S0031182012002259.

157. Perez-Orella, C. and Schulte-Hostedde, A. I. (2005). Effects of sex and body size on ectoparasite loads in the northern flying squirrel (*Glaucomys sabrinus*). *Canadian Journal of Zoology*, 83, 1381-1385. doi:10.1139/Z05-126.

158. Pergam, S. A., Schmidt, D. W., Nofchissey, R. A., Hunt, W. C., Harford, A. H., and Goade, D. E. (2009). Potential renal sequelae in survivors of Hantavirus cardiopulmonary syndrome. *American Journal of Tropical Medicine and Hygiene*, 80(2), 279-285.

159. Peters, C. J., and Khan, A. S. (2002). Hantavirus pulmonary syndrome: The new American hemorrhagic fever. *Clinical Infectious diseases*, 34(9), 1224-1231.

160. Plyusnin, A., Vapalahti, O., Lankinen, H., Lehväslaiho, H., Apekina, N., Myasnikov, Y., Kallio-Kokko, H., Henttonen, H., Lundkvist, Å., Brummer-Korvenkontio, M., Gavrilovskaya, I., and Baher, A. (1994). Tula virus: a newly detected Hantavirus carried by European common voles. *Journal of virology*, 68(12), 7833-7839.153.

161. Price, J. L. (1978). Serological evidence of infection of Tacaribe virus and arboviruses in Trinidadian bats. *American Journal of Tropical Medicine and Hygiene*, 27(1), 162-167.

162. Price, M. E., Fisher-Hock, S. P., Craven, R. B., and McCormick, J. B. (1988). A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. *British Medical Journal*, 297(6648), 584-587.

163. Price, P. W. (1984). *Insect Ecology* (2nd Edition). John Wiley and Sons, Inc. New York, United States of America.

164. Poulin, R. and Morand, S. (2001). The diversity of parasites. *The Quarterly Review of Biology*, 75(3), 277-293.

165. Poulin, R., Krasnov, B. R., and Shenbrot, G. I. (2008). Interaction frequency across the geographical range as a determinant of host specialisation in generalist fleas. *International Journal for Parasitology*, 38, 989–997. doi:10.1016/j.ijpara.2008.01.001.

166. Quan, P. L., Firth, C., Conte, J. M., Williams, S. H., Zambrana-Torrelio, C. M., Anthony, S. J., Ellison, J. A., Gilbert, A. T., Kuzmin, I. V., Niezgoda, M., Osinub, M. O. V., Recuenco, S., Markotter, W., Breiman, R. F., Kalemba, L., Malekani, J., Lindblade, K. A., Rostal, M. K., Ojeda-Flores, R., Suzan, G., Davis, L B., Blau, D. M., Ogunkoya, A. B., Epstein, J. H., Daszak, P., Rupprecht, C. E., Holmes, E. C., and Lipkin, W. I. (2013). Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proceedings of the National Academy of Sciences*, 110(20), 8194-8199.

167. Quinn, G. P. and Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge University Press. New York, United States of America.

168. Rafinejad, J., Piazak, N., Dehghan, A., Shemshad, K., and Basseri, H. R. (2013). Affect of some environmental parameters on fleas density in human and animal shelters. *American Journal of Research Communication* ISSN: 2325-4076.

169. Rahbari, S., Nabian, S., Nourolahi, F., Arabkhazaeli, F., and Ebrahimzadeh, E. (2008). Flea Infestation in Farm Animals and Its Health Implication. *Iranian Journal of Parasitology*, 3(2), 43-47.

170. Rasmuso, J., Andersson, C., Norman, E., Haney, M., Evander, M., and Ahlm, C. (2011). Time to revise the paradigm of Hantavirus syndrome? Hantavirus pulmonary syndrome caused by European Hantavirus. *European Journal of Clinical Microbiology and Infectious Diseases*, 30, 685-690. doi: 10.1007/510096-010-1141-6.

171. Rowe, W. P., Murphy, F. A., Bergold, G. H., Casals, J., Hotchin, J., Johnson,K. M., Lehmann-Grube, Mims, C. A., Traub, E., and Webb, P. A. (1970). Arenoviruses: Proposed name for a newly defined virus group. *Journal of Virology*, 5(5), 651-652.

172. Rueesch S., Lemoine, M. and Richner, H. (2012). Ectoparasite reproductive performance when host condition varies. *Parasitology Research*, 111, 1193–1203. doi:10.1007/s00436-012-2953-3.

173. Salas, R., de Manzione, N., Tesh, R. B., Rico-Hesse, R., Shope, R. E., Betancourt, A., Godoy, O., Bruzual, R., Pacheco, M. E., Ramos, B., Tamayo, J. G., Jaimes, E., Vasquez, C., Araoz, F., and Querales, J. (1991). Venezuelan haemorrhagic fever. *Lancet*, 338(8774), 1033-1036.

174. Salvato, M. S., and Shimomaye, E. M. (1989). The complete sequence of Lymphocytic choriomeningitis virus reveals a unique RNA structure and a gene for a zinc finger protein. *Virology*, 173(1), 1-10.

175. Salvato, M. S., Shimomaye, E. M., and Oldstone, M. B. (1989). The primary Structure of the Lymphocytic choriomeningitis virus L gene encodes A putative RNA polymerase. *Virology*, 169(2), 377-384.

176. Sánchez, A. B., and de la Torre, J. C. (2005). Genetic and Biochemical Evidence for an Oligometic Structure of the Function L Polymerase of the Prototypic Arenavirs Lymphocytic Choriomeningitis Virus. *The Journal of Virology*, 79(11), 7262-7268.

177. Scharninghausen, J. J., Meyer, H., Pfeffer, M., Davis, D. S., and Honeycutt, R. L. (1999). Genetic evidence of Dobrava virus in Apodemus agrarius in Hungary. *Emerging Infectious Diseases*, 5(3), 468-470.

178. Schmaljohn, C. S., and Dalrymple, J. M. (1983). Analysis of Hantaan virus RNA: evidence for a new genus of Bunyaviridae. *Virology*, 131(2), 428-491

179. Schmaljohn, C. and Hjelle, B. (1997). Hantaviruses: A Global Disease Problem. *Emerging Infectious Diseases*, 3(2), 95-104.

180. Segerman, J. (1995). Siphonaptera of Southern Africa: handbook for the identification of fleas. Department of Medical Entomology, South African Institute for Medical Research, Johannesburg, South Africa.

181. Scholtz, H. and Holm. (1985). *Insects of Southern Africa*, (pp. 322-326). Butter worth Publishers (PTY) LTD. Durban, South Africa.

182. Schmaljohn, C. S., and Dalrymple, J. M. (1983). Analysis of Hantaan virus RNA: evidence for a new genus of Bunyaviridae. *Virology*, 131(2), 428-491.

183. Schmaljohn, C. S., Hasty, S. E., Dalrymple, J. M., LeDuc, J. W., Lee, H. W., von Bonsdorff, C. H., Brummer-Korvenkontio, M., Vaheri, A., Tsai, T. F., Regnery, H. L., Goldgaber, D., and Lee, P. W. (1985). Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science*, 227, 1041-1044. doi: 10.1126/science.2858126.

184. Schmaljohn, C. S. (1996). Molecular Biology of Hantaviruses. In R. M. Elliott (Ed), *The Bunyaviridae*. (pp. 63-83). Plenum Press, New York.

185. Schmaljohn, C. and Hjelle, B. (1997). Hantaviruses: A Global Disease Problem. *Emerging Infectious Diseases*, 3(2), 95-104.

186. Sewell, D. L. (1995). Laboratory-associated infections and biosafety. *Clinical Microbiology Reviews*, 8(3), 389-405.

187. Shipley, A. E. (1914). Insects and war: III. Fleas. *British Medical Journal*, 2(2805), 568-569.

188. Shihepo, F. G., Eiseb, S., and Cunningham, P. (2008). Fleas (Insecta: Siphonaptera) associated with small mammals in selected areas in northern Namibia. *Journal of the Namibian Scientific Society*, 56, 5-23.

189. Sikes, R. S., Gannon, W. L., and the animal care and use committee of the American society of mammalogists. (2011). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, 92(1), 235-253.

190. Simond, M., Godley, M. L., Mouriquand, P. D. (1998). Paul-Louis Simond and his discovery of plague transmission by rat fleas: a centenary. *Journal of the Royal Society of Medicine*, 91(2), 101-104.

191. Simpson, D. I. H. (1978). Viral haemorrhagic fevers of man. *Bulletin of the World Health Organization, 56(6), 819-832.*

192. Sirola, H., Kallio, E. R., Koistinen, V., Kuronen, I, Lundkvist, A., Vaheri, A., Vapalahti, O., Henttonen, H., and Närvänen, A. (2004). Rapid field test for detection of hantavirus antibodies in rodents. *Epidemiology and Infectection*, 132(3), 549-553.

193. Song, J., Gu, S. H., Bennett, S. N., Arai, S., Puorger, M., Hilbe, M. and Yanagihara. (2007). Seewis virus, a genetically distinct Hantavirus in the Eurasian common shrew (*Sorex araneus*). *Virology Journal*, 4, 1-5. doi:10.1186/1743-422X-4-114.

194. Song, J. W., Kang, H. J., Song, K. J., Truong, T. T., Bennett, S. N., Arai, S., Truong, N. U., and Yanagihara, R. (2007). Newfound Hantavirus in Chinese mole shrew, Vietnam. *Emerging Infectious Diseases*, 13(11), 1784-1787.

195. Stuart C. and Stuart T. (2007). *Field Guide to Mammals of Southern Africa.* Revised Edition. Struik Nature, Cape Town. South Africa.

196. Sumibcay, L., Kadjo, B., Gu, S. H., Kang, H. J., Lim, B. K., Cook, J. A., Song, J., and Yanagihara, R. (2012). Divergent lineage of a novel hantavirus in the banana pipstrelle (*Neoromicia nanus*) in Côte d'Ivoire. *Virology Journal*, 9, 34-46. doi:10.1186/1743-422X-9-34.

197. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28, 2731-2739. doi:10.1093/molbev/msr121.

198. Ter Meulen, J., Lukashevich, I., Sidibe, K., Inapogui, A., Marx, M., Dorlemann, A., Yansane, M. L., Koulemou, K., Change-Claude, J., and Schmitz, H. (1996). Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. *American Journal of Tropical Medicine and Hygiene*, 55(6), 661-666.

199. Towner, J. S., Pourrut, X., Albariño, C. G., Nkogue, C. N., Bird, B. H., Grard, G., Ksiazek, T. G., Gonzalez, J. P., Nichol, S. T., and Leroy, E. M. (2007). Marburg virus infection detected in a common African bat. *PLoS One*, 2(8), e74.

200. Traavik, T., Sommer, A. I., Mehl, R., Berdal, B. P., Stavem, K., Hunderi, O. H., and Dalrymple, J. M. (1984). Nephropathia epidemica in Norway: antigen and antibodies in rodent reservoirs and antibodies in selected human populations. *Journal of Hygiene (London)*, 93(1), 139-146.

201. Tsai, T. F. (1987) Hemorrhagic fever with renal syndrome: mode of transmission to humans. *Lab Anim. Science*, 37(4), 419-427.

202. Ulrich, R. G., Schmidt-Chanasit, J., Schlegel, M., Jacob, J., Petz, H. L., Mertens, M., Wenk, M., Büchner, T., Masur, D., Sevke, K., Groschup, M. H., Gerstengarbe, F. W., Pfeffer, M., Oehme, R., Wegener, W., Bemmann, M., Ohlmeyer, L., Wolf, R., Zoller, H., Koch, J., Brockmann, S., Heckel, G., and Essbauer, S. S. (2008). Network "Rodent-borne pathogens" in Germany: longitudinal studies on the geographical distribution and prevalence of Hantavirus infections. *Parasitology Research*, 103, S121-S129. doi:10.1007/s00436-008-1054-9.

203. Uusiku, S. S. (2007). *Seasonal occurrence of fleas and other ectoparasites on small mammals at Waterberg Plateau, Namibia* (Unpublished master's thesis). University of Namibia, Windhoek, Namibia.

204. Vezza, A. C., Gard, G. P., Compans, R. W., and Bishop, D. H. L. (1977). Structural components and Arenavirus Pichnide. *Journal of Virology*, 23(3), 776-786.

205. Vieth S., Drosten, C., Lenz, O., Vincent, Omilabu, M. S., Hass, M., Becker-Ziaj, B., ter Meulen, J., Nichol, S. T, Schmitz, H., and Günthera, S. (2007). RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 101, 1253—1264. doi:10.1016/j.trstmh.2005.03.018.

206. Viljoen, H., Bennett, N. C., Ueckermann, E. A., and Lutermann. (2011). The role of host traits, season and group size on parasite burdens in a cooperative mammal. *PLoS One*, 6(11), 1-8.

207. Vincent, M. J., Zuiroz, E., Gracia, F., Sanchez, A. J., Ksiazek, T. G., Kitsutani, P. T., Ruedas, L. A., Tinnin, D. S., Caceres, L., Gracia, A., Rollin, P. E., Mills, J. N., Peters, C. J., and Nichol, S. T. (2001). HPS in Panama: identification of novel Hantaviruses and their likely reservoirs. *Virology*, 277, 14-19. doi: 10.1006/viro.2000.0563.

208. Vitullo, A. D., and Merani, M. S. (1988). Is vertical transmission sufficient to maintain Junin virus in Nature? *Journal of General Virology*, 69, 1437-1440. doi: 10.1099/0022-1317-69-6-1437.

209. Wang, H., Yashimatsu, K., Ebihara, G., Ogino, M., Araki, K., Kariwa, H., Wang, Z, Luo, Z Li, D., Hang, C., and Arikawa, J. (2000). Genetic diversity of Hantaviruses isolated in China and characterization of novel Hantaviruses isolated from *Niviventer confucianus* and *Rattus rattus*. *Virology*, 278, 332-345. doi: 10.1006/viro.2000.06.30.

210. Webb, P. A., Justines, G., and Johnson, K. M. (1975). Infection of wild and laboratory animals with Machupo and Latino viruses. *Bulletin of the World Health Organization*, 52(4-6), 493-499.

211. Weiss, N. A. (2002). *Elementary Statistics* (5th ed.). Boston, United States of America: Pearson Education, Inc.

212. Weiss, S., Witkowski, P. T., Auste, B., Nowak, K., Weber, N., Fahr, J., Mombouli, J., Wolfe, N. D., Drexler, J. F., Drosten, C., Klempa, B., Leendertz, F. L. and Krüger, D. H. (2012). Hantavirus in Bat, Sierra Leone. *Emergent Infectious Diseases*, 18, 159–161. doi:10.3201/eid1801.111026.

213. Wells, R. M., Estani, S. S., Yadon, Z. E., Enria, D., Padula, P., Pini, N., Mills, J. N., Peters, C. J., Segura, E. L. and the Hantavirus Pulmonary Syndrome Study Group for Patagonia. (1997). An Unusual Hantavirus Outbreak in Southern Argentina: Person-to-Person Transmission? *Emerging Infectious Diseases*, 3(2), 171-174.

214. Wichmann, D., Gröne, H., Frese, M., Pavlovic, J., Anheier, B., Haller, O., Klenk, H., and Feldmann, H. (2002). Hantaan virus infection causes an acute neurological disease that is fatal in adult laboratory mice. *Journal of Virology*, 76, 8890-8899. doi: 10.1128/JVI.76.17.8890-8899.2002.

215. Wink, M. (Ed). (2006). *An Introduction to Molecular Biotechnology: Molecular Fundamentals, Methods and Application in Modern Biotechnology.* WILEY-VCH Verlag GmbH & Co. kGaA, Weinheim, Germany.

216. Woodruff, A. W., Monath, T. P., Mahmoud, A. A., Pain, A. K., and Morris, C. A. (1973). Lassa fever in Britain: an imported case. *British Medical Journal*, 3(5881), 616-617.

217. Wulff, H., McIntosh, B. M., Hammer, D. B., and Johnson, K. M. (1977). Isolation of an arenavirus closely related to Lassa virus from *Mastomys natalensis* in South-east Africa. *Bulletin of the World Health Organization*, 55(4), 441-444.

218. Yadav, P. D., Vincent, M. J. and Nichol, S. T. (2007). Thottapalayam virus is genetically distant to the rodent-borne hantaviruses, consistent with its isolation from the Asian house shrew (*Suncus murinus*). *Virology Journal*, 4, 1-5. doi:11.1186/1743-422X-4-80.

219. Yamanishi, K., Dantas, J. R., Takahashi, M., Yamanouch, T., Domae, K., Takahashi, Y. and Tanishita, O. (1984). Antigenic differences between two viruses, isolated in Japan and Korea, that causes hemorrhagic fever with renal syndrome. *Journal of Virology*, 52(1), 231-237.

220. Yanagihara, R., Amyx, H. L., and Gajdusek, D. C. (1985). Experimental infection with Puumala virus, the etiologic agent of Nephropathia Epidemica, in Bank vole (*Clethrionomys glareolus*). *Journal of Virology*, 55(1), 34-38.

221. Zapata, J.C., and Salvato, M. S. (2013). Arenavirus variation due to host-specific adaptation. *Viruses*, 5, 241-278. doi:10.3390/v5010241.

222. Zimba, M., Pfukenyi, D., Loveridge, J., and Mukaratirwa, S. (2011). Seasonal abundance of plague vector *Xenopsylla brasiliensis* from rodents captured in three habitat types of periurban suburbs of Harare, Zimbabwe. *Vector Borne Zoonotic Diseases*, 11, 1187-1192. doi: 10.1089/vbz.2010.0095.

APPENDICES

APPENDIX 1: Data sheet for small mammals trapped in Rundu.

Date	Animal #	Species	Ref. #	Sex	Mass (g)
29/01/2011	1	*Gerbilliscus leucogaster*	RDTl1	F	72
29/01/2011	2	*Gerbilliscus leucogaster*	RDTl2	M	73
29/01/2011	3	*Gerbilliscus leucogaster*	RDTl3	M	49
29/01/2011	4	*Gerbilliscus leucogaster*	RDTl4	F	66
29/01/2011	5	*Gerbilliscus leucogaster*	RDTl5	F	39
29/01/2011	6	*Saccostomus campestris*	RDSc1	M	39
29/01/2011	7	*Mastomys* sp.	RVMsp1	F	29
29/01/2011	8	*Mastomys* sp.	RVMsp2	F	54
29/01/2011	9	*Mastomys* sp.	RVMsp3	F	34
29/01/2011	10	*Mastomys* sp.	RVMsp4	M	50
29/01/2011	11	*Mastomys* sp.	RVMsp5	F	24
29/01/2011	12	*Mastomys* sp.	RVMsp6	F	32
29/01/2011	13	*Mastomys* sp.	RVMsp7	F	38
29/01/2011	14	*Mastomys* sp.	RVMsp8	M	53
29/01/2011	15	*Aethomys namaquensis*	RVAn1	F	45
29/01/2011	16	*Mastomys* sp.	RVMsp9	M	34
29/01/2011	17	*Mastomys* sp.	RVMsp10	F	25
29/01/2011	18	*Aethomys namaquensis*	RVAn2	F	23
29/01/2011	19	*Mastomys* sp.	RVMsp11	F	30
29/01/2011	20	*Mastomys* sp.	RVMsp12	F	20
29/01/2011	21	*Mastomys* sp.	RVMsp13	F	29
29/01/2011	22	*Mastomys* sp.	RVMsp14	F	26
29/01/2011	23	*Mastomys* sp.	RVMsp15	M	59
29/01/2011	24	*Mastomys* sp.	RVMsp16	F	38
29/01/2011	25	*Mastomys* sp.	RVMsp17	M	27
29/01/2011	26	*Mastomys* sp.	RVMsp18	M	42
30/01/2011	27	*Gerbilliscus leucogaster*	RDTl6	F	64
30/01/2011	28	*Gerbilliscus leucogaster*	RDTl7	M	70
30/01/2011	29	*Gerbilliscus leucogaster*	RDTl8	F	23
30/01/2011	30	*Saccostomus campestris*	RDSc2	M	23
30/01/2011	31	*Gerbilliscus leucogaster*	RDTl9	F	14
30/01/2011	32	*Gerbilliscus leucogaster*	RDTl10	M	94
30/01/2011	33	*Gerbilliscus leucogaster*	RDTl11	M	59
30/01/2011	34	*Gerbilliscus leucogaster*	RDTl12	F	61
30/01/2011	35	*Gerbilliscus leucogaster*	RDTl13	F	83

30/01/2011	36	*Mastomys* sp.	RVMsp19	F	29
30/01/2011	37	*Mastomys* sp.	RVMsp20	M	64
30/01/2011	38	*Mastomys* sp.	RVMsp21	F	30
30/01/2011	39	*Mastomys* sp.	RVMsp22	F	17
30/01/2011	40	*Mastomys* sp.	RVMsp23	M	41
30/01/2011	41	*Mastomys* sp.	RVMsp24	M	40
30/01/2011	42	*Mastomys* sp.	RVMsp25	F	46
31/01/2011	43	*Gerbilliscus leucogaster*	RDTl14	F	78
31/01/2011	44	*Gerbilliscus leucogaster*	RDTl15	F	54
31/01/2011	45	*Gerbilliscus leucogaster*	RDTl16	F	24

APPENDIX 2: Data sheet for small mammals trapped in Palmwag.

Date	**Animal #**	**Species**	**Ref. #**	**Sex**	**Mass (g)**
04-04-11	1	*Aethomys namaquensis*	PRMn1	F	42
04-04-11	2	*Aethomys namaquensis*	PRMn2	F	50
04-04-11	3	*Aethomys namaquensis*	PRMn3	F	26
04-04-11	4	*Thallomys paedulcus*	PRTp1	M	102
05-04-11	5	*Thallomys paedulcus*	PRTp2	F	86
05-04-11	6	*Aethomys namaquensis*	PRMn4	F	16
05-04-11	7	*Aethomys namaquensis*	PRMn5	M	60
05-04-11	8	*Gerbirullus paeba*	PRGp1	F	58
05-04-11	9	*Aethomys namaquensis*	PRMn6	M	40
05-04-11	10	*Aethomys namaquensis*	PRMn7	M	43
05-04-11	11	*Aethomys namaquensis*	PRMn8	M	34
05-04-11	12	*Pedromyscus collinus*	PRPc1	M	24
05-04-11	13	*Pedromyscus collinus*	PRPc2	M	22

APPENDIX 3: Data sheet for small mammals trapped in CCF.

Date	**Animal #**	**Species**	**Ref. #**	**Sex**	**Mass (g)**
03-02-11	1	*Aethomys namaquensis*	CCCFAn1	F	25
03-02-11	2	*Saccostomus campestris*	CCCFSc1	F	60
03-02-11	3	*Mastomys* sp.	CCCFMsp1	F	7
03-02-11	4	*Aethomys chrysophilus*	CCCFAc1	M	38
03-02-11	5	*Aethomys namaquensis*	CCCFAn2	F	35
04-02-11	6	*Saccostomus campestris*	CCCFSc2	F	70
04-02-11	7	*Aethomys namaquensis*	CCCFAn3	M	43
04-02-11	8	*Aethomys namaquensis*	CCCFAn4	F	83
04-02-11	9	*Saccostomus campestris*	CCCFSc3	F	64
04-02-11	10	*Aethomys namaquensis*	CCCFAn5	M	39
04-02-11	11	*Aethomys namaquensis*	CCCFAn6	M	88
04-02-11	12	*Aethomys namaquensis*	CCCFAn7	M	87
04-02-11	13	*Aethomys namaquensis*	CCCFAn8	M	88
04-02-11	14	*Aethomys namaquensis*	CCCFAn9	M	52
04-02-11	15	*Aethomys namaquensis*	CCCFAn10	F	45
04-02-11	16	*Crocidura fuscomurina*	WCCFCf1	F	4
04-02-11	17	*Aethomys namaquensis*	WCCFAn1	F	88
04-02-11	18	*Aethomys namaquensis*	WCCFAn2	M	72
04-02-11	19	*Aethomys namaquensis*	WCCFAn3	F	70
04-02-11	20	*Aethomys namaquensis*	WCCFAn4	M	90
04-02-11	21	*Gerbilliscus leucogaster*	WCCFTl1	F	82
04-02-11	22	*Saccostomus campestris*	WCCFSc1	F	34
04-02-11	23	*Aethomys namaquensis*	WCCFAn5	M	73
04-02-11	24	*Aethomys namaquensis*	WCCFAn6	M	42
04-02-11	25	*Aethomys namaquensis*	WCCFAn7	F	36
04-02-11	26	*Aethomys namaquensis*	WCCFAn8	F	80
04-02-11	27	*Aethomys namaquensis*	WCCFAn9	F	66
04-02-11	28	*Aethomys namaquensis*	WCCFAn10	M	32
04-02-11	29	*Saccostomus campestris*	WCCFSc2	F	56
04-02-11	30	*Saccostomus campestris*	WCCFSc3	M	53
05-02-11	31	*Elephantulus intufi*	WCCFEi1	F	36
05-02-11	32	*Aethomys chrysophilus*	WCCFAc1	F	82
05-02-11	33	*Aethomys namaquensis*	WCCFAn11	M	97
05-02-11	34	*Aethomys namaquensis*	WCCFAn12	F	31
05-02-11	35	*Saccostomus campestris*	WCCFSc4	F	38
05-02-11	36	*Aethomys namaquensis*	WCCFAn13	F	72
05-02-11	37	*Crocidura fuscomurina*	WCCFCf2	F	4

05-02-11	38	*Saccostomus campestris*	WCCFSc5	M	39
05-02-11	39	*Saccostomus campestris*	WCCFSc6	F	52
06-02-11	40	*Aethomys chrysophilus*	MFCCFAc1	M	90
06-02-11	41	*Aethomys namaquensis*	MFCCFAn1	F	69
06-02-11	42	*Mastomys* sp.	MFCCFMn1	F	30
06-02-11	43	*Aethomys chrysophilus*	MFCCFAc2	M	64
06-02-11	44	*Aethomys chrysophilus*	MFCCFAc3	M	57
06-02-11	45	*Mastomys* sp.	GCCFMn1	F	30
06-02-11	46	*Mastomys* sp.	GCCFMn2	M	42
06-02-11	47	*Mastomys* sp.	GCCFMn3	F	36
07-02-11	48	*Aethomys chrysophilus*	HCCFAc1	F	82
07-02-11	49	*Mastomys* sp.	HCCFMn1	M	51
07-02-11	50	*Saccostomus campestris*	HCCFSc1	M	23
07-02-11	51	*Aethomys chrysophilus*	HCCFAc2	M	83
07-02-11	52	*Saccostomus campestris*	HCCFSc2	M	49
07-02-11	53	*Aethomys chrysophilus*	HCCFAc3	M	64
07-02-11	54	*Saccostomus campestris*	HCCFSc3	M	52
07-02-11	55	*Aethomys chrysophilus*	HCCFAc4	M	97
07-02-11	56	*Mastomys* sp.	HCCFMn2	F	66
07-02-11	57	*Rhabdomys pumilio*	G2CCFRp1	F	38
07-02-11	58	*Mastomys* sp.	G2CCFMsp1	F	47
07-02-11	59	*Mastomys* sp.	G2CCFMsp2	F	48

APPENDIX 4: Data sheet for small mammals trapped in the Okahandja.

Date	**Animal #**	**Species**	**Ref. #**	**Sex**	**Mass (g)**
19-06-12	1	*Gerbilliscus leucogaster*	OkhGL1	M	46
19-06-12	2	*Elephantulus intufi*	OkhEi1	F	44
19-06-12	3	*Micaelamys namaquensis*	OkhMi.n1	F	40
19-06-12	4	*Elephantulus intufi*	OkhEi2	F	43
19-06-12	5	*Micaelamys namaquensis*	OkhMi.n2	F	19
19-06-12	6	*Micaelamys namaquensis*	OkhMi.n3	M	32
19-06-12	7	*Elephantulus intufi*	OkhEi3	F	48
19-06-12	8	*Aethomys chrysophilus*	OkhAc1	F	61
19-06-12	9	*Micaelamys namaquensis*	OkhMi.n4	M	15
19-06-12	10	*Aethomys chrysophilus*	OkhAc2	F	49
19-06-12	11	*Mastomys* sp.	OkhMn17	F	19
19-06-12	12	*Gerbilliscus leucogaster*	OkhGL5	F	47
19-06-12	13	*Gerbilliscus leucogaster*	OkhGL6	F	48
19-06-12	14	*Gerbilliscus leucogaster*	OkhGL7	F	27
19-06-12	15	*Gerbilliscus leucogaster*	OkhGL8	F	48
19-06-12	16	*Gerbilliscus leucogaster*	OkhGL9	F	19
19-06-12	17	*Gerbilliscus leucogaster*	OkhGL10	F	47
19-06-12	18	*Micaelamys namaquensis*	OkhMi.n4	M	31
19-06-12	19	*Mastomys natalensis*	OkhMn18	F	32
19-06-12	20	*Gerbilliscus leucogaster*	OkhGL11	M	71
19-06-12	21	*Micaelamys namaquensis*	OkhMi.n5	M	38
19-06-12	22	*Mastomys* sp.	OkhMn19	F	18
19-06-12	23	*Micaelamys namaquensis*	OkhMi.n6	M	39
19-06-12	24	*Aethomys chrysophilus*	OkhAc4	M	72
19-06-12	25	*Micaelamys namaquensis*	OkhMi.n7	M	21
19-06-12	26	*Mastomys* sp.	OkhMn20	M	18
19-06-12	27	*Micaelamys namaquensis*	OkhMi.n8	M	35
19-06-12	28	*Gerbilliscus leucogaster*	OkhGL12	M	51
19-06-12	29	*Gerbilliscus leucogaster*	OkhGL13	F	50
19-06-12	30	*Gerbilliscus leucogaster*	OkhGL14	F	29
19-06-12	31	*Mastomys* sp.	OkhMn1	M	15
19-06-12	32	*Mastomys* sp.	OkhMn2	M	21
19-06-12	33	*Mastomys* sp.	OkhMn3	M	19
19-06-12	34	*Mastomys* sp.	OkhMn4	M	39
19-06-12	35	*Mastomys* sp.	OkhMn5	M	14
19-06-12	36	*Mastomys* sp.	OkhMn6	F	20
19-06-12	37	*Gerbilliscus leucogaster*	OkhGL2	F	70
19-06-12	38	*Mastomys* sp.	OkhMn7	M	22

19-06-12	39	*Mastomys* sp.	OkhMn8	M	19
19-06-12	40	*Mastomys* sp.	OkhMn9	F	13
19-06-12	41	*Mastomys* sp.	OkhMn10	F	12
19-06-12	42	*Mastomys* sp.	OkhMn11	M	11
19-06-12	43	*Aethomys chrysophilus*	OkhAc3	F	62
19-06-12	44	*Mastomys* sp.	OkhMn12	M	13
19-06-12	45	*Mastomys* sp.	OkhMn13	F	19
19-06-12	46	*Mastomys* sp.	OkhMn14	M	30
19-06-12	47	*Mastomys* sp.	OkhMn15	M	17
19-06-12	48	*Gerbilliscus leucogaster*	OkhGL3	M	87
19-06-12	49	*Mastomys* sp.	OkhMn16	F	17
19-06-12	50	*Gerbilliscus leucogaster*	OkhGL4	F	26
20-06-12	51	*Micaelamys namaquensis*	OkhMi.n9	F	34
20-06-12	52	*Micaelamys namaquensis*	OkhMi.n10	F	41
20-06-12	53	*Micaelamys namaquensis*	OkhMi.n11	M	31
20-06-12	54	*Mastomys* sp.	OkhMn21	M	44
20-06-12	55	*Mastomys* sp.	OkhMn22	M	22
20-06-12	56	*Gerbilliscus leucogaster*	OkhGL15	F	54
20-06-12	57	*Gerbilliscus leucogaster*	OkhGL16	M	55
20-06-12	58	*Mastomys* sp.	OkhMn23	M	28
20-06-12	59	*Gerbilliscus leucogaster*	OkhGL17	F	50
20-06-12	60	*Micaelamys namaquensis*	OkhMi.n12	M	20
20-06-12	61	*Micaelamys namaquensis*	OkhMi.n13	F	31
20-06-12	62	*Gerbilliscus leucogaster*	OkhGL18	F	47
20-06-12	63	*Mastomys* sp.	OkhMn24	M	12
20-06-12	64	*Gerbilliscus leucogaster*	OkhGL19	M	39
20-06-12	65	*Micaelamys namaquensis*	OkhMi.n14	F	37
20-06-12	66	*Micaelamys namaquensis*	OkhMi.n15	M	30
20-06-12	67	*Micaelamys namaquensis*	OkhMi.n16	M	28
20-06-12	68	*Micaelamys namaquensis*	OkhMi.n17	M	31
20-06-12	69	*Gerbilliscus leucogaster*	OkhGL20	F	50
20-06-12	70	*Micaelamys namaquensis*	OkhMi.n18	F	19
20-06-12	71	*Micaelamys namaquensis*	OkhMi.n19	F	46
20-06-12	72	*Micaelamys namaquensis*	OkhMi.n20	M	30
20-06-12	73	*Mastomys* sp.	OkhMn25	M	15
20-06-12	74	*Gerbilliscus leucogaster*	OkhGL21	F	63
20-06-12	75	*Gerbilliscus leucogaster*	OkhGL22	F	69
20-06-12	76	*Mastomys* sp.	OkhMn26	F	19
20-06-12	77	*Micaelamys namaquensis*	OkhMi.n21	F	47
20-06-12	78	*Micaelamys namaquensis*	OkhMi.n22	F	44
20-06-12	79	*Gerbilliscus leucogaster*	OkhGL23	F	50

20-06-12	80	*Micaelamys namaquensis*	OkhMi.n23	F	34
20-06-12	81	*Aethomys chrysophilus*	OkhAc5	M	68
20-06-12	82	*Gerbilliscus leucogaster*	OkhGL24	F	60
20-06-12	83	*Micaelamys namaquensis*	OkhMi.n24	M	31
20-06-12	84	*Gerbilliscus leucogaster*	OkhGL25	F	38
20-06-12	85	*Mastomys* sp.	OkhMn27	F	19
20-06-12	86	*Micaelamys namaquensis*	OkhMi.n25	F	16
20-06-12	87	*Mastomys* sp.	OkhMn28	F	15
20-06-12	88	*Aethomys chrysophilus*	OkhAc6	M	78
20-06-12	89	*Micaelamys namaquensis*	OkhMi.n26	M	28
20-06-12	90	*Micaelamys namaquensis*	OkhMi.n27	F	19
20-06-12	91	*Micaelamys namaquensis*	OkhMi.n28	F	24
20-06-12	92	*Gerbilliscus leucogaster*	OkhGL26	M	66
20-06-12	93	*Micaelamys namaquensis*	OkhMi.n29	F	32
20-06-12	94	*Micaelamys namaquensis*	OkhMi.n30	M	31
20-06-12	95	*Aethomys chrysophilus*	OkhAc7	F	60
20-06-12	96	*Mastomys* sp.	OkhMn29	M	13
20-06-12	97	*Micaelamys namaquensis*	OkhMi.n31	M	14
20-06-12	98	*Micaelamys namaquensis*	OkhMi.n32	M	16
20-06-12	99	*Gerbilliscus leucogaster*	OkhGL26	F	51
20-06-12	100	*Micaelamys namaquensis*	OkhMi.n33	M	22
20-06-12	101	*Micaelamys namaquensis*	OkhMi.n34	M	22
20-06-12	102	*Gerbilliscus leucogaster*	OkhGL27	F	68
20-06-12	103	*Gerbilliscus leucogaster*	OkhGL28	M	17
20-06-12	104	*Micaelamys namaquensis*	OkhMi.n35	M	21
20-06-12	105	*Micaelamys namaquensis*	OkhMi.n36	M	23
20-06-12	106	*Gerbilliscus leucogaster*	OkhGL29	F	59

APPENDIX 5: Data sheet for small mammals trapped in Neudamm.

Date	**Animal #**	**Species**	**Ref. #**	**Sex**	**Mass (g)**
06-09-10	1	*Rhabdomys pumilio*	WHRp1	M	30
06-09-10	2	*Rhabdomys pumilio*	WHRp2	F	40
06-09-10	3	*Rhabdomys pumilio*	WHRp3	F	24
06-09-10	4	*Rhabdomys pumilio*	WHRp4	F	41
06-09-10	5	*Rhabdomys pumilio*	WHRp5	M	35
06-09-10	6	*Rhabdomys pumilio*	WHRp6	M	22
06-09-10	7	*Rhabdomys pumilio*	WHRp7	F	25
06-09-10	8	*Rhabdomys pumilio*	WHRp8	F	25
06-09-10	9	*Rhabdomys pumilio*	WHRp9	F	18
06-09-10	10	*Rhabdomys pumilio*	WHRp10	F	18
06-09-10	11	*Rhabdomys pumilio*	WHRp11	F	24
06-09-10	12	*Shrew* sp.	WHSh1	M	28
06-09-10	13	*Gerbilliscus leucogaster*	WHGs1	F	45
06-09-10	14	*Rhabdomys pumilio*	WHRp12	F	16
06-09-10	15	*Rhabdomys pumilio*	WHRp13	F	25
06-09-10	16	*Rhabdomys pumilio*	WHRp14	M	20
06-09-10	17	*Rhabdomys pumilio*	WHRp15	F	28
06-09-10	18	*Rhabdomys pumilio*	WHRp16	M	32
06-09-10	19	*Rhabdomys pumilio*	WHRp17	M	26
06-09-10	20	*Rhabdomys pumilio*	WHRp18	M	19
06-09-10	21	*Rhabdomys pumilio*	WHRp19	F	24
06-09-10	22	*Rhabdomys pumilio*	WHRp20	M	27
06-09-10	23	*Rhabdomys pumilio*	WHRp21	M	26
06-09-10	24	*Rhabdomys pumilio*	WHRp22	M	20
06-09-10	25	*Rhabdomys pumilio*	WHRp23	M	26
06-09-10	26	*Rhabdomys pumilio*	WHRp24	F	21
06-09-10	27	*Rhabdomys pumilio*	WHRp25	M	18
06-09-10	28	*Rhabdomys pumilio*	WHRp26	M	24
06-09-10	29	*Rhabdomys pumilio*	WHRp27	F	33
06-09-10	30	*Rhabdomys pumilio*	WHRp28	M	25
06-09-10	31	*Rhabdomys pumilio*	WHRp29	F	28
06-09-10	32	*Rhabdomys pumilio*	WHRp30	F	27
06-09-10	33	*Rhabdomys pumilio*	WHRp31	M	26
06-09-10	34	*Rhabdomys pumilio*	WHRp32	F	24
06-09-10	35	*Rhabdomys pumilio*	WHRp33	F	16
06-09-10	36	*Crocidura fuscomurina*	WHSh2	F	4
08-09-10	37	*Gerbilliscus leucogaster*	WHGs2	F	49

08-09-10	38	*Gerbilliscus leucogaster*	WHGs3	F	52
08-09-10	39	*Gerbilliscus leucogaster*	WHGs4	F	52
08-09-10	40	*Gerbilliscus leucogaster*	WHGs5	F	47
08-09-10	41	*Gerbilliscus leucogaster*	WHGs6	M	64
08-09-10	42	*Micaelamys namaquensis*	WHAc1	M	26
08-09-10	43	*Micaelamys namaquensis*	WHAc2	M	16
08-09-10	44	*Micaelamys namaquensis*	WHAc3	F	32
25-01-12	45	*Mastomys* sp.	NDMn1	M	44
25-01-12	46	*Mastomys* sp.	NDMn2	M	30
25-01-12	47	*Micaelamys namaquensis*	NDMi.n1	M	39
25-01-12	48	*Rhabdomys pumilio*	NDRp1	F	26
25-01-12	49	*Micaelamys namaquensis*	NDMi.n2	M	40
25-01-12	50	*Micaelamys namaquensis*	NDMi.n3	M	36
25-01-12	51	*Rhabdomys pumilio*	NDRp2	M	39
25-01-12	52	*Micaelamys namaquensis*	NDMi.n4	F	22
25-01-12	53	*Micaelamys namaquensis*	NDMi.n5	M	36
25-01-12	54	*Micaelamys namaquensis*	NDMi.n6	M	39
25-01-12	55	*Micaelamys namaquensis*	NDMi.n7	M	39
26-01-12	56	*Micaelamys namaquensis*	NDMi.n8	M	40
26-01-12	57	*Gerbilliscus leucogaster*	NDGl1	M	49
26-01-12	58	*Rhabdomys pumilio*	NDRp3	F	28
26-01-12	59	*Rhabdomys pumilio*	NDRp4	F	27
26-01-12	60	*Micaelamys namaquensis*	NDMi.n9	F	22
26-01-12	61	*Micaelamys namaquensis*	NDMi.n10	F	18
26-01-12	62	*Rhabdomys pumilio*	NDRp5	M	34
26-01-12	63	*Rhabdomys pumilio*	NDRp6	M	34
26-01-12	64	*Rhabdomys pumilio*	NDRp7	F	40
26-01-12	65	*Micaelamys namaquensis*	NDMi.n11	M	30
26-01-12	66	*Micaelamys namaquensis*	NDMi.n12	M	34
26-01-12	67	*Gerbilliscus leucogaster*	NDGl2	M	68
26-01-12	68	*Micaelamys namaquensis*	NDMi.n13	M	38
26-01-12	69	*Crocidura fuscomurina*	NDCf1	F	5
26-01-12	70	*Mus indutus*	NDMi1	F	3
27-01-12	71	*Micaelamys namaquensis*	NDMi.n14	M	33
27-01-12	72	*Rhabdomys pumilio*	NDRp8	M	32
27-01-12	73	*Micaelamys namaquensis*	NDMi.n15	F	23
27-01-12	74	*Rhabdomys pumilio*	NDRp9	M	25
27-01-12	75	*Micaelamys namaquensis*	NDMi.n16	M	25
27-01-12	76	*Micaelamys namaquensis*	NDMi.n17	M	30
27-01-12	77	*Aethomys chrysophilus*	NDAc1	M	42

27-01-12	78	*Aethomys chrysophilus*	NDAc2	F	28
27-01-12	79	*Aethomys chrysophilus*	NDAc3	M	47
27-01-12	80	*Mastomys* sp.	NDMn3	M	38
27-01-12	81	*Micaelamys namaquensis*	NDMi.n18	M	30
27-01-12	82	*Rhabdomys pumilio*	NDRp10	F	33
27-01-12	83	*Micaelamys namaquensis*	NDMi.n19	F	22
27-01-12	84	*Micaelamys namaquensis*	NDMi.n20	F	22
27-01-12	85	*Micaelamys namaquensis*	NDMi.n21	F	21
27-01-12	86	*Micaelamys namaquensis*	NDMi.n22	F	22
27-01-12	87	*Micaelamys namaquensis*	NDMi.n23	F	25
27-01-12	88	*Micaelamys namaquensis*	NDMi.n24	F	17
27-01-12	89	*Micaelamys namaquensis*	NDMi.n25	M	34
31-01-12	90	*Gerbilliscus leucogaster*	NDGl3	F	57
31-01-12	91	*Rhabdomys pumilio*	NDRp11	M	45
31-01-12	92	*Rhabdomys pumilio*	NDRp12	F	34
31-01-12	93	*Micaelamys namaquensis*	NDMi.n26	M	37
31-01-12	94	*Micaelamys namaquensis*	NDMi.n27	F	22
31-01-12	95	*Micaelamys namaquensis*	NDMi.n28	F	23
31-01-12	96	*Micaelamys namaquensis*	NDMi.n29	F	23
31-01-12	97	*Micaelamys namaquensis*	NDMi.n30	M	38
31-01-12	98	*Micaelamys namaquensis*	NDMi.n31	M	38
31-01-12	99	*Micaelamys namaquensis*	NDMi.n32	F	29
31-01-12	100	*Micaelamys namaquensis*	NDMi.n33	F	27
31-01-12	101	*Micaelamys namaquensis*	NDMi.n34	M	34
31-01-12	102	*Micaelamys namaquensis*	NDMi.n35	F	30
31-01-12	103	*Micaelamys namaquensis*	NDMi.n36	F	20
31-01-12	104	*Micaelamys namaquensis*	NDMi.n37	F	24
31-01-12	105	*Rhabdomys pumilio*	NDRp13	M	29
31-01-12	106	*Micaelamys namaquensis*	NDMi.n38	F	20
31-01-12	107	*Micaelamys namaquensis*	NDMi.n39	M	32
31-01-12	108	*Gerbilliscus leucogaster*	NDGl4	F	63
31-01-12	109	*Rhabdomys pumilio*	NDRp14	M	36
31-01-12	110	*Rhabdomys pumilio*	NDRp15	M	40
31-01-12	111	*Micaelamys namaquensis*	NDMi.n40	F	40
31-01-12	112	*Micaelamys namaquensis*	NDMi.n41	M	34
31-01-12	113	*Gerbilliscus leucogaster*	NDGl5	F	39
31-01-12	114	*Micaelamys namaquensis*	NDMi.n42	M	37
31-01-12	115	*Micaelamys namaquensis*	NDMi.n43	F	27
31-01-12	116	*Micaelamys namaquensis*	NDMi.n45	F	25
31-01-12	117	*Micaelamys namaquensis*	NDMi.n46	M	28

31-01-12	118	*Micaelamys namaquensis*	NDMi.n47	F	29
31-01-12	119	*Micaelamys namaquensis*	NDMi.n48	F	20
31-01-12	120	*Gerbilliscus leucogaster*	NDGl6	F	63
31-01-12	121	*Saccostomus campestris*	NDSc1	F	66
31-01-12	122	*Gerbilliscus leucogaster*	NDGl7	F	60
31-01-12	123	*Micaelamys namaquensis*	NDMi.n49	M	36
31-01-12	124	*Gerbilliscus leucogaster*	NDGl8	F	66
31-01-12	125	*Micaelamys namaquensis*	NDMi.n50	M	52
31-01-12	126	*Rhabdomys pumilio*	NDRp15	F	28
31-01-12	127	*Micaelamys namaquensis*	NDMi.n51	F	38
31-01-12	128	*Micaelamys namaquensis*	NDMi.n52	F	24
31-01-12	129	*Micaelamys namaquensis*	NDMi.n53	M	32
31-01-12	130	*Rhabdomys pumilio*	NDRp16	M	44
31-01-12	131	*Rhabdomys pumilio*	NDRp17	F	42
31-01-12	132	*Rhabdomys pumilio*	NDRp18	F	20
31-01-12	133	*Micaelamys namaquensis*	NDMi.n54	M	26
31-01-12	134	*Micaelamys namaquensis*	NDMi.n55	F	29
31-01-12	135	*Micaelamys namaquensis*	NDMi.n56	F	18
31-01-12	136	*Micaelamys namaquensis*	NDMi.n57	M	30
31-01-12	137	*Micaelamys namaquensis*	NDMi.n58	M	42
31-01-12	138	*Gerbilliscus leucogaster*	NDGl9	F	65
31-01-12	139	*Micaelamys namaquensis*	NDMi.n59	F	18
31-01-12	140	*Micaelamys namaquensis*	NDMi.n60	M	40
31-01-12	141	*Micaelamys namaquensis*	NDMi.n61	M	35
31-01-12	142	*Rhabdomys pumilio*	NDRp19	F	36
31-01-12	143	*Micaelamys namaquensis*	NDMi.n62	F	22
31-01-12	144	*Micaelamys namaquensis*	NDMi.n63	F	24
31-01-12	145	*Rhabdomys pumilio*	NDRp20	M	40
31-01-12	146	*Micaelamys namaquensis*	NDMi.n64	M	34
31-01-12	147	*Rhabdomys pumilio*	NDRp21	F	36
31-01-12	148	*Gerbilliscus leucogaster*	NDGl10	F	59
31-01-12	149	*Crocidura fuscomurina*	NDCf2	F	4
31-01-12	150	*Rhabdomys pumilio*	NDRp22	F	36
31-01-12	151	*Micaelamys namaquensis*	NDMi.n65	M	31
31-01-12	152	*Micaelamys namaquensis*	NDMi.n66	F	20
31-01-12	153	*Gerbilliscus leucogaster*	NDGl21	F	43

APPENDIX 6: Data sheet for small mammals trapped in Ben-hur.

Date	**Animal #**	**Species**	**Ref #**	**Sex**	**Mass (g)**
15-11-11	1	*Gerbilliscus leucogaster*	Bh1Ac1	F	70
15-11-11	2	*Micaelamys namaquensis*	Bh1Mi.n1	M	27
15-11-11	3	*Micaelamys namaquensis*	Bh1Mi.n2	F	28
15-11-11	4	*Gerbilliscus leucogaster*	Bh2(Ac1)Gl1	F	79
15-11-11	5	*Lemniscomys rosalia*	Bh2Lr1	F	49
15-11-11	6	*Gerbilliscus leucogaster*	Bh2Gl1	M	75
15-11-11	7	*Gerbilliscus leucogaster*	Bh2Gl2	M	76
15-11-11	8	*Gerbilliscus leucogaster*	Bh2Gl3	M	86
15-11-11	9	*Gerbilliscus leucogaster*	Bh2Gl4	M	72
15-11-11	10	*Gerbilliscus leucogaster*	Bh2Gl5	F	74
15-11-11	11	*Gerbilliscus leucogaster*	Bh2Gl6	F	73
15-11-11	12	*Gerbilliscus leucogaster*	Bh3Gl1	M	81
15-11-11	13	*Micaelamys namaquensis*	Bh3Mn1	F	39
15-11-11	14	*Micaelamys namaquensis*	Bh3Mn2	M	45
15-11-11	15	*Gerbilliscus leucogaster*	Bh3Gl3	M	78
15-11-11	16	*Mastomys* sp.	Bh3Mas.n1	F	16
16-11-11	17	*Lemniscomys rosalia*	Bh2Lr1	F	50
16-11-11	18	*Gerbilliscus leucogaster*	Bh2Gl7	M	59
16-11-11	19	*Saccostomus campestris*	EmSc1	M	51
16-11-11	20	*Micaelamys namaquensis*	EmMi.n1	F	38
16-11-11	21	*Micaelamys namaquensis*	EmMi.n2	M	39
16-11-11	22	*Gerbillurus setzeri*	EmGs1	M	14
17-11-11	23	*Gerbilliscus leucogaster*	TWGl1	M	74
17-11-11	24	*Gerbilliscus leucogaster*	TWGl2	M	73
17-11-11	25	*Gerbilliscus leucogaster*	TWGl3	F	61
17-11-11	26	*Gerbilliscus leucogaster*	TWGl4	F	57
17-11-11	27	*Gerbilliscus leucogaster*	TWGl5	M	90
17-11-11	28	*Gerbilliscus leucogaster*	TWGl6	F	77
17-11-11	29	*Gerbilliscus leucogaster*	TWGl7	M	69
18-11-11	30	*Gerbillurus paeba*	TWGp1	F	12
18-11-11	31	*Gerbilliscus leucogaster*	TWGl8	M	72
18-11-11	32	*Aethomys chrysophilus*	TWAc1	M	77
18-11-11	33	*Gerbilliscus leucogaster*	TWGl9	M	74
18-11-11	34	*Gerbilliscus leucogaster*	TWGl10	M	66
18-11-11	35	*Gerbilliscus leucogaster*	TWGl11	F	82
18-11-11	36	*Aethomys chrysophilus*	TWAc2	F	63

18-11-11	37	*Gerbilliscus leucogaster*	TWGl12	F	72
18-11-11	38	*Gerbilliscus leucogaster*	TWGl13	F	62
18-11-11	39	*Aethomys chrysophilus*	TWAc3	M	73
18-11-11	40	*Gerbilliscus leucogaster*	TWGl14	F	60
18-11-11	41	*Gerbilliscus leucogaster*	TWGl15	M	81
18-11-11	42	*Aethomys chrysophilus*	TWAc4	F	58
18-11-11	43	*Gerbilliscus leucogaster*	TWGl16	F	55
18-11-11	44	*Micaelamys namaquensis*	TWMn1	F	19
18-11-11	45	*Gerbillurus paeba*	TWGp2	M	24
18-11-11	46	*Gerbilliscus leucogaster*	TWGl17	M	64
18-11-11	47	*Aethomys chrysophilus*	TWAc5	F	73

APPENDIX 7: Data sheet for small mammals trapped in Talismanus.

Date	**Animal #**	**Species**	**Ref. #**	**Sex**	**Mass (g)**
06-12-11	1	*Aethomys chrysophilus*	StAc1	M	92
06-12-11	2	*Gerbilliscus leucogaster*	StGl1	F	78
06-12-11	3	*Gerbilliscus leucogaster*	StGl2	M	76
06-12-11	4	*Gerbilliscus leucogaster*	StGl3	M	68
06-12-11	5	*Gerbilliscus leucogaster*	StGl4	M	84
06-12-11	6	*Aethomys chrysophilus*	StAc2	F	73
06-12-11	7	*Gerbilliscus leucogaster*	StGl5	F	57
06-12-11	8	*Gerbilliscus leucogaster*	StGl6	M	97
06-12-11	9	*Gerbilliscus leucogaster*	StGl7	F	72
06-12-11	10	*Gerbilliscus leucogaster*	StGl8	M	78
07-12-11	11	*Gerbilliscus leucogaster*	StGl9	M	74
07-12-11	12	*Gerbilliscus leucogaster*	StGl10	F	68
08-12-11	13	*Gerbilliscus leucogaster*	StGl11	M	69
08-12-11	14	*Gerbilliscus leucogaster*	StGl12	M	76
08-12-11	15	*Gerbilliscus leucogaster*	StGl13	F	57
08-12-11	16	*Gerbilliscus leucogaster*	StGl14	F	68
08-12-11	17	*Gerbilliscus leucogaster*	StGl15	M	91
08-12-11	18	*Gerbilliscus leucogaster*	StGl16	F	72
08-12-11	19	*Gerbilliscus leucogaster*	StGl17	M	80
08-12-11	20	*Gerbilliscus leucogaster*	StGl18	M	93
08-12-11	21	*Gerbilliscus leucogaster*	StGl19	M	68
08-12-11	22	*Dendromus melanotis*	StDm1	F	5
09-12-11	23	*Gerbilliscus leucogaster*	StGl20	F	61
09-12-11	24	*Aethomys chrysophilus*	StAc3	M	54
09-12-11	25	*Gerbilliscus leucogaster*	StGl21	F	67
09-12-11	26	*Gerbilliscus leucogaster*	StGl22	F	67
09-12-11	27	*Gerbilliscus leucogaster*	StGl23	F	72
09-12-11	28	*Gerbilliscus leucogaster*	StGl24	F	75
09-12-11	29	*Micaelamys namaquensis*	StMi.n1	M	34
09-12-11	30	*Gerbilliscus leucogaster*	StGl25	M	79
09-12-11	31	*Gerbilliscus leucogaster*	StGl26	M	76

APPENDIX 8: Data sheet for small mammals trapped in Mariental.

Date	**Animal #**	**Species**	**Ref #**	**Sex**	**Mass (g)**
02-04-12	1	*Gerbilliscus leucogaster*	MR Gl1	F	34
02-04-12	2	*Micaelamys namaquensis*	MR Mi.n1	F	34
02-04-12	3	*Rhabdomys pumilio*	MR Rp1	M	31
02-04-12	4	*Mastomys* sp.	MR Mn1	M	48
02-04-12	5	*Rhabdomys pumilio*	MR Rp2	M	25
02-04-12	6	*Mastomys* sp.	MR Mn2	F	38
02-04-12	7	*Micaelamys namaquensis*	MR Mi.n2	F	39
02-04-12	8	*Gerbilliscus leucogaster*	MR Gl2	F	53
02-04-12	9	*Elephantulus intufi*	MR Ei1	F	47
02-04-12	10	*Micaelamys namaquensis*	MR Mi.n3	M	46
02-04-12	11	*Micaelamys namaquensis*	MR Mi.n4	F	37
02-04-12	12	*Micaelamys namaquensis*	MR Mi.n5	M	49
02-04-12	13	*Micaelamys namaquensis*	MR Mi.n6	F	41
02-04-12	14	*Micaelamys namaquensis*	MR Mi.n7	F	19
03-04-12	15	*Rhabdomys pumilio*	MR Rp3	F	40
03-04-12	16	*Rhabdomys pumilio*	MR Rp4	F	37
03-04-12	17	*Mastomys* sp.	MR Mn3	F	35
03-04-12	18	*Gerbilliscus leucogaster*	MR Gl2	F	60
03-04-12	19	*Gerbilliscus leucogaster*	MR Gl3	F	50
03-04-12	20	*Elephantulus intufi*	MR Ei2	F	39
03-04-12	21	*Rhabdomys pumilio*	MR Rp5	M	39
03-04-12	22	*Rhabdomys pumilio*	MR Rp6	M	40
03-04-12	23	*Mus indutus*	MR Mi1	F	9
03-04-12	24	*Micaelamys namaquensis*	MR Mi.n8	F	21
03-04-12	25	*Petromyscus collinus*	MR Pc1	M	10
03-04-12	26	*Crocidura hirta*	MR Ch1	F	5
03-04-12	27	*Micaelamys namaquensis*	MR Mi.n9	M	33
03-04-12	28	*Micaelamys namaquensis*	MR Mi.n10	M	22
03-04-12	29	*Micaelamys namaquensis*	MR Mi.n11	M	22
03-04-12	30	*Mastomys* sp.	MR Mn4	M	15
03-04-12	31	*Rhabdomys pumilio*	MR Rp7	M	32
04-04-12	32	*Mus indutus*	MR Mi2	F	7
04-04-12	33	*Gerbilliscus leucogaster*	MR Gl4	M	58
04-04-12	34	*Mastomys* sp.	MR Mn5	F	26
04-04-12	35	*Gerbilliscus leucogaster*	MR Gl5	F	26
04-04-12	36	*Gerbilliscus leucogaster*	MR Gl6	F	56
04-04-12	37	*Gerbilliscus leucogaster*	MR Gl7	F	28

04-04-12	38	*Rhabdomys pumilio*	MR Rp8	F	34
04-04-12	39	*Gerbilliscus leucogaster*	MR Gl8	F	48
04-04-12	40	*Gerbilliscus leucogaster*	MR Gl9	F	28
04-04-12	40	*Crocidura hirta*	MR Ch2	F	4
04-04-12	42	*Mastomys* sp.	MR Mn6	F	32
04-04-12	43	*Gerbilliscus leucogaster*	MR Gl10	F	46
04-04-12	44	*Gerbilliscus leucogaster*	MR Gl11	F	49
04-04-12	45	*Mastomys* sp.	MR Mn7	M	41
04-04-12	46	*Gerbilliscus leucogaster*	MR Gl12	F	58
04-04-12	47	*Rhabdomys pumilio*	MR Rp9	F	33
04-04-12	48	*Mastomys* sp.	MR Mn8	F	32
04-04-12	49	*Gerbilliscus leucogaster*	MR Gl13	F	64
04-04-12	50	*Gerbilliscus leucogaster*	MR Gl14	F	26
04-04-12	51	*Gerbilliscus leucogaster*	MR Gl15	F	28
04-04-12	52	*Gerbilliscus leucogaster*	MR Gl16	F	27
04-04-12	53	*Gerbilliscus leucogaster*	MR Gl17	F	27
04-04-12	54	*Gerbilliscus leucogaster*	MR Gl18	F	50
04-04-12	55	*Gerbilliscus leucogaster*	MR Gl19	F	61
04-04-12	56	*Gerbilliscus leucogaster*	MR Gl20	F	52
04-04-12	57	*Mus indutus*	MR Mi3	F	4
04-04-12	58	*Mastomys* sp.	MR Mn9	F	28
04-04-12	59	*Gerbilliscus leucogaster*	MR Gl21	M	53
04-04-12	60	*Crocidura hirta*	MR Ch3	F	3
04-04-12	61	*Mastomys* sp.	MR Mn10	M	11
04-04-12	62	*Mastomys* sp.	MR Mn11	M	30
04-04-12	63	*Crocidura hirta*	MR Ch4	F	4
04-04-12	64	*Crocidura hirta*	MR Ch5	F	4

APPENDIX 9: Data sheet of all flea species recovered from small mammals in Rundu.

Date	Host species	Ref. #	Host sex	Family	Subfamily	Genus	Species	# of fleas	Flea sex
29-01-11	*Gerbilliscus leucogaster*	RDT.l1(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	1	M
29-01-11	*Gerbilliscus leucogaster*	RDT.l2(1)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	3	F
29-01-11	*Gerbilliscus leucogaster*	RDT.l2(2)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		F
29-01-11	*Gerbilliscus leucogaster*	RDT.l2(3)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		F
29-01-11	*Gerbilliscus leucogaster*	RDT.l3(1)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	2	M
29-01-11	*Gerbilliscus leucogaster*	RDT.l3(2)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*		M
29-01-11	*Gerbilliscus leucogaster*	RDT.l4(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	5	M
29-01-11	*Gerbilliscus leucogaster*	RDT.l4(2)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*		F
29-01-11	*Gerbilliscus leucogaster*	RDT.l4(3)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
29-01-11	*Gerbilliscus leucogaster*	RDT.l4(4)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		F
29-01-11	*Gerbilliscus leucogaster*	RDT.l4(5)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
29-01-11	*Gerbilliscus leucogaster*	RDT.l5()	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	2	F
29-01-11	*Gerbilliscus leucogaster*	RDT.l5()	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
30-01-11	*Gerbilliscus leucogaster*	RDT.l7(1)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	1	F
29-01-11	*Micaelamys namaquensis*	RVAn2(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	2	M
29-01-11	*Micaelamys namaquensis*	RVAn2(2)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
29-01-11	*Mastomys* sp.	RVMsp1(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	5	M
29-01-11	*Mastomys* sp.	RVMsp1(2)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*hirsuta*		F
29-01-11	*Mastomys* sp.	RVMsp1(3)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*hirsuta*		F
29-01-11	*Mastomys* sp.	RVMsp1(4)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*hirsuta*		F
29-01-11	*Mastomys* sp.	RVMsp1(5)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
29-01-11	*Mastomys* sp.	RVMsp3(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	1	F
29-01-11	*Mastomys* sp.	RVMsp4(1)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	1	F

29-01-11	*Mastomys* sp.	RVMsp7(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	4	M
29-01-11	*Mastomys* sp.	RVMsp7(2)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*		F
29-01-11	*Mastomys* sp.	RVMsp7(3)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		F
29-01-11	*Mastomys* sp.	RVMsp7(4)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*		F
29-01-11	*Mastomys* sp.	RVMsp9(1)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	1	M
29-01-11	*Mastomys* sp.	RVMsp11(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	1	F
29-01-11	*Mastomys* sp.	RVMsp15(1)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	3	F
29-01-11	*Mastomys* sp.	RVMsp15(2)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
29-01-11	*Mastomys* sp.	RVMsp15(3)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*		M
29-01-11	*Mastomys* sp.	RVMsp16(1)	F	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*brasiliensis*	1	F
30-01-11	*Mastomys* sp.	RVMsp23(2)	M	Pulicidae	*Xenopsyllinae*	*Xenopsylla*	*cheopis*	1	M

APPENDIX 10: Data sheet of all flea species recovered from small mammals in Neudamm.

Date	Host species	Reference	Host sex	Family	Subfamily	Genus	Species	# of fleas	Flea sex
25-01-12	*Mastomys* sp.	Mn 2(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	21	F
25-01-12	*Mastomys* sp.	Mn 2(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*		F
25-01-12	*Mastomys* sp.	Mn 2(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 2(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 2(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(8)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 2(9)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(10)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(11)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(12)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(13)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(14)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 2(15)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 2(16)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 2(17)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(18)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(19)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(20)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 2(21)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n3(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	M

25-01-12	*Micaelamys namaquensis*	Mi n3(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n3(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n1(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	M
25-01-12	*Micaelamys namaquensis*	Mi n1(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n1(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n6(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	M
25-01-12	*Micaelamys namaquensis*	Mi n6(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	10	M
25-01-12	*Micaelamys namaquensis*	Mi n5(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n5(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n5(8)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(9)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n5(10)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Rhabdomys pumilio*	Rp 1(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	6	F
25-01-12	*Rhabdomys pumilio*	Rp 1(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Rhabdomys pumilio*	Rp 1(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Rhabdomys pumilio*	Rp 1(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Rhabdomys pumilio*	Rp 1(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Rhabdomys pumilio*	Rp 1(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Rhabdomys pumilio*	Rp 2(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	5	F
25-01-12	*Rhabdomys pumilio*	Rp 2(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M

25-01-12	*Rhabdomys pumilio*	Rp 2(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Rhabdomys pumilio*	Rp 2(4)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*		F
25-01-12	*Rhabdomys pumilio*	Rp 2(5)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
25-01-12	*Mastomys* sp.	Mn 1(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	9	F
25-01-12	*Mastomys* sp.	Mn 1(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Mastomys* sp.	Mn 1(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 1(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 1(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 1(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 1(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 1(8)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Mastomys* sp.	Mn 1(9)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Gerbilliscus leucogaster*	Gl 2(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
25-01-12	*Micaelamys namaquensis*	Mi n4(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	19	F
25-01-12	*Micaelamys namaquensis*	Mi n4(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(5)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(6)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(7)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(8)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(9)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(10)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n4(11)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(12)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M

25-01-12	*Micaelamys namaquensis*	Mi n4(13)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(14)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(15)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n4(16)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
25-01-12	*Micaelamys namaquensis*	Mi n4(17)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(18)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n4(19)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
25-01-12	*Micaelamys namaquensis*	Mi n7(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
26-01-12	*Rhabdomys pumilio*	Rp5(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	4	F
26-01-12	*Rhabdomys pumilio*	Rp5(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
26-01-12	*Rhabdomys pumilio*	Rp5(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Rhabdomys pumilio*	Rp5(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Micaelamys namaquensis*	Mi n11(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	8	M
26-01-12	*Micaelamys namaquensis*	Mi n11(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
26-01-12	*Micaelamys namaquensis*	Mi n11(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Micaelamys namaquensis*	Mi n11(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
26-01-12	*Micaelamys namaquensis*	Mi n11(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Micaelamys namaquensis*	Mi n11(6)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
26-01-12	*Micaelamys namaquensis*	Mi n11(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Micaelamys namaquensis*	Mi n11(8)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Rhabdomys pumilio*	Rp 4(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	1	M
26-01-12	*Rhabdomys pumilio*	Rp 6(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	M
26-01-12	*Rhabdomys pumilio*	Rp 6(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
26-01-12	*Rhabdomys pumilio*	Rp 6(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
26-01-12	*Rhabdomys pumilio*	Rp 8(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F

26-01-12	*Rhabdomys pumilio*	Rp 7(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	M
26-01-12	*Rhabdomys pumilio*	Rp 7(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
26-01-12	*Micaelamys namaquensis*	Mi n12(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F
26-01-12	*Micaelamys namaquensis*	Mi n12(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
26-01-12	*Micaelamys namaquensis*	Mi n12(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
27-01-12	*Micaelamys namaquensis*	Mi n3(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
27-01-12	*Micaelamys namaquensis*	Mi n25(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	4	M
27-01-12	*Micaelamys namaquensis*	Mi n25(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
27-01-12	*Micaelamys namaquensis*	Mi n25(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
27-01-12	*Micaelamys namaquensis*	Mi n25(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n24(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n39(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	6	F
31-01-12	*Micaelamys namaquensis*	Mi n39(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n39(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n39(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n39(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n39(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n54(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F
31-01-12	*Micaelamys namaquensis*	Mi n54(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n54(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 10(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	1	F
31-01-12	*Micaelamys namaquensis*	Mi n65(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n66(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	3	F
31-01-12	*Micaelamys namaquensis*	Mi n66(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n66(3)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*		F

31-01-12	*Micaelamys namaquensis*	Mi n38(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	6	M
31-01-12	*Micaelamys namaquensis*	Mi n38(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n38(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n38(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n38(5)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n38(6)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n53(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	M
31-01-12	*Micaelamys namaquensis*	Mi n53(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n48(1)	M	Pulicidae	Xenopsyllinae	Xenopsylla	brasiliensis	3	F
31-01-12	*Micaelamys namaquensis*	Mi n48(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n48(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n29(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n46(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	M
31-01-12	*Micaelamys namaquensis*	Mi n46(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n46(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n47(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	F
31-01-12	*Micaelamys namaquensis*	Mi n47(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n13(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n42(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	6	F
31-01-12	*Micaelamys namaquensis*	Mi n42(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n42(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n42(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n42(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n42(6)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
31-01-12	*Micaelamys namaquensis*	Mi n23(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F

31-01-12	*Micaelamys namaquensis*	Mi n23(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n23(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n56(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	F
31-01-12	*Micaelamys namaquensis*	Mi n56(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 14(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n49(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	11	M
31-01-12	*Micaelamys namaquensis*	Mi n49(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(3)	M	Chimaeropsyllinae	Chiastopsyllinae	*Chiastopsylla*	*rossi*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(8)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(9)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(10)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n49(11)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Min 51(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	4	M
31-01-12	*Micaelamys namaquensis*	Mi n51(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Min 51(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n51(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Min 45(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n37(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F
31-01-12	*Micaelamys namaquensis*	Mi n57(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F
31-01-12	*Micaelamys namaquensis*	Mi n57(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n57(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M

31-01-12	*Micaelamys namaquensis*	Mi n59(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	5	M
31-01-12	*Micaelamys namaquensis*	Mi n59(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n59(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n59(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n59(5)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Gerbilliscus leucogaster*	Gl 9(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	M
31-01-12	*Gerbilliscus leucogaster*	Gl 9(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Gerbilliscus leucogaster*	Gl 9(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n32(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F
31-01-12	*Micaelamys namaquensis*	Mi n32(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n32(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n36(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	M
31-01-12	*Micaelamys namaquensis*	Mi n36(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Gerbilliscus leucogaster*	Gl 4(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	4	F
31-01-12	*Gerbilliscus leucogaster*	Gl 4(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Gerbilliscus leucogaster*	Gl 4(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Gerbilliscus leucogaster*	Gl 4(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n27(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	M
31-01-12	*Micaelamys namaquensis*	Mi n27(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n50(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	4	M
31-01-12	*Micaelamys namaquensis*	Mi n50(2)	M	Pulicidae	Xenopsyllinae	Xenopsylla	brasiliensis		M
31-01-12	*Micaelamys namaquensis*	Mi n50(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n50(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Gerbilliscus leucogaster*	Gl 8(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n60(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	5	M

31-01-12	*Micaelamys namaquensis*	Mi n60(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
31-01-12	*Micaelamys namaquensis*	Mi n60(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n60(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n60(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n31(1)	M	Chimaeropsyllinae	Chiastopsyllinae	*Chiastopsylla*	*rossi*	1	M
31-01-12	*Rhabdomys pumilio*	Rp 18(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	2	M
31-01-12	*Rhabdomys pumilio*	Rp 18(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n52(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	3	M
31-01-12	*Micaelamys namaquensis*	Mi n52(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n52(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Gerbilliscus leucogaster*	Gl3(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	F
31-01-12	*Gerbilliscus leucogaster*	Gl3(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 16(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	4	M
31-01-12	*Rhabdomys pumilio*	Rp 16(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 16(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 16(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 20(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F
31-01-12	*Micaelamys namaquensis*	Mi n2(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n28(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	M
31-01-12	*Micaelamys namaquensis*	Mi n28(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n26(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F
31-01-12	*Micaelamys namaquensis*	Mi n26(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n26(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Gerbilliscus leucogaster*	Gl 5(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F
31-01-12	*Rhabdomys pumilio*	Rp 11(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	1	M

31-01-12	*Rhabdomys pumilio*	Rp 17(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	1	F
31-01-12	*Saccostomus campestris*	Sc1(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Gerbilliscus leucogaster*	Gl 7(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	M
31-01-12	*Micaelamys namaquensis*	Mi n30(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	8	F
31-01-12	*Micaelamys namaquensis*	Mi n30(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n30(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n30(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n30(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n30(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n30(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n30(8)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n61(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	5	M
31-01-12	*Micaelamys namaquensis*	Mi n61(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n61(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n61(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n61(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Rhabdomys pumilio*	Rp 15(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F
31-01-12	*Micaelamys namaquensis*	Mi n63(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F
31-01-12	*Micaelamys namaquensis*	Mi n40(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	5	M
31-01-12	*Micaelamys namaquensis*	Mi n40(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*		F
31-01-12	*Micaelamys namaquensis*	Mi n40(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n40(4)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n40(5)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n43(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F
31-01-12	*Gerbilliscus leucogaster*	Gl 10(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	3	F

31-01-12	*Gerbilliscus leucogaster*	Gl 10(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Gerbilliscus leucogaster*	Gl 10(3)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		M
31-01-12	*Micaelamys namaquensis*	Mi n57(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	7	F
31-01-12	*Micaelamys namaquensis*	Mi n57(2)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n57(3)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n57(4)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n57(5)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n57(6)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n57(7)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Rhabdomys pumilio*	Rp 22(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*	4	F
31-01-12	*Rhabdomys pumilio*	Rp 22(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*philoxera*		F
31-01-12	*Rhabdomys pumilio*	Rp 22(3)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
31-01-12	*Rhabdomys pumilio*	Rp 22(4)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
31-01-12	*Micaelamys namaquensis*	Mi n33(1)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	2	F
31-01-12	*Micaelamys namaquensis*	Mi n33(2)	F	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*		F
31-01-12	*Micaelamys namaquensis*	Mi n58(1)	M	Pulicidae	*Listropsyllinae*	*Listropsylla*	*aricinae*	1	F

APPENDIX 11: Data sheet of all flea species recovered from small mammals in Mariental.

Date	Host species	Reference	Host sex	Family	Subfamily	Genus	Species	# of fleas	Flea sex
02-04-12	*Micaelamys namaquensis*	MRMi.n1(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*	2	M
02-04-12	*Micaelamys namaquensis*	MRMi.n1(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n2(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	2	F
02-04-12	*Micaelamys namaquensis*	MRMi.n2(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n3(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*	9	F
02-04-12	*Micaelamys namaquensis*	MRMi.n3(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n3(3)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n3(4)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n3(5)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n3(6)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n3(7)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n3(8)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n3(9)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n4(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	5	F
02-04-12	*Micaelamys namaquensis*	MRMi.n4(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n4(3)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n4(4)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n4(5)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n5(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*	8	M
02-04-12	*Micaelamys namaquensis*	MRMi.n5(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n5(3)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n5(4)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n5(5)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M

02-04-12	*Micaelamys namaquensis*	MRMi.n5(6)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
02-04-12	*Micaelamys namaquensis*	MRMi.n5(7)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		F
02-04-12	*Micaelamys namaquensis*	MRMi.n5(8)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
02-04-12	*Rhabdomys pumilio*	MRRp1(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	3	F
02-04-12	*Rhabdomys pumilio*	MRRp1(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
02-04-12	*Rhabdomys pumilio*	MRRp1(3)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
02-04-12	*Rhabdomys pumilio*	MRRP2(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*	2	F
02-04-12	*Rhabdomys pumilio*	MRRP2(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		F
02-04-12	*Mastomys* sp.	MRMn1(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	1	M
03-04-12	*Micaelamys namaquensis*	MRMi.n9(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	4	F
03-04-12	*Micaelamys namaquensis*	MRMi.n9(2)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
03-04-12	*Micaelamys namaquensis*	MRMi.n9(3)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*nubica*		M
03-04-12	*Micaelamys namaquensis*	MRMi.n9(4)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*hirsuta*		F
03-04-12	*Rhabdomys pumilio*	MRRp4(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	1	F
04-04-12	*Mastomys* sp.	MRMn7(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	1	M
04-04-12	*Mastomys* sp.	MRMn8(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*	1	M
04-04-12	*Mastomys* sp.	MRMn9(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	3	F
04-04-12	*Mastomys* sp.	MRMn9(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
04-04-12	*Mastomys* sp.	MRMn9(3)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
04-04-12	*Mastomys* sp.	MRMn10(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	1	M
04-04-12	*Rhabdomys pumilio*	MRRp8(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	4	F
04-04-12	*Rhabdomys pumilio*	MRRp8(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
04-04-12	*Rhabdomys pumilio*	MRRp8(3)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
04-04-12	*Rhabdomys pumilio*	MRRp8(4)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		F
04-04-12	*Rhabdomys pumilio*	MRRp9(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*	1	M
04-04-12	*Gerbilliscus leucogaster*	MRGl4(1)	M	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*	1	F

04-04-12	*Gerbilliscus leucogaster*	MRGl5(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	1	F
04-04-12	*Gerbilliscus leucogaster*	MRGl6(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	2	F
04-04-12	*Gerbilliscus leucogaster*	MRGl6(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*		F
04-04-12	*Gerbilliscus leucogaster*	MRGl7(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	1	F
04-04-12	*Gerbilliscus leucogaster*	MRGl9I1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*	1	F
04-04-12	*Gerbilliscus leucogaster*	MRGl12(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	1	F
04-04-12	*Gerbilliscus leucogaster*	MRGl13(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*cheopis*	1	F
04-04-12	*Gerbilliscus leucogaster*	MRGl19(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*	4	M
04-04-12	*Gerbilliscus leucogaster*	MRGl19(2)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*		F
04-04-12	*Gerbilliscus leucogaster*	MRGl19(3)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*versuta*		M
04-04-12	*Gerbilliscus leucogaster*	MRGl19(4)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*		M
04-04-12	*Gerbilliscus leucogaster*	MRGl20(1)	F	Pulicidae	Xenopsyllinae	*Xenopsylla*	*piriei*	1	M

APPENDIX 12: Fleas infesting the small mammal species in the four regions of study.

Region	Host species	Total number of fleas collected (number of fleas/hosts examined)								
		Cr	**Lar**	**Xb**	**Xc**	**Xh**	**Xn**	**Xph**	**Xpi**	**Xv**
Okavango	*G. leucogaster* (6)	0 (0.00)	0 (0.00)	4 (0.67)	10 (1.67)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Mastomys sp. (9)	0 (0.00)	0 (0.00)	7 (0.78)	8 (0.89)	3 (0.33)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	M. namaquensis (1)	0 (0.00)	0 (0.00)	1 (1.00)	1 (1.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Khomas	*G. leucogaster* (8)	0 (0.00)	16 (2.0)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Mastomys sp. (2)	0 (0.00)	29 (14.5)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.2)	0 (0.00)	0 (0.00)
	M. namaquensis (49)	2 (0.04)	161 (3.29)	2 (0.04)	2 (0.04)	0 (0.00)	0 (0.00)	4 (0.08)	0 (0.00)	7 (0.14)
	R. pumilio (38)	0 (0.00)	23 (0.61)	0 (0.00)	1 (0.026)	0 (0.00)	0 (0.00)	8 (0.21)	0 (0.00)	6 (0.16)
	S. campestris (1)	0 (0.00)	1 (1.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Hardap	*G. leucogaster* (9)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.67)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.67)	1 (0.11)
	Mastomys sp. (5)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.20)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.20)	5 (1.00)
	M. namaquensis (6)	0 (0.00)	0 (0.00)	0 (0.00)	7 (1.17)	1 (0.17)	12 (2.00)	0 (0.00)	1 (0.17)	9 (1.50)
	R. pumilio (5)	0 (0.00)	0 (0.00)	0 (0.00)	5 (1.00)	0 (0.00)	2 (0.40)	0 (0.00)	0 (0.00)	4 (0.80)