DIVERSITY OF TERRESTRIAL SMALL MAMMALS AND PREVALENCE OF HAEMOPATHOGENS IN *RATTUS RATTUS* OF MAFIA ISLAND, TANZANIA

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THE DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER DEGREE OF SCIENCE IN WILDLIFE MANAGEMENTAND CONSERVATION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

2022

EXTENDED ABSTRACT

This study presents the diversity of terrestrial small mammals and prevalence of haemopathogens in Mafia Islands and the small islands (Chole, Jibondo, Juani and Shungimbili) which are associated with Mafia. Terrestrial small mammals comprise a group of mammals including volant and non-volant which are cosmopolitan and successful due to their wide range of feeding, adaptive reproduction strategies and habitat occupation. A study on the diversity of small mammals and their haemopathogens prevalence was conducted in Mafia Island and small islands associated with it from August to October 2021. A total of 120 rodents were captured by direct method using Sherman traps and indirectly using camera traps. Each directly trapped individual was immobilized with ethanol and parameters such as weight and sex were recorded. The samples that were taken include an earpiece which was preserved in Eppendorf tube containing 90% ethanol for further confirmatory species identification using molecular techniques. For the purpose of molecularly identifying the hemopathogens, 0.5 ml of blood was collected from the retro-orbital sinus using a capillary tube and kept as a dried blood spot on filter paper (Whatman paper). According to the findings, all of the collected rodents were classified as Rattus rattus and belonged to the RrC lineage I. One Crocidura hildegardae shrew was also trapped.Our camera traps recorded black and rufous sengi *Rhynchocyon petersi*, blue monkey *Cercopithecus mitis,* blue duiker *Cephalophus monticola*, the introduced Small Indian Genet *Viverricula indica,* and the red bush squirrel *Paraxerus palliatus*. Of the 120 *Rattus rattus,* 13.33% tested positive for *Bartonella* DNA. Prevalence between the six studied sites was significantly different (df = 5 and p-value <0.001). None of the rickettsia, leptospira, brucella, anaplasma, coxiella and trypanosoma was detected. Despite the limited time of data collection, our results show that there is low diversity of small mammals on the Mafia islands, with *R. rattus* dominating in all small islands. Also, the results show the presence of *Uncultured Bartonella spp* among other haemopathogen being obtained in *R. rattus*. The study recommends further studies in the rest of forest patches in Mafia and to also explore the diversity of flying mammals which was not included in this study but also to explore more on the prevalence of haemopathogens within the island.

Key Words: Diversity, Small mammals, *Rattus rattus*, Haemopathogens, Bartonella*,* Mafia

DECLARATION

I, TECLA MAGENI SIRILO, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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ACKNOWLEDGEMENT

My gratitude appreciation is highly conveyed to Prof. Dr. Herwig Leirs, for giving me the chance to perform this study through conducting research and data processing in the University of Antwerp in Belgium and for providing guidance and feedback. In addition, a great word of appreciation goes to supervisors Dr. Christopher Sabuni, Dr. Nsajigwa Emmanuel Mbije and Dr. Anne Laudisoit who gave valuable and helpful advice and who shared extensive in-field knowledge during the fieldwork in Mafia Island, Tanzania. In addition, I also appreciate the efforts that Natalie Van Houtte made in showing me the way in the laboratory and Prof. Dr. Sophie Gryseels for the feedback on the molecular methods.
For their willingness to work together to complete this project, I would especially want to thank the Evolutionary Ecology Group at the University of Antwerp and the Department of Wildlife Management at the Sokoine University of Agriculture (SUA) in Tanzania. Additionally, I would like to express my gratitude to the Mafia Island Marine Park administration for granting me permission to carry out this fieldwork and to Mr. Mshangama and Mr. Meza Abubakari for their assistance and sharing of their local expertise.To my fellow master's students from the University of Antwerp Ms Evy Pauly and Ms Maud Van Haarlem I really appreciate their valuable contributions and support in the field. Further, I thank Dr. Charles Lyimo for his valuable observations and input to this study. Finally, I extend my sincere thanks to my mother and my husband for being with me throughout this journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMOVA	:	Analysis of molecular variance
BLAST:		Basic Local Alignment Search Tool
CTR	:	Camera Trap Rate
Cytb	:	Cytochrome b
DNA	:	Deoxyrebosenucleic Acid
DNTPs:		Deoxynucleotide Triphosphates
EVECO	:	Evolutionary Ecology Group
H	:	Shannon wiener diversity index
MEGA	:	Molecular Evolutionary Genetic Analysis
MtDNA:		Mitochondria Deoxyribonucleic acid
NC	:	Number of captures
NCBI	:	National Center for Biotechnology Information, GenBank
NP	:	Number of Photos
NT	:	Number of trap night
PCR	:	Polymerase Chain Reaction
P_i	:	Proportional of individual species
RrC	:	Rattus rattus Complex
TN	:	Trap Night
TS	:	Trapping success
VIB	:	Vlaams institute for Biotechnology

STRUCTURE OF THE DISSERTATION

This dissertation consists of FOUR chapters.

Chapter ONE: Describes background information on diversity of terrestrial small mammals and prevalence of haemopathogens in mafia island, problem statement and justification, research objectives, and the list of manuscripts.

Chapter TWO: (Manuscript ONE) describes the Diversity of terrestrial small mammals in Mafia Island, Tanzania, submitted to the ACTA TROPICA JOURNAL on 28/09/2022.

Chapter THREE: (Manuscript TWO) describes the Prevalence of haemopathogens in *Rattus rattus* of Mafia Island, Tanzania, in preparation for submission

Chapter FOUR: Consists of general discussion, conclusion, and recommendations.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Small mammals are classified as individual mammal species that weigh up to 5 kg and can be found in both terrestrial and aquatic settings. Volant and non-volant small mammals are included in this category (Hafner, 2007). According to some authors, small mammals are any mammal species weighing less than 1 kg (Happold, 2013; Gurnell and Flowerdew, 2006; Michael *et al*., 2016). The definition of a tiny animal in this study will be based on Hafner's (2007).

Small mammals include both flying (such as the Chiroptera) and non-flying (such as rodents such squirrels, mongooses, hedgehogs, and shrews, as well as elephant shrews or sengis (Macroscelidae), and hares (Lagomorpha)) (Kingdon, 2015). More over half of all known extant species of mammals are members of the small mammal group, which is the largest among mammals in terms of abundance and species diversity (Happold, 2013).

The majority of these species have developed successful nighttime behaviors, effective body temperature regulation, and quick reproduction after drought. Even though small mammals make up a substantial portion of the ecosystem's fauna, they are frequently ignored when conservation policies are put into place (Avenant & Cavallini, 2007). Small mammals are indeed essential animals that help to maintain the energy and carbon cycles, soil fertility, and act as sensitive bioindicators of environmental deterioration (Avenant & Cavallini,2007, Michael *et al*., 2016).

Due to climatic and vegetation changes in the past, tiny mammals' distribution patterns frequently varied (Happold, 2013). Studies on the variety and composition of small mammals have mostly been undertaken in vegetation types that are unlike to coastal forests and home to the savanna biome, such as dry woodland, grassland, and shrub land (Carey and Harrington, 2001; Kock and Stanley, 2009; Merritt, 2010; Michael *et al*., 2016).

In Tanzania however, very few studies have tried to explore on small mammals of coastal forests for example the influence of floristic and distribution of small mammals and study on the ecology of sympatric species in Zaraninge and in coastal forests in Saadani National Park respectively (Kiwia 2000; Sabuni *et al*, 2015).

The importance of small mammals where rodents comprise a large group are found to be reservoir hosts for about 60 zoonotic diseases (Gratz, 1997; Taylor *et al.*, 2008) which participate in the transmission of diseases in different ways (Meerburg *et al.*, 2009, Buckle and Smith, 2014). Some of the zoonotic diseases includes Plague (Kilonzo 1992) and Leptospirosis (Mgode *et al*., 2021).

Mafia island being one of the Districts in Coastal region comprise remnant of coastal forest. This isolation makes the coastal forests to be different from that of offshore such as Zaraninge coastal forest that is linked to large savanna land and hence could have connectivity of small mammal and hence allow gene exchange flow. Despite this isolation there is paucity of information on diversity and species composition on non-flying small mammals (Kock and Stanley, 2009) also no record on diseases associated with small mammal particular rodents. This study therefore aims at determining the current small

mammals' status of species diversity that exists in the Mafia Island forests and associated diseases. The information obtained will be used as a base line for further studies and knowing the existing species will help in mitigation measure for conservation purpose.

1.2 Problem Statement and Justification

Coastal forests have been known to harbor and support large number of small and large mammal species, of which they include, rodent species, primates, elephant shrews and carnivorous mammals (Kiwia, 2000). In East Africa, a number of endemic species are being observed in Kenyan and Tanzanian coasts where in Tanzania this endemism is concentrated largely in Pemba and Mafia islands (Afican *et al.*, 1998). This endemism is attributed by the presence of diverse microhabitats supporting different endemic and cosmopolitan species of mammals (Pires *et al.*, 2002). However, endemic species of mammals in these Islands are subjected to vanishing population following imposition of unregulated human activities which have impacts on habitat loss inhabited by many livings' fauna (Rickart *et al.*, 2011).

Many studies in small mammals have been conducted in different vegetation including woodland, savanna and mountain vegetation but little is known on the diversity of terrestrial small mammals and prevalence of haemopathogens in mafia island .This then proposes for a need of exploring and gathering more information on small mammals' composition, diversity and presence of haemopathogens which will include their ecology, species richness and abundance in different coastal forest habitats within Mafia Island.

1.3 Objectives

1.3.1 General objective

The main objective of the study was to assess the diversity and composition of terrestrial small mammals, and prevalence of haemopathogens on *Rattus rattus* in selected coastal forests of Mafia Island

1.3.2 Specific objective

i. To assess small mammal's species richness and diversity in the coastal forest of Mafia Island
ii. To estimate the prevalence of haemopathogens of public health importance in *Rattus rattus* of Mafia Island

1.3.3 List of Manuscripts

This dissertation is based on two manuscripts titled.

i. Diversity of terrestrial small mammals in Mafia Island, Tanzania
ii. Prevalence of haemopathogens in *Rattus rattus* of Mafia Island, Tanzania

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CHAPTER TWO

2.0 DIVERSITY OF TERRESTRIAL SMALL MAMMALS IN MAFIA ISLAND, TANZANIA

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ABSTRACT
From August to October 2021, research focusing on the diversity of small mammals was carried out in Mafia Island's Chole, Jibondo, and Juani. Sherman traps, which were used to catch small rodents and shrews, and camera traps, which were used to record medium-sized mammals that are too big to be trapped in Sherman traps, were both methods employed in the study. Each detained individual was recognized, weighed, and sexed before being rendered immobile with ethanol. For additional confirmation of the species using molecular methods, a little portion of each collected animal's ear was cut under aseptic circumstances and kept in 1.5 ml screw-capped Eppendorf tubes containing 90% ethanol. All captured rodents in Mafia Island were identified as *Rattusrattus* (*R. rattus*) based on the results obtained from cytochrome b (cytb) gene sequences. The identified *Rattus rattus* were observed to fall within the *Rattus rattus* Complex Lineage I. Only one shrew *Crocidura* sp. was captured which is yet to be characterized. Results from the camera traps include, *Rhynchocyon petersi*, *Cercopithecus mitis, Cephalophus monticola*, *Viverricula indica,* and the *Paraxerus palliatus*. The findings indicate a limited diversity of small mammals in the Mafia islands despite the temporal constraints on data collection, with *R. rattus* as the dominant species there. This study recommends additional research on the remaining forest sections and examining the diversity of flying mammals, which was not covered in this study.
Keywords: Diversity, Small mammals, *Rattus rattus*, Mafia

2.1 Introduction

Small mammals comprise many animal groups including flying (e.g., Chiroptera) and non-flying (e.g., rodents, mongoose, hedgehogs, shrews (Soricidae), elephant shrews or sengis (Macroscelidae), squirrels and hares (Kingdon, 2015). This group is the largest among mammals in terms of abundance and species diversity, comprising about 47% of all known mammals (Happold, 2013).

The majority of these species have evolved to be active at night, to effectively conserve water, to regulate their body temperatures, and to quickly begin reproducing following a drought season. Despite being an important component of the ecosystem's fauna, tiny animals are sometimes overlooked when conservation plans are put into place (Avenant and Cavallini, 2007). Small mammals do really play a crucial role in the carbon cycle, energy flow, soil fertility, and the function of sensitive bio-indicators of environmental deterioration (Avenant and Cavallini, 2007, Michael *et al*., 2016).

The small mammals' communities' interactions with forest ecosystems are important in determining biodiversity ecosystem health (Carey and Harrington, 2001). They contribute to seed dissemination of vascular plants, decomposing organic matter and litter, regulating

some invertebrates' populations and serve as food for predators such as birds of prey, snakes and medium to large size carnivorous mammals (Carey and Harrington, 2001).

The disappearance of coastal habitats is now a major subject of concern on a global scale. Coastal ecosystems frequently offer resources that the local population relies on for both survival and subsistence (Salvat, 1978). In order to meet human needs and demands, there has been an increase in demand for forest products for centuries. This demand is linked to massive ecosystem destruction on islands, which results in habitat loss and a decrease in faunal biodiversity because the island flora can no longer support the animals (Kueffer*et al*., 2014).

Mafia island forms part of a coastal tourism destination with unique biodiversity along the East African coast which is made up of coastal forests recognized as one of the major 25 biodiversity hotspots worldwide (Myers *et al.*, 2000).Even though during the recent past, numerous studies of small mammals have been conducted in various habitats in Tanzania, including both montane and coastal forests, as well as non-forested biotopes (Stanley *et al*., 1998, 2005; Kiwia, 2000; Cordeiro *et al.,* 2005; Mulungu *et al*., 2008; Kock and Stanley, 2009 and Sabuni *et al*.,2016), yet there is still a paucity of information on diversity of small mammals in the Mafia islands which consists also the remnants of coastal forests (Kock and Stanley, 2009). Nevertheless, the previous surveys have increased our knowledge of understanding the status, composition, and species diversity of small mammals on a large scale and some of these have yielded the discovery of new species (Verheyen *et al*., 2007). Generally, the study on small mammals in Mafia Island aimed at first; providing a list on small mammal species that are available in the area and establishing the diversity in the existing habitats where the study was conducted.

2.2 Materials and Methods

2.2.1 Study Area

This study was conducted in various habitats of Mafia Island including Jibondo, Chole, and Juani (Kock and Stanley, 2009) between August and October 2021. The islands, covering about 440km^2 of land, occupying latitudes 7^0 and 8^0 south, and longitude 39^0 and 40^0 east, approximately 20 km east of the mouth of the Rufiji River, and about 120 km south of Dar-es-Salaam City (Kock and Stanley, 2009) (Figure 2.1).

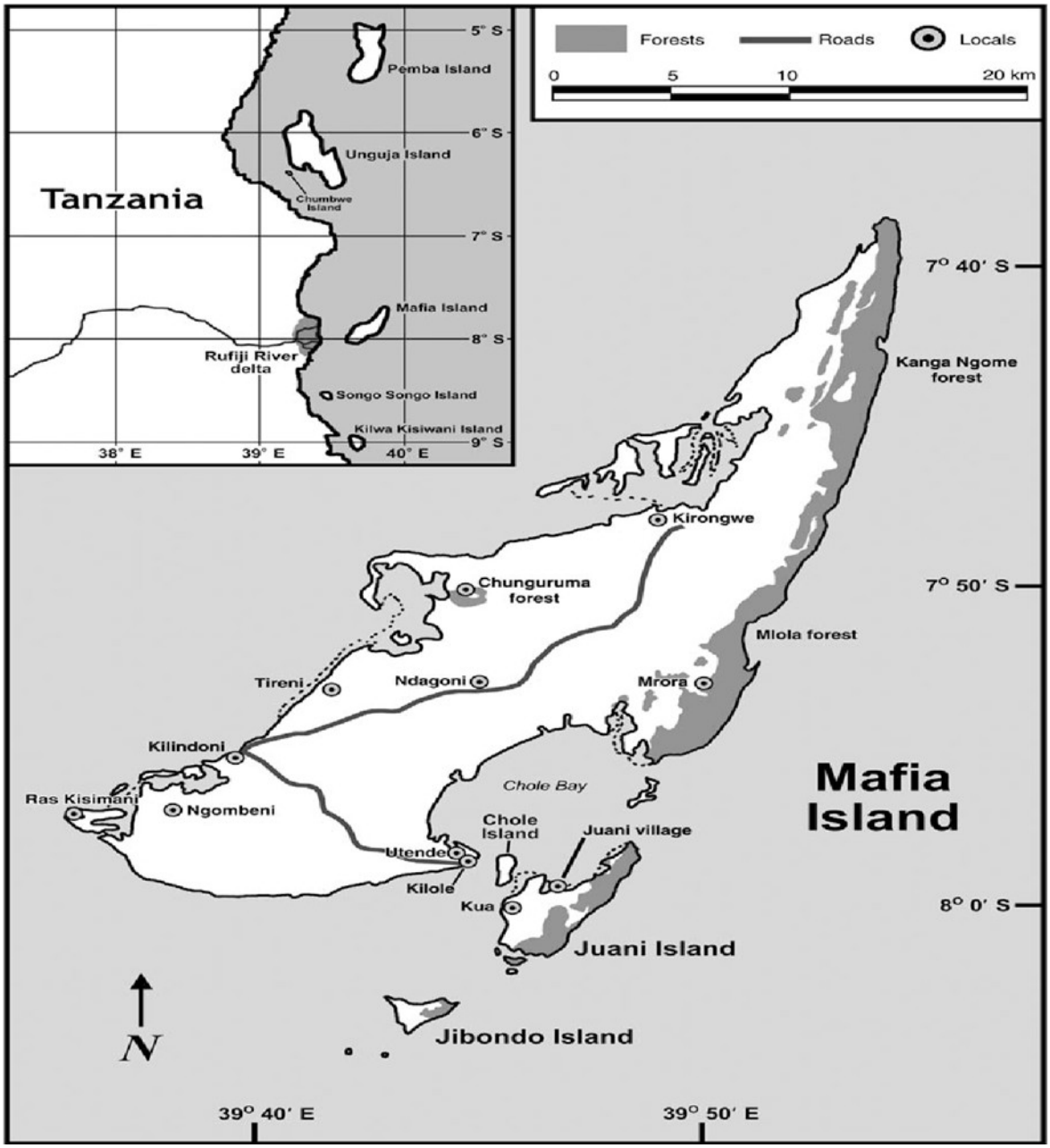

Figure 2.1:Map showing the group of Mafia Island (Juani, Chole and Jibondo)
Source: Kock and Stanley (2009)

The natural vegetation on Mafia Island, which consists of lowland rainforest, scrubby coastal moorlands, and tidal mangrove thickets, is what gives the island its name. Additionally, baobab trees flourish among native Albizia (Swai *et al*., 2011). The forests on the islands are small, fragmented, and surrounded by underprivileged communities who significantly rely on the land and forest resources of the islands, such as the timber and charcoal manufacturing seen in the Kua and Mlola forests (Kock and Stanley, 2009). The study was conducted in areas with natural vegetation, in which Mlola and Kua-Juani were covered by forests and woodlands; Utende and, Chole was dominated by mangroves, and Juani occupied by shrubs.

2.2.2 Trapping of Small Mammal

Using live folding aluminum-galvanized box traps measuring 23 x 8 x 9 cm from H.B Sherman Traps, Inc. in Tallahassee, Florida, the small mammals were captured (Sabuni *et al*, 2015). Traps were placed using two layouts; one was a transect layout (20m x 500m) with 150 traps; each trap was placed at a distance of 10m, and there were 10m between each transect line. In accordance with Mulungu *et al*., (2008), a grid layout (70 m x 70 m) with 49 traps, each trap was placed at 10 m .The grid layout was set in Utende, Chole, and Jibondo, while the transect layout was set at Mlola and Juani. Based on the topography and vegetation patches in the area, the two Sherman trap configurations in the field were suggested.

According to Sabuni *et al*., (2015), the bait used was a mixture of peanut butter and maize, and the traps were checked for catches each morning for five straight nights. Following capture, the small mammals were field identified (Happold, 2013). An ear tissue sample was collected from each seized animal and preserved in 1.5 ml screw-capped Eppendorf tubes containing 90% ethanol for further confirmation of the species using molecular methods. The animal was then released in the same location where it had been taken (Cordeiro *et al*., 2005; Stanley *et al*., 2005 and Sabuni *et al*., 2015).

2.2.3 Camera Trapping

For each forest, 10 camera traps (Reconyx Hyper fire HC 500 (Reconyx, Inc) semi–covert infrared emitters) were set spaced at about 100-200m apart. Around the specific camera locations, indicative signs were searched before setting the camera to maximize photographic capture rate, for example, distinct paths used by elephant shrews (Sabuni *et al.*, 2015).

2.2.4 Trapping Success

The trapping success of captured animals was computed using the following formular.

$$TS = \frac{NC}{NT} \times 100\,\%$$

In which; **TS** = trapping success of captured animal, **NC** = number of captured animals, **NT** = Number of trap night.

2.2.5 Camera Trap Rate

The Camera trap rate was computed using the following formular.

$$CTR = \frac{NP}{TN}$$

In which **CTR** = Camera Trap Rate, **NP** = Number of Photos, **TN** = Trap Night.

2.2.6 Species Abundance

Small mammal's Relative abundance in Sherman trap was determined using trapping success while for the Camera traps we used camera trap rate.

2.2.7 Species Richness

This was determined as number of species existing in the study site; this was obtained as sum of species of small mammals recorded in field.

2.2.8 Species Diversity

Diversity of small mammals captured in Sherman traps and camera was computed using Shannon Wiener diversity index by using the following formula.

$$H' = \sum_{i=1}^{s} Pi \ln(Pi) \quad ¿$$

In which; H= Shannon wiener diversity index

P_i = Proportional of individual species.

2.2.9 Molecular Identification of Species

Species confirmatory was carried using a single mitochondrial marker (cytb gene). To avoid contamination, pre-amplification procedures and post-amplification analyses were performed in separate rooms in the Evolutionary Ecology Group (EVECO) Laboratory at the University of Antwerp in Belgium (Mariën *et al*., 2022). DNA was extracted from the tissue with NucleoSpin Tissue Kit (Macherey-Nagel) following manufacturer's instructions. Primer sets used to amplify the cytb genes are listed in Table 2.1.

All amplifications were conducted in 15µL reactions containing about 1.5µL of extracted DNA, 0.3µL of dNTP, 0.8µM of each primer, and 0.2 units of Taq polymerase, 3µL of 10X buffer, 0.9mM of MgCl2 and 7.5µL of Nuclease free water.

Cycling conditions were as follows: one activation step at 94°C for 5min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30s, elongation at 72°C for 90s, final extension at 72°C for 5min and lastly it ended with a final temperature of 22°C. Sequencing was done at Neuromics Support Facility (part of VIB, Vlaams institute for Biotechnology).

In this study, twenty-five (25) nucleotide sequences of rodents from five islands were subjected to Basic Local Alignment Search Tool (BLAST) to determine species identity compared with other nucleotides of published rodent species available in GenBank database. Alignments of cytb gene sequences were performed using bioinformatics software platform Geneious Prime® 2022.2.1.

A BLAST search was performed for each sequence to locate nucleotide sequences of the related species available in the National Center for Biotechnology Information (NCBI) GenBank.

Table 2.1: Primers and PCR cycling conditions used in this study

Designation	Gene name	Nucleotide sequence 5' T 3'	Annealing temperature	Fragment length
Cytb				
L14723	Cytochrome b	ACCAATGACATGAAAAATCATCGTT	52°C	1140bp
H15915		TCTCCATTTCTGGTTTACAAGAC	52°C	1140bp

2.2.10 Molecular Analysis

Using Arlequin Version 3.0, analysis of molecular variance (AMOVA) and population pairwise Fst were used to ascertain the genetic relationships between rodent populations from various habitatss (Excoffier and Lischer, 2010; Shadia *et al*., 2022). Using the Excoffier and Lischer (2010) technique, the genetic distance for each population was estimated at $p< 0.05$.

The genetic differentiation was assessed using the Pairwise Fst test using 10 000 permutations. Wright's fixation index was estimated by Weir and Cockerham (1984), and this value was used to gauge the degree of genetic divergence. Using Network 4.6.1.0 software (https://www.fluxus-engineering.com/sharenet.htm), median-joining networks were created to determine the evolutionary relationships of mitochondrial Deoxyribonucleic Acid (mtDNA) rodent's haplotypes using the techniques of Bandelt *et al*. (1995). Maximum Likelihood was used for the phylogenetic analysis, and MEGA's bootstrap test method with 100 000 repeats was used (Shadia *et al*.,2022).

The evolutionary history of rodents found at Mafia Island was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The percentage of a replicate of the optimal trees in which the associated taxa clustered are shown next to the branches. There were a total of 1 124 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.,* 2021). Sequences that were outside the nucleotide sequences from 1 124 bp of the Cytb region were excluded from the molecular analysis. Sequences were submitted to GenBank under accession numbers OP485588 to OP485612 (Table 2.2).

Table 2.2: The GenBank accession numbers with the study sites

LOCATION	LAB ID	ACCESSION NUMBER
MLOLA	101	OP485588
MLOLA	110	OP485589
JUANI	130	OP485590
JUANI	132	OP485591
UTENDE	155	OP485592
UTENDE	156	OP485593
UTENDE	161	OP485594
UTENDE	163	OP485595
UTENDE	165	OP485596
UTENDE	168	OP485597
CHOLE	173	OP485598
CHOLE	174	OP485599
CHOLE	177	OP485600
CHOLE	178	OP485601
CHOLE	181	OP485602
CHOLE	182	OP485603
CHOLE	185	OP485604
CHOLE	187	OP485605
CHOLE	188	OP485606
JIBONDO	190	OP485607
JIBONDO	196	OP485608
JIBONDO	199	OP485609
JIBONDO	201	OP485610
JIBONDO	210	OP485611
JIBONDO	221	OP485612

2.3 Results

2.3.1 Live Trapping Success

A total of 168 small mammals belonging to the family Muridae, *R. rattus* species, were captured in the five studied sites Mlola(n=41), Kua-Juani(n=37), Utende(n=25), Chole(n=20) and Jibondo(n=45).

There was a significant variation on the trapping successes of the small mammals in Mafia Island in the selected fives sites (Figure 2.2). The variation in the trapping success ranged from 2.72%, in Chole, to 6.12% in Jibondo Island.

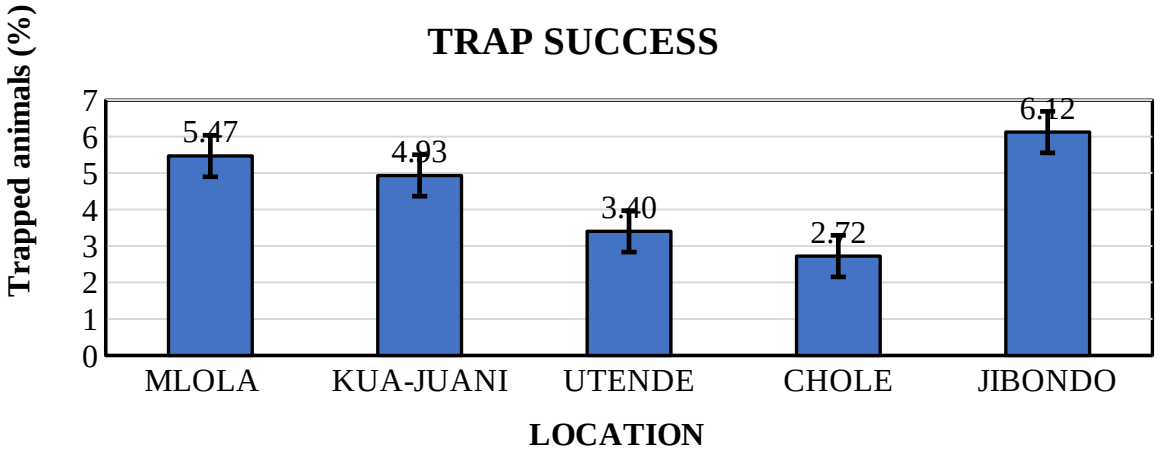

Figure 2.2: Trapping success of small mammals in Mafia Island

2.3.2 Camera Trap Rate

The camera trap rate results from the five studied sites in Mafia Island show a slight significant variation from one site to another (Figure 2.3).

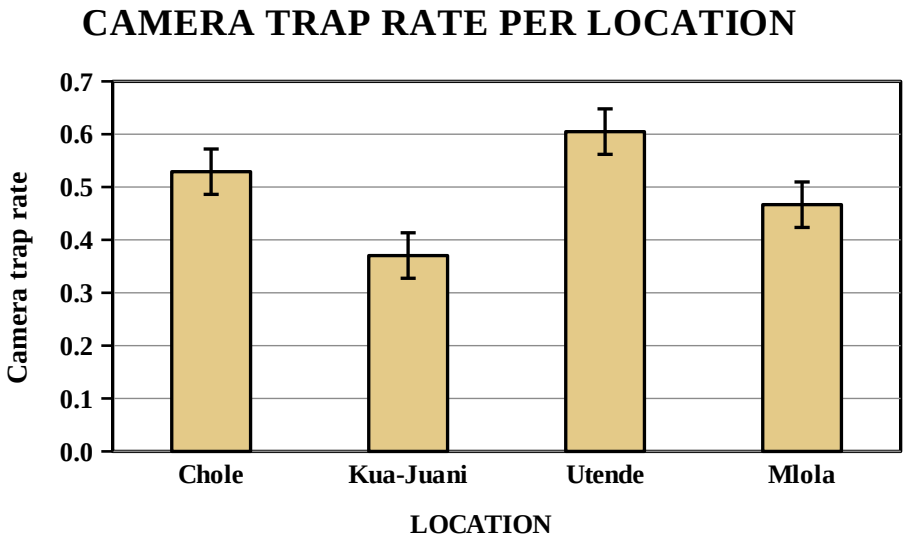

Figure 2.3: Camera trapping rate of small mammals in Mafia Island

2.3.3 Species Abundance

Species abundance of the five studied sites of Mafia Island was obtained showing that there was a clear distinction on the abundance of animals captured in the studied sites (Figure 2.4).

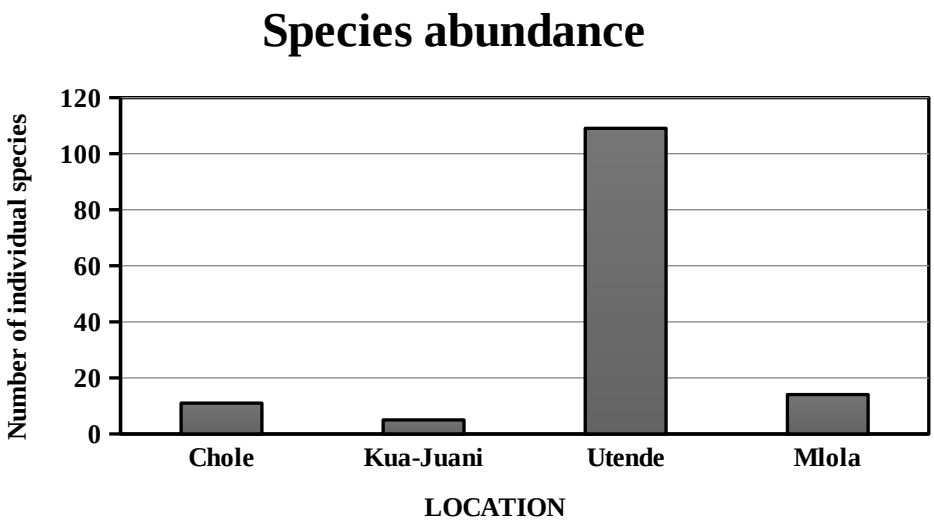

Figure 2.4: Species relative abundance of small mammals in Mafia Island

Five species from five genera and five families were recorded from the camera traps (Table2.3). From Sherman trapping, family Muridae was dominant with *R. rattus* strikingly accounting for all (n=120) rodents captured in the five study sites.

Table 2.3: Species abundance of mammals captured indirectly using Camera trap

Common Name	**Scientific name**	**Family**	**Abundance**
Blue monkey	*Cercopithecusmitis*	Cercopithecidae	36
Civet cat	*Civettictiscivetta*	Viverridae	11
Squirrel	*Sundasciurussp*	Sciuridae	27
Duiker	*Philantombamonticola*	Bovidae	17
Sengis	*Rhynchocyonsp*	Macroscelidae	48
	Genera (5)	**Family (5)**	

2.3.4 Species Richness

The small mammals' species richness was obtained as shown in Figure 2.5 and Table 2.4.

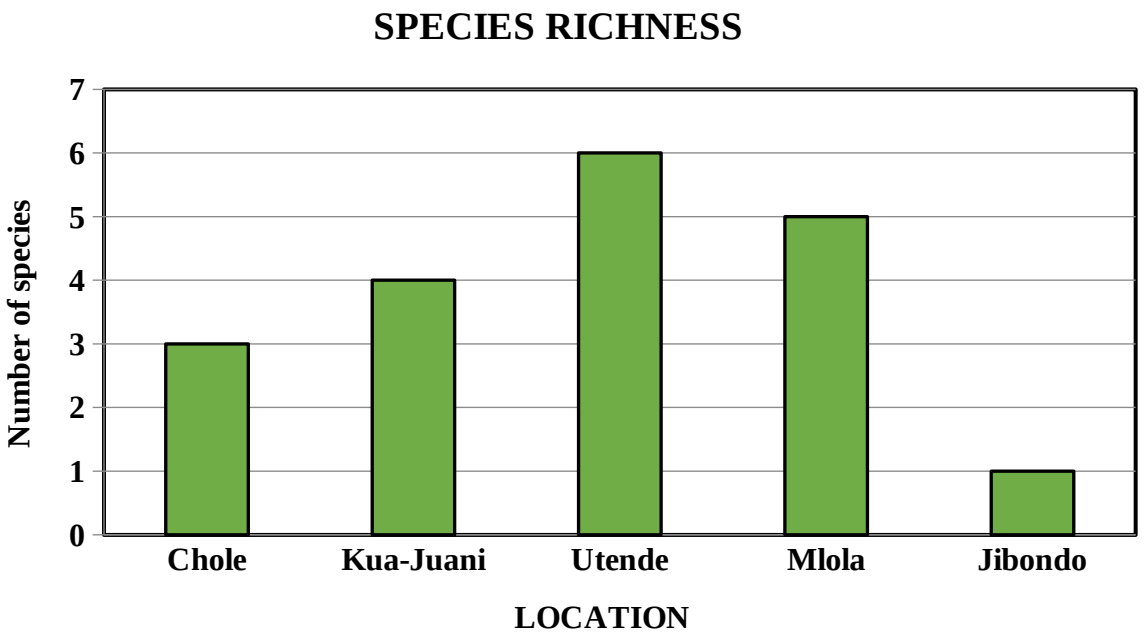

Figure 2.5: Species richness of small mammals in Mafia Island

Table 2.4: Species richness in Chole, Kua-Juani, Utende, Mlola and Jibondo islands

Common Name	Chole	Kua-Juani	Utende	Mlola	Jibondo
Rat	1	1	1	1	1
Blue monkey	1	1	1	1	0
Civet cat	1	1	1	0	0
Squirrel	0	0	1	1	0
Duiker	0	1	1	1	0
Sengis	0	0	1	1	0
Species richness	**3**	**4**	**6**	**5**	**1**

2.3.5 Species Diversity

The species diversity in the selected five sites showed a slightly significant difference between Utende and the other four sites. However, there was no a clear significant difference on the species diversity between Mlola, Kua-juani, and Chole (Figure 2.6).

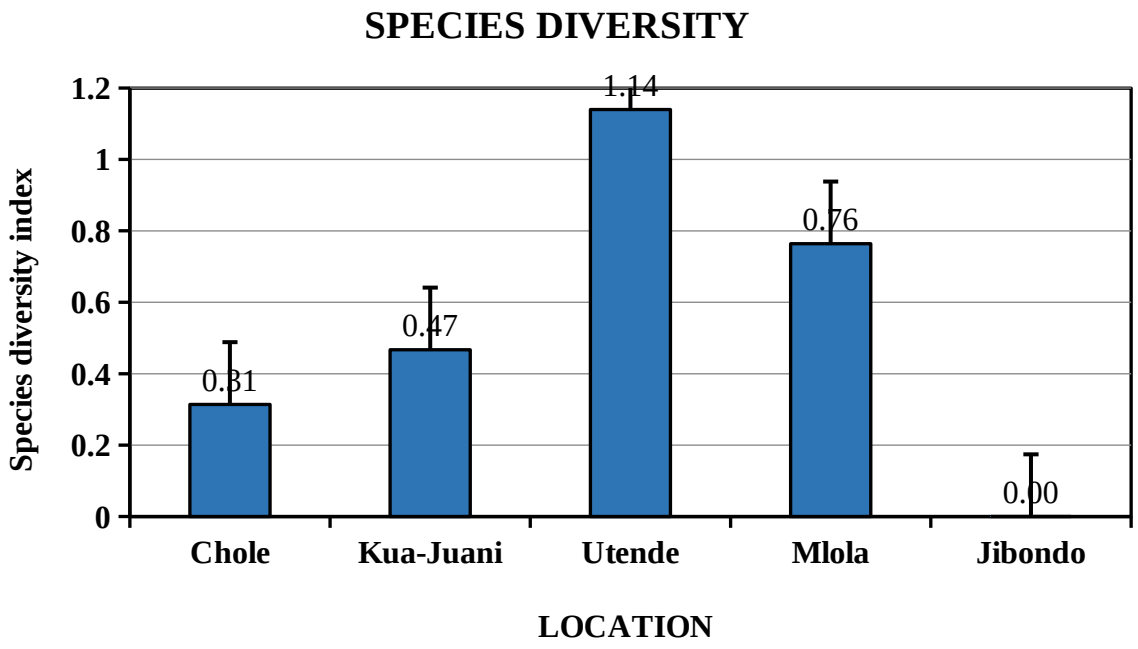

Figure 2.6: Species diversity of small mammals in Mafia Island

2.3.6 Molecular Identification of Species

The obtained nucleotide sequences from Cytb mitochondrial gene, subjected to BLAST, were compared with previously published data from five different habitats in Mafia Island. They were identified as *Rattus rattus* falling within the RrC lineage I (Aplin*et al*., 2011) as shown by the Maximum Likelihood phylogenetic tree (Figure 2.7).

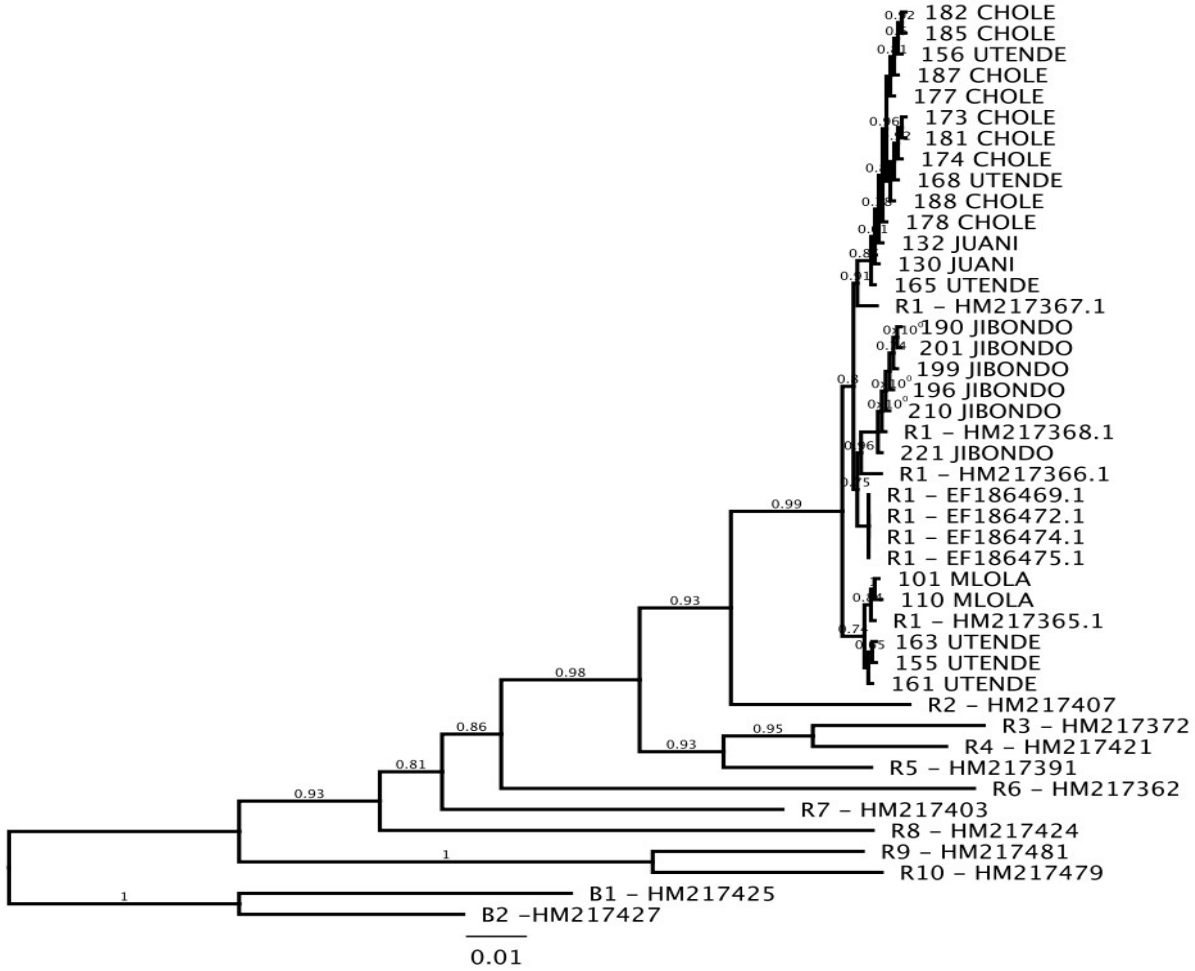

Figure 2.7: Maximum likelihood phylogenetic tree of the *Rattus rattus* Cytb sequences

The analysis of molecular variance revealed that 76.96% of the genetic variation occurred between populations, whereas only 23.04% of the genetic variation occurred within populations (Table 2.5). High genetic differentiation (Fst 0.76961) among the *R. rattus* populations was found. Strongly genetic structuring of the populations was revealed by Fst value ($p<0.0001$), suggesting that there was greater genetic divergence between rodent populations found in different habitats at Mafia Island.

Table 2.5: Molecular Variance (AMOVA) of the *Rattus rattus*

Source of Variation	**Degree of freedom**	**Sum of Squares**	**Variance Components**	**Percentage of Variations**	**F_{ST}**	**P Value**
Between Populations	4	6.6	0.33405 Va	76.96	0.76961	0.0001
Within Population	20	2	0.10000 Vb	23.04		
Total	**24**	**8.6**	**213.98582**			

2.3.7 Evolutionary Relationship

In the evolutionary relationship, the median-joining network was constructed to understand the relationship of haplotypes of rodents from Mafia Island. The haplotype distribution revealed six distinct haplogroups of the *R. rattus* population found in the Mafia Islands (Figure. 8). High haplotype diversity was observed between Jibondo, Chole, Mlola, and Juani populations.

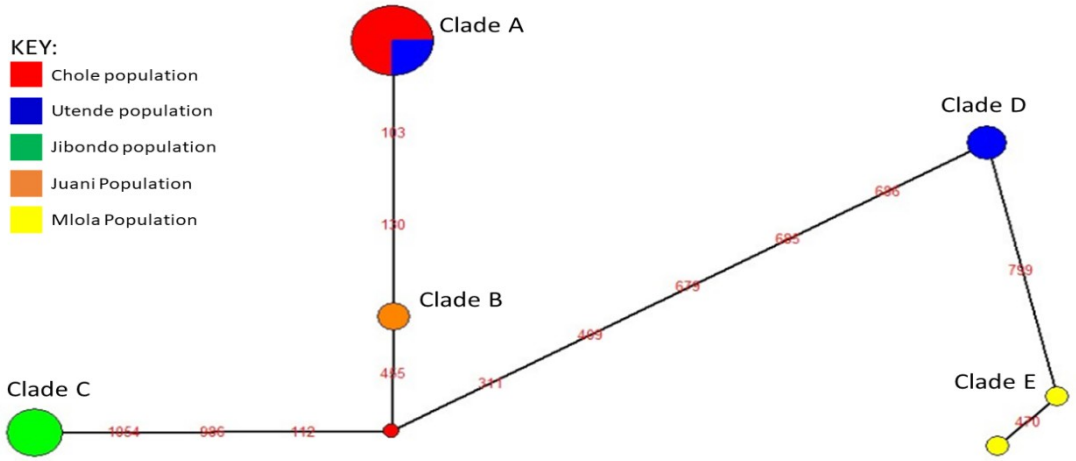

Figure 2.8: Median-joining network of six haplotypes observed in the *R. rattus* populations found in Mafia Island based on the polymorphic sites of the mitochondrial Cytochrome b gene

2.4 Discussion

Despite the trapping efforts employed on family Muridae with single species of *rattus* from RrC Lineage I was captured. For camera trap, five terrestrial small mammals were captured, which included the Black and Rufous Sengi, Blue Duiker, the Small Indian Genet, blue monkey, and the Red Bush squirrel.The biogeographic pattern of RrC Lineage I is available in Western India with wide distribution outside of mainland Asia in Europe, the Americas, Africa, and Madagascar, Australia, and various Pacific Islands (Aplin *et al.*, 2011).

Rattus rattus is extensively disseminated as a household as well as agricultural pest impacting cereals, vegetables, and palm plantations. It is also a dominant wild rodent in natural habitat (Atkinson, 1985; Goodman, 1994; Barnett, 2001; Meerburg *et al* 2009). Trapping of small mammals on Mafia Island biotopes was low especially for Sherman traps. This could be due to the area to be dominated by *Rattus rattus* and in some site traps were found capturing non-target land hermit crabs (*Coenobita rugosus*) and the most common species of shell (*Terebralia palustris*) which are abundant in the study areas.

2.5 Small mammals' species abundance, richness, and diversity

Species abundance, richness and diversity in Mafia Island is influenced by the type of habitat and human activities in an area. Small mammal abundance and diversity in various locations is attributed to the type of vegetation, food resource availability, cover, and human activity (Kingdon, 2015; Michael *et al*., 2016; Sabuni*et al.*, 2015). The same appears to be the case with Mafia and neighboring small islands.

The variation in species abundance in the five sites was likely due to the nature of the vegetation which limited some methods of capturing small mammals to be employed, hence the low trapping success of the traps. For instance, the transect layout was set at Mlola and Juani, with 150 traps for each site, since the areas had fewer physical trapping obstacles. The transect layout was therefore easy to apply. On the contrary, the grid layout at Utende, Chole and Jibondo, was more difficult because the areas were congested with vegetation and igneous rocks.

Indeed, species richness for terrestrial small mammals was recorded as low in Jibondo due to the failure of the camera traps to capture animals, because of high temperature during the day. This area was also predominantly rocky thus hindering good capturing by the camera. It is striking, however, that only *R. rattus* was captured, which is more commonly an indoor small mammal. It is possible that other small mammal species were missed in other habitats given the limited time of data collection.

2.6 Evolutionary Relationship and Molecular Identification

Haplotypes from this study provide additional information on the colonization pathways of *R. rattus* in the Western Indian Ocean region (Walley, 2004). It is sought that during the medieval maritime, trade routes by traders in the Indian Ocean caused further spread of RrC lineage I *R. rattus* from India into East Africa, the Indian Ocean Islands, and whole of the Mediterranean region (Schwarz, 1934; Walley, 2004; Aplin *et al*., 2011).

Historically, in the18th and19th centuries, there was a strong interaction of the ocean-centered world trade, which took the East African coast, including the Mafia, as a trade center (Walley, 2004). It is plausible, however, that the colonization by *R. rattus* in the islands originated from East Africa, Madagascar, and the Arabian Peninsula and through independent shipping from all over the Western Indian Ocean (Tollenaere *et al*., 2010; Aplin *et al.,* 2011). The reason for this variation is also based on different colonization that occurred in the Island.

There was also an ambiguity in the morphological identification of rodents based on coloration, with *R. rattus* being confused with *Aethomys spp*, which has not been observed in the Western Indian Ocean. The rodent described to be *Aethomys spp* has a yellowish/grayish ventral coloration and a greyish dorsal part. This ambiguity was, however, cleared through DNA molecular identification of the species.

2.7 Conclusion

The broad range of interspecies morphological variation makes morphological criteria insufficient for accurate small mammal species identification because it has led to an over-description of species and confusing taxonomy, particularly because of an overabundance of synonyms.

Thus, using DNA molecular techniques for species identification, this study has managed to describe the taxonomy of one of the most difficult groups of mammals, *Rattus rattus* (Robins *et al.,* 2007; Pagès *et al.*, 2010), as most species of rodents expected within the area were retrieved and turned to fall within the *Rattus* genus. Future studies should comprehensively include morphological, karyological, mitochondrial and nuclear markers data.

Lastly the study suggests that further findings should focus on the rest of forest patches in Mafia and to explore the flying mammal's diversity, which was not included in this study. More studies based on DNA molecular identification of the small mammals should be done given the high accuracy of genetical approach in species identification.

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CHAPTER THREE

3.0 PREVALENCE OF HAEMOPATHOGENS IN *RATTUS RATTUS* OF MAFIA ISLAND, TANZANIA

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ABSTRACT
Findings on the prevalence of haemopathogens in small mammals(rodents) was conducted in Mafia Island for the period of two months consecutively. Small rodents were trapped using Sherman traps in selected sites of Chole, Utende, Jibondo, Mlola, Juani and Shungimbili. For Each captured rodent, immobilization was done using with halothane, and field identification was made. For molecular identification of hemopathogens, blood from the retro-orbital sinus was collected and preserved as dried blood spots on filter paper. 13.33 percent of the 120 *Rattus rattus* tested positive for Bartonella spp. The prevalence between the six study sites differed considerably (df = 5 and p-value <0.001) from one another. No Leptospira, Anaplasma, Coxiella, or Rickettsia species were found. Despite the short period of data collection, the findings indicate that Bartonella species were present in *R. rattus*, the sole rodent species found on the island. For a better understanding of the hemopathogens found in rats on Mafia Island, more research is required.
Key words: Haemopathogens, *Rattusrattus*, *Bartonella,* Mafia

3.1 INTRODUCTION

With over 2000 species now known, spread across 33 families, rodents make up the biggest order of living mammals and account for 42% of all mammalian species worldwide. Worldwide distribution and excellent habitat adaptation make rodents a common species (Capizzi *et al*., 2014; Dalecky *et al*., 2015). They stand for the mammals that have traveled the world with humans the most frequently. Due to their synanthropic nature, changes in land use and urbanization play a significant role in the spread of many rodent species outside of their normal distribution ranges (Dalecky *et al*., 2015).

In fact, rodents are known reservoir hosts for a number of zoonotic pathogens (Gratz, 1997; Taylor *et al*., 2008; Pagès *et al*., 2010), and they help spread harmful diseases through a variety of means (Meerburg *et al*., 2009, Buckle and Smith, 2014). Salmonellosis, plague, leptospirosis, leishmaniasis, toxoplasmosis, rat-bite fever, taeniasis, zoonotic babesiosis, Lassa fever, hemorrhagic fever with renal syndrome (HFRS), and the hantavirus cardiopulmonary syndrome (HCPS) are some of the most significant diseases of public health concern (Chomel *et al*., 2004; Meerburg *et al*., 2009; Gundi *et al*., 2010; Dahmana *et al*., 2020).

Over the time, it has been observed that there is an increase in human diseases and pathogens associated with the presence of rodents (Meerburg *et al*., 2009). Many of these pathogens are transmitted to humans and domestic animals through contamination of stored

produce and animal feed with rodent urine and faeces (Singleton 2003; Meerburg *et al*.,2009;Stuart *et al*., 2011).

In most cases, rodent outbreaks are associated commonly with social-economic challenges such as poor hygiene, poverty as well as low level of education (Brown *et al*. 2008; Meerburg *et al*.,2009). According to reports, some Tanzanian tribes have resorted to destroy entire hamlets in the past to combat the rodent-borne epidemic (Kilonzo *et al*., 2005). Leptospirosis and bartonellosis are two examples of zoonotic diseases that can infect humans and livestock and cause major morbidity and mortality (Gratz, 1997; Machang'u *et al*., 1997).

Mafia is an island close to Tanzania mainland in which little is known of rodent borne haemopathogens of pathogenic significance. Given the increase, in recent years, of movement of goods and people between mainland Tanzania and the Mafia Island, it driven us to conduct a study on Mafia Island, focusing on rodent borne haemopathogens that are potentially pathogenic to humans and other animals.

3.2 Materials and Method

3.2.1 Study area

The study was conducted in the Mafia Island in the island and neighboring small islands in Mafia, where the Main Island was Mafia itself and the smaller islands included Jibondo, Chole and Juani. Mafia island is in the eastern coast of Indian Ocean in Tanzania and covers about 440km^2 with the latitude7^0 and 8^0 south between longitude 39^0 and 40^0 east. It also lies nearly 20 km east of the mouth of the Rufiji River and 120 km south of the Dar municipal (Kock and Stanley, 2009).

Mafia Island is characterized by the natural vegetation, which includes tidal mangrove thickets and scrubby coastal moorlands to palm wooden grassland and lowland rainforest, also baobab trees and the native Albizia are commonly found (Swai *et al.*, 2011). The forests within the island are generally fragmented, small, and surrounded by marginalized communities that heavily depend on these Islands for land and forest resources, such as timber and the production of charcoal, in Kua and Mlola forests (Kock and Stanley, 2009).
Concern over the global loss of coastal habitats, especially in the tropics, is growing. Coastal ecosystems frequently supply the resources that the local population relies on to survive and make a living (Salvat, 1978). According to Greenway *et al*. (1988), Mafia Island's coastal forest were abundant in the 1930s, with the last one being cleared in the late 1980s to make way for coconut plantations. However, by the early 1990s, there were only a few densely packed little patches of forest along a 40 km by 1 km strip of coral rag along the eastern side of the island. Since then, remnants of coastal forest have persisted in locales all across Mafia Island. The thicket persisted as one main forest, Mlola forest at the southeast side of the island, being characterized by dense tree canopy of palms, lianas and epiphytes and dense underbrush of ferns (Clarke, 2000).

3.2.2 Data Collection

3.2.2.1 Rodent trapping

The Capture Mark Recapture (CMR) method was used on collecting data of rodents between August 2021 to October 2021. Trapping was conducted using Sherman® traps (Standard medium size LFA: 7.6 X 8.9 X 23 cm). The trapping was conducted for five consecutive nights and inspected every morning for captures.

A mixture of peanut butter and maize flour was used as the bait. Captured rodents were anaesthetized by halothane, and blood was drawn using a capillary tube from the retro-orbital sinus and dropped on Whatman filter paper and let to dry. The dried bloods on the filter papers were stored for further molecular detection of haemopathogens (Cordeiro *et al.*, 2005 and Sabuni *et al.*, 2015).

3.2.2.2 Molecular identification

3.2.2.2.1 DNA extraction

Genomic DNA was extracted from eluted blood using the RTP® pathogen kit (INVITEK) and DNeasy Blood &Tissue kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) following the manufactures instructions. The DNA extracts were then stored at -20°C.
Amplification reactions of all haemopathogens were conducted in a final volume of 10 µL containing 5 µL of 2xEurogentec TakyonTM Mix (Eurogentec, Liège, Belgium), 0.5 µL of each primer (10 µM), 0.25 µL of Probe (5 µL), 1.25 µL of DNase-free water, and 2.5 µL of DNA template (Table 1).

The RT-qPCR was performed on the StepOneTM Real-Time PCR system (Thermo Fisher Scientific) using the following thermal profile: an incubation step at 50 °C for two minutes for eliminating PCR amplicons' contaminant, then an activation step at 95 °C for three minutes followed by 40 cycles of denaturation at 95 °C for 15 seconds and an annealing-extension at 60 °C for 30 seconds while annealing temperature for Anaplasma was 55°C. Samples with a Ct value below 35 were screened a second time (duplicates). An individual was considered positive on the RT-qPCR if tested positive during both runs (Mariën *et al*., 2022).

Further amplification of the ITS region (453–780 bp) (Böge *et al.*, 2021) was done using conventional PCR system before sequencing . The amplification reactions were conducted in a final volume of 15 µL, containing 7.5 µL of Hot Goldstar master mix, 0.3 µL of each primer, 5.4 µL of DNA free water and 2.5 µL of DNA template. Reactions were conducted in a thermal cycler (TProfessional Basic Thermocycler by Biometra) under the following amplification conditions; 40 cycles for 30 s at 94 °C, for 30 s at 66 °C, for 50 s at 72 °C. PCR products were prepared with DNA Gel Loading Dye (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) for gel electrophoresis in 1% agarose. Visualization was done using UV light. Amplicons of positive samples were purified and sent to the Neuromics Support Facility-Vlaams Institute of Biotechnologie for Sanger Sequencing with forward and reversed primers.

Individuals were positive for *Bartonella* if they were positive on the RT-qPCR after replication, and we obtained Sanger sequences from the conventional PCR for at least one gene. The categorization was made because the RT-qPCR is generally a more sensitive approach than the conventional PCR (which targets larger DNA amplicons). DNA extracts from blood were also screened for other potentially rodent pathogens including *Rickettsia spp, Leptospira spp, Anaplasma spp* and*Coxiella spp* by real time qPCR and were all found negative.

3.3 Data Analysis

Sequences were trimmed using Geneious Prime® 2022.2.1. and compared to available data in GenBank with BLASTn. Prevalence was computed as number of individuals infected against total number of individuals examined times 100 and expressed in terms of percentage. Confidence intervals (95% CI) for the prevalence of *Bartonella* spp in *R.rattus* was determined at the level of alpha of 0.05 (Böge*et al*., 2021).

Targets	**Targeted gene**	**Name**	**Primers (5'-3') & Probe**	**Annealing Temperature** (Mariën *et al*., 2022).
Leptospira		LIP32BF	AGCTCTTTTGTTCTGAGCGA	
		LIP32BR	TACGAACTCCCATTTCAGCGATTA	60°C
		LIP32P	AAAGCCAGGACAAGCGCCG-BHQ1	
Bartonella		Barto_ITS3_F	GATGCCGGGGAAGGTTTTC	
	ITS	Barto_ITS3_R	GCCTGGGAGGACTTGAACCT	
		Barto_ITS3_P	6FAM-GCGCGCGCTTGATAAGCGTG-TAMRA	60°C
	ITS gen (16S-23SrRNA)	Ba325S	CTTCAGATGATGATCCCAAGCCTTCTTCTGGCG	
		Ba1100aS	GAACCGACGACCCCCTGCTTGCAAAGC	
Rickettsia		RKND03_F	GTGAATGAAAGATTACACTATTTAT	
		RKND03_R	GTATCTTAGCAATCATTCTAATAGC	60°C
		RKND03_P	6FAM-CTATTATGCTTGCGGCTGTCGGTTC-TAMRA	
Coxiella burnetii		CB_IS1111_0706F	CAAGAAACGTATCGCTGTGGC	
	IS1111A	CB_IS1111_0706R	CACAGAGCCACCGTATGAATC	60°C
		CB_IS1111_0706P	6FAM_CCGAGGTTCGAAACAATGATTC	
Anaplasma		TtAna_F	TGACAGCGTACCTTTTGCAT	
	23S	TtAna_R	GTAACAGGTTCGGTCCTCCA	55°C
		TtAna_P	6FAM_GGATTAGACCCGAAACCAAG	

Table 3.1: Oligonucleotide Sequences of Primers and Probe used for QPCRs and conventional PCRs in this study

3.4 RESULTS

3.4.1 Pathogen Identification and prevalence Results

Among the tested pathogens from 120 *R. rattus,* 13.33% tested positive for uncultured *Bartonella*. Samples from Chole, Jibondo and Shungimbili areas were shown positive (Figure 3.1).

The prevalence of pathogens between locations were significantly different p-value <0.001, df = 5. This significant difference showed that there was a variation in host-parasite association, where Jibondo, Chole and Shungimbili had a high prevalence of *Bartonella*spp from the tested rodent samples.

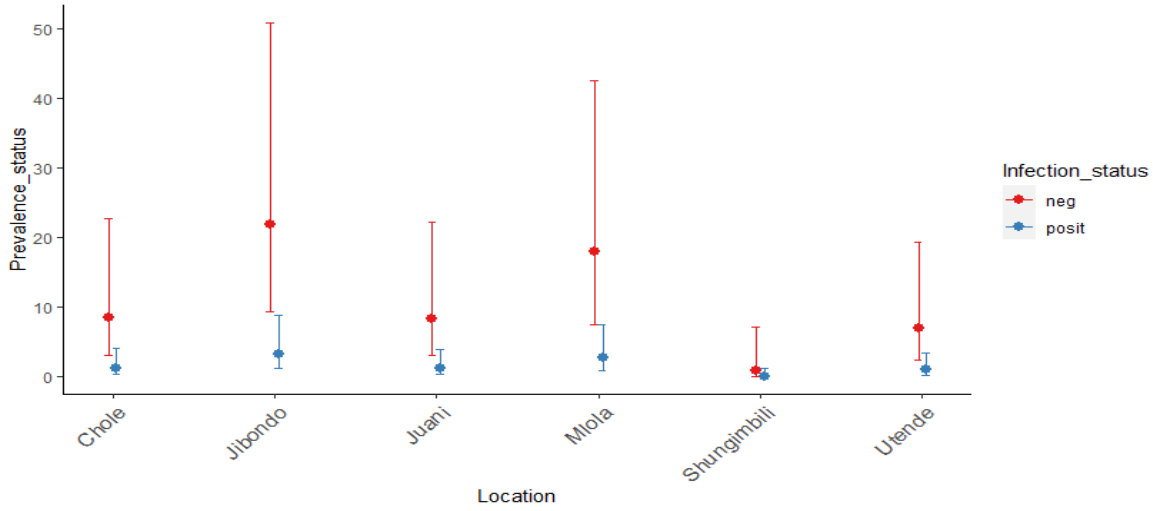

Figure 3.1: Prevalence of Haemopathogens in Mafia Island

3.5 DISCUSSION

In this study, *Bartonella* spp were detected in blood samples of rodents from six selected sites of Mafia Island, which were Chole, Utende, Mlola, Jibondo, Juani and Shungimbili. Of the six sites only Chole, Jibondo and Shungimbili tested positive for Bartonella, implying that some rodents from Chole, Jibondo and Shungimbili were reservoirs of Bartonella pathogens.

Bartonella spp was found in *R. rattus* population of three out of the six studied sites, suggesting that the presence of *Bartonella* spp in Mafia Island may lead to an emergence of Bartonellosis. It is yet to be determined in the island whether the *Bartonella* species can infect and cause clinical disease in humans and other animals. Nonetheless rodents that were Bartonella positive serve as a warning of the potential of infection with this pathogen in Mafia Island.

Based on this study, *R rattus* could have the potential of supporting different assemblages of *Bartonella* spp, including pathogenic species of concern in an area with interaction of different people like Mafia Island. An understanding of host–parasite interactions is important as it can help reducing the risk of an infection. Consideration should be given in reducing the interaction between humans and small mammals, inclusively, which are known to be important reservoir hosts of disease-causing agents.

Mafia island is one among the best tourist destinations with influx and outflux of people throughout the year. Further studies are recommended to determine the potential of rodents particularly rodents and other small mammal as potential reservoir of Bartonella.

3.6 CONCLUSION AND RECCOMENDATION

This study lays the foundation to better investigate rodent-borne diseases in Mafia Island and establish their prevalence for public health interest. Despite the comparatively low *Bartonella* spp prevalence in the *R. rattu s*of Mafia Island, further work is required to investigate bartonellosis prevalence in humans and to characterize the pathogenic species.

The study also recommends a wider scope of studies in the rest of forest patches of Mafia and to also explore the prevalence of other pathogens associated with small mammals including the flying mammals which were not included in this study.

Furthermore, it is recommended that stakeholders in public health, tourism, agriculture, and environment should team up as “One Health” in the management of rodents and other small mammals that are potential vectors of pathogens in Mafia Island and elsewhere.

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CHAPTER FOUR

4.0 GENERAL DISCUSSION, CONLCUSION AND RECOMMENDATIONS

4.1 General discussion

Mitochondrial DNA sequence data confirms the presence of R. rattus in Mafia Island. The distribution of R. rattus seems restricted to the western Indian Ocean although more complete sampling throughout the Mafia Island should be conducted to confirm this.

Rattus rattus was found throughout the Mafia Island being captured in less disturbed area. When food is available and since they are omnivorous this specie has been shown to be highly adaptive with a wide tolerance for different habitat type making them less dependent on agricultural food sources as is the case for Mus.

Having Mafia with different haplotypes from East Africa, Madagascar, and Arabian Peninsula shows that the area had multiple colonization hence the observed variation.

4.2 Conclusion

This study contributes to a growing body of literature that documents the usefulness of DNA in establishing the taxonomy of the most difficult taxa to identify morphologically. We provide the first molecular evidence for the presence of one invasive rodent species (R. rattus) in Mafia Island. Using molecular data as well as historical records for Mafia Island we can see that the species were introduced into the Mafia via different routes. The first is via the western seaport where specimens of *Rattus rattus* on the western and northwestern side of Mafia show close ties with East Africa, and Madagascar haplotypes. The second is via the east where specimens of *Rattus rattus* are closely tied to Arab and southeast Asian haplotypes (likely the slave trade). Future work should consider more comprehensive sampling throughout the area to investigate accurately the occurrence of invasive species as well as extend sampling to other African countries and other Western Indian Ocean Islands given the threat that invasive species pose to local biodiversity, agricultural yield, and food security.

4.3 Challenges

The challenges, faced in this study include theft of storage equipment that composed of external device; laptop as well as SD Cards were all data that was collected on that time frame was lost and thus, we had to repeat to collect again the same information hence time consuming. However, trapping of unexpected individuals occurred whereby instead of capturing rodents in the Sherman traps we captured the land hermits and crab thus lowering the number of rodents caught in the area.

4.4 Recommendation

This study recommends that further studies should focus on trapping rodent samples from both wilderness area as well as the homesteads, to get the full picture of what is available within the island.