



Faculty of Resource Science and Technology

**Rodents and The Associated Parasites from Different Habitat Types in
Sarawak**

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Rodents and The Associated Parasites from Different Habitat Types in Sarawak

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Rodents are the most successful mammalian order and are well distributed globally, including Sarawak. However, habitat destruction and alteration may influence the rodents' population structures and distributions. Rodents can serve as hosts for medically concerning parasites that might act as vectors for zoonotic diseases. This has highlighted the importance of understanding the distribution and diversity of rodents in different habitat types and the parasites they carry. This study is intended to: 1) study the distribution and diversity of rodents in different habitat types in Sarawak; 2) determine the parasite prevalence and habitat preference of rodents in different habitat types in Sarawak; and 3) discover the host-parasite relationship between rodents and their parasites in Sarawak. Samplings were conducted between November 2018 and December 2019, which resulted in 241 individuals of rodents captured, comprising eight species. Of this, 78 individuals were captured from urban areas, followed by oil palm plantations (72 individuals), rural areas (65 individuals), and forest areas (26 individuals). Statistical analysis showed that the forest areas have the most diverse rodent' species among all habitats ($H' = 1.91$, $p < 0.05$), followed by oil palm plantations ($H' = 0.93$), urban ($H' = 0.66$) and rural areas ($H' = 0.40$). A total of 236 rodents were then examined for parasites, and 218 of them were found to be infected with at least one parasite. A total of 41 species of parasites were recovered in this study, and the rodent community was mainly dominated by ectoparasites (77.12%), followed by endoparasites (61.44%), and hemoparasites (30.51%). From the habitat viewpoint, overall parasite prevalence was highest in forest areas (100%). A significant difference between habitat types was detected in the prevalence of hemoparasites and endoparasites (both $p < 0.001$) believed to be influenced by humidity and temperature as well as other factors. This study also discovered 129 host-parasite interactions between 41 parasite species and eight murid rodents. Goodness-of-fit

indicates ($m2XY=183.3784$, $p= 0.035$, $nperm=1000$) resulted significant congruence between the host and parasite distance matrices. Meanwhile, bipartite analysis indicates a low level of specialization for all parasites, or, in other words, parasites in this study are not host-specific based on bipartite analysis. These results provide information on the structure of rodents as well as the population of their parasites in Sarawak. This study also depicts Borneo's present parasite infection status and rodent-parasite relationship, which may help in deeper research on parasite and host relationships as well as be part of the effort in forecasting human health risk in this region.

Keywords: Habitat gradient, host-parasites interaction, parasite, rodents, zoonoses.

Roden dan Parasitnya dari Pelbagai Jenis Habitat di Sarawak

ABSTRAK

Tikus merupakan sejenis mamalia yang paling berjaya dan tersebar luas di seluruh dunia, termasuk di Sarawak. Walau bagaimanapun, kemusnahan dan perubahan habitat boleh menjejaskan struktur dan kepelbagaian populasi tikus. Tikus boleh berperanan sebagai tuan rumah untuk parasit yang berkaitan dengan perubatan dan boleh bertindak sebagai vektor untuk penyakit zoonotik. Ini membuktikan kepentingan untuk memahami penyebaran dan kepelbagaian tikus dalam habitat yang berbeza dan parasit yang mereka bawa. Kajian ini bertujuan untuk: 1) mengkaji penyebaran dan kepelbagaian tikus dalam habitat yang berbeza di Sarawak; 2) menentukan kelaziman parasit dan keutamaan habitat tikus di pelbagai jenis habitat di Sarawak dan 3) mengkaji hubungan tuan rumah-parasit antara tikus dan parasit di Sarawak. Pengumpulan sampel telah dijalankan antara November 2018 dan Disember 2019, dimana 241 individu tikus telah ditangkap terdiri daripada lapan spesies. Sebanyak 78 ekor tikus dari jumlah tersebut ditangkap dari kawasan bandar, diikuti oleh ladang kelapa sawit (72 ekor), kawasan perkampungan (65 ekor), dan kawasan hutan (26 ekor). Analisis statistik menunjukkan bahawa kawasan hutan mempunyai spesies tikus dengan kepelbagaian paling tinggi di antara semua habitat ($H' = 1.91$, $p < 0.05$), diikuti oleh ladang kelapa sawit ($H' = 0.93$), bandar ($H' = 0.66$) dan kawasan perkampungan ($H' = 0.40$). Sebanyak 236 tikus kemudian diperiksa untuk jangkitan parasit, dan 218 daripada tikus ini telah dijangkiti oleh sekurang-kurangnya satu parasit. Sebanyak 41 spesies parasit telah diperoleh dari kajian ini, dan komuniti tikus ini didominasi oleh ektoparasit (77.12%), diikuti oleh endoparasit (61.44%), dan hemoparasit (30.51%). Dari sudut pandang habitat, penyebaran parasit secara keseluruhan adalah tertinggi di kawasan hutan (100%). Perbezaan yang ketara antara jenis habitat ditemui dalam kelaziman hemoparasit dan

endoparasit (kedua $p < 0.001$) yang diyakini dipengaruhi oleh kelembapan dan suhu serta faktor-faktor lain. Kajian ini juga mendapati 129 interaksi tuan rumah dan parasit antara 41 spesies parasit dan lapan tikus Murid. Indikator kesesuaian yang baik ($m2XY = 183.3784$, $p = 0.035$, $nperm = 1000$) menghasilkan kongruen yang ketara antara matriks jarak tuan rumah dan parasit. Sementara itu, analisis bipartit menunjukkan tahap pengkhususan yang rendah untuk semua parasit, atau, dengan kata lain, parasit dalam kajian ini tidak spesifik kepada tuan rumah berdasarkan analisis bipartit. Hasil ini telah memberikan maklumat tentang struktur tikus serta populasi parasit mereka di Sarawak. Kajian ini juga menggambarkan status jangkitan parasit Borneo yang sedia ada dan hubungan tikus dan parasit, yang boleh membantu dalam penyelidikan yang lebih mendalam mengenai hubungan parasit dan tuan rumah serta menjadi sebahagian daripada usaha dalam meramalkan risiko kesihatan manusia di rantau ini.

Kata kunci: Kecerunan habitat, interaksi perumah-parasit, parasit, tikus, zoonosis.

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LIST OF ABBREVIATIONS

CA	Correspondence analysis
cm	centimetre
DI	Desa Ilmu
EDTA	Ethylenediamine tetraacetic acid
FA	Forest areas
KB	Kampung Baru
KNP	Kubah National Park
KSM	Kampung Sebat Melayu
LC	Least Concern
m	meter
NaCl	sodium chloride
NNP	Niah National Park
OP	Oil palm plantation
PCA	Principle Components Analysis
PDI	Paired Difference Index
PJ	Petrajaya
RA	Rural areas
SJOP	Sadong Jaya Oil Palm Plantation
UA	Urban areas
UNIMAS	Universiti Malaysia Sarawak
VU	Vulnerable
WOP	Wilmar Oil Palm Plantation

CHAPTER 1

INTRODUCTION

1.1 Study Background

Rodent is a diverse group of mammals that exploit a wide range of habitats (Yihune & Bekele, 2012). They make up an important group in the forest, cultivated land and towns, extending from the shore to mountain top and from the ground to the forest (Paramasvaran et al., 2013). They covered nearly every continent in the world except Antarctica and certain oceanic islands (Bonney et al., 2008). They include 29 families, 468 genera, and over 2052 species worldwide (Ardianyah & Mahrin, 2022). A total of 66 species of them are listed in Borneo that belonging to three families, Sciuridae, Muridae and Hystricidae (Phillips & Phillips, 2018). Among those families, Muridae is the most medically concerning group and the most successful family in the mammalian order with a global distribution (Telmadarray et al., 2007).

Their broad distribution has been the main concern on human health risk. The growth of economy and agriculture in Malaysia has forced Malaysia to experience rapid deforestation and habitat degradation (Madinah et al., 2014). Sarawak is not exempted to be part in this development since forests were reduced by 0.62% annually (Kamlun et al., 2012). Rodent was the one that most affected by these activities as they are response quickly to environmental changes owing to their short lifespan and prolific reproduction capabilities. (Wan et al., 2022). The increased of the habitat destruction and the human population in Sarawak may lead to near extinction of some conservation important species and increase exposure of either direct or indirect transmission of diseases by the rodents. Yet, rodents are good ecological engineer in natural habitat and it also medically important as it is vector for

various zoonotic diseases (Rabiee et al., 2018). Eventhough their population response quickly to the environmental changes, some of them are highly adaptable to the unpredictable environment combined with other factors that favour the parasitic growth (Chuluun et al., 2005; Premaalatha et al., 2017). Therefore, information on distribution, diversity and abundance of rodents are crucial for conservation purposes and to aid in disease surveillance.

A large proportion of the disease from rodent was led by the parasitic infections based on Rabiee et al. (2018). Rodents are hosts to an impressive diversity of parasites because they are able to provide a favourable habitat for many species of parasites on an individual (Madinah et al., 2014; Nadchatram, 2008; Nava et al., 2003). Parasites are often associated with harmful diseases.

Apart from parasite diversity, there is a paucity of information on the host-parasite relationship between rodent and parasites in Sarawak. The information on factors affecting rodent and parasite relationship is important in understanding disease emergence and transmission and the roles of parasites in host adaptation process. Even though the investigation of host-parasite associations might be dreary with given the generally low parasite prevalence and infestation rates, there is still a desire to make up an inference on prevalence and host-specificity patterns (Wells et al., 2013). This underline the need of a study on of the host-parasite relationship between rodents and the associated parasites in Sarawak as well as to understand the disease transmission by the rodent, specifically from the family Muridae. This study provides an information that aid authorities in improving their management on health risk management as well as educate and create awareness among communities on the potential reservoirs of infectious diseases in this region. This study documents the distribution of rodent as a potential zoonotic disease reservoir and its diversity

of parasite in contrasting habitats in Sarawak. The relationship and host specificity between host and parasite that provide crucial information on the health risk status of human interactions with rodent was also being discussed herein.

1.2 Problem Statement

Land-used intensification is the main causes in structuring rodent population (Morand et al., 2019; Palmeirim et al., 2020; Zhang et al., 2016). Massive habitat alterations may cause disappearing of native species and may increase the interaction between human and rodent. Most of the previous studies conducted in Malaysia were focused on species diversity of rodents in forest and human settlement, rarely combined the oil palm plantation.

Forecasting zoonotic disease reservoir is a stressing public health priority (Han et al., 2015). Parasites study is always being important in the medical field. There are many parasite species have been reported in Malaysia and some of them were medically concerning species due to their association of disease transmission to human. A large proportion of them have contributed in zoonoses worldwide. The studies on parasite infections of small mammals in Peninsular Malaysia from contrasting habitats ranging from forested to urban areas have been conducted over the years (Madinah et al., 2011; Paramasvaran et al., 2009; Priscilla et al., 2015; Zain, 2008). In addition, the studies of rodent parasites in Malaysia have focused on arthropods or ectoparasites (Adrus et al., 2011; Chuluun et al., 2005; Hafidzi et al., 2007; Madinah et al., 2011; Madinah et al., 2013; Mariana et al., 2011; Mariana et al., 2005, 2008; Mariana et al., 2007; Mustapha et al., 2019; Ng et al., 2017; Paramasvaran et al., 2009; Razali et al., 2018). Although the parasites of rodents were studied substantially in Malaysia, these studies rarely include population from agricultural areas, particularly from oil palm plantations as the largest growing crop in Malaysia. Moreover, the parasite diversity in

Sarawak is seldom addressed. Consequently, there is a lack of information regarding the ecology of rodent parasites in Sarawak.

There are poor of information regarding the distribution rodents and its parasite from the different habitats in Sarawak. The host parasite interaction in Sarawak also still poorly understood. Thus, this study contribute to the information regarding the current status of parasites and its host infection in Borneo. The expansion and urbanisation have resulted in a serious ecological changes that have irreversibly altered the composition and structure of terrestrial rodents as well as their parasite populations. Since, Sarawak is facing rapid development, the effect of this development needs to be taken into action and attention.

1.3 Objectives

Thus, the objectives of this study were:

- i. To study the distribution and diversity of rodent in different habitat types in Sarawak
- ii. To determine the prevalence of parasites and habitat preference on rats in different habitat types in Sarawak
- iii. To discover the host-parasite relationship between rodent and their parasites in Sarawak

1.4 Hypothesis

The hypothesis tested are:

- i. H_0 : There is no significant difference in host distributions in different habitat types in Sarawak
 H_A : There is a significant difference in host distributions in different habitat types in Sarawak. (Chapter 4).
- ii. H_0 : There is no significant difference of rodent parasite in contrasting habitats in Sarawak
 H_A : There is a significant difference of rodent parasite in contrasting habitats in Sarawak (Chapter 5).

1.5 Chapter Summary

This thesis is divided into six chapters. First chapter comprises of the general introduction and objectives of this research study. Second chapter reviews the previous studies and information relevant to this research study. Chapter 3 looks into the finding of rodents distributions and species diversity in different habitat types in Sarawak. Chapter 4 provides and discusses the result of the rodent parasites diversity in different habitat types in Sarawak and compares the results between habitats. Chapter 5 discusses the host-parasite relationship emphasize to rodent and their parasites in Sarawak. Chapter 6 summaries the results of this research study with recommendation for forthcoming research on parasite of rodents. Lastly, Chapter 7 comprises the overall conclusion of this study and recommendation for future parasite studies.

CHAPTER 2

LITERATURE REVIEWS

2.1 Taxonomy of rodent

The classical rodent classification divided into three suborders which are Sciuromorpha (resembling squirrels), Myomorpha (resembling mice), and Hystricomorpha (resembling porcupines) originate from Brandt (1855). This classification relies on the examination of the jaw and skull structure, as well as the positioning of the masticatory muscles. Another classification was made by Tullberg (1899) who combined the mandibular conformation and Brandtian zygomaseteric criteria. Hence, this classification divided into two major group namely Sciurognathi and Hystricognathi. Rodent authorities (Landry, 1999; Wood, 1985) strongly advise against using Brandtian descriptions as argument rise on the descriptive meaning of the terms do not strictly agree with the morphologies of included members, which leads to confusion. However, the suborders Sciuromorpha, Myomopha, and Hystricomorpha have strong evidence supporting their classification as monophyletic taxa. Meanwhile, the evidence for classifying Castorimorpha and Anomaluromorpha is less convincing (Donnelly, 2015).

The suborders Sciuromorpha and Hystricomorpha each consist of one family, namely Sciuridae (squirrel family) and Hystricidae (porcupine family), respectively (Tripathi, 2014). On the other hand, the suborder Myomorpha is further divided into five families namely Dipodidae (birch mice), Platacanthomyidae (dormice), Spalacidae/Rhizomyidae (bamboo rats), Cricetidae (hamsters), and Muridae (voles, rats, mice, gerbils, etc.) (Tripathi, 2014).

Malaysian rodents consist of squirrels (Sciuridae), porcupines (Hystricidae), rats and mice (Muridae).

2.2 Rodent as important host to zoonotic diseases

Rodents are small mammals from order Rodentia that are characterised by their dentition that consist of a pair of incisors in both upper and lower jaws. According to Nieri-Bastos et al. (2004) and Madinah et al. (2013), Rodentia is the most species-rich order among mammalian (Meltesen et al., 2023). They are divided into three major families which are Sciuridae (squirrels), Muridae (rats) and Hystricidae (porcupines) (Hawkey, 2017). Several studies have stated that family Muridae are known as the largest family among the other mammalian families (Hamdan et al., 2016; Wilson & Reeder, 2005). Approximately 27 species of Muridae, 36 species of Sciuridae and three species of Hystricidae have been recorded in Borneo (Phillipps & Phillipps, 2018) specifically.

Despite being an essential part of natural communities, they also could cause damage to human-made structures, food resources and increase health risk by being a highly potential vector of diseases. Telmadarraiy et al. (2007) highlighted that Muridae is the most medically important family of rodent. Hence, this study has been conducted with a specific focus on Muridae, commonly referred to as rats and mice. Based on a study by Sengupta (2013), rodent grow rapidly and attain sexual maturity as early as the sixth week. They are able to deliver litters from one to 13 pups per pregnancy (Chahoud & Paumgarten, 2009) with an average gestation period of 22 days (Berman, 2020).

According to Johnson and Horn (2008), the distribution of rodents is influenced by several environmental factors which includes the habitat exploitation by human. These have been supported by Yihune and Bekele (2012) since the distribution of rodent in their study

were affected by vegetation, food availability and human impact. Their incredible adaptation to various lifestyles and environments (Rabiee et al., 2018) enables them to thrive in the new human-made habitats such as residential areas and plantation areas. The combination of a short reproductive cycle and big litters per pregnancy will lead to a rapid growth in their population. This, in turn, can result in an increase in zoonotic diseases that can infect the human community (Hafidzi et al., 2007). Therefore, the information of this significant host of transmissible zoonotic diseases could not be neglected.

2.3 Rodents parasites

Rodents are notorious hosts to numbers of zoonotic diseases that serve a genuine risk to human health (Luis et al., 2013; Meerburg et al., 2009; Morand et al., 2015). Many cases have been reported on zoonotic disease transmitted to human by the rodent such as toxoplasmosis, plague, hymenolepiasis, and others (Tijjani et al., 2020). Rodent-borne diseases are a huge issue that cannot be simply deal with due to the fluctuating occurrence rates and their correlation with the fast expansion of industrial and agricultural sectors, as well as global climate change (Rabiee et al., 2018). Rabiee et al. (2018) has been gathering diseases data from worldwide and found about 70 diseases were known as rodent-borne disease. In addition, study by Rabiee et al. (2018) proved that 50% of the rodent-borne disease were caused by parasitic infection, followed by bacterial and viral infections.

Three main classes of parasites that have been noted to directly cause disease in humans are protozoa, ectoparasites and helminth (Castro & Olson, 1996). Generally, parasites utilise host as their both nutrient bank and habitat for shelter, but transmission to another host has sometimes been crucial for the development and survival (Moore, 2010). Transmission of parasite falls into those general categories: the transmission of propagules (direct transmission), transmission by living vectors (usually by hematophagous insect), and

transmission by intermediate hosts (Moore, 2010) which may allow the transmission of zoonosis to human. Parasitic zoonoses have been contributed over 60% of all human infectious diseases worldwide (Tijjani et al., 2020). This is an indication that the study of parasites is vital since they possess a huge potential threat to human health.

2.3.1 Rodents blood parasites

There is a scarcity of published information about blood parasites in Malaysia. The majority of research articles in Malaysia and elsewhere have been focused on studying ectoparasites, while blood parasites have been largely overlooked. Protozoa are the parasite that commonly found in blood and transmitted by bloodsucking infected vector or in other cases it can be transmitted through the oral route (Shafiyyah et al., 2012).

Table 2.1 establishes the blood parasites found in rodents that have been discovered in Malaysia from about 20 years ago until recently. There are composed of *Plasmodium* sp., *Trypanosoma* sp., and *Trypanosoma lewisi* (Table 2.1). McConnaughey (2014) states that the genus *Plasmodium* can cause malaria, which is characterised by the symptoms of chills, fever, and anaemia. Furthermore, there are four species that infect humans, which known as *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* (McConnaughey, 2014).

In the other hand, genus *Trypanosoma* is widespread among the vertebrate host and primarily transmitted via arthropod vectors. Several species have been identified as agents of disease such as *T. brucei* (human trypanosomoses) and *T. cruzi* (Chagas Disease) (Hamilton et al., 2007; Truc et al., 2013). In the case of *T. lewisi*, they are often found in rodent, namely genus *Rattus* and generally non-pathogenic to human (Tatard et al., 2017). However, ephemeral and lethal infections have been reported in human from Asia and Africa

(Howie et al., 2006; Raminder et al., 2007; Sarataphan et al., 2007; Truc et al., 2013; Verma et al., 2011).

Based on studies in Malaysia, the potential infectious blood parasites were inhabited human settlement where people prone to the infection (Table 2.1). These findings may be due to most of the listed studies were focused on urban, commercial and residential areas including a study in Sarawak by Perison et al. (2022). Neither of the studies has been reported in agricultural areas and forested area in Sarawak region. Therefore, the potential threat to humans in Sarawak covering different habitat types need to be identified.

Table 2.1: List of blood parasites found on murid rodents recorded in Malaysia from 1999 until recently.

Species	Host								References
	<i>R. annandalei</i>	<i>R. argentiventer</i>	<i>R. exulans</i>	<i>R. norvegicus</i>	<i>R. rattus diardii</i>	<i>R. tanezumi</i>	<i>R. tiomanicus</i>	Rodent spp.	
PROTOZOA									
<i>Babesia</i> sp.					L		L		7
<i>Hepatozoon</i> sp.					L		L		7
<i>H. ophisauri</i>						U			8
<i>Hepatocystis</i> sp.						U			8
<i>Plasmodium</i> sp.	U, K	U, K, R	U, K, R	U, K, R	U, K, R		U, K, R		5, 7
<i>Toxoplasma</i> sp.					L		L		
<i>Trypanosoma</i> sp.	U, K, R	U, K, R	U, K, R	U, K, R	U, K, R		U, K, R	U	5, 6
<i>T. lewisi</i>								U	1, 4
No parasite found								X, U	2, 3

Note: L- lake; K- wet market; R- residential area; U- urban area; X- unmentioned habitat 1- Zainal Abidin and Noor Azmi (1999); 2- Paramasvaran et al. (2003); 3-Premaalatha et al. (2010); 4-Shafiyyah et al. (2012); 5-Siti Norliyanti (2017); 6-Premaalatha et al. (2018); 7-Kamil et al. (2021); and (Perison et al., 2022).

2.3.2 Rodents ectoparasites

A considerable amount of literature has been published on rodent ectoparasites both worldwide and locally (citation). However, there has been relatively little publication available in Sarawak, Borneo. There are four groups of ectoparasite that have been classified as rodent ectoparasite particularly ticks, mites, lice as well as fleas and relatively large proportion of them are zoonoses vectors (Mašán & Stanko, 2005). Ahmad et al. (2020) indicated that ticks and chiggers are the main reservoirs of vector-borne diseases found on small mammals in Malaysia. Based on those studies in Malaysia, several species have been pointed as medically important due to its cause of disease to human such as the tick namely *Ixodes granulatus* and the chigger, *Leptotrombidium deliense* (Madinah et al., 2011).

According to Mariana et al. (2008), *I. granulatus* harbours the virus causing Langat encephalitis (Hoogstraal, 1966), Lanjan Virus (Hoogstraal et al., 1972) and also involved in the tick typhus cycles and Q-fever in the forests of Malaysia (Marchette, 1966). To add, *I. granulatus* is also the most common tick species infested on rodents in Malaysia (Madinah et al., 2011; Nadchatram, 2008) (Table 2.2). On the other hand, *L. deliense* has been noted as a medically importance ectoparasite as it belongs to genus of an important vector of scrub typhus (Madinah et al., 2011). This vector can be found in various of environmental conditions, including the tropical atmospheres, forested and coastal areas of the Malay Peninsula and Indonesia (Kelly et al., 2009; Paramasvaran et al., 2009; Mariana et al., 2011).

The detection of ectoparasite of rodent in Sarawak needs to be extended due to the great number of the medically importance ectoparasites present in different habitat type in Sarawak that might be a threat to human community. Although many studies have been done, there are still many rodent ectoparasites with potential medical importance that need

to be elucidated and this study will provide an additional literature of ectoparasite study in Sarawak.

Table 2.2: List of ectoparasites on murid rodents recorded in Malaysia between 2000 until recent.

Ectoparasite species	<i>Leopoldamys sabanus</i>	<i>Maxomys alticola</i>	<i>Maxomys ochraceiventer</i>	<i>Maxomys rajah</i>	<i>Maxomys surifer</i>	<i>Maxomys whiteheadi</i>	<i>Mus musculus</i>	<i>Niviventer cremoriventer</i>	<i>Niviventer rapit</i>	<i>Rattus argentiventer</i>	<i>Rattus bowersi</i>	<i>Rattus exulans</i>	<i>Rattus norvegicus</i>	<i>Rattus rattus diardi</i>	<i>Rattus tanezumi</i>	<i>Rattus tiomanicus</i>	<i>Rattus</i> sp.	<i>Sundamys infraluteus</i>	<i>Sundamys muelleri</i>	Rodent spp.	References
Tick																					
<i>Amblyomma</i> sp.	F			F										B							2, 5, 12
<i>A. testudinarium</i>																			F		13
<i>Dermacentor</i> sp.	F			F	F	F					F					F		F	F		2, 4, 5, 13, 21
<i>D. atrosignatus</i>	F			F	F									F							18
<i>Haemaphysalis</i> sp.	F			F, W	F	F		F			F		R	R	R	F, R			F	F	2, 4, 5, 7, 8, 13, 15, 18, 19, 21
<i>H. bispinosa</i>																			F		13

Table 2.2 continued

<i>H. cornigera</i>														F	13	
<i>H. koningsbergeri</i>														F	13	
<i>H. papuana</i>														F	13	
<i>H. semermis</i>														F	13	
<i>H. traguli</i>														F	13	
<i>Ixodes</i> sp.	F		F		F									F	2, 4, 21	
<i>I. granulatus</i>	F, W		F, W	F	F		F	F	R	R		R, U	F, R	F	F, O	2, 4, 5, 8, 9, 11, 12, 13, 18, 19, 21
<i>Rhipicephalus</i> sp.			F											F	8, 21	
<i>R.</i> <i>haemaphysaloides</i>														F	13	
<i>R. sanguineus</i>			F												18	

Table 2.2 continued

Fleas						
<i>Sigmactenus</i>	F				F	10
<i>alticola</i>						
<i>Xenopsylla cheopis</i>			R, U	R, U	R	5, 19
Lice						
<i>Antarctophthirus</i> sp.				R		3
<i>Ancistroplax</i>	F					21
<i>nasuta</i>						
<i>Hoplopleura</i> sp.				R, U	U	15, 17, 21
<i>H. acanthopus</i>			R	R		3

Table 2.2 continued

<i>H. dissicula</i>										F	O, R	11, 12, 21	
<i>H. pacifica</i>					M	R	R, U		R		O, R	1, 5, 12, 19	
<i>H. pectinata</i>			M									1	
<i>Linognathus</i> sp.											U	17	
<i>Polyplax</i> sp.											U	17	
<i>P. spinulosa</i>	F, D		M				R, U, K	R, U	U	R	K	O	1, 4, 5, 12, 14, 16, 19, 20, 21
<i>Sathrax</i> sp.					F								21
<i>Goniocotes</i> <i>gallinae</i>							R, K						3

Table 2.2 continued

Mite									
<i>Listrophoroides</i> sp.	F	F	F, P	F				F	O, R 8, 11, 12, 21
<i>Myocoptes</i>							U		16
<i>musculus</i>									
<i>Notoedres muris</i>							U		5
<i>Sarcoptes scabiei</i>								F	21
<i>Echinolalelaps</i>							R, R,		
<i>echidninus</i>	D			R	A		K K		3, 14
<i>Haemolaelaps</i> sp.								F	21
<i>Haemolaelaps</i>								F	21
<i>traubi</i>									
<i>Laelaps</i> sp.	F		F F F		F			F	7, 18

Table 2.2 continued

[illegible]

Table 2.2 continued

							C,												
				F,			M	R,	R,	R,	F,		F,	O,			1, 2, 4, 5, 11,		
L. nutalli	F		F	F		F	,	K	U	U	R		K	R			12, 15, 19, 20,		
				M			K,										21		
							F												
L. rex			F														21		
				F,															
L. sanguisugus			P,	F	W												4, 5, 8, 21		
			W																
			F,																
L. sculpturata	F	F		F		F	F						F	O,			2, 4, 5, 8, 21		
			P											R					
L. sedlaceki			F		F		F				R,	F,			F		2, 4, 11, 15, 21		
											U	R							

Table 2.2 continued

<i>L. turkestanicus</i>				F	F			F	F	21
<i>Longolaelaps</i>										
<i>longulus</i>	F	F	F	F		F				2, 4, 5, 21
<i>L. whartoni</i>	F, W	F								4, 8
<i>Dermanyssus</i>							R,			3
<i>gallinae</i>							K			
<i>Ornithonyssus</i>							U,		O,	
<i>bacoti</i>						R	R	R		5, 12, 19
<i>Myobia</i> sp.						R,	R,			3
						U	K			
<i>Radfordia</i> sp.									U	17

Table 2.2 continued

Chigger									
<i>Ascoschoengastia</i>				F				F	21
sp.									
<i>A. indica</i>				M		C	U	U	1, 5
<i>Eutrombicula</i>									
<i>wichmanni</i>	F							F	2
<i>Gahrlepiea</i>									
(<i>Walchia</i>) sp.								F	21
<i>Gahrlepiea fletcheri</i>	F	F	F	F,	F			F	1, 2, 4, 5
				M					
<i>G. naniparma</i>	F		F	F				F	2, 4
<i>Leptotrombidium</i>									
<i>deliense</i>	F					C		F	2, 4, 5, 21

Table 2.2 continued

<i>Microtrombicula</i>					
sp.	F				21
<i>Siseca</i> sp.	F				21
<i>Walchiella</i>					
<i>oudemansi</i>	F		C	F	4, 5
<i>Walchiella</i> spp.		F		F	21
<i>Demodex</i> sp.				X	8

Keys: A- agricultural area; B- rice field; C- coastal area; D- waste disposal area; F- forest; K- wet market; M- mangrove; O- oil palm plantation; P- peat swamp; R- residential area; U- urban; W- wetland; U-unmentioned habitat; 1- Chuluun et al. (2005); 2- Mariana et al. (2005); 3- Hafidzi et al. (2007); 4- Mariana et al. (2008); 5- Paramasvaran et al. (2009); 6- Premaalatha et al. (2010); 7- Adrus et al. (2011); 8- Madinah et al. (2011); 9- Mariana et al. (2011); 10- Wells et al. (2011); 11- Madinah et al. (2013); 12- Nursyazana et al. (2013); 13- Wells et al. (2013); 14- Priscilla et al. (2015); 15- Ng et al. (2017); 16- Premaalatha et al. (2017); 17- Premaalatha et al. (2018); 18- Razali et al. (2018); 19- Mustapha et al. (2019); 20- Ibrahim (2020); 21- Adrus et al. (2021).

2.3.3 Rodents endoparasites

In Malaysia, a few studies have been carried out on rodent endoparasites especially in Sarawak. Table 2.3 shows only one study has been reported in Sarawak between the year of 2000 and recent study which only covering forested area (Hamdan et al., 2016). While, the rest of the listed studies were from Peninsula Malaysia.

Endoparasite is the parasite that lives inside the body of their host either in internal organ or tissue (Mayer & Donnelly, 2012). Mayer and Donnelly (2012) also added that word of endoparasite was most commonly refer to helminth. The helminths are divided into roundworm (Nematode), and flatworm which further divided into a tapeworm (Cestode) and fluke (Trematode) (Engelkirk & Duben-Engelkirk, 2008). Goering et al. (2018) highlighted that many helminths lived in the intestine but some of them also found in tissues. There are four ways of helminths invasion, which are by swallowing eggs and larvae through fecal-oral route, by swallowing the larvae in tissue of infected hosts, by active penetration of the skin and by the bite of bloodsucking infected vector (Goering et al., 2018).

Genus *Hymenolepis* is one of the helminths that commonly found worldwide associated with murid rodent as a host (Jarošová et al., 2020). According to Tijjani et al. (2020), genus *Hymenolepis* are known to pose a serious health threat through Hymenolepiasis. However, Hymenolepiasis is classified as neglected diseases and approximately 3800 million people worldwide were suffering from the infection (Fitte et al., 2018). Hymenolepiasis is more prevalent among children and most infections was asymptomatic and if it does, the symptoms include abdominal pain, diarrhea, and vomiting (Kandi et al., 2019b). Based on Table 2.3, genus *Hymenolepis* mostly reported in human-dwelling areas which put human populations in Malaysia to the risk of infection.

Generally, infections of helminth are widely distributed in tropical and subtropical areas with moist and warm climate condition (Amenu, 2014). The climate conditions of Malaysia might favourable to those zoonotic parasites that can high potentially be transmitted to human. Thus, detection of endoparasite in Sarawak is fundamental since little publication is available in Borneo.

Table 2.3: List of endoparasites on murid rodents recorded in Malaysia from 2000 until recent.

Species	<i>Leopoldamys sabanus</i>	<i>Maxomys rajah</i>	<i>Maxomys surifer</i>	<i>Maxomys whiteheadi</i>	<i>Niviventer cremoriventer</i>	<i>Rattus argenteiventer</i>	<i>Rattus exulans</i>	<i>Rattus norvegicus</i>	<i>Rattus rattus diardii</i>	<i>Rattus tiomanicus</i>	<i>Sundamys muelleri</i>	Rodent host	References
Nematode													
<i>Angiostrongylus cantonensis</i>												R	13
<i>A. malaysiensis</i>							F	U	U	F		O, R	1, 6, 7
<i>Ascaridida</i> worm												U	11
<i>Aspicularis tetraptera</i>								U, K					10
<i>Capillaria</i> sp.												U	11
<i>C. hepatica</i>		F	F	F			F			F	F	U, K, R	1, 2, 4, 12

Table 2.3 continued

[illegible]

Table 2.3 continued

<i>Mastophorus</i>						I, O,	
<i>muris</i>	F		F	U	U	F	1, 3, 4, 6,
							R, U,
							7, 9
							K
<i>Nippostrongylus</i>							
sp.						U	11
						K, O,	
<i>N. brasiliensis</i>				U	U		
						R, U,	4, 6, 7, 12
						K	
Oxyurida worm	F		F				2
<i>Physolaptera</i> sp.						U, K	4
<i>Pterogodermais</i>							
sp.						U, K	

Table 2.3 continued

<i>Pterygodermatites</i>										
<i>whartoni/tani</i>										
						U	U			6
<i>Reticularia tanii</i>										
	F	F							U, K	1, 4
Spirurida worm	F			F						2
Strongylida worm	F			F						2
<i>Strongyloides</i> sp.										
	F	F	F		F	U, K		F	F	U
										1, 5, 9, 10, 11
<i>Subulura</i>										
	F	F								1
<i>andersoni</i>										
<i>Syphacia muris</i>										
	F	F					U	F	F	O, R, U, K
										1, 4, 6, 7
<i>Toxocara</i> sp.										
	F	F	F	F						9

Table 2.3 continued

Trichocephalida worm	D											8
<i>Trichuris</i> sp.						U, K				U, R		4, 10, 11, 13
Cestode												
Cyclophyllidea worm	F, D		A									2, 8
<i>Hymenolepis</i> sp.										U, K		4
<i>H. dimunita</i>	F	F				U	U	F		O, R, U, K, K, R		1, 4, 6, 7, 12, 13
<i>H. nana</i>						U	U			U, K, R		4, 6, 13

Table 2.3 continued

<i>H. sapnema</i>								U, K	4
<i>Raillietina</i> sp.								U, K	4
<i>Rodentolepis</i> sp.								U	11
<i>R. nana</i>	F		F				F	I	1, 3
<i>Taenia</i>								O, R,	1, 4, 6, 7,
<i>taeniaformis</i>		F	F		F		U	F	U, K,
								U, R	11, 13
Trematode									
<i>Fibricola</i>							F		1
<i>ramachandrani</i>									
<i>Zonorchis</i> sp.	F	F							1

Table 2.3 continued

Pentastomide				
<i>Armillifer</i>				
<i>moniliformis</i>	F		F	1
Acantocephala				
<i>Moniliformis</i>				U, K,
<i>moniliformis</i>		U	U	4, 6, 13
				R
Protozoan				
<i>Blastocystis</i> sp.		U, K		U 10, 11
Coccidian worm				U 11
<i>Trichomonas</i> sp.		U, K		U 10, 11

Keys: A– farm; D- waste disposal area; F- forest; I- island; K- wet market; O- oil palm plantation; R- residential area; U- urban; W- wetland; X- unmentioned habitat; 1- Ask and Mohd Zain (2006); 2- Wells et al. (2007); 3- Mohd Zain (2008); 4- Paramasvaran et al. (2009b); 5- Premaalatha et al. (2010) ;6- Mohd Zain et al. (2012); 7- Nursyazana et al. (2013); 8- Priscilla et al. (2015); 9- Hamdan et al. (2016); 10- Premaalatha et al. (2017); 11- Premaalatha et al. (2018); 12- Ibrahim (2020); and 13- Tijjani et al. (2020).

2.4 Parasites and host relationship

Host parasite relationships appear due to the associations between organisms that living in an environment. The host reacts to parasitism in various methods from morphological to the behavioural (Solomon et al., 2015). The main determinants explaining parasite richness among host species of rodent in most comparative analyses are consistently shown to be the host density and host geographic range (Kamiya et al., 2014; Morand, 2015; Morand & Bordes, 2015). In addition, Morand and Bordes (2015) stated that parasite species richness also related to host investment in immune defence, basal metabolism, trade-off in life-history traits, and sexual size dimorphism (SSD). The higher immunogenetic diversity, higher basal metabolism, longer host life spans and male-biased infection may contribute to a greater parasite species richness (Morand et al., 2004; Cooper et al., 2012; Morand, 2015).

Parasites show a high level of specialization and have faster reproduction rate than their hosts (Solomon et al., 2015). The host specialization of parasite may be due to general territorial social structure of host that limits the opportunities for transmission to different species (Poulin et al., 2006). Other variables may be involved in host specialization such as the structural of the habitat, the dispersal and the colonising abilities of parasites, as well as the features of the parasites (such as their mode of transmission) (Poulin et al., 2006). Base on the study by Pedersen et al. (2005), parasites with direct (sexual or physical) contact transmission mode are highly specific, while, parasites transmitted via food and via intermediate hosts are prone to be less specific.

2.5 Impact of habitat environment on parasites species richness and abundance

Host and habitat or landscape are the two important factors that always to be considered in understanding disease ecology and parasite transmission (Bordes et al., 2013).

The influence of habitats on disease ecology draws extra attention when the environmental changes are stimulated by deforestation (Bordes et al., 2013) which prone to alter the distributions and abundance of parasites, hosts, or vectors (Bordes et al., 2013; Cottontail et al., 2009; Gillespie & Chapman, 2008; Goldberg et al., 2008). In Sarawak, large of the forest area has been cleared since two decades ago for urban development and oil palm plantation (Tsuyuki et al., 2011) which eventually force the alteration of the parasite population in this region.

Bonneaud et al. (2009) once stated forest is more likely to be the favourable habitat for parasite transmission. Other studies have proven this statement with the positive result of higher infection in the natural habitats rather than disturbed or deforested habitats (Chasar et al., 2009; Durden et al., 2004). However, it seems to contradict to other helminth study with the result of the richness, prevalence and abundance were positively inclining towards the crop fragments than natural landscapes (Froeschke & Matthee, 2014). The diverse outcomes seen in parasite research have highlight the significance of environmental factors, such as humidity and temperature, in the developmental processes of eggs and larvae (Brouat et al., 2007; Froeschke & Matthee, 2014; Hulbert & Boag, 2001; Mas-Coma et al., 2008). Dry open areas tend to serve an excessive heat that can adverse the survival of helminth larvae which means they need moist areas for successful development of larval stage (Munang'andu et al., 2012). Another example can be shown on mite study, which they need warmer and less humid environment for the successful population where too low temperature may delay the egg development while too high may slay the eggs (Dube et al., 2018). However, every research findings may differ due to different habitats environment involve in each research.

In addition, the nest substrate also influences the parasite population since a study revealed that wooden structure is more favourable for mite than concrete or metal structure (Dube et al., 2018). An explanation has been provide by the author as the wood is a better thermal insulator than metal or concrete in order to avoid temperature fluctuation (Ankersmit & Stappers, 2017). Other than that, ground cover of habitat also plays an important role in parasite development. For Ixodid tick, 90% of them were characterised in three-host life cycle, which means they need to detach and find shelter such as under leaf litter on the forest floor and soil for molting after feeding (Nicholson et al., 2009). These response have indicate the study of different habitat type on parasite population is essential in understanding parasite ecology (Chakraborty et al., 2019).

According to Chakraborty et al. (2019) and Gottdenker et al. (2014), the effect of plantation on parasites is one type of habitat that has not been studied well. As in Sarawak, the oil palm plantation has been conquered large area of the land cover and has been noted as the fastest growing crop with 77% of area increment annually (Tsuyuki et al., 2011) but their effect on parasite was still poor studied not only in Sarawak but also in Malaysia. Therefore, this study intended to include oil palm plantation to see the effect of habitat type on the parasite in Malaysia or in Sarawak specifically.

CHAPTER 3

THE DISTRIBUTION AND HABITAT PREFERENCE OF RODENT IN DIFFERENT HABITAT TYPES IN SARAWAK

3.1 Introduction

Rodents are important contributors to the biodiversity ecosystems as its are important primary consumers of seeds and herbage (Krebs 2001). They not only serve as food for avian, reptiles and mammalian predators but also being trap by some local people in Sarawak for personal consumption (Yi & Mohd-Azlan, 2020). Rodents are susceptible to being carriers of zoonotic diseases, which raises concerns over the transmission of such diseases to humans. They comprise of 42% of mammalian fauna recorded worldwide which is the largest mammalian order (Chen et al., 2022) and 66 species has been recorded in Borneo (Phillipps & Phillipps, 2018). They are divided in three major families namely Muridae, Sciuridae and Hystricidae (Hawkey, 2017).

The family Muridae is well known as the most dominant family (Paramasvaran et al., 2012; Timbuka & Kabigumila, 2006) and the most medically important family compared to the others family in the order Rodentia (Telmadarrai et al., 2007). The remarkable adaptability of many rodent groups has contributed to their widespread success across diverse habitats, hence raising concerns about the global transmission of diseases to humans. (Paramasvaran et al., 2012).

Distribution and abundance of rodents depend on vegetation for food and shelter (Cramer & Willig, 2002; Fitzherbert et al., 2007). Because of food and cover requirements, rodents are likely to be adversely impacted by overuse of habitat. To date, over 1.5 million hectares of land area of Sarawak have been turn into oil palm plantation (Jaafar et al., 2020;

Mohd-Azlan et al., 2019). This massive land-use intensification may have influenced the habitat preference, diversity, distribution, and population of rodents (Morand et al., 2019; Palmeirim et al., 2020; Zhang et al., 2016).

This highlights the need of understanding their distribution in different stages of habitat disturbance to provide reliable information which can be used by various stakeholders to enhance existing management strategies for conservation of rare species, action plans of pest control and provide basic checklist of rodent species that maybe related to zoonotic disease in Malaysia. In view of this, the objectives of this study are to determine the distribution of rodents in contrasting habitats and determine the habitat preferences of rodents in Sarawak, Malaysian Borneo. This study predicted that there were less species living in disturbed or modified habitats.

3.2 Materials and Methods

3.2.1 Study areas

This study was conducted in eight localities in Sarawak (Figure 3.1) consists of four habitat types which are forest (FA), oil palm plantation (OP), rural (RA) and urban areas (UA). The forest areas consist of a mix-dipterocarp forest, high density of trees with low human activities and far from human settlement. Meanwhile, the oil palm plantation is cultivated oil palm area with limited human activities and far from human settlement. The rural area is described as scattered human residential areas with small mixed crops and moderate human activities while the urban area is described as the lowest vegetation cover, highest human activities and density of human settlement.

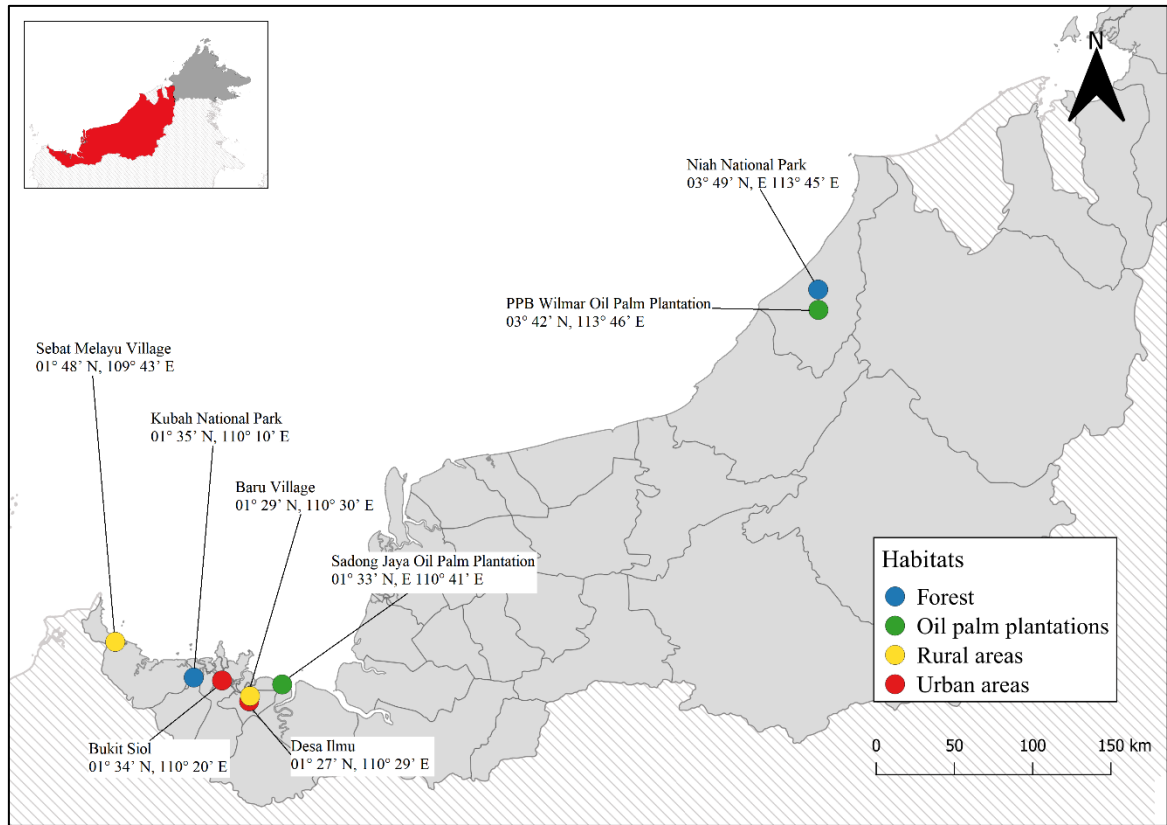


Figure 3.1: Rodent trapping in eight locations consist of four different habitats in Sarawak, Malaysian Borneo.

3.2.2 Rodents Sampling

Rodents were sampled from November 2018 to December 2019. Total sampling efforts for all habitats are same (1000 hours) except for oil palm plantation areas (1300 hours) (Table 3.1).

This study used Sherman's and cage traps (as per Table 3.1) to catch rodents. In the forested areas, traps were set up on the forest floor along defined routes and established trails. Traps also were deployed in oil palm plantations, with trees ranging in age from one to eight years old, surrounded by sparse bush cover. In rural areas, trapping activities were carried out in the yards of the villagers' house. Meanwhile, rodent trappings in urban areas were carried out in the residential, storefronts, and disposal sites. Cage and Sherman's traps were baited with bananas, roasted palm kernels, coconut flesh, dried salted fish, and mixture

of peanut butter and oat. Traps were set in each study area with 10 m apart from each trap and kept open for up to five consecutive nights. The traps were checked twice a day, once in the morning (0700–0900) and once in the evening (1600–1800) to prevent stress and death of the rodents. The rodents were trapped alive and brought to the base camp or laboratory for identification. The rodents were euthanised with 30% isoflurane and cervical dislocation following the procedure by Carbone et al. (2012). Morphological measurements, sexes, and life stages were recorded for species identification. The rodents were identified to species level based on Phillipps and Phillipps (2018). The dead rodents were preserved in 70% ethanol and deposited in the Zoological Museum, Universiti Malaysia Sarawak as voucher specimens.

Table 3.1: Summary of sampling efforts in 10 localities in Sarawak.

Study sites	Date sampling	Total sampling days	No. of trap used
Forest areas (FA)			
Kubah National Park	4-8 November 2018	5	100
Niah National Park	2 - 6 December 2019	5	140
Oil palm plantation (OP)			
Wilmar Oil Palm Plantation	28 February - 8 March 2019	8	100
Sadong Jaya Oil Palm Plantation	18 - 22 July 2019	5	100
Rural Areas (RA)			
Kampung Sebat Melayu	12 - 16 July 2019	5	100
Kampung Baru	16 - 20 August 2019	5	100
Urban Areas (UA)			
Desa Ilmu	18 - 22 November 2018	5	100
Petrajaya	27 Jun - 1 July 2019	5	100

3.2.3 Statistical analyses

All data obtained in this study were compiled in Excel spreadsheet and a table constructed from captured rodent data to document their distribution in different habitat types in Sarawak. Species diversity was estimated using Shannon's (H') and Simpson's (D) Index, where H' value shows information of richness and abundance while D value shows information of evenness in each habitat. Shannon's Index (H') has a moderate ability to discriminate between communities and low sensitivity to sample size (Maguran, 2004). Rarefaction and extrapolation were used to compute expected species richness since total sampling efforts vary among habitats. Rarefaction allow to standardise the different in sampling effort to provide fair comparison of species richness between habitats. The species richness was estimated from the number of species with the individuals accumulation (Colwell et al., 2012; Mohd-Azlan et al., 2019). These diversity indices and rarefaction were estimated using 'iNEXT' package (Chao et al., 2014; Hsieh et al., 2016).

Indicator value analysis (*IndVal*) was used to measure the uniqueness of species occurrence in the habitats by rank and it is also frequently used to express the importance of species at a community level (Podani & Csányi, 2010). *Pearson's phi coefficient* of association was used to estimate the habitat preference of the rodents. Output from *phi coefficient* ranged from -1 to 1 which correspond to the strength of the relationships. The positive value indicates the positive relationship while the negative value indicates the strength of avoidance of the species to a particular habitats (Cáceres & Legendre, 2009). This analysis was conducted using 'indicspecies' package (version 1.7.8). All analysis was conducted using R Statistical Software version 4.0.4 (R Core Team, 2021).

Both native and commensal rodents have been listed to calculate the composition in different habitat type in Sarawak (Figure 3.4). A native species is defined as one that exists within its natural habitat (Crees & Turvey, 2022). While commensal species is rodents that coexist with humans, utilising the resources such as food, water, and habitats that are made available by human (Phuckett et al., 2020).

3.3 Results

3.3.1 Rodent species distribution and diversity in Sarawak

A total of 241 individuals representing eight species of rodents were successfully captured in four different habitat types in Sarawak. Seven out of eight species were recorded in forest areas namely *Leopoldamys sabanus*, *Maxomys rajah*, *M. whiteheadi*, *Niviventer cremoriventer*, *Rattus exulans*, *R. tanezumi* and *Sundamys muelleri*. Three species found in oil palm plantation areas were *R. exulans*, *R. tanezumi* and *R. tiomanicus*. Two species found in rural areas namely *R. tanezumi* and *R. tiomanicus*. Two species found in urban areas were *R. tanezumi* and *S. muelleri* (Table 3.2).

Rattus tanezumi was the most abundant species (N = 149) and the only species recorded in all habitats. Meanwhile, *L. sabanus*, *M. rajah*, *M. whiteheadi* and *N. cremoriventer* were exclusively recorded in a single habitat (forest areas) (Table 2). The oil palm plantation areas recorded three commensal rats namely *R. tanezumi* (N=41), *R. tiomanicus* (N=23) and *R. exulans* (N=8) (Table 3.2).

The most diverse habitat was the forest areas that reported significantly higher species diversity ($H'=1.84$, $D=0.83$, $p<0.05$) compared to the other habitat types (Figure 2). Rural areas recorded the lowest species diversity in this study. Species diversity decreased with habitat gradient and slightly increase in urban areas (Figure 3.2). However, species

richness shows decreasing trend along habitat gradient, changes in habitat structure that related to vegetation cover and human activity in this study (Table 3.2, Figure 3.2). This richness trend was further confirmed by rarefaction curve (Figure 3.3) as sampling efforts varied among habitats and rarefaction were used to standardise the sampling effort and compute the expected richness after the standardisation. Figure 3.3 shows the species richness is highest in forest area followed by oil palm plantation, rural and urban area.

Five out of eight species from this study were noted as native namely *L. sabanus*, *M. rajah*, *M. whiteheadi*, *N. cremoriventer* and *S. muelleri*. The other three species namely *R. exulans*, *R. tanezumi* and *R. tiomanicus* were commensal species. Only 73% and 37 % of rodent from forest and urban areas respectively were native species. Meanwhile, oil palm plantation and rural areas were fully dominated by commensal species. Native species disappeared from disturbed habitats except for *S. muelleri* (Figure 3.4).

Table 3.2: Distribution of rodents in different habitat types from Sarawak.

Habitat	Locality	Rodent species								Total Individuals (N)	N per habitat	Total species recorded
		<i>Leopoldamys sahanus</i>	<i>Maxomys rajah</i>	<i>Maxomys whiteheadi</i>	<i>Niviventer cremoriventer</i>	<i>Rattus exulans</i>	<i>Rattus tanezumii</i>	<i>Rattus tiomanicus</i>	<i>Sundamys muelleri</i>			
Forest areas	KNP	4	3	3		3			3	16	26	7
	NNP			1	1	1	3		4	10		
Oil palm plantations	WOP					8	31	3		42	72	3
	SOP						10	20		30		
Rural areas	KSM						26	9		35	65	3
	KB						30			30		
Urban areas	PJ						9		29	38	78	2
	DI						40			40		
Total		4	3	4	1	12	149	32	36	241	241	8
IUCN status		LC	VU	VU	LC	LC	LC	LC	LC			

Keys: KNP- Kubah National Park; NNP- Niah National Park; WOP- Wilmar Oil Palm; SOP- Sadong Oil Palm; KSM- Kampung Sebat Melayu; KB- Kampung Baru; PJ- Petrajaya; DI- Desa Ilmu; LC- Least Concern; VU- Vulnerable; and N- number of rodent captured.

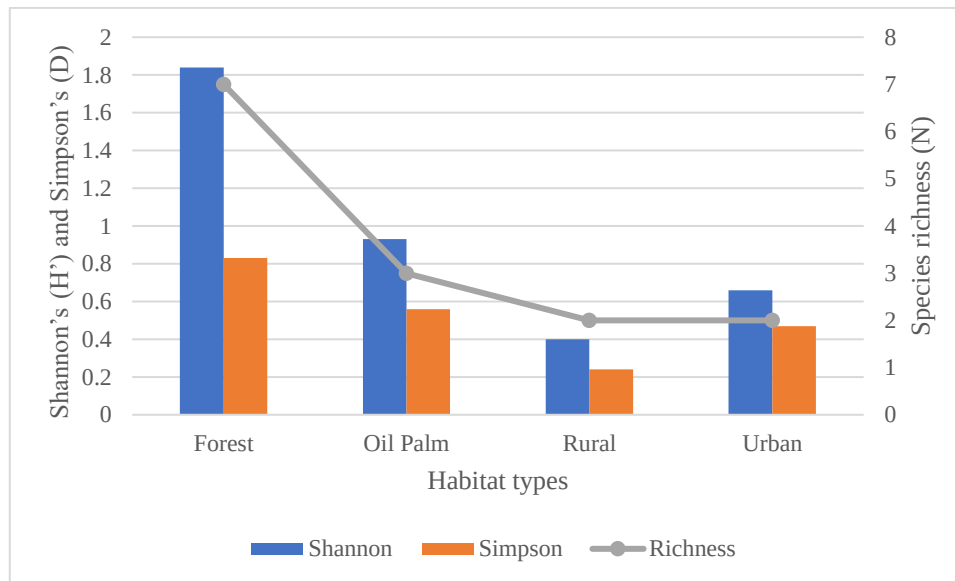


Figure 3.2: The Shannon's (H'), Simpson's (D) Diversity Indices and species richness based on total rodent captured in forest, oil palm plantation, rural and urban areas.

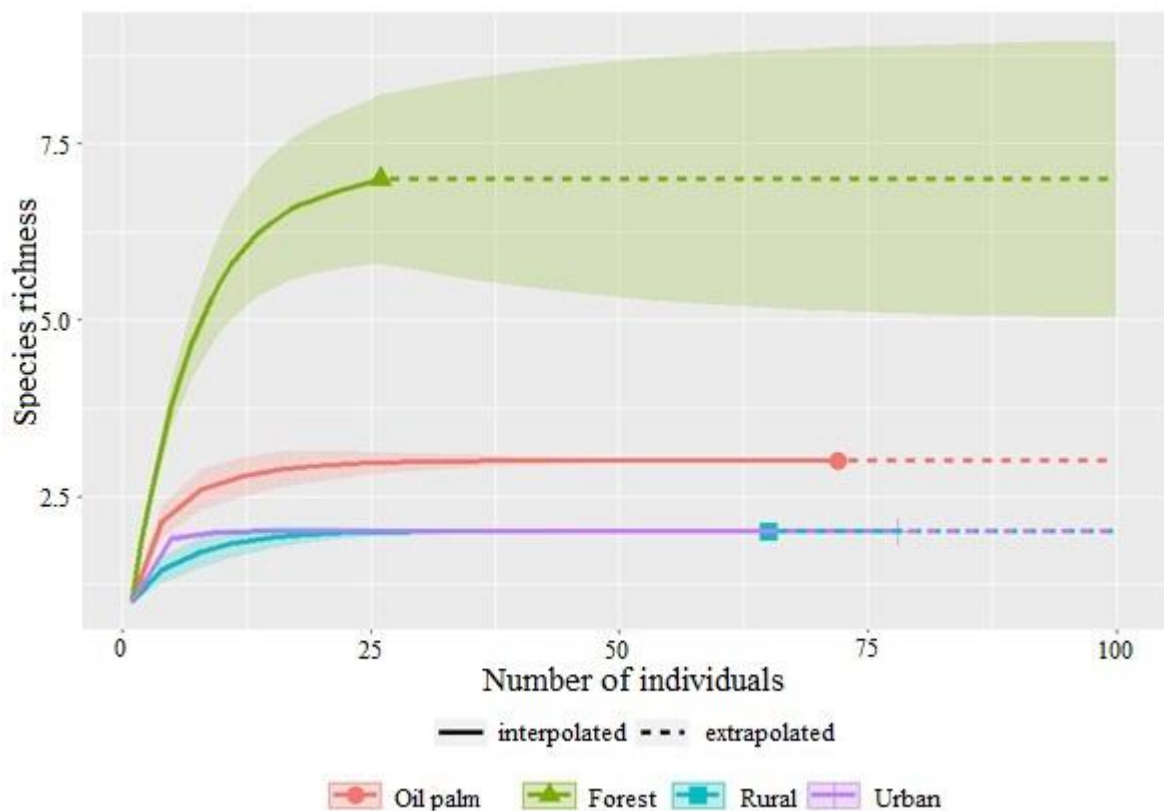


Figure 3.3: The species richness rarefaction curves habitats comparison. Curves show species accumulations and the species richness rarefied estimation by number of individual caught.

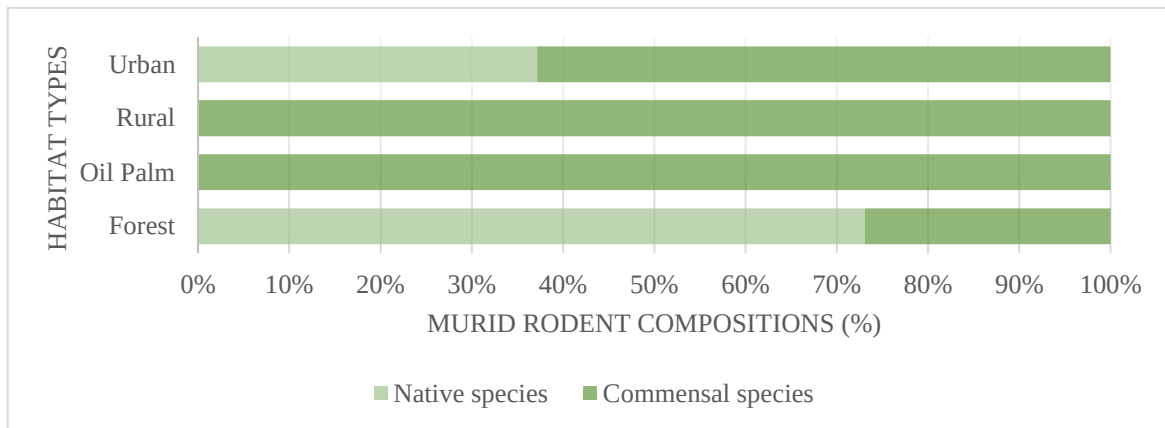


Figure 3.4: Murid rodent compositions from forest, oil palm plantation, rural and urban areas in Sarawak.

3.3.2 Habitat preference of rodent in Sarawak

Based on Figure 3.5, four species of rodents are only subjected to single habitats *L. sabanus*, *M. rajah*, *M. whiteheadi* and *N. cremoriventer* considered as habitat specialist. Meanwhile, other species can be found to multiple habitats combination which considered as habitat generalist. All native species shows a high preference on forest habitat ranging from 0.66 to 0.75 (*phi* index) (Figure 3.5). *Rattus* species seems favour oil palm plantation areas (*phi* range=0.15-0.75). *Sundamys muelleri* showed habitat references not only to forest areas (*phi*=0.75), but also to urban areas (*phi*=0.15) (Figure 3.5).

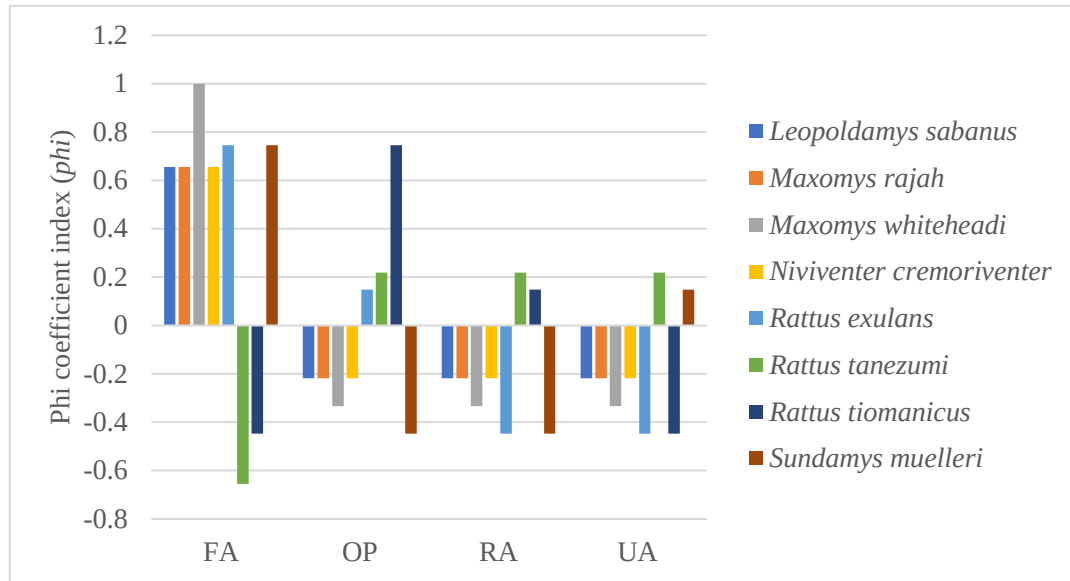


Figure 3.5: Habitat preference of rodent in Sarawak, Malaysian Borneo generated from *phi* coefficient. FA, forest areas; OP, oil palm; RA, rural areas; and UA, urban areas.

3.4 Discussions

Cumulative species richness (8 species) nearly matched to the findings by Wells et al. (2014) (9 species Muridae) from the Sabah, Malaysian Borneo. Some species may have strong preferences for specific habitat such as rice field, coastal area, bamboo tree area and more. Exclusion of such habitats during surveys may affect species richness in a study.

Rattus tanezumi, the dominant species has a wide distribution across the Indo-Malayan region (Aplin et al., 2011; Guo et al., 2019). This generalist species is considered as an invasive species in human settlement as well as in plantation areas (Phillipps & Phillipps, 2018). It was reported in all habitats, but with low frequency in forest habitat consistent to that of Mulungu et al. (2008). The lack of food availability may be the major factor that contribute to this. This species have been reported to migrate to other habitats in order to survive from food insufficiency (Guo et al., 2019).

In this study, *N. cremoriventer* is the least captured species throughout this study. *Niviventer cremoriventer* is an arboreal rat, the only rat species that usually construct their

nest on a tree (Phillipps & Phillipps, 2018; Wells, Lakim, et al., 2006). Trapping activity in this study was mostly conducted on the ground which may reduce the chances of *N. cremoriventer* to be captured. An additional sampling in forest areas may increase captures of this species and enhance more species detection.

Unlike *N. cremoriventer*, *Maxomys* and *Leopoldamys* genus were commonly utilized subterranean burrows for constructing their nests (Wells et al., 2006). This nesting strategy able to provide as food storage (Wells & Bagchi, 2005), reduced predation risk and protect these species from exposure of the sun and rain (Wells et al., 2006). Furthermore, they are also associated coarse woody debris in the forest and this is important for moving and travelling through the forest undergrowth (Cusack et al., 2015; Wells, Pfeiffer, et al., 2006).

The expected species richness was estimated by rarefaction to standardise the differences in total sampling efforts across habitats. The results further confirm the diversity trend that the forest area has the highest species diversity, followed by the oil palm plantations, rural areas, and urban areas. Rarefaction suggested a higher individual count needed to compute species richness, thus extending sampling efforts in forest area is recommended for future rodent diversity studies.

Higher species diversity recorded in forest areas compared to all other habitats was expected as forest is known high quality habitat which could provide unique resources to support array of rodent species. This was comparable to study by Hamdan et al. (2017) who reported a high diversity of rodent in forested areas from the same region ($H'=2.24$, $p<0.05$). Increase habitat heterogeneity such as logs and branches, vegetation cover and shrub layer as well as high flora and fauna diversity in mixed-dipterocarp forest are able to provide greater food sources (Nurqamareena et al., 2018) and cover from predators to wide-range of

small mammal species (Ecke et al., 2001). Forested habitat contains more resources that provides various ecological niches than the other habitats attracting more rodent species (Rajpar, 2018).

The decrease in rodent diversity along the gradient can potentially increase disease transmission (Morand et al., 2019). Transmission rates and the success reproduction of pathogen are often related to abundance of host. High diversity regulates the number of the reservoir species, hence lower the risk of pathogens transmission and diseases Suzán et al. (2009). Suzán et al. (2009) have demonstrated that the infection prevalence of hantavirus increased to 35% in low species richness communities. Their finding indicates rodent is high potentially cause major consequences for public health (Blasdell et al., 2011; Blasdell et al., 2019; Guo et al., 2019; Huang et al., 2013; Ishak et al., 2018; Lerdthusnee et al., 2003; Lerdthusnee et al., 2008; Mohamed-Hassan et al., 2010; Plyusnina et al., 2009). However, the low rodent diversity in disturbed habitats in this study insufficient to prove potential of disease transmission by the rodent as this study only perform basic parasite detection on host and further study on disease detection is needed to support this diversity trend.

The lowest species diversity ($H'=0.4$, $p<0.05$) was recorded in rural areas. In this habitat, *Rattus tiomanicus* was recorded only in Kg. Sebat Melayu. A proportion of this rural residential area was covered by small patches of mixed crop such as oil palm, 'buah kristal', papaya, banana, coconut et cetera as well as surrounded by secondary forest. This surrounding habitat matrix may provide suitable habitat for *R. tiomanicus* as it is associated to secondary forest and oil palm plantation areas (Phillipps & Phillipps, 2018). Meanwhile, Kampung Baru only has small patch of banana crops plant by the villagers. Lee and Goh (2000) found this species preferred fresh coconut and less preferred banana and jackfruit.

This suggest that *R. tiomanicus* able to thrive in residential area with an available of their food preference.

As in oil palm plantation areas, only three commensal species were recorded and none of any forest were detected in this habitat. Cultivated plantation at oil palm plantation provide homogeneous vegetation. Hence, there are a restricted range of resources that are only accessible to specific species. However, this finding contrast to the seven species of Muridae recorded in the same oil palm plantation (PPB Wilmar oil plantation) (Mohd-Azlan et al., 2019) that succeed capturing native species in oil palm plantation areas. Forest species from nearby secondary forest able utilised the oil palm plantation for unlimited food resource (Mohd-Azlan et al., 2019). This suggest that increased sampling period, type of bait used, avoid wet seasons, and include fruiting seasons may help in capturing more species in this study area. According to Khan et al. (2017), a longer sampling duration can potentially lead to a higher capture rate. Utilise a variety of bait types can enhance the capture of different rodent species as Muridae showed a greater preference for sweet potato with peanut butter compared to bananas, oil palm, and dried fish used in current study (Mohd-Taib and Ishak, 2021). The wet season might reduce the activity of non-volant small animals, leading to a decrease in capture rates (Baharudin et al., 2023). Other than that, during fruiting seasons, rodent attracted to the area due to the availability of abundant food resource, thereby increasing the success of trapping rodents (Wells & Bagchi, 2005).

Many native species appear to avoid disturbed habitat partly responding to the heterogeneous distribution of resources. This suggests the lack of complexity (such as canopy and ground cover, vegetation and microhabitat diversity) and susceptibility to predation in disturbed habitats (oil palm plantation, rural, and urban areas) may have been hostile to many forest and native species (Laurance, 2008; Mohd-Azlan et al., 2019). Similar

study also showed native small mammals disappeared from disturbed or modified landscapes and replaced by invasive and commensal species (Wells et al., 2014) as shown in oil palm plantation and rural areas in this study. In addition, rodent communities had lower species diversity and richness in urban conditions where it is exposed to roads and high human activities (Cavia et al., 2009; William-Dee et al., 2019). This indicate that forested areas are crucial for sustaining more species, especially forest species assemblage where many of these species cannot thrive in a disturbed habitat.

In this study, the only native species that could adapt anthropogenic habitat is *Sundamys muelleri*. The detection of this species also have been reported by Wells et al. (2014) and Hamdan et al. (2017) in residential areas (urban and sub-urban) of this region. However, this is inconsistent to Payne et al. (2005) and Phillipps and Phillipps (2018) who reported this species as a common resident in forested areas. Current study shows that *Sundamys muelleri* displays positive preference to both forest ($\phi=0.476$) and urban area ($\phi=0.265$). This species have been known to lower their occurrence probability with low land-use intensity and prefers urban housing areas with vegetation remnants (Wells et al., 2014). Other study found this species is actually associated with water bodies such as rivers and lakes (Jayaraj et al., 2012; Khan et al., 2019). However, the least water body present in the study area was the residential water drainage instead of rivers or lakes. This water source may be made this opportunistic species able to expand its habitat range to urban habitats (Hamdan et al., 2017; Wells et al., 2014).

Risk of predation also may affect habitat preference (Cramer & Willig, 2002). Predation by domestic cat may be the most plausible explanation for most rodent species to avoid rural and urban areas in this study (Piontek et al., 2021). Domestic cats activities are heavily reliant on human settlements (Ferreira et al., 2011). Low natural land cover alongside

with highly dense human settlement in urban areas in this study may increase predation risk by domestic cat in the study areas (Hong & Mohd-Azlan, 2018; Krauze-Gryz et al., 2017).

3.5 Conclusions

In conclusion, the distribution of rodent in different habitat types in Sarawak has been determined. Additionally, the habitat preference of rodent in Sarawak also been discovered. This study showed habitats alteration and interference results in changing the distribution of rodent and habitat preference of rodent species. The number of species decreased as habitat disturbance increased, and lesser habitat preference in places where habitat disturbance is greater. Highest rodent species diversity was recorded in forest areas. All native species were highly preferred forest areas while *Rattus* species mostly preferred for disturbed habitat. Most of natives species were habitat specialist therefore retention of forest habitat may benefit native and species of conservation importance that can perhaps regulate the population of commensal or invasive species. Further study to understand zoonotic disease prevalence among the rodent community should be considered to predict the risk of disease transmission to human.

CHAPTER 4

THE PREVALENCE AND HABITAT PREFERENCE OF PARASITE FROM RODENT IN DIFFERENT HABITAT TYPES IN SARAWAK

4.1 Introduction

Rodents are commonly known as important disease reservoir due to their ability to harbor zoonoses and greatly adapted to human dwelling and environmental transition (Chaisiri et al., 2012). The predominant factors contributing to rodent-borne diseases have been identified as parasitic infections, rather than bacterial and viral infections (Rabiee et al., 2018). Parasites in rodents may infect in various form such as blood, insect, acarine and helminth. The family Muridae is the most widely distributed and medically significant group of organisms (Rabiee et al., 2018), making it a great model for studying parasite ecology.

The study of parasite is relevant from an ecological, evolutionary, epidemiological, and conservationist standpoint (Morand et al., 2007). Parasites infection can act as indicator in forecasting disease transmission risk, status of conservation importance species and as bio-indicators to an environment condition (Altizer et al., 2003; Gómez & Nichols, 2013; Omalu et al., 2017). Parasite response towards different environmental may provide valuable information on ecosystem health (Marcogliese, 2005). Therefore, current parasite study is attempting to understand which factors, whether host or the environment, are the most appropriate predictors of parasite species prevalence.

More than 20% of parasitic infections are known contributor to global disease (Engels & Savioli, 2006). Urbanisation has been identified as a significant contributing factor to the emergence of these diseases (Connolly et al., 2021; Gubler, 2011; Wolf, 2016). The process of urbanisation has led to the efficient occupation of land by humans, animals,

and vectors, resulting in high population densities. This phenomenon has disrupted the balance of ecosystems (Wilcox & Gubler, 2005; Wu et al., 2017). As a result, it increase contact frequency and raising the risk of infection (Feng et al., 2022; Tong et al., 2015).

Therefore, it should be a wakeup call for Sarawak government and community since urban growth includes land use change in Sarawak has reached over 70% (Ali et al., 2021). Changes in the structure and composition of the environment can have a significant impact on the parasite's prevalence (Gillespie et al., 2005). Despite their relevance to the environment, parasites are frequently overlooked in the management and maintenance of biological resources and ecosystems (Marcogliese, 2004).

This chapter intended to determine the prevalence of parasites recovered from rodents in different habitat types Sarawak. Comparison in parasite prevalence also been made to see if there is a significance difference between different habitats types; forest, oil palm plantation, rural and urban areas. Furthermore, the habitat preference of parasites species also been illustrated and explained.

4.2 Materials and Methods

4.2.1 Study areas

The study areas have been mentioned and described in Chapter 3. There were eight sampling locations involved in this study consist of four different habitats namely forest, oil palm plantation, rural and urban areas.

4.2.2 Rodent trapping

Rodents trapping procedure were explained in Chapter 3. There were 241 individuals of rodents successfully caught during sampling occasions, five individuals were escape during handling for euthanisation. There were 236 individuals of rodents were euthanized

with 30% isoflurane and cervical dislocation following Carbone *et al.* (2012) and examined for parasites.

4.2.3 Parasitological analysis

4.2.3.1 Haemoparasites examination

Blood samples were collected following Protocol for Cardiac Puncture by Parasuraman *et al.* (2010) which 1ml syringe with 25G needle was used for the blood withdrawal from the heart. On separate blood slides, blood was immediately smeared for thin and thick smears and allowed to air dry. The thin smear was fix with methanol and air dried before staining. Remaining blood was immediately stored in a blood collection tube containing Ethylenediamine tetraacetic acid (EDTA) under 4 C°. Blood smears were stained following procedure of Quick Stain Giemsa by Brown *et al.* (1993). The smears were placed in undiluted Giemsa Stain for 1-2 minutes, then placed in deionised water for 2-4 minutes. After that, the smear was rinsed with deionised water to remove remaining stain. The smears were air dried before evaluated under 1000x magnification of compound microscope. Culleton (2005), Wellborn and Rogers (1970), Mathison *et al.* (2019) and some other illustrations were used for haemoparasite identification.

4.2.3.2 Ectoparasites examination

The rodent was placed on white tray and dust-ruffling method following Clayton and Drown (2001) was applied. Then, the host was combed to let the remaining ectoparasite fall on the white tray. The ectoparasites were collected from the tray using forceps and preserved in a vial containing 70% ethanol separately for each host. The collected ectoparasites were sort accordingly to their morphology under 40x of stereomicroscope in Parasitology Laboratory. The mounting process of ectoparasites were modified from Madinah *et al.* (2011) where listrophorid mites were not subjected to process of soak in lactic acid and not

been heated on 200°C prior to mounting in Hoyer's medium. Instead, all ectoparasites (excluding ticks) were prepared by soaking in a clearing agent, lactophenol solution at room temperature for a week (depending on their size) before mounting in Canada Balsam's medium. The mounted slides were incubated at 40 °C for a week to harden the mounting medium (Paramasvaran et al., 2009). Then, mounted ectoparasites were observed under a compound microscope and identified up to genera and species level as possible referring to Fritz and Pratt (1947), Kohls (1957), Strandtmann and Mitchell (1963), Nava et al. (2017) and some other available keys and illustrations.

4.2.3.3 Endoparasites examination

After collecting all ectoparasites and blood samples, the rodents were dissected to expose the gastrointestinal tract and other internal organs. The gastrointestinal tract was cut out and tie at the end of the stomach, small intestine and large intestine prevent mixing of the content. Body cavity and other organs also been observes for visible helminth parasites. The gastrointestinal tract was also preserved in 70% ethanol solution before cut opened to examine any visible parasite in the lab. The visible parasite that was found will be stored in 70% ethanol solution separately for the stomach, small intestine and large intestine. The intestinal contents were subjected to simple floatation using technique by Sheater (1923) and sedimentation technique by Lutz (1919) for parasite eggs or oocyst detections. The content were mixed with saturated sodium chloride (NaCl) solution and the suspension was poured into a test tube until upper meniscus. A cover slip was placed onto the mouth of the tube and left for five minutes. The cover slip were lifted off vertically up from the tube and immediately place on a microscope glass slide that has a mix of glycerol and methylene blue. The slide were examined under a compound microscope for the presence of nematode eggs and coccidian oocyst. For sedimentation method, the faecal samples were mixed with tab

water, then the suspension poured into a test tube through a filter paper to separate debris from the suspension and allowed to sediment for 5 minutes. The supernatant was discarded and the sediment was re-suspended for 5 minutes. A drop of sediment was mixed with a drop of methylene blue and glycerol on a microscope glass slide then covered with a cover slip before examining under a compound microscope for the detection of trematode eggs. Parasite identification was followed to Hartwich et al. (1974) illustration. The rodent specimens were deposited into Zoological Museum, Universiti Malaysia Sarawak.

4.2.4 Additional data on abiotic factors

Humidity and temperature were measure for each habitats using environmental meter device during sampling occasions. Readings were taken in the morning approximately nine o'clock. Humidity was presented in percent (%) meanwhile the temperature was taken in Fahrenheit (F).

4.2.5 Statistical analyses

An excel spreadsheet was used for data recorded. The parasites prevalence from different habitat types were calculated following equation below:

$$\text{Parasite prevalence} = \frac{\text{Number of rodents infected with parasite group}}{\text{A total of rodents individuals captured}} \times 100 \quad \text{Equation 4.1}$$

The mean differences in parasitic infection between habitats were compared using non-parametric tests, the Kruskal Wallis tests where the significance was defined as $P > 0.05$. The Kruskal Wallis was calculated using R Statistical Software version 4.0.4 (R Core Team, 2021).

The parasites' habitat preferences were assessed using Correspondence Analysis (CA) following (Kassambara, 2017). The CA defines similarity as the presence or absence

of parasite, as well as the frequency of infection (number of infected rodents by a particular parasites species). Correspondence analysis (CA) is an extension to Principle Components Analysis (PCA) that is used to analyse the relationships between qualitative variables and does not yield a specific hypothesis from it (Doey & Kurta, 2011; Kassambara, 2017). This analysis was performed using ‘FactoMineR’ and ‘factoextra’ packages in R Statistical Software version 4.0.4 (R Core Team, 2021).

4.3 Results

4.3.1 Prevalence of parasite in four different habitat types

A total of 41 species of parasite were recovered from rodent in this study belong to heamoparasites (six species), ectoparasites (21 species) and endoparasites (14 species). Noted that each parasites categories in this study were consist of several parasite types, haemoparasites (protozoa, bacteria and filarial), ectoparasites (ticks, mites, lice and chiggers) and endoparasites (cestodes and nematodes) (Table 4.1). Unfortunately, this study failed to record any fleas infested on captured rodent. Figure 4.2 shows common species of parasites recovered from this study.

A total of 218 (92.37%) rodents were found infected at least by one parasites. Seventy-two out of 236 (30.51%) individuals of rodents were infected by haemoparasites, 182(77.12%) individuals of rodent were infested by ectoparasites and 145(61.44%) individuals were infected by endoparasite (Table 4.1).

Forest areas had the highest prevalence of parasites (100%, n=23), followed by urban areas (97.40%, n=75), rural areas (89.23%, n=58), and oil palm plantations (87.32%, n=62). The highest prevalence of haemoparasite was seen in forest areas (65.22%, n=15), followed by oil palm plantations (33.80%, n=24), urban areas (33.77%, n=26), and rural areas

(10.77%, n=7). Ectoparasites showed a decreasing infection trend from forest areas to urban areas (86.96%, 80.28%, 75.38%, 72.73%). Meanwhile, the highest prevalence of endoparasites was found in urban areas (83.12%, n=64), followed by forest areas (65.22%, n=15), rural areas (58.46%, n=38), and oil palm plantations (39.44%, n=28) (Figure 4.1).

The haemoparasite, protozoa (26.09%) and bacteria (34.78%), the ectoparasite, ticks (56.52%), mites (82.61%) and lice (30.43%), as well as the endoparasite, cestodes (30.43%) recorded highest infection in forest areas. The filarial infection (15.49%) was highest in oil palm plantation. Chiggers (4.62%) infection was highest in rural areas. Meanwhile, nematodes (81.82%) infection was highest in urban areas. From habitat types viewpoint, all habitat were largely dominated by mites except for urban areas which is dominated by nematodes (Table 4.1).

Figure 4.2 shows the common parasites infected rodent in this study. All parasite infection has significant difference between habitat types ($p < 0.001$). Similar to result obtained for haemoparasites and endoparasites were significantly different between habitat types (both $p < 0.001$) (Figure 4.1). On the other hand, there was no significant difference between habitat types in ectoparasites infestation ($p = 0.307$).

The highest mean humidity was recorded in forest areas. Mean humidity shows decreasing pattern towards disturbed habitats (oil palm and rural) and slightly increase in urban areas. Meanwhile, mean temperature shows increasing trend towards habitat disturbance with urban areas recorded the highest mean temperature (Figure 4.3).

Table 4.1: Prevalence of parasite infection in four different habitat types.

Parasites species	Codes	Prevalence of parasite infection %				
		FA	OP	RA	UA	Total
		n=23	n=71	n=65	n=77	n=236
A. Haemoparasites						
Protozoa						
<i>Hepatocystis</i> sp.	H1	-	-	-	14.29	4.66
<i>Hepatozoon ophisauri</i>	H2	-	-	-	5.19	1.69
<i>Plasmodium</i> sp.	H3	-	9.86	7.69	3.90	6.36
<i>Trypanosoma</i> sp.	H4	26.09	11.27	1.54	-	6.36
Bacteria						
<i>Streptococcus</i> sp.	H5	34.78	2.82	1.54	5.19	6.36
Filaria						
<i>Brugia</i> sp.	H6	4.35	15.49	-	9.09	8.05
B. Ectoparasites						
Ticks						
<i>Amblyomma</i> sp.	T1	26.09	-	3.08	16.88	8.90
<i>Haemaphysalis</i> sp.	T2	-	12.68	1.54	20.78	11.02
<i>Ixodes granulatus</i>	T3	34.78	5.63	1.54	2.60	6.36
<i>Rhipicephalus</i> sp.	T4	4.35	-	-	-	0.42
Mites						
<i>Listrophoroides</i> sp.	M5	43.48	12.68	4.62	23.38	16.95
<i>Laelaps</i> spp.	M14	21.74	8.45	18.46	3.90	11.02

Table 4.1 continued

<i>Laelaps aingworthae</i>	M6	8.70	-	-	-	0.85
<i>Laelaps echidninus</i>	M7	21.74	19.72	41.54	18.18	25.42
<i>Laelaps insignis</i>	M8	13.04	-	-	-	1.27
<i>Laelaps mercedae</i>	M9	13.04	-	-	-	1.27
<i>Laelaps nuttalli</i>	M10	8.70	49.30	26.15	22.08	30.08
<i>Laelaps sanguisugus</i>	M11	8.70	-	-	-	0.85
<i>Laelaps sculpturatus</i>	M12	13.04	-	-	-	1.27
<i>Laelaps sedlaceki</i>	M13	43.48	8.45	10.77	50.65	26.27
<i>Laelaps turkestanicus</i>	M15	17.39	22.54	4.62	15.58	14.83
<i>Longolaelaps</i> sp.	M16	4.35	-	-	-	0.42
Chiggers						
<i>Ascoschoengastia</i> sp.	C17	-	-	1.54	-	0.42
<i>Leptotrombidium</i> sp.	C18	-	4.23	3.08	-	2.12
Lice						
<i>Hoplopleura</i> sp.	L20	30.43	11.27	13.85	18.18	16.10
<i>Polyplax spinulosa</i>	L21	-	1.41	10.77	7.79	5.93
<i>Sathrax</i> sp,	L22	-	4.23	-	5.19	2.97
C. Endoparasites						
Cestodes						
<i>Hymenolepis diminuta</i>	C1	17.39	-	1.54	5.19	3.81
<i>Hymenolepis nana</i>	C2	13.04	1.41	29.23	14.29	14.41
<i>Taenia taeniaformis</i>	C3	-	1.41	9.23	3.90	4.24

Table 4.1 continued

Nematodes						
<i>Capillaria</i> sp.	N4	-	-	-	9.09	2.97
<i>Gongylonema</i>	N5					
<i>neoplasticum</i>		8.70	36.62	7.69	9.09	16.95
<i>Hepatojarakus malayae</i>	N6	-	-	-	3.90	1.27
<i>Heterakis</i> sp.	N7	-	-	34.21	6.25	11.72
<i>Heterakis spumosa</i>	N8	-	-	3.08	-	0.85
<i>Mastophorus muris</i>	N9	8.70	-	23.08	-	7.20
<i>Strongyloides</i> sp.	N10	13.04	1.41	-	57.14	20.34
<i>Syphacia</i> sp.	N11	4.35	-	-	-	0.76
<i>Syphacia muris</i>	N12	8.70	-	1.54	-	1.27
<i>Toxocara</i> sp.	N13	4.35	-	-	-	0.42
<i>Trichuris muris</i>	N14	4.35	-	-	27.27	9.32
A. Haemoparasites						
Total protozoa infection		26.09	19.72	9.23	20.78	17.80
Total bacterial infection		34.78	2.82	1.54	5.19	6.36
Total filarial infection		4.35	15.49	-	9.09	8.05
Total haemoparasite infection		65.22	33.80	10.77	33.77	30.51

Table 4.1 continued

B. Ectoparasites					
Total tick infestation	56.52	16.90	6.15	31.17	22.46
Total mite infestation	82.61	76.06	70.77	67.53	72.46
Total chigger infestation	-	4.23	4.62	-	2.54
Total lice infestation	30.43	12.68	21.54	25.97	21.19
Total ectoparasite infestation	86.96	80.28	75.38	72.73	77.12
C. Endoparasites					
Total cestodes infection	30.43	2.82	32.31	20.78	19.49
Total nematodes infection	47.83	36.62	49.23	81.82	55.93
Total endoparasites infection	65.22	39.44	58.46	83.12	61.44
Overall parasites infection	100.00 (23)	88.73 (63)	89.23 (58)	97.40 (75)	92.37 (218)

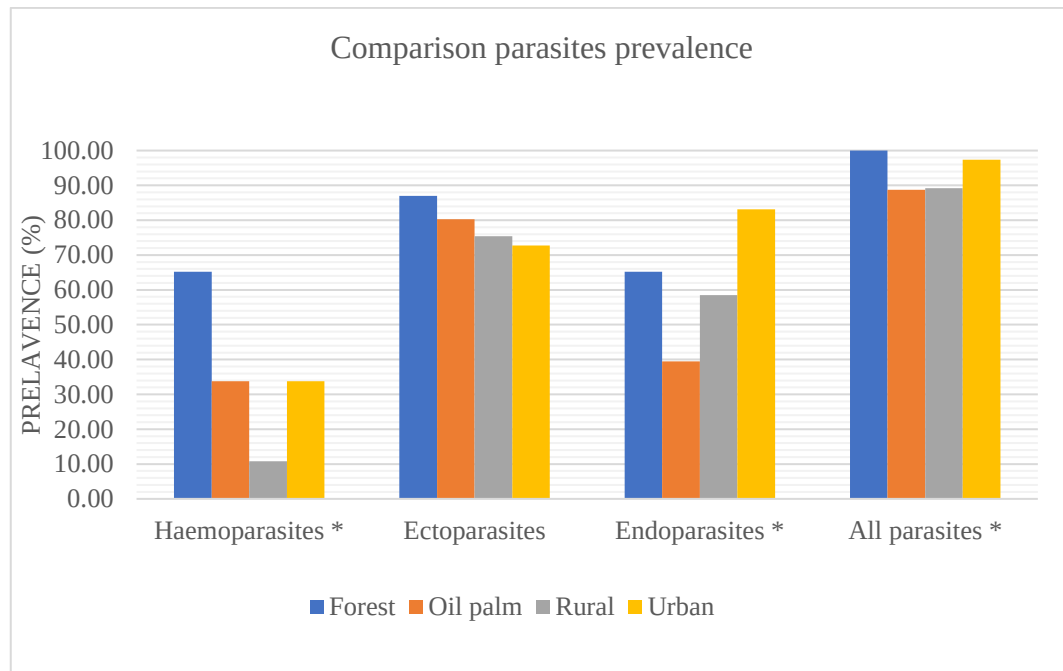


Figure 4.1: Comparison of parasites prevalence in four different habitat types; forest, oil palm plantation, rural and urban areas. * indicates significant difference between habitats (Kruskal Wallis $p < 0.05$).

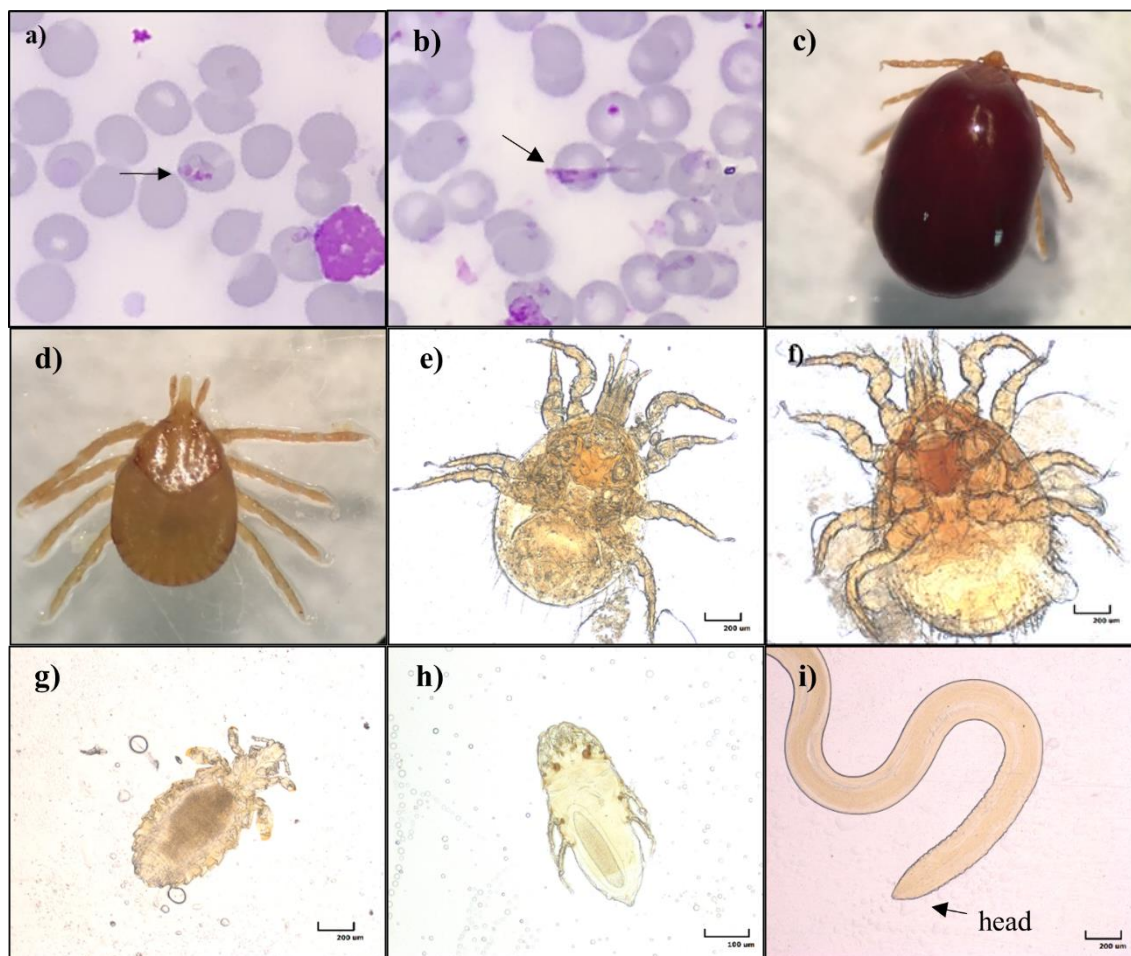


Figure 4.2: Common parasites found in four different habitat type in Sarawak. a-b are haemoparasites, c-h are ectoparasites and i are endoparasites. a) *Plasmodium* sp., b) *Trypanosoma* sp., c) *Haemaphysalis* sp. d) *Amblyomma* sp. e) *Laelaps echidninus*, f) *Laelaps sedlaceki*, g) *Hoplopleura* sp., h) *Listrophoroides* sp., and i) *Gongylonema neoplasticum*.

Additional data on abiotic factors in four different habitats

Since parasites infections were influence by environmental factors, this additional data maybe crucial for parasite infection analysis. Collecting abiotic data at different times may affect the readings since temperature and humidity fluctuate throughout the day due to sunlight exposure. Therefore, abiotic data were collected at the same time (9 a.m.) in each sampling location to standardise the data collection. Figure 4.3 shows humidity declined from forest to rural areas and slightly increase in urban area. Forest areas recorded highest

mean humidity meanwhile, rural areas recorded the lowest mean humidity in this study. The mean temperature inclined towards habitat disturbance. Urban areas recorded the highest mean temperature meanwhile, forest area recorded the lowest mean temperature.

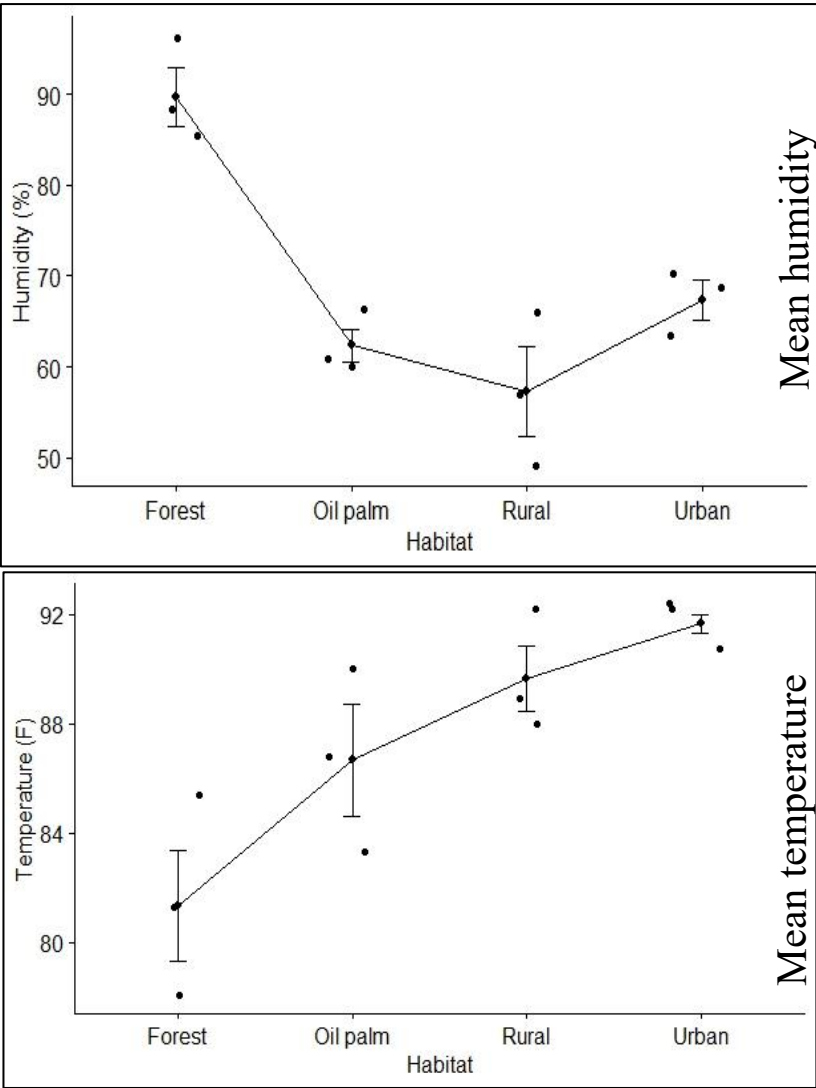


Figure 4.3: Mean humidity and mean temperature obtained in four habitats; forest, oil palm, rural and urban areas.

4.3.2 Habitat preference of parasites

The $\cos^2$ (quality of representation) measures the degree of association between parasites and the dimensions. The parasites are well represented as $\cos^2$ close to one. The close proximity between points (by angle from the origin) in CA plot implies that the parasite species have a similarity in habitat preferences (Figure 4.4). $\cos^2$ represents the strength of parasite association with habitats. The data on parasite infection in each habitat were used to compute $\cos^2$.

Based on Figure 4.4, most parasites shows their preference to disturbed habitat. Certain parasites, such as bacteria and ticks, continue to exhibit a preference for forested area. Haemoparasites show clear habitat partitioning where *Hepatocystis* sp. (H1) and *Hepatozoon ophisauri* (H2), found in urban areas meanwhile *Plasmodium* sp. (H3) found in oil palm plantations and rural areas. *Trypanosoma* sp. (H4) and *Streptococcus* sp. (H5) shows preference to forest areas. and *Brugia* sp. (H6) seems has low degree of association to any habitats. Most ectoparasites shows preference to disturbed habitat with some of them remained to prefer forest areas such as *Ixodes granulatus* (T3), *Rhipicephalus* sp. (T4), *Laelaps aingworthae* (M7), *Laelaps insignis* (M9), *Laelaps mercedae* (M10), *Laelaps sanguisugus* (M12), *Laelaps sculpturatus* (M13), *Longolaelaps* sp. (M16). As for endoparasites, five species have been identified preferred for urban areas namely *Capilaria* sp. (N4), *Hepatojarakus malayae* (N6), *Strongyloides* sp. (N10) and *Trichuris muris* (N14). One species preferred for oil palm plantation was *Gongylonema neoplasticum* (N5) and others were preferred for rural or forest or both.

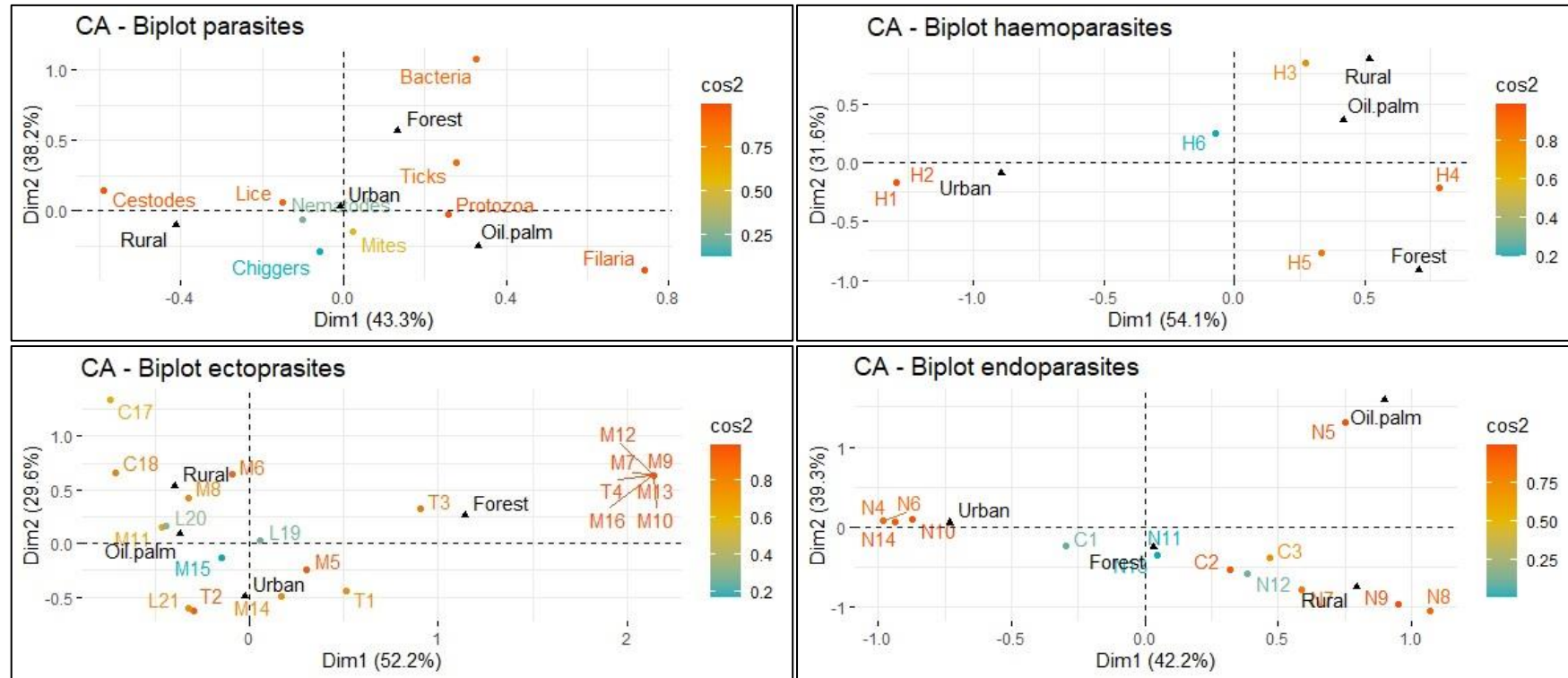


Figure 4.4: Correspondence analysis shows the habitat preferences of parasite species of rodent in Sarawak. H1 to H6 represented haemoparasites species, T1 to T4 represented ticks species, M4 to M16 represented mite species, C17 to C18 represented chigger species, L19-L21 represented lice species, C1 to C3 represented cestodes species, and N4 to N14 represented nematodes species (refer Table 4.1).

4.4 Discussions

The ongoing occurrence of habitat change has raised concerns on the implications of transmission and infectious diseases. Understanding and measuring parasites and their hosts respond to environmental changes has thus become critical (Chasar et al., 2009). A few studies on parasites of small mammals have been conducted in Sarawak but failed to find any literature that includes all parasites (haemoparasite, ectoparasite and endoparasite) into a research discussion, mostly were focusing on one parasites category. This study tried to cover infection of parasites which includes haemo, ecto, and endoparasite of rodent in Sarawak. Three major groups of haemoparasites (protozoa, bacteria and filaria), four of ectoparasites (ticks, mites, chiggers and lice) and two of endoparasites (nematodes and cestodes) found in this study were previously reported infecting rodent species in other parts Malaysia such as Selangor, Kuala Lumpur, Melaka, Pahang, Penang, Perak, Kelantan, Negeri Sembilan and more (Adrus et al., 2011; Ask & Mohd Zain, 2006; Ibrahim, 2020; Kamil et al., 2021; Mariana et al., 2005; Mohd Zain et al., 2012; Mustapha et al., 2019; Ng et al., 2017; Nursyazana et al., 2013; Paramasvaran et al., 2009; Premaalatha et al., 2018; Razali et al., 2018; Siti Norliyanti, 2017; Tijjani et al., 2020; Wells et al., 2013) and some of them were recorded worldwide (Archer et al., 2017; Bonfim, 2001; Pakdel et al., 2013; Seifollahi et al., 2016; Sumangali et al., 2007). Therefore, all of the identified parasites discovered in this study were commonly infected in rodents and other animals. Some of them are among the zoonotic importance parasites such as *Plasmodium* sp., *Trypanosoma* sp., *Ixodes granulatus*, *Leptotrombidium* sp., *Cysticercus fasciolaris*, *Hymenolepis* spp., *Capillaria* sp., and *Toxocara* sp. with reported cases infecting human (Hussin et al., 2020; Joshi et al., 2005; Macpherson, 2013; Oryan & Alidadi, 2015; Santibáñez et al., 2015; Sharma et al., 2017).

Overall parasites prevalence were significantly difference between habitats. On that account, the determination of parasite prevalence to a smaller scale (haemo, ecto and endoparasites) was made in accessing the risk of disease transmission. Haemoparasites were relatively low prevalence (30.51%) among parasites categories in this study, which is consistent with previous studies that found low prevalence of haemoparasites in rodent from Malaysia (Premaalatha et al., 2018; Shafiyyah et al., 2012). Other authors, on the other hand, have observed varying or even contradictory results with high prevalence of the haemoparasite (Kamil et al., 2021; Paramasvaran et al., 2003; Premaalatha et al., 2010). The low prevalence of haemoparasites could be driven by the absence of insect vector. This case was referring to *Trypanosoma* sp. which the infection was always linked to flea insect vector. This study failed to find any fleas species infested on rodent in all habitat types which may lower the probability of *Trypanosoma* sp. transmission among the rodent. In other haemoparasite studies, *Trypanosoma lewisi* is exclusively found in rodent and fortunately it is not pathogenic to human (Lin et al., 2015). *T. lewisi* even unable to undergo development in mice (Verma et al., 2011). The presence of parasites in the blood is sporadic and the development maybe depending on the strain of the rodent (Verma et al., 2011). The morphology identification seems insufficient to identify haemoparasites to species level. Therefore, molecular identification is recommended for parasite identification in order to verify species identification and hence assess the potential risk of the parasite infection to humans.

Despite of the low prevalence of haemoparasite infecting rodent in this study, there are significantly different in the haemoparasite prevalence between habitat types. Forest areas show the highest prevalence of haemoparasite compared to other habitats. This high prevalence was largely contributed by the high prevalence of bacteria, *Streptococcus* sp.

Infection can occur in respiratory tract, blood stream or skin infection (Rohde & Cleary, 2016), through direct contact, aerosolisation or on fomites and it also can be transmitted by asymptomatic carrier (O'Rourke, 2004). The high prevalence of this bacteria in forest areas could not be concluded since it should also be well distributed to other habitat due to its spontaneous infection. However, temperature and humidity in the forest areas maybe is at optimum condition for this species (Cruz-Paredes et al., 2021; Hoeksma et al., 2015).

Ectoparasites infestation recorded considerably high in this study, and other similar studies were reported even lower and higher ectoparasite infestation of rodent in different habitat (Obiegala et al., 2021; Paramasvaran et al., 2009). This difference may due to set of habitat types involves in those studies are different. Therefore, habitats may influence the parasite infestation. Most ectoparasites have direct transmission modes and so have a higher transmission efficiency (Barrientos et al., 2014). However, there was no significant difference found in the ectoparasite prevalence between habitat types in this study. This suggest that most ectoparasites was host-specific or habitat generalist.

Nonetheless, there is still some ectoparasites population response to surrounding habitat. Even though the decreasing of prevalence pattern from forest to urban areas in this study was contradict to other ectoparasites studies that shows infection rate increase with habitat disturbance (Madinah et al., 2014), result from this study might explain some insight of why ectoparasite still shows little differences between habitats. As tick, chiggers and fleas were not considerably host-specific parasites (Maaz et al., 2018), they rely on habitat environment to survive, reproduce and disperse. The high temperatures and low humidity in disturbed areas (subjected to urban followed by rural and oil palm plantation) may decelerate reproduction and development of ectoparasites, as well as increasing adult mortality, especially in the case of temporary parasites (Maaz et al., 2018).

In the case of tick, *Ixodes* species prevalence were influenced by relative humidity of habitat surrounding (Berger et al., 2014; Kiffner et al., 2011). *Ixodes* species are inactive at relative humidity levels below 70%, therefore they require high relative humidity for development and dispersal (Kiffner et al., 2011). The influence of relative humidity appears to be convincing, since the prevalence of *Ixodes granulatus* decreased with lower relative humidity in oil palm plantations and rural areas, and increased somewhat with higher relative humidity in urban areas in this study. This conclusion contrasts with findings from other research that found the higher *Ixodes* spp. population in drier environment (Boyard et al., 2008; Kiffner et al., 2011). *Ixodes granulatus* typically found in forested area and dwells on the forest floor (Masuzawa et al, 2004). High relative humidity in forest areas in this may be caused by vegetation cover, which is generally positively related to relative humidity on the forest floor (Kiffner et al., 2011), affecting *Ixodes granulatus* prevalence.

As for chigger, previous literature mentioned that they are habitat-dependent compare to host-dependent, owing to their reliance on habitat choices (Maaz et al., 2018). Chiggers survive and thrive well at high relative humidity levels (>50%) and has been proven by the increase in scrub typhus transmission with humidity (Li et al., 2014; Rubio & Simonetti, 2009; Sun et al., 2017; Wei et al., 2017). However, rodent host in forest and urban areas were free from chigger infestation. The absent of chigger in urban areas in this study is inconclusive since high temperature, humidity and abundance of host (rodent) should support the transmission and reproduction of chiggers (Kuo et al., 2011). Therefore, further research is essential to includes more habitat parameters in assessing their prevalence.

Similar to ticks and chiggers, fleas were also influenced by surrounding humidity and temperature, but there is disparities of result on favourable condition for flea (Maaz et al., 2018; Tran et al., 2021). Unfortunately, this study unable to determine habitat preference

of flea since no flea infestation on rodent detected in any habitat in this study. Fleas are haematophagous parasites that alternate between feeding on their hosts' bodies and hiding in their burrows or nests (Hawlena et al., 2006). The low occurrence of fleas on Southeast Asian rat may imposed by the warm and humid climate in Southeast Asia, and unfavourable conditions for fleas in underground rat burrows could be the factors (Wells et al., 2011). Burrow sometimes located close to stream and wet floor inside the burrow maybe not suitable for flea development (Wells et al., 2006).

Parasites have a strong bond with not only their hosts, but also with their surroundings, therefore habitat disturbance is likely to have an impact on parasite infestation levels (Froeschke et al., 2013). Endoparasite infection rely on factors including host diet, host local aggregation and intermediate hosts (Bordes et al., 2007; Vicente et al., 2006). In this case, the existence of open waste disposal areas in residential areas, commercial premises, and wet markets serve as foraging site or the main route for foraging activities for the rodent in urban areas as a number of traps were successful to catch rodent in those areas. There are also a number of studies have reported the same high prevalence of endoparasite in dumpsite area (Ogolla et al., 2019; Premaalatha et al., 2017). This study suggest poor quality food resource from the dumpsite may increase transmission of endoparasite among rodents. Evidence from other study have shown dietary deficits induced by a low food resources increase the adverse effects of helminth parasitism by compromising the immune response (Ezenwa, 2004; Santicchia et al., 2015). This may be explained the high prevalence of endoparasite infecting rodent in urban area.

Endoparasites infection is further influenced by the intermediate hosts. *Hymenolepis diminuta*, *Gongylonema neoplasticum*, *Heterakis spumosa*, *Heterakis* sp., and *Mastophorus muris* are among endoparasites that rely on intermediate host. Abundance of intermediate

hosts may increase the transmission rate of parasite infection (Stien et al., 2010). Certain endoparasite species utilises a variety of invertebrates as intermediate hosts such as insect, earthworm, and snails, with egg ingestion resulting in growth and development in the gastrointestinal or body cavity of a rodent host (Wharton, 2002). Considering that, the prevalence of these type of endoparasites maybe varied with different habitat depending on their vector host abundance and ecology.

The habitat preference pattern of parasites can be seen via CA illustrations. There are two possibility of low representation of parasites (Cos2 with blue colour) in CA all map, they maybe too low in number of infection, or they are a habitat generalist. While, others (Parasites with other than blue coloured Cos2) are well represented their preference towards habitat types. Most parasite preferred to disturbed environments (oil palm, rural, urban areas) are an evidence that urbanization does increase risk disease transmission. *Plasmodium* sp. was found infected *Rattus tanezumi*, *R. tiomanicus* and *Sundamys muelleri*, as well as preferred for oil palm plantatiton and rural areas. The fact that this genus is responsible for transmitting malaria disease (Sato, 2021), thus create an alert for the risk of infection in oil palm plantatiton and rural areas. Based on malaria incidence and profile in Malaysia, malaria is common in Sabah and Sarawak (77.7%), and Sarawak has reported the highest average incidence rate (0.42/1000) within 2013-2017 (Hussin et al., 2020). Therefore, it is a risk alert to oil palm plantation workers and rural resident community in Sarawak since this study is an evidence of *Plasmodium* sp. preferred for oil palm and rural areas as these areas may provide diversity of habitat for their insect vector as a result from poor sanitation activities.

Despite of the large proportion of ectoparasites species preference for disturbed habitat (oil palm, rural, urban areas), there is one ectoparasite that need to be highlighted due to their potential medical risk. The *Leptotrombidium* sp. was found on *Rattus tanezumi* and

Rattus tiomanicus, and as well as for rural and oil palm plantation. This scrub typhus vector can transmit disease via biting (Santibáñez et al., 2014). Scrub typhus has been identified as a public health issue in Southeast Asia's rural areas (Tay et al., 2013). Furthermore, scrub typhus cases also has been reported in Malaysia infected the oil palm worker (Yuhana et al., 2019). This means rural community as well as oil palm labourer in Sarawak are expose to the risk of potential scrub typhus disease. Education on disease associated with *Leptotrombidium* sp. should be expose to them so that they are able to seek an earlier treatment if they were infected through chiggers bites.

Most of the endoparasites discovered in this study are classed as soil transmitted helminth where they require soil as a medium for their development and continued their life cycle. *Capillaria* sp., *Hepatojarakus malayae*, *Strongyloides* sp., *Trichuris muris*, *Heterakis spumosa*, *Heterakis* sp., *Hymenolepis nana* are among soil transmitted helminth (Adu-Gyasi et al., 2018; Fernández-Soto et al., 2016; Franssen et al., 2016; Steinbaum et al., 2017; Xie et al., 2018) that observed to be preferred for urban and rural areas. They can be transmitted either through their larval forms penetrating the skin or through consumption of contaminated foods and drinks (Strunz et al., 2014). Infected rodent may passed the eggs through feces. As rodent foraging and left the dropping in area with human activities, transmission of the helminth can occur to human through skin penetration or food contamination. As a result, they are growing more prevalent and even spreading diseases. Some of them are non-pathogenic and some of those mentioned parasites have been reported for disease infections such as Strongyloidiasis, Hymenolepiasis, Trichuriasis and Capillariasis in Malaysia and overseas (Boulware et al., 2007; Kandi et al., 2019a; Tee et al., 2013; Vasantha et al., 2012). Therefore, sanitisation are important especially in human

dwelling areas to minimise risk of disease transmission since medically important parasites have shown their preference to habitat associated human activities.

4.5 Conclusions

There is significant difference in overall parasite prevalence between the four habitat types in Sarawak. There is no significant difference in the prevalence of ectoparasites among the four habitat types in Sarawak. However, there is a significant difference in the prevalence of haemoparasites and endoparasites among the four habitat categories in Sarawak. The habitat preferences of rodent's parasites in Sarawak was determined and illustrated using an analysis. Most parasites preferred disturbed habitat (oil palm, rural, and urban). Some of them were medical concern parasites such as *Plasmodium* sp., *Trypanosoma* sp., *Ixodes granulatus*, and *Leptotrombidium* sp.

Further research are recommended for deeper discovery on the health risks assesment of rodents' parasite infections in Sarawak. Molecular identification of parasites and detection of diseases from the parasites as well as adding more detailed habitat parameters may help for future research. Information from this study may contribute to knowledge in rodents' parasite infection and their habitat preference in Sarawak.

CHAPTER 5

THE HOST-PARASITES RELATIONSHIP BETWEEN RODENT AND THEIR PARASITES IN SARAWAK

5.1 Introduction

Due to parasite's diversity and reliance on other species, they are technically found in every ecosystem (Dobson et al., 2008; Wood & Johnson, 2015). Parasites may put substantial pressure on their host selection and population may vary regardless of the number of hosts present. Parasites may also be missing or restricted in a host populations as a result of various factor such as competitive interactions and host immunological responses, which prevent specific parasite species from co-existing (Kołodziej-Sobocińska, 2019; Rovenolt & Tate, 2022). These emphasise the significance of addressing host–parasite interactions at local and regional settings.

Availability of host species were contribute to parasite host specificity, refers to a parasite's proclivity to infect range of host species, and it is the result of parasite and host lineages co-existence (Seneviratne et al., 2009). It is a critical indicator of a parasite's ability to shift hosts and cause disease onset (Wells & Clark, 2019). Parasites that can infect several host species have a big role in the range of infectious diseases that impact human and animals. It was estimated 70% of human parasites are of zoonotic multi-host parasites, rather than parasites that exclusively infect single host species (Wells & Clark, 2019).

Host-parasite relationship considered barely explored in this region. Most relationship between rodents and parasites were related to ectoparasites (Madinah et al., 2014; Blasdell et al., 2022). This study try to cover a larger subjects which include haemoparasite, ectoparasites, and endoparasites. Therefore, the objectives of this study are

to determine the prevalence of parasites species based on host species and determine the host-parasite relationship in Sarawak through relationship congruence and host specificity.

5.2 Materials and Methods

5.2.1 Statistical analyses

Rodent trapping and parasite examination have been described in Chapter 3 and Chapter 4. Both data were arranged in excel database. The parasites prevalence of parasites infections from different host species were calculated in percentage.

The ‘ape’ and ‘phytools’ packages were used to construct a tanglegram of host-parasite associations. The host and parasite tree topologies were created using their higher classification and morphology binary data (e.g., brown-bodied, ten nipples, six legs, etc.). 0 indicates absence, and 1 indicates presence. A binary data table was then run in the mentioned R packages to construct tree topologies for rodents and parasites. The trees were sorted based on their distance matrix. Then, the parasite and host were linked by line projection using the parasite's infection binary matrix. 0 indicates not infected, and 1 indicates infected. The line was only projected to the infected host. Procrustes cophylogenetic analysis were performed to determine the phylogenetic congruence (cophylogeny) in the hosts and parasites relationship (Balbuena et al., 2013) with the aid of ‘paco’ package. Goodness-of-fit statistic was tested using the residual sum of squares (m^2_{XY}) under 1000 permutations to test the congruence of host-parasite associations (Balbuena et al., 2013). The parasite phylogeny significantly depends on the host phylogeny if p value is smaller than 0.05. At the same time, squared residuals were generated. These squared residuals were extracted to analyse the contribution of each host-parasite link to the global congruence.

Specialisation index (H2) were computed in four separated categories consist of overall, haemoparasite, ectoparasites and endoparasites infections. Then, Paired Difference Index (PDI) was chosen to measure the specificity of parasite on species level towards the rodent host in this case. It takes into account that the performances might have a variety of statistical distributions in which no measure of variability was applied equally to the data sets (Poisot et al., 2011) and it displayed higher robustness to sampling error and most informative (Poisot et al., 2012). The highest output value of complete 1 is considered as a specialist, reduction of the value indicates reduced in specificity towards host selection. These indices were computed using 'bipartite' package (version 2.16) in R Statistical Software version 4.0.4 (R Core Team, 2021).

5.3 Results

5.3.1 Prevalence of parasites by host species

A total of 100% parasite prevalence were recorded by all rodent except for *Rattus* species, *R. tanezumi* (89.26%), *R. exulans* (91.67%) and *R. tiomanicus* (96.77%). *Niviventer cremoriventer* recorded 100% prevalence for all three parasites categories. *Maxomys whiteheadi* recorded zero haemoparasite infections and 100% ectoparasites infestation. Meanwhile, *Leopoldamys sabanus* recorded lowest ectoparasites infections (66.67%) and highest endoparasites infections (100%).

The highest prevalence among all parasites species were recorded by *Laelaps nuttali* (30.08%) followed by *Laelaps sedlaceki* (26.69%), and *Laelaps echidninus* (25.42%). Meanwhile, *Rhipicephalus* sp., *Longolaelaps whartoni*, *Ascoschoengastia* sp., and *Toxocara* sp. (0.42%) recorded the lowest prevalence among all parasites

Table 5.1: Prevalence of parasites infected different species of rodent host.

Prevalence %		Host species								
Parasite species	Codes	<i>Leopoldamys sabanus</i>	<i>Maxomys rajab</i>	<i>Maxomys whiteheadi</i>	<i>Niviventer cremoriventer</i>	<i>Rattus exulans</i>	<i>Rattus tanezumi</i>	<i>Rattus tiomanicus</i>	<i>Sundamys muelleri</i>	Total
A. HAEMOPARASITES										
Protozoa										
<i>Hepatocystis</i> sp.	Hepat	-	-	-	-	-	7.38%	-	-	4.66%
<i>Hepatozoon ophisauri</i>	Heoph	-	-	-	-	-	2.68%	-	-	1.69%
<i>Plasmodium</i> sp.	Plasm	-	-	-	-	-	6.71%	6.45%	9.09%	6.36%
<i>Trypanosoma</i> sp.	Trypa	33.33%	33.33%	-	-	25.00%	2.68%	16.13%	3.03%	6.36%

Table 5.1 continued

Bacteria										
<i>Streptococcus</i> spp.	Strep	-	-	-	100.00%	8.33%	3.36%	3.23%	21.21%	6.36%
Nematode										
<i>Brugia</i> sp.	Brugi	33.33%	-	-	-	-	3.36%	22.58%	18.18%	8.05%
B. ECTOPARASITES										
Ticks										
<i>Amblyomma</i> sp.	Ambly	-	-	25.00%	100.00%	-	3.36%	-	42.42%	8.90%
<i>Haemaphysalis</i> sp.	Haema	-	-	-	-	25.00%	6.71%	3.23%	36.36%	11.02%
<i>Ixodes granulatus</i>	Ixgra	33.33%	33.33%	25.00%	100.00%	8.33%	4.03%	-	12.12%	6.36%
<i>Rhipicephalus</i> sp.	Rhipi	-	-	-	-	-	-	-	3.03%	0.42%

Table 5.1 continued

Mites										
<i>Listrophoroides</i> sp.	Listro	33.33%	33.33%	25.00%	100.00%	16.67%	8.05%	9.68%	57.58%	16.95%
<i>Laelaps</i> spp.	Laela	66.67%	-	50.00%	-	16.67%	4.70%	9.68%	12.12%	8.47%
<i>Laelaps aingworthae</i>	Laain	-	66.67%	-	-	-	-	-	-	0.85%
<i>Laelaps echidninus</i>	Laech	-	66.67%	25.00%	-	25.00%	24.16%	22.58%	33.33%	25.42%
<i>Laelaps insignis</i>	Lains	-	66.67%	25.00%	-	-	-	-	-	1.27%
<i>Laelaps mercedae</i>	Lamer	-	66.67%	25.00%	-	-	-	-	-	1.27%
<i>Laelaps nuttalli</i>	Lanut	-	-	-	-	58.33%	22.15%	61.29%	36.36%	30.08%
<i>Laelaps sanguisugus</i>	Lasan	-	66.67%	-	-	-	-	-	-	0.85%
<i>Laelaps sculpturatus</i>	Lascu	-	33.33%	50.00%	-	-	-	-	-	1.27%

Table 5.1 continued

<i>Laelaps sedlaceki</i>	Lased	-	33.33%	-	100.00%	8.33%	22.82%	16.13%	63.64%	26.69%
<i>Laelaps turkestanicus</i>	Latur	-	33.33%	25.00%	100.00%	8.33%	10.74%	12.90%	33.33%	14.83%
<i>Longolaelaps</i> sp.	Longo	-	-	25.00%	-	-	-	-	-	0.42%
Chigger										
<i>Ascoschoengastia</i> sp.	Ascos	-	-	-	-	-	0.67%	-	-	0.42%
<i>Leptotrombidium</i> sp.	Lepto	-	-	-	-	-	0.67%	6.45%	-	1.27%
Lice										
<i>Hoplopleura</i> sp.	Hoplo	-	-	25.00%	-	-	14.77%	12.90%	33.33%	16.10%
<i>Polyplax spinulosa</i>	Pospi	-	-	-	-	-	9.40%	-	-	5.93%
<i>Sathrax</i> sp,	Sathr	-	-	-	-	-	2.68%	-	9.09%	2.97%

Table 5.1 continued

C. ENDOPARASITES

Cestode

<i>Hymenolepis diminuta</i>	Hydim	66.67%	-	-	-	-	0.67%	-	18.18%	3.81%
<i>Hymenolepis nana</i>	Hynan	33.33%	-	50.00%	-	8.33%	19.46%	3.23%	-	14.41%
<i>Taenia taeniaformis</i>	Tatae	-	-	-	-	-	2.68%	9.68%	9.09%	4.24%

Nematode

<i>Capilaria</i> sp.	Capil	-	-	-	-	-	0.67%	-	18.18%	2.97%
<i>Gongylonema neoplasticum</i>	Goneo	-	-	25.00%	-	16.67%	14.09%	29.03%	21.21%	16.95%
<i>Hepatojarakus malayae</i>	Hemal	-	-	-	-	-	-	-	9.09%	1.27%
<i>Heterakis</i> sp.	Heter	-	-	-	-	-	10.07%	-	6.06%	7.20%

Table 5.1 continued

<i>Heterakis spumosa</i>	Hespu	-	-	-	-	-	1.34%	-	-	0.85%
<i>Mastophorus muris</i>	Masmu	33.33%	-	-	-	-	8.05%	9.68%	3.03%	7.20%
<i>Strongyloides</i> spp.	Stron	33.33%	33.33%	-	-	-	24.83%	-	27.27%	20.34%
<i>Syphacia</i> sp.	Sypha	-	-	-	-	-	0.67%	-	-	0.42%
<i>Syphacia muris</i>	Sypmu	-	-	-	100.00%	8.33%	-	3.23%	-	1.27%
<i>Toxocara</i> sp.	Toxoc	-	33.33%	-	-	-	-	-	-	0.42%
<i>Trichuris muris</i>	Trimu	-	-	-	-	-	7.38%	-	33.33%	9.32%
Haemoparasites infection		66.67%	33.33%	-	100.00%	33.33%	24.16%	41.94%	45.45%	30.51%
Ectoparasites infestation		66.67%	100.00%	100.00%	100.00%	75.00%	69.13%	90.32%	96.97%	77.12%
Endoparasites infection		100.00%	33.33%	75.00%	100.00%	33.33%	59.73%	45.16%	90.91%	61.44%
All parasites infection		100.00%	100.00%	100.00%	100.00%	91.67%	89.26%	96.77%	100.00%	92.37%

5.3.2 Rodents-Parasites Interaction network

This study discovered 143 interactions between 41 parasites species and eight Murid rodents (Figure 5.1). The blue lines indicate links between parasite and rodent associations. Many linkages connected to a rodent species illustrate multiple parasite species infections. Sixteen parasites species were found interacted with only one host species. Goodness-of-fit indicates ($m^2_{XY}=286.61$, $p= 0.001$, $nperm=1000$) significant congruence between the host and parasites distance matrices as analysed with *paco*, which means proportion of closely related parasites species was depends on closely related host species.

5.3.3 Specialization and specificity

Bipartite analysis shows $H2=0.25$, indicating low specialisation of overall parasite species infections. Bipartite analysis also was run for haemoparasites, ectoparasites and endoparasites, omitted $H2$ values of 0.25, 0.18, and 0.29 respectively (Table 5.2), indicating low level of specialisation.

The higher the specificity index value (PDI, $max=1$) indicate the high specificity of parasites to their host. Two species of haemoparasite, six species of ectoparasites (includes tick, mites and louse) and four species of endoparasites (all nematodes) were found interacted with only one host species (all $PDI=1.00$) which considered as specialist (Table 5.3 and Table 5.1). They are *Hepatocystis* sp., *Hepatozoon ophisauri*, *Rhipicephalus* sp., *Laelaps aingworthae*, *Laelaps sanguisugus*, *Longolaelaps* sp., *Ascoschoengastia* sp., *Polyplax spinulosa*, as well as *Hepatojarakus malayae*, *Heterakis spumosa*, *Syphacia*.sp. and *Toxocara* sp. (Table 5.3)

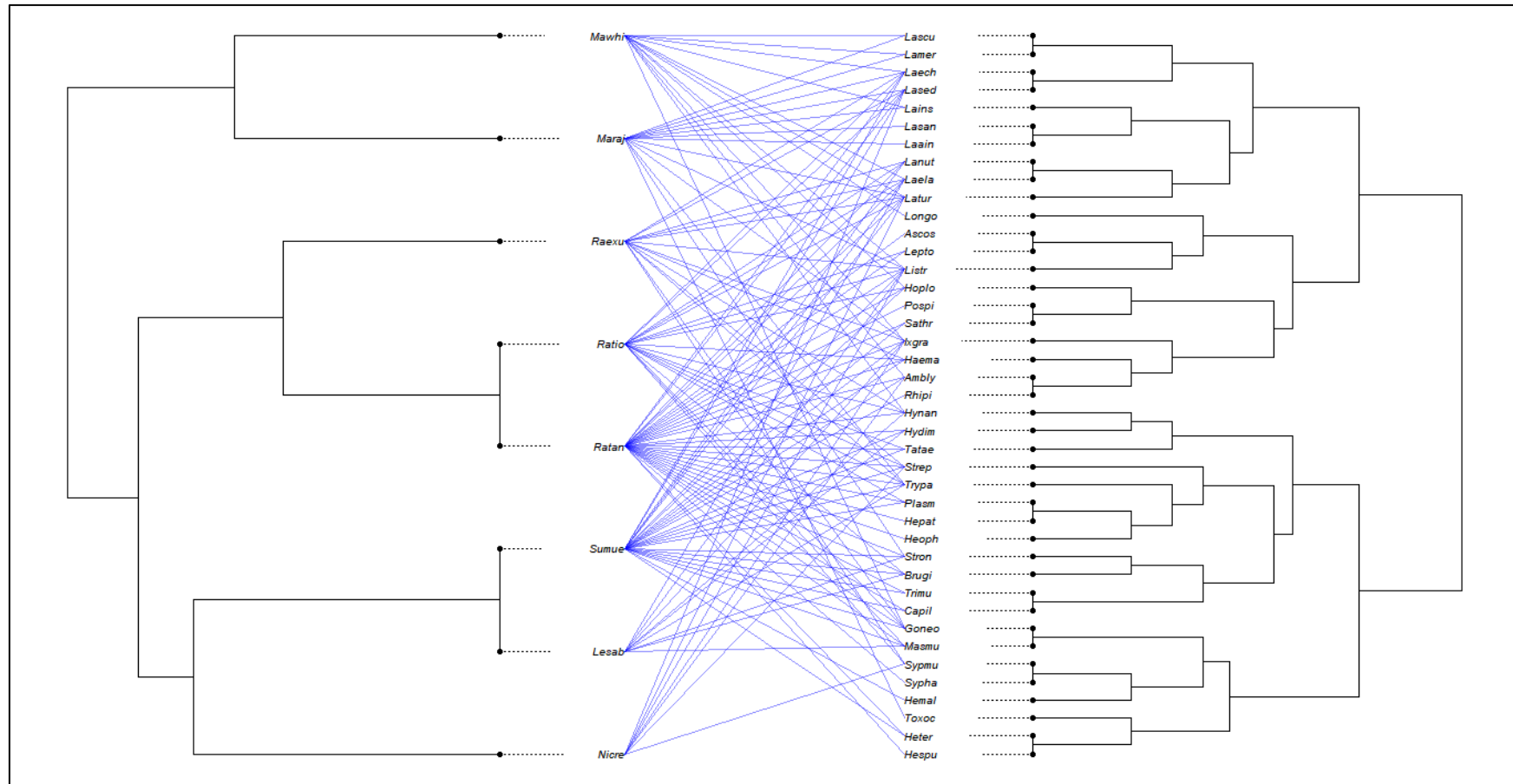


Figure 5.1: Tanglegram generated from R software showing the relationships between 41 parasites and eight different species of Murid rodent. Parasites-rodent interactions network generated based on present-absence of parasites infection. Trees of rodent (right) and parasite (left) were built based on classification and morphological characteristics. Rodent and parasite naming codes were provided in Table 5.1.

Table 5.2: Niche overlap and specialization index for parasite categories.

Parasites categories	No. of parasite species	N0	H2'
All parasites	41	0.46	0.22
Haemoparasites	6	0.65	0.25
Ectoparasites	21	0.41	0.18
Endoparasites	14	0.46	0.29

N0 is Niche overlap and H2' is specialization index.

Table 5.3: Host specificity index (Paired Difference Index, PDI) of all parasites species recovered in this study.

Parasite species	PDI
A. Haemoparasites	
<i>Hepatocystis</i> sp.	1
<i>Hepatozoon ophisauri</i>	1
<i>Plasmodium</i> sp.	0.93
<i>Trypanosoma</i> sp.	0.71
<i>Streptococcus</i> sp.	0.84
<i>Brugia</i> sp.	0.76
B. Ectoparasites	
<i>Amblyomma</i> sp.	0.91
<i>Haemaphysalis</i> sp.	0.83
<i>Ixodes granulatus</i>	0.79
<i>Rhipicephalus</i> sp.	1

Table 5.3 continued

<i>Listrophoroides</i> spp.	0.84
<i>Laelaps</i> spp.	0.73
<i>Laelaps aingworthae</i>	1
<i>Laelaps echidninus</i>	0.90
<i>Laelaps insignis</i>	0.93
<i>Laelaps mercedae</i>	0.93
<i>Laelaps nuttalli</i>	0.84
<i>Laelaps sanguisugus</i>	1
<i>Laelaps sculpturatus</i>	0.93
<i>Laelaps sedlaceki</i>	0.89
<i>Laelaps turkestanicus</i>	0.83
<i>Longolaelaps</i> sp.	1
<i>Ascoschoengastia</i> sp.	1
<i>Leptotrombidium</i> sp.	0.93
<i>Hoplopleura</i> sp.	0.89
<i>Polyplax spinulosa</i>	1
<i>Sathrax</i> sp.	0.89
C. Endoparasites	
<i>Hymenolepis diminuta</i>	0.93
<i>Hymenolepis nana</i>	0.98
<i>Taenia.taeniaformis</i>	0.79
<i>Capilaria</i> sp.	0.98
<i>Gongylonema neoplasticum</i>	0.89

Table 5.3 continued

<i>Hepatojarakus malayae</i>	1
<i>Heterakis</i> sp.	0.98
<i>Heterakis spumosa</i>	1
<i>Mastophorus muris</i>	0.94
<i>Strongyloides</i> spp.	0.96
<i>Syphacia</i> sp.	1
<i>Syphacia muris</i>	0.71
<i>Toxocara</i> sp.	1
<i>Trichuri muris</i>	0.86

5.4 Discussions

The study of host-parasite relationships between parasite and their rodent hosts in Sarawak may not be the first time. Therefore, this study try to include and combine more parasite categories than the previous rodent-parasite study in Sarawak. Only eight species out of 61 species of rodent reported in Borneo were examined for parasites in this study. Collecting study material of rodent can be challenging or even impossible due to their protected status or rarity (Kołodziej-Sobocińska, 2019). Therefore, the diversity of mammalian hosts is one of the major challenges in characterising and systematising the parasite of wild mammals (Kołodziej-Sobocińska, 2019).

A total of six out of eight rodent species had 100% parasite infection. Despite being the lowest prevalence of overall parasite infection, *R. tanezumi*, *R. exulans* and *R. tiomanicus* recorded high number of host infected (n=133, n=11 and n=30 respectively). *Rattus* genus

has been well known to be the reservoir to wide range of parasite species. The fact that both species hosted high diversity of parasites has been reported by other studies (Ng et al., 2017; Sepe et al., 2020) and is explained that these rodents can live in a broad range of environments (Aplin et al., 2011; Rizwar, 2019). These must have made them susceptible to parasites infections in their native settings (Kołodziej-Sobocińska, 2019).

The highest prevalence of haemoparasites in *Niviventer cremoriventer* was most likely attributed to the fact that only one individual of this species was discovered during this study. As a result, this species has a high chance of becoming the highest prevalence of parasite infection (Hamdan et al., 2016). Meanwhile, *Maxomys whiteheadi* recorded zero prevalence of haemoparasite infection. Similar result was responded with negative antibody's result for the presence of *T. gondii* by Normaznah et al. (2015). Hence, this study is the second study to report on haemoparasite prevalence in *Maxomys whiteheadi* in Malaysia.

Ectoparasites prevalence were expected to be high since ectoparasites found in this study were commonly infested on rodent. However, prevalence of ectoparasite infestation on *Leopoldamys sabanus* was the lowest among the other rodent species with only three species of ectoparasites infested on them. This finding were comparable to Razali et al. (2018) and Thanee et al. (2009) who reported low prevalence of ectoparasites with only one to two ectoparasites species infested on *L. sabanus*. The reasons behind this seems unclear because their forest habitat preference, larger body size, and larger home range distance (Phillipps & Phillipps, 2018; Wells et al., 2008) should support higher ectoparasites infestations (Fernandes et al., 2021; Paramasvaran et al., 2009; Razali et al., 2018).

The highest prevalence of endoparasites were recorded in *Niviventer cremoriventer* and *Leopoldamys sabanus*. As has been mentioned earlier, the high prevalence of endoparasite in *N. cremoriventer* was contribute by the single individual representative of the species. A high prevalence of endoparasite in *L. sabanus* was previously recorded by Ask and Mohd Zain (2006) and Wells et al. (2007), in Johor and Sabah, respectively. Meanwhile, low endoparasite prevalence in *L. sabanus* have been documented in Sarawak and Selangor (Hamdan et al., 2016; Priscilla et al., 2015). Despite of the inconsistent of endoparasites prevalence in this species, those previous studies and this study considered have less recorded parasite species which may be linked to host (*L. sabanus*) genetic factor related parasite resistance (Bordes & Morand, 2011). Lenz et al. (2009) has proven the high diversity of major histo-compatibility complex (MHC) in *L. sabanus* resulted in their good body condition and helminth parasites resistance. Additionally, the linked of genetic factor of MHC diversity in parasites resistance also have been proven by several studies using different rodent species elsewhere (Harf & Sommer, 2005; Meyer-Lucht et al., 2010; Meyer-Lucht & Sommer, 2005). Endoparasite that infested *L. sabanus* maybe able to adapt with this genetic factor of *L. sabanus* and infect multiple number of this host species resulting in high prevalence of parasite infection.

Despite the feeble taxonomic linkages and interaction closeness in the rodent-parasite community, paco analysis reveals the significant congeruence between rodent and parasite phylogenies, which shows dependency on each other. However, congruence at poor representation of rodent-parasite respective clades most likely denotes lineages within those clades that have congruent evolutionary connections instead of depicting the phylogenetic congruence between these specific species (Hutchinson et al., 2017).

This study shows the haemoparasites were low in specialisation indicating most of the haemoparasites species are generalist. Despite the low network specialisation in haemoparasite infection, *Hepatocystis* sp. and *Hepatozoon ophisauri* were specific to only *Rattus tanezumi*. *Hepatocystis* sp. is known to be closely related to genus *Plasmodium*, malarial parasites that has long been acknowledged for its role in the worldwide burden of human disease (Guinovart et al., 2006). The fact that both malarial parasites have narrow specificity vertebrate host selection (Déchamps et al., 2010; Martinsen et al., 2008), *Hepatocystis* sp. was infected only on mammals primarily bats, non-human primate, and rodent with the aid of insect vector, *Culicoides* genus (Low et al., 2021; Perison et al., 2022; Schaer et al., 2013). Therefore, *Hepatocystis* sp. in this study maybe a host specific since its been detected in only one host species and has been supported by Low et al. (2021) and (Perison et al., 2022) that also recorded this parasite in only one host species.

Similar to *Hepatocystis* sp., *Hepatozoon ophisauri* was detected only in one host, *R. tanezumi* with low prevalence. Previous study in Malaysia has reported *Hepatozoon* genus in *R. rattus*, *R. tiomanicus* and *R. tanezumi* (Kamil et al., 2021; Perison et al., 2022). Its even not host-specific as it also infect host from other lineage such as birds, reptiles, and amphibians (Perles et al., 2019). In Malaysia, this genus often been observed in cats and dogs in peri-urban areas (Masrin et al., 2019; Prakash et al., 2018). Despite the fact that no zoonotic relevance has yet been established, *Hepatozoon* sp. infections in rodent can be pathogenic but often moderate and asymptomatic (Baker et al., 2006). *Hepatozoon* spp., on the other hand, may impair immunological state in other host lineage, predisposing it to coinfection by other infections such as *Toxocara* sp. and *Angiostrongylus* sp. (Diakou et al., 2020; Ferrari et al., 2022). Thus, result from the relationship of *Hepatozoon* sp. in this study

could possibly raise the risk of incidence involving domestic and wild animals as well as conservation importance species since *R. tanezumi* is well dominated in any habitats.

Additional highlight in this study is infection of *Plasmodium* sp. focused on *R. tanezumi*, *R. tiomanicus* and *S. muelleri*. As far as has been acknowledge for the recent two decades, this is the first study recorded *Plasmodium* sp. infected in *S. muelleri* in Malaysia. Previous study in Malaysia was only recorded *Plasmodium* sp. infected genus *Rattus* (Siti Norliyanti, 2017). Their host selection may influence by red blood cell specificity (Déchamps et al., 2010; Martinsen et al., 2008). This finding indicate that *S. muelleri* has met the criteria for *Plasmodium* infection and *Plasmodium* has adapt and able to perform host switching. Eventhough the high association of those host species with human may not aid in *Plasmodium* transmission to human since neither mice nor rats could be infected by any of the human malaria agents (Déchamps et al., 2010), the information from this study may trigger warning for the risk of malaria infection since rodent and human's malaria parasites using same insect vector, *Anopheles* sp. to facilitate the malaria transmission (Khan et al., 2021; Marinotti et al., 2013; Matsuoka et al., 2002; Spence et al., 2012).

Ixodid ticks are usually generalist and have been documented by other studies (Madinah et al., 2014; Obiegala et al., 2021), but different cases for *Rhipicepalus* sp. discovered in this study that infect only *S. muelleri*. Madinah et al. (2011) and Razali et al. (2018) both documented the presence of *Rhipicepalus* ticks infested on a specific rodent species, *Maxomys rajah* in both cases. Ticks of the genus *Rhipicepalus* are mainly two- or three-host ticks and one-host ticks for certain *Rhipicepalus* species. Species that parasitised wildlife and even human are actually those with broader host range (Tan et al., 2021), not host specific. According to Islam et al. (2006), albeit Ixodid ticks may have a stronger predilection for one host over another, they are not necessarily host-specific. Their flexibility

in host specificity could be one of their survival and adaptation strategies when environmental upheavals might have a significant impact on them (Islam et al., 2006).

The finding of this study shows that chigger and most laelapid mites are not host specific, in line with research by Madinah et al. (2014), Martins-Hatano et al. (2002) and Zhan et al. (2013) except for *Laelaps aingworthae*, *Laelaps sanguisugus* and *Longolaelaps* sp. Infection of these laelapid mites in this study were subjected to only *Maxomys rajah* and *M. whiteheadi*. Previous studies have documented these species on varied host species include *Leopoldamys sabanus*, *M. Surifer*, *Niviventer cremoriventer*, *Rattus exulans*, *Sundamys muelleri* and even on bats (Adrus et al., 2021; Ahamad et al., 2013; Madinah et al., 2011; Mariana et al., 2005, 2008; Paramasvaran et al., 2009). The high specificity of these species may be related to host home range. Genus *Maxomys* in this study is the most common species in forest areas and there is an evidence shows that *Maxomys rajah* has reasonably larger home range compared to other rodent species studied here (Nakagawa et al., 2007; Wells et al., 2008). This larger home range size may influence the frequency of host–parasite contacts and interspecific interactions that enhance ectoparasite infection (Fernandes et al., 2021).

While, *Ascoschoengastia* sp. infection was specific to only *R. tanezumi* as has been observed in this study. Similar to *Laelaps aingworthae*, *L. sanguisugus*, and *Longolaelaps* sp., *Ascoschoengastia* sp. also has been reported on different host previously (Adrus et al., 2021; Chuluun et al., 2005; Paramasvaran et al., 2009). Based on previous study, trombiculid mites are associated with specific settings and often form ‘mite island’ in the areas thus create patchy distribution on the vegetation (Cakiroglu et al., 2008; Moss & Beeching, 2003; Shatrov & Kudryashova, 2006). Furthermore, they will infest the first animal that come across their favourable habitat (e.g. humid, tall grass area and forest border) (Alkathiry et

al., 2022; Cakiroglu et al., 2008; Santibáñez et al., 2015; Shatrov & Kudryashova, 2006). In this case, *Ascoschoengastia* sp. only found on one individual of *Rattus tanezumi* that maybe accidentally cross over *Ascoschoengastia* sp. habitat and lead to high specificity reading. Eventhough this relationship is likely by chance, human and wildlife are alike at risk as a result of this rodent and chiggers coexistent because chiggers have been found to be a possible vector of Scrub typhus, Hantavirus and Trombiculiasis disease in Malaysia (Santibáñez et al., 2015). Additionally, *R. tanezumi* are highly adaptable in human dwelling which may increase the interaction possibility to disease infection to human (Griffiths et al., 2022).

As endoparasites are normally low in specialisation, the high host specificity of *Hepatojarakus malayae*, *Heterakis spumosa* and *Toxocara* sp. may considered rare cases. The fact, these species has been documented in multiple host species by several studies in Malaysia (Ask & Mohd Zain, 2006; Hamdan et al., 2016; Mohd Zain, 2008; Mohd Zain et al., 2012; Paramasvaran et al., 2009b). This suggest the specificity of these endoparasite species maybe influenced by interspecific competition between parasite species or influences by host microhabitat.. The deteriorated feature of parasite have made them difficult to identify up to species level and sometimes left them in a very small number of individuals resulted in a very low prevalence and high host specificity. Hence, good sample handling and molecular identification may aid in the identification process as it important in parasite studies (Poulin & Leung, 2010).

5.5 Conclusion

The prevalence of parasites by host species were determined and reported in this study. The interaction of rodent's parasites and host specificity have been determined and validated by the paco and specialisation index analysis. The low specialisation for overall, haemoparasites, ectoparasite and endoparasites infections were reported in this study. Six species of ectoparasites and 10 species of endoparasites are discovered as specialist. The explanation provided in this study for specialisation of each parasite species and their associated rodent species in Sarawak may alleviate the understanding of the host-parasites interaction.

As this study using morphological binary characteristics to create parasite and rodent phylogram trees which resulting in weak representation of clade, other data types were recommended to facilitate in calculating precise distance matrix and tree presentation. On the other hand, it seems difficult to determine parasite host-specificity with unidentified sample. Therefore, proper sample handling and sufficient key identification are crucial in parasite study. This study provide information on host-parasite relationship that may be a potential risk of disease transmission.

CHAPTER 6

GENERAL DISCUSSION

6.1 General discussion

Anthropogenic activities have created new altered habitats for many rodent species. As forest has been considered as the least disturbed environment in this study, it was expected to have the most diversified rodents species. Forest habitat offers a suitable environment with natural shelter and food for these small animals, especially for conservation importance species. Four rodent species are found to preferred for a single habitat (only forest areas) namely, *Leopoldamys sabanus*, *Maxomys rajah*, *Maxomys whiteheadi* and *Niviventer cremoriventer*. These species are highly habitat specialist and important forest dispersal agent. Hence, the preservation of forest habitat is crucial for the conservation of these wild populations since forest modification may destroy their shelter and food source as well as reduce their survivability. The preferable bait (banana and sweet potato with peanut butter) can be consider and diversified the type of bait for future sampling in order to attract more species into the trap.

Rattus tanezumi thriving to human dwelling areas (rural and urban areas) has been reported elsewhere, and colonize with high number of individual in those study areas. This finding is comparable to those of Hamdan et al. (2017) and Ng et al. (2017). Anthropogenic activities also has made *Sundamys muelleri* expand their habitat preference to urban areas. The presence of favourable environmental element such as water source and abandoned patches of vegetation cover has open an opportunity for them to thrive in urban areas. Overall, rodent species diversity and habitat preferences in this study may influence by food

resources, vegetation cover for shelter as well as intraspecific competition between rodent species.

Although this study found that parasites were common among murid rodent, the prevalence of these parasites are varied by habitats. It is reasonable to anticipate that the parasite community detected from this study would be influenced by the environmental condition (temperature and humidity), and behaviour and vulnerability to parasite infection since they were found from different habitat. This study demonstrated that endoparasites were most prevalent in urban areas. Meanwhile, haemoparasite and ectoparasite had the highest prevalence in forest habitats. Different temperature and humidity in different habitat types have influence parasite prevalence pattern in each habitat. The low temperature and high humidity of the surrounding environment have promote activities and development of many parasites species (Short et al., 2017). The foraging behaviour in unhygienic areas has increase the possibility of parasite infection, and the existence of potential habitat for intermediate host such as mosquitoes has creates risk of infection, both resulted from anthropogenic activities (Plaza & Lambertucci, 2017; Wilke et al., 2019).

Laelaps spp. (*L. nuttali*, *L. sedlaceki*, and *L. echidninus*) found abundant on *Rattus* spp. (*R. exulans*, *R. tanezumi*, *R. tiomanicus*) and *Sundamys muelleri* which these host species subjected to abundant in disturbed habitat in this study. Apart from habitat, host species are also the main factor that influence parasites infection. This study showed that rodents are great in providing habitat for parasite since they obtained high prevalence for overall parasites infection with five out of eight species were 100% infected by parasites. However, certain factors force parasites to participate in specific host infection events such as due limited host species available in a habitat, accidental infection and may also be host immune competence. Paco analysis omitted the significant congruence between rodent and

parasite phylogenies suggest lineages within both clades (rodent and parasite) have congruent evolutionary connections. Besides, high specificity index also been displayed by 14 parasites species portray that parasites really have strong tendency in host selection. However, unable to identify parasites to species level may reduce the accuracy of the result. Therefore, identification is important in parasites study and in understanding host-parasite relationship (Poulin & Leung, 2010).

Overall, results from all chapters were linked to each other resolve the infections trend in this region. Even though there is no explicit habitat or animal species effect, habitats do have much influence not only on the prevalence of parasites infection on rodent, but also on number of parasite species and the diversity of rodents.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

This study has successfully determined the rodent distribution and discovered habitat preference in different habitat types namely forest, oil palm plantation, rural and urban areas. This study revealed that habitat alteration and interference influence rodent distribution and habitat preferences. The rodent species diversity was significantly difference in the four habitat types in Sarawak. The rodent species diversity decreased towards urban area, as habitat disturbance increased. Meanwhile, habitat preference of most rodent decreased with higher habitat disturbance. Anthropogenic activities has successfully eliminate native species in disturbed habitat.

The prevalence of rodent parasites in different habitat types (forest, oil palm plantation, rural and urban areas) across Sarawak was effectively identified in this study. There is no significant difference in overall parasite prevalence between the four habitat types in Sarawak. There was also no significant difference in the prevalence of ectoparasites among the four habitat types, but the prevalence of haemoparasites and endoparasites were significantly different among the four habitat types in Sarawak. The majority of parasites preferred a disturbed environment (oil palm, rural, and urban). *Plasmodium* sp., *Trypanosoma* sp., *Ixodes granulatus*, *Leptotrombidium* sp., and the Trombiculid mite were among the parasites found to be medically important.

On the other hand, this study revealed the prevalence of parasites in different host species and the specificity of these parasites to their respective hosts. All rodent species recorded 100% parasite infections except for *Rattus tanezumi* and *R. tiomanicus*. This study

also found that there was low specialisation for all parasite categories. Specialists have been recognised in six ectoparasite species and ten endoparasite species. Most medically concerning species were generalist. The explanations presented in this study for the specialisation of each parasite species and their related rodent species in Sarawak may help to clarify the host-parasite relationship.

The key finding is that the environment has an obvious modifying effect on rodent diversity, haemoparasites and endoparasites infection rates, less obvious for ectoparasites. Besides, host species factor also can be seen through parasite infections and parasites specificity. The findings of this study might help researchers better understand rodent parasite infection in various habitat in Sarawak. This study also provide information on host-parasite relationship that expected to be a potential risk of disease transmission.

7.2 Recommendations

It seems difficult to determine parasite with missing or damage of morphological features as well as inadequate source of identifications. Therefore, proper sample handling and sufficient key identification are crucial in parasite study. In addition, identification through molecular approach may aid in these inconvenience. The accurate identifications are also useful in better analysis especially in establishing the relationship of parasite, host and habitats. The precise data might also provide information on medically concern parasites that could be transferred to humans if the host species is well-adapted to human-modified environments.

The continuous growth of human population and anthropogenic activities may drive rodent populations away from their natural environment allows spreading of invasive species and intensify of humans and rodent interaction in the future. Since forest habitat capable in

holding most native species and more diversity of life, retention forest habitat would be the most effective effort in preserving native and species of conservation importance that can perhaps regulate the population of commensal or invasive species. Furthermore, more research on zoonotic disease prevalence in rodents should be explored to forecast the likelihood of disease transmit to humans. Further study is needed to have a better understanding of the health concerns associated with rodent parasite infections in Sarawak. Molecular parasite identification and disease detection from parasites, as well as more comprehensive ecological factors, may aid future parasite studies. More habitat type should be explored for rodent-parasite studies such as coastal area, mangrove, cave and many more as an effort to grow the number of literatures and reveal the health risk status in Sarawak.

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APPENDICES

Appendix A: Table of Kruskal-Wallis test comparison of parasite infection between habitats.

Parasite categories	<i>H (Chi2)</i>	df	P value
Haemoparasites	25.655	3	1.13E-05
Ectoparasites	3.6106	3	0.3067
Endoparasites	30.036	3	1.36E-06
Overall parasites	27.092	3	5.63E-06

Appendix B: Tables of correspondence analysis for overall parasites, haemoparasites, ectoparasites and endoparasites.

CA analysis overall parasite

	Eigenvalue	Variance percent	Cumulative variance percent
Dim.1	0.070013	43.31767	43.31767
Dim.2	0.061739	38.19851	81.51618
Dim.3	0.029875	18.48382	100

CA analysis haemoparasites

	Eigenvalue	Variance percent	Cumulative variance percent
Dim.1	0.473648	54.14644	54.14644
Dim.2	0.276543	31.61386	85.7603
Dim.3	0.124562	14.2397	100

CA analysis ectoparasites

	Eigenvalue	Variance percent	Cumulative variance percent
Dim.1	0.284449	52.20348	52.20348
Dim.2	0.161494	29.63821	81.84169
Dim.3	0.098942	18.15831	100

CA analysis endoparasites

	Eigenvalue	Variance percent	Cumulative variance percent
Dim.1	0.553555	42.22587	42.22587
Dim.2	0.51479	39.26879	81.49466
Dim.3	0.242594	18.50534	100

Appendix C: Distance matric of rodent and parasites trees

	Lesab	Maraj	Mawhi	Nicre	Raexu	Ratan	Ratio
Maraj	2.828427						
Mawhi	3	1.732051					
Nicre	2	2.828427	2.645751				
Raexu	2.236068	2.645751	2.44949	1.732051			
Ratan	2.645751	3	2.828427	2.236068	1.414214		
Ratio	2.645751	3	2.828427	2.236068	1.414214	0	
Sumue	0	2.828427	3	2	2.236068	2.645751	2.645751

Figure 1: Rodent distance matric

	Hepat	Heoph	Plasm	Trypa	Strep	Brugi	Ambly	Haema	Ixgra	Rhipi	Listr	Laela	Laain	Laech	Lains	Lamer	Lanut	Lasan	Lascu	Lased
Heoph	1																			
Plasm	0	1																		
Trypa	1.414214	1	1.414214																	
Strep	1.414214	1.732051	1.414214	1.414214																
Brugi	2	2.236068	2	2	2															
Ambly	3	3.162278	3	3	3	3														
Haema	3.162278	3.316625	3.162278	3.162278	3.162278	3.162278	1													
Ixgra	2.828427	3	2.828427	2.828427	2.828427	2.828427	1	1.414214												
Rhipi	3	3.162278	3	3	3	3	0	1	1											
Listr	2.828427	3	2.828427	2.828427	2.828427	2.828427	1.732051	2	1.414214	1.732051										
Laela	3.872983	4	3.872983	3.872983	3.872983	3.872983	3.162278	3.316625	3	3.162278	2.645751									
Laain	3.872983	4	3.872983	3.872983	3.872983	3.872983	3.162278	3.316625	3	3.162278	2.645751	2								
Laech	3.741657	3.872983	3.741657	3.741657	3.741657	3.741657	3	3.162278	2.828427	3	2.44949	2.236068	2.236068							
Lains	3.741657	3.872983	3.741657	3.741657	3.741657	3.741657	3	3.162278	2.828427	3	2.44949	1.732051	1	2						
Lamer	3.741657	3.872983	3.741657	3.741657	3.741657	3.741657	3	3.162278	2.828427	3	2.44949	2.236068	2.236068	1.414214	2					
Lanut	3.872983	4	3.872983	3.872983	3.872983	3.872983	3.162278	3.316625	3	3.162278	2.645751	0	2	2.236068	1.732051	2.236068				
Lasan	3.872983	4	3.872983	3.872983	3.872983	3.872983	3.162278	3.316625	3	3.162278	2.645751	2	0	2.236068	1	2.236068	2			
Lascu	3.741657	3.872983	3.741657	3.741657	3.741657	3.741657	3	3.162278	2.828427	3	2.44949	2.236068	2.236068	1.414214	2	0	2.236068	2.236068		
Lased	3.741657	3.872983	3.741657	3.741657	3.741657	3.741657	3	3.162278	2.828427	3	2.44949	2.236068	2.236068	0	2	1.414214	2.236068	2.236068	1.414214	
Latur	3.872983	4	3.872983	3.872983	3.872983	3.872983	3.162278	3.316625	3	3.162278	2.645751	1.414214	2	2.236068	1.732051	2.236068	1.414214	2	2.236068	2.236068
Longo	3.316625	3.464102	3.316625	3.316625	3.316625	3.316625	2.44949	2.645751	2.236068	2.44949	1.732051	2.44949	2.44949	2.236068	2.236068	2.236068	2.44949	2.44949	2.236068	2.236068
Ascos	3.162278	3.316625	3.162278	3.162278	3.162278	3.162278	2.236068	2.44949	2	2.236068	1.414214	2.645751	2.645751	2.44949	2.44949	2.44949	2.645751	2.645751	2.44949	2.44949
Lepto	3.162278	3.316625	3.162278	3.162278	3.162278	3.162278	2.236068	2.44949	2	2.236068	1.414214	2.645751	2.645751	2.44949	2.44949	2.44949	2.645751	2.645751	2.44949	2.44949
Hoplo	2.645751	2.828427	2.645751	2.645751	2.645751	2.645751	2	2.236068	1.732051	2	1.732051	3.162278	3.162278	3	3	3	3.162278	3.162278	3	3
Pospi	2.828427	3	2.828427	2.828427	2.828427	2.828427	2.236068	2.44949	2	2.236068	2	3.316625	3.316625	3.162278	3.162278	3.162278	3.316625	3.316625	3.162278	3.162278
Sathr	2.828427	3	2.828427	2.828427	2.828427	2.828427	2.236068	2.44949	2	2.236068	2	3.316625	3.316625	3.162278	3.162278	3.162278	3.316625	3.316625	3.162278	3.162278
Hydim	2.236068	2.44949	2.236068	2.236068	2.236068	2.236068	2.828427	3	2.645751	2.828427	2.645751	3.741657	3.741657	3.605551	3.605551	3.605551	3.741657	3.741657	3.605551	3.605551
Hynan	2.44949	2.645751	2.44949	2.44949	2.44949	2.44949	3	3.162278	2.828427	3	2.828427	3.872983	3.872983	3.741657	3.741657	3.741657	3.872983	3.872983	3.741657	3.741657
Tatae	2	2.236068	2	2	2	2	2.645751	2.828427	2.44949	2.645751	2.44949	3.605551	3.605551	3.464102	3.464102	3.464102	3.605551	3.605551	3.464102	3.464102
Capil	2.236068	2.44949	2.236068	2.236068	2.236068	1.732051	2.828427	3	2.645751	2.828427	2.645751	3.741657	3.741657	3.605551	3.605551	3.605551	3.741657	3.741657	3.605551	3.605551
Goneo	3	3.162278	3	3	3	2.236068	3.464102	3.605551	3.316625	3.464102	3.316625	4.242641	4.242641	4.123106	4.123106	4.123106	4.242641	4.242641	4.123106	4.123106
Hemal	2.44949	2.645751	2.44949	2.44949	2.44949	1.414214	3	3.162278	2.828427	3	2.828427	3.872983	3.872983	3.741657	3.741657	3.741657	3.872983	3.872983	3.741657	3.741657
Heter	3.162278	3.316625	3.162278	3.162278	3.162278	2.44949	3.605551	3.741657	3.464102	3.605551	3.464102	4.358899	4.358899	4.242641	4.242641	4.242641	4.358899	4.358899	4.242641	4.242641
Hespu	3.162278	3.316625	3.162278	3.162278	3.162278	2.44949	3.605551	3.741657	3.464102	3.605551	3.464102	4.358899	4.358899	4.242641	4.242641	4.242641	4.358899	4.358899	4.242641	4.242641
Masmu	3	3.162278	3	3	3	2.236068	3.464102	3.605551	3.316625	3.464102	3.316625	4.242641	4.242641	4.123106	4.123106	4.123106	4.242641	4.242641	4.123106	4.123106
Stron	2.236068	2.44949	2.236068	2.236068	2.236068	1	2.828427	3	2.645751	2.828427	2.645751	3.741657	3.741657	3.605551	3.605551	3.605551	3.741657	3.741657	3.605551	3.605551
Sypha	2.645751	2.828427	2.645751	2.645751	2.645751	1.732051	3.162278	3.316625	3	3.162278	3	4	4	3.872983	3.872983	3.872983	4	4	3.872983	3.872983
Sypmu	2.645751	2.828427	2.645751	2.645751	2.645751	1.732051	3.162278	3.316625	3	3.162278	3	4	4	3.872983	3.872983	3.872983	4	4	3.872983	3.872983
Toxoc	3	3.162278	3	3	3	2.236068	3.464102	3.605551	3.316625	3.464102	3.316625	4.242641	4.242641	4.123106	4.123106	4.123106	4.242641	4.242641	4.123106	4.123106
Trimu	2.236068	2.44949	2.236068	2.236068	2.236068	1.732051	2.828427	3	2.645751	2.828427	2.645751	3.741657	3.741657	3.605551	3.605551	3.605551	3.741657	3.741657	3.605551	3.605551

Figure 2: Parasite distance matrix

	Latur	Longo	Ascos	Lepto	Hoplo	Pospi	Sathr	Hydim	Hynan	Tatae	Capil	Goneo	Hemal	Heter	Hespu	Masmu	Stron	Sypha	Sypmu	Toxoc
Heoph																				
Plasm																				
Trypa																				
Strep																				
Brugi																				
Ambly																				
Haema																				
Ixgra																				
Rhipi																				
Listr																				
Laela																				
Lain																				
Laech																				
Lains																				
Lamer																				
Lanut																				
Lasan																				
Lascu																				
Lased																				
Latur																				
Longo	2.44949																			
Ascos	2.645751	1.732051																		
Lepto	2.645751	1.732051	0																	
Hoplo	3.162278	2.44949	2.236068	2.236068																
Pospi	3.316625	2.645751	2.44949	2.44949	1															
Sathr	3.316625	2.645751	2.44949	2.44949	1	0														
Hydim	3.741657	3.162278	3	3	2.44949	2.645751	2.645751													
Hynan	3.872983	3.316625	3.162278	3.162278	2.645751	2.828427	2.828427	1												
Tatae	3.605551	3	2.828427	2.828427	2.236068	2.44949	2.44949	1	1.414214											
Capil	3.741657	3.162278	3	3	2.44949	2.645751	2.645751	2	2.236068	1.732051										
Goneo	4.242641	3.741657	3.605551	3.605551	3.162278	3.316625	3.316625	2.828427	3	2.645751	2.44949									
Hemal	3.872983	3.316625	3.162278	3.162278	2.645751	2.828427	2.828427	2.236068	2.44949	2	1.732051	1.732051								
Heter	4.358899	3.872983	3.741657	3.741657	3.316625	3.464102	3.464102	3	3.162278	2.828427	2.645751	1.732051	2							
Hespu	4.358899	3.872983	3.741657	3.741657	3.316625	3.464102	3.464102	3	3.162278	2.828427	2.645751	1.732051	2	0						
Masmu	4.242641	3.741657	3.605551	3.605551	3.162278	3.316625	3.316625	2.828427	3	2.645751	2.44949	0	1.732051	1.732051	1.732051					
Stron	3.741657	3.162278	3	3	2.44949	2.645751	2.645751	2	2.236068	1.732051	1.414214	2	1	2.236068	2.236068	2				
Sypha	4	3.464102	3.316625	3.316625	2.828427	3	3	2.44949	2.645751	2.236068	2	1.414214	1	1.732051	1.732051	1.414214	1.414214			
Sypmu	4	3.464102	3.316625	3.316625	2.828427	3	3	2.44949	2.645751	2.236068	2	1.414214	1	1.732051	1.732051	1.414214	1.414214	0		
Toxoc	4.242641	3.741657	3.605551	3.605551	3.162278	3.316625	3.316625	2.828427	3	2.645751	2.44949	1.414214	1.732051	1	1	1.414214	2	1.414214	1.414214	
Trimu	3.741657	3.162278	3	3	2.44949	2.645751	2.645751	2	2.236068	1.732051	0	2.44949	1.732051	2.645751	2.645751	2.44949	1.414214	2	2	2.44949

Figure 3: Continued parasite distance matrix

Appendix D: Table of rodent infected by parasites in different habitat types in Sarawak.

No. of host infected															
Parasites	Forest areas							Oil palm plantations			Rural areas		Urban areas		Total
species	Lesab N=3	Maraj N=3	Mawhi N=4	Nicre N=1	Raexu N=4	Ratan N=3	Sumue N=5	Raexu N=8	Ratan N=41	Ratio N=22	Ratan N=56	Ratio N=9	Ratan N=49	Sumue N=28	N=236
A. Haemoparasites															
Hepat													11		0
Heoph													4		0
Plasm									7		3	2		3	15
Trypa	1	1			3		1		3	5	1				15
Strep				1	1	2	4		1	1			2	3	15
Brugi	1								4	7			1	6	19
B. Ectoparasites															
Ambly			1	1		2	2				2		1	12	21
Haema								3	5	1	1		4	12	26

Appendix D continued

Ixgra	1	1	1	1	1		3		4		1		1	1	15
Rhipi							1								1
Listro	1	1	1	1		3	3	2	5	2	3	1	1	16	40
Laela	2		2				1	2	3	1	4	2		3	20
Laain		2													2
Laech		2	1			1	1	3	7	4	24	3	4	10	60
Lains		2	1												3
Lamer		2	1												3
Lanut							2	7	13	15	13	4	7	10	71
Lasan		2													2
Lascu		1	2												3
Lased		1		1	1	3	4		2	4	6	1	23	17	63
Latur		1	1	1			1	1	11	4	3		2	10	35
Longo			1												1

Appendix D continued

Ascosp									1				1
Leptos						1	2	2					5
Hoplost	1		3	1		6	4	9		4	10		38
Pospi						1		7		6			8
Sathr						2		1		1	3		7
C. Endoparasites													
Hydim	2			2				1			4		9
Hynan	1	2			1			18	1	11			23
Tatae							1	4	2		3		10
Capil										1	6		7
Goneo		1		1	2	16	8	4	1	1	6		40
Hemal											3		3
Heter								13		2	2		17
Hespu								2					2

Appendix D continued

Masmu	1					1			12	3		17	
Stron	1	1				1		1			36	8	48
Sypha							1						1
Sypmu				1	1					1			3
Toxoc		1											1
Trimu							1				11	10	22