DEMOGRAPHIC STUDY AND IDENTIFICATION OF PARASITE FAUNA RECOVERED IN WILD RATS IN PENINSULAR MALAYSIA

NURHALIMAH BINTI MOHD SAAD

FACULTY OF SCIENCE

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NURHALIMAH BINTI MOHD SAAD

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DEMOGRAPHIC STUDY AND IDENTIFICATION OF PARASITE FAUNA RECOVERED IN WILD RATS IN PENINSULAR MALAYSIA

ABSTRACT

The discovery of parasitic fauna in wild rats in Peninsular Malaysia is very important for scientific research as it involves animal and human health. It also affects humans negatively, including agricultural pests, food spoilage and contamination, and disease transmission to humans, livestock, and domestic animals. Murids or known as rat and mice are widespread in all environments and have a high potential to be in areas where people live. Rat act as reservoir hosts for zoonotic helminths and some ectoparasites. Thus, the purpose of this study is to determine the prevalence of ectoparasites and helminths endoparasite infections found in wild rats by examining the demographic distribution of wild rats from various types of forests and their faunal parasites with a focus to identify zoonotic parasite fauna infections in rats as hosts and to use molecular techniques to confirm the species of cyclophyllidean cestodes. This study covers forest reserves, ecotourism forests/recreational parks, and modified or agricultural forests involving oil palm plantations located in Peninsular Malaysia. Wild rats were captured and parasites were extracted. The infestation and infection rates were calculated while cestode molecular identification was inferred from two markers (18SrDNA and COX1). Collectively, ten species of wild rats were captured with twenty-two fauna parasites species recovered where some of the listed fauna parasites were known as zoonotic species namely, three species of ectoparasites (*Laelaps* spp., *Leptotrombidium* sp., and *Ixodes granulatus*) and five endoparasites species (*Capillaria* sp., *Angiostongylus malaysensis*, *Raillietina* spp., *Hymenolepis diminuta* and *Hydatigera taeniaeformis*). Following this, the phylogeny of tapeworms was investigated as vast of information remain unknown particularly with molecular identification of cestodes. All the sequences were successfully amplified with product with total length of 205 and 1202 base pairs (bp), respectively. Four cestode species from the Family Hymenolepididae (*Hymenolepis*

diminuta), Family Taeniidae (*Hydatigera parva*; *Hydatigera taeniaeformis*) and Family Davaineidae (*Raillietina* spp.) were successfully characterized using phylogenetic analyses and haplotype networking. The molecular approaches have made significant progress in detecting complex species and very useful for studying intra-specific variation in helminths, especially the cestode group. In summary, this research will increase public knowledge and awareness of fauna parasite infestation and infections, which are important to human health.

Keywords: parasite, prevalence, wild rats, zoonotic, Peninsular Malaysia

KAJIAN DEMOGRAFI DAN PENGENALAN FAUNA PARASIT YANG DI BAWA DALAM TIKUS LIAR DI SEMENANJUNG MALAYSIA

ABSTRAK

Penemuan fauna parasit dalam tikus liar di Semenanjung Malaysia sangat penting untuk penyelidikan saintifik kerana ia melibatkan kesihatan haiwan dan manusia. Ia juga memberi kesan negatif kepada manusia, termasuk perosak pertanian, kerosakan dan pencemaran makanan, dan penularan penyakit kepada manusia, ternakan dan haiwan peliharaan. *Murids* atau dikenali sebagai tikus tersebar luas di semua persekitaran dan berpotensi tinggi untuk berada di kawasan tempat tinggal manusia. Tikus bertindak sebagai perumah takungan untuk helmint zoonotik dan beberapa ektoparasit. Justeru, tujuan kajian ini adalah untuk menentukan kelaziman jangkitan ektoparasit dan endoparasit helmint yang terdapat pada tikus liar dengan meneliti taburan demografi tikus liar dari pelbagai jenis hutan dan parasit faunanya dengan fokus adalah untuk mengenalpasti jangkitan fauna parasit zoonotik di tikus sebagai perumah dan menggunakan teknik molekul untuk mengesahkan spesies sestod cyclophyllidean. Kajian ini meliputi hutan simpan, hutan ekopelancongan/taman rekreasi, dan hutan terubah suai atau pertanian yang melibatkan ladang kelapa sawit yang terletak di Semenanjung Malaysia. Tikus liar telah ditangkap dan parasite diekstrak. Kadar serangan dan jangkitan dikira manakala pengenalpastian molekul sestod disimpulkan daripada dua penanda genetic (18SrDNA dan COX1). Secara kolektif, sepuluh spesies tikus liar telah ditangkap dengan dua puluh dua spesies parasit fauna ditemui semula di mana beberapa parasit fauna yang disenaraikan dikenali sebagai spesies zoonotik iaitu; tiga spesies ektoparasit (*Laelaps* spp., *Leptotrombidium* sp., dan *Ixodes granulatus*) dan lima spesies endoparasit (*Capillaria* sp., *Angiostongylus malaysensis*, *Raillietina* spp., *Hymenolepis diminuta* dan *Hydatigera taeniaeformis*). Berikutan itu, filogeni cacing pita telah disiasat kerana terdapat banyak maklumat yang masih tidak diketahui terutamanya dengan pengenalpastian molekul sestod. Semua jujukan telah berjaya diamplifikasi dengan

jumlah panjang masing-masing 205 dan 1202 pasangan asas. Empat spesies sestod daripada famili Hymenolepididae (*Hymenolepis diminuta*), famili Taeniidae (*Hydatigera parva*; *Hydatigera taeniaeformis*), dan famili Davaineidae (*Raillietina* spp.) berjaya dicirikan menggunakan analisis filogenetik dan rangkaian haplotip. Pendekatan molekul telah mencapai kemajuan yang ketara dalam mengesan spesies kompleks dan sangat berguna untuk mengkaji variasi intra-spesifik dalam helmint, terutamanya kumpulan sestod. Secara ringkasnya, penyelidikan ini akan meningkatkan pengetahuan dan kesedaran orang ramai tentang serangan dan jangkitan parasit fauna, yang penting kepada kesihatan manusia.

Kata kunci: parasit, kelaziman, tikus liar, zoonotik, Peninsular Malaysia

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LIST OF SYMBOLS AND ABBREVIATIONS

~	:	Approximately
χ^2	:	Chi-square
°	:	Degree
°C	:	Degree Celsius
♀	:	Female gender
∞	:	Infinity
♂	:	Male gender
µl	:	Microlitre
µM	:	Micromolar
%	:	Percentage
P	:	Probability
18SrDNA	:	18 small subunit ribosomal deoxyribonucleic acid
a.s.l.	:	Above sea level
et al.	:	And others
BLAST	:	Basic Local Alignment Search Tool
cm	:	Centimetre
COX1	:	Cytochrome *c* oxidase subunit 1
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxyribonucleotide triphosphate
ddH_2O	:	Double distilled water
EDTA	:	Ethylenediamine tetraacetic acid
g	:	Gram
h	:	Hectare
kb	:	Kilobase
km	:	Kilometre

K2P	:	Kimura-2 parameter
$MgCl_2$	:	Magnesium chloride
ML	:	Maximum-Likelihood
m	:	Metre
mg	:	Milligram
ml	:	Millilitre
min	:	Minutes
MEGA	:	Molecular evolutionary genetics analysis
ng	:	Nanogram
nm	:	Nanometres
NJ	:	Neighbour-Joining
PBS	:	Phosphate-buffered saline
PCR	:	Polymerase chain reaction
rpm	:	Revolutions per minute
s	:	Second
spp.		Species (plural)
sp.	:	Species (singular)
km^2	:	Square kilometre
i.e.	:	That is
TAE	:	Tris acetate EDTA
UPW	:	Ultra-pure water
UV	:	Ultraviolet
V	:	Volt

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Rats (family: Muridae)

Over 42% of all the mammal species were belong to rodents (rats and mice) with more than 2,700 species (Musser & Carleton, 2005). About 27 species of rats were identified in Peninsular Malaysia (Francis, 2008; Lim, 2015). Rat act as reservoir hosts for diverse zoonotic pathogens and can transmit several diseases to human and animals, including leptospirosis, helminthic diseases, hantaviral diseases, plague, and murine typhus. Rats are well-known for their geographical spread and ability to adapt in wide range of environments, allowing them to quickly exploit new habitats and become the most destructive pests. The presence of rats in almost all environments makes human interaction even more dangerous. As it concludes that rats are an important small mammal with several major impact on humans (Aplin *et al*., 2003) and considered a serious agricultural pest in Malaysia which decrease about 5 to 10 % in rice, 5 % in oil palm, and 6 % in pineapple production (Aplin *et al*., 2003; Joomwong, 2007).

Knowledge of wild rat distribution and their association with fauna parasites of Peninsular Malaysia remain limited due to most studies focus on urban rats and covered urban geographical region. Other species of rats, rather than *Rattus* sp., are important for studying the distribution pattern of hosts and parasites, even though those hosts interacted heavily with humans. Rat surveys in Peninsular Malaysia in relation with their parasite infection and diseases have been began since 1933 till now. However, there are gaps in this subject due to the scarcity of current research on murids and parasites. As the problem of re-emerging and new diseases has expanded in recent years.

Knowing the rat species found in the diverse region is crucial to know the parasites being carried which provide important information in terms of environmental health. Rats in Peninsular Malaysia have been recorded to harbour ectoparasite such as chigger mites, mesostigmatid mites, ticks, lice, and fleas, while endoparasite helminths such as

tapeworm, roundworm and hookworm (Mohd Zain *et al*., 2008; Mohd Zain *et al*., 2015; Premaalatha *et al*., 2017; Tijjani *et al*., 2020). Several studies have demostrated on the study of murids in terms of parasite infestation and diseases (Ahmad *et al*., 2020; Ghazali *et al*., 2017; Ibrahim, 2020; Ikbal *et al*., 2019b; Ishak *et al*., 2018; Loong *et al*., 2018; Mohd-Taib *et al*., 2018; Premaalatha *et al*., 2017; Razali *et al*., 2018; Tijjani *et al*., 2020). Therefore, understanding the murids and parasite relationship is necessary to fully understanding the association and interactions of host and parasites.

To summarise, continuing to research the murid parasite in relation to human and veterinary medicine, as well as their biology, is vital since it promotes human health. This research can also be utilised to enhance public awareness of parasite infestations, allowing for a more effective preventative and control campaign.

1.2 Research objectives

The primary aim of this thesis is to investigate the demographic distribution of wild rats, and their parasite fauna in Peninsular Malaysia involved the association between host and parasite. Besides, this study also included the genetic relationships of cestode worm inferred from COX1 and 18SrDNA genes which infects wild rats with referring to the below research questions:

1.2.1 What is the distribution pattern of wild rats and their parasite fauna in Peninsular Malaysia?

The distribution of rats and their fauna parasites in Peninsular Malaysia was reviewed in this study. The objectives were: (1) to review demographic distribution of the wild rat species in the diverse forest types which are still limited, (2) to create the list of fauna parasites harboured in diverse rat species other than *Rattus* spp. from Peninsular Malaysia and (3) to create a networking between host rats and parasites in term of rats species. Part of this project has been published as Saarani *et al*. (2021).

1.2.2 What is the genetic relationship of cestode worms found in wild rat based on the nuclear 18SrDNA and mitochondrial COX1 genes?

The genetic relationships of cestode worms harboured in wild rats in Peninsular Malaysia was examined in this study. The objectives were: (1) to clarify the Cyclophyllidean helminths recovered from wild rats captured from various forest types based on the 18SrDNA and COX1 genes and (2) to verify whether molecular identification of this helminths are highly similar with the recorded genetic sequences in other region from GenBank. However, this cestode only focus on cestode found in wild rats as host animals. This project has been published as Mohd-Saad *et al*. (2022). Molecular characterisation and genetic affinities of Cyclophyllidean cestodes infecting wild rats in Peninsular Malaysia. *Tropical Biomedicine,* 39(2): 170-178.

CHAPTER 2: LITERATURE REVIEW

2.1 Murids of Southeast Asia

Rats and mice (also known murids or muroid rodents) is a major mammalian order. (Wilson & Reeder, 2005). The family Muridae, subfamily Murinae, is the largest rodent family with 126 genera and 561 species widely distributed throughout the world (Musser & Carleton, 2005). It represents approximately two-thirds of all living species in the order of Rodentia. Over thirty-nine (39) species belonging to twelve (12) genera have been recorded in Southeast Asia as followed *Bandicota bengalensis*, *Bandicota indica, Bandicota savilei, Berylmys berdmorei*, *Berylmys bowersi, Chiropodamys gliroides, Hapalomys longicaudatus, Lenothrix canus, Leopoldamys ciliatus, Leopoldamys edwardsi, Leopoldamys sabanus, Maxomys bartelsii, Maxomys inas, Maxomys rajah, Maxomys surifer, Maxomys whiteheadi, Mus caroli, Mus cervicolor, Mus cookii, Mus musculus, Niviventer cameroni, Niviventer cremoriventer, Niviventer fulvescens, Niviventer lepturus, Pithecheir parvus, Rattus andamanensis, Rattus annadalei, Rattus argentiventer, Rattus exulans, Rattus hoffmanni, Rattus losea, Rattus nitidus, Rattus norvegicus, Rattus rattus diardii, Rattus sakeratensis, Rattus tanezumi, Rattus tanezumi R3, Rattus tiomanicus,* and *Sundamys muelleri* (Blasdell *et al*., 2015; Pakdeenarong *et al*., 2014; Palmeirim *et al*., 2014; Pimsai *et al*., 2014).

Murid genera such as *Bandicota*, *Chiropodomys*, *Hapalomys*, *Lenothrix*, *Leopoldamys*, *Maxomys*, *Mus*, *Niviventer*, *Pithecheir*, *Rattus*, *Rhizomys*, and *Sundamys* also have been described in Malaysia. The taxonomic classification of murid rats is as follows list below:

Table 2.1: The taxonomic class of murid rats in Peninssular Malaysia.

Kingdom:	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Rodentia
Family:	Muridae
Subfamily:	Murinae
Genus:	*Bandicota*
	Bandicota indica
	Bandicota bengalensis
Genus:	*Berylmys*
	Berylmys bowersi
Genus:	*Chiropodomys*
	Chiropodomys gliroides
Genus:	*Hapalomys*
	Hapalomys longicaudatus
Genus:	*Lenothrix*
	Lenothrix canus
Genus:	*Leopoldamys*
	Leopoldamys ciliatus
	Leopoldamys sabanus
Genus:	*Maxomys*
	Maxomys rajah
	Maxomys surifer
	Maxomys inas
	Maxomys whiteheadi
Genus:	*Mus*
	Mus caroli
	Mus musculus
Genus:	*Niviventer*

Niviventer cameroni

Niviventer cremoriventer

Niviventer fulvescens

Genus: *Pithecheir*

Pithecheir parvus

Genus: *Rattus*

Rattus annandalei

Rattus argentiventer

Rattus exulans

Rattus norvegicus

Rattus rattus

Genus: *Rhizomys*

Rhizomys pruinosus

Rhizomys sumatrensis

Genus: *Sundamys*

Sundamys muelleri

Therefore, revising the key identification by combining a set of keys was helpful in solving the issues of murine identification (Pagès *et al*., 2010; Pimsai *et al*., 2014). Challenges in the identification of rodents have most likely occured, as indicated by misnamed (Pimsai *et al*., 2014). The classification of rodents were being changed, especially in the *Rattus rattus* complex, where some species are difficult to distinguish. As a result of synonym names and species complexes, rat taxonomy challenges have evolved. The modified name is then redescribed as shown in Table 2.2 using Wilson & Reeder's (2005) Mammal Species of the World as a guide:

Table 2.2: List of updated taxonomic names in the rodent taxonomy (Table sourced from Wilson and Reeder's Mammal Species of the World, 2005).

Updated taxonomic name	Previous taxonomic name
Berylmys bowersi	*Rattus bowersi*
Leopoldamys sabanus	*Rattus sabanus*
Maxomys rajah	*Rattus rajah*
Maxomys surifer	*Rattus surifer*
Maxomys whiteheadi	*Rattus whiteheadi*
Niviventer cremoriventer	*Rattus cremoriventer*
Niviventer fulvescens	*Rattus bukit gracilis*
Rattus tanezumi	*Rattus rattus tanezumi*

More future research related to rat should focus on taxonomy (including both morphometrics and genetic studies), phylogeny, phylogeography and palaeontology (looking at evolutionary histories), ecology and behaviour (particularly in relation to ecosystem services), and the role of rats in disease transmission, not only in the peninsula but throughout Southeast Asia (Pimsai *et al*., 2014). To have a better understanding of the potential epidemiology of a certain rat-borne disease, the rat host must be accurately identified at the species level (Blasdell *et al*., 2015).

Murids have the most individuals, diversity, and biomass, significantly impacting humans (Pimsai *et al*., 2014). Murids tend to have their habitat specifications which sometimes have been related to human habitation such as primary forests, agricultural areas, or human settlements (Adler 1995; Alder *et al*., 1999; Jittapalapong *et al*., 2009; Suntsov *et al*., 2003). According to McFarlane *et al*. (2012), there were two emerged

concepts known as ‘synanthropic’ species and ‘generalist’ species. Synanthropic species are species that prefer to be ecologically related with humans such as agricultural environments, while generalist species are species that live in peridomestic habitats or commonly invade disturbed environments. Based on Blasdell *et al*. (2015), there are several species of murids that have specialized habitats preferences such as *Rattus exulans*, *R. norvegicus* and *R. tanezumi* in human habitations; *Bandicota indica*, *Bandicota savilei*, *Mus caroli*, *Rattus argentiventer*, and *Rattus sakerantensis* in paddy fields; *Rattus nitidus* in dry land or paddy fields; *Berylmys berdmorei*, *Berylmys bowersi*, *Mus cervicolor*, and *M. cookii* in dry lands; *Niviventer fulvescens* in forest or dry lands; and *Leopoldamys edwarsi*, *Maxomys surifer* and *Rattus andamanensis* in forest. Charles & Ang (2010) found *Leopoldamys sabanus*, *Maxomys rajah*, *Maxomys whiteheadi*, and *Niviventer cremoriventer* as Brunei's pristine forest species while *Sundamys muelleri* as disturbed forest species. According to Paramasvaran *et al*. (2013), *R. argentiventer*, *R. exulans*, *R. norvegicus*, *R. rattus diardii*, and *R. tiomanicus* are among species in urban and agricultural habitats while *Berylmys bowersi*, *Leopoldamys sabanus*, *Maxomys rajah*, *Maxomys whiteheadi*, and *Sundamys muelleri* are species found in forest habitat in Malaysia.

Murids are typically nocturnal and exhibit a variety of behaviours. It can be seen on the ground foraging and resting in burrows, climbing trees and nesting in tree holes in the middle forest, and far up in the canopy. From the coast to the mountain, from terrestrial to arboreal, and even from the island to the mainland, which appears to be a significant and diverse population in urban, agricultural areas, and forests. A high level of habitat selectivity, and when these habitats are altered or destroyed because of development such as agriculture, deforestation, or even urbanization, it can result in a diversity of species (Paramasvaran *et al*., 2012).

2.2 Murids as carrier of pathogenic organisms

Since we know how significant rats as pathogenic carriers are, rat-borne diseases can affect humans in two ways (i.e., direct, and indirect transmission) (Meerburg *et al*., 2009). Direct transmission is one of the spreading methods of pathogens. Humans can receive infections from rats by biting or consuming any of the contaminated food or water with rat waste. Furthermore, humans probably by chance contact with rat urine contaminated surface water such as leptospirosis or breathe in microorganisms found in rodent faeces such as hantaviruses. Besides humans, rats were also addressed in reference to causing animal diseases, which indirectly resulted in significant economic losses and a bad reputation for livestock farming. Rats' ability to transmit diseases is facilitated by their scavenging habits on food waste and sewage, as well as their mobility and physiologies that appear to be shared by humans and rats (Hamidi, 2018). Infectious spread not only through direct contact with the body, excretions, bodily fluids, and bites but also through rat-contaminated food, water pollution, and soil contamination. Moreover, indirect transmission of rodent-borne diseases to humans also occurs. Rats can however act as host of diseases, carrying pathogens in direct contact with humans through vectors of ectoparasitic arthropods (fleas, mites, and ticks). In the situation where livestock are consuming rodents, either intentionally or by chance, can transmit diseases that can cause harm to humans if the meats are not properly cooked or eaten raw. The disease transmission cycle was maintained by rodents in various habitats, ranging from highly populated urban habitation to the rural and forest environments.

Rats transmit a variety of diseases, including bacterial (leptospirosis, plague, salmonellosis, tularaemia, scrub typhus, and murine typhus, known as rickettsioses), protozoal (babesiosis, cryptosporidiosis, leishmaniosis), and viral diseases (arenavirus, hantavirus, and rabies). Leptospirosis, on the other hand, is one of Malaysia's most reported infections. Leptospirosis is considered an endemic disease in Malaysia. The

disease's prevalence increased from 12.5 per 100,000 to 15.0 per 100,000 in 2013, according to Malaysia's Ministry of Health, with 71 of 4,457 cases resulting in death (Benacer *et al*., 2017).

About 60 zoonotic diseases are known to infect murids, posing a major hazard to human health (Meerburg *et al*., 2009). List of diseases transmitted by murids were attached in Appendix A. In tropical areas, parasitic diseases acquired by wild rat transmission are a major problem (Brown *et al*., 1978; Singh, 1990; Tan, 1979; Traub *et al*., 1974).

2.3 Rats and parasite fauna association

Murid are known to be carriers or reservoirs of parasites, that can be transmitted to humans and animals. The distribution and populations of parasites, hosts, and vectors are affected by environmental change, such as habitat disturbance, urbanisation, and biodiversity loss (Gillespie & Chapman, 2008; Ostfeld *et al*., 2005; Ostfeld, 2009). Ectoparasite and gastrointestinal helminths are the most common parasitic fauna infected in Malaysian rats. Helminths such as roundworms, tapeworms, and flatworms, as well as ectoparasite arthropods for example chigger mites, mesostigmatid mites, fleas, lice, and ticks act as microparasite vectors which known as macroparasite. Arthropod vectors that feed on wild rats and can transmit parasites to humans are another mode of transmission.

Macroparasite have more complex genomic and life cycles that require the presence of either vectors or intermediate hosts to complete their life. Among the macroparasite recorded, there were several species of helminths that could be classified as neglected tropical diseases (NTDs) by rats, for example toxoplasmosis (*Toxoplasma gondii*), babesiosis (*Babesia* sp.), cryptosporidiosis (*Cryptosporidium*), Chagas disease (*Trypanosoma cruzi*), leishmaniasis (*Leishmania* sp.), giardiasis (*Giardia lamblia*), taeniasis (*Taenia (=Hydatigera) taeniaeformis*), rodentolepiasis (*Rodentolepis* (=*Hymenolepis*) *microstoma*), echinococcosis (*Echinococcus multilocularis*),

schistosomiasis (*Schistosoma mansoni*), human fasciolosis (*Fasciola hepatica*), brachylaimiasis (Trematoda: family Brachylaimidae), alariasis (*Alaria* spp.), echinostomiasis (Trematoda: family Echinostomatidae), trichinosis (*Trichinella* spp.), capillariasis (*Capillaria hepatica*), angiostrongylosis (*Angiostrongylus cantonensis*), toxascariasis (*Toxascaris leonina*), baylisascariasis (*Baylisascaris procyonis*), aelurostrongylosis (*Aelurostrongylus abstrusus*), amoebic dysentery (*Entamoeba histolytica*), and neosporosis (*Neospora caninum*). However, not all listed diseases have been recorded in Malaysia. *Angiostrongylus cantonensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Gongylonema neoplasticum*, *Rictularia tani*, *Syphacia muris*, *Trichuris muris*, *Trichuris trichiura*, *Hymenolepis diminuta*, *Hymenolepis nana*, *Raillietina* sp., *Hydatigera taeniaeformis* and *Moniliformis moniliformis* are among the zoonotic helminth parasites found in Malaysian rats (Hamdan *et al*., 2016; Paramasvaran *et al*., 2009a; Syed-Arnez & Mohd Zain, 2006; Tijjani *et al*., 2020).

In addition, various ectoparasitic arthropods have been found infesting rats in Malaysia, including chigger mites (*Ascoschoengastia indica*, *Doloisia* spp., *Gahrliepia* (*Walchia*) spp., *Gahrliepia* (*Gahrliepia*) spp., *Leptotrombidium deliense*, and *Leptotrombidium* spp.), mesostigmatid mites (*Echinolaelaps echidninus*, *Echinonyssus nasutus*, *Laelaps aingworthae*, *Laelaps nuttali*, *Laelaps echidninus*, *Laelaps sculpturatus*, *Laelaps sanguisugus*, *Laelaps sedlaceki*, *Longolaelaps longulus*, *Longolaelaps whartonii*, and *Ornithonyssus bacoti*), fleas (*Xenopsylla cheopis*), lice (*Hoplopluera dissicula*, *Hoplopleura pacifica*, and *Polyplax spinulosa*) and ticks (*Amblyomma testudinarium*, *Dermacentor atrosignatus*, *Haemaphysalis* spp., *Ixodes granulatus*, and *Rhipicephalus sanguineus*), all of which are medically significant species (Madinah *et al*., 2014; Mariana *et al*., 2005; Mariana *et al*., 2008; Mohd Zain *et al*., 2015; Paramasvaran *et al*., 2009b; Razali *et al*., 2018).

2.3.1 Ectoparasite infestation on rats

Ectoparasites can be a vector arthropod that can cause illness indirectly in humans or domestic animals such as plague and scrub typhus. In 1961, Dhaliwal stated the *Trombicula deliensis* and *T. akamushi* were the species of mites that act as vectors for the scrub typhus virus hosted by *Rattus rattus*. However, scrub typhus only can be transmitted by certain species of mites from family Trombiculidae, in which the larval stage only is known to be parasitic (Nadchatram, 1970). *Leptotrombidium deliense* appeared to be the strongest species which are widely dispersed and abundant in birds and even rats as *Rattus tiomanicus jalorensis* that known to be distributed throughout agricultural habitats and secondary forest in Malaya (Nadchatram, 1970).

According to Muul & Lim (1974), the highest chigger infestation occurred in *Sundamys muelleri*. Muul *et al*. (1977) have studied the infection of scrub typhus occurred in rats which divided into four different habitats in Peninsular Malaysia as the isolation of *Rickettsia tsutsugamushi* from *L. deliense* have been done on four species of rats (*Leopoldamys sabanus*, *Rattus argentiventer*, *R. exulans*, and *R. tiomanicus*) obtained from the village, Lalang, edge forest, and forest. In Malaysia, Murine typhus was recorded by Tay *et al*. (1999) and Tay *et al*. (2000). *Leptotrombidium deliense* is one of the vectors known for scrub typhus which probably became one of the potential health risks to Kuala Selangor Nature Park visitors (Chuluun *et al*., 2005).

Ectoparasites such as Acari (mites and ticks) are known to be the most common and abundant in rodents and carry various types of human pathogens (Houck *et al*., 2001). In Sarawak, Muul & Lim (1974) discovered the habitat distribution and ectoparasite infestation in small mammals with different trapping areas including primary forest, secondary forest, swamp forest, and edge habitats. A total of 502 of arboreal and terrestrial small mammals were trapped which comprise of *Callosciurus notatus*, *C. prevostii*, *Hylopetes lepidus*, *Tupaia tana*, *T. glis*, *T. minor*, *T. gracilis*, *Rattus*

cremoriventer, *R. sabanus*, *R. muelleri*, *R. rajah*, *R. whiteheadi*, *R. tiomanicus*, *R. rattus* and *R. exulans* (Muul & Lim, 1974).

Two species of rodents (*R. exulans* and *M. whiteheadi*) were caught in Kuala Selangor Nature Park where they found seven species of ectoparasites comprise of *Ascoschoengastia indica*, *Hoplopleura pacifica*, *Hoplopleura pectinate*, *Laelaps echidninus*, *Laelaps nuttalli*, *Leptotrombidium deliense* and *Polyplax spinulosa* (Chuluun *et al*., 2005).

Mariana *et al*. (2005) underwent the first survey of ectoparasites in Gunung Stong Forest Reserve, Kelantan and enable to trap about seven species of rodents (*L. sabanus*, *M. rajah*, *M. surifer*, *M. whiteheadi*, *N. cremoriventer*, *R. tiomanicus*, and *S. muelleri*) which infested by fifteen species of ectoparasite including ticks (*Dermacentor* spp., *Haemaphysalis* spp., *Ixodes granulatus*, *Ixodes* spp.), Mesostigmatids (*Laelaps aingworthae*, *L. echidninus*, *L. insignis*, *L nuttalli*, *L. scuplturatus*, *L. sedlaceki*, *Longolaelaps longulus*) and chiggers (*Eutrombicula wichmanni*, *Gahrliepia* (G) *fletcheri*, *Gahrliepia* (*Walchia*) *naniparma*, *Leptotrombidium* (*L.*) *deliense*, *Leptotrombidium* spp.).

Mariana *et al*. (2008) was recovered the ectoparasites belong to ticks, mesostigmatids, and chiggers where some of them categories as public health important such as *D. auratus*, *I. granulatus* and *Leptotrombidium deliense* that infested a total of 161 animals including six species of non-volant small mammals (*L. sabanus*, *M. surifer*, *M. whiteheadi*, *M. rajah*, *S. muelleri*, and *T. glis*) in Ulu Muda Forest Reserve, Kedah.

A total of 20 species of ectoparasites comprising of mites, chiggers, ticks, louse, and fleas found infested nine species of rodents and shrews such as *M. whiteheadi*, *M. rajah*, *R. bowersi*, *R. exulans*, *R. norvegicus*, *R. rattus diardii*, *S. muelleri*, *T. glis*, and *Lariscus*

insignis which were caught from four habitats in Kuala Lumpur, Selangor and Negeri Sembilan (Paramasvaran *et al*., 2009b).

In Peninsular Malaysia, four wildlife reserves were surveyed for ectoparasitic infestation of small mammals as Endau Kluang Wildlife Reserve, Johore (EKWR), Tasek Bera, Pahang (TBRS), Sungai Dusun Wildlife Reserve, Selangor (SDWR) and Lata Bujang Krau Wildlife Reserve, Pahang (LBKWR) which caught five species of the host (*M. whiteheadi*, *M. rajah*, *L. sabanus*, *Lariscus insignis*, and *T. glis*) that infested by 14 species of ectoparasites from the family of Ixodidae (three species of ticks), Laelaptidae (seven species of mesostigmatid mite), Listrophoriidae (one species of listrophorid mites) and Trombiculidae (three species of chiggers) were examined (Madinah *et al*., 2011). The survey of acarine ectoparasites was investigated by Mariana *et al*. (2011) at Panti Forest Reserve, in Johor where a total of 140 animals consists of bats, birds, rodents, tree shrew and myriapods. Species of rodents such as *M. whiteheadi*, *M. surifer*, *M. rajah*, and *L. sabanus* were infested by ticks, mesostigmatids mite, and chiggers (Mariana *et al*., 2011).

Previously, there was research conducted to determine the prevalence of small mammals and their tick's infestations in the recreational forest and semi-urban residential areas collected from Selangor (Ishak *et al*., 2018). A total of 15 species of small mammals were examined with the presence of ticks namely *Amblyomma* sp., *Dermacentor* sp., and *I. granulatus* that collected from the forest and human settlement in Selangor, Malaysia (Ishak *et al*., 2018). Besides, there were also studies conducted on island habitat by Mohd-Taib *et al*. (2018) as they did the survey of ectoparasite of small mammals in Pangkor Island, Perak which captured four species of small mammals (*Callosciurus notatus*, *M. surifer*, *M. rajah* and *R. tiomanicus*) infested with only mites, namely as *Laelaps* species. *I. granulatus* was known to be the common infesting the small mammals in monoculture plantation areas based on a previous study (Razali *et al*., 2018). Small mammals belong to genus *Maxomys* such as *M. rajah*, *M. surifer* and *M. whiteheadi* were the common

species with the greatest number of mite infestation (Razali *et al*., 2018). Five species of ticks known as *Amblyomma testudinarium*, *Dermacentor atrosignatus*, *Haemaphysalis* sp., *Ixodes granulatus*, and *Rhipicephalus sanguineus* were ectoparasite species found in Kemasul Forest Reserve, Pahang (Razali *et al*., 2018).

2.3.2 Helminths worm infection on rats

Helminths comprise a notable number of parasitic diseases, in which most of the cases come from cestodes (Verma *et al*., 2018). Endoparasite can be found in the brain, bladder, esophagus, lymphatic, lung, heart, trachea, muscle, subcutaneous tissue and internal structures can be transmitted to humans (Robert & Janovy, 2006). Cestoda tapeworms were normally attached to the intestinal wall of the small intestine by anchoring their scolex (William *et al*., 2011). Various organs can be affected by cestodes including skin, subcutaneous tissue, muscle, heart, liver, gastrointestinal tract, orbit, brain, and spinal cord (Verma *et al*., 2018). Host-parasite interactions such as helminth parasite may sometimes cause changes in the host tissues and organs, which could induce their defence mechanisms and from the direct effect of the parasite (Kapczuk *et al*. 2018). The presence of intermediate hosts are very important indicator as it could be one of the factors that influence the rate of intensity of various helminth species, especially the cestode groups.

Several studies on endoparasites of forest and commensal rats have been carried out in East and West Malaysia. Adam (1933) was the first researcher to report the studies on the prevalence of nematodes collected in wild rats in Malaysia. Dunn *et al*. (1968) studied about helminth-host and blood parasite-host in relation to host food preferences and habitat in Southern Malay Peninsular recorded from lowland rainforest and commensal mammals.

In Malaysia, oil palm plantations face a problem of heavy infestation of rats that can transmit the number of parasites to humans. Sinniah *et al*. (1978) were the first to record the human case of *H. diminuta* in Malaysia which involved the data from examination of

oil palm plantation workers. The commonly found parasites among the workers of oil palm plantation were *A. lumbricoides*, *Entamoeba coli*, *Giardia lamblia*, hookworm, and *T. trichiura* (Sinniah *et al*., 1978). They also found one case of *H. nana* and *H. diminuta* with both egg morphology and adult worms (Sinniah *et al*., 1978).

Paramasvaran *et al*. (2009a) was discovered 17 species of parasites and 11 of the parasites identified as zoonotic which harboured from three species of rats (*R. rattus diardii*, *R. norvegicus and R. exulans*) consists of ten nematodes (*C. hepatica*, *G. neoplasticum*, *Rictularia tani*, *S. muris*, *He. spumosa*, *Heterakis* sp., *M. muris*, *N. brasiliensis*, *Pterogodermatis* sp. and *Physaloptera* sp.), six cestodes (*H. diminuta*, *H. nana*, *H. sabnema*, *Hymenolepis* sp., *Raillietina* sp., and *T. taeniaeformis*) and one acanthocephalan (*Moniliformis moniliformis*). Endoparasite fauna of rats was investigated in five wet markets located in Kuala Lumpur known as Chow kit, Dato Keramat, Jinjang, Kepong, and Setapak which trapped 97 rats within a month (Paramasvaran *et al*., 2009a).

A total of eleven species of helminth were identified from 346 *R. rattus* and 104 *R. norvegicus* population in Kuala Lumpur which comprise of seven species of nematodes (*An. malaysiensis*, *N. brasiliensis*, *He. spumosum*, *M. muris*, *S. muris*, *Pteygodermatites tani*/*whartoni*, *G. neoplasticum*), three species of cestodes (*T. taeniaeformis*, *H. diminuta*, *H.* (*Rodentolepis*) *nana*) and one acanthocephalan (*M. moniliformis*) (Mohd Zain *et al*., 2012). Shafiyyah *et al*. (2012) have been discovered four species of helminth consist of two nematodes (*N. brasiliensis*, and *C. hepatica*) and two cestodes (*H. diminuta*, and *H. nana*), one intestinal protozoa (*Entamoeba histolytica*/*E. dispar*) and one blood protozoa (*Trypanosoma lewisi*) among *R. rattus diardii*, *R. norvegicus*, *R. argentiventer*, *R. tiomanicus*, and *R. exulans* in Kuala Lumpur.

There were 186 individuals' gastrointestinal helminths consist of nematodes and cestodes infected six species of murids namely, *S. muelleri*, *L. sabanus*, *N. cremoriventer*,

M. whiteheadi, *M. surifer* and *M. rajah* at five different localities in Western Sarawak (Hamdan *et al*., 2016). Recently, there were studies of rodent-borne parasitic pathogens of *R. rattus diardii* and *R. norvegicus* in Serdang Selangor, Malaysia which discovered twelve species of parasites namely *T. taeniaeformis*, *H. nana*, *H. diminuta*, *An. cantonensis*, *Sarcocystis* spp., *C. hepatica*, *Cryptosporidium* spp., *Toxoplasma gondii*, *Trichuris* spp., *Giardia* spp., *M. moniliformis*, and *Entamoeba histolytica*/ *Entamoeba dispar* (Tijjani *et al*., 2020).

There were many other human diseases such as helminths that originated from commensal rodents (Antoniou *et al*., 2010; Davis & Calvet, 2005; Elsheikha *et al*., 2009; Jansen & Schnieder, 2011; Meerburg *et al*., 2009; Mohd Zain *et al*., 2012).

CHAPTER 3: METHODOLOGY

3.1 Ethic and Permit Approval

All protocols involved in the handling of animals were followed in the Eighth Edition of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The Institutional Animal Care and Use Committee of the University of Malaya (IACUC UM) approved this study with ethics clearance number: S/18092020/28072020-01/R. The research also approved by the Department of Wildlife and National Park (DWNP) of Peninsular Malaysia and the Department of Forestry Malaysia for their permit permission with permit reference number JPHL&TN(IP): 100-34/1.24 Jld 14(66) and JH/100 Jld 23(3) respectively.

3.2 Sampling and study area

Peninsular Malaysia is located north of the equator, between 1.27^{o} and 6.72^{o} north latitude and 99.64^{o} and 104.53^{o} east longitude of Greenwich. Malaysia is a tropical rainforest region with a hot and humid climate all year round. Two seasons are influenced by monsoon wind systems that involve the rainy season from November to March (Northeast Monsoon) and the dry season from May to September (Southwest Monsoon).

The study was conducted at eight localities in Peninsular Malaysia which involve diverse types of forests (i.e., forest reserve, recreational forest, and modifies forest). Trapping was conducted for five days and four nights for each locality, with a total of 100 cages distributed evenly between March 2019 and February 2020.

The sites were represented in four different regions: south, east, central, and north of Peninsular Malaysia. The first areas were situated at southern region (Segamat oil palm plantation, which divided into three estates plantations; Gunung Arong, Mersing), east coast region (Pulau Tioman, Pahang; Lubuk Yu Eco Park, Pahang), central region (Ulu

Gombak Forest Reserve, Selangor) and northern region (Ulu Muda Forest Reserve, Kedah) Peninsular Malaysia (Figure 3.1).

Segamat is a northern district located in the state of Johor. Segamat has located 95 km from Johor Bahru to Yong Peng via the North-South Expressway. It borders two other states, namely Negeri Sembilan in the north and Pahang in the east. The district has an area of 2,807.29 km^2. Segamat is where the agricultural sector proliferates, such as oil palm and rubber, mostly grown on estates. Agriculture is the key contributor to the economy of Segamat, followed by the industrial sector and government service sector. The main oil palm plantation belongs to the IOI Plantation Group and Sime Darby Plantation. As in this sampling, there were three different oil palm plantation stages known as matured, young-mature, and immature oil palm plantation, as in Table 3.1.

Gunung Arong Forest Eco Park is in the Mersing district in the state of Johor. Mersing is the eastern half of the state of Johor, which links the south with the state of Pahang on the east coast. It is also part of Gunung Arong Reserved Forest, about 17 km from Mersing Town and 127 km from Johor Bahru. The town of Mersing occupies an area of 761 km^2. The third lowest mountain in the State of Johor with 273 m a.s.l with lowland dipterocarp forest. This forest reserve is one of the recreational forests that provides a magnificent view of the horizon of the South China Sea. The trail is clear, well-marked, and takes about an hour to reach the top of Gunung Arong.

Pulau Tioman is an island in the Rompin district located in Pahang. Geographically, this island is closer to mainland Johor, and however, it is under Pahang territory. It is 32 km off the state's east coast and approximately 39 km long and 12 km wide, located in the South China Sea. This island has an area of 136 km^2 with four main villages known as Air Batang, Juara, Salang, and Tekek. The ecotourism island with densely forested areas and surrounded by coral reefs is a great attraction for tourism activities.

Lubuk Yu Forest Eco Park is a forest located in the Berkelah Forest Reserve. It is known as a lowland dipterocarp forest 224 m a.s.l. About 100 km from Kuantan Town and 30 km from Maran Town in the state of Pahang. The recreational forest with waterfall and campsite for the adventurer.

Ulu Gombak Forest Reserve is a secondary rainforest in the Gombak district in Selangor and the north part of Kuala Lumpur. It is situated on the southern edge of the old Kuala Lumpur highway to Bentong, Pahang, with selectively forest-logged, mixed, and lowland dipterocarp forest with 132.8 km^2. The forest is the Ulu Gombak Field Study Center of Universiti Malaya at the western edge of the reserve.

Ulu Muda Forest Reserve is a reserved forest in the northern state of Kedah, and it is in the three districts known as Baling, Padang Terap, and Sik. The forest cover about 100,000 hectares of area with various type of forest, including lowland dipterocarp forest, hill dipterocarp forest, and the upper hill dipterocarp forest with an elevation range from 97 to 1256 m a.s.l. This forest is a massive rainforest that protects various flora and fauna.

Table 3.1: The localities and sample sizes (N) from which wild rats' population were collected in Peninsular Malaysia.

Locality	Latitude	Longitude	Forest structure	Sample sizes (N)
Modified forest				
Pukin Plantation, Segamat	2.700° N	102.900° E	Agricultural land with mature oil palm tree	42 (23♂, 19♀)
Paya Lang Plantation, Segamat	2.768° N	102.705° E	Agricultural land with young-mature oil palm tree	17 (10♂, 7♀)
Tambang Plantation, Segamat	2.632° N	102.716° E	Agricultural land with immature oil palm tree	6 (4♂, 2♀)
Recreational forest				
Gunung Arong Forest Eco Park, Mersing	2.550° N	103.800° E	Recreational mountain forest (273 m)	7 (2♂, 5♀)
Lubuk Yu Forest Eco Park, Pahang	3.756° N	102.652° E	Recreational forest with waterfall (200 m)	11 (6♂, 5♀)
Pulau Tioman Juara Trails, Pahang	2.823° N	104.164° E	Recreational island forest (200 m)	12 (4♂, 8♀)
Forest reserve				
Ulu Gombak Forest Reserve, Selangor	3.317° N	101.750° E	Secondary lowland dipterocarp forest (100 m)	6 (3♂, 3♀)
Ulu Muda Forest Reserve, Kedah	6.100° N	100.950° E	Primary lowland dipterocarp forest (150 m)	23 (15♂, 8♀)

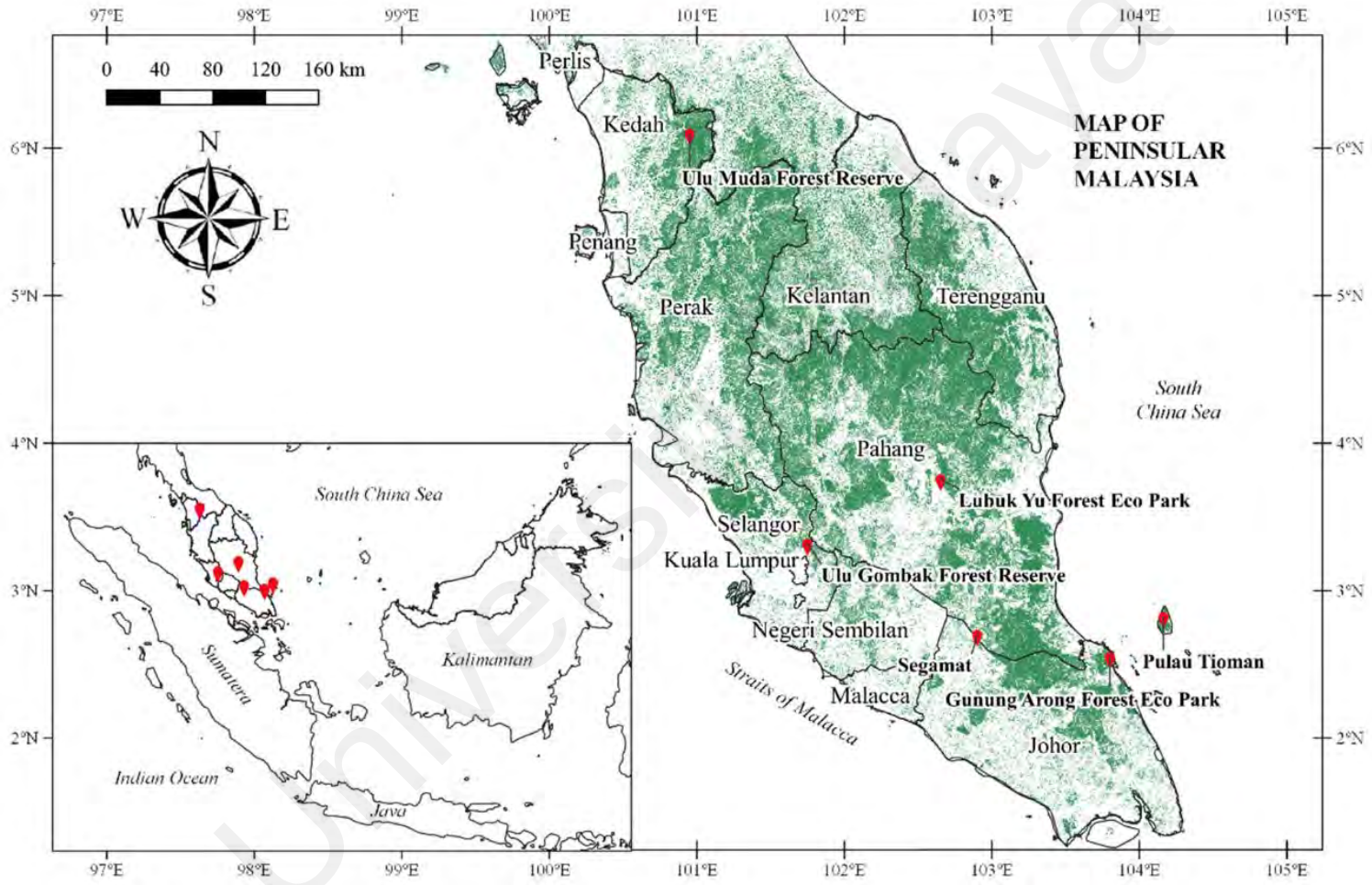


Figure 3.1: Map showing the eight sampling sites located in the states of Johor, Pahang, Selangor, and Kedah in Peninsular Malaysia as each of the location was pointed in red pointer (QGIS version 3.10.14-A Coruña).

3.3 Rat

3.3.1 Rat collections

Rats collections were carried out using single-capture live-traps known as cage-traps (27 cm × 14 cm × 14 cm). Random sampling techniques were applied as all the cages were distributed randomly in different topographies, such as along the river or stream, forest trails, vegetation, and elevation. About one hundred single door cage traps were used to catch rats in each sampling site selected in this study. Besides, physical environmental factors were encountered in the selection of trapping location, in which the cage traps should be deployed in a shaded area to avoid stress and exposure from the sunlight and predators of trapped animals such as rats. Oil palm kernel, banana with peanut butter, and salted fish were used to trap the rats. Cage traps were left overnight for four consecutive days. Trapped rodents were collected in the morning and brought to a suitable field site for the following procedure.

3.3.2 Identification of rat species

In order to identify the rat species, the morphological measurements were recorded, and their phenotypic characteristic was observed based on Francis (2008). All the sex, weight, and external measurement, including the length of head-body (HB), tail length (TL), hindfoot (HF), and ear (E), were taken as this information for morphological identification. Developing reproductive organs such as testes for males and vaginas for females can also determine whether the individual rodent was either a juvenile or maturely adult rat (Aplin *et al*., 2003).

3.3.3 Euthanasia of rat

Euthanasia was necessary for this research study as we needed to isolate endoparasites from internal organs that infected rodent hosts. All trapped rats were sacrificed by placing them inside a yellow bag containing cotton wool soaked with isoflurane. This overdose

of inhalant anaesthetics such as isoflurane was commonly applied and approved in laboratory animal medicine and veterinary practice (Seymour & Nagamine, 2016). The prerequisite of scarifying rodents was performed accordingly with the reference guidelines (American Veterinary Medical Association Council on Research, 2007). All the muscle tissue and liver samples of rats were collected and preserved in 90 % ethanol for molecular study. The rats were then labelled and preserved in 70 % ethanol as wet specimens and kept as a museum specimen in the Museum of Zoology, Universiti Malaya.

3.4 Collection and identification of fauna parasites

3.4.1 Ectoparasites

The rat was collected and placed on a white plate, where it was visually checked for ectoparasites from head to tail. The ectoparasites were removed by combing vigorously throughout the fur with a fine comb and searching using fine forceps (Herbreteau *et al*., 2011). Ticks were usually dislodged in-between the fur, including around the ear lobe, by picking with forceps as it attached firmly on the skin of host rodents. Fleas and lice on the lower and underbody of rats were quickly removed, as were chigger mites in the ears and around the anal. All ectoparasites obtained from each host were preserved in labelled vials with 70 % ethanol for further morphological analysis. The identification of ectoparasite was made to genera or species level using identification keys and illustration provided by taxonomists (Guglielmone *et al*., 2014; Mathison & Pritt, 2014; Mullen & OConnor, 2019; Tanskul & Inlao, 1989; Varma, 1993; Voltzit & Keirans, 2002; Walker *et al*., 2003; Yamaguti *et al*., 1971).

3.4.2 Autopsy and helminths collection

The rat was placed in a dissecting tray with a ventral side up position, fixed the limbs with dissecting pins, pinched the skin with forceps, and cut through from the posterior to anterior regions until the thoracic region of the rat with blunt-end scissors. The skin and

muscle body were opened, and other rat organs were exposed and examined for endoparasites. An autopsy was conducted by removing all internal organs such as the liver, lungs, heart, and whole gastrointestinal tract, including the esophagus, stomach, small intestine, large intestine, and anus. Each organ was examined for the presence of helminths worm. For example, metacestodes *Taenia* spp. and other tapeworm cysts can be found in the liver and in body cavities. Species such as *Angiostrongylus* sp. can be obtained from the lungs and Filaridae in the thoracic cavity of rats.

The body cavity of the rat was flushed with 10 % phosphate-buffered saline (PBS) to remove excess blood and scanned for endoparasite in their body. All gastrointestinal tract sections were separated accordingly into different petri dishes that contained 10 % PBS. The excessive content of the stomach and caecal was filtered for better searching of helminths. The helminths isolation and collection protocol were followed by Henttonen & Haukisalmi (2008) and Herbreteau *et al*. (2011). All the collected helminths from each rat were counted and preserved in labelled vials containing 70 % ethanol before further identification. The endoparasite helminths species found in various locations known as microhabitats were kept separately into different vials and recorded. Each vial should be carefully labelled with species of rat and the name of organ or microhabitat. Identification of cestode and nematode were done using a few identification guide book provided by (Anderson *et al.*, 2009; Bhaibulaya & Cross, 1971; Jones *et al*., 1994; Ow-Yang, 1971; Schmidt Gerald, 1934). Species such as *Angiostrongylus malaysiensis* was identified and described in Bhaibulaya & Cross (1971) as first recorded in Malaysia. The cestode vials were kept in a cool place or -20 ^{o}C refrigerators in the laboratory to prevent the disintegration and contamination of samples, especially for molecular identification purposes.

3.5 Statistical analyses

The data were recorded according to the number of species obtained and the presence or absence of ectoparasite and helminths for each rat host. Factors such as species, sex, maturity, and localities were used to determine the distribution pattern and infection rate of ectoparasites and helminth parasites. The prevalence, intensity, and abundance were calculated and assessed (Mangolis *et al*., 1982). The prevalence means and median intensity were obtained in this study.

Ectoparasite and helminth species abundance were calculated using the 'Vegan' package (Oksanen *et al*., 2013) implemented in R freeware version 3.0.1 for total individual counts of ectoparasite and helminth species detected in each host. Kruskal-Wallis chi-squared was applied for each of the forest type to obtain the differences between the host-parasite interaction. A species accumulation curve was also created using species abundance to assess rat sample size sufficiency between ectoparasite and helminth parasites by using the PAST program version 3.26 (Hammer *et al*., 2011).

To investigate host-parasite interactions, we used the 'bipartite' packages (Dormann *et al*., 2009) also implemented in R freeware version 3.0.1 to conduct bipartite network analysis of the helminth assemblage in rat hosts. Only host species with more than five individual sample sizes were considered in the analysis. The functions 'visweb' and 'plotweb' were used to visualise host-parasite interaction matrices based on absence/presence (bipartite network and nestedness matrices) that combined the three habitat types.

3.6 Cestode Molecular Identification

3.6.1 Extraction of genomic DNA

Genomic DNA from the tissue samples (a whole individual for small specimens or 1-2 proglottids for the larger specimens) of the cestode obtained from rats' host were extracted using the GF-1 Tissue DNA Extraction Kit (Vivantis Technologies Sdn. Bhd).

About 30 milligram (mg) tissue samples were weighed and put into 2 milliliters (ml) vial tubes. A total of 250 microliters (µl) buffer TL and 20 µl proteinase K were added to the sample vial. The sample vials were vortexed for about 5 seconds. Then, another 12 µl lysis enhancer was added and mixed immediately. The sample vials were incubated at 65^{o}C for 1-3 hours or overnight to ensure all the sample tissues were fully lysis into the solution. After incubation, 560 µl buffer TB was homogenized into sample vials and incubated for another 10 minutes at 65^{o}C in a water bath incubator. 200 µl of absolute ethanol was added, and the sample vials were pulse-vortexed for 5 seconds. Then, 650 µl of the sample mixture was loaded into a provided column tube and centrifuged at 5000 revolutions per minute (rpm) for one minute at 25^{o}C. The filtrates were discarded, and the spin filter was inserted back to the tube.

For the washing step, 650 µl of wash buffer was inserted into the spin filter and centrifuged at 5000 rpm for one minute, and this step was repeated twice. After the column washing, the column tube was centrifuged for one minute at 1000 rpm to dry it. The spin filter was changed with a new 1.5 ml vial tube, and the filters were discarded. 50 µl of elution buffer was added to the DNA elution stages and incubated at room temperature for two minutes. After two minutes, the sample tubes were centrifuged for one minute at 5000 rpm. Then, the extracted DNA was stored at -20^{o}C for subsequent use in polymerase chain reaction (PCR) processes.

3.6.2 Determination of DNA quality and concentration

The DNA product was analysed on 1 % agarose gel electrophoresis to verify its quality. Five µl of DNA product were combined with one µl of loading dye and placed into a 1 % agarose gel containing gel stain. The first well was then loaded with five µl of 1 kilobase (kb) DNA ladder for DNA quantification. For 30 minutes, electrophoresis was performed in 1 X TAE buffer at a voltage of 100 Volts (V) and a current of 200 milliamperes (mA). The gel was then visualised under ultraviolet (UV) light. The

NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, USA) was used to investigate the concentration of extracted DNA. Purification was determined using a 1:10 (DNA: buffer) dilution and the A260/A280 nanometres (nm) ratio value.

3.6.3 DNA amplification of polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was used to amplify the desired genes, which required thermal cycling, done with the Mastercycler® Nexus (Eppendorf North America, Inc.) since the processes included denaturing, annealing, and extending. The PCR was carried out with a set of primers from the 18SrDNA gene and previously described primers corresponding to the COX1 gene (Appendix B). For PCR, a total of 50 l reaction mixture was utilised, which included 2 µl of DNA template, 2 µl of each primer, 19µl of ultra-pure water, and 25 µl of 2X Power Taq PCR MasterMix (BioTeke, Beijing) (Appendix C). Each PCR reaction had a negative control of ultra-pure water (UPW) or double-distilled water (ddH2O). The PCR amplification profile consisted of one cycle of initial denaturation at 94 °C for 4 minutes, followed by 35 cycles of 94 °C for 30 seconds (denaturation), 47 – 54 °C for 30 seconds (annealing), and 72°C for 1 minute (extension), with a final extension at 72 °C for 10 minutes (Appendix D).

3.6.4 Gel electrophoresis

A 2 % agarose gel was used to electrophoresis the PCR product. All the chemicals needed for the gel were measured as 0.8 g of agarose powder and 40 ml of 1 X TAE buffer. Both chemicals were combined and microwaved for three minutes in a beaker or until no precipitate formed in the solution. After that, 40 ml of agarose gel mixture was stained with 4.0 µl of gel dye. The gel was put into a gel casting tray with the sample comb. The gel was left in the tray for around 40 minutes, or until it was completely solidified.

The comb was carefully removed from the gel casting tray once the gel had solidified to ensure the gel wells were in good condition. The gel tray was taken from the tray support and placed in a 1 X TAE buffer electrophoresis tank. Each gel well was added with five µl of PCR product and two µl of loading dye at the end of the gel well. The electrophoresis was carried out in a 1 X TAE buffer for approximately 35 minutes at 80 V and 180 mA. The gel was removed from the electrophoresis tank after 45 minutes. The gels were then illuminated using an ultraviolet (UV) transilluminator.

3.6.5 Purification of PCR products and DNA sequencing

The PCR products were submitted to Apical Scientific Sdn. Bhd. in Seri Kembangan, Selangor, where purification and sequencing DNA were performed. A set of forward and reverse primers was sent as required in the nucleotide sequencing procedure. The Applied Biosystems 3730 XL Genetic Analyzer was utilised to obtain the targeted gene locus using the Sanger Sequencing technique.

3.6.6 Sequence editing and alignment

Visual and computer software editing was used to edit the DNA sequences obtained. The validity of the DNA sequencing data was confirmed by looking at the chromatogram file to ensure there were no errors in the base's interpretation supplied by the sequencing machines. Both forward and reverse sequences were edited and aligned using MEGAX (Molecular Evolutionary Genetics Analysis Version X) (Kumar *et al*., 2018). For protein-coding genes such as COX1, the stop codon (*) was checked in the translated protein sequences. The altered sequences were matched with similar sequence searches using the GenBank BLAST application. Using the MEGAX ClustalW multiple alignment algorithms, all sequences were aligned with the selected DNA sequences, and an outgroup sequence was retrieved from GenBank. The sequences discovered in this study were all deposited in the GenBank database (Appendix F).

3.6.7 Sequence and phylogenetic analysis

The 18SrDNA and COX1 sequences of the cestode worm were downloaded from GenBank and compared to the sequences generated in this study. The list of reference sequences used in the analysis is shown in Appendix E. The ClustalW multiple alignment methods of MEGAX were used to align the nucleotide sequences of cestode worms available from GenBank and those collected from gastrointestinal tracts, body cavity, and liver following the editing procedure. MEGAX software was used to check the nucleotide composition percentage, sequence divergences, and pairwise analyses calculated using the Kimura 2-Parameter (K2P) algorithm model.

The construction of a phylogenetic tree is a part of phylogenetic analysis. Neighbour-joining tree (NJ) and maximum-likelihood tree (ML) was used to construct the phylogenetic trees. The phylogenetic tree for all cestode worms was constructed using MEGAX. In NJ and ML tree construction, the K2P model was utilised with a bootstrap value of 1000.

DNA sequence polymorphism version 6.12.03 (DnaSP v6) was used to create haplotype data within and between species. A minimum-spanning network (MSN) was generated using Network 10.2.0.0 (Bandelt *et al*., 1999) to show the haplotype connections. A minimum spanning network was built for each species, and the haplotype was identified.

CHAPTER 4:RESULTS

4.1 Distribution and population of wild rats in Peninsular Malaysia

One hundred twenty-four wild rats were successfully captured from several type of forest in Peninsular Malaysia. All rats were caught from six locations of four states in Peninsular Malaysia, i.e., Johor, Pahang, Selangor, and Kedah. These locations cover forest reserves, recreational forests, and modified forests. Ten species of rats caught from the study sites were identified following Aplin *et al*. (2003) and Francis (2008) which are all known as *Leopoldamys sabanus*, *Maxomys surifer*, *Maxomys* sp., *Maxomys whiteheadi*, *Niviventer cremoriventer*, *Niviventer fulvescens*, *Rattus argentiventer*, *Rattus rattus diardii*, *Rattus tiomanicus*, and *Sundamys muelleri.* These species are divided into two groups: forest species *L. sabanus* (N = 9), *Maxomys* sp. (N = 1), *M. surifer* (N = 24), *M. whiteheadi* (N = 6), *N. cremoriventer* (N = 2), *N. fulvescens* (N = 2), and *S. muelleri* (N = 5) and commensal rat species (*R. argentiventer* (N = 12), *R. rattus diardii* (N = 7), *R. tiomanicus* (N = 56).

Rattus tiomanicus (N = 56, 45.2 %) was the most common rat species (Table 4.1). Male rats (N = 65, 54.03 %) were numerous than female rats (N = 57, 45.97 %), with adults (N = 108, 87.2 %) more numbers than juveniles (N = 12.8 %) (Table 4.1). In Ulu Muda Forest Reserve, six forest species were caught as listed: *M. surifer* (N = 10, 43.5 %), *M. whiteheadi* (N = 6, 26.1 %), *N. cremoriventer* (N = 2, 8.7 %), *N. fulvescens* (N = 2, 8.7 %), *R. tiomanicus* (N = 2, 8.7 %), and *S. muelleri* (N = 1, 4.3 %). Male rats were captured in more significant numbers than female rats, with 15 males and 8 females captured. There were fewer juveniles (N = 3, 13 %) captured than adults (N = 20, 87 %) based on host age.

Sundamys muelleri (N = 4, 66.7 %) and *L. sabanus* (N = 2, 33.3 %) were among the six rats captured from the Ulu Gombak Forest Reserve. Male and female rats were found

in three equal numbers in both sexes. They were all adult rats (N = 6, 100 %), according to host age.

Twelve wild rats were caught in the predominantly forested region of Pulau Tioman. The most common species was *L. sabanus* (50.0 %), followed by *R. tiomanicus* (33.3 %) and *M. surifer* (16.7 %). Females (N = 8) were collected more significantly than males (N = 4). No juveniles were found because all the murid rats (N = 12) were adults.

Lubuk Yu and Gunung Arong were two separate sites in the forest eco-park. *Maxomys surifer* (N = 5, 45.5 %), *R. tiomanicus* (N = 3, 27.3 %), *L. sabanus*, *Maxomys* sp., and *R. rattus diardii* each had one individual in Lubuk Yu (9.1 %). Furthermore, only *Maxomys surifer* (N = 7, 100 %) was successfully obtained on Gunung Arong. Only adult rats, comprising 18 individuals, were caught in both forest eco-parks.

Besides, in the modified forest, the rat population comprised of wild rats from species of *R. tiomanicus* (N = 47, 72.3 %), *R. argentiventer* (N = 12, 18.5 %), and *R. rattus diardii* (N = 6, 9.2 %). Male rats dominated female rats slightly differently, with 37 males and 28 females. There were fewer juveniles (N = 12, 18.5 %) than adults (N = 53, 81.5 %) based on host age.

Table 4.1 summarized the general rat population by host sex and host age. Male and female rats were captured in nearly equal numbers, with adults vastly outnumbering juveniles. Table 4.2 summarizes the rat population in Peninsular Malaysia based on sampling sites. Meanwhile, as shown in Table 4.3, more adults were captured than juveniles across all sites.

Table 4.1: The wild rat population according to host sex and host age in Peninsular Malaysia.

Rat species	Host sex		Host age	
	Male	Female	Adult	Juvenile
L. sabanus	3	6	8	1
Maxomys sp.	1	0	1	0
M. surifer	10	14	23	1
M. whiteheadi	5	1	4	2
N. cremoriventer	2	0	2	0
N. fluvescens	1	1	2	0
R. argentiventer	3	9	11	1
R. rattus diardii	5	2	6	1
R. tiomanicus	35	21	46	10
S. muelleri	2	3	5	0
TOTAL	67	57	108	16

Table 4.2: The wild rat population according to host sex in each location.

	Location											
	UMFR		UGFR		PTP		LYFEP		GAFEP		SGT	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
L. sabanus	-	-	1	1	2	4	0	1	-	-	-	-
Maxomys sp.	-	-	-	-	-	-	1	0	-	-	-	-
M. surifer	5	5	-	-	2	0	1	4	2	5	-	-
M. whiteheadi	5	1	-	-	-	-	-	-	-	-	-	-
N. cremoriventer	2	0	-	-	-	-	-	-	-	-	-	-
N. fluvescens	1	1	-	-	-	-	-	-	-	-	-	-
R. argentiventer	-	-	-	-	-	-	-	-	-	-	3	9
R. rattus diardii	-	-	-	-	-	-	1	0	-	-	4	2
R. tiomanicus	2	0	-	-	0	4	3	0	-	-	30	17
S. muelleri	0	1	2	2	-	-	-	-	-	-	-	-
TOTAL	15	8	3	3	4	8	6	5	2	5	37	28
	23		6		12		11		7		65	

Notes: UMFR (Ulu Muda Forest Reserve), UGFR (Ulu Gombak Forest Reserve), PTP (Pulau Tioman Pahang), LYFEP (Lubuk Yu Forest Eco Park), GAFEP (Gunung Arong Forest Eco Park), SGT (Segamat), M (male), F (female).

Table 4.3: The wild rat population according to host age in each location.

	Location											
	UMFR		UGFR		PTP		LYFEP		GAFEP		SGT	
Host age	A	J	A	J	A	J	A	J	A	J	A	J
L. sabanus	-	-	2	0	6	0	1	0	-	-	-	-
Maxomys sp.	-	-	-	-	-	-	1	0	-	-	-	-
M. surifer	9	1	-	-	2	0	5	0	7	0	-	-
M. whiteheadi	4	2	-	-	-	-	-	-	-	-	-	-
N. cremoriventer	2	0	-	-	-	-	-	-	-	-	-	-
N. fluvescens	2	0	-	-	-	-	-	-	-	-	-	-
R. argentiventer	-	-	-	-	-	-	-	-	-	-	10	2
R. rattus diardii	-	-	-	-	-	-	1	0	-	-	5	1
R. tiomanicus	2	0	-	-	4	0	3	0	-	-	38	9
S. muelleri	1	0	4	0	-	-	-	-	-	-	-	-
TOTAL	20	3	6	0	12	0	11	0	7	0	53	12
	23		6		12		11		7		65	

Notes: UMFR (Ulu Muda Forest Reserve), UGFR (Ulu Gombak Forest Reserve), PTP (Pulau Tioman Pahang), LYFEP (Lubuk Yu Forest Eco Park), GAFEP (Gunung Arong Forest Eco Park), SGT (Segamat), A (adult), J (juvenile).

4.2 Distribution pattern of parasite fauna in wild rat populations

4.2.1 Frequency distribution of ectoparasite infestation

Eighty-six wild rats (69.35 %) were diagnosed infested with ectoparasites out of a total of 124 collected in three types of forests. Three tick species (*Ambylomma* sp., *Haemaphysalis* sp., and *Ixodes granulatus*) (N = 52, 41.94 %), one mite (*Laelaps* spp.) (N = 63, 50.81 %), one chigger (*Leptotrombidium* sp.) (N = 7, 5.65 %), and two lice (*Haplopleura pacifica* and *Polyplax spinulosa*) (N = 14, 11.29 %) were discovered in this study. Each rats was infected with at least one or a maximum of four types of ectoparasite at the same time. Forty-five wild rats (36.29 %) infected with a single or multiple (N = 41, 33.06 %) ectoparasites infestation.

According to the host species, four groups of ectoparasites were discovered in rats: ticks, mesostigmatids mite, chigger's mite, and lice (Table 4.4) . Among the rats captured in Peninsular Malaysia, *R. tiomanicus* had the lowest prevalence. *Leopaldamys sabanus* and *R. tiomanicus*, on the other hand, were discovered to be infested by all seven ectoparasite species (Table 4.5). Most forest rat species from genera such as *Leopoldamys*, *Maxomys*, *Niviventer*, and *Sundamys* were infested 100 % with ectoparasite. Meanwhile, the highest infestation from genus *Rattus* was shown in *R. argentiventer* (69.23 %).

Table 4.4: Ectoparasite infestation rate according to host species based on ectoparasites group.

Rat species	n	Examined +ve with ectoparasite (%)	Number of hosts infested by ectoparasites group			
			Ticks X (%)	Mesostigmatids X (%)	Chiggers X (%)	Lice X (%)
L. sabanus	9	9 (100)	7 (77.8)	6 (66.7)	1 (11.1)	4 (57.1)
Maxomys sp.	1	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)
M. surifer	24	23 (95.8)	13 (54.2)	23 (95.8)	0 (0)	0 (0)
M. whiteheadi	6	6 (100)	2 (33.3)	6 (100)	0 (0)	0 (0)
N. cremoriventer	2	2 (100)	1 (50)	2 (100)	0 (0)	0 (0)
N. fluvescens	2	2 (100)	2 (100)	2 (100)	0 (0)	0 (0)
R. argentiventer	12	8 (66.7)	3 (25)	3 (25)	1 (8.3)	2 (16.7)
R. rattus diardii	7	4 (57.1)	4 (57.1)	1 (14.3)	0 (0)	1 (14.3)
R. tiomanicus	56	26 (46.4)	16 (28.6)	14 (25)	5 (8.9)	5 (8.9)
S. muelleri	5	5 (100)	4 (80)	5 (100)	0 (0)	2 (40)
TOTAL	124	86 (69.4)	52 (41.9)	63 (50.8)	7 (5.6)	14 (11.3)

Notes: X: Number of positive infected rats; (%): Prevalence of ecto infestation; n: Total number of rats examined according to species.

Table 4.5: Ectoparasite infestation rate based on host species in Peninsular Malaysia.

Rat (n)	Number of infested rats (% prevalence)				
	0	1	2	3	4
L. sabanus (9)	0	33.33	22.22	22.22	22.22
Maxomys sp. (1)*	0	100	0	0	0
M. surifer (24)	4.17	41.67	37.50	16.67	0
M. whiteheadi (6)	0	83.33	16.67	0	0
N. cremoriventer (2)*	0	50	50	0	0
N. fulvescens (2)*	0	0	100	0	0
R. argentiventer (12)	33.33	50	16.67	0	0
R. rattus diardii (7)	42.86	14.29	42.86	0	0
R. tiomanicus (56)	53.57	30.36	12.50	1.79	1.79
S. muelleri (5)	0	20	20	40	20
Total (124)	30.65	36.29	22.58	7.26	3.23

*Rat species had a small sample size (< 5).

Table 4.6 shows the infestation data for wild rats harboured in Peninsular Malaysia according to study sites. Twenty-nine rats (100 %) were infested with ectoparasites in the forest reserve. All forest rats were shown to have ectoparasite infestations. Each infested rat harboured a minimum of one and a maximum of four ectoparasite species. The equivalently same number of rats harboured a single and double species of ectoparasite (Table 4.7).

Table 4.6: Ectoparasite infestation rate in wild rat population according to sites.

Sites		Number of ectoparasites on host					Total
		0	1	2	3	4	
UMFR	No. of host	0	11	10	2	0	23
	Prevalence (%)	0	47.83*	43.48	8.70	0	100
UGFR	No. of host	0	1	2	2	1	6
	Prevalence (%)	0	16.67	33.33*	33.33*	16.67	100
PTP	No. of host	1	4	2	2	3	12
	Prevalence (%)	8.33	33.33*	16.67	16.67	25.00	100
LYFEP	No. of host	2	1	5	3	0	11
	Prevalence (%)	18.18	9.10	45.45*	27.27	0	100
GAFEP	No. of host	1	6	0	0	0	7
	Prevalence (%)	14.29	85.71*	0	0	0	100
SGT	No. of host	34	22	9	0	0	65
	Prevalence (%)	52.31*	33.85	13.85	0	0	100

Notes: UMFR, Ulu Muda; UGFR, Ulu Gombak; PTP, Pulau Tioman; LYFEP, Lubuk Yu; GAFEP, Gunung Arong; SGT, Segamat. (*) indicate the highest prevalence of infestation by sites.

Table 4.7: Ectoparasite infestation rate harboured from wild rat populations in forest reserves.

Host species		Number of ectoparasites on host					Total
		0	1	2	3	4	
*L. sabanus**	No. of host	0	1	1	0	0	2
	Prevalence (%)	0	50.00	50.00	0	0	100
M. surifer	No. of host	0	2	6	2	0	10
	Prevalence (%)	0	20.00	60.00	20.00	0	100
M. whiteheadi	No. of host	0	5	1	0	0	6
	Prevalence (%)	0	83.33	16.67	0	0	100
*N. cremoriventer**	No. of host	0	1	1	0	0	2
	Prevalence (%)	0	50.00	50.00	0	0	100
*N. fulvescens**	No. of host	0	0	2	0	0	2
	Prevalence (%)	0	0	100.00	0	0	100
*R. tiomanicus**	No. of host	0	2	0	0	0	2
	Prevalence (%)	0	100.00	0	0	0	100
S. muelleri	No. of host	0	1	1	2	1	5
	Prevalence (%)	0	20.00	20.00	40.00	20.00	100

*Rat species had a small sample size (< 5).

From a total of 30 rats captured in the recreational forest, 86.67 % of rats were infested with a minimum of one and a maximum of four species of ectoparasite species. Major infestations were shown in *L. sabanus* (100 %), followed by *M. surifer*. Most rats harboured either one or two ectoparasites species (Table 4.8).

Table 4.8: Ectoparasite infestation rate harboured from wild rat populations in recreational forests.

Host		Number of ectoparasites on host					Total
		0	1	2	3	4	
L. sabanus	No. of host	0	2	1	2	2	7
	Prevalence (%)	0	28.57	14.29	28.57	28.57	100
Maxomys sp.*	No. of host	0	1	0	0	0	1
	Prevalence (%)	0	100.00	0	0	0	100
M. surifer	No. of host	1	8	3	2	0	14
	Prevalence (%)	7.14	57.14	21.43	14.29	0	100
*R. rattus diardii**	No. of host	0	0	1	0	0	1
	Prevalence (%)	0	0	100	0	0	100
R. tiomanicus	No. of host	3	0	2	1	1	7
	Prevalence (%)	42.86	0	28.57	14.29	14.29	100

*Rat species had a small sample size (< 5)

In modified forests such as agricultural land, 31 out of 65 rats captured (47.69 %) were infested with ectoparasites with *R. argentiventer* (69.23 %), the highest infestation compared to *R. rattus diardii* and *R. tiomanicus.* Most agricultural land rats harboured one or two ectoparasite species (Table 4.9).

Table 4.9: Ectoparasite infestation rate harboured from wild rat populations in modified forest.

Host		Number of ectoparasites on host					Total
		0	1	2	3	4	
R. argentiventer	No. of host	4	7	2	0	0	13
	Prevalence (%)	30.77	53.85	15.38	0	0	100
R. rattus diardii	No. of host	3	1	2	0	0	6
	Prevalence (%)	50.00	16.67	33.33	0	0	100
R, tiomanicus	No. of host	27	14	5	0	0	46
	Prevalence (%)	60.87	28.26	10.87	0	0	100

* Rat species had a small sample size (< 5).

In terms of host sex, there was no massive difference between the male and female, with a similar number of 48.84 % and 51.16 %, respectively. More *R. tiomanicus* males (60 %) were captured infested with single ectoparasites species. All *L. sabanus* were infested with ectoparasites as most females (57.89 %) found had a minimum of one and a maximum of four ectoparasite species (Figure 4.10).

Table 4.10: Ectoparasite infestation rate in wild rat populations according to host sex.

Rat (n)		Number of ectoparasites on host					Total host
	Prevalence (%)	0	1	2	3	4	
L. sabanus (9)	♂	0	66.67	0	0	33.33	3
	♀	0	16.67	33.33	33.33	16.67	6
Maxomys sp. (1)*	♂	0	100	0	0	0	1
	♀	0	0	0	0	0	0
M. surifer (24)	♂	10	30	50	10	0	10
	♀	0	50	28.57	21.43	0	14
M. whiteheadi (6)	♂	80	20	0	0	0	5
	♀	100	0	0	0	0	1
N. cremoriventer (2)*	♂	0	50	50	0	0	2
	♀	0	0	0	0	0	0
N. fulvescens (2)*	♂	0	0	100	0	0	1
	♀	0	0	100	0	0	1
R. argentiventer (12)	♂	33.33	0	66.67	0	0	3
	♀	33.33	66.67	0	0	0	9
R. rattus diardii (7)	♂	40	0	60	0	0	5
	♀	50	50	0	0	0	2
R. tiomanicus (56)	♂	57.14	34.29	8.57	0	0	35
	♀	47.62	23.82	19.05	4.76	4.76	21
S. muelleri (5)	♂	0	0	0	100	0	2
	♀	0	33.33	33.33	0	33.33	3

*Rat species had a small sample size (< 5).

In terms of host ages, adult rats were highly infested (91.86 %) with ectoparasite with mostly single infestation. A high number of adult *L. sabanus*, *M. surifer*, and *R. tiomanicus* were infested with ectoparasites compared to juveniles (100 %, 92.31 %, and 92 %, respectively). Table 4.11 showed the infestation rate of ectoparasites in the wild rat population according to host age.

Table 4.11 Ectoparasite infestation rate in wild rat populations according to host age.

Rat (n)	Prevalence (%)	Number of ectoparasites on host					Total host
		0	1	2	3	4	
L. sabanus (9)	Adult	0	33.33	22.22	22.22	22.22	9
	Juvenile	0	0	0	0	0	0
Maxomys sp. (1)*	Adult	0	100	0	0	0	1
	Juvenile	0	0	0	0	0	0
M. surifer (24)	Adult	4.35	39.13	39.13	17.39	0	23
	Juvenile	0	100	0	0	0	1
M whiteheadi (6)	Adult	0	75	25	0	0	4
	Juvenile	0	100	0	0	0	2
N. cremoriventer (2)*	Adult	0	50	50	0	0	2
	Juvenile	0	0	0	0	0	0
N. fulvescens (2)*	Adult	0	0	100	0	0	2
	Juvenile	0	0	0	0	0	0
R. argentiventer (12)	Adult	33.33	50	16.67	0	0	10
	Juvenile	0	0	0	0	0	2
R. rattus diardii (7)	Adult	40	20	40	0	0	6
	Juvenile	50	0	50	0	0	1
R. tiomanicus (56)	Adult	50	32.61	15.22	2.17	0	47
	Juvenile	70	20	0	0	10	9
S. muelleri (5)	Adult	0	20	20	40	20	5
	Juvenile	0	0	0	0	0	0

*Rat species had a small sample size (< 5).

4.2.2 Frequency distribution of helminth parasite infection

Thirty-nine wild rats (31.45 %) were discovered to be infected with endoparasites helminths from a total of 124 wild rats captured in the forest reserve, recreational forest, and modified forest. Each infected wild rat harboured a minimum of and a maximum of five one endoparasite helminth.

In wild rats, two types of endoparasites helminth were discovered: nematodes and cestodes (Table 4.12). In total, fifteen species were recovered from this study, including eleven nematode species (22.58 %), *Ancylostoma brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria* sp., *Gongylonema* sp., *Heterakis spumosa*, *Mastophorus muris*, *Nippostrongylus brasiliensis*., *Protospirura muris*, *Syphacia muris*, *Syphacia obvelata*, and *Trichuris muris* and four species of cestodes (18.55 %), *Hy. parva*, *Hy. taeniaeformis*, *Raillietina* sp. and *Hymenolepis diminuta*. The accounted of some species such as *Angiostrongylus malaysiensis* in host lungs and *Ancylostoma brasiliensis* in host intestinal were identified using identification keys by Anderson *et al.* (2009). Research done by Lim *et al.* (1976) was recorded *An. malaysiensis* as one of the commonly found nematodes in rat host. Besides, genus *Ancylostoma* was a hookworm species that attached to the intestinal wall of host species Reynoldson *et al.* (1997).

Table 4.12: Endoparasites helminth infection rate according to host species based on helminth group.

Rat species	n	Examined +ve (%)	Number of hosts infected	
			Nematode X (%)	Cestode X (%)
L. sabanus	9	6 (66.67)	3 (33.33)	5 (55.56)
Maxomys sp.	1	0 (0)	0 (0)	0 (0)
M. surifer	24	6 (25.0)	6 (25.0)	1 (4.17)
M. whiteheadi	6	1 (16.67)	1 (16.67)	1 (16.67)
N. cremoriventer	2	2 (100)	2 (100)	1 (50)
N. fluvescens	2	2 (100)	2 (100)	1 (50)
R. argentiventer	12	3 (25)	3 (25)	2 (16.67)
R. rattus diardii	7	3 (42.86)	2 (28.57)	1 (14.29)
R. tiomanicus	56	13 (23.21)	6 (10.71)	10 (17.86)
S. muelleri	5	3 (60)	3 (60)	1 (20)
TOTAL	124	39 (31.45)	28 (22.58)	23 (18.55)

Notes: X: Number of positive infected rats; (%): Prevalence of helminth infection; n: Total number of rats examined according to species.

Most wild rats were captured with a single endoparasite species (58.97 %), followed by two, three, four, and five endoparasite species, respectively. The frequency distribution of endoparasite helminths in the wild rat population of Peninsular Malaysia is represented in Table 4.13. *Leopoldamys sabanus* had the highest infection rate (66.67 %), followed by *S. muelleri* (60 %) and *R. rattus diardii* (42.86 %).

Table 4.13: Endoparasite helminth infection rate based on host species in Peninsular Malaysia.

Host species (n)	Number of endoparasites on host (%)					
	0	1	2	3	4	5
L. sabanus (9)	33.33	33.33	11.11	11.11	0	11.11
Maxomys sp. (1)*	100	0	0	0	0	0
M. surifer (24)	75	16.67	8.33	0	0	0
M. whiteheadi (6)	83.33	0	16.67	0	0	0
N. cremoriventer (2)*	0	50	50	0	0	0
N. fulvescens (2)*	0	50	50	0	0	0
R. argentiventer (12)	75	8.33	8.33	0	8.33	0
R. rattus diardii (7)	57.14	42.86	0	0	0	0
R. tiomanicus (56)	76.79	16.07	5.36	1.79	0	0
S. muelleri (5)	40	20	40	0	0	0
Total (124)	68.55	18.55	9.68	1.61	0.81	0.81

*Host species had a small sample size (< 5).

Table 4.14 summarises the infection rates of endoparasites helminths by the site. Lubuk Yu Forest Eco Park had the highest endoparasite helminth infection (63.64 %), followed by Ulu Muda Forest Reserve (43.48 %).

Table 4.14: Endoparasite helminth infection rate in wild rat population according to sites.

Sites		Number of endoparasites						Total
		0	1	2	3	4	5	
UMFR	No. of host	13	5	5	0	0	0	23
	Prevalence (%)	56.52	21.74	21.74	0	0	0	100
UGFR*	No. of host	1	1	3	0	0	1	6
	Prevalence (%)	1.67	1.67	50	0	0	1.67	100
PTP	No. of host	7	4	1	0	0	0	12
	Prevalence (%)	58.33	33.33	8.33	0	0	0	100
LYFEP	No. of host	4	5	1	1	0	0	11
	Prevalence (%)	36.36	45.45	9.10	9.10	0	0	100
GAFEP*	No. of host	7	0	0	0	0	0	7
	Prevalence (%)	100	0	0	0	0	0	100
SGT	No. of host	53	8	2	1	1	0	65
	Prevalence (%)	81.54	12.31	3.08	1.54	1.54	0	100

*Sites had a small sample size (< 10).

In two forest reserves, Ulu Muda Forest Reserve (UMFR) and Ulu Gombak Forest Reserve (UGFR), a total of 15 rats (51.72 %) were found infected with endoparasite helminth. *Sundamys muelleri* was found to have the highest infection rate (60 %). Each infected wild rat carried between one and five endoparasite helminth species. Most wild

rats were found to be infected with two species, while only *L. sabanus* was found to be infected with five species (Table 4.15).

Table 4.15: Endoparasite helminth infection rate harboured from wild rat populations in forest reserves.

Host species		Number of endoparasites						Total
		0	1	2	3	4	5	
*L. sabanus**	No. of host	0	0	1	0	0	1	2
	Prevalence (%)	0	0	50	0	0	50	100
M. surifer	No. of host	7	2	1	0	0	0	10
	Prevalence (%)	70	20	10	0	0	0	100
M. whiteheadi	No. of host	5	0	1	0	0	0	6
	Prevalence (%)	83.33	0	16.67	0	0	0	100
*N. cremoriventer**	No. of host	0	1	1	0	0	0	2
	Prevalence (%)	0	50*	50*	0	0	0	100
*N. fulvescens**	No. of host	0	1	1	0	0	0	2
	Prevalence (%)	0	50*	50*	0	0	0	100
*R. tiomanicus**	No. of host	0	1	1	0	0	0	2
	Prevalence (%)	0	50*	50*	0	0	0	100
S. muelleri	No. of host	2	1	2	0	0	0	5
	Prevalence (%)	40	20	40	0	0	0	100

*Host species had a small sample size (< 5).

Twelve (40 %) of the thirty wild rats captured in recreational forests were infected with a minimum of one and a maximum of three endoparasite helminth species. *Leopoldamys sabanus* and *R. tiomanicus* had the same infection rate (57.14 %), whereas *M. surifer* had only 21.43 %. Most rats were parasitized by a single endoparasite helminth species (Table 4.16).

Table 4.16: Endoparasite helminth infection rate harboured from rat populations in recreational forests.

Host species		Number of endoparasites						Total
		0	1	2	3	4	5	
L. sabanus	No. of host	3	3	0	1	0	0	7
	Prevalence (%)	42.86	42.86	0	14.29	0	0	100
Maxomys sp.*	No. of host	1	0	0	0	0	0	1
	Prevalence (%)	100*	0	0	0	0	0	100
M. surifer	No. of host	11	2	1	0	0	0	14
	Prevalence (%)	78.57	14.29	7.14	0	0	0	100
*R. rattus diardii**	No. of host	0	1	0	0	0	0	1
	Prevalence (%)	0	100*	0	0	0	0	100
R. tiomanicus	No. of host	3	3	1	0	0	0	7
	Prevalence (%)	42.86	42.86	14.29	0	0	0	100

*Host species had a small sample size (< 5).

Only 12 wild rats (18.46 %) captured in the modified forest were infected with endoparasite helminths. A single endoparasite helminth species primarily parasitizes the majority of wild rats. *Rattus rattus diardii* had the highest infection rate (N = 2, 33.33 %), followed by *R. argentiventer* (N = 3, 25 %) and *R. tiomanicus* (N = 7, 14.89 %). Each

wild rat infected comprised a minimum of one and a maximum of four species of endoparasite helminth (Table 4.17).

Table 4.17: Endoparasite helminth infection rate harboured from rat populations in modified forest.

Host species		Number of endoparasites						Total
		0	1	2	3	4	5	
R. argentiventer	No. of host	9	1	1	0	1	0	12
	Prevalence (%)	75.0	8.33	8.33	0	8.33	0	100
R. rattus diardii	No. of host	4	2	0	0	0	0	6
	Prevalence (%)	66.67	33.33	0	0	0	0	100
R. tiomanicus	No. of host	40	5	1	1	0	0	47
	Prevalence (%)	85.11	10.64	2.13	2.13	0	0	100

*Host species had a small sample size (< 5).

Males and females were infected with endoparasite helminths at similar infection rates, 31.34 % and 31.58 %, respectively. Both sexes are parasitized by one or two different endoparasite helminth species. Male *Maxomys* sp. and female *M. whiteheadi* were not infected with endoparasite helminth species. As shown in Table 4.18, the endoparasite helminth infection rate varies by host sex in the wild rat population.

Table 4.18: Endoparasite helminth infection rate in wild rat populations according to host sex.

Host species (n)	Sex	Number of endoparasites on host (%)						Total host
		0	1	2	3	4	5	
L. sabanus (9)	♂	66.67	0	33.33	0	0	0	3
	♀	16.67	50	0	16.67	0	16.67	6
Maxomys sp. (1)	♂	100	0	0	0	0	0	1
	♀	0	0	0	0	0	0	0
M. surifer (24)	♂	80	20	0	0	0	0	10
	♀	71.43	14.29	14.29	0	0	0	14
M. whiteheadi (6)	♂	80	0	20	0	0	0	5
	♀	100	0	0	0	0	0	1
N. cremoriventer (2)	♂	0	50	50	0	0	0	2
	♀	0	0	0	0	0	0	0
N. fulvescens (2)	♂	0	100	0	0	0	0	1
	♀	0	0	100	0	0	0	1
R. argentiventer (12)	♂	33.33	0	33.33	0	33.33	0	3
	♀	88.89	11.11	0	0	0	0	9
R. rattus diardii	♂	60	40	0	0	0	0	5
	♀	50	50	0	0	0	0	2
R. tiomanicus (56)	♂	74.29	20	5.71	0	0	0	35
	♀	80.95	9.52	4.76	4.76	0	0	21
S. muelleri (5)	♂	50	50	0	0	0	0	2
	♀	33.33	0	66.67	0	0	0	3

Adult wild rats were found to have a highly infected than juveniles with a single or double endoparasite helminth species. Only juvenile *R. rattus diardii* (100 %) and *R. tiomanicus* (33.33 %) were found to be infected with a single endoparasite helminth species, however. As shown in Table 4.19, the endoparasite helminth infection rate varies according to host age in the wild rat populations.

Table 4.19 Endoparasite helminth infection rate in wild rat populations according to host age.

Host species (n)	Age	Number of endoparasites on host (%)						Total host
		0	1	2	3	4	5	
L. sabanus (9)	Adult	33.33	33.33	11.11	11.11	0	11.11	9
	Juvenile	0	0	0	0	0	0	0
Maxomys sp. (1)	Adult	100	0	0	0	0	0	1
	Juvenile	0	0	0	0	0	0	0
M. surifer (24)	Adult	73.91	17.39	8.70	0	0	0	23
	Juvenile	100	0	0	0	0	0	1
M. whiteheadi (6)	Adult	75	0	25	0	0	0	4
	Juvenile	100	0	0	0	0	0	2
N. cremoriventer (2)	Adult	0	50	50	0	0	0	2
	Juvenile	0	0	0	0	0	0	0
N. fulvescens (2)	Adult	0	50	50	0	0	0	2
	Juvenile	0	0	0	0	0	0	0
R. argentiventer (12)	Adult	70	10	10	0	10	0	10
	Juvenile	100	0	0	0	0	0	2
R. rattus diardii (7)	Adult	66.67	33.33	0	0	0	0	6
	Juvenile	0	100	0	0	0	0	1
R. tiomanicus (56)	Adult	80.85	12.77	6.38	2.13	0	0	47
	Juvenile	66.67	33.33	0	0	0	0	9
S. muelleri (5)	Adult	40	20	40	0	0	0	5
	Juvenile	0	0	0	0	0	0	0

4.2.3 Host-parasite network analysis

The accumulation curve of ectoparasite and endoparasite helminth species increased steadily in the early phase and began to be in the plateau when 40 and 120 rodents were examined respectively, indicating that an adequate sample size had been achieved (Figure 4.1 and Figure 4.2).

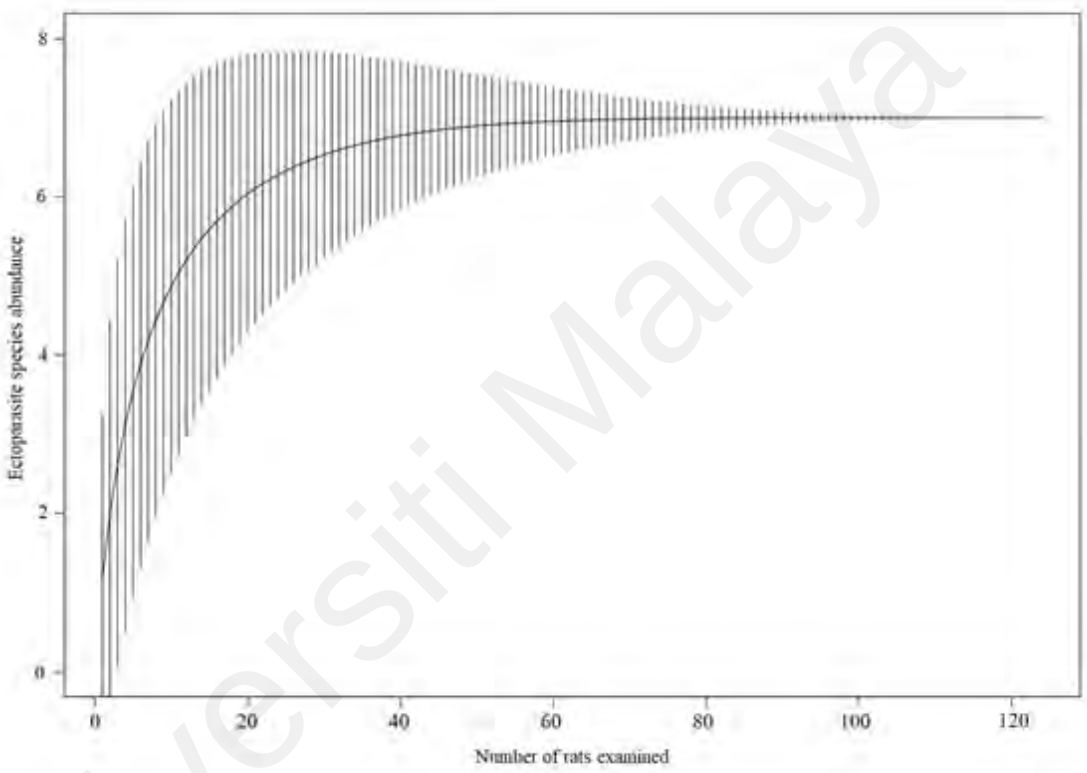


Figure 4.1: Species accumulation curve of ectoparasite recovered from 124 rat hosts.

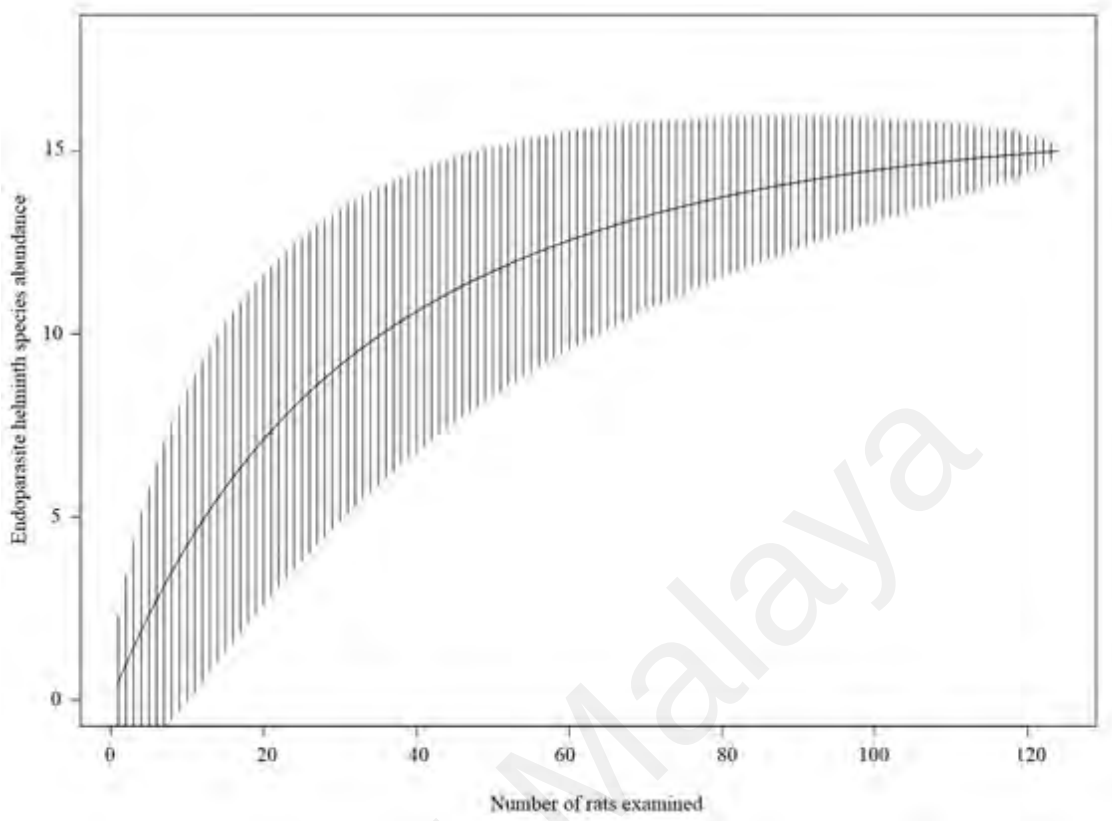


Figure 4.2: Species accumulation curve of endoparasite helminth recovered from 124 rat hosts.

Prevalence of ectoparasite infestation according to forest types was 51.72 % in forest reserve followed by recreational forest and modified forest (Kruskal-Wallis chi-squared = 0.58703, df = 2, p-value = 0.7456). Meanwhile, infection for endoparasite helminth based on forest type was 25% in the modified forest followed by recreational forest and forest reserve (Kruskal-Wallis chi-squared = 0.58503, df = 2, p-value = 0.7464). Figure 4.3 and 4.4 showed the mean abundance of fauna parasites in three different forest types in Peninsular Malaysia.

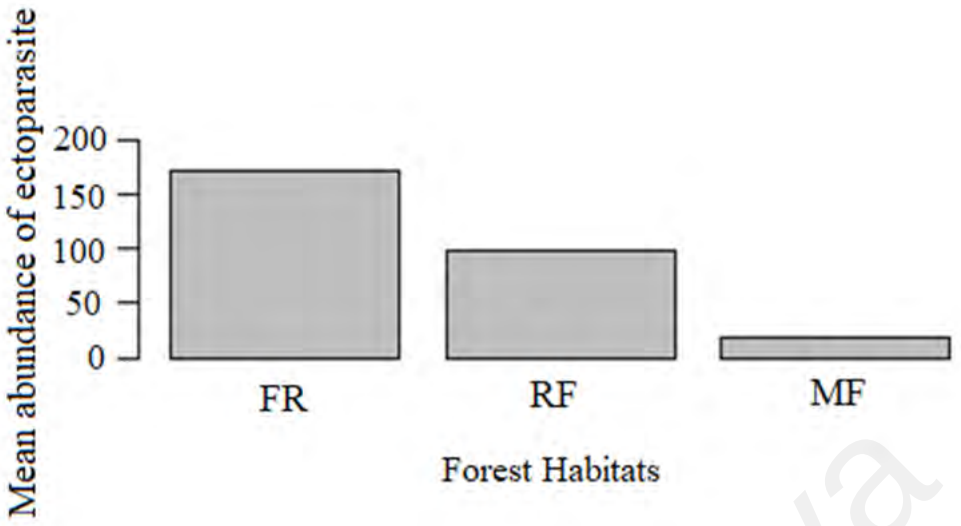


Figure 4.3: Analysis of differences in mean of ectoparasite abundance found at the host individual among the three forest habitats; FR (Forest Reserve); RF (Recreational Forest); MF (Modified Forest).

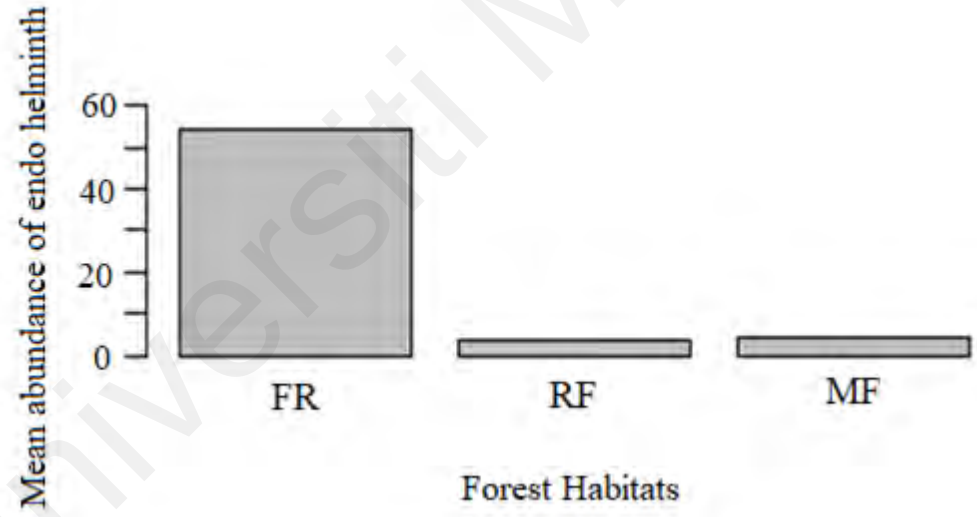


Figure 4.4: Analysis of differences in mean of endoparasite helminth abundance foumd at the host individual among the three forest habitats; FR (Forest Reserve); RF (Recreational Forest); MF (Modified Forest).

We performed bipartite network analysis on the assesmblages of wild rats and fauna parasites in Peninsular Malaysia (Figure 4.5 and Figure 4.6, respectively). *Leopoldamys sabanus* and *R. tiomanicus* were placed to the top rows of the bipartite matrix because they harboured large communities of ectoparasite species. Alternatively, the ectoparasites *Haemaphysalis* sp. and *Laelaps* sp. were placed on the right-most side

of the matrix and were found in most of host species; the two ectoparasite species on the left-side infected three host species: *P. spinulosa* and *Leptotrombidium* sp. in *L. sabanus*, *R. tiomanicus*, and *R. argentiventer* (Figure 4.7).

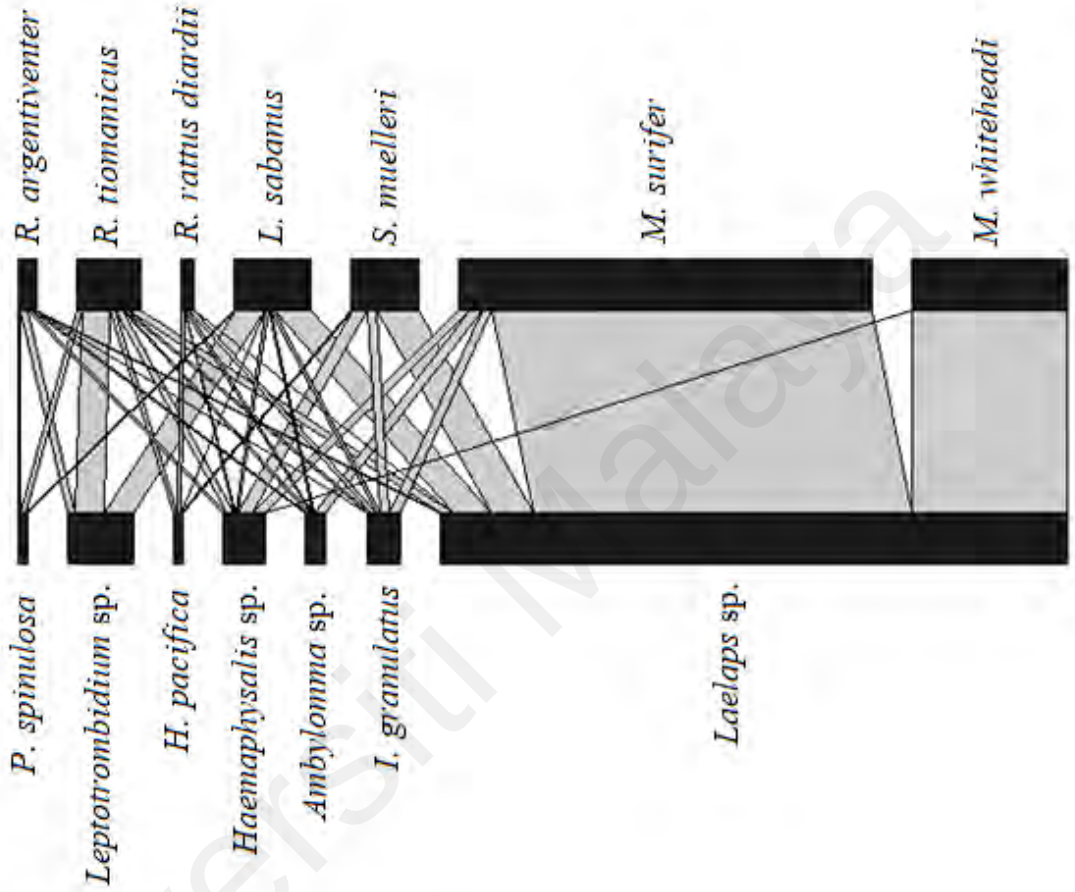


Figure 4.5: Bipartite network analysis demonstrates the relationship between wild rats and ectoparasites in Peninsular Malaysia, as this bipartite graph indicates the number of observations by the strength of the interaction lines.

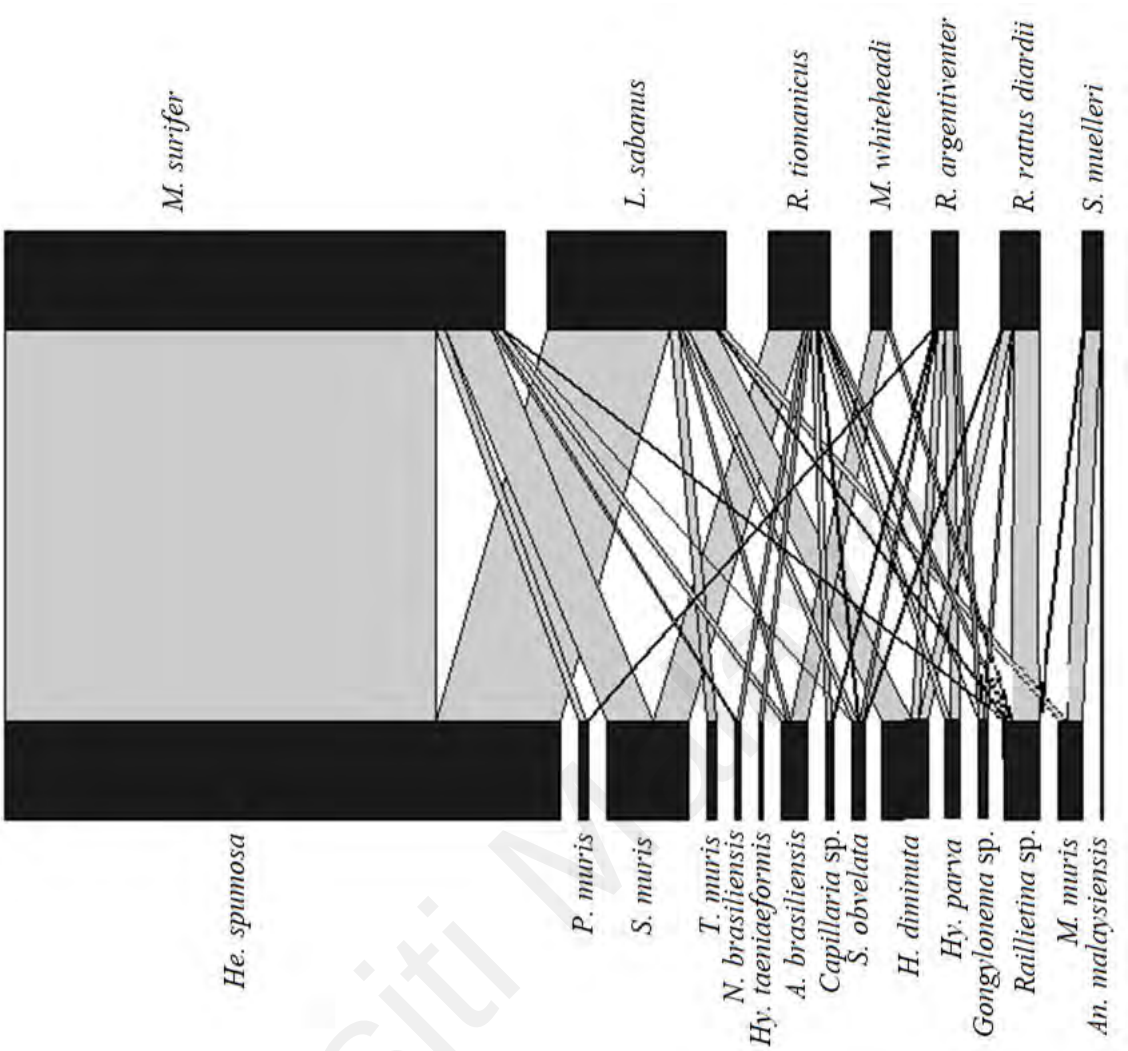


Figure 4.6: Bipartite network analysis demonstrates the relationship between wild rats and endoparasite helminth in Peninsular Malaysia, as this bipartite graph indicates the number of observations by the strength of the interaction lines.

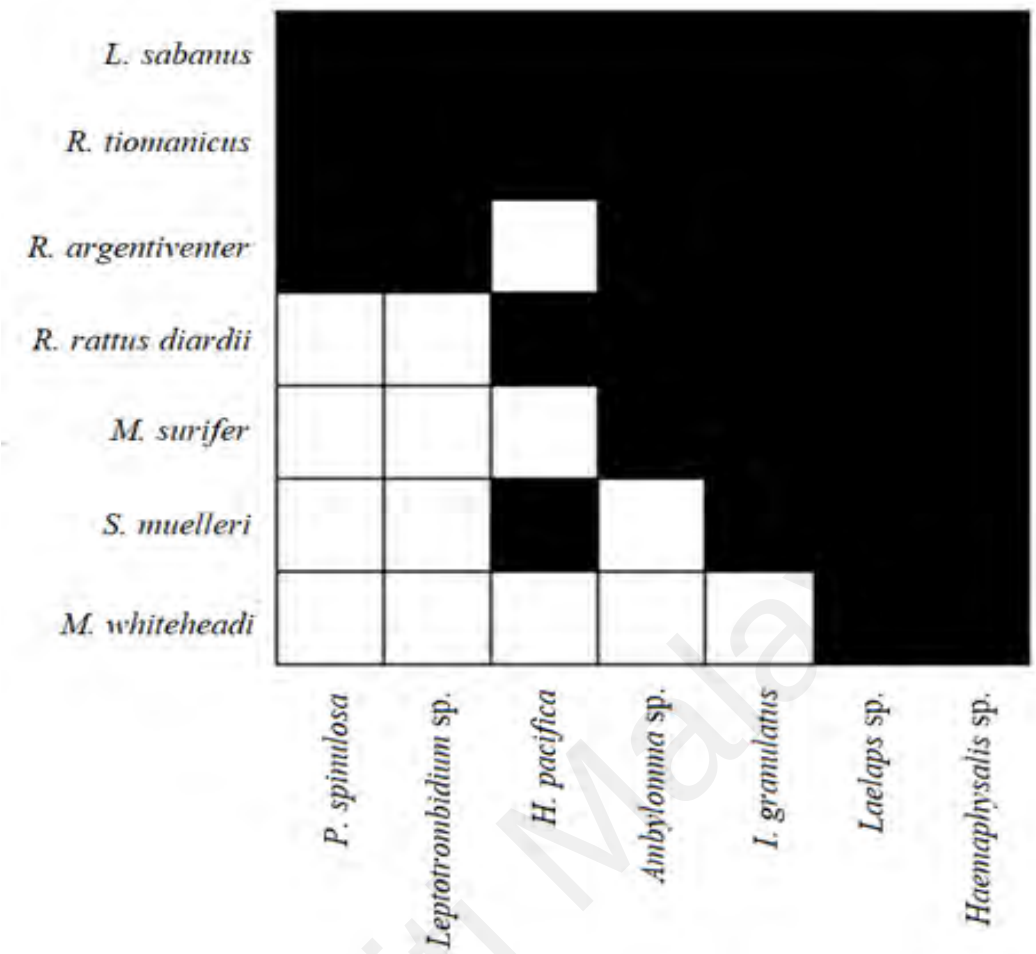


Figure 4.7: Network matrix based on presence and absence data for wild rat and ectoparasite associations. We included only host species with sample sizes of ≥ 5 individuals.

In addition to the bipartite matrix, the bipartite modules generate four modules or subgroups, as illustrated in Figure 4.8. Group 1: two ectoparasite species (*H. pacifica* and *I. granulatus*) were discovered in *R. rattus diardii* and *S. muelleri*; group 2: two ectoparasite species (*P. spinulosa* and *Leptotrombidium* sp.) infected one forest rat, *L. sabanus*, and two agricultural rats, *R. argentiventer* and *R. tiomanicus*; group 3: two ectoparasite species (*Laelaps* sp. and *Haemaphysalis* sp.) found mainly in *M. whiteheadi* and group 4: one ectoparasite (*Ambylomma* sp.) found highly in *M. surifer*.

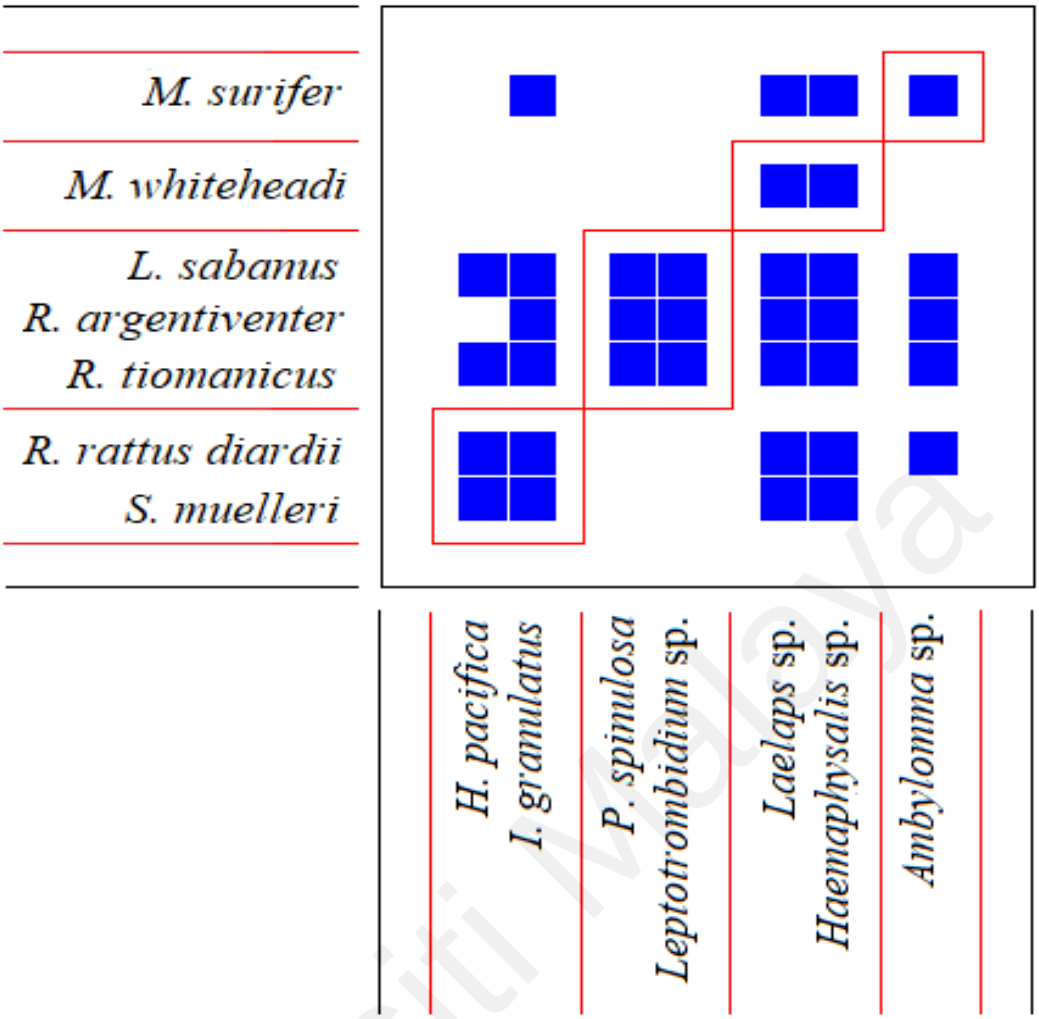


Figure 4.8: Composite panels of modules revealed from bipartite network analysis of ectoparasite species shared by wild rat hosts.

However, for the host-endoparasite helminth association in the bipartite matrix, *R. tiomanicus*, and *L. sabanus* were located in the top row because they have a large group of endoparasite helminth species. On the other hand, *Raillietina* sp., and *S. obvelata* helminths were placed on the right side of the matrix because they can be found in almost all host species; the three endoparasitic helminth species on the left infect only one host species each, *Hy. taeniaeformis* in *R. tiomanicus*, *T. muris* in *L. sabanus*, and *An. malaysiensis* in *S. muelleri* (Figure 4.9).

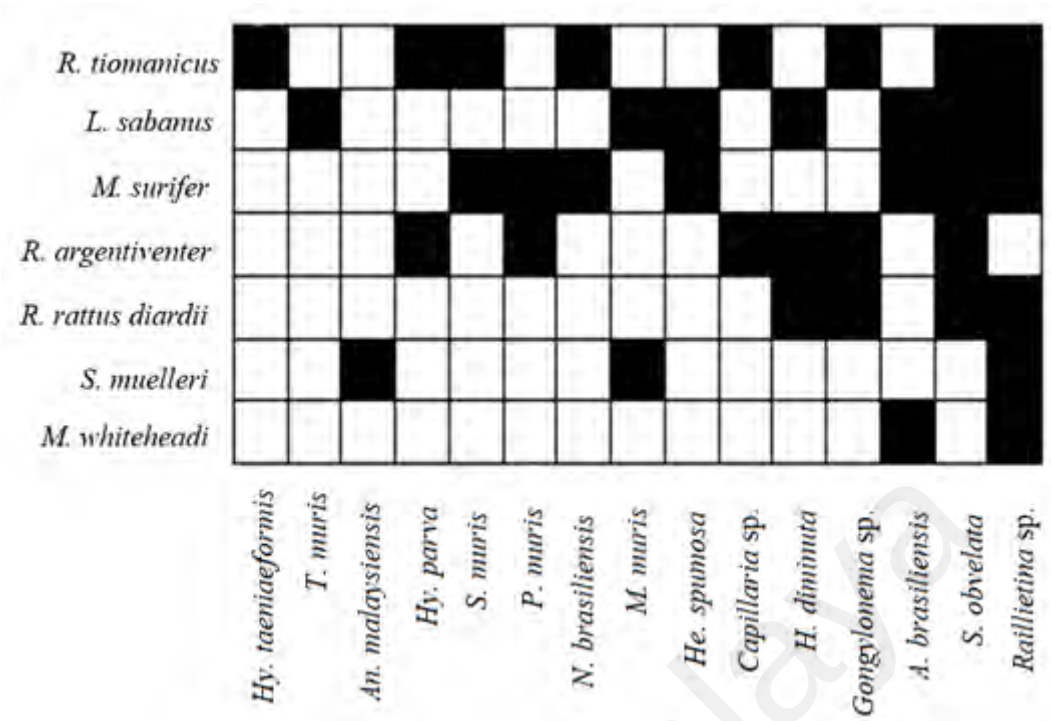


Figure 4.9: Network matrix based on absence and presence data for wild rat and endoparasite helminth associations. We included only host species with sample sizes of ≥ 5 individuals.

Besides, the bipartite matrix creates three modules or subgroups, as referred to in Figure 4.10. As in group 1: helminth species (6) found in *R. argentiventer*, only three helminth species (*H. diminuta*, *S. obvelata*, *Gongylonema* sp.) also infected in *R. rattus diardii*; group 2: three helminth species (*Hy. taeniaeformis*, *S. muris*, and *N. brasiliensis*) but only two were found abundant in *M. surifer* and *R. tiomanicus*, and group 3: six helminth species (*Raillietina* spp., *T. muris*, *M. muris*, *He. spumosa*, *An. malaysiensis*, and *A. brasiliensis*) found in forest rat species.

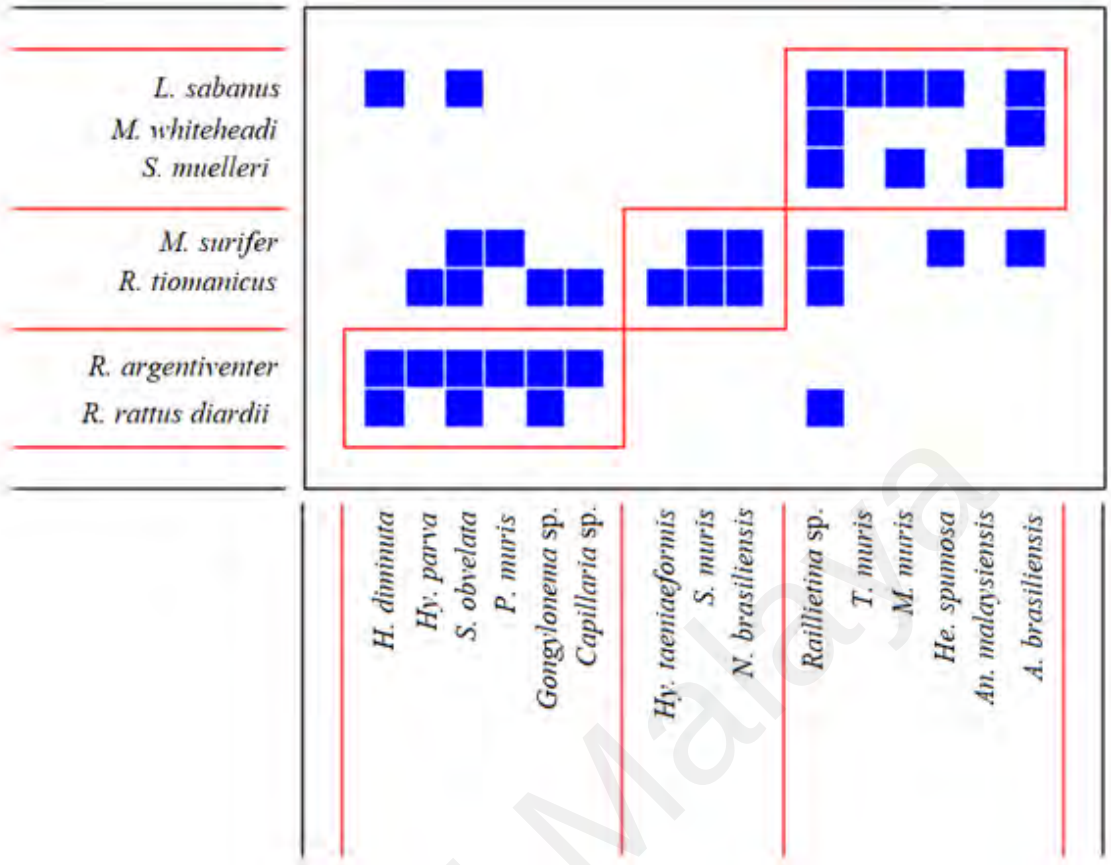


Figure 4.10: Composite panels of modules revealed from bipartite network analysis of helminth species shared by wild rat host.

4.3 Molecular characterization of cestode in wild rat

4.3.1 Occurrence of cestode infection in wild rats

A total of 124 wild rats were successfully collected, with a cestode infection incidence of 16.93% across all study sites. The two most common cestode species recovered were *Hymenolepis diminuta* (N = 31 individuals) and *Raillietina* sp. (N = 31 individuals), followed by *Hydatigera parva* (N = 6 individuals) and *Hydatigera taeniaeformis* (N = 3 individuals).

Raillietina sp. infected all individual rats in this study, as opposed to *H. diminuta*, *Hy. parva*, and *Hy. taeniaeformis*. *Leopoldamys sabanus*, *Maxomys surifer*, *Maxomys whiteheadi*, *Niviventer cremoriventer*, *Rattus rattus diardii*, *Rattus tiomanicus*, and *Sundamys muelleri* are the known rat species, while *H. diminuta* can be found in *L. sabanus*, *M. surifer*, and *R. tiomanicus*. It's detected in *R. argentiventer* and *R. tiomanicus* for *Hy. parva*. According to our findings, *Hy. taeniaeformis* is the smaller cestode that only infects *R. tiomanicus*. *Rattus tiomanicus* has the highest percentage of rats that are positively infected (47.62 %), followed by *L. sabanus* (19.05 %), and *M. surifer* (9.52 %). Two *R. tiomanicus* individuals hosted two cestode species, *Raillietina* sp. and *Hy. taeniaeformis*, and *Hy. taeniaeformis* and *Hy. parva*, respectively. Meanwhile, just one individual of each of the host species, *M. whiteheadi*, *N. cremoriventer*, *R. argentiventer*, *R. rattus diardii*, and *S. muelleri*, was infected with cestode (4.76 %).

4.3.2 Phylogenetic relationships

Each cestode species were identified using cestode's universal primers from mitochondrial regions such as the cytochrome c oxidase subunit one gene (COX1) and the 18S ribosomal DNA gene (18SrDNA). All the sequences were amplified with 127 and 1255 base pairs (bp) total lengths, respectively. To compare with the 23 sequences of each gene utilised in this investigation, a total of 15 COX1 sequences and 19 sequences

of 18SrDNA were acquired from GenBank. All the GenBank accession numbers were included, as well as for Peninsular Malaysia samples in appendix F. The COX1 data set (204 bp) had 31 conserved and 173 variable sites, with 140 of them being parsimony-informative, while the 18SrDNA data set (1200 bp) had 86 conserved and 1114 variable sites, with 1021 of them being parsimony-informative.

For both mitochondrial genes, COX1 (Figure 4.11A and Figure 4.11B) and 18SrDNA (Figure 4.12A and Figure 4.12B), we used the Kimura 2-parameter model with gamma distribution (K2 + G) and indicated 1000 replicates of bootstrap value for both NJ and ML phylogenetic trees. *Hymenolepis diminuta* of the Hymenolepididae group (e.g., Lubuk Yu, Pulau Tioman, Segamat, and one sample from Sichuan) constitutes a monophyletic group, according to the COX1 and 18SrDNA genes. In NJ and ML analyses, the bootstrap values for the COX1 gene were greater than 80 %. In the meantime, the 18SrDNA gene results in the ML tree revealed greater than 90 % bootstrap support, but ambiguous bootstrap values in the NJ tree analysis. *Leopoldamys sabanus*, *R. tiomanicus*, and *M. surifer* are three host species connected with *H. diminuta*, according to our phylogenetic analyses.

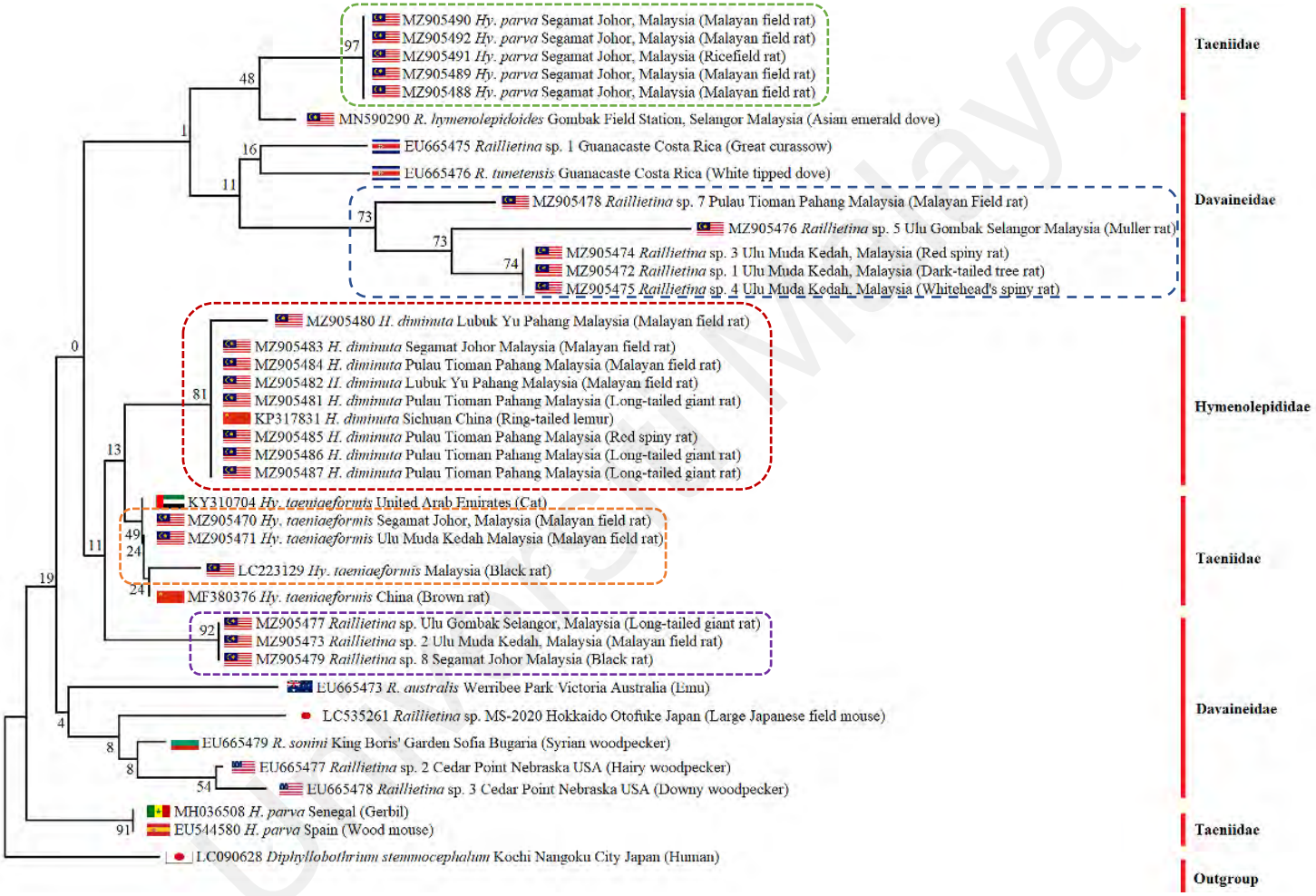


Figure 4.11A: The neighbour-joining phylogenetic tree estimated using the Kimura-2 parameter with gamma distribution (K2 + G) and 1000 bootstrap replications, based on COX1 gene.

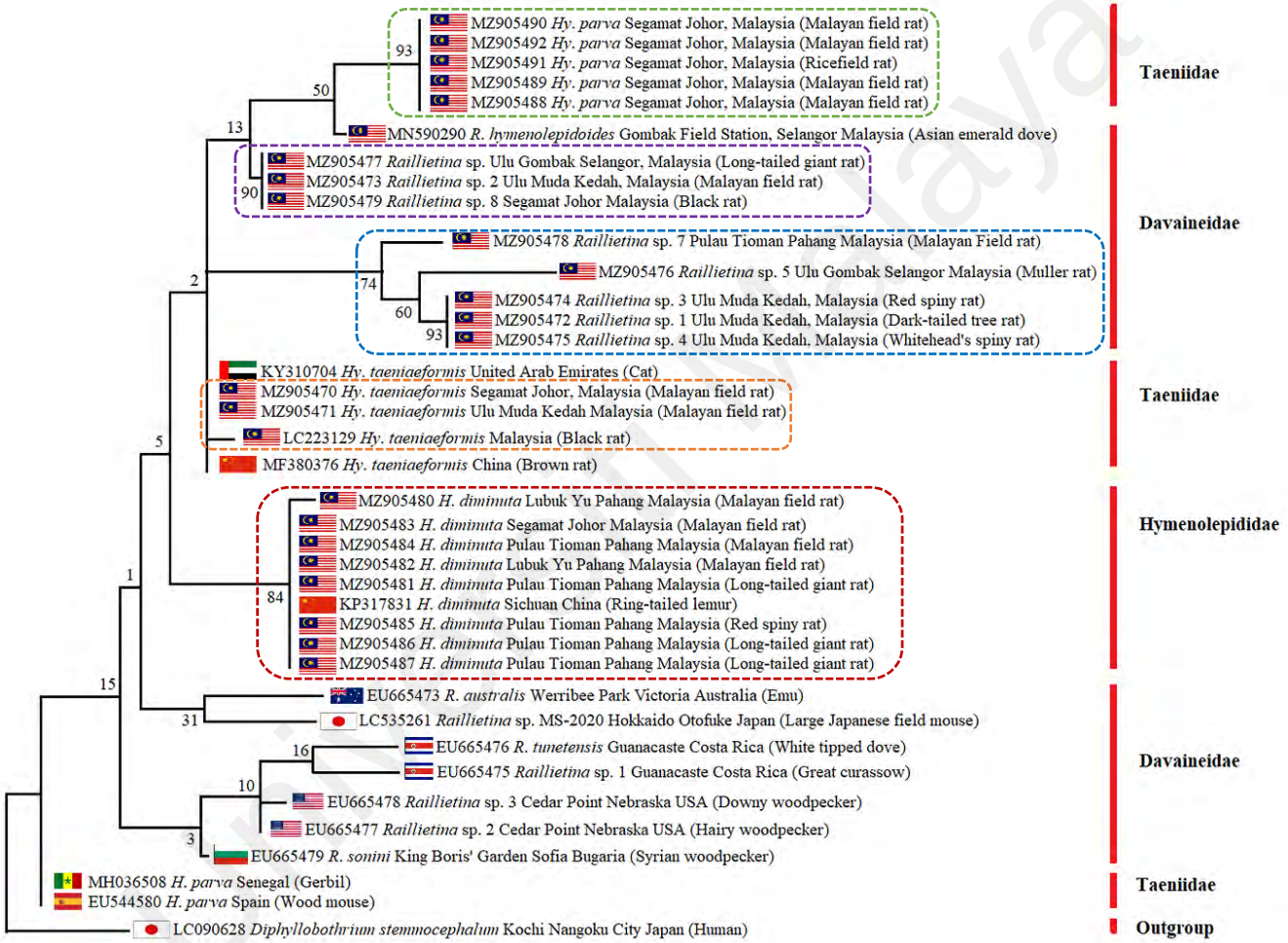


Figure 4.11B: Maximum-likelihood phylogenetic tree estimated using the Kimura-2 parameter with gamma distribution (K2 + G) and 1000 bootstrap replications, based on COX1 gene.

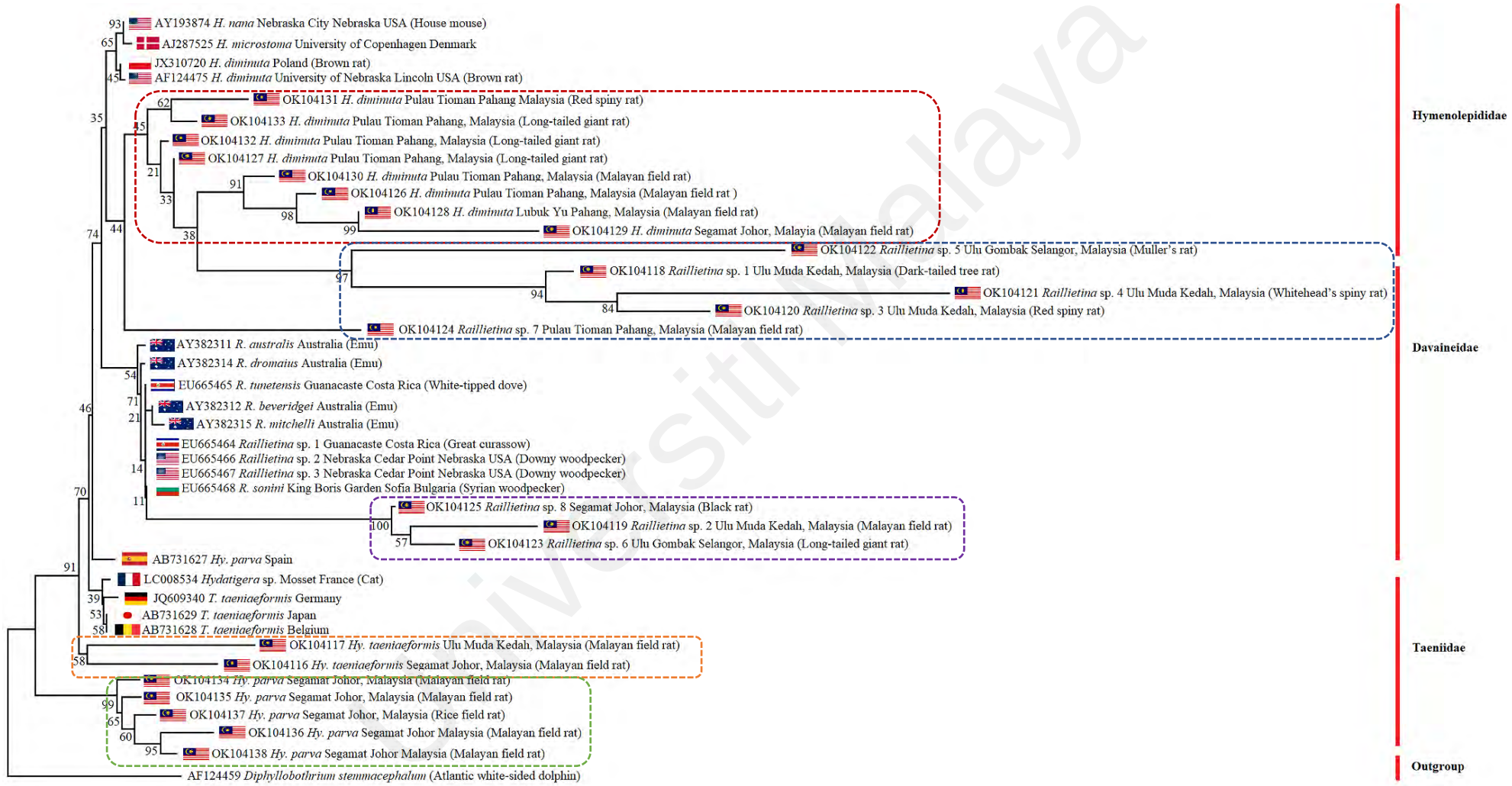


Figure 4.12A: The neighbour-joining phylogenetic tree estimated using the Kimura 2-parameter with gamma distribution (K2P + G) and 1,000 bootstrap replications, based on the 18SrDNA gene.

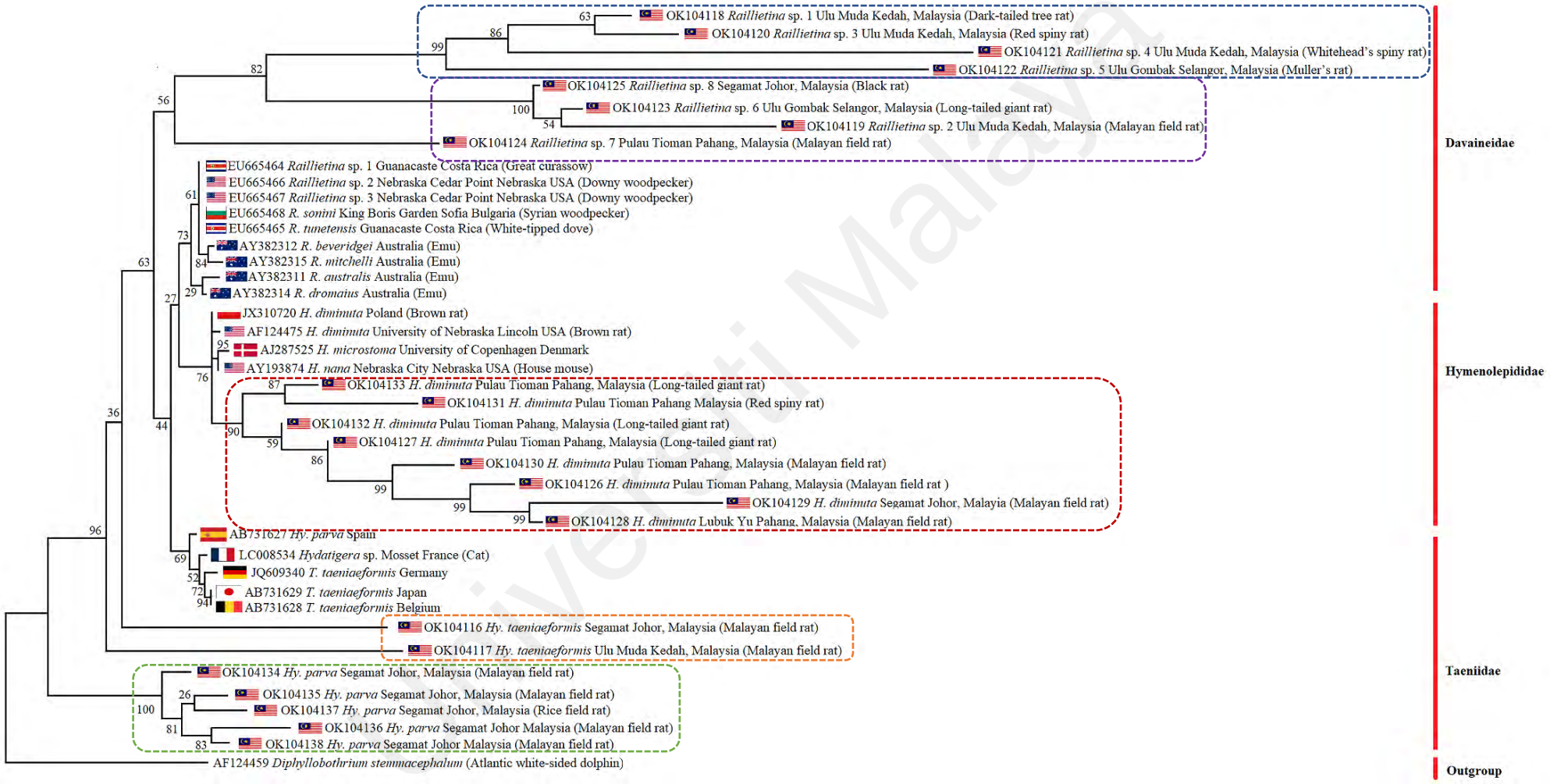


Figure 4.12B: The maximum-likelihood phylogenetic tree estimated using the Kimura 2-parameter with gamma distribution (K2P + G) and 1,000 bootstrap replications, based on the 18SrDNA gene.

Hydatigera parva (*Hy. parva*) and *Hydatigera taeniaeformis* (*Hy. taeniaeformis*) are the two Taeniidae species investigated in this study, and they constituted a monophyletic group in both mitochondrial genes for all analyses. In COX1, *Hy. parva* from Segamat, Johor was polyphyletic with *Hy. parva* clades from Spain (mouse host) and Senegal (gerbil host), but *Hy. taeniaeformis* from Ulu Muda, Kedah, and Segamat were monophyletic with the United Arab Emirates (cat host); Malaysia and China (rat host). Our *Hy. taeniaeformis* samples, unlike 18SrDNA, form independent branches that are distinct from *Hy. taeniaeformis* Germany, Japan, and Belgium. *Hydatigera parva* clade from Peninsular Malaysia is unique from the *Hy. parva* from Spain. In contrast to the clade of *Hy. taeniaeformis* and *Hy. parva* from Peninsular Malaysia, it appears that *Hy. taeniaeformis* (Germany, Japan, and Belgium) and *Hy. parva* (Spain and France) formed into the same branch. *Rattus argentiventer* was exclusively detected in this family with the accession codes MZ905491 in COX1 and OK104137 in 18SrDNA as *R. tiomanicus* was mostly seen infecting Taeniidae cestodes.

Our research shows that the *Raillietina* species from Peninsular Malaysia belong to a polyphyletic group within the Davaineidae family. *Raillietina* spp. samples 1, 3, 4, 5 and 7 were assigned to the clade group A, whereas *Raillietina* spp. samples 2, 6, 8 were assigned from the clade group B. In both genes, group B had a strong support value in all analyses (COX1, 92 % in NJ and 90 % in ML; 18SrDNA, 100 % in NJ and ML). Meanwhile, for group A, all lineages were performed based on the location of the study sites, which were located on Ulu Gombak, Ulu Muda, and Pulau Tioman, and all analyses yielded consistent results. Surprisingly, this species of cestode was discovered in all of the rats used in this study (Appendix F).

4.3.3 Genetic Divergence and Diversity

The K2P distance model was used to examine the genetic relationships of cyclophyllidean worms utilising the COX1 and 18SrDNA mitochondrial DNA sequences used in this study. The mean percentage pairwise K2P distance (%) and standard error of means (±SE) for the Taeniidae and Davaineidae families have been summarised using the genetic divergence acquired from both genes (Table 4.20 and Table 4.21). Phylogenetic relationship analysis revealed that these families belonged to a polyphyletic group. In terms of data, our Hymenolepididae family has less issues to compare, with *H. diminuta* forming monophyletic with a bootstrap value of above 85 %. As a result, we do not use K2P distance to compare this species.

The COX1 mean distance between *Hy. taeniaeformis* from Malaysia (Table 4.20A) and *Hy. taeniaeformis* from China was 8.62 ± 3.27 % (±SE) K2P distance, whereas the distance between *Hy. taeniaeformis* from the United Arab Emirates (UAE) was 19.54 ± 6.23 % (±SE). In the meantime, as shown in Table 4B, the mean intra-specific distance for *Hy. taeniaeformis* Peninsular Malaysia was false-positive inter-specific mean distance with other countries such as UAE, France, Spain, Germany, Japan, and Belgium, forming a high mean distance. In addition to *Hy. taeniaeformis*, our Malaysian *Hy. parva* samples revealed a high K2P distance (more than 25 %) with other *Hy. parva* samples in the COX1 and 18SrDNA genes (Table 4.20A and 4.20B).

Table 4.20A: Mean percentage (%) of pairwise genetic divergence based on the Kimura 2-parameter model (K2P) are below the diagonal, and their standard errors of mean (±SE) are given above the diagonal among *Hydatigera* spp., namely as *Hydatigera taeniaeformis* Malaysia: *Hyta* MY and *Hydatigera parva* Malaysia: *Hypa* MY with another two *Hydatigera* species obtained from GenBank and outgroup respectively (*Hydatigera taeniaeformis* United Arab Emirates: *Hyta* UAE; *Hy. Taeniaeformis* China: *Hyta* CN; *Hy. parva* Spain & Senegal: *Hypa* ES & SN) and *Diphyllobothrium stemmacephalum: Ds* (LC090628) as outgroup of targeted COX1 gene.

	Hyta UAE	*Hyta* MY	*Hyta* CN	*Hypa* ES & SN	*Hypa* MY	*Ds*
Hyta UAE		6.23	4.06	7.70	11.21	11.61
Hyta MY	19.54		3.27	8.59	9.49	12.25
Hyta CN	8.73	8.62		5.48	8.48	9.82
Hypa ES & SN	22.96	27.32	14.86		10.81	9.67
Hypa MY	35.66	30.83	25.39	34.45		13.61
Ds	36.78	41.28	31.32	30.29	42.02	

Table 4.20B: Mean percentage (%) of pairwise genetic divergence based on the Kimura 2-parameter model (K2P) are below the diagonal, and their standard errors of means (±SE) are given above the diagonal among *Hydatigera* spp., namely as *Hydatigera taeniaeformis* Malaysia: *Hyta* MY and *Hydatigera parva* Malaysia: *Hypa* MY with another two *Hydatigera* species obtained from GenBank and outgroup respectively (*Hydatigera* sp. France: *Hy*sp FR; *Hy. taeniaeformis* Germany, Japan, Belgium: *Hyta* GR, JP, BE; *Hy. parva* Spain: *Hypa* ES) and *Diphyllobothrium stemmacephalum: Ds* (AF124459) as outgroup of targeted 18SrDNA gene.

	*Hy*sp. FR	*Hyta* GR, JP, BE	*Hyta* MY	*Hypa* ES	*Hypa* MY	*Ds*
*Hy*sp FR		0.43	2.55	0.61	2.91	3.65
Hyta GR, JP, BE	1.10		2.45	0.65	2.93	3.74
Hyta MY	25.07	24.31		2.65	4.18	5.30
Hypa ES	1.56	2.25	25.96		3.03	3.84
Hypa MY	27.19	27.38	44.84	28.46		4.18
Ds	31.81	32.86	53.40	33.18	37.84	

As shown in Table 4.21A for the COX1 gene for *Raillietina* spp., the mean inter-specific distance between *Raillietina* sp. 2 and *Raillietina* sp. 3 from the United States was (8.23 ± 3.59 % (±SE)), indicating a false-negative. Malaysian *Raillietina* spp. (*Raillietina* sp. 1; *Raillietina* sp. 3; *Raillietina* sp. 4) and Costa Rica *R. tunetensis* had the largest mean K2P genetic divergence, with values of 9.48 ± 10.73 % (±SE). Despite the fact that *R. hymenolepidoides* is also from Malaysia, it has the highest mean K2P genetic divergence with *Raillietina* spp. from this study, with 39.02 ± 10.17 % (±SE) (*Raillietina* sp. 1; *Raillietina* sp. 3; *Raillietina* sp. 4), 29.29 ± 8.37 % (±SE) (*Raillietina* sp. 5), 29.29 ± 8.24 % (±SE) (*Raillietina* sp. 7), and 23.05 ± 6.12 % (±SE) (*Raillietina* sp.2; *Raillietina* sp. 6; *Raillietina* sp. 8), respectively. There were false-negative species identification for inter-specific variation with the smallest value below 2.0 % OT for absolutely zero percent as indicated among *Raillietina* sp. 1, *Raillietina* sp. 2, and *Raillietina* sp. 3, which were found in the United States and Costa Rica as their avian host. *Raillietina* spp.

Malaysia has the highest mean K2P genetic divergence for inter-specific variation, with a value of 126.71 ± 17.26 % (±SE) (Table 4.21B). In this study, none of the *Raillietina* species showed intra-specific variation when compared to other *Raillietina* species found in GenBank with distance values less than 2.0 % OT values.

According to Zhang *et al.* (2014), the mean percentage of K2P genetic divergence of Taeniid species based on COX1 gene analysis, mean intra-specific variation is 0.71 ± 0.17 % (±SE), whereas 15.97 ± 0.22 % (±SE) and higher distances are considered inter-specific variance. This study was based on Galimberti *et al.* (2012), who defined optimal barcoding threshold (OT) values as 2.0 % or higher of the mean K2P distance.

Table 4.21A: Mean percentage (%) of pairwise genetic divergence based on the Kimura 2-parameter model (K2P) are below the diagonal, and their standard errors of mean (±SE) are given above the diagonal among *Raillietina* spp. Malaysia, namely as *Raillietina* sp. 2, *Raillietina* sp. 6, *Raillietina* sp. 8: *R* (2,6,8) (Group B); *Raillietina* sp. 1, *Raillietina* sp. 3, *Raillietina* sp. 4: *R* (1,3,4) (Group A); *Raillietina* sp. 7: *R* (7) (Group A); *Raillietina* sp. 5: *R* (5) (Group A) with another eight *Raillietina* species obtained from GenBank and outgroup respectively (*Raillietina* sp. 1: *R*sp1; *Raillietina* sp. 2: *R*sp2; *Raillietina* sp. 3: *R*sp3; *Raillietina* sp. MS-2020: *R*sp; *R. australis*: *Ra*; *R. tunetensis*: *Rt*; *R. sonini: Rs*; *R. hymenolepidoides: Rh*) and *Diphyllobothrium stemmacephalum: Ds* (LC090628) as outgroup of targeted COX1 gene.

Raillietina sp.	*R* (2,6,8) (Group B)	*R* (1,3,4) (Group A)	*R* (7) (Group A)	*R* (5) (Group A)	*R*sp1	*R*sp2	*R*sp3	*R*sp	*Ra*	*Rt*	*Rs*	*Rh*	*Ds*
R (2,6,8) (Group B)		9.30	6.82	8.11	7.57	7.02	5.69	8.01	6.55	9.27	6.63	6.12	8.75
R (1,3,4) (Group A)	35.10		5.48	4.73	7.63	9.62	9.74	10.16	8.23	10.73	9.61	10.17	13.41
R (7) (Group A)	25.79	17.92		6.15	5.67	7.69	8.33	9.06	8.01	8.49	8.33	8.24	10.07
R (5) (Group A)	30.36	15.44	20.00		7.51	8.72	8.19	9.67	8.01	10.00	9.00	8.37	13.34
*R*sp1	28.10	28.04	18.34	23.70		5.12	5.65	7.04	8.43	6.46	4.77	7.59	8.93
*R*sp2	25.45	34.86	25.73	29.40	13.90		3.59	4.78	5.91	4.25	3.50	6.89	7.86
*R*sp3	21.25	36.74	29.29	27.35	16.74	8.23		4.99	5.39	4.27	3.70	5.82	7.58
*R*sp	30.71	38.21	31.39	33.51	23.57	13.74	14.19		5.47	5.75	4.34	6.80	7.24
Ra	23.24	30.77	27.28	27.28	27.29	18.40	16.74	16.75		6.13	5.80	6.52	7.31
Rt	34.73	39.48	29.60	33.59	20.03	12.25	12.26	18.34	20.03		5.25	7.32	6.61
Rs	24.22	36.64	29.35	31.53	13.70	8.23	9.53	12.32	17.13	16.75		6.75	7.41
Rh	23.05	39.02	29.29	29.29	25.39	21.72	18.35	21.72	20.32	23.52	21.78		7.27
Ds	31.01	47.86	33.51	43.28	29.35	25.35	25.39	23.52	23.52	21.92	23.70	23.51	

Table 4.21B: Mean percentage (%) of pairwise genetic divergence based on the Kimura 2-parameter model (K2P) are below the diagonal, and their standard errors of means (±SE) are given above the diagonal among *Raillietina* spp. Malaysia, namely as *Raillietina* sp. 1, *Raillietina* sp. 3, *Raillietina* sp. 4: *R* (1, 3, 4) (Group A); *Raillietina* sp. 2, *Raillietina* sp. 6, *Raillietina* sp. 8: *R* (2, 6, 8) (Group B); *Raillietina* sp. 5: *R* (5) (Group A); *Raillietina* sp. 7: *R* (7) (Group A) with another nine *Raillietina* species obtained from GenBank and outgroup respectively (*Raillietina* sp. 1: *R*sp1; *Raillietina* sp. 2: *R*sp2; *Raillietina* sp. 3: *R*sp3; *R. australis*: *Ra*; *R. beveridgei*: *Rb*; *R. dromaius*: *Rd*; *R. mitchelli*: *Rm*; *R. tunetensis*: *Rt*; *R. sonini: Rs*;) and *Diphyllobothrium stemmacephalum: Ds* (AF124459) as outgroup of targeted 18SrDNA gene.

Raillietina spp.	*R* (1,3,4) (Group A)	*R* (2,6,8) (Group B)	*R* (5) (Group A)	*R* (7) (Group A)	*R*sp1	*R*sp2	*R*sp3	*Ra*	*Rb*	*Rd*	*Rm*	*Rt*	*Rs*	*Ds*
R (1,3,4) (Group A)		11.30	13.22	11.63	9.22	9.22	9.22	9.78	9.65	9.43	9.04	9.33	9.09	21.97
R (2,6,8) (Group B)	103.28		17.26	8.39	4.33	4.33	4.33	4.34	4.41	4.37	4.57	4.36	4.36	11.36
R (5) (Group A)	111.29	126.71		15.99	11.49	11.49	11.49	11.14	12.00	11.73	11.44	11.66	11.39	31.33
R (7) (Group A)	93.23	72.87	110.25		3.21	3.21	3.21	3.21	3.33	3.20	3.43	3.17	3.23	6.69
*R*sp1	77.40	40.77	86.65	25.64		0.00	0.00	0.57	0.35	0.35	0.53	0.20	0.20	3.84
*R*sp3	77.40	40.77	86.65	25.64	0.00		0.00	0.57	0.35	0.35	0.53	0.20	0.20	3.84
*R*sp2	77.40	40.77	86.65	25.64	0.00	0.00		0.57	0.35	0.35	0.53	0.20	0.20	3.84
Ra	80.43	41.36	87.27	26.05	1.55	1.55	1.55		0.61	0.52	0.72	0.60	0.60	3.94
Rb	79.73	41.49	88.84	26.85	0.66	0.66	0.66	1.78		0.51	0.46	0.41	0.40	3.93
Rd	78.13	41.12	87.90	25.64	0.66	0.66	0.66	1.33	1.33		0.67	0.35	0.42	3.94
Rm	76.48	43.17	86.98	27.61	1.33	1.33	1.33	2.46	1.10	2.01		0.59	0.58	4.12
Rt	77.99	41.28	87.58	25.26	0.22	0.22	0.22	1.78	0.88	0.66	1.55		0.31	3.90
Rs	77.07	41.10	86.34	26.03	0.22	0.22	0.22	1.78	0.88	0.88	1.55	0.44		3.92
Ds	137.43	93.85	171.72	58.88	31.46	31.46	31.46	32.78	32.35	32.37	33.65	31.89	31.89	

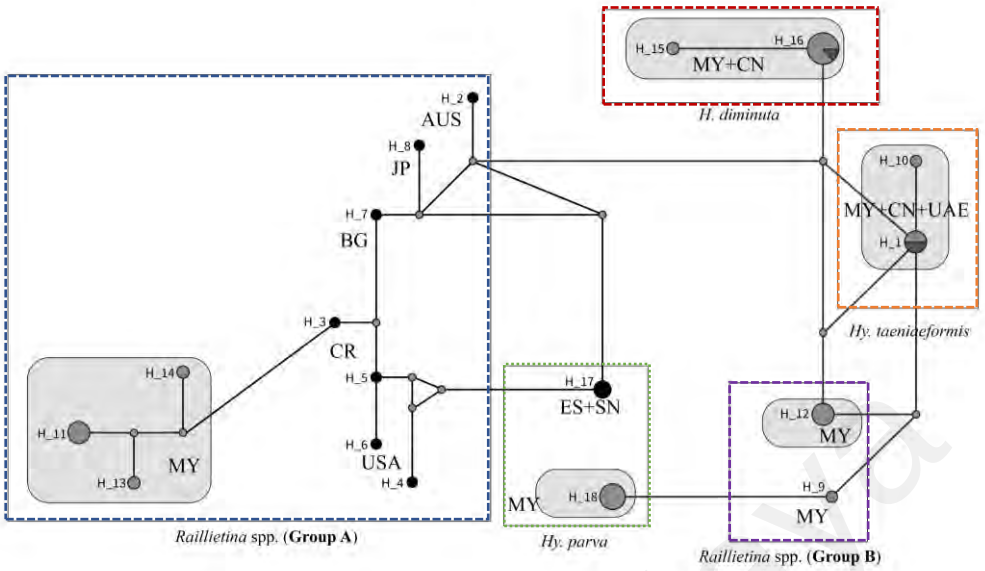
Cestode sequences based on the COX1 and 18SrDNA genes were subjected to DNA single polymorphism analysis. In the COX1 gene, there were eighteen haplotypes found in 37 isolates, resulting in a haplotype diversity (Hd) value of 0. 0.923 and nucleotide diversity (π) value of 0.128, whereas in the 18SrDNA gene, there were 38 haplotypes found in 41 isolates, resulting in a haplotype diversity (Hd) value of of 0.995 and nucleotide diversity (π) value of 0.212. For both COX1 and 18SrDNA, the neutrality test revealed no significant differences between Tajima's D (-0.22890 and -1.55945) ($P > 0.10$) Fu and Li's D* (0.22731 and -0.92840) ($P > 0.10$) and Fu and Li's F* (0.08758 and -1.38287) ($P > 0.10$).

The minimum-spanning network (MSN) was built using the haplotype data. This MSN was created to show the relationships between four different cestode species. For COX1, *H. diminuta* from Malaysia shared a haplotype with *H. diminuta* from China (H_16), as well as *H. diminuta* Poland (H_35) and *H. diminuta* USA (H_34) in 18SrDNA.

In the case of *Hydatigera* spp., the Malaysian *Hy. taeniaeformis* shared a haplotype with *Hy. taeniaeformis* from China and a link with *Hy. taeniaeformis* from the United Arab Emirates in the COX1 gene. *Hy. taeniaeformis* from Malaysia was classified as H_2 (Ulu Muda) and H_14 (Segamat) for 18SrDNA. Apart from this species, all *Hy. parva* from Malaysia had the same haplotype (H_18) in the COX1 gene, whereas it had its unique haplotypes in the 18SrDNA gene (Figure 4.13B).

In phylogenetic analyses, our *Raillietina* spp. displayed two cluster networking groupings, which we called Group A and Group B. Except for *Raillietina hymenolepidoides* from Malaysia, which was obtained in an avian host in the COX1 gene (H_9, see Figure 4.13A), Group A and Group B were obviously distinct and formed a tight haplotype with Australia, Bulgaria, Costa Rica, Japan, and the United States.

(A) COX1



(B) 18S rRNA

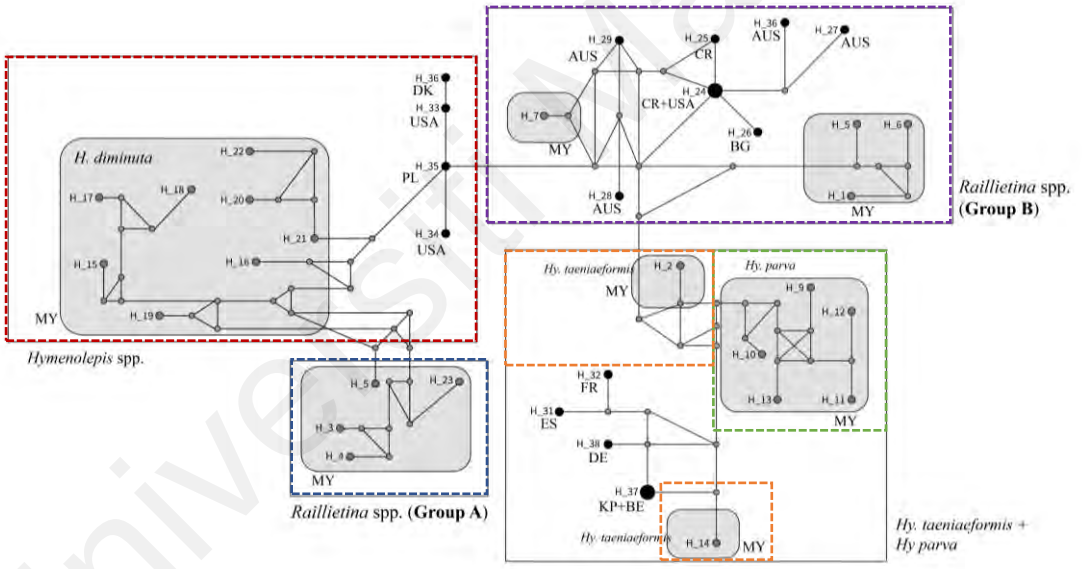


Figure 4.13: Minimum-spanning network of cestodes inferred from COX1 (A) and 18SrDNA (B) haplotypes using NETWORK 10.2.0.0. The size of the circle represents the number of individuals per haplotype (37 taxa and 18 haplotypes) and (41 taxa and 38 haplotypes) for COX1 and 18SrRNA, respectively. Shaded circles (grey boxes) belong to sequences from this study while the black colour from GenBank sequences. Notes: MY (Malaysia); AUS (Australia); BE (Belgium); BG (Bulgaria); CN (China); CR (Costa Rica); DE (Germany); DK (Denmark); ES (Spain); FR (France); JP (Japan); PL (Poland); SN (Senegal); UAE (United Arab Emirates); USA (United State of America); Group A (*Raillietina* spp. Malaysia A); Group B (*Raillietina* spp. Malaysia B).

CHAPTER 5: DISCUSSION

5.1 Wild rat distribution in Peninsular Malaysia

Verifying the distribution pattern of wild rat is neccesary for creating checklist of rat species in different forest types and indirectly framing their interaction with parasite. Rats are parasitized by a varied parasites species, some of which are zoonotic and can cause great distress to the animals and even death to human. Due to rats and human live in close proximity, it is critical to understand the consequences that the parasites they harbour can be transmitted to human and spread to surrondings. The quantity of wild rats in these areas has probably been maintained by ongoing human activity, an abundant food source, and enough shelter or infrastructure in the surrounding area. Yet studying the wild rats remains a challenge often due to some studies were constraint at only one particular localities or habitats.

In general, the composition of the wild rats populations in this three forest environment varied in term of microhabitats. Ten species from five genera of murids were identified in this study. One species of commensal rat was found to inhabit all forest types but could be vary in term of number of individual captured. *Rattus tiomanicus* was the most prevalent commensal rat species, while *M. surifer* was the most prevalence forest rat species.

This literature review has produced a checklist of wild rats species for Peninsular Malaysia (Pagès *et al*., 2010; Pimsai *et al*., 2014). According to Pimsai *et al*. (2014) about 12 genera and 28 species of murids were recorded from Peninsular Malaysia, Myanmar, Thailand and Singapore. Sinniah (1979) identified a greater number of rat species, capturing up to nine species from a variety of environments in Peninsular Malaysia, including oil palm plantations. Meanwhile, Mohd Zain (2008) examined the murids population on Pulau Jarak and Langkawi which discovered three commensal rat species (*R. tiomanicus*, *R. rattus diardii*, and *R. exulans*) that live on the island. Besides,

Paramasvaran *et al*. (2009b) discovered three rat species in rice paddy fields, coastal areas, and urban areas: *R. exulans*, *R. norvegicus*, and *R. rattus diardii*.

The present of murid rats in Ulu Muda Forest Reserve (UMFR) was represented by one commensal rat and five forest rats respectively, *R. tiomanicus*, *M. surifer*, *M. whiteheadi*, *N. cremoriventer*, *N. fluvescens*, and *S. muelleri* with *M. surifer*, the dominant species. *Maxomys surifer* was frequently obtained in two forest habitats (UMFR and LYFEP) except oil palm plantation in Segamat (SGT) and only this species found in Gunung Arong Forest Eco Park (GAFEP). Categories as a forest rat that can be found in reserve and recreational forest which it is common in the forest of the lowlands and hills (Medway, 1969). *Maxomys surifer* is widely distributed throughout Peninsular Malaysia including south-west-China to Indonesia and Myanmar to Indochina with a 'Least Concern' record in IUCN (Pimsai *et al*., 2014). Insects, snails, and small vertebrates are included as the diet instead of fruits (Pimsai *et al*., 2014). It is a nocturnal and terrestrial who live in burrows at forested area including in agricultural land and gardens (Francis, 2008). According to Razali *et al*. (2018), *M. surifer* was the most abundant species captured in Chemomoi, Pahang. The murid data at UMFR has been published by Saarani *et al*. (2021) which *N. fulvescens* was the first records for the study area.

Leopoldamys sabanus, *M. surifer*, and *R. tiomanicus* were found in Pulau Tioman (PTP), with *L. sabanus*, the dominant species. The same species found by Lim *et al*. (1999) in PTP with additional species recorded, *N. cremoriventer* and *R. exulans*. Similarly, *L. sabanus* was discovered in forest reserves and recreational areas. *Leopoldamys sabanus* was primarily obtained in dipterocarp forests and throughout the Peninsular Malaysia from varies of an ecological aspect (Pimsai *et al*., 2014). Taxonomic have been classified *Leopoldamys* spp. in Peninsular Malaysia can be varied in different geologically as *L. ciliatus* occurs in montane forest while *L. vociferans* can be a mainland form (Yong, 1970). The diet of this species consists of vegetable matter including snails,

insects, and some of fruits (Lim, 1970). Behaviourally, it was a semi-arboreal murid that able to climb freely as well as ground dwelling on the ground (Medway, 1969).

Rattus tiomanicus was generalist rats species which captured in all three forest types (UMFR, PTP, LYFEP, and SGT) in this study. About 47 individual captured from modified forest or known as oil plam plantation (SGT). Sundamic species with a range that includes Bali, Borneo, Sumatra, Java, and Palawan in addition to Peninsular Thailand (Musser & Carleton, 2005) and known to be listed as a 'Least Concern' in IUCN. This wild rat species was found in a wide variety of habitats, including agricultural region, coastal scrub vegetation, and secondary forest (Ow-Yang, 1971; Paramasvaran *et al*., 2013), as well as in primary forest at different elevations (Chasen, 1933). Due to their adaptability, it can be serious nuisance species in oil palm plantations. It commonly find shelter in piles of chopped fronds, though less frequently in cut stumps or fallen logs (Medway, 1969).

However, for Ulu Gombak Forest Reserve (UGFR) only two forest species collected, *L. sabanus* and *S. muelleri*, with *S. muelleri* the dominant species. Paramasvaran *et al*. (2005) were recorded the highest murids species yielded, with three other species of *R. tiomanicus*, *M. rajah*, and *M. whiteheadi*. *Sundamys muelleri* is the most widespread, listed as 'Least Concern' in IUCN (Pimsai *et al*., 2014). The decline of species in UGFR can be due to deforestration and development occurred by human activities at this region.

Other than these major species, we also recorded species such as *M. whiteheadi*, *N. cremoriventer*, *N. fluvescens*, *R. argentiventer*, and *R. rattus diardii*. *Maxomys whiteheadi*, *N. cremoriventer*, and *N. fluvescens* are known to be a nocturnal and mainly forest murids which live in tall and secondary-growth forests, gardens, and distributed areas with vegetation (Aplin *et al*., 2008; Pimsai *et al*., 2014). In conservation status, *M. whiteheadi* and *N. cremoriventer* were listed as 'Vulnerable' in IUCN, while *N. fluvescens* and *S. muelleri* were listed as 'Least Concern' in IUCN since its wide

distribution. The diet includes ants, other insects, and small snails as well as plant based (Francis, 2008; Pimsai *et al*., 2014).

Besides, *R*. *argentiventer* is restricted to agricultural land, such as rice fields and oil palm plantation and absent in forest habitations as only found in Segamat in this study. In rice-growing regions, it often causes crop losses and feeds on young rice plants, grasses, paddy seeds, and grains, as well as some animals, insects, and snails (Medway, 1969). Meanwhile, *R*. *rattus diardii* is known to be Malaysian house rat and morphologically, synonym of *R*. *tanezumi* (Pagès *et al*., 2010). Well known species with a vast field of studies involved from morphology to genetic and disease carried in Malaysia (Mohd Zain *et al*., 2012; Paramasvaran *et al*., 2013; Ikbal *et al*., 2019a; Ikbal *et al*., 2019b; Tijjani *et al*., 2020). Feed on almost all edible materials from insects to small vertebrates and fruits which can be found in most habitations, include urban, sub-urban, recreational forest and agricultural lands. Both of this species were recorded as 'Least Concern' in IUCN.

In term of sexes, all male and female wild rats were captured in all forest types. The capture of both sexes demonstrates that wild rats' scavenging behaviour is independent of one another. Mohd Zain (2008) and Mohd Zain *et al*. (2012) shown that there is no substantial difference between males and females. However, Tijjani *et al*. (2020) captured more female rats than male rats, implying that female rats travel and are more active foraging for food, possibly occupying a larger home range than male rats.

Additionally, the low capture rate of juvenile rats can be related to their fundamentally limited activity and home range. Mohd Zain (2008), Mohd Zain *et al*. (2012), and Tijjani *et al*. (2020) have all published comparable findings. Adult wild rats are thought to be more dominating, active, and able to travel farther than immature wild rats. Additionally, adult wild rats were apprehended more frequently than juvenile wild rats due to their

narrower home ranges. Other possible explanations include adults' proclivity to investigate areas near their nests or disperse to other areas.

5.2 The prevalence and distribution of fauna parasite

A large proportion of forest rat species were found to be infested with ectoparasites, particularly in forest reserves (UMFR and UGFR) with single or double infestations. In general, the diversity of ectoparasites was rather low in comparison to earlier studies conducted by Nursyazana *et al*. (2013) and Razali *et al.* (2018). Ectoparasites discovered included common species that are often found in rats. Ectoparasite infestations could theoretically grow when huge populations of wild rat's share habitats, promoting transmission and increasing the likelihood of numerous infestations via interaction between and within species.

Many wild rats from forest reserves were infested with endoparasite helminths, followed by those from recreational and modified forest. It is a measure of the extent of helminth infection among wild rats living in specific habitats. Additionally, the existence of intermediate hosts contributes to the spread of cestode infections, whereas the direct lifecycle happens rather easily and presumably more consistently in nematode infections. Malaysia's relatively high rainfall and humidity levels provide an ideal environment for the eggs to hatch before the infective larvae penetrate or are accidentally consumed by the host species.

Most of the wild rat in this study was infected with between one and two endoparasite helminth species. Additionally, a recent study by Tijjani *et al*. (2020) indicated that 17.1% of wild rats caught were infected. The infection rate as a percentage in this study was most likely impacted by the host's consumption and lifestyle. Additionally, their foraging behaviour and nutrition may expose them to a range of endoparasite illnesses and populations.

Females were found to be more infected with both ectoparasite and endoparasite helminths as Nursyazana *et al*. (2013) demonstrated a similar outcome in this study. However, Tijjani *et al*. (2020) reported that male rats had a slightly greater prevalence of endoparasites than female rats. This variance in infection prevalence may be related to the immunosuppressive impact of male sex hormones, explaining why there is a larger proclivity for parasite infection (Mohd Zain *et al*., 2012).

On the other hand, host age shown substantial differences between adults and juveniles in all rat species except *R. argentiventer* and *R. tiomanicus*, which were infected with endoparasite helminths. Adult wild rats were also captured in greater numbers than juveniles in this study. The difference in parasitic loads between host ages is related to elder rats' prolonged exposure to infective stages (Krishnasamy *et al*., 1980). *Rattus argentiventer* and *R. tiomanicus* were shown to be biased in terms of the number of juveniles infected with endoparasite helminths, owing to the increased likelihood of transmission and hence the degree of infection.

About 7 ectoparasites and 15 endoparasite helminths including two cysticerci species of genus *Hydatigera* were found in 10 species of wild rats, indicating murid rats from these parts of Peninsular Malaysia. Some of these fauna parasites which have been found in previous studies from Malaysia (Mariana *et al*., 2008; Paramasvaran *et al*., 2009a; Madinah *et al*., 2011; Mariana *et al*., 2011; Mohd Zain *et al*.,2012; Shafiyyah *et al*., 2012; Hamdan *et al*., 2016; Ishak *et al*., 2018; Razali *et al*., 2018; Mohd-Taib *et al*., 2018; Tijjani *et al*., 2020). However, *Hy. parva* found in this study have been rarely reported among Malaysian rats.

5.2.1 Distribution pattern of ectoparasite infestation

Ectoparasites infested an exceptionally higher of the wild rat population in the forest reserve, recreational forest, and modified forest. However, the overall diversity of

ectoparasites was limited, and it is comparable with previous findings by Razali *et al.* (2018), Mohd-Taib *et al*. (2018), and Ibrahim *et al*. (2018). *Ambylomma* sp., *Haemaphysalis* sp., *Ixodes granulatus*, *Laelaps* spp., *Leptotrombidium* sp., *Hoplopleura pacifica*, and *Polyplax spinulosa* have all been implicated as significant vectors or mechanical carriers for the spread of zoonotic illnesses. Only seven ectoparasite species were identified in this study. Ectoparasite species diversity was nearly identical across the three forest types, with only five species reported in UMFR and UGFR. This demonstrated that the disproportionately large number of rats sharing a niche facilitates transmissions and allows for several infestations via inter- and intraspecific interactions.

The greatest diversity was recovered in recreational forest and modified forest, with seven ectoparasite encountered. This is due to both of forest types, such as oil palm plantations have a larger niche, which directly increase the diversity of ectoparasites. However, there was no noticeable difference in ectoparasite diversity between these three forests.

In term of localities, all study sites had a high prevalence of ectoparasites, as UMFR and UGFR were the most infested. Ectoparasites obtained from forest reserve also had the highest individual count. Forest reserve being the most suitable habitats for all animals (host and ectoparasites) due to favorable environment such as humidity, temperature, and diversity of animals (Stanko *et al*., 2006; Johnson *et al*., 2013).

In general, intrinsic factors such as host and age had no significant effect on the distribution of ectoparasites in wild rat populations, except for the rat louse (*Polyplax spinulosa*), which showed a significant difference between male and female rats, as more female was heavily infested by *P. spinulosa* (Nursyazana *et al*., 2013). This tendency can be explained by life of females which tend to travel more frequent, and directly exposure them to infestation. These blood-sucking lice parasites are more prevalent in young, neglected, and malnourished rats (Durden, 2019).

The most prevalent mite was the mesostigmatid, followed by ticks, lice, and chiggers. Additionally, *Laelaps* sp. infestation was highest in *M. surifer*, *S. muelleri*, and *L. sabanus* in this research. Razali *et al*. (2018) suggested that *Laelaps* sp. is a rodent generalist mite, based on their observations in *L. sabanus*, *M. rajah*, *M. surifer*, and *M. whiteheadi*, whereas Mohd-Taib *et al*. (2018) noted that *Laelaps* sp. was the most abundant mite in *M. rajah*. *Laelaps echidninus*, *Laelaps nutalli*, *Laelaps sedlaceki L. turkestanicus*, *Laelaps sanguisugus*, and *Longolaelaps whartoni* were among the more prevalent mesostigmatids carried by rats in Peninsular Malaysia (Paramasvaran *et al*., 2009b; Madinah *et al*., 2014; Mohd Zain *et al*., 2015; Ibrahim, 2020). Additionally, an earlier study revealed high mite infestations in four habitats: forest, urban, coastal, and rice field (Paramasvaran *et al*., 2009b).

Numerous studies on the distribution of mesostigmatid mites have been published in Peninsular Malaysia (Chuluun *et al*., 2005; Mariana *et al*., 2008; Mariana *et al*., 2011; Madinah *et al*., 2014; Mohd-Taib *et al*., 2018; Razali *et al*., 2018; Ibrahim, 2020). Mites thrive in damp environments and cannot survive in low humidity for more than a few days (Nursyazana *et al*., 2013). Thus, it is more significant when *L. sabanus* or any other forest rat species are abundant since they reside in logged areas with high humidity, as Malaysia is a tropical rainfall forest.

Ambylomma sp., *Haemaphysalis* sp., and *Ixodes granulatus* were found to be infested on rats in this study. Except for *Maxomys* sp., all ticks infested most wild rat species. However, only *Haemaphysalis* sp. infested *Niviventer cremoriventer*, *N. fluvescens*, and *M. whiteheadi*. Most ticks extracted were young, which corresponds to the findings of Mariana *et al*. (2011), who collected four tick species (*Ambylomma* sp., *Dermacentor* sp., *Haemaphysalis* sp., and *I. granulatus*) from rats in Panti Forest Reserve. Extensive collections have showed that wild rats are widespread and may serve as a favoured host for juvenile ticks such as *Ambylomma* spp., *Haemaphysalis* spp., and *Dermacentor* spp.,

although *I. granulatus* was the only adult tick discovered on rats (Domrow & Nadchatram, 1963; Ho *et al*., 1990; Nadchatram & Ng, 1966; Lim, 1972; Trevor & James, 1994).

The great prevalence of *I. granulatus* infesting *L. sabanus* is owing to the perfect environment and behaviour of this host species in forest habitats, where it is a predominantly land-dwelling parasite. The identification of *I. granulatus* is consistent with reports that *I. granulatus* is commonly seen in rats. *Ixodes granulatus* is significant medically because it is the primary vector of the Langat Virus, which is closely related to the Russian Spring-Summer Encephalitis Complex (RSSE) (Smith, 1956). Additionally, it is known to transmit pathogens and spread tick typus and Q fever in Peninsular Malaysia's climax forest (Marcheette, 1966).

Hoplopleura pacifica and *P. spinulosa* were the two most often observed lice species in this study. This louse is known to be host specific, infesting only specific species of rats (Shabrina, 1990). Both, however, were discovered infested *L. sabanus* and *R. tiomanicus* in this research, whereas *P. spinulosa* was found infesting only *R. argentiventer* and *H. pacifica* was found infesting only *R. rattus diardii* and *S. muelleri*. Ibrahim (2020) also observed that the most abundant *P. spinulosa* population in Terengganu was infested by *R. norvegicus*, *R. exulans*, and *S. muelleri*. According to Chuluun *et al*. (2005), *H. pacifica* infested only *R. exulans*, while *P. spinulosa* was discovered in *M. whiteheadi*. The high prevalence of lice infestations in forest reserves may be a result of environmental changes. It created an ideal environment for rapid rat lice reproduction and growth, resulting in an increase in intensity. *Polyplax spinulosa* is a lice species of public health concern since it is known to harbour plague bacilli, transmit tularemia and bartonellosis to humans, and act as an adjuvant in the transmission of murine typhus and plague from rat to rat (Zahedi *et al*., 1984; Shabrina, 1990).

In this investigation, the only chigger detected on *L. sabanus*, *R. argentiventer*, and *R. tiomanicus* was *Leptotrombidium* spp. This species is one of the most often encountered chigger species in Malaysia rats, according to a comprehensive investigation (Mariana *et al*., 2005; Paramasvaran *et al*., 2009b; Mariana *et al*., 2011, Mohd Zain *et al*., 2015; Ahmad *et al*., 2020). However, low overall intensity indicates a limited spread, which could be attributed to the rat and chigger's effectiveness. In Peninsular Malaysia, the chigger, particularly *L. deliense*, is a major vector of scrub typhus (*Orientia tsutsugamushi*). The presence of this vector species indicates a risk of infection by scrub typhus in humans bitten by chiggers, particularly in places with human occupation or activity, such as plantation areas.

Rapid environmental degradation as a result of current global climate change may alter the ecology of rats and establish new foci for the profiling of vectors that transmit rodent-borne parasitic illnesses. Ecological variety will enhance contact between humans and rats, leading in a greater disease load that will put public health services under risk.

5.2.2 Distribution pattern of endoparasite helminth infections

In Peninsular Malaysia, majority of wild rats are infected with endoparasite helminths. This demonstrates the enormous magnitude of helminth infectious among wild rats sharing a habitat. However, helminth species diversity was lower in comparison to another research (Paramasvaran *et al*., 2009a). Sinniah (1979) discovered 18 species of helminths infecting rats from variety of habitats, whereas this research identified only fifteen species. The community of endoparasite helminth consisted of eleven nematodes (*Ancylostoma brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria* sp., *Gongylonema* sp., *Heterakis spumosa*, *Mastophorus muris*, *Nippostrongylus brasiliensis*, *Protospirura muris*, *Syphacia muris*, *Syphacia obvelata*, and *Trichuris muris*) and four cestodes (*H. diminuta*, *Hy. taeniaeformis*, *Hy. parva*, and *Raillietina* sp.). Mohd Zain *et al*. (2012) also identified a similar number of helminth species, but the overall infection rate for *R.*

tiomanicus was 51.5 %, significantly greater than the previous study, showing high in infection over time in urban areas.

Although *R. tiomanicus* dominated the rat population in terms of total wild rats collected, *L. sabanus* was the most heavily infected host species in our study. As a result, it is surprising that this species had a greater rate of helminth infection than other species, given that species richness is highly dependent on host density and sampling effort (Feliu *et al*., 1997). In reserve and modified forest, the highest diversity of helminth species was recorded, with eight species recovered, compared to recreational forest. However, Sinniah (1979) documented 19 species infecting a variety of environments, including an oil palm estate. Similarly, Krishnasamy *et al*. (1980) identified the same number of helminth species but found a significantly lower prevalence of infection in the most prevalent rat species, *R. tiomanicus*. This could be due to the presence of commensal rats in the ecosystem, as well as their high population density, which contributes to widespread and high endoparasitic helminth infection.

Lubuk Yu Forest Eco Park (LYFEP) had the highest prevalence of endoparasite helminth infections in rats, followed by UMFR, PTP, and SGT oil palm plantation. Helminth infection was most severe in forest reserves, followed by modified and recreational forests. The presence of high infection rates in forest regions suggested that the forest environment facilitated the transmission of endoparasites helminths among the wild rat population.

The nematode *Syphacia obvelata* was the most prevalent species found in Peninsular Malaysia's wild rat species, although had a lower intensity than *S. muris* (Mohd Zain *et al*., 2012; Paramasvaran *et al.,* 2012). Adam (1933) described *S. obvelata* from unidentified rats in Pahang and Taiping, but did not provide any details on its morphology, which suggests it is *S. muris*. Ow-Yang (1971), Adams (1933), Schacher & Chee-Hock (1960), and Yoshida *et al*. (1985) were the earliest reported the presence of

Syphacia in host rats. Additionally, Dewi *et al*. (2016) conducted a review of the genus *Syphacia* from rats in Southeast Asia and Indonesia. According to Dunn *et al*. (1968), the frequency of this nematode was negatively related to the amount of plant food consumed.

Another nematode, *Heterakis spumosa*, was supposed to be the most abundant endoparasite helminth, found only in *M. surifer* and *L. sabanus* forest reserve areas. Similarly, it had previously been observed in disturbed forest habitats (Singh & Krishnasamy, 1979) and lowland forest (Singh & Krishnasamy, 1979; Ow-Yang, 1971). The infection of rats to endoparasite helminths can be explained by their lifestyle. Clearly, environmental conditions such as rainfall and humidity support an optimal hatching of eggs prior to infective larvae penetration or host meal consumption (Charlier & Claerebout, 2022).

The presence of *Angiostrongylus malaysiensis* in only *S. muelleri* suggested that the snail's diet consists of intermediate hosts. *Sundamys muelleri* was abundantly collected ecologically along streamlines or small rivers with moist microhabitats. *Angiostrongylus cantonensis* was first discovered in Peninsular Malaysia in *R. rattus diardii* and *R. exulans* (Schacher & Cheong, 1960). None of the *R. rattus diardii* in this study, however, were infected with this species. This could be because of the smaller number of *R. rattus diardii* collected, particularly and none of them caught in forested areas. Additionally, this nematode species was frequently harboured by commensal rats belonging to the genus *Rattus* (Lim *et al*, 1976; Mohd Zain *et al*., 2012).

Nippostrongylus brasiliensis, the most prevalent species of rat in Peninsular Malaysia, was also found in this study. This species was found in *L. sabanus* and *M. surifer*. *Ancylostoma brasiliensis* was also found in these two species. The direct lifecycle of this *N. brasiliensis* was relatively simple due to Malaysia's warm and humid climate, which lacks distinct seasons, which provides ideal circumstances for egg survival and hatching. In *L. sabanus*, *M. rajah*, *M. whiteheadi*, *R. tiomanicus*, and *S. muelleri*, Singh & Cheong

(1971) described *N. brasiliensis*. Nursyazana *et al*. (2013), on the other hand, described infection in commensal rats in coastal and island settings. Additionally, Paramasvaran *et al*. (2009a) detected *N. brasiliensis* in rats from five wet marketplaces.

Mastophorus muris, on the other hand, was discovered in rat populations from *L. sabanus* and *S. muelleri*. Additionally, previous research has documented this species infecting wild rats in a variety of Malaysian habitats, from urban to forest to coastal (Leong *et al*., 1979; Krishnansamy *et al*.,1980; Paramasvaran *et al*., 2005, 2009a; Syed Arnez & Mohd Zain, 2006; Mohd Zain *et al*., 2012). *Trichuris muris* was isolated from *L. sabanus* in the UGFR. Schacher & Cheong (1960) also described an unintentional infection of *R. rattus diardii* in Kuala Lumpur. This species appears to be extremely rare in the Peninsular Malaysia, where it is restricted to lowland and freshwater-swamp forests rodents (Ow-Yang, 1971). *Trichuris muris* is known to infect mice and has also been observed in wild rats in Iran (Kia *et al*., 2010) and Serdang, Selangor (Tijjani *et al*., 2020). Human trichuriases were believed to be responsible for around 500 million instances, with these cases being linked to *Trichuris trichiura* infections.

In this study, commensal rats were infected with *Capillaria* species. Rats only infected with this parasite if the infective stages are available. Since most of the rats feed and dwell in the same region, infection spread readily within the population. *Capillaria hepatica* was shown to be widespread in rats of the genus *Rattus* in many studies (Sinniah, 1979; Paramasvaran *et al*., 2005; Syed-Arnez & Mohd Zain, 2006; Paramasvaran *et al*., 2009a; Nursyazana *et al*., 2013). Apparently, foraging and feeding preferences were substantially correlated, suggesting that they were one of the factors contributing to the infection's diverse nature. This parasite appears to be ubiquitous in Malaysia, with a wide variety of rat hosts. Additionally, it can produce Hepatic capillariasis, a zoonosis that affects primarily rats and is only rarely observed in humans (Shafiyyah *et al*., 2012). Regrettably,

this parasite can induce hepatitis in humans, characterised by a high level of eosinophilia and a protracted fever (Paramasvaran *et al*., 2009a).

Only four cestodes were identified in this study: *Hy. parva* (syn *T. parva*), *Hy. taeniaeformis* (syn *T. taeniaeformis*), and *H. diminuta*. *Raillietina* sp. and *H. diminuta* were discovered in considerable abundance on UMFR and PTP, respectively. In the forest reserve, no *H. diminuta* were detected (UMFR and UGFR). Meanwhile, *Hy*. *parva* was isolated to an oil palm field in SGT. Mohd Zain *et al*. (2012) identified two cestode species in wild rats in Kuala Lumpur, whereas Nursyazana *et al*. (2013) identified two cestode species in wild rats in coastal and urban areas. Paramasvaran *et al*. (2009a) observed a larger diversity of cestodes obtained from urban rats, including *H. nana*, *H. diminuta*, *H. sabnema*, *Hymenolepis* sp., *Raillietina* sp., and *T. taeniaeformis*. As a result, certain cestodes species were more frequently associated with commensal rats but were also detected in forest rats in this investigation. Paramasvaran *et al*. (2009a) stated that there was a significant increase.

Hydatigera parva infections were shown to be significantly lower across the two rat species (*R. argentiventer* and *R. tiomanicus*) than *Hy. taeniaeformis* infections, which were discovered exclusively in *R. tiomanicus*. Adult tapeworms of this parasite live in the small intestines of carnivores (cats), which act as definitive hosts. However, infection is acquired by carnivores, when cats feed on rodents (intermediate hosts), or by consuming infected food or soil with cat excrement. *Taenia taeniaeformis*, more specifically the larval stage, has previously been recorded from a variety of locations worldwide (Mcinnes *et al*., 2014; Zhang *et al*., 2012).

Intermediate hosts have been observed to include rats, birds, insectivores, and even humans (Bowman *et al*., 2002; Mino *et al*., 2013; Sterba & Barus, 1976). However, *Hy*. *parva* (syn *T*. *parva*) was rarely recorded in Peninsular Malaysia but has been described in Sudan (Elowni & Abu Samra, 1988), Nigeria (George *et al*., 1990), Tunisia (Bernard,

1963), South Africa (Julius *et al*., 2018), and Guinea (M*astomys erythroleucus*) from Sierra Leone and the Democratic Republic of the Congo (Southwell & Kirshner, 1937; Mahon, 1954).

Leopoldamys sabanus, *R. argentiventer*, and *R. tiomanicus* were infected with *H. diminuta*. Sinniah *et al*. (1978) observed *H. diminuta* and *H. nana* infections in oil palm estate workers, demonstrating that these parasites are transmitted to humans. For *H. diminuta* to complete its life cycle, it required the consumption of an intermediate host such as fleas or cockroaches. It is not necessarily required, however, for the lifecycle to be completed by an intermediate host.

Raillietina sp. was identified as the most generalist cestode in this study, as it can infect all wild rat species except *R*. *argentiventer*. *Raillietina* sp. infection was recovered in wild rats caught in urban areas of Kuala Lumpur, Negeri Sembilan, and the Ulu Gombak Forest Reserve in Selangor by Paramasvaran *et al.* (2005) and Paramasvaran *et al*. (2009).

In general, the intrinsic factor and host sex did not differ amongst endoparasite species, except for *Raillietina* sp., which was shown to be more prevalent in females than in males. This tendency may be explained by females foraging more extensively and the availability of intermediate hosts, both of which result in an increase in infection exposure. Previously, we noted a female host bias (Wertheim, 1963). Males, on the other hand, were more likely to be infected with *Syphacia muris*. Males are believed to have developed such a widespread and pervasive infection as a result of frequent, long-term exposure to polluted food, water, and environment.

Between host ages, no significant differences were identified. Adults had a higher rate of infection than juveniles, implying that adults were foraging more energetically, increasing their exposure to infection. However, in this investigation, only *Raillietina* sp., *H. diminuta*, *H. parva*, and *S. obvelata* were shown to infect juvenile. In this investigation,

most of the rats infected were adult. At forest reserves and modified forest, the diversity of endoparasite helminths was greater. Additionally, there are no obvious differences in endoparasite abundance across these three habitats.

5.2.3 Fauna parasite and wild rat's associations

From previous to recent studies, the parasite-rodent relationship has been studied in Malaysia by some researchers. However, only a few studies have conducted research of wild rats and parasites from various forests, starting with forest reserves (Gunung Stong Forest Reserve, Kelantan (Mariana *et al*., 2005), Ulu Gombak Forest Reserve, Selangor (Paramasvaran *et al*., 2005; Paramasvaran *et al*., 2009), Endau Rompin National Park, Johor (Syed-Arnez & Mohd Zain, 2006), Ulu Muda Forest Reserve, Kedah (Mariana *et al*., 2008), Panti Forest Reserve, Johor (Mariana *et al*., 2011), Kemasul Forest Reserve, Pahang (Razali *et al*., 2018) and Hulu Terengganu, Tasik Kenyir (Ahmad *et al*., 2020)), recreational forest (Kuala Selangor Nature Park, Selangor (Chuluun *et al*., 2005), Langkawi Island and Jarak Island (Mohd Zain, 2008), Pangkor Island, Perak (Mohd-Taib *et al*., 2008), Carey Island (Nursyazana *et al*., 2013)) includes some covered wildlife reserve, wildlife sanctuary and National Park (Madinah *et al*., 2014; Hamdan *et al*., 2016) and modified forest in Hulu Langat, Selangor (Ishak *et al*., 2018).

Throughout all the research localities, including in this study, the number of rat species recovered were almost the same (*L. sabanus*, *Maxomys* sp., *M*. surifer, *M. whiteheadi*, *N. cremoriventer*, *R. argentiventer*, *R. rattus diardii*, *R. tiomanicus*, *S. muelleri*) except for *N. fulvescens* was not recorded previously while *R. exulans*, *M. rajah* and *Mus musculus* were not found in this research. The present of ectoparasites and endoparasites in forest reserve were known to be highest as compared to recreational forest and modified forest. For examples, Syed-Arnez & Mohd Zain (2008) identified 8 rat species (*L. sabanus, M. rajah, M. surifer, M. whiteheadi, R. exulans, R. rattus diardii, R. tiomanicus,* and *S. muelleri*) which harboured about 23 nematode species, 3 cestode species, 2 trematode

species and 1 pentastomide species from Endau Rompin National Park, Johor. Hamdan *et al*. (2016) found *L. sabanus*, *M. rajah*, *M. surifer*, *M. whiteheadi*, *N. cremoriventer*, and *S. mulleri* were positively infected with gastrointestinal helminth in five localities (National Park) in Western Sarawak and *M. surifer* was the highest infection rate. *Strongyloides* sp. and *Toxocara* sp. were identified in rats host at Sarawak which known to be zoonotic species.

The distribution of ectoparasites in most islands had more or less the same genus, namely *Laelaps* spp. which infested *M. surifer*, *M. rajah*, *R. tiomanicus, R. rattus diardii*, *R. argentiventer* and *R. norvegicus* (Nursyazana *et al*., 2013; Mohd-Taib *et al*., 2018). Madinah *et al*. (2014) covered over 16 localities of Wildlife Reserve, Wildlife Santuary and National Park included Peninsular and Borneo (Sabah and Sarawak) which captured 16 species of rodents species with ticks, chiggers, mesostigmatids, listrophorids, astigmatid mites, and lice from year 2008 to 2010. Besides, in Terengganu with the mixed dipterocarp forest structure, there were group of ectoparasite such as fleas, tick, mesostigmatid, and chigger infested rat species (*L. sabanus, M. rajah, M. surifer, M. whiteheadi, Mus musculus, R. argentiventer, R. exulans, R. tiomanicus, R. rattus* and *S. muelleri*) except *Mus musculus* (Ahmad *et al*., 2020). *Rattus tiomanicus* was the most infested species in coastal forest, insular forest (Ahmad *et al*., 2020) as the same outcome in this study.

Habitat appeared to be a critical element influencing helminth infection and variation in Malaysian rodents. Habitat shifts which occur in hosts can cause the nestedness of parasite collection in community (Timi & Poulin, 2003). In comparison to rodents in other contexts, particularly forest reserve rats, agricultural mice showed a significantly lower ectoparasite and endoparasite helminth species richness and were more related to recreational forest rodents. This research confirms recent findings from other Southeast Asian nations (Chaisiri *et al*. 2017; Chaisiri *et al*. 2012; Pakdeenarong *et al.* 2014) that

development reduces the diversity of rodent helminth species. This study discovered that the diversity of vertebrate and invertebrate species reduced in urban regions. Development and other human intervention may influence the host-parasite network, impairing initial parasite transmission (Bordes *et al*. 2015). According to Palmeirim *et al*., (2014) rats found in forests have the highest helminth burden when compared to rat found in anthropized environments, similarly with ectoparasite existence.

We investigated the rat-fauna parasite assemblage using host-parasite network analysis, combining the eight locations analysed. The first discovery was that host-parasite interactions are nested. Certain assumptions regarding the differing abilities of hosts and parasites to interact could account for the structure. Intrinsic properties of a parasite, such as its life cycle complexity, may affect its capacity to infect and survive a new host (Goüy de Bellocq *et al*., 2003). Simple or direct life cycles, which require only one or two intermediate hosts, may be more adaptable to new hosts than parasites with more complex (indirect) life cycles (figure 5B). Additionally, depending on feeding habits and climatic variables, host environment variation might result in nested parasite assemblages within a community (Timi & Poulin, 2003). This hypothesis is consistent with another theory explaining how nestedness structures formed, which states that when a new species enters a community, interactions within the network tend to become nested (Bastolla *et al*., 2009; Joppa *et al*., 2010).

Additionally, the unipartite network revealed that *R. tiomanicus* was the network's most core host based on both ecto and endoparasite helminth species sharing, but with additional *L. sabanus* for ectoparasite. *Rattus tiomanicus* is a cosmopolitan species with a low habitat preference that can be found in a wide variety of habitats (Palmeirim *et al*., 2014; Blasdell *et al*., 2015; Morand *et al*., 2015). This species appears to be adapting to Southeast Asia's changing environment, and it may serve as a 'bridge species' connecting rodent-parasite families found in forest and agricultural zones. Based on our findings, we

hypothesise that *R. tiomanicus* may play a substantial role in host-parasite interactions and parasite transmission of helminths and other illnesses to other rodent hosts and possibly humans.

5.3 Cestode molecular characterization

With the advent of molecular phylogenetic investigations, the interrelationships of tapeworms have been largely resolved in terms of their hierarchy. However, phylogenetic studies on cestodes in rats are scarce in Malaysia and even more so in Southeast Asia. Due to a scarcity of molecular data on cestodes in Malaysia, this cestode species has been molecularly identified. As a result, this study employed mitochondrial genetic markers such as COX1 and 18SrDNA genes. The 18SrDNA gene was identified and used as a universal primer, meanwhile the COX1 gene was employed to amplify the sequences as previously described by Poon *et al.* (2017). The outcomes of this study indicate that 18SrDNA may be used to characterise the majority of cestode species identified in rats, apart from *Raillietina* sp., as well as the genetic marker COX1.

Using DNA sequences obtained from GenBank, we can clarify the newly discovered species their respective families, namely Davaineidae, Hymenolepidae, and Taeniidae. Additionally, the trees constructed for these two molecular mitochondria markers demonstrate that *Raillietina* sp. are unable to be categorised to the species level. One of the primary reasons for the species' inability to be further explained is a lack of molecular data in GenBank. Although some DNA data are available in GenBank, they are not from the *Raillietina* sp. identified in rats, but rather from avian, which is their most common host species. *Raillietina* sp. discovered in rats in Malaysia are almost certainly cryptic. This is because host changes might influence parasite species. Unfortunately, morphological characterization cannot be utilised to support this study because several critical features were not apparent under the microscope and some specimens had been degraded. As a result, sufficient molecular data should be added to the GenBank in order

to conduct a more extensive investigation of this species. Not just in Malaysia, but throughout Southeast Asia as a whole.

The 18SrDNA gene was found to be useful molecular tool for resolving the phylogenetic connections in a variety of taxa, including parasitic parasitic platyhelminths with high taxonomic level such as cestode (Mariaux, 1996; Foronda *et al*., 2004; Yan *et al*., 2013; Tanaka *et al.* 2014). In comparison to the COX1 gene, a more relevant phylogenetic tree was created using the 18SrDNA gene. Only a few cestode species, such as *Hydatigera* spp. and *H. diminuta*, were amplified correctly utilising the COX1 gene, but not *Raillietina* species. This could be because the sequences amplified during the sequence amplification process are short in length. While identifying specimens to the species level may be problematic with sequence lengths less than 200 bp, we were able to classify all specimens into their genus as belonging to the order Cyclophyllidea. Additionally, a well-organized database is critical. When the sequences in GenBank are not well-organized, misidentifications can occur.

All commonly used molecular regions are insufficient for recognising known cestode species in the context of helminths. Through and generally, universal primers are an advantageous alternative for decreasing the expense and labour associated with producing species-specific primers, particularly when a collection of organisms encompasses a diverse range of species, such as cestode. It is vital, however, to produce a good universal primer capable of binding a vast array of species. Because we were utilising primers obtained by Poon *et al*. (2017), he also noted that the COX1 primer contained just three tapeworm genera (*Anoplocephala*, *Hydatigera*, and *Taenia*), but no *Raillietina* species. This may be one of the reasons why *Raillietina* sp. cannot be properly defined as another species, even if the COX1 gene was one of the previously identified DNA barcoding genes. Alternatively, *Raillietina* species specific primers should be created in order to

establish a more precise genetic categorization by acquiring all available sequences in GenBank.

The order cyclophyllidea is found in a variety of hosts, including mammals, birds, reptiles, and amphibians. However, the transitions of tetrapod parasitism remain confusing due to the lack of fossil evidence in even well-supported phylogenies (Littlewood *et al*., 2015). Nonetheless, our study is the first to do molecular analyses on cestodes obtained from rats, and the phylogenetic tree was able to convincingly define *H. diminuta* as a species. Lavikainen *et al*. (2016) updated and reclassified *T*. *taeniaeformis* as *Hy. taeniaeformis*. Nowadays, *Hy*. *taeniaeformis* is one of the most studied species, owing to its widespread distribution and the presence of three morphologically cryptic species (Lavikainen *et al*., 2016; Alvi *et al*., 2021). Thus, for the family Davaineidae, additional study is required to define the genus *Raillietina* at the species level utilising additional DNA markers in order to more precisely identify this cestode species.

The identification of *Raillietina* sp. in rats is not new in Malaysia; it has been described in various earlier studies (Jeffery *et al*., 1986; Paramasvaran *et al*. 2005, 2009b). On the other hand, the molecular data were the first to be documented beside published by Mariaux *et al*. (2021). Jeffery *et al.* (1986) reported the discovery of the first *Raillietina* (*Raillietina*) *celebensis* (Janicki 1902) Fuhrmann 1920 in Peninsular Malaysia in a new host of *Rhizomys sumatrensis* (large bamboo rat). *Raillietina* sp. has infectious cysticercoid intermediate hosts such as ants and beetles. *Raillietina* is a zoonotic species that has been associated with human infection in China, Costa Rica, France, Polynesia, Indonesia, Japan, and Thailand (Chaisiri *et al*., 2015).

CHAPTER 6: CONCLUSION

The relationship between two living organisms (host and parasite) in which one supports the other is known as parasitism (generally). In environments with diverse hosts, parasites are more prevalent, while in environments with diversified hosts, parasite diversity is greater. One of the important features of host-parasite interactions is the so-called co-evolutionary process. The interpretation of the data may have been altered by the several limitations of this parasitology study in this murids research. Nevertheless, the distribution of wild rats in Peninsular Malaysia has highlighted the fauna parasite infested and revealed some of parasite species harboured in murid species. Altogether these findings have important implication for the disease or parasite transmitted by murid rats in term of murid species and their habitats in the region.

6.1 Employing the population of wild rats in different forest types from Peninsular Malaysia

Knowing which species are found in different forest areas in Peninsular Malaysia and their distribution across the region is important to estimate the environmental health of several localities for wild rats. Through this study, 10 species of murid rats have been documented in Peninsular Malaysia. However, most wild rat species lack and/or accurate locality records and are listed as "Least Concern" by the IUCN, further suggesting the need for further rat surveys and reconsideration of the conservation status of these species in the region. Identification is done through morphological identification, where their taxonomic status and presence in the region is somewhat unresolved due to the lack of DNA barcodes. Future work including more study sites should be involved to access the distribution of wild rodents in a wider range in the region. As a result, there is a need to raise awareness about rat population and control not only in this research area, but in all habitats with human contact and settlements.

6.2 Understanding host-parasites relationships harboured in wild rats

The host-parasite has a diverse relationship. Various factors affect the presence of parasites on rats, one of which is the existence of a variety of species in an area with high humidity, most preferred by cysts of worms. The researchers discovered that wild rats collected in the research area were infected with zoonotic parasites from a variety of different species. There are seven ectoparasites and fifteen endoparasite worms, and some of them are known to be zoonotic species, *Laelaps* sp., *Leptotrombidium* sp., *Ixodes granulatus*, *Polyplax spinulosa,* several genera, such as *Capillaria*, *Trichuris*, *Hydatigera* (syn *Taenia*), *Hymenolepis*, and *Raillietina*.

The trends of infection between parasite-rat associations went downward, with fewer host species diversity between the different forested areas. Although the number of host species was almost the same, there were decreases in abundance in each location. Forest rat species are more likely to be infected with parasites than commensal rat species. The diversity of other animals in the forest affects this situation; parasites such as tapeworms have a more complicated life cycle, and rats as the intermediate host. The discovery of parasitic cestodes has resulted in the recognition of cestodes as significant zoonotic agents for humans and animals in Southeast Asia.

Additional infectious diseases associated with rats, such as bacteria, fungi, and viruses, should be investigated. This research can be used to increase public awareness about parasite infections, allowing for a more effective prevention and control programme to be implemented. This is because the parasites carried by rats may pose a threat to public health.

6.3 Utilising molecular-based identification to analyse cyclophyllidean worms in wild rats

The characterization of cestode worms from wild rats at the molecular level is not well established. This study has demonstrated the utility of DNA techniques in documenting

the cyclophyllidean cestode infected in wild rats from Peninsular Malaysia. One of universal primer from 18SrDNA genes has been designated to identify the cestodes species and COX1 genes was also used from published paper. Thus, the 18SrDNA gene was found to be an effective marker for establishing and clarifying the phylogenetic relationships between all cestode worms. As a result, four tapeworm species which harboured in seven species of murids. This eventually updates the molecular data presence in GenBank as the cyclophyllidean molecular data is still limited, mostly in Peninsular Malaysia. Research or molecular characterization of *Raillietina* spp. is necessary to clarify their classification in terms of phylogenetic analyses and future results. Future research on molecular systematics and taxonomy in the order of Cyclophyllidean cestodes is critical for deciphering complex species-specific linkages.

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