

Small mammal assemblages and their ectoparasite prevalence (Acarina) in mangrove forests of Peninsular Malaysia

Farah Shafawati Mohd-Taib*, Rosha Asyikha and Shukor Md. Nor

Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

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Abstract: The mangrove forest is located in the coastal intertidal zone and plays a vital role in buffering against erosion from storm surges, currents, waves, and tides. This ecosystem also serves for ecotourism due to its heterogeneous habitats, which supported a high diversity of plants and animals. Small mammals are among the abundant but poorly recognised inhabitants in mangrove forests. Apart from their role as primary consumer and prey, they are host to various ectoparasites, which potentially become the primary vector for tick-borne diseases among visitors. Therefore, this study aims to determine the small mammal assemblages and their ectoparasite prevalence in mangroves forests of Peninsular Malaysia. The capture-release method was applied for small mammal sampling, and each captured individual was screened and collected for ectoparasites before released. DNA barcoding using CO1 genes was performed for small mammal species verification, whereby ectoparasite identification was based on morphological identification and molecular verification using 16S rDNA genes. A total of 94 small mammals from 6 species were captured across sites, dominated by *Rattus tiomanicus* (n=74). From this number, 15 individuals (16% prevalence) were infested by ticks, 22 individuals (23%) were infested by mites, whereas 4 individuals (4%) were infested by both ticks and mites. Five species of ticks and one mite species were identified; *Amblyomma cordiferum*, *Ixodes granulatus*, *Haemaphysalis hystricis*, *Dermacentor auratus*, *Dermacentor atrosignatus*, and *Laelaps echidninus*. This study reveals a relatively poor diversity of small mammals in the mangroves forest, of which highly infested with a diversity of ectoparasites, elucidating the relationship of host-ectoparasite associations in the riparian zone. This information is crucial to inform visitors to these areas, ultimately safeguard against ectoparasite-borne disease.

Key words: Ectoparasite; rodent; riparian zone; PCR detection; prevalence; Malaysia.

Introduction

The mangrove forest is a wetland ecosystem that plays a crucial role in stabilising and protecting the shorelines from increased storm surge, flooding, and hurricanes. It is also

*Corresponding author. E-mail: farah_sh@ukm.edu.my

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considered one of the most productive ecosystems, which hosts a diversity of flora and fauna (Sundararaman et al. 2007; Jusoff 2013). This ecosystem provides economic and social benefits (Ashton and Macintosh 2002) to human livelihood. However, the unique coastal mangrove resources have been exploited for many purposes, such as timber harvesting for the charcoal industry, aquaculture, agriculture, fisheries, residential, industrial, and urbanisation (Chong 2006). These anthropogenic disturbances, along with natural events, have consequently threatened mangrove ecosystems worldwide (Schipper et al. 2008; Polidoro et al. 2010).

Mangroves serve as habitat, nurseries, and breeding grounds for various marine animals, including crustaceans and fishes (Barbier and Strand 1998), as well as terrestrial animals. Many animals are highly dependent on mangroves, particularly concerning food resources, shelter, and their interaction with non-living components in the mangrove ecosystems (Kathiresan 2000). For instance, large predators such as snakes, owls, and eagles hunt a wide variety of preys like rats and small birds. Small mammals like rodents thrive in this ecosystem due to their high adaptability to a variety of living conditions (Tripathi 2014). This group of animals also plays a vital role in mangrove ecosystems as pollinating agents (squirrels and bats), pest control among insects and molluscs, as well as biological indicators of forest ecosystem health (Khan 2012). Nonetheless, the literature on the assemblage of terrestrial vertebrates, especially on small mammals in mangrove forests, is scarce (Luther and Greenberg 2009; Hogarth 2015). Thus, it is imperative to understand the species assemblages present. Besides, anthropogenic disturbances, including deforestation, habitat destruction, and unsustainable harvesting of the forest products pose significant threats to mangrove-dependent species (Kairo et al. 2001), including small mammals and other wildlife species (Kathiresan and Bingham 2001).

Ectoparasites, including mites, lice, fleas, and ticks are often associated with small mammals as their main hosts (Eslami et al. 2018). Several studies have revealed that small mammals like rodents and tree shrews are important hosts for ectoparasites (Paramasvaran et al. 2009; Madinah et al. 2011; Mohd Zain et al. 2015; Ernieenor et al. 2016; Ishak et al. 2018b; Razali et al. 2018). Ishak et al. (2018b) reported relatively low diversity of ticks in small mammals in a tropical lowland dipterocarp forest. Meanwhile, Eslami et al. (2018) revealed that a rodent species, *Rattus rattus* (Linnaeus, 1758), was highly infested by three ectoparasite species from the mangrove forest in Qeshm Island, Iran. Nevertheless, there are still no detailed studies on the prevalence of ectoparasites on small mammals in mangrove forests, specifically in Southeast Asia. The presence of ectoparasites in an area is influenced by the composition and abundance of the small mammal inhabitants (Dobson et al. 2016). Therefore, ectoparasite movement is highly dependent on the community and distribution of their hosts (Estrada-Peña and de la Fuente 2014). Ticks (Acari: Ixodida) are obligate blood-feeding parasites of terrestrial vertebrates, grouped into three families, i.e., Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttalliellidae (Horak et al. 2002). Hard ticks could be found in areas with abundant vegetation and more temperate climates, whereas soft ticks are more resilient and live in a more barren and arid zone (Kröber and Guerin 2007). Both groups are important vectors of various pathogens of veterinary and medical concern (Sarwar 2017). Mites are smaller Acari under the class Arachnida. They are divided into four types, namely mesostigmatid, chiggers (larvae), myobiids and listrophorids (Mohd-Zain et al. 2015). *Laelaps echidninus* Berlese, 1887 is one of the mite species in the family Laelapidae that is often associated with small mammals, where this species tends to infect rodent species like *Rattus norvegicus* (Berkenhout, 1769) (Baak-Baak et al. 2016).

Small mammals have also been reported as primary hosts of various pathogens vectored by these ectoparasites, which can also be transmitted to humans (Tripathi 2014). Many species of ectoparasites are of substantial interest and value as vectors of a wide variety of pathogens, such as the Lyme disease, meningoencephalitis, babesiosis, ehrlichiosis, and tularaemia to both humans and animals (Pulscher *et al.* 2018; Heukelbach *et al.* 2012). For instance, *Rickettsia helvetica* is spread primarily by tick species and is responsible for spotted fever (Dobec *et al.* 2009), whereas *Anaplasma phagocytophilum* (human granulocytic anaplasmosis agent) transmission occurs by Ixodid ticks, with rodents as host (de la Fuente *et al.* 2016). *Orientia tsutsugamushi* is responsible for scrub typhus, which is transmitted by mites (chiggers; Rodkvamtook *et al.* 2018). Apart from that, Haddad *et al.* (2018) described that the small bites from ticks could cause severe injury and direct damage, leading to toxic circumstances like paralysis, irritation, and allergies in animals and humans.

In the present study, we determined the ectoparasite diversity and prevalence from small mammals captured in three mangrove areas in Peninsular Malaysia. These mangrove sites have undergone varying anthropogenic pressures, which resulted in a reduced mangrove cover, and increased anthropogenic activities (horticulture, urbanisation, and monoculture plantation activities). Apart from tourism, these habitats continue to provide resources for the local community's livelihood, including seafood products and logs for charcoal production. This study is pioneer in documenting the ectoparasite assemblages infesting small mammals in mangrove ecosystem; thus, the potential infestation to the local communities, as well as visitors to these areas.

Material and methods

Study sites

The study was conducted at three mangrove forests of Peninsular Malaysia, in the state of Terengganu (KSY), Negeri Sembilan (KST), and Perak (KDP) (Figure 1). Sampling was carried out in two phases at each location, between December 2017 and August 2018, with seven days of continuous trapping per phase, at each location. Sampling was conducted at five stations along a 1 km stretch from the jetty, following the river at each mangrove site. Each station was not further than 300 m from the riverbank.

Small mammals trapping and species identification

A total of 125 wired mesh cage traps (28 cm × 15 cm × 12.5 cm) were deployed randomly at five research stations in each mangrove forest (25 cages/station). These cages were baited with different types of aromatic baits, including banana, oil palm fruit, and jackfruit (Bernard *et al.* 2004) in equal amounts, and were placed on tree stumps, fallen logs, and at ground level (Balete *et al.* 2009; Rickart *et al.* 2011). All traps were checked twice a day at 0900 hours and 1700 hours. Captured individuals were marked with an ear tag with an ID number. However, in this study, recaptured animals were not considered as a new individual. All captured individuals were anaesthetised (Massolo *et al.* 2003), with a volume between 0.1-0.3 ml of Zoletil 100® injected intramuscularly according to the weight of the animal. As soon as the animals lost consciousness, the standard external measurements were recorded, including the length of tail, whole body, head, ears, and foot patch using a Vernier calliper and measuring tape. Species identifications were based on the morphological measurements, as well as physical features such as fur and tail colour, following Francis (2008). In addition, the tip of the tail (tissue samples) was cut off from

each individual, for species verification using barcoding, targeting on the CO1 genes, according to Li et al. (2014). Small mammal trapping and handling procedures were approved by the animal research ethics committee of Universiti Kebangsaan Malaysia (FST/2020/FARAH SHAFAWATI/16-JAN./1080-FEB.-2020-FEB.-2022-NAR-CAT2).

Ectoparasite collection and morphological identification

Ectoparasites were identified based on morphological observations using a stereomicroscope and further verified with molecular methods. Each host was carefully combed for ectoparasites (ticks and mites) and collected using tweezers and placed individually in labelled cryo-vials containing 70% alcohol for preservation. For morphological identification, the ectoparasites were observed under a stereomicroscope (Motic SMZ-168 Stereo Zoom microscope; Motic, Hong Kong) to inspect the basic features, and identify the samples up to genus level, as well as to classify the developmental stage (larvae, nymph, or adult) and sex followed Strandtmann and Mitchell 1963; Yamaguti et al. 1971; Baker 1999; Walker et al. 2007). Next, ectoparasite species identifications were confirmed by a molecular approach based on 16S rRNA gene (Black and Piesman 1994). Prior to this, each ectoparasite individual was surface sterilised by rinsing with 70% ethanol solution, followed by washing in sterile distilled water to eliminate debris from possible environmental pollution (Carpi et al. 2011).

DNA extraction and PCR analysis

DNA from ticks and mites was extracted using the HiYield Plus Genomic DNA Mini Kit (Real Biotech Corporation [RBC], Taiwan), according to the manufacturer's protocol.

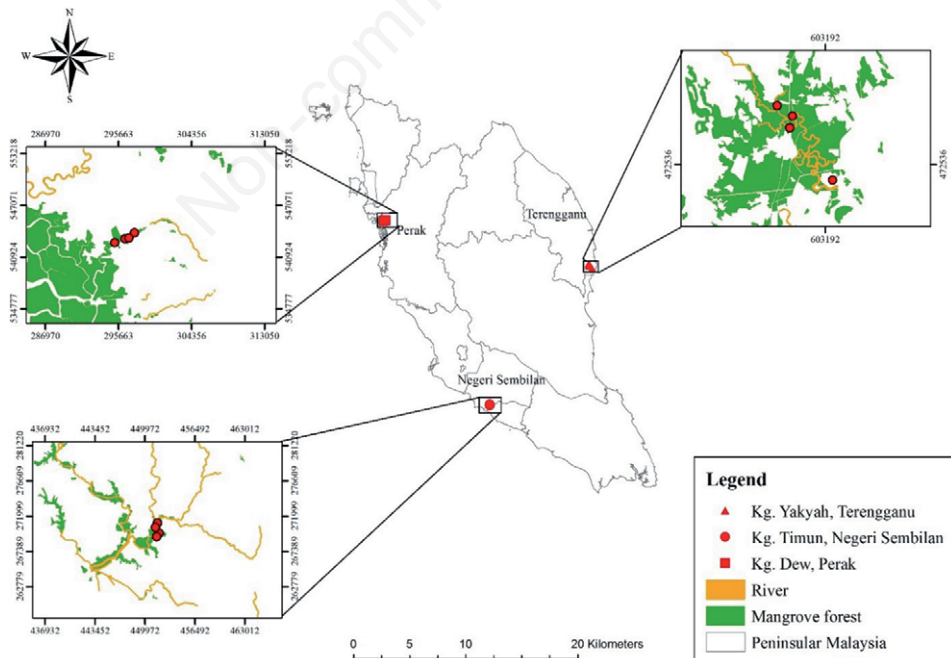


Figure 1. Location of mangrove sites at Kg. Dew, Perak (KDP), Kg. Yakyah, Terengganu (KSY) and Kg. Timun, Negeri Sembilan (KST) in Peninsular Malaysia.

DNA was extracted by adding 500 μL of phosphate-buffered saline (PBS) to the sample. Then, 200 μL of QGT Buffer and 20 μL of Proteinase K were added. The samples were vortexed and incubated at 60°C overnight until the sample lysate became clear. PCR amplification was initially performed to amplify mitochondrial rDNA gene for the ectoparasites, with the primer set designed by Black and Piesman (1994), namely, 16S+1 for the forward strand (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') and 16S-1 for the reverse strand (5'-CCGGTCTGAACTCAGATCAAGT-3'). The PCR was performed using an Alpha Cycler PCRmax machine (PCRmax, UK). The 16S rDNA gene amplification program was conducted as follows; initial denaturation at 95°C (5 min), followed by 10 cycles of denaturation at 92°C (1 min), 48°C (1 min), and 72°C (1.5 min). Then, continued with 32 cycles of 92°C (1 min), 54°C (35 sec), 72°C (1.5 min), and followed by a final extension step at 72°C (7 min). Nuclease free water was used as the negative control to replace the DNA template. Next, the amplified products were electrophoresed on 1.0% agarose gel and viewed under an ultraviolet (UV) transilluminator. DNA purification and sequencing analysis were performed by MyTACG Bioscience Enterprise (Malaysia).

Sequencing alignment and phylogenetic analysis

Representative DNA sequences of ectoparasite species, animal host species, and the sampling sites, were used in a BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST>) and aligned with other ectoparasite reference sequences available in the GenBank. The new accession numbers of all ectoparasite sequences created from the GenBank were mentioned. Analysis of multiple sequence alignment of 16S rDNA sequences was generated with the Muscle software tool in the MEGA (Molecular Evolutionary Genetics Analysis) software version 7, as defined by Kumar *et al.* (2016). The results of DNA sequences were exported as FASTA format files. A phylogenetic tree was constructed by neighbour-joining (NJ) based on the Kimura two-parameter model (K2) to conclude the relationships within and between the ectoparasite species. NJ tree was performed using both sequences of tick and mite samples in this study and other reference sequences from the GenBank. Pairwise sequence comparison was performed using the MEGA software version 7 based on 16S rRNA genes.

Data analysis

To distinguish the completeness of sampling effort, individual-based rarefaction curves were constructed (Entsminger 2012; Mohd-Taib *et al.* 2020) for each site using EcoSim 700 software version 7.71 (Acquired Intelligence Inc. 2018). Ectoparasite abundance was calculated for every small mammal individual captured. In epidemiological studies, prevalence is calculated based on all individuals affected by the disease at a time (Shields and Twycross 2003) and stretches a figure for a factor at a single point in time (Jekel *et al.* 2001). From this study, the prevalence of small mammal species infested with ticks and mites was calculated according to Akucewich *et al.* (2002), using the formula below:

$$= \frac{\text{Number of infested small mammals}}{\text{Total number of small mammals captured}} \times 100\%$$

Next, an independent sample t-test was used to ascertain the differences between ectoparasite infestations on male and female hosts, using SPSS version 23 (IBM Corporation).

Results

Small mammal assemblages and their ectoparasite prevalence at mangrove areas

A total of 94 small mammals belonging to six species [*Rattus tiomanicus* (Miller, 1900), *Rattus rattus diardii* (Linnaeus, 1758), *Maxomys whiteheadi* (Thomas, 1894), *Sundamys muelleri* (Jentink, 1879), *Tupaia glis* (Diard, 1820), and *Callosciurus notatus* (Boddaert, 1785)] and two orders (Rodentia and Scandentia) were trapped from the three different mangrove sites. The barcoding results of different species are illustrated in Table 1, and the distribution of small mammal species are listed in Table 2. The most abundant small mammal species trapped from this study is *R. tiomanicus* (Malaysian wood rat; 78.7%, n=74), followed by *T. glis* (9.6%, n=9), and *S. muelleri* (5.3%, n=5). The least abundant host species was *M. whiteheadi* (1.06%, n=1). Each mangroves site recorded three small mammal species, with KDP recording the highest abundance with 42 individuals, followed by KST (36 individuals), and the least at KSY, with 16 individuals. The Shannon diversity index was slightly higher at KSY (0.7029), followed by KDP (0.6948), and KST (0.5503). In addition, rarefaction curves demonstrated that nearly all study sites

Table 1. List of the different species of small mammals and BLAST results from the GenBank.

ID code	Mangrove area	Host species (Identification)		Similarity with GenBank, %
		Morphology	Molecular	
PKY010	KSY	<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98
PKY012		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98
PKY002		<i>Rattus rattus</i>	<i>Rattus rattus diardii</i>	99
PKY009		<i>Rattus rattus</i>	<i>Rattus rattus diardii</i>	98
PKY004		<i>Maxomys whiteheadi</i>	<i>Maxomys whiteheadi</i>	99
STM032	KST	<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	99
STM001		<i>Tupaia glis</i>	<i>Tupaia glis</i>	99
STM014		<i>Tupaia glis</i>	<i>Tupaia glis</i>	99
STM015		<i>Callosciurus notatus</i>	<i>Callosciurus notatus</i>	99
STM020		<i>Callosciurus notatus</i>	<i>Callosciurus notatus</i>	99
KDM016	KDP	<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	99
KDM006		<i>Sundamys muelleri</i>	<i>Sundamys muelleri</i>	99
KDM036		<i>Sundamys muelleri</i>	<i>Sundamys muelleri</i>	99
KDM004		<i>Tupaia glis</i>	<i>Tupaia glis</i>	99
KDM012		<i>Tupaia glis</i>	<i>Tupaia glis</i>	100

Table 2. Species distribution of small mammals at different mangrove sites.

Small mammal species	KSY	KST	KDP	Total
<i>Rattus tiomanicus</i>	12	27	35	74
<i>Tupaia glis</i>	0	7	2	9
<i>Sundamys muelleri</i>	0	0	5	5
<i>Rattus rattus diardii</i>	3	0	0	3
<i>Callosciurus notatus</i>	0	2	0	2
<i>Maxomys whiteheadi</i>	1	0	0	1
Total	16	36	42	94
Diversity	0.7029	0.5503	0.6948	

approached an asymptote, indicating the sampling relatively covered all species present (Figure 2).

From the 94 individuals captured, 15 individuals from 3 species (16% prevalence) were infested with ticks. Table 3 lists the number of small mammal individuals captured and the number of individuals infested with ticks and mites (with % prevalence). *R. tiomanicus* has the highest rate of ectoparasite infestation, with 12 individuals infested (12.8% prevalence), followed by *S. muelleri* (n=2, 2.1%), and *Rattus rattus diardii* (n=1, 1.1%). On the other hand, 22 individuals from 4 species were infested with mites (23.4%). Similar to ticks, the highest small mammal species infested with mites was *R. tiomanicus* (n=18, 19.1%), followed by *S. muelleri* (n=2, 2.1%), while *Rattus rattus diardii* and *M. whiteheadi* shared a similar prevalence percentage (n=1, 1.1%). All the infested hosts belonged to the same family, Muridae. On the other hand, *T. glis* and *C. notatus* were not infested with either ticks or mites, while three individuals of *R. tiomanicus* and one individual of *Rattus rattus diardii* were infested by both ticks and mites. Although ectoparasite infestation was not significantly different between host sex ($p>0.05$), female hosts exhibit higher infestation than male hosts. Table 4 shows the distribution of male and female small mammals infested by both ticks and mites across the study sites.

Table 3. Number of small mammals infested by ticks and mites and their prevalence (%) in all study sites.

Order	Family	Small mammal species	Number of individuals	Number of hosts infested by ticks (%)	Number of hosts infested by mites (%)
Rodentia	<i>Muridae</i>	<i>Rattus tiomanicus</i>	74	29/12 (12.8)	46/18 (19.1)
		<i>Sundamys muelleri</i>	5	8/2 (2.1)	7/2 (2.1)
		<i>Rattus rattus diardii</i>	3	1/1 (1.1)	1/1 (1.1)
		<i>Maxomys whiteheadi</i>	1	0 (0)	6/1 (1.1)
		<i>Sciuridae</i>	<i>Callosciurus notatus</i>	2	0 (0)
Scandetia	<i>Tupaiaidae</i>	<i>Tupaia glis</i>	9	0 (0)	0 (0)
Total			94	38/15 (16)	60/22 (23.4)

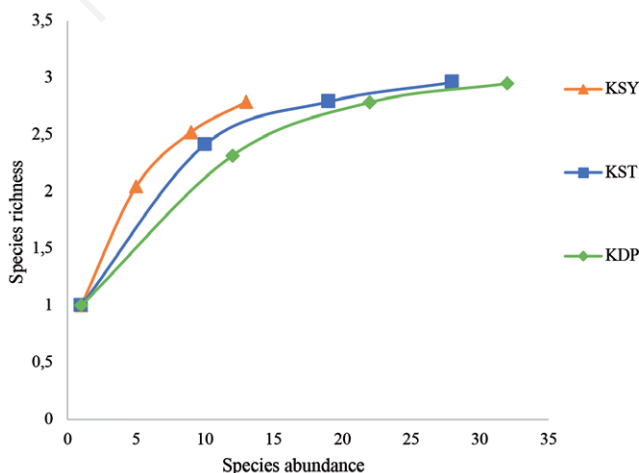


Figure 2. Rarefaction curve of small mammals in all study sites.

Morphological and molecular identification of ectoparasite samples

A total of 60 mites and 38 ticks were collected from the 33 infested small mammals belonging to 4 host species. From the stereomicroscopic examination, four tick species were identified, i.e., *Ixodes granulatus* Supino, 1897, *Dermacentor* sp., *Haemaphysalis* sp., and *Amblyomma* sp., according to their morphology and external characteristics. The amplified DNA sequence of ectoparasites species on 16S rRNA genes were approximately 460 bp in length. The similarity of the genes with the available sequence in the NCBI GenBank and the newly generated GenBank accession numbers are provided in Table 5. The

Table 4. Number of male and female hosts, and their infestation rate of ticks and mites.

Ectoparasites	Male host	Male infested (%)	Female hosts	Female infested (%)
Ticks		6 (6.4)		7 (7.4)
Mites	51	10 (10.6)	43	12 (12.8)
Total		16 (17.0)		19 (20.2)

Table 5. List of representative ectoparasite sequences with their host species, BLAST results and newly generated accession numbers from the GenBank.

ID code (sample)	Host species	Species (Identification)		Similarity with GenBank, %	Accession number
		Morphology	Molecular		
STM005-02	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	97	MT912952
STM009-02	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912961
STM010-03	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912962
STM021-01	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912964
STM021-03	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912965
STM021-04	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912966
STM021-06	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912967
KDM032-01	<i>R. tiomanicus</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912980
KDM037-01	<i>S. muelleri</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	98	MT912981
KDM037-06	<i>S. muelleri</i>	<i>Amblyomma</i> sp.	<i>A. cordiferum</i>	97	MT912986
STM016-01	<i>R. tiomanicus</i>	<i>Haemaphysalis</i> sp.	<i>H. hystricis</i>	98	MT912970
PKY016-03	<i>R. tiomanicus</i>	<i>Dermacentor</i> sp.	<i>D. auratus</i>	99	MT914183
PKY016-04	<i>R. tiomanicus</i>	<i>Dermacentor</i> sp.	<i>D. auratus</i>	98	MT914184
STM010-04	<i>R. tiomanicus</i>	<i>Dermacentor</i> sp.	<i>D. atrosignatus</i>	99	MT912973
STM010-05	<i>R. tiomanicus</i>	<i>Dermacentor</i> sp.	<i>D. atrosignatus</i>	99	MT912974
STM012-01	<i>R. tiomanicus</i>	<i>Dermacentor</i> sp.	<i>D. atrosignatus</i>	98	MT912975
STM012-02	<i>R. tiomanicus</i>	<i>Dermacentor</i> sp.	<i>D. atrosignatus</i>	98	MT912976
PKY002-01	<i>Rattus rattus diardii</i>	<i>Ixodes</i> sp.	<i>I. granulatus</i>	98	MT914179
PKY003-01	<i>R. tiomanicus</i>	<i>Ixodes</i> sp.	<i>I. granulatus</i>	98	MT914180
STM023-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	89	MT808284
STM033-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	88	MT808284
KDM001-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	87	MT808284
KDM015-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	88	MT808284
KDM016-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	87	MT808284
KDM018-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	88	MT808284
KDM020-01	<i>R. tiomanicus</i>	<i>L. echidninus</i>	<i>Androlaelaps casalis</i>	88	MT808284

BLAST search result revealed 5 tick species, namely *I. granulatus* (n=2), *Dermacentor auratus* Supino, 1897 (n=4), *Dermacentor atrosignatus* Neumann, 1906 (n=5), *Amblyomma cordiferum* Neumann, 1899 (n=26), and *Haemaphysalis hystricis* Supino, 1897 (n=1). The external morphological characteristic of mite species was first observed and identified under the stereomicroscope as *Laelaps* sp. based on published taxonomic keys, and further identified as *L. echidninus* after confirmation with the mite expert, Dr John McGarry (personal communication). However, BLAST results showed that the mite samples were *Androlaelaps casalis* (Berlese, 1887) through molecular identification on 16S rRNA genes, but with low per cent similarity value between DNA sequences and the closest match in GenBank (87% to 89%). We concluded that the species of mite found in small mammal host is recognised as *L. echidninus* based on their specific morphological features and a large number of this species have been recorded infecting small mammal hosts, according to previous studies (Montasser 2006; Baak-Baak *et al.* 2016).

From 38 individual tick samples, 19 DNA sequences of 16S rDNA genes from the present study were selected for phylogenetic analysis based on the Kimura two-parameter model (K2). In general, the per cent similarity between tick sequences and the closest match in NCBI GenBank is between 97-99% (Table 5). NJ trees are based on DNA sequences of partial 16S rDNA genes on ticks, as shown in Figure 3. The figure shows the construction

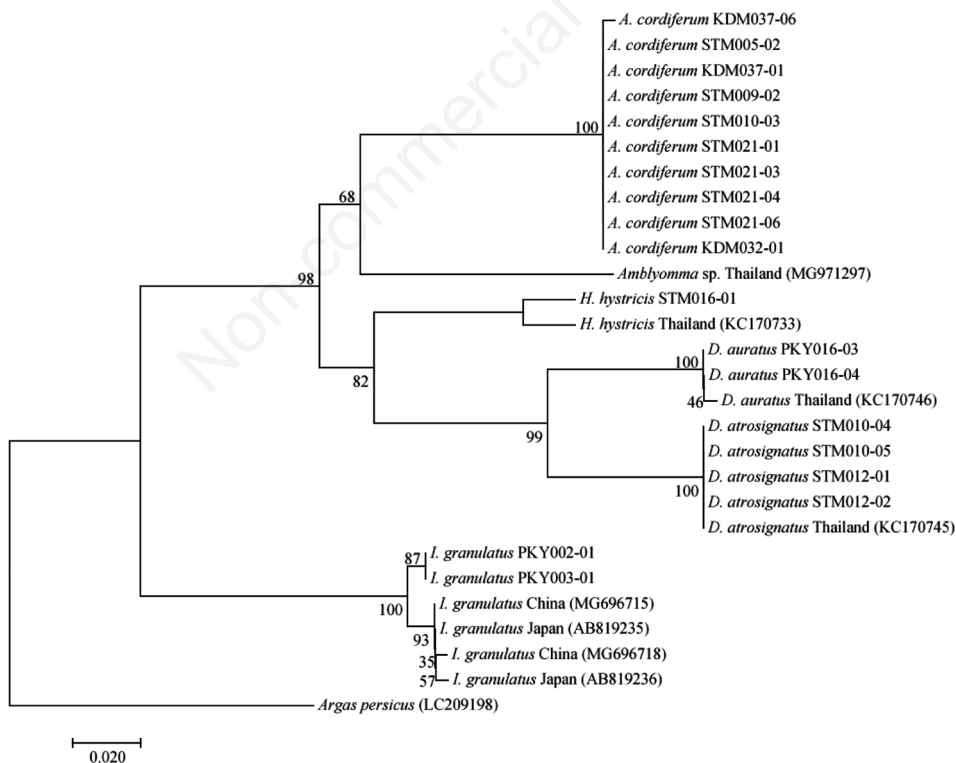


Figure 3. Phylogenetic relationships of 19 mitochondrial 16S rDNA genes of *Amblyomma* sp., *Haemaphysalis* sp., *Dermacentor* sp., and *Ixodes* sp., rooted with the reference sequences (including 1 outgroup (*Argas persicus*)) available in the GenBank. The tree was constructed and analysed with the neighbour-joining method with 1000 bootstrap replications.

of different major clades of tick species, i.e., *I. granulatus*, *A. cordiferum*, *D. auratus*, *D. atrosignatus*, and *H. hystricis*. In this analysis, *Argas persicus* is used as an outgroup and was highly separated from the other five major clades.

All *A. cordiferum* ticks from this study are grouped in the same clade, separated from ticks of the *Amblyomma* sp. from Thailand (bootstrap value = 70%). *H. hystricis*, STM016-01 is clustered together with *H. hystricis* from Thailand (bootstrap value = 100%), whereby *D. atrosignatus* samples are grouped in the same clade with *D. atrosignatus* from Thailand (bootstrap value = 100%). Both *D. auratus* sequences (PKY016-03 and PKY016-04) are clustered together in the same clade, but are distantly grouped with *D. auratus* from Thailand (bootstrap value = 46%), corresponding to a lower per cent identity from sequence similarity comparison. Moreover, both *I. granulatus* ticks (PKY002-01 and PKY003-01) formed a monophyletic clade separate from the *I. granulatus* ticks from Japan and China (bootstrap value = 100%). Pairwise distance analysis and comparison of *A. cordiferum* ticks show that the local species is genetically different from *Amblyomma* sp. from Thailand (0.15%). No significant difference in genetic distance is observed between *H. hystricis* in this study and *H. hystricis* from Thailand. Meanwhile, *I. granulatus* ticks are slightly different in the genetic distance value ranging between 0.01% to 0.02% compared to *I. granulatus* from China and Japan.

Ten individual mite samples from different hosts were chosen for the phylogenetic analysis. The partial 16S rDNA showed only 87%–89% similarities to existing mite sequences (*A. casalis*) in the NCBI GenBank (Table 5). NJ tree was generated using sequences of mite samples compared to other reference sequences. Pairwise sequence comparison of all *A. casalis* in this study showed intraspecific variation from 0% to 0.01% for partial 16S rDNA genes sequences. These species were close to *Gigantolaelaps wolffosohni* Fonseca, 1939 (HM059822) isolated from Brazil. Unfortunately, we could not obtain the sequence of *Laelaps* sp. and made a comparison with that species due to the lack of data in the GenBank database. Thus, the outcome of this analysis could not be extended due to the lack of mites' species validation from other molecular studies.

The intensity of ectoparasite infestation from different host species

From the five-tick species identified, *A. cordiferum* (n=26) is the most common and abundant tick species infesting two small mammal species, *R. tiomanicus* and *S. muelleri* (Table 6). Meanwhile, only one individual of *H. hystricis* is found infesting *R. tiomanicus*. In addition, one individual of *R. tiomanicus* is found co-infested with *Dermacentor cordiferum* and *A. cordiferum*. *L. echidninus* (n=60) is found in 4 out of 6 infested small mammal species from all mangrove sites. The most mites collected infested *R. tiomanicus* (n=46), and only one individual species of *L. echidninus* is collected from *Rattus rattus diardii*.

Table 6. Ectoparasite species load in different host species at each study site.

Host species	Total tick load	Ticks					Mites
		<i>I. granulatus</i>	<i>D. auratus</i>	<i>D. atrosignatus</i>	<i>H. hystricis</i>	<i>A. cordiferum</i>	<i>L. echidninus</i>
<i>Rattus tiomanicus</i>	29	1	4	5	1	18	46
<i>Rattus rattus diardii</i>	1	1	0	0	0	0	1
<i>Maxomys whiteheadi</i>	0	0	0	0	0	0	6
<i>Sundamys muelleri</i>	8	0	0	0	0	8	7
Total	38	2	4	5	1	26	60

Discussion

In this study, we present the diversity of small mammal inhabitants and the prevalence of ectoparasites in three mangroves areas in Peninsular Malaysia; Kg. Sg. Yak Yah, Terengganu (KSY); Kg. Dew, Perak (KDP), and Kg. Sg. Timun, Negeri Sembilan (KST). The ectoparasite microscopic identification was also validated through genetic analyses, including the construction of phylogenetic trees to recognise the phylogenetic relationships between each group of ectoparasites species. Six species of small mammals were captured from all study sites, dominated by *R. tiomanicus*. From this number, three host species were infested by ticks and mites (*R. tiomanicus*, *Rattus rattus diardii*, and *S. muelleri*), whereas *M. whiteheadi* was only infested with mites. These small mammals were hosts to five tick species (*I. granulatus*, *D. auratus*, *D. atrosignatus*, *A. cordiferum*, and *H. hystricis*), and one mite species (*L. echidninus*). Among these ectoparasites, *L. echidninus* mite and *A. cordiferum* tick were highly prevalent among the small mammals.

Overall, the diversity of small mammals in the mangrove forests was relatively poor (Shannon index of <1.0), compared to lowland (Yusof *et al.* 2019) and highland (Shukor *et al.* 2001) forests in the Malaysian region. From this study, the least diverse small mammals were recorded at KST, which corresponded to the higher level of anthropogenic pressure, as indicated in Mohd-Taib *et al.* (2020). This finding is parallel with Carugati *et al.* (2018), who stated that the diversity of fauna was significantly lesser in disturbed habitats, due to their extreme conditions (irradiation and high temperature) and anthropogenic disturbances. In addition, Friggens and Beier (2010) and Young *et al.* (2015) implied that the ectoparasite prevalence among small mammal hosts was associated with the intensity of habitat disturbance, where high host diversity and low levels of anthropogenic disturbances led to a lower intensity of ectoparasite prevalence. In contrast, our study did not show a correlation between parasite prevalence and anthropogenic disturbances. Other environmental factors like the rainfall that led to flooding may suppress the development of ectoparasites and their larvae (Krasnov *et al.* 2001), subsequently reducing the prevalence of ectoparasites. On the other hand, incidental effects of the environment on ectoparasites involved changes in host behaviour (grooming rates, burrowing behaviour, physiology, and community density; Young *et al.* 2015), which may influence the ectoparasites community. However, the growing adaptability of the host population in exploiting the areas with high anthropogenic disturbance may facilitate the transmission of ectoparasite (Pearce and O'Shea 2007).

Unlike the four infested species that belong to the family Muridae, two species were not infested by any ectoparasites, namely *T. glis* and *C. notatus* from the families Tupaiidae and Sciuridae. Similar to Madinah *et al.* (2014) and Ishak *et al.* (2018b), these species exhibited a significantly low number of ectoparasite loads compared to Muridae. Rodents from the family Sciuridae are tree-top squirrel and spent most of the time on tree canopies, instead of on the ground (Thanee *et al.* 2009). Thus, the chance of getting infested with ectoparasite, especially in the nymph stage and contact with other mammal hosts are lower, resulting in a low infestation rate (Nicholson *et al.* 2019). Meanwhile, the lack of ectoparasite loads on *T. glis* (ground squirrel) could be due to high frequency of grooming activities, which was established as the most effective way in reducing the prevalence of ectoparasite on this species (Sulaiman *et al.* 2016). Moreover, Maaz *et al.* (2018) stated that the absence of ectoparasites on host species could be due to the survival of ectoparasite itself in the surrounding, low rate of infestation, and low host specificity. On another note, there were no records of other ectoparasites, such as flea or louse present in all small mammals captured in this habitat, which concurs with other previous studies of Salleh *et al.*

(2003) and Paramasvaran et al. (2009). We suggest that the dynamics of host-parasite relations, including host immunity on parasite fitness and host preferences, contribute to the absence of these ectoparasites. In addition, some ectoparasites like flea and louse, which are often associated with hosts, are living selectively in specific resting sites with different nest and burrows environment (Sparagano et al. 1999; Razali et al. 2018) and the tendency to develop well in warmer surroundings (Krasnov et al. 2001).

Rattus tiomanicus, a highly abundant species captured in our study, exhibited a high prevalence of ticks and mites' infestation. This could be due to social and behavioural aspects of the hosts, which include social group size, and home range that covers riparian habitat (Ng et al. 2017). This species is nocturnal and often found in typical habitats (primary and secondary forest), including coastal forest (Payne et al. 1985). It is also known as a commensal, as they also inhabit human habitation (Harrison 1957). Besides that, this species is recognised as the predominant domestic pest in an agricultural area (Medway 1983) and build spherical nests in recesses of tree stumps in which they directly tend to harbour a high abundance of ectoparasite species from the vegetation (Mohamed-Hassan et al. 2012; Kim et al. 2013). The ecology and distribution of *R. tiomanicus* encompassed the Indonesian island of Sumatra, southern Thailand (Wood 1972), and was reportedly widespread throughout Peninsular Malaysia as early as the 1930s (Wood and Chung 2003). Lim et al. (1977) related the foraging and omnivorous behaviour of commensal rats, contributing to their pervasive infection. Mariana et al. (2005) reported six tick species, including *Haemaphysalis* spp., *Dermacentor* spp., and *Ixodes* spp., and two species of mites (*Laelaps echidninus* and *Laelaps nuttali* Hirst, 1915) collected on-host of *R. tiomanicus* from Gunung Stong forest reserve, Kedah, Malaysia. Furthermore, Ng et al. (2017) also documented multiple infections of ectoparasite species (*Haemaphysalis* sp., *Laelaps* spp., and chiggers) on *R. tiomanicus* from residential areas in western Sarawak.

The number of male hosts collected in all mangrove sites was higher than female hosts. Kowalski et al. (2015) mentioned that males of various small mammal species move over longer distances and significantly farther than females host, increasing the chance of getting captured in traps. However, female hosts harboured twice the number of ectoparasites compared to the male species. Females of several small mammal species typically solitarily nurse their offspring, which eases the vertical spreading of ectoparasites to their juveniles (Yamamura 1993; Ebert and Herre 1996). Gorrell and Schulte-Hostedde (2008) strongly demonstrated that the ectoparasites (females) lay their eggs in the host nest, directly consuming a higher nutritional resource than a solitary male nest. Therefore, it may reveal higher ectoparasite infestation levels on female individuals host than male hosts. However, the findings contradicted the later studies of Kowalski et al. (2015) and Ishak et al. (2018b), where the infestation rate of ectoparasites on male hosts was higher than females of small mammals. Bantihun and Bekele (2015) reported that males often have a more extensive home range and can travel further, therefore, easily exposed to ectoparasites than females. Differences in host body mass, movement patterns, spatial behaviour, and minor immunocompetence of males could provide a greater variety of niches for ectoparasites infestation, as they can withstand a higher number of arthropods (Balashov et al. 2007). Our study showed no record of male tick species, as mentioned by Durden et al. (2018) that they naturally do not feed on host skin.

Few studies have examined the morphological and molecular characteristics of arthropods, particularly ticks and mites (Chuluun et al. 2005; Paramasvaran et al. 2009; Madinah et al. 2011; Sponchiado et al. 2015; Ernieenor et al. 2016; Ishak et al. 2018a). According

to their external features, tick species are more likely to be recognised and identified based on specific published taxonomic keys up to the species level. Mite observation under the stereomicroscope, on the other hand, is more challenging due to their tiny size, the lack of taxonomic keys, and the fact that the information of mite species is scarce and outdated. Based on the morphological and physical characteristics of the reference keys (Baker 1999), the mite species was initially identified as *Laelaps* sp., which was then confirmed to be *L. echidninus*. This species is occasionally found in rodents and as a parasite of the Norway rat (brown rat), *R. norvegicus* (Montasser 2006; Baak-Baak *et al.* 2016). Besides that, the study on *L. echidninus* was vital and primarily concerned investigations of host-parasite relationships (Soliman *et al.* 2001). Nevertheless, according to the BLAST results obtained, the gene similarity of the mite sequences was matched to *A. casalis*, with low per cent similarity value through molecular identification on 16S rRNA genes. The low per cent similarity value could be due to the lack of studies and reference sequence in the GenBank database using partial 16S rDNA genes. Thus, we suggest using other target markers, such as 28S and 18S rDNA genes (Machida and Knowlton 2015) for identifying mite samples at specific DNA region for future complementary analysis. This was supported by Matsuda *et al.* (2014) and Famah Sourassou *et al.* (2015), as the genes of nuclear ribosomal DNA have been considered valuable for determining high phylogeny level of mite species. Meanwhile, a total of five different tick species were identified up to the genus level (*Ixodes* sp., *Amblyomma* sp., *Dermacentor* sp., and *Haemaphysalis* sp.). Other than that, the DNA sequences belonging to all tick species presented high similarity values of about 97%–99% through the BLAST search. The results from this study further established the achievement of molecular data of tick species through DNA barcoding for this region, including South-east Asia.

Our study indicates that the genetic characterisation is concurrent with the geographical distribution of each ectoparasite species. *Haemaphysalis hystricis*, *Dermacentor auratus*, and *Dermacentor atrosignatus* are grouped with the same species in Thailand. The findings proved that tick species from different Southeast Asian countries shared the same genetic clade. *Ixodes granulatus* formed a monophyletic clade with the same species from Japan and China. Meanwhile, *A. cordiferum* tick was distantly separated from *Amblyomma* sp. of Thailand, which concluded that there were some differences in genetic identity between them. *H. hystricis* ticks have also been occasionally found from barking deer and wild boar (Petney *et al.* 2019), while their nymphs have been collected from the black rat, *R. rattus*, and the Asian palm and civet squirrels (Khoo *et al.* 2016; Petney *et al.* 2019). The nymph stage of *D. auratus* is commonly found on small to medium mammals, other rodents, porcupine, wild hens, and monkeys (Hoogstraal and Wassef 1985). In addition, *D. atrosignatus* is also found in wild pigs, reptiles, and mammals (Mariana *et al.* 2005). All ticks from the genus *Dermacentor* are a likely vector to zoonotic diseases, such as the Lanjan virus (Karabatsos 1985). *I. granulatus* infested small mammals, especially rodents in Peninsular Malaysia (Ernieenor *et al.* 2016; Madinah *et al.* 2011; Ishak *et al.* 2018b). The larvae or immature stages of *A. cordiferum* tick feed on small mammals (Barnard and Durden 2000), and this tick prefers to feed on ophidian hosts during the adult stage (Ho and Ismail 1984). On the other hand, little is known about the mite of small mammals in Peninsular Malaysia; thus, it is often underestimated. Laelapidae mites were reported as obligate or facultative parasites in small mammal nests in various habitats (Martins-Hatano *et al.* 2011). Our findings revealed that the mite load was much higher than that of the tick, as mites were frequently found infesting commensal wild rodents at night, in their nests (Mullen and O'Connor 2002). Klein *et al.* (2018) mentioned that host's nests provide food and nutri-

tional supply to parasitic mites through excretions and scabs of the hosts, incidentally facilitating dispersal to other nests, as well as increasing the number of mite infestation among hosts. Besides, the fruiting season might induce mite infestation among rodent hosts due to increasing resource accessibility (Wells et al. 2014). *L. echidninus* is also frequently found in rodents such as *Rattus* sp., as reported in Mexico (Baak-baak et al. 2016), and capable of transmitting the Junin virus and lethal apicomplexan protozoan to animals, especially rats (McLay et al. 2014). In short, small mammals associated with ectoparasites, especially ticks, act as a vector to many zoonotic pathogens, like the spotted fever group *Rickettsia*, *Bartonella* spp., *Borrelia burgdorferi*, *Ehrlichia* spp., and tick-borne encephalitis virus (Comer et al. 2001). Rodents living near human population act as a vital reservoir in spreading vector-borne diseases to humans and animals (Meerburg et al. 2009). Krasnov et al. (2004) described that the diversity of small mammal hosts in a habitat could also be crucial in defining the species richness of ticks and mites instead of the intrinsic influences (body size of the host).

Conclusions

The low diversity of small mammals in the mangrove ecosystem were heavily infested with ectoparasites, including ticks and mites. The development of each life-stage of these ectoparasites was dependent on the habitat quality and their countless hosts (Maaz et al. 2018). Validation for each ectoparasite species from both morphological and molecular approaches should be improved and refined to avoid misinterpretation of the information. Besides, the total prevalence of small mammals infested by mites was higher than ticks at all study sites. It indicates that mites colonised a greater niche range in mangrove forests. The study of ectoparasites in small mammals remains preliminary, with a further assessment about the exposure yet to be explored. Besides, small mammals are reportedly important hosts for the immature development of the most imperative vector-borne pathogens in many countries over the world (Klein et al. 2018). Ectoparasite-borne disease investigation can offer a picture of the relative abundance and diversity of small mammal inhabitants, as well as their important epidemiologic data on the host preference and distribution of associated ectoparasites. Further surveillances of the ectoparasite-host relationships are essential to better understand the dynamics of their population and distributions, the prevalence of arthropod-borne pathogens, and their potential transmission, including health risk in areas close to human settlements and nearby attraction areas. Apart from that, a comprehensive investigation on ecological requirements of ectoparasites, the intensity of small mammal-associated ectoparasites on wild rodents, and how they could colonise an anthropologically disturbed area close to people, need to be addressed in future studies.

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Contributions

FSM-T, RA and SMN conceived and designed the study; RA collected data; FSM-T and RA did the analysis, interpreted the results and prepared the draft manuscript. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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