

Occurrence of Ectoparasite on Small Mammals between Disturbed and Undisturbed Area of Peat Swamp Forest in Ayer Hitam Utara Reserve Forest

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Abstract

The Ayer Hitam Utara Forest Reserve (AHUFR) is situated in Peninsular Malaysia's Muar district in Johor (1° 52' North, 103° 11' East) and this region, which spans around 3,797 hectares (ha), is recognised as the largest and last peat swamp forest. This study aimed to identify the various species of small mammals and ectoparasites and evaluate ectoparasites prevalence on ectoparasites between disturbed and undisturbed areas of AHUFR for a total of 12 sampling days. Sampling involved 24 cage traps and four mist nets across two sampling sites in each disturbed and undisturbed areas. Four species were recorded: *Tupaia glis* (Common Treeshrew), *Leopoldamys sabanus* (Long-tailed Giant Rat), *Maxomys rajah* (Rajah Spiny Rat), and *Rhinolophus trifolius* (Trefoil Horseshoe Bat). Total of seven different species of ectoparasites were sampled which are *Haemaphysalis concinna*, *Amblyomma sp.*, *Haemaphysalis sp.*, *Laelaps nutalli*, *Ixodes granulatus*, *Ixodes sp.*, and unknown species. While undisturbed area has higher individual number of ectoparasites (59) while only 2 species, disturbed area has higher species number (7) with 21 total individuals. The disturbed area exhibited a higher Shannon-Weiner index and evenness and a T-test indicated no statistically significant difference between the areas. The findings can provide baseline data for future research and conservation efforts.

1. Introduction

Peatlands are habitats distinguished by the buildup of partially decomposed organic matter, or "peat," which is created from plant debris under wet circumstances [1]. Peatlands make up about 11% of the world's total peatland area and span more than 440 000 km² in tropical regions [2]. The largest and last peat swamp forest is the Ayer Hitam Utara Forest Reserve (AHUFR), which is in the Muar district of Johor, Peninsular Malaysia (1° 52' North, 103° 11' East). The area is around 3,797 hectares (ha) [3].

Merritt, (2010) classified "small mammals" in The Ecology of Small Mammals as "insectivores" and rodents weighing no more than 120 g (4.2 oz) [4]. The first record of *Tupaia glis* in 1780 was reported by William Ellis which is called ground squirrel in Malaysia [5]. Tree shrews use a variety of habitats and are typically found close to human populations, plantations, fruit orchards, secondary woods, and dipterocarp forests [5]. In local groups of small mammals, the common, generalist species is the long-tailed giant rat, *Leopoldamys sabanus* [6]. This species can be found all over Southeast Asia's Sunda area including both logged and unlogged forest.

Ectoparasites are organisms that live on the surface or on the protrusions of another creature (the host) for varying lengths of time, often to the host's harm [7]. The species *Ixodes granulatus* is unique to Asia, with a range of Southeast Asia, Japan, India, and China to the west [8]. Additional research conducted over a long period of time in Peninsular Malaysia has also shown that *Ixodes granulatus* is the most prevalent and numerous species infesting mammals, particularly rats, in all active stages [9, 10]. Small mammals, especially rodents, are frequently ectoparasite by mites of the genus *Laelaps* (Mesostigmata: Laelapidae) [11].

The objectives of this study are to identify the various species of small mammals and ectoparasites of undisturbed and disturbed area of Ayer Hitam Utara Reserve Forest in Johor to evaluate ectoparasites prevalence on ectoparasites in North Ayer Hitam Forest Reserve.

1.1 Comparison of Current Study with Past Study (Tasek Bera, Pahang)

First Malaysian Ramsar site in Tasek Bera, Pahang (TBRS) (N 02°58' E 102°36') encompasses a 6,150 ha (61.5 km²) alluvial riparian peat swamp and 61,380 ha (613.8 km²) of water catchment area [12]. The largest freshwater wetland in Peninsular Malaysia's Pahang state is TBRS [13]. However, when comparing species richness of ectoparasite, AHUFR recorded seven species, indicating higher species richness than TBRS's five species (Table 4.5). This difference may be attributed to more less sampling efforts and less type of host sampled in TBRS. The survey in TBRS only check twice per day while the study in AHUFR checked every two hours from 7:00 a.m. to 5:00 p.m., while the type of host sampled in TBRS were *Maxomys whiteheadi*, *Maxomys rajah* and *Leopoldamys sabanus* while study in AHUFR sampled four which were *Tupaia glis*, *Leopoldamys sabanus*, *Maxomys rajah* and *Rhinolophus trifoliatus*.

Table 1 List of ectoparasites between AHUFR and TBRS

AHUFR		TBRS	
Host	Scientific name	Common name	Scientific name
<i>Tupaia glis</i>	<i>Haemaphysalis concinna</i>	<i>Maxomys whiteheadi</i>	<i>Laelaps sanguisugus</i>
	<i>Amblyomma sp.</i>		
	<i>Haemaphysalis sp.</i>		
	Unknown		
<i>Leopoldamys sabanus</i>	<i>Laelaps nutalli</i>	<i>Leopoldamys sabanus</i>	<i>Ixodes granulatus</i>
	<i>Ixodes granulatus</i>		
	<i>Ixodes sp</i>		
	Unknown		
<i>Maxomys rajah</i>	<i>Laelaps nutalli</i>	<i>Maxomys rajah</i>	<i>Ixodes granulatus</i>
			<i>Haemaphysalis sp</i>
			<i>Laelaps aingworthae</i>
			<i>Laelaps sanguisugus</i>
<i>Rhinolophus trifoliatus</i>	-		<i>Longolaelaps whartoni</i>

2. Methodology

2.1 Study Site

The Ayer Hitam Utara Forest Reserve is situated in Peninsular Malaysia's Muar district in Johor (1° 52' North, 103° 11' East) and this region, which spans around 3,797 hectares (ha), is recognized as the largest and last peat swamp forest [3]. The study was conducted at two sampling sites in disturbed and undisturbed areas within the peat swamp forest at AHUFR (Fig. 1, Fig. 2), with the site coordinates provided in Table 1.



(a) (b)
Fig. 1 (a) Site 1; (b) Site 2 identified as disturbed areas



(a) (b)
Fig. 2 (a) Site 1; (b) Site 2 identified as undisturbed areas

Table 2 Coordinates and date visit for each site

<i>Coordinate and date visit</i>	<i>Disturbed area</i>	<i>Undisturbed area</i>
<i>Site 1</i>	$2^{\circ} 03' 02.49'' N$ $102^{\circ} 49' 38.43'' E$ 1 to 3 November 2024 3 days	$2^{\circ} 03' 04.05'' N$ $102^{\circ} 49' 37.45'' E$ 7 to 9 November 2024 3 days
<i>Site 2</i>	$2^{\circ} 03' 17.45'' N$ $102^{\circ} 49' 44.66'' E$ 4 to 6 November 2024 3 days	$2^{\circ} 03' 08.69'' N$ $102^{\circ} 49' 40.43'' E$ 14 to 16 November 2024 3 days

2.2 Sampling Method

The techniques that applied are adapted from [14]. The two different habitat types where the traps were placed were the disturbed region and the undisturbed area. Each habitat was placed 12 cage traps baited with bananas along 600m transect line and 2 mist nets were installed for volant small mammals. For three days each site, the traps were examined for every 2 hours 7 a.m. to 5 p.m. The baits were changed when necessary for moist mixes

and as needed for dry one. The captured tiny animals were examined for their physical traits, noted, marked with hair paint, and then released to prevent recapture.

2.3 Data Collection

The sampling methods and species identification used are followed and modified from [15]. Every ectoparasite that had adhered to the coat of the host animal was removed using a fine toothcomb. Using a wet sharpening stick, ectoparasites fell during combing were collected and kept in collecting vials containing 70% ethanol, one vial for each host small mammals. Ectoparasites obtained from various small mammals were placed in separate vials and labelled with a code that matches the data in the notebooks, such as the host species, collection location, date, collector name, quantity of ectoparasites, and sex [16]. After the vials were properly tagged, they were returned to the laboratory of Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Campus for further analysis so that the species could be identified. The ectoparasites, which include mesotigmatid mites, ticks, chiggers, and insect ectoparasites including lice, fleas, and bat flies, were prepared for mounting on slides and identified based on their taxonomic families with the use of microscope [17]. The specimen is carefully positioned on the slide with its key anatomical structures exposed such as shape and segmentation of the body, presence and structure of legs, antennae, or mouthparts, or specialized adaptations like claws, suckers, or bristles [17].

2.4 Data Analysis

The number of species and individual per species (species abundance) of small mammals and ectoparasites, species diversity (species richness of ectoparasites) and occurrence of ectoparasites on small mammal species are among the quantitative data that will be needed for analysis.

First, the statistics used in data analysis are the measures of frequency, which often access the data in the form of counting, percentage, and frequency. To generate further data statistics, the number of small animals and ectoparasite species, together with the number of individuals within each species, will be counted and entered SPSS as main data. The Shannon Weiner Index (H') will be used to quantify the species diversity, which includes species richness, which is defined as the total number of species [14]. Occurrence is the percentage of a population that possesses a particular attribute throughout a given period. Therefore, the equation (1) given will be used to represent it in percentage [18].

$$\text{Shannon Weiner Diversity Index } (H') = - \sum_{i=1}^S (\pi_i) \ln \pi_i \quad (1)$$

where:

H' : Shannon Wiener diversity index

S : number of types

π : proportion of the i -th individual count (n_i/N)

\ln : natural log

Meanwhile, the Shannon Index has the following indicators:

$H' < 1.5$ = low diversity level

$1.5 \leq H' \leq 3.5$ = moderate diversity level

$H' > 3.5$ = high diversity level

$$\text{Occurrence} = \frac{\text{Total number of infested small mammals}}{\text{Total number of small mammals captured}} \times 100\% \quad (2)$$

3. Result and Discussion

3.1 Composition of Small Mammals and Ectoparasites in Disturbed and Undisturbed Areas

Four species of small mammals were caught during the study in Ayer Hitam Utara Forest Reserve. Three species were recorded for the non-volant group which are the Common Treeshrew (*Tupaia glis*) from the family Tupaiidae, the Long-tailed Giant Rat (*Leopoldamys sabanus*), and the Rajah Spiny Rat (*Maxomys rajah*) from the

family Muridae. Only one species was identified in the volant group which is the Trefoil Horseshoe Bat (*Rhinolophus trifolius*) from the family Rhinolophidae.

Seven different species of ectoparasites were discovered during the study in Ayer Hitam Utara Forest Reserve. The species that recorded are *Haemaphysalis concinna* from Ixodidae family, *Amblyomma* sp. from Ixodidae family, *Haemaphysalis* sp. from Ixodidae family, *Laelaps nutalli* from Ixodidae family, *Ixodes granulatus* from Ixodidae family, *Ixodes* sp. from Ixodidae family, and lastly the unknown species. The ectoparasites were shown in Fig. 3, Fig. 4, Fig. 5 and Fig. 6.



(a)



(b)

Fig. 3 (a) *Haemaphysalis concinna* (b) *Amblyomma* sp



(a)



(b)

Fig. 4 (a) *Haemaphysalis* sp. (b) *Laelaps nutalli*



(a)



(b)

Fig. 5 (a) *Ixodes granulatus* (b) *Ixodes* sp



Fig. 6 Unknown species

The study highlights a stark contrast between disturbed and undisturbed habitats. While species richness and diversity were higher in the disturbed area, the undisturbed area exhibited a higher total number of individuals, predominantly due to the infestation of *Laelaps nutalli* on *Maxomys rajah*. This suggests that habitat disturbance fosters diverse ectoparasite communities but reduces overall host abundance, potentially due to increased environmental stress or reduced habitat suitability for host species [19, 20].

The findings underscore the critical role of habitat quality in shaping host-parasite dynamics. Disturbed areas appear to support a broader range of ectoparasite species, possibly due to the influx of generalist parasites or increased host susceptibility under stress [19, 20]. The result might be biased due to unstable weather conditions during period of sampling. Regardless of the land usage, the ectoparasite burden should be higher during the dry season than during the wet one [19]. Besides that, more ectoparasites should infest adult males than adult females [19]. Male hosts were dominated our sampling data due to females appear to be more cautious around traps than males [21]. Hence, biased data of ectoparasites on male hosts happened.

3.2 Species distribution and abundance ectoparasites on host in both areas

Table 2 highlights how ectoparasites are distributed and abundant in disturbed areas in three different mammalian hosts which are *Tupaia glis*, *Maxomys rajah*, and *Leopoldamys sabanus*. Total of seven different species of ectoparasites were sampled which are *Haemaphysalis concinna*, *Amblyomma sp.*, *Haemaphysalis sp.*, *Laelaps nutalli*, *Ixodes granulatus*, *Ixodes sp.*, and unknown species. Each host shows unique patterns of association with these ectoparasites. *Tupaia glis* hosts a moderate diversity of ectoparasites, including *Haemaphysalis concinna* (2 individuals), *Haemaphysalis sp.* (2 individuals), and *Amblyomma sp.* (1 individual). However, it does not host any *Laelaps nutalli*, *Ixodes granulatus*, *Ixodes sp.* and unknown species. On the other hand, *Maxomys rajah*, which hosts three individuals, is solely linked to the *Laelaps nutalli* and has no connection to any of the other ectoparasites on the list. This suggests a limited interaction between the parasite and its host. Ectoparasite diversity and abundance are maximum in *Leopoldamys sabanus*. It is primarily associated with *Ixodes granulatus* (5 individuals) and *Ixodes sp.* (5 individuals), along with a single unidentified ectoparasite. However, it does not host any *Haemaphysalis* or *Amblyomma* species, suggesting a preference or ecological compatibility with *Ixodes* species.

Table 3 Species distribution and abundance across disturbed areas

Host	Ectoparasite						
	<i>Haemaphysalis concinna</i>	<i>Amblyomma sp.</i>	<i>Haemaphysalis sp.</i>	<i>Laelaps nutalli</i>	<i>Ixodes granulatus</i>	<i>Ixodes sp.</i>	unknown
<i>Tupaia glis</i>	2	1	2	-	-	-	-
<i>Maxomys rajah</i>	-	-	-	3	-	-	-
<i>Leopoldamys sabanus</i>	-	-	-	2	5	5	1

Table 3 highlights how ectoparasites are distributed and abundant in undisturbed areas among the three host species (*Rhinolophus trifolius*, *Maxomys rajah*, and *Tupaia glis*). Only two different species of ectoparasite were sampled during the period of study. It shows that *Rhinolophus trifolius* does not host ectoparasites in these habitats. In contrast, *Maxomys rajah* has 58 individuals and is severely infested with the *Laelaps nutalli*, suggesting

a robust and specialised host-parasite association. Finally, *Tupaia glis* has a low ectoparasite burden compared to *Maxomys rajah*, as it only hosts one individual of ectoparasite from the unknown species.

Table 4 Species distribution and abundance across undisturbed areas

Host	Ectoparasite	
	unknown	<i>Laelaps nutalli</i>
<i>Rhinolophus trifoliatus</i>	-	-
<i>Maxomys rajah</i>	-	58
<i>Tupaia glis</i>	1	-

The host-parasite associations revealed unique patterns influenced by habitat conditions. *Tupaia glis* hosted moderate ectoparasite diversity in the disturbed area but had minimal parasitism in the undisturbed area. *Maxomys rajah* demonstrated a strong association with *Laelaps nutalli* in both habitats, suggesting that this species of ectoparasites is common and able to thrive in different condition of area. This have been proven by past studies [9, 22, 23]. This can be explained by the ectoparasite have more generalist food habits that range and considered facultative nest or hair parasites, the Laelapidae family can take advantage of a broad range of hosts [21]. Besides that, this family of ectoparasites have the process of ecological fitting which a process by which organisms use new resources, colonise and stay in new settings, or establish new relationships with other species [23, 24]. Conversely, *Leopoldamys sabanus* in the disturbed area harboured a higher diversity of ectoparasites, including Ixodes species, indicative of broader ecological interactions.

There were no ectoparasites founded on the volant small mammals. This is the lack of sample caught during this study. The low on data highly due to only installed two mist net in each area. It is advised to set up at least three mist net stations per night and to move the nets to new locations each day to increase capture rates. Bats rapidly adjust to the net placements, which can lower capture rates, therefore this is crucial [26]. Next, short period of sampling time is also one of the reasons the caught rate of volant small mammals was low. According to Larsen (2016), at least five nights should be spent sampling each venue, ideally for a full week. Lastly, the unpredictable weather patterns also a cause for concern in this study [26]. Rainfall, wind, and temperature variations during the day can impact small animals' activity levels, which in turn can impact their catchability [27].

3.3 Comparison of the composition of small mammals between disturbed and undisturbed areas

The species richness of ectoparasites in disturbed and undisturbed areas was recorded as the seven and two respectively. The total number of individuals is higher in the undisturbed area (N = 59) compared to the disturbed area (N = 21). Despite this, the Shannon-Weiner Index shows higher diversity in the disturbed area ($H' = 1.76$) compared to the undisturbed area ($H' = 0.0859$). The Shannon-Weiner equitability index (E) recorded that the disturbed area has a higher value (E = 0.906) compared to the undisturbed area (E = 0.1239). This indicates that the ectoparasites in the disturbed area are more evenly distributed, whereas the undisturbed area exhibits greater dominance by *Laelaps nutalli* (Table 5).

Table 5 Species richness, Shannon-Wiener Index and Evenness in both areas

	Disturbed area	Undisturbed area
Species richness	7	2
Total of individuals (N)	21	59
Shannon-Wiener Index (H')	1.76	0.09
Shannon-Wiener equitability index (E)	0.91	0.12

Table 6 Descriptive statistics of T-test

Study site	N	Mean	Standard Deviation	Standard Error of the Mean
Disturbed area	7	3.00	1.91	0.72
Undisturbed area	2	29.50	40.31	28.50

T-test in this study showed that the result was no statistically significant difference in the composition of ectoparasites between the disturbed and undisturbed areas, as the p-value is higher than the significant level of 0.05. This is because peat swamp forests are difficult habitats with special traits that may affect small mammals and their ectoparasites. Ectoparasite patterns may overlap between disturbed and undisturbed peat swamp environments if some undisturbed-like traits are still present. Hence, it was difficult to distinguish between disturbed and undisturbed areas in this study. Undisturbed regions can support more species since they often have higher vegetation densities and diversity. On the other hand, disturbed landscapes have higher levels of human activity, such as development of area for industrial and agricultural activities, deforestation and air, water and land pollution which are easier to reach by humans. Because there are more open spaces, these locations usually receive more light and have less trees [28].

Table 7 Occurrence of Ectoparasites

Study site	Small Mammals Sampled	Infected host	Occurrence
Disturbed area	6	4	66.67%
Undisturbed area	7	2	28.57%

The overall occurrence of ectoparasites in disturbed area is higher (66.67%) compared to undisturbed area (28.5%). A wider variety of ectoparasite species seem to be found in disturbed regions, maybe because of the introduction of generalist parasites or heightened host vulnerability to stress [28].

4. Conclusion

By compiling a list of small mammals that serve as hosts and ectoparasites and comparing their composition in disturbed and undisturbed sections of AHUFR, this study effectively met all its objectives. A total of four species of small mammals were identified in AHUFR, including three non-volant species and one volant species. The recorded species were *Tupaia glis*, *Leopoldamys sabanus*, *Maxomys rajah*, and *Rhinolophus trifoliatus*. Among these, *Tupaia glis* was the most abundant, while *Rhinolophus trifoliatus* was the least common and the only volant species recorded. Seven different species of ectoparasites were discovered during the study in Ayer Hitam Utara Forest Reserve. The species that recorded are *Haemaphysalis concinna*, *Amblyomma sp.*, *Haemaphysalis sp.*, *Laelaps nutalli*, *Ixodes granulatus*, *Ixodes sp.*, and lastly unknown species.

The comparison of the composition of ectoparasites between the disturbed and undisturbed areas showed that the undisturbed areas had a higher abundance of ectoparasites, with 59 individuals recorded, compared to 21 individuals in disturbed areas. *Laelaps nutalli* was particularly dominant in undisturbed areas, where 58 individuals were captured, while the remaining individuals were one unknown species. However, the disturbed areas showed a higher Shannon-Weiner diversity index and the species were more evenly distributed compared to the undisturbed area as have variety of ectoparasite species.

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Conflict of Interest

The author confirms that there are no conflicts of interest regarding the publication of the paper.

Author Contribution

The authors confirm contributions to the paper as follows: **study conception and design:** Kong Khei Khiong; **data collection:** Kong Khei Khiong; **analysis and interpretation of results:** Kong Khei Khiong, Arney Sapaat; **draft manuscript preparation:** Kong Khei Khiong, Arney Sapaat. All authors reviewed the results and approved the final version of the manuscript.

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