ECTOPARASITES (TICKS AND MITES) PREVALENCE ON SMALL TO MEDIUM-SIZED MAMMALS ASSOCIATED WITH HABITAT CONDITION IN KEMASUL, PAHANG

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ABSTRACT

Ectoparasites of small mammals and medium mammals are divided into two main classes which are Insecta and Arachnida. The members of the class Arachnida including order Ixodida (ticks) and Mesostigmata (mites) meanwhile class Insecta comprising Phthiraptera (lice) and Siphonaptera (fleas). This study was conducted to determine tick's and mite's prevalence on the small to medium-sized mammals in Kemasul Forest Reserve, Pahang. This forest has undergone rapid deforestation for agricultural purposes. Two study sites were chosen which represented by a forest remnant surrounded with different matrix of monoculture plantation; Jambu Rias (JR) (*Elaeis guineensis*) and Chemomoi (CM) (*Acacia mangium*). Three hundred wired mesh cage traps sized (28 cm × 15 cm × 12.5 cm) and forty wired mesh cage traps sized (60 cm × 40 cm × 40 cm) were deployed at the study area and ectoparasites were extracted from each host using a fine comb. Identification was based on morphology and molecular using cytochrome oxidase 1 for confirmation. Mites only represented by *Laelaps* sp. which shows 95% and 70% prevalence in JR and CM respectively. Ticks were represented by five species, namely *Ixodes granulatus, Dermacentor atrosignatus, Rhipicephalus sanguineus, Amblyomma testudinarium* and *Haemaphysalis* sp. JR comprise of five species while CM with two species. *I. granulatus* was the most common infesting the small mammals in both sites. The highest parasite load was found on small mammals which were *Maxomys surifer, M. rajah* and *M. whiteheadi* in both study sites, particularly with mites. The study indicates that habitat condition significantly affects parasite prevalence in small to medium-sized mammal population, which could be due to the resilience of an individual to persist in disturbed habitat.

Keywords: Agriculture, monoculture plantation, small mammals, prevalence, Pahang.

INTRODUCTION

Disturbance is an important ecological factor affecting species diversity in natural environments (Sousa 1984). Increasing anthropogenic activities towards the forest has resulted in intense habitat degradation and rapid deforestation (Myers et al. 2000). These practices have resulted in modified ecosystem that are characterized by a high level of disturbance and homogeneity (Gentili et al. 2014). Deforestation has brought about habitat loss, resource exhaustion and subsequently declining in wildlife population. Agriculture has been known as the major cause of forest loss, having been estimated about 90 per cent of all deforestation in the tropics (Benhin 2006). Agricultural intensity strongly influences ecosystem service and biodiversity (Kleijin et al. 2009). In Southeast Asia, the conversion of forest (both primary and logged) to oil palm agriculture has been uncontrolled (Reynolds et al. 2011).

Ticks are easily adapted group of insects that infest the external body surface of vertebrates (Hanafi-Bojd et al. 2007). Most ticks usually can be found during critical stages of the host's life cycle such as lactation or gestation (Czenze & Broders 2011). Ticks have an important role in the transmission of a wide range of diseases and a main vector for pathogenic organisms such as protozoa, rickettsia, bacteria and viruses that can cause zoonotic diseases (Madinah et al. 2011). These organisms cause serious and life-threatening illness in humans and animals (Chul-Min et al. 2006). Large number of ticks' species used human as intermittent host. Some ticks parasitize wide variety of hosts, while others are extremely selective and feed solely on one host species (Oliver 1989). Fever, Rocky Mountain spotted fever and typhus fever are known to be transmitted by ticks (Ansari 1953). Ticks species *Dermacentor marginatus* also play important role in the epidemiology of the brucellosis (*Brucella melitensis* and *B. abortus*) and can even acts as reservoirs of the infective agent (Galuzo & Rementsova 1955). The infection persists in the adult ticks at the temperature of the laboratory for 120-150 days and declines after 200-300 days. Mites are highly diverse and specialized group that can be further divided into chiggers, mesostigmatid mites, listrophorids and myobiids (Mohd Zain et al. 2015). Most kinds of mites are free-living; they are not parasitic but a few kinds become specialized to feed upon or infest animal (Walter & Proctor 2013). Chiggers are known as a vector for scrub typhus disease where *Leptotrobidium chiangraiensis* and *L. deliense* are the vector for *Orientia tsutsugamushi* (Lerdthusnee et al. 2003; Madinah et al. 2011; Weitzel et al. 2016). *Ornithonyssus bacoti*, is mesostigmatid mites that can cause dermatitis in human (Baumstark et al. 2007; Reeves et al. 2007; Rosen et al. 2002).

The distribution of ticks and mites are affected by the host's microhabitat as ticks and mites may encounter other hosts especially when habitats are disturbed, resulting in the change of the host assemblage (Bittencourt & Rocha 2003). Habitat loss and fragmentation can also contribute to disease outbreaks indirectly through the loss of biodiversity (Gentili et al. 2014). It has been suggested that biodiversity plays an important role in decreasing the prevalence of infection disease among hosts due to the dilution effect (Ostfeld & Keesing 2000). Hence, the diversity and structure of host communities in different environmental settings can influence tick's dynamics and the transmission of diseases (Wells et al. 2011), and the presence of parasite can give important impacts either to host populations or communities (Hudson et al. 2006). The distribution of mites can be further classified according to their species of specialised; *Dermanyssus gallinae* and *Ornithonyssus sylvarium* can migrate from birds to human, grain mites and mushroom mites are found in food materials or stored products, chigger mite is found in lawns and open woodlands, while *Sarcoptes scabiei* and *Demodex folliculorum* are permanent residents on humans (Dhooria 2016).

Small mammals are considered to any non-volant mammals weighing less than 1 kg when adult (Barnett & Dutton 1995), while medium-sized mammals weighing less than 5 kg. Rodents, bats and some carnivores fall under the category of small mammals, whereas medium-sized could refer to porcupines, and civets. The skin and its outgrowth form the physical environment in which ticks and mites spend much of their lives and it directly supplies their food (Marshall 1981). These mammals play a significant role in maintaining ecosystem functionality as seed dispersal agents and arthropod control, and are medically important as reservoirs of zoonotic diseases (Chaisiri et al. 2010; Thanee et al. 2009). Rodents and scandents are host that can carry many different ectoparasites (Acari and Insecta) (Nadchatram 2008). Rodents are considered as the most important hosts because taxonomically this group had the largest number of species (Nieri-Bastos et al. 2004).

The aim of this study was to determine tick's and mesotigmatid mite's prevalence on the non-volant small to medium-sized in relation to different habitat condition, in Kemasul Forest Reserve, Pahang. We hypothesized that forest remnant with more disturbed surrounding areas contribute to higher intensity of ectoparasites, as an indication of a stressful condition of the animals. Generally, this study can help to gain a better understanding about intensity or prevalence of ticks and mites infestation in relation to habitat condition. Understanding the richness of tick's species provides valuable insights into the ecological roles they play in the regulation of their host population and communities (Wei et al. 2010). Thus, the management of hosts can be done effectively to control the spreading of diseases. Information on host-parasite-pathogen interaction is important as it is related to management of zoonotic diseases (Madinah et al. 2014).

MATERIAL AND METHODS

The study was carried out in Kemasul Forest Reserve, Pahang (03°22'34.2" N, 102° 16'01.6" E) (Fig. 1). This forest is lowland dipterocarp forest types, but majority of the areas has been logged to give way for monoculture plantation, such as oil palm. Apart from that, some part of the forest has been converted to forest farm with the plantation of fast-growing acacia trees (*Acacia mangium*). Sampling was conducted in a long strip of forest fragment with different matrix types; Jambu Rias (JR) with oil palm and Chemomoi (CM) with *A. mangium* plantation (Lim et al. 2011) (Fig. 2**)** from August to November 2015. Kemasul Forest Reserve has an area of 39,000 hectares and was gazetted as forest farm project handled by Pahang Forestry Department to provide continuous supplication of timbers apart from providing jobs for farmers in Pahang (Malaysia Auditor General's Report 2012).

A total of three hundred wired mesh cage traps sized (28 cm × 15 cm × 12.5 cm) and forty wired mesh cage traps sized (60 cm × 40 cm × 40 cm) were deployed in each study sites. Banana, oil palm fruit and jackfruit were used as bait in the study (Bernard et al. 2004). At each study sites, there were six different trails where fifty wired mesh cage traps sized (28 cm × 15 cm × 12.5 cm) and five wired mesh cage traps sized (60 cm × 40 cm × 40 cm) were deployed at 5 m interval. The placements of traps were random which located on tree stumps, fallen logs (Balete et al. 2009), and at ground level (Rickart et al. 2011). All traps were checked once daily at (0900 hrs-1600 hrs). Every individual that have been caught were given anaesthetic injection using Zoletil 100 (Massolo et al. 2003) and species identification followed Francis (2008). All procedures were approved by The National University of Malaysia Animal Ethic Committee with approval number FST/2016/SHUKOR/18-MAY/750-MAY-2016-SEPT.-2018-AR-CATS.

The mammals caught were inspected for ticks and mites by combing with a fine-tooth comb and picking using fine forceps. Each individual of ticks and mites found were preserved in 90% ethanol and stored in labelled individual's vials. Ticks were brought to the lab for classification and identification up to genus and species level by using taxonomic keys and published pictorial data. Ticks were first sorted into their respective order under dissecting microscope. The technique for mounting acarines (ticks and mites) followed Mariana et al. 2005 and Chuulun et al. 2005. Slides were prepared only for the larval stages as adults can be identified without mounting. Stereo and compound microscope were used in this study. The slides were examined under compound microscope (magnification 40x, 100x, 400x, and 100x) for identification. Acarines were identified according to the keys and descriptions given by Arthur (1962) and Gullan & Cranston (2010). Mites are brought back to the lab for further identification using molecular procedures. The extraction of DNA was using the DNeasy® Blood & Tissue (Qiagen USA) kit. Polymerase Chain Reaction (PCR) was done using COI gene to identify the mite's individuals up to species level.

Data Analysis

Prevalence gives a figure for a factor at single point in time (Jekel et al. 2001). In epidemiological study, prevalence is a measurement of all individuals affected by the disease at a particular time (Shields & Twycross 2003). In this study, overall prevalence of infected small mammals and prevalence of infected small mammals with ectoparasite species X were calculated.

Prevalence of infected small mammals were calculated by using formula:

$$= \frac{\text{Total no. of infected small mammals}}{\text{Total number of small mammals captured}} \times 100\%$$

Prevalence of infected small mammals with ectoparasite species X were calculated by this formula:

$$= \frac{\text{Total no. of infected small mammals with ectoparasites X}}{\text{Total no. of infected small mammals with all ectoparasites}} \times 100\%$$

RESULTS

A total of 30 and 25 individuals of small mammals were captured in JR and CM respectively that make the overall total individual of small to medium-sized mammals captured, 55 as shown in Table 1. JR comprised of four families, six genera and eight species. Muridae was the dominant family and represent 66.7% of all individual captured at JR, followed by Sciuridae (10%), Tupaiidae (10%) and Hystricidae (6.7%). Out of eight species, two of them have been listed as Vulnerable by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, which are *Maxomys rajah* and *M. whiteheadi.* CM on the other hand recorded five families, seven genera and 10 species. Muridae was also dominant family found in both study areas. *Maxomys surifer* was found to be the most dominant species which represent 20% of total small mammals captured at Chemomoi.

From 55 individuals host captured, 32 were infected by ectoparasites where 20 host were captured in JR and 12 host were captured in CM (Table 2). *Maxomys* spp. *(M. rajah, M. whiteheadi* and *M. surifer*) were highly infested host in both localities. *Leopoldamys sabanus* and *Hystrix brachyura* were infested in JR, meanwhile *Rattus rattus* and *Viverra tangalunga* were infested in CM. A total of five species of ticks were identified in both study sites, namely *Ixodes granulatus, Dermacentor atrosignatus, Rhipicephalus saguineus, Amblyomma testudinarium* and *Haemaphysalis* sp. Mites was only represented by one species, which was *Laelaps* sp. Prevalence of ectoparasite were higher in JR with 67%, compared to CM with 48%. Although JR shows contrastingly higher host infestation compared to CM, Man-Whitney U test reveal no significant difference between the number of infested host ($U = 18$, $z = -0.7942$, $p = 0.4271$) in both study areas. Similarly, parasite load was higher in JR with 198 individual's parasites collected, compared to CM with 79, particularly for mites (*Laelaps* sp.). Parasite load of *Laelaps* sp. was also generally high in several species.

Ticks prevalence was slightly higher in JR with 35% compared to CM with 33.3% (Table 3). In JR, the highest prevalence was from *Dermacentor atrosignatus* and *Ixodes granulatus* with 15%. *Haemaphysalis* sp. was only recorded in single individual in *Maxomys rajah*, as well as *Amblyomma testudinarium* which only infest *Hystrix brachyura* and *Rhipicephalus saguineus* which only infested *M. rajah*. CM on the other hand, only recorded 3 species of ticks, and dominated with *Ixodes granulatus* (25%) which infested *M. surifer*, *M. whiteheadi* whereby and *Viverra tangalunga*. Next, *Dermacentor atrosignatus* recorded 8.3% prevalence was found in *Rattus rattus* and *M. surifer* respectively.

The study reveal that mites only infested specific species in high abundance, namely all *Maxomys* spp. in both locations while *Leopoldamys sabanus* also infested with only one individuals of mites (Table 3). Prevalence of mites were higher in JR with 95%, compared to CM with 70% (Table 4).

DISCUSSIONS

The higher infestation rate in JR and certain species might be attributed to various important factors including tick's distribution, host's preference, host's distribution and microhabitat. *Ixodes granulatus* found to be the dominant species for both study site because this species has wide distribution throughout the continent (Trevor & James 1994). Other species such as *Dermacentor atrosignatus* (Wassef & Hoogstraal, 1984), *Rhipicephalus sanguineus* (Cooley 1946) and *Amblyomma testudinarium* (Voltzit & Keirans 2002) that was found in the study was also widely distributed across South-East Asia region. As for *Haemaphysalis* sp. that cannot be identified to species level due to lack of keys, however, the distribution of genus *Haemaphysalis* has its center of distribution in South-East Asia (Petney 1993). The result of this study was supported by Mariana et al. (2008) which also recorded three genera of Ixodid ticks in non-volant small mammals, similar to this study which were *Dermacentor*, *Haemphysalis*, and *Ixodes*. In general, ticks' species documented in this study feed on small mammals, such as *Haemaphysalis* sp. (Mohd Zain et al. 2015), *Ixodes granulatus* (Ho & Krishnasamy 1990; Nadchatram et al. 1996) and *Amblyomma testudinarium* (Voltzit & Keirans 2002).

Host's distribution and habitat condition are among the main factor influencing tick's distribution (Engelbrecht 2016). According Madinah et al. (2014), the distribution of ectoparasites may have been influenced by the sampling site selection. In this study, JR and CM were surrounded with different matrix types, with oil palm plantation and Acacia plantation respectively. The distribution of host was higher in JR due to the higher availability of food resources compared to CM with less availability of food resource, instead providing buffer zone for wildlife movement (Razali et. al. unpubl). Some ectoparasites will associate with hosts of a certain resting site or habitat like whether the area is small or with a distinct environment nest, burrows, caves, rock piles, thickets, dense reverine forests, and wet lowlands (Sparagano, 1999). Kitron et al. (1992) found a strong correlation between tick infestation on white tailed deer and the occurrence of sandy oil and hardwoods (Ostfeld et al. 1995). According to Zumpt 1961, *Laelaps* sp. geographically widespread in Sub-saharan Africa. However, there is no current study about this mite's species in Malaysia or South-east Asia. Mites also have a strong correlation with host's habitats as recorded in another study by Russo et al. (2010) and Engelbrecht (2016) where *Laelaps* sp. can be found on two murids hosts namely *Mastomys coucha* (grass/ plain dwelling rodent) and *Micaelamys namaquensis* (rocky habitats). Members of the parasitc mite genus *Laelaps* complete majority of their life cycle in the nest of the host (Radovsky 1994). It is evident that habitat disturbance may increase the prevalence of hosts and ectoparasites and affect the structure of small mammal's communities (Madinah et al. 2014), and therefore, induce parasite host-switches (Wells et al. 2007). Loss of biodiversity will occur due to the highly-fragmented landscape (Blake & Karr 1987) thus reduces the abundance of parasite-hosts and lead to another factor such as limited area for dispersal and survival of ticks on smaller patches (Werden 2012).

Maxomys whiteheadi had the highest prevalence of *Ixodes granulatus* especially in JR. Madinah et al. (2014) use network analysis to determine the host specialization of ticks, in order to observe patterns of parasites and their hosts (Poulin 2010). Like non-volant small mammals, ectoparasites also can be divided to specialist and generalist depends on its host. Most species in genus *Ixodes* are generally host specific (Mccoy et al. 2013) except *Ixodes granulatus* and *I. ovatus* (Petney 1993). Thus, the distribution of ticks is tightly linked to the ecological characteristics of the host such as host's microhabitat (Mccoy et al. 2013). On the other hand, *Laelaps* sp. tend to be rodent generalist mites such as found in *Maxomys rajah, M. whiteheadi, M. surifer* and *Leopoldamys sabanus*. This result was supported by Zumpt (1961) who reported infestation of *Laelaps* sp. from multiple rodent species in Sub-saharan Africa. Other mite's species such as *Laelaps giganteus* is a host specific mite which occurs on the four-stripped mouse genus *Rhabdomys* in southern Africa (Matthee et al. 2007).

Other factors that contribute to higher infestation rate in JR are host's sex, body mass, and size of body's host. These factors are interrelated in affecting infestation rate of ectoparasite. Usually male hosts have higher body mass and larger body size compared to female host. Large body mass resulted in large size of host. Many studies showed that the greater the size of the animal may provide a greater surface for ectoparasites to attached due to greater area it will sweep (Arneberg et al., 1998; Milne 1949; Mysterud et al. 2015). This study showed that most of the small to medium size mammals captured which weighted more than 10g threshold (Mysterud et al. 2015) have infested by ectoparasites. Similar to this study, other studies explained that increasing body mass of host will increase the ectoparasites loads (Mysterud et al. 2015; Perkin et al. 2003). This statement explained some of the species in this study such as *Maxomys rajah, M. whiteheadi* and *M. surifer* at JR have high ectoparasites loads on them as shown in Table 2.

However, medium-sized mammals in this study which were female *Hystrix brachyura* and female *Viverra tangalunga* had the least number of tick's load. Both females are assumed lactating due to its morphological and have young. Typically, female mammals and their young's self-groomed and groomed their young at a higher rate. In behavioural study of Impala, (*Aepyceros melampus*) have observed that both young and their mothers are consistently groomed have low ticks load (Hart et al. 1992). Thus, the infestation rates of ticks on small mammals can serves as ecological labels related to the patterns of distribution and behaviour of the host (Audy 1954).

Out of six species of ticks, four of them were reported as vector for Rickettsiae that caused spotted fever which are *Haemaphysalis* sp., *Dermacentor atrosignatus* and *Ixodes granulatus* (Marchette, 1965). *Ixodes granulatus* is also a vector of Langat Virus (Smith 1956) and is involved in the cycle of tick typhus and Q fever in Peninsular Malaysia (Marchette 1965). Langat Virus is similar to Russian Spring-Summer Encephalitis Complex (RSSE) and was first recognized in 1956 in *Ixodes granulatus* infesting *Sundamys muelleri* and *Leopoldamys* *sabanus* (Madinah et al. 2011). Apart from that, *Rhipicephalus sannguineus* is responsible for the transmission of *Babesia gibsoni*, to dogs in this region as well as other parts of the world (Mokhtar et al. 2013). However, there is still no further study on *Laelaps* sp. as a vector for zoonotic disease.

CONCLUSION

In conclusion, this study indicates that habitat condition will significantly affect parasite prevalence in small to medium-sized mammal population. Factor that greatly caused this could be due to the resilience of an individual to persist in disturbed habitat. It is also important to understand the links between prevalence and rate of infestation of ectoparasites to predict tick population dynamics and epidemiology of tick-borne disease. This study can also serve as the stepping stone to create awareness among public on the tick-borne disease for a better prevention control program.

ACKNOWLEDGEMENT

We would like to thank Department of Wildlife and National Parks (DWNP) to conduct this research at Kemasul, Pahang. Our sincere appreciation to DWNP staff for their assistance during sampling. We also want to extend our gratitude to Tenaga Nasional Berhad Research for providing research grant to conduct this research under research code ST 2015-001.

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APPENDICES

Table 1 Total number of individual's host captured at both study sites (LC=Least Concerned; V=Vulnerable; NT=Near Threatened)

Family	Species	Common Name	IUCN Status	Sites		Total
				Jambu Rias	Chemomoi	
Muridae	*Maxomys rajah*	Rajah Maxomys	V	12	1	13
	Maxomys surifer	Red Spiny Maxomys	LC	4	5	9
	Maxomys whiteheadi	Whitehead's Maxomys	V	4	3	7
	Rattus rattus	House Rat	LC	1	2	3
	Leopoldamys sabanus	Long-Tailed Giant Rat	LC	1	0	1
Sciuridae	*Callosciurus nigrovittatus*	Grey-bellied Squirrel	NT	0	3	3
	Callosciurus notatus	Plaintain Squirrel	LC	3	1	4
Tupaiidae	*Tupaia glis*	Common Treeshrew	LC	3	4	7
Hystricidae	*Hystrix brachyura*	Malayan Porcupine	LC	2	2	4
Viverridae	*Viverra tangalunga*	Malay Civet	LC	0	3	3
	Paradoxurus hermaphroditus	Common Palm Civet	LC	0	1	1
Total				**30**	**25**	**55**

Table 2 Number of infected host, and parasite load on each parasites species in both study areas.

Site	Host sp	Num. of infested host	Mites / Ticks	Infected host with parasite X	Parasite load
JR	*Maxomys rajah*	11	*Laelaps* sp.	10	88
			Haemaphysalis sp.	1	1
			Dermacentor atrosignatus	1	1
			Rhipicephalus saguineus	1	1
	Maxomys whiteheadi	4	*Laelaps* sp.	4	41
			Ixodes granulatus	3	34
	Maxomys surifer	3	*Laelaps* sp.	3	27
			Dermacentor atrosignatus	1	1
	Leopoldamys sabanus	1	*Laelaps* sp.	1	2
			Dermacentor atrosignatus	1	1

	Hystrix brachyura	1	*Amblyomma testudinarium*	1	1
Total / prevalence (%)		**20**		**67%**	**198**
CM	*Rattus rattus*	1	*Dermacentor atrosignatus*	1	1
	Maxomys surifer	5	*Laelaps* sp.	3	41
			Ixodes granulatus	1	7
	Maxomys whiteheadi	4	*Laelaps* sp.	3	20
			Ixodes granulatus	1	1
	Maxomys rajah	1	*Laelaps* sp.	1	8
	Viverra tangalunga	1	*Ixodes granulatus*	1	1
Total / prevalence (%)		**12**		**48%**	**79**

Table 3 Prevalence (%) of ticks in both study sites.

Ticks	JR	CM
Haemaphysalis sp.	5	0
Dermacentor atrosignatus	15	8.3
Rhipicephalus sanguineus	5	0
Ixodes granulatus	15	25
Amblyomma testudinarium	5	0
Total prevalence (%)	35	33.3

Table 4 Number of individual's host infected by mites and the prevalence in both study sites.

Site	Host species	No. of infested host	No. of infected host with parasite X	Prevalence of mites (%)
JM	*Maxomys rajah*	11	10	91
	Maxomys whiteheadi	4	4	100
	Maxomys surifer	3	3	100
	Leopoldamys sabanus	1	1	100
Mites prevalence (%)				95
CM	*Maxomys surifer*	5	3	60
	Maxomys whiteheadi	4	3	75
	Maxomys rajah	1	1	100
Mites prevalence (%)				70

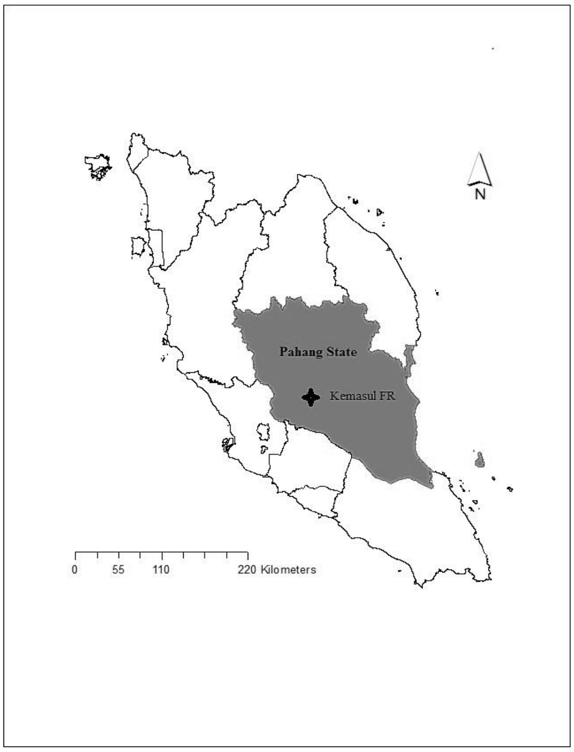

Figure 1 Location of Kemasul Forest Reserve, Pahang in Peninsular Malaysia.

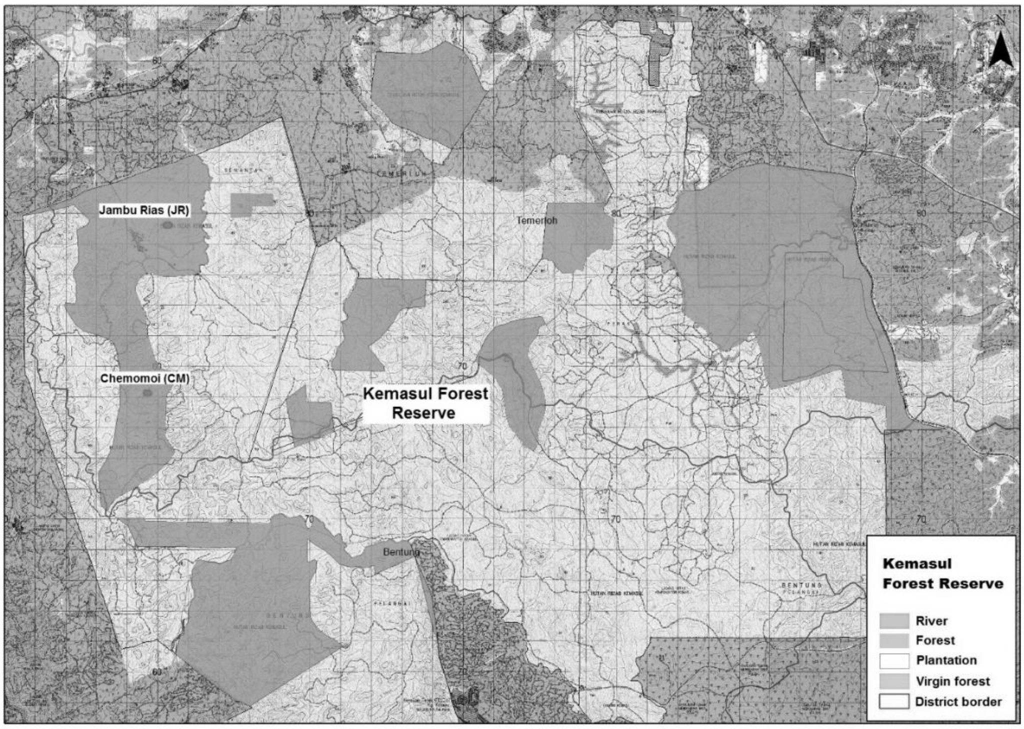

Figure 2 Sampling sites in Kemasul Forest Reserve, Pahang.