ECTOPARASITES OF SMALL MAMMALS IN FOUR LOCALITIES OF WILDLIFE RESERVES IN PENINSULAR MALAYSIA

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Abstract. Field surveys of ectoparasites on rodents and scandents were conducted in four localities of wildlife reserves in Peninsular Malaysia from October 2008 to November 2009. A total of 16 animals comprising 5 species of hosts were caught and examined for ectoparasites. The hosts examined were *Maxomys rajah, Maxomys whiteheadi, Leopoldamys sabanus, Lariscus insignis* and *Tupaia glis*. Of these hosts, 9 genera, consisting of 14 species of ectoparasites were extracted. Three species of ticks (Ixodidae), 7 species of mesostigmatid mites (Laelaptidae), 3 species of chiggers (Trombiculidae) and 1 species of listrophorid mites (Listrophoriidae) were identified. The infestation rate of ectoparasites observed ranged from 12.5% to 62.5%. Among the ectoparasites found, *Ixodes granulatus* and *Leptotrombidium deliense* are of known medical importance.

Keywords: ectoparasites, small mammals, wildlife reserves, Peninsular Malaysia

INTRODUCTION

Ectoparasites are a diverse and highly adapted group of animals that infest the external body surface of vertebrates (Hanafi-Bojd *et al,* 2007). They are considered a main vector of zoonotic diseases and play an important role in the transmission of a wide variety of diseases. Ticks are notorious vectors of mumerous pathogenic organisms, such as protozoa, rickettsiae, bacteria and viruses. These organisms cause serious and life-threatening illnesses in humans and animals (Chul-Min *et al,* 2006). Animals and their ectoparasites play important roles in distribution of arboviruses, streptococcal infections, choriomeningitis, plague, tularemia, leptospirosis and spirochetosis (Manson and Stanko, 2005). In Peninsular Malaysia, information regarding ectoparasites on small mammals has been studied by the Institute for Medical Research (IMR) (Leong and Marshall, 1968; Mariana *et al,* 1996; Chuulun *et al,* 2005; Mariana *et al,* 2005a,b; Mariana *et al,* 2006a,b; Mariana *et al,* 2007; Mariana *et al,* 2008a,b; Nadchatram, 2008; Mariana *et al,* 2009).

The tropical rainforest of Malaysia is rich in flora and fauna species diversity (Harrisson, 1962; Fleming, 1973). In Peninsular Malaysia, the Department of Wildlife and National Park (DWNP) is the management authority responsible for

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conservation of fauna and all protected areas. The DWNP had organized field inventories of flora and fauna since 2008 and the Universiti Malaysia Sarawak (UNIMAS) was involved in the study of small mammals and their ectoparasites during four inventories from October 2008 to November 2009. The inventories were conducted in Endau Kluang Wildlife Reserve, Johore (EKWR); Tasek Bera Ramsar Site, Pahang (TBRS); Sungai Dusun Wildlife Reserve, Selangor (SDWR) and Lata Bujang Krau Wildlife Reserve, Pahang (LBKWR).

Some ectoparasites of rodents and scandents in Malaysia are known to be of public health importance (Nadchatram, 2008). However, there is little quantitative information available regarding ectoparasites on small mammals, as hosts of zoonotic pathogens or their vectors (Muul and Lim, 1974). There have been no ectoparasite surveys of rodents or scandents conducted in wildlife reserves in Peninsular Malaysia, in EKWR, TBRS, SDWR and LBKWR. Thus, the aim of this study was to identify the presence of ectoparasites of known public health importance. We also determined the distribution and host interaction with ectoparasites from rodents and scandents in the four different sites of wildlife reserves in Peninsular Malaysia.

MATERIALS AND METHODS

Study areas

Four wildlife reserves in Peninsular Malaysia were surveyed (Fig 1). The first site surveyed was Endau Kluang Wildlife Reserve, Johore (EKWR) conducted from October to November 2008. The area (N 02º22′ E 103º07′ and N 02º35′ E 103º25′) was established in 1933 as a part of Endau Rompin National Park (ERNP). This particular park is an extension from EKFR to Lesong Forest Reserve in Pahang. The ERNP, which covers an area of approximately 800 km^2, is the second largest national park in Peninsular Malaysia after Taman Negara. It is mostly hilly with some prominent sand plateaus made up of a lush, pristine tropical rainforest and home of several rare and endangered species of flora and fauna (Anonymous, 2010).

The second area surveyed was the first Malaysian Ramsar site in Tasek Bera, Pahang (TBRS) (N 02º58′ E 102º36′). The survey was conducted in May 2009. It contains the largest natural freshwater lake in Malaysia covering an area of approximately 38,446 ha (384.46 km^2) of forest that stretches over the low east-west watershed of Peninsular Malaysia (Furtado and Mori, 1982). It is a water catchment area of 61, 380 ha (613.8 km^2) and covers a 6,150 ha (61.5 km^2) alluvial riparian peat swamp (Furtado and Mori, 1982). The TBRS is of great importance not only because of the richness in biodiversity but also in scientific, educational, recreational, and economic points of view. This alluvial peat swamp supports biologically diverse community.

Sungai Dusun Wildlife Reserve, Selangor (SDWR) was the third site surveyed in October 2009. It is located about 120 km from Kuala Lumpur at latitude 03º 35′ to 03º 40′ N and longitude 101º 23′ to 101º 27′ E (Muda and Suib, 1989). The reserve area is about 4,208.7 ha (42.087 km^2) in size and covers a peat swamp and lowland dipterocarp forest (Mohamad and Romo, 2002). The reserve is drained by Sg Bernam (boundary of Selangor and Perak State) in the north and Sg Tengi in the south. It bordered by Federal Land Development Authority (FELDA) schemes on the northern and eastern side; and by peat swamp forest on the western and

Fig 1–Map of the four sampling sites in Peninsular Malaysia: 1 Endau Kluang Wildlife Reserve, Johore (EKWR); 2 Tasek Bera Ramsar Site, Pahang (TBRS); 3 Sungai Dusun Wildlife Reserve, Selangor (SDWR); 4 Lata Bujang Krau Wildlife Reserve, Pahang (LBKWR).

southern side. Since 1985, the SDWR has been known as a center for protecting the habitat of the endangered Sumatran rhinoceros, *Dicerorhinus sumatraensis;* it also serves as a conservation center for the Malayan tapir, *Tapirus indicus* (Hassan *et al*, 2008).

The fourth site surveyed was the Lata Bujang Krau Wildlife Reserve, Pahang (LBKWR) (N 03º50′ E 102º06′). The survey was conducted in November 2009. LBKWR is located at the foot hill of Gunung Benom in the Krau Wildlife Reserve with an elevation of 200 m above Mean Sea Level (MSL). Sungai Terboi and Lata Bujang waterfalls have been water sources and landmarks for the area. Lata Bujang consists mainly of pristine tropical lowland rainforest. The area is covered by lowland dipterocarp, peat swamp and lower montane forest. Gunung Benom (2,107 m) is the ninth highest peak in Peninsular Malaysia and located in northern Krau Wildlife Reserve. It is an isolated mountain located in central Peninsular Malaysia and is predominantly made up of Permian-Triassic Marine sediments surrounded by Jurassic-Cretaceous granite.

Host collection

One hundred cage traps were used to capture the rodents and scandents at each study site except LBKWR, where 50 traps were used. Cage traps were placed on the ground and tree branches along the existing trails at approximately five meter intervals. Cage traps were baited with bananas, oil palm fruit, tapioca or salted fish and checked twice daily. Payne *et al* (2005) found a variety of different foods are suitable for baiting small mammals, including fruit and salted fish. Bananas were frequently used in this study because it is effective bait and an easily available fruit (Lim, 1973; Azmin *et al*, 1988; Andrew *et al*, 2002; Payne *et al*, 2005). Bait was replenished every morning and evening. Trapped animals were placed in cloth bags and brought back to a field laboratory for further processing.

Processing of animals

Trapped animals were identified following Medway (1983), Francis (2008) and Payne *et al* (2005). The ectoparasites were collected using various methods. Ani-

Table 1
Taxonomic list of small mammals trapped in four localities in Peninsular Malaysia (October 2008-November 2009).

Species of host	Number of individuals	Total	Locality	Habitat
Lariscus insignis	1	4	Endau Kluang Wildlife Reserve, Johore	Lowland dipterocarp forest
Maxomys rajah	2			
Tupaia glis	1			
Leopoldamys sabanus	1	4	Tasek Bera Ramsar Site, Pahang	Peat swamp
Maxomys whiteheadi	1			
Maxomys rajah	2			
Maxomys rajah	2	5	Sungai Dusun Wildlife Reserve, Selangor	Lowland dipterocarp forest
Tupaia glis	3			
Maxomys rajah	3	3	Lata Bujang Krau Wildlife Reserve, Pahang	Lower mountain forest
	Total	16		

mals selected for molecular phylogenetic studies and biomedical investigations were anesthetized with chloroform in individual cloth bags before screening for ectoparasites (Gannon and Willig, 1995). This is because many ectoparasites are host-specific (Upton, 1991) and separation can avoid contamination among hosts (Bittercourt and Rocha, 2002). Anesthetized animals were removed from the bags, placed on a white enamel tray and combed thoroughly with a fine tooth comb (Mariana *et al*, 2005a). The contents of the tray were carefully examined for ectoparasites which were collected with a sharpened wooden applicator stick. The ectoparasites were placed in collection vials containing 70% ethanol. A separate vial was used for each animal. The vials containing ectoparasites were labeled with sufficient information, such as host species, location, ecology, sex and date of collection. For unanesthetized animals, ectoparasites were collected directly from eye-lids, ear-lobes, ear fringes, chins, muzzles and other parts of the body using fine forceps. The animals were released after the species, weight, sex and morphological data were recorded. Captured animals were marked with a necklace containing a numbered Unimas metal tag around the animal's neck to prevent reevaluation. All ectoparasites were brought to the Ecology Laboratory of the Zoological Museum, UNIMAS for further identification. All preserved ectoparasites, excluding ticks, were later mounted prior to identification.

Mounting of ectoparasites

Preliminary identification of preserved ectoparasites was made under a dissecting microscope. Preserved ectoparasites were sorted based on their morphology. The technique for mounting of acarine ectoparasites (ticks and mites) followed the methods of Mariana *et al* (2005a) and Chuulun *et al* (2005). The different ectoparasites were further processed as follows: mesostigmatid mites were first cleared in lactophenol before mounted in Hoyer's medium. Listrophorid mites were placed in lactic acid and heated on a hot

Table 2
Ectoparasitic infestation rates on rodents and scandents in four localities in Peninsular Malaysia (October 2008 - November 2009).

Species	No. caught	No. of hosts infested			
		Ticks	Mesostigmatids	Chiggers	Listrophorids
Rodentia					
Lariscus insignis	1	1	-	-	-
Leopoldamys sabanus	1	1	-	-	-
Maxomys rajah	9	6	9	-	2
Maxomys whiteheadi	1	-	1	-	-
Scandentia					
Tupaia glis	4	-	-	3	-
Grand total	16	8 (50.0%)	10 (62.5%)	3 (18.8%)	2 (12.5%)

(-) means no ectoparasites were collected from that particular animal.

plate to 200ºC for 5 minutes before being mounted in Hoyer's medium while chiggers were mounted directly in Hoyer's medium and gently cleared by warming over an open flame. For ticks, slides were not prepared except for the larval stages. Mounted slides were incubated at 40ºC for a week to harden the mounting medium. The coverslips were ringed with nail polish to prevent desiccation of the medium during storage.

Identification of ectoparasites

The slides were examined under a compound microscope (magnification 40x, 100x, 400x and 1,000x) for identification. Ticks were identified using a stereo microscope. Ectoparasites were identified to genera and species levels when possible using available keys, published taxonomic drawings and references (Kohl, 1957; Strandtmann and Mitchell, 1963).

RESULTS

A total of 16 animals, comprised of 3 species of rats (*Maxomys rajah, Maxomys whiteheadi* and *Leopoldamys sabanus*), 1 species of squirrel (*Lariscus insignis*) and 1 species of tree shrew (*Tupaia glis)* were examined for ectoparasites. The types of rodents and scandents caught in all four wildlife reserves are shown in Table 1.

Four groups of ectoparasites were recovered from the rodents and scandents caught. The ectoparasites were ticks, mesostigmatid mites, chiggers (larval trombiculid mites) and listrophorid mites. Nine genera of ectoparasites consisting of 14 species were found. Three species of ticks, 7 species of mesostigmatid mites, 3 species of chiggers (larval trombiculid mites) and 1 species of Listrophorid mite were collected (Table 3). The rates of infestation of each ectoparasite ranged from 12.5% to 62.5%. The greatest infestation was with mesostigmatid mites whilst the least infestation occurred with listrophorid mites (Table 2). These ectoparasites were recovered from five host species, but not every species was infested.

Ticks

Two genera and one species of hard ticks (Ixodidae) were found on animals in all four wildlife reserves. *Rhipicephalus* sp was found only in LBKWR. *Haemaphysalis*

Fig 1–Hard tick (*Ixodes granulatus*) (of medical importance).

Fig 2–Chigger mite (*Leptotrombidium deliense*) (of medical importance).

sp was found in TBRS and SDWR and *Ixodes granulatus* was found in EKWR, TBRS and LBKWR (Table 3).

Mesostigmatid mites

Seven Laelapid species of mesostigmatid mites were recovered from rodents in all study areas (Table 3). Those species were *Laelaps aingworthae, Laelaps flagellifer, Laelaps insignis, Laelaps mercedae, Laelaps sanguisugus, Laelaps sculpturatus* and *Longolaelaps whartoni*. In this survey, *Laelaps sanguisugus* was found in all localities while *L. sculpturatus* was found only in SDWR.

Chiggers

Three species of chiggers (Trombiculidae) were recovered from the ear-lobes, eye-lids and bodies of the rodents and scandents. The species were *Gahrliepia* (*walchia)* sp, *Leptotrombidium deliense* and *Ascoschoengastia* sp (Table 3). All chiggers were found on *T. glis* in SDWR.

Listrophorid mites

Only one genus of listrophorid mites, *Listrophoroides* sp, was identified on rodents at study sites EKWR and SDWR (Table 3).

DISCUSSION

In this study, only *M. rajah* was caught at all study sites. This species is a common forest rat expected to be caught due to their wide distribution throughout Southeast Asia (Francis, 2008). The small number of animal species recorded was probably due to poor weather conditions, such as a large number of rainy days and the types of bait used may not have been attractive to the animals. Weather can affect trapping success: good weather (dry days) can increased the yield of trapping (Kelly and Caro, 2003; McCain, 2007; Paschal, 2007). The types of bait used is important since it can affect the types of species and the numbers of animals captured (Zakaria *et al*, 2001; Abdullah *et al*, 2006). Careful selection of bait can increase the number of animals caught in relation to the habitat sampled (Lim, 1973).

Studies over many years in Malaysia, particularly in Peninsular Malaysia, have

Table 3
Number of ectoparasites found on rodents and scandents in four localities of Peninsular Malaysia (October 2008 - November 2009).

Species	Endau Kluang Wildlife Reserve			Tasek Bera Ramsar Site			Sg Dusun Wildlife Reserve		Lata Bujang Krau Wildlife Reserve	Medical importance
	Lariscus insignis	*Maxomys rajah*	*Tupaia glis*	*Maxomys whiteheadi*	*Maxomys rajah*	*Leopoldamys sabanus*	*Maxomys rajah*	*Tupaia glis*	*Maxomys rajah*	
Ticks										
Ixodes granulatus	1	-	-	-	1	10	-	-	1	√
Haemaphysalis sp					2		9			
Rhipicephalus sp									1	
Mesostigmatids										
Laelaps aingworthae		51			39					
Laelaps sanguisugus		12		4	45		21		46	
Laelaps sculpturatus							1			
Laelaps flagellifer		1					24		8	
Laelaps insignis		6					14		51	
Laelaps mercedae		3					2		19	
Longolaelaps whartoni		14			1					
Chiggers										
Gahrliepia (walchia) sp								27		
Leptotrombidium deliense								29		√
Ascoschoengastia sp								1		
Listrophorid mites										
Listrophoroides sp		2					2			

(-) means no ecoparasites were collected from that particular animal; (√) indicates medical importance.

shown *I. granulatus* (Fig 1) in all its stages continues to be one of the most common species of ticks on rodents (Nadchatram, 2008). It is a common acarine ectoparasite of rodents in Malaysia and its distribution extends from Southeast Asia to eastern India and China (Nadchatram, 2008). Finding *I. granulatus* agrees with the report the commonest host of *I. granulatus* is rodents. This was confirmed by other recent studies where *I. granulatus* was also found on rodents (Mariana *et al,* 2005a, 2008a).

Ixodes granulatus is a vector of public health importance (Mariana *et al,* 2005a, 2008b). It is a vector of Langat Virus (Smith, 1956), is able to transmit the pathogen (Marchette, 1965) and is involved in the cycle of tick typhus and Q fever in

the climax forest of Peninsular Malaysia (Marchette, 1965). Langat Virus is similar to Russian Spring-Summer Encephalitis Complex (RSSE), and was first recognized in 1956 in *I. granulatus* infesting *S. muelleri* and *L. sabanus*.

Previous studies in Peninsular Malaysia have shown *L. sanguisugus* can be found on *M. rajah* (Strandmann and Mitchell, 1963; Ho *et al*, 1985; Shabrina, 1990; Mariana *et al*, 2006a, 2007; Paramasyaran *et al*, 2009). This species can also be found on other species of rodents, such as *M. whiteheadi*. Although *L. sanguisugus* was found on *M. rajah* caught in all sites surveyed, it was also extracted from *M. whiteheadi* in one individual in TBRS. Mesostigmatid mites were most frequently found on rats but nearly absent on tree shrews and squirrels. This is in accordance with the findings of Muul and Lim (1974). However, none of the Mesostigmatid mites recovered was of known medical importance.

There have been no authenticated reports of mites serving as vectors of human infection, although many species of Mesostigmatid mites have been reported to be associated with humans (Nadchatram, 2008). It is likely for them to be an enzoonotic cycle including humans (Nadchatram, 2008). This is because mesostigmatids are parasites of wild rodents in the forest and their territory overlaps other rodent species that contact humans.

Chiggers, from the genus *Gahrliepia*, are known as denizens of the tropical rainforest, where they parasitized a variety of ground rats that nest in burrows; their nidicolous habitat was discovered in Malaysian forests (Kudin *et al*, 1966). Hence, the occurrence of this chigger in our study is not surprising. *Gahrliepiine* chiggers are known to attain their maximum development in the oriental region (Audy, 1954; Traub and Morrow, 1955) and Southeast Asia is believed to be the center of evolution for this genus (Nadchatram, 1970).

A species of chiggers, *L. deliense* (Fig 2), which is of known medical importance, was identified in this study. The species was found in SDWR. The genus, *Leptotrombidium* is an important vector of scrub typhus (Shabrina *et al*, 1992/1993; Chuulun *et al*, 2005; Mariana *et al*, 2005a, 2007, 2008a). In this study, it was found on *T. glis* in SDWR. This species can be found on various hosts and can become an efficient vector of scrub typhus from one rodent to another (Domrow and Nadchatram, 1963). Hence, *T. glis* has the potential to become a natural host for rickettsia causing scrub typhus (Mariana *et al*, 2007).

Listrophorid mites are obligate parasites and live on the fur of mammals. They are also associated with rodents and are widely distributed throughout the world (Fain and Bochkov, 2004).

Although the number of hosts and ectoparasites collected in this study was insufficient for further analysis, the presence of ectoparasites in these mammals is important, since information about the distribution of these parasites and their hosts speaks to the potential transmitting newly emerging diseases in this area (Smith, 1956; Shabrina, 1989; Nadchatram, 2008).

A total of 3 species of ticks, 7 species of Mesostigmatid mites, 3 species of chiggers and 1 species of Listrophorid mite were identified from 16 animals comprising 4 species of rodents and 1 species of scandents. The species of hosts examined for ectoparasites were *M. rajah, M. whiteheadi, L. sabanus, L. insignis* and *T. glis*. Of the ectoparasites found, *I. granulatus* and *L. deliense* are of known public health

importance.

Further surveys are recommended to develop a more comprehensive inventory of ectoparasites in different locations and habitats which can improve knowledge regarding host-parasite relationships, biology and ecology.

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