Tropical Biomedicine 34(3): 723–731 (2017)

Short Communication

Co-infections of ectoparasite species in synanthropic rodents of western Sarawak, Malaysian Borneo

Ng, Y.L.[1,2], Hamdan, N.E.S.[1], Tuen, A.A.[1], Mohd-Azlan, J.[1] and Chong, Y.L.[1*]
[1]Department of Zoology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[2]Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[*]Corresponding author e-mail: ylchong@unimas.my; yeelingchong@gmail.com
Received 24 November 2016; received in revised form 23 January 2017; accepted 1 February 2017

Abstract. Little is known regarding infestation of ectoparasites in synanthropic rodents in Sarawak, Malaysia. A total of 44 rodents from three species (*Rattus rattus*, *Rattus tiomanicus* and *Sundamys muelleri*) were trapped from four residential areas in western Sarawak, Malaysia, for ectoparasites screening. A total of 117 ectoparasites from three hard tick species (*Ixodes granulatus*, *Haemaphysalis* sp. 1, *Haemaphysalis* sp. 2), three mesostigmatid mite species (*Laelaps echidninus*, *Laelaps sedlaceki*, and *Laelaps nuttalli*), one trombiculid mite (chigger species), and one louse species (*Hoplopleura* sp.), were recovered from 32 infected rodents (72.73% infestation). Infestations by multiple ectoparasite species on the same rodent individuals were recorded in *R. rattus* and *R. tiomanicus* (28.1%, n=9) in this study, while *Sundamys muelleri* was only infested with *L. echidninus*. One *R. rattus* individual was co-infected with ticks, louse, and mesostigmatid mite. *L. echidninus* was the generalist ectoparasite species that infected all three rodent species in three of the residential areas studied. Ectoparasite species diversity was significantly different among four residential areas based on Shannon index and diversity t-test (p-value <0.05). This study provides the first record of the association of synanthropic rodents with multiple ectoparasite infections in residential areas of western Sarawak, Malaysia.

Synanthropic rodents living in close proximity with humans are comprehensively known as vertebrate pests. They damage food crops that lead to economic losses (Brown & Khamphoukeo, 2007) and serve as reservoir hosts for viral and bacterial pathogens that may be a source of concerns in both public and veterinary health. Rodent-borne diseases in humans such as Lanjan virus, Bartonellae, Hantavirus and Rickettsiosis (tick typhus, Q fever, or urban typhus) are transmitted by ectoparasitic arthropod vectors infesting rodents (Marchette, 1966; Tan *et al.*, 1967; Woolhouse *et al.*, 2001; Stojčević *et al.*, 2004; De Sousa *et al.*, 2006; Reeves *et al.*, 2007; Tay *et al.*, 2014; Yu & Tesh, 2014).

The occurrence and population distribution of rodent ectoparasites are associated with their hosts and habitats (Luyon and Salibay, 2007; Thanee *et al.*, 2009). Rodent species sharing the similar microhabitats are more likely to harbour the same ectoparasite species (Nava *et al.*, 2003). The co-existence among the parasites are also one of the important factors contribute to the distribution pattern of ectoparasite populations (Stojčević *et al.*, 2004; Thanee *et al.*, 2009).

Several studies on ectoparasite infestation and species composition in association with the rodent populations were reported in Peninsular Malaysia

(Chuluun *et al.*, 2005; Mariana *et al.*, 2005, 2008, 2011; Paramasvaran *et al.*, 2009; Madinah *et al.*, 2011, 2013). Related ectoparasites studies on rodents were also conducted in several neighbouring countries such as Philippines (Luyon and Salibay, 2007) and Thailand (Changbunjong *et al.*, 2010). There are relatively few records of ectoparasites and their native rodents in western Sarawak, Malaysia (Madinah *et al.*, 2013, 2014a, 2014b). However, record of interrelationship between ectoparasites and synanthropic rodents living in close contact with human populations in this region is lacking. In this study, the ectoparasite infestation and species diversity of synanthropic rodent populations at selected residential areas in western Sarawak, Malaysia were investigated.

Rodents used in this study were previously described in Hamdan *et al.* (2017). In brief, rodents were trapped at four suburban and rural residential areas in Western Sarawak, Malaysia. The rodent sampling locations and GPS readings include: suburban residential area at Sebayor Village, Kota Samarahan (SEB) (1º27'34"N, 110º29' 56"E); three rural residential areas at Bako Hulu Village, Kuching (SBK) (1º39'45"N, 110º25'56"E), Krusen Kranji Village, Serian (SER) (1º5'15"N, 110º30'40"E), Serian Ulu Village, Betong (SEU) (1º50'0"N, 111º40'0"E).

The rodents were captured from September 2014 to March 2015 (7 months). The rodent trapping method was adopted from the techniques of Herbreteau (2011) and Payne *et al.* (2007) with slight modification. A total of 50 cage traps (35 cm x 17 cm x 17 cm) were randomly deployed (10 m apart from each cage trap) at each sampling site at 6.00 pm and checked on the next morning at 7.00 am. The total sampling efforts for this study was 1,000 trap days. Traps were baited with both banana and dried salted fish. Trapped animals were identified based on the morphological characteristics, maturity determination and developmental stage following several references (McKenna *et al.*, 1997; Payne *et al.*, 2007; Francis, 2008; Herbreteau, 2011). The morphological measurements such as head and body length (HB), weight (WT), tail length (TL), ear length (EL), head length (HL), hind foot length (HF) and sex were recorded.

Rodents were euthanized with chloroform in plastic bag and the fallen ectoparasites were collected. The animals were then inspected with fine tooth comb to obtain more ectoparasites. The ectoparasites were collected using dissecting forceps into collection tube with 70% ethanol solution for preservation and mounting (Herbreteau, 2011). The preserved ectoparasites were sorted based on their morphology under the dissecting microscope before mounting on permanent slides. The mounting technique for acarines (ticks and mites) was modified following Chuluun *et al.* (2005) and Mariana *et al.* (2005). The slides for ticks were not prepared except for the larval stages. Mesostigmatid mites were first immersed in lactophenol for one hour, punctured with a minute needle at the lateral sides of the body, and immersed again in lactophenol overnight to allow the entry of lactophenol to clear the internal body, and lastly mounted with Canada balsam. Trombiculid mite was directly mounted with Canada balsam. A cover slip was placed on the specimen and gradually warmed over an open flame to allow the clearing of the chigger. All specimens were then identified to the species level where possible using available identification keys, published taxonomic figures and other related references following Strandtmann and Mitchell (1963), Flynn *et al.* (2007), and Herbreteau (2011).

Mean abundance (MA) of the ectoparasites was calculated based on the total number of individuals of a particular parasite species in a sample of a particular host species/total number of hosts of that species, including both infected and non-infected hosts, following Nava *et al.* (2003). The species diversity of the ectoparasites on residential areas was calculated using Shannon index and compared using diversity t-test implemented in PAST version 2.17c (Hammer *et al.*, 2001). The common ectoparasite species recovered from rodents between two localities (community similarity) were compared based on Sorenson's Coefficient (CC). CC = 2C/S1 + S2, where C is the number of ectoparasite species the two localities have in common, S1 is the total number of ectoparasite species found in Locality 1 and S2 is the total number of ectoparasite species found in Locality 2.

A total of 44 rodents comprising three species [*Rattus rattus* (n=35), *Rattus tiomanicus* (n=4) and *Sundamys muelleri* (n=5)] were trapped from four selected residential areas in Western Sarawak. In total, 117 individuals of ectoparasites were recovered from 32 rodents (72.7% ectoparasites infestation). These include two genera of three hard ticks species (*Ixodes granulatus*, *Haemaphysalis* sp. 1 and *Haemaphysalis* sp. 2); three mesostigmatid mites species from genus *Laelaps* (*Laelaps echidninus*, *L. nuttalli*, *L. sedlaceki*); one trombiculid mite (chigger species); and one lice species (*Hoplopleura* sp.) (Table 1). *R. rattus* was highly infected with ectoparasites (71.4% prevalence; MA=2.49). A total of 87 ectoparasites from seven species were identified in *R. rattus*, except chigger mites. *I. granulatus*. *Haemaphysalis* sp. 2, *L. nutalli*, and *Hoplopleura* sp. infestations were only recorded in *R. rattus*. Seven of the 25 infected *R. rattus* individuals were co-infected with more than one ectoparasite species (Table 2). On the other hand, all four individuals of *R. tiomanicus* were infected with at least one ectoparasite species. A total of 14 individuals of ectoparasites were collected from this rodent species (MA=3.50). Interestingly, one *R. tiomanicus* individual was co-infected with *Haemaphysalis* sp. 1 and one chigger species (Table 2). None of the other rodents from any species were infected with this chigger mite. Three individuals of *Sundamys muelleri* captured were infected with only *L. echidninus* (n=16). No other ectoparasite species were recovered from *Sundamys muelleri*.

From the ectoparasites collected, *L. echidninus* was the only generalist ectoparasite species found parasitising all three rodent host species with the highest infestation percentage of 56.41% (n=66). This was followed by *L. sedlaceki* (25.64%; n=30), *L. nuttalli* (6.84%; n=8), *Hoplopleura* sp. (3.42%; n=4), and Chiggers sp. (2.56%; n=3). While *I. granulatus*, *Haemaphysalis* sp. 1, and *Haemaphysalis* sp. 2 recorded the least prevalence of 1.71% respectively (with only two individuals captured per species).

Infested rodents showed the highest species diversity of ectoparasites at Sebayor Village (H=1.361), followed by Serian Ulu Village (H=1.013), Bako Hulu Village (H=0.525), and Krusen Kranji Village (H<0.01). The overlapped territories between human and rodents in Sebayor Village, with high density of residential houses, where the availabilities of food and shelter may increase the chances of trapping these synanthropic rodents, hence more ectoparasite species recovered. There is a significant difference in the species diversity of ectoparasites between each pair of the four residential areas based on diversity t-test (p-value <0.05), with the exception of that between Sebayor Village and Ulu Serian Village. Bako Hulu Village shared two common ectoparasite species (i.e. *L. echidninus* & *L. sedlaceki*) with Sebayor Village (CC=0.500) and Serian Ulu Village (CC=0.667). While three ectoparasite species (i.e. *Haemaphysalis* sp. 1, *L. echidninus* & *L. sedlaceki*) were mutually recovered in rodents at both Sebayor Village and Serian Ulu Village (CC=0.600). Krusen Kranji Village did not share common ectoparasite species compared to other localities (CC=0.000). The only two individuals of *Ixodes granulatus* recovered were infesting single *R. rattus* individual caught at Krusen Kranji Village.

In this present study, three synanthropic rodent species were captured from four residential areas in Western Sarawak, Malaysia for ectoparasites screening. *Rattus rattus* was captured at all four residential areas in this study and it is a well-documented urban pest in Malaysia and worldwide (Zahedi *et al.*, 1984; Battersby *et al.*, 2008; Lim, 2015). This species was highly infested with the most ectoparasite species, including tick, louse and mites via single or multiple-infection. Multiple-infection of ectoparasites was common in this study. One *R. rattus* individual was co-infected with tick, louse and mesostigmatid mite simultaneously. It is worth noting that *I. granulatus*. *Haemaphysalis* sp. 2, *L.*

Table 1. Ectoparasites mean abundance, infestation number and prevalence associated with rodent species from three residential areas in western Sarawak

Ectoparasites	Rodent species								
	Rattus rattus (n=35)			*Rattus tiomanicus* (n=4)			*Sundamys muelleri* (n=5)		
	MA	I	P(%)	MA	I	P(%)	MA	I	P(%)
Mesostigmatid mite									
Laelaps echidninus	1.29	15	60.00	1.25	2	50.00	3.2	3	60.00
Laelaps sedlaceki	0.71	5	14.29	1.25	2	50.00	–	–	–
Laelaps nutalli	0.23	7	20.00	–	–	–	–	–	–
Trombiculid mite									
Chigger (unidentified)	–	–	–	0.75	1	25.00	–	–	–
Tick									
Ixodes granulatus	0.06	1	2.86	–	–	–	–	–	–
Haemaphysalis sp. 1	0.03	1	2.86	0.25	1	25.00	–	–	–
Haemaphysalis sp. 2	0.06	1	2.86	–	–	–	–	–	–
Lice									
Hoplopleura sp.	0.11	3	8.57	–	–	–	–	–	–
Total	2.49[a]	25[b]	71.43[c]	3.50[a]	4[b]	100.00[c]	3.2[a]	3[b]	60.00[c]

MA (Mean Abundance) = number of individuals of a particular parasite species in a sample of a particular host species/total number of hosts of that species, including both infected and non-infected hosts [following Nava *et al.* (2003)].
I (Infestation number) = number of rodent hosts infected with a particular parasite species.
P (Prevalence) = number of rodent hosts infected with a particular parasite species/total number of rodent hosts examined for that parasite species x 100.
'–' indicates no rodents infected.
[a] MA is calculated based on the total number of parasite individuals/total number of host of that species.
[b] I is calculated based on the total number of infected rodent host of a particular species.
[c] P is calculated based on the total number of infected rodent hosts of a particular species/total number of rodent hosts examined x 100.

Table 2. Single and multiple infections of ectoparasites associated with rodent species from residential areas in western Sarawak. A total of 44 rodents were inspected

Ectoparasites	Infected rodent individuals (ectoparasite individuals recovered)		
	Rattus rattus	*Rattus tiomanicus*	*Sundamys muelleri*
Laelaps echidninus	12(35)	1(1)	3(16)
Laelaps sedlaceki	2(15)	1(3)	
Laelaps nuttalli	2(2)		
Ixodes granulatus	1(2)		
Hoplopleura sp.	1(1)		
Laelaps echidninus & *Laelaps nuttalli*	2(6)		
Laelaps echidninus & *Laelaps sedlaceki*	1(14)	1(6)	
Laelaps nuttalli & *Laelaps sedlaceki*	2(5)		
Haemaphysalis sp. 1 & *Hoplopleura* sp.	1(3)		
Haemaphysalis sp. 1 & *Chigger* sp.		1(4)	
Haemaphysalis sp. 2 & *Hoplopleura* sp. & *Laelaps nuttalli*	1(4)		
Total infected	25(87)	4(14)	3(16)

nutalli, and *Hoplopleura* sp. infestations were only recovered from *R. rattus* in this study. In Peninsular Malaysia, Paramasvaran *et al.* (2009) also detected multiple infestations of *R. rattus* from urban habitats with *L. echidninus*, *L. nuttali* and *Hoplopleura* sp. While this non-native synanthropic rodent species has been intermittently detected and moved across various types of urban, suburban habitats and old-growth forests in Borneo (Wells, 2006), this may increase the chance of interaction with various native rodent species, hence high risk to ectoparasitism.

Five *S. muelleri* were caught at three residential areas in this study, and they were found only parasitized by *L. echidninus*. *S. muelleri* is a native rodent species with occurrence probability from forests to urban habitats (Wells *et al.*, 2014). *S. muelleri* captured from forested areas were commonly co-infested with multiple ectoparasite species including hard ticks, mites, chiggers, and lice (e.g. *I. granulatus*, *Haemaphysalis* sp., *L. sedlaceki*, *L. nuttalli*) (Mariana *et al.*, 2005, 2008; Paramasvaran *et al.*, 2009; Madinah *et al.*, 2013). Little is known about the co-infection on *S. muelleri* in urban habitats. Our study may only provide a partial picture of the ectoparasite infestation characteristics in this rodent species because of the low number of captures.

R. tiomanicus was only found in Serian Ulu Village, the only rural residential area that was surrounded by paddy field, which was an agricultural pest in Peninsular Malaysia (Wood and Fee, 2003). The four captures were either single or multiple-infected with ectoparasite species. One *R. tiomanicus* was also coinfected with *Haemaphysalis* sp. 1 and chigger, whereas the later was seldom reported at paddy fields but urban, forested and coastal areas in Malaysia (Mariana *et al.*, 2005; Paramasvaran *et al.*, 2009).

Two genera of ticks (Ixodidae), namely *Ixodes* and *Haemaphysalis*, were recovered from *R. rattus* and *R. tiomanicus* in this study. It is worth noting that *I. granulatus* was recovered only from one individual *R. rattus* caught at Krusen Kranji Village, a remote rural area surrounded with forests. This hard tick was not found from the same rodent species at other residential areas with higher human population density. Previous ectoparasite studies demonstrated *I. granulatus* was one of the most common species of ticks infesting wild rodents in forests of Malaysia (Mariana *et al.*, 2005; Paramasvaran *et al.*, 2009; Madinah *et al.*, 2011). This tick species can infest both small and larger mammals (Lah *et al.*, 2015). The relatively low prevalence of tick infestation in this study might due to the lack of tick-host interaction or absence among the rodents being examined at the residential areas (Cumming, 2004; Petney *et al.*, 2007). Host behaviours such as social group size (Ezenwa, 2004), habitat (Madinah *et al.*, 2014a) and home range size (Bordes *et al.*, 2009) may play important roles in mediating the exposure of ectoparasites. Microclimate (e.g. relative humidity) differences may directly affect the tick population in the hosts. High abundance of vegetation in forested area usually will have a slightly higher relative humidity compared to urban areas (Laurance, 2004).

Mesostigmatid mites (Laelaptidae) were the predominant ectoparasite species recovered from synanthropic rodents in this study. This is consistent with studies by Paramasvaran *et al.* (2009) and Madinah *et al.* (2011) where mites have the highest infestation rate compared to other ecto-parasites. To note, *L. echidninus* was the most abundant ectoparasite species recovered from all rodent species in this study, comparable with the studies by others (Zahedi *et al.*, 1996; Thanee *et al.*, 2009). Previous studies showed aggregated distribution of *L. echidninus* on their hosts and represented the lowest host-specificity ectoparasite recovered from a wide niche range (Guo, 1997, 1998). *L. echidninus* was found infesting mostly on commensal and wild rodents, but sometimes also cause skin irritation to man (Azad, 1986). *L. echidninus* usually infest on their rodent host at night, where they hide around their host nesting and resting places (Mullen and O'Connor, 2002). In forested habitats, *L. echidninus* has been found to infect many other native rodent species (Zahedi *et al.*, 1996; Chuulun *et al.*, 2005; Mariana *et al.*, 2005, 2008; Paramasvaran *et al.*, 2009). In this study, rodents infested with *L. echidninus* came from three residential areas that were all in the vicinity to cultivated areas. Environmental factors such as fruiting or harvesting season may be directly related with the foraging success of rodents at the community level due to resource availability, and this might induce the mite infestation to rodents (Wells *et al.*, 2014).

Hoplopleura sp. was the only sucking louse species recovered from three individuals of *R. rattus* from Sebayor Village, Kota Samarahan. *Hoplopleura* spp. were commonly infesting *Rattus* spp. all over the world, mainly tropical, subtropical and warm temperate regions (Durden, 2001; Kim, 2006). In general, sucking lice species that infest on rodent host are usually highly specific, where they parasitize on single host species, or closely related groups of rodent hosts, while parasitism on heterospecific hosts are uncommon. This might be one of the reasons why only single species of louse (*Hoplopleura* sp.) was recovered from single host species (*R. rattus*). To note, lice infestations have been previously reported in domestic animals such as cats and dogs (Wiebe, 2015), as well as farm animals (George *et al.*, 1992).

This is the first documentation of ectoparasites infestation and species diversity from synanthropic rodents at residential areas in western Sarawak, Malaysia. Little is known about the micro-habitats and ecological interactions between ectoparasite species and rodent hosts. The multiple infestations of *R. rattus* and *R. tiomanicus* with several ectoparasite species on the same rodent individuals recorded in this study warrant further investigations. As certain ticks and mites recorded in this study are important arthropod vectors of pathogens that can cause diseases in animals and human, there is a need to further study the pathogens associated with ectoparasites recovered from these synanthropic rodents.

This study was partially supported by the Malaysia Ministry of Education under Niche Research Grant Scheme (NRGS/1088/2013(02)). Permission for rodents sampling was obtained from the head of villages and research permits were obtained from Sarawak Forestry Corporation (SFC) and Sarawak Forestry Department (SFD) [NCCD.907.4.4 (Jld.12)-12]. Gratitude to the Department of Zoology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, for technical assistance, transportation support and administrations provided. Special thanks go to Mr. Lee Wei Bin for his field and technical assistance.

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