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Prevalence of on-host ticks (Acari: Ixodidae) in small mammals collected from forest near to human vicinity in Selangor, Malaysia

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Abstract

Ticks are important vectors that transmit a variety of pathogenic microorganisms known to be medically important worldwide. Many vertebrate groups have become host to this organism, and their presence and abundance are an indicator of the condition of both host and its habitat. This study was conducted to determine tick's infestation and its prevalence on small mammal's residing in the recreational forests (RF) and semi-urban (SU) residential areas which have encountered Leptospirosis outbreak and cases in Hulu Langat, Selangor Malaysia. Trapping of the small mammals involved deploying two hundred cage traps in a systematic one-hectare plot (100 m x 100 m), as well as along the stream and forest trails at random. Ticks were extracted from the captured individual hosts. Identification of the tick species was performed based on morphological features and molecular approach using 16S rDNA and COI (cytochrome oxidase subunit I) genes. A total of 278 individuals of small mammals belonging to 15 species (13 Rodentia, 1 Scandentia and 1 Insectivora) were captured in the study areas. From these, 34 individuals from eight small mammal species were infested with ticks. The most infested host species was Muller's giant Sunda rat (*Sundamys muelleri*) with 5.80% (n=16). Ticks prevalence was slightly higher in RF with 6.40% (n=18) compared to SU with 5.80% (n=16). A total of 107 adult ticks (103 female and 4 male) were collected from the infested host. *Ixodes granulatus* was the most dominant tick species encountered (70.40%, n=85), followed by *Dermacentor* sp. (18.60%, n=20), while *Amblyomma* sp. was the least abundant (2%, n=2). This study provides information on tick species present and tick burden on small mammal hosts within the study areas. Our findings suggest that the visitors to the recreational forests and the residents of the semi-urban area were not only exposed to Leptospirosis bacteria but also tick bites and potentially tick-borne disease, therefore, precaution should be taken to avoid contact with small mammal hosts.

Keywords: Hard-ticks, Rodents, *Ixodes granulatus*, Small mammals, Infectious diseases, Tick-borne diseases

Introduction

Many human and animal infectious diseases are transmitted through arthropod vectors such as lice, fleas, and ticks (Jongejan & Uilenberg 2004). Disease transmission is largely affected by environmental conditions (Rogers & Randolph 2006). In the last few decades, tick-borne diseases have become a growing concern and numerous studies have been conducted to identify tick-borne pathogens as well as their hosts especially in Asian countries such as China and Taiwan (Niu *et al*. 2011; Chao *et al*. 2012; Wu *et al.* 2013). Ticks are among the most widely distributed blood feeding

arthropods and vectors of various pathogens (Jongejan & Uilenberg 2004). One of the most important tick-borne disease is the Lyme disease (Lyme borreliosis) caused by the spirochete bacteria *Borrelia burgdorferi*, which is typically transmitted by ixodid ticks (Burgdorfer *et al*. 1982; Morshed *et al*. 2005; Chao *et al*. 2012). The hard ticks of *I. granulatus*, *H. longicornis*, and *H. bispinosa*, for instance, were suggested as the principal vectors for the transmission of *B. burgdorferi* spirochetes in China (Chu *et al*. 2008).

Ticks have a diverse range of vertebrate hosts from which they feed on, affecting 240 species of wild and domesticated animals, including many species of birds and reptiles (Greenfield 2011). Besides, ticks have the ability to survive in different habitat types and parasitize variety of hosts, making them a disease vector of interest in recent years (Greenfield 2011). Host-parasite associations for ticks range across a spectrum, with some ticks species being host specialists or generalists. Many species of ticks are opportunistic feeders, in which they feed on any animals, without any evident of host selection (Krasnov *et al*. 2004; Brunner *et al.* 2008). Ticks and host associations are driven by several factors such as tick life history, climate and host factors, including sex, age, and behaviour (Randolph & Storey 1999).

Generally, small mammals such as rodents and shrews are recognized as key hosts for many tick species and regarded as important vector in the transmission of several tick-borne pathogens (Manneli *et al.* 2012; Ostfield *et al.* 2014; Cull *et al.* 2017). Small mammals commonly exist in high densities in forest habitats and the utilization of small mammals by ticks has been previously investigated in forests (Dantas-Torres *et al*. 2012).

Understanding of the host-parasite relationship is crucial, not only for the ecology of both hosts and parasites but also for its importance in public health due to the potential for disease transmission. In years 2016 to 2017, an epidemiological study was undertaken to investigate leptospirosis cases in a number of recreational forests and semi-urban residential areas in Hulu Langat, Selangor state, Malaysia, which involved the capture of small mammals such as wild rodents as potential carriers of *Leptospira* bacteria. These current study leverages on the capacity of the above study to investigate the host-parasite associations within small mammal communities in the affected areas. The small mammal species inhabiting forests near human vicinity, along with the infesting tick species, was identified in this study. We hypothesize that the composition of the small mammal community and the associated ticks would vary by different habitat areas. In addition, prevalence of tick infestation among these small mammal hosts could give information on which host species and which habitat type is prone to tick infestation, so specific strategies to control tick population can be implemented.

Materials and methods

Study areas

The study was conducted in the forest near human vicinity as part of a larger epidemiological study for leptospirosis cases in Hulu Langat, Selangor Malaysia. These areas can be categorized as recreational forest (RF) and semi-urban residential areas (SU) which are located adjacent to the forest. These site categories were represented by two locations each. The recreational forest sites receive a huge number of visitors daily as it provides space for outdoor activities such as hiking, swimming, and picnic. Thus, they are attractive to wild rodents due to the dumping of garbage and food leftovers from visitors, which become their food source. Meanwhile, the selected semi-urban residential sites have many houses scattered in a village-like fashion. The nearby forest supports the livelihoods of the semi-urban households by providing vital ecosystem services in the forms of water catchments. The households receive water directly from the forest, subjecting them to the risks of leptospirosis.

Small mammals sampling

Two hundred cage traps were used to capture small mammals in each habitat. Two sampling methods were deployed: (1) a systematic one-hectare plot (100 m x 100 m) which consists of 100 traps each, (2) random sampling where 100 traps were placed randomly along the stream or forest trails. Cage traps were baited with oil palm fruits, sweet potatoes with peanut butter, salted fish or a special type of aromatic banana as this was shown to be effective to attract small mammals such as rodents, squirrels and tree shrews (Shahrul *et al.* 2008). Trappings was checked once daily in the morning for five consecutive nights. Trapped animals were brought to the research station prior to sample collection. Morphological measurements were taken and identification from physical appearance was based on Francis (2008). Before handling, all animals were anesthetized with an intramuscular injection of Zoletil® 50 as previously described (Rivas *et al*. 2015) and after gaining consciousness, they were released back at their captured sites. Before releasing, the animals were marked with numbered ear tags. Trapping and handling procedures for small mammals have been approved by the animal research ethics committee at Universiti Kebangsaan Malaysia (FST/2016/ SHUKOR/18-MAY/750-MAY-2016-SEPT.-2018-AR-CAT2).

Ticks sampling and identification

Each host was carefully combed for ticks, which were collected with fine forceps before storing individually in labeled cryo-vials containing 70% alcohol for preservation. Ticks were first identified morphologically up to genus's level, and classified based on the developmental stage (larvae, nymph, or adult) and sex, following previously published taxonomic keys (Yamaguti *et al*. 1971 and Walker *et al*. 2003). Tick species was further confirmed by the molecular approach. Tick samples were first washed thrice in 70% ethanol followed by sterile deionized water to remove environmental debris and disinfect the surface (Capri *et al*. 2011). The extraction of DNA was performed using the MN-NucleoSpin® Tissue kit (MN Germany). Polymerase chain reaction (PCR) was performed to amplify the partial 16S rDNA and COI (cytochrome oxidase subunit I) genes as described by Black & Piesman (1994) and Folmer *et al*. (1994) for the confirmation of tick species.

Data analysis

In an epidemiological study, prevalence is a measurement of all individuals affected by the disease at a time (Shields & Twycross 2003). Prevalence gives a figure for a factor at a single point in time (Jekel *et al*. 2001). In this study, the overall prevalence of infested small mammals and prevalence of infested small mammals with ectoparasites was calculated.

Prevalence of infested host was calculated by using the formula below:

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

Next, data were checked for normality test to determine whether the sample data fits a standard normal distribution or not. Non-parametric Man-Whitney U test was performed to identify the differences in prevalence of tick's infestation in relation to site category (RF and SU). Next, one sample Chi-square analysis was performed to determine the association between host gender and tick's infestation. One-way analysis of variance (ANOVA) was used to determine whether there are any differences between tick species in different host species.

Sequencing alignment and phylogenetic analysis

Selected DNA sequences representative of tick species, animal host species in which the tick was collected from, and the sampling location, were used in a BLAST search (http:// www.ncbi.nlm.nih.gov/BLAST) and aligned with other tick reference sequences that were available

in the GenBank. Analysis of multiple sequence alignment of 16S rDNA and COI sequences were generated with Muscle software tool in MEGA (Molecular Evolutionary Genetic Analysis) software version 7 as described by Kumar *et al*. (2016). The alignment and trimming process was manually edited to remove any alignment errors and exported as MEGA and FASTA format files. Phylogenetic tree was performed by neighbour-joining (NJ) based on Kimura two-parameter model (K2) to infer the relationships within and between tick species. Pairwise sequence comparison was performed using MEGA software version 7.

Results

Prevalence of ticks in each host species in different study sites

A total of 278 small mammals belonging to 15 species from two different habitats were captured and examined for tick's infestation. Table 1 listed the number of individuals examined for each host species, and the number of individuals infested with ticks. 15 host species were examined in this study, namely *Sundamys muelleri* (Muller's giant Sunda rat), *Maxomys whiteheadi* (Whitehead's maxomys), *Leopoldamys sabanus* (Long-tailed giant rat), *Maxomys rajah* (Rajah maxomys), *Maxomys surifer* (Red spiny maxomys), *Rattus norvegicus* (Norway rat), *Rattus rattus* (House rat), *Rattus tiomanicus* (Malaysian wood rat), *Sundasciurus lowii* (Low's squirrel), *Callosciurus notatus* (Inornate squirrel), *Callosciurus caniceps* (Grey-bellied squirrel), *Sundasciurus lowii* (Low's squirrel), *Lariscus insignis* (Three striped ground squirrel), *Tupaia glis* (Common treeshrew) and *Suncus murinus* (House shrew).

TABLE 1. Prevalence of ticks in each small mammals' species in all study areas.

Host species	Examined	Infested	Prevalence (%)	Ticks Load
Sundamys muelleri	42	16	5.80	75
Maxomys whiteheadi	59	4	1.40	8
Rattus tiomanicus	57	2	0.70	6
Tupaia glis	31	6	2.20	6
Rattus rattus	39	2	0.70	4
Maxomys rajah	8	2	0.70	4
Leopoldamys sabanus	11	1	0.35	3
Sundasciurus tenuis	3	1	0.35	1
Callosciurus notatus	12	0	0	0
Rattus norvegicus	4	0	0	0
Sundasciurus lowii	4	0	0	0
Suncus murinus	3	0	0	0
Callosciurus caniceps	2	0	0	0
Maxomys surifer	2	0	0	0
Lariscus insignis	1	0	0	0
Total	**278**	**34**	**12.20**	**107**

The most abundant host species captured were *Maxomys whiteheadi* (21%, n=59), *Sundamys muelleri* (15%, n=42) and *Rattus rattus* (14%, n=39). The least abundant host species were *Suncus*

murinus (1%, n=3), *Callosciurus caniceps* (0.7%, n=2), *Maxomys surifer* (0.7%, n=2 and *Lariscus insignis* (0.3%, n=1). Among these, 34 individual hosts from eight species were infested with ticks, which equals to 12.20% prevalence of ticks in all the small mammals captured here. Muller's giant Sunda rat (*Sundamys muelleri*) was the host species with the highest infestation, in which 16 out of 42 individuals were infested with ticks (5.80%). This was followed by *Tupaia glis* (2.20%), *Maxomys whiteheadi* (1.40%), *Rattus tiomanicus* (0.70%), *Rattus rattus* (0.70%), *Maxomys rajah* (0.70%), *Leopoldamys sabanus* (0.35%) and *Sundasciurus tenuis* (0.35%). There were no ticks observed in the following seven small mammal species: *Rattus norvegicus*, *Suncus murinus, Maxomys surifer*, *Callosciurus notatus, Sundasciurus lowii, Callosciurus caniceps,* and *Lariscus insignis*.

From 278 small mammals captured, SU recorded 189 individuals small mammals (12 species) compared to RF with 89 individuals (11 species). The prevalence of infested small mammals was higher in RF with 6.40% (n=18) compared to SU with 5.80% (n=16) as shown in Table 2. However, there was no significant difference in prevalence of tick's infestation in relation to site category (Man-Whitney U Test, U=3.00, N=4, P=0.102). Species representation of small mammals was similar for both sites, except *Leopoldamys sabanus* which was found only in SU and *Sundasciurus tenuis* in RF.

TABLE 2. Prevalence of ticks for each small mammal species according to study areas.

Site	No. of host	Host species	Infested	Prevalence (%)
RF	1	*Maxomys whiteheadi*	1	0.35
	2	*Sundamys muelleri*	12	4.30
	3	*Rattus rattus*	1	0.35
	4	*Rattus tiomanicus*	1	0.35
	5	*Maxomys rajah*	1	0.35
	6	*Tupaia glis*	1	0.35
	7	*Sundasciurus tenuis*	1	0.35
		Total	18	6.40
SU	1	*Maxomys whiteheadi*	3	1.01
	2	*Sundamys muelleri*	4	1.44
	3	*Rattus rattus*	1	0.35
	4	*Rattus tiomanicus*	1	0.35
	5	*Maxomys rajah*	1	0.35
	6	*Tupaia glis*	5	1.80
	8	*Leopoldamys sabanus*	1	0.35
		Total	16	5.80

RF: Recreational forests
SU: Semi-urban residential areas

Identification of tick samples

A total of 107 adult ticks of which 103 were females and only four males were collected from the 34 infested small mammals belonging to eight host species. External morphological examination of tick samples identified only one genus (*Ixodes*) and other ticks identified as unknown due to lack of morphology features such as missing part of mouthpiece, legs and fully engorged with blood. In order to confirm the genetic identities of tick species in Selangor, all tick sample of 16S rDNA and

COI sequences were aligned and compared with the downloaded sequences from the GenBank. BLAST search result revealed three different tick species which were *Ixodes granulatus* (n=85), *Dermacentor* sp. (n=20) and *Amblyomma* sp. (n=2).

TABLE 3. List of tick samples, host species and BLAST results from the GenBank.

Sample ID Code	Host species	Tick species (Morphology)	Tick species (Molecular)	
			% Similarity with GenBank (16S rDNA)	% Similarity with GenBank (COI)
112-SU003	*S. muelleri*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
220-SU003	*S. muelleri*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
278-SU001	*L. sabanus*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
283-SU001	*M. whiteheadi*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
243-RF001	*M. rajah*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
289-RF001	*S. muelleri*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
289-RF010	*S. muelleri*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
294-RF002	*S. muelleri*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
351-RF002	*R. rattus*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
351-RF003	*R. rattus*	*Ixodes* sp.	*I. granulatus* (99)	*I. granulatus* (94)
275-SU002	*S. muelleri*	Unknown	*Dermacentor* sp. (99)	*Dermacentor* sp. (87)
283-SU002	*M. whiteheadi*	Unknown	*Dermacentor* sp. (100)	*Dermacentor* sp. (87)
289-RF005	*S. muelleri*	Unknown	*Dermacentor* sp. (99)	*Dermacentor* sp. (87)
365-RF001	*S. muelleri*	Unknown	*Dermacentor* sp. (100)	*Dermacentor* sp. (87)
122-SU001	*T. glis*	Unknown	*Amblyomma* sp. (97)	*Amblyomma* sp. (91)

RF: Recreational forests
SU: Semi-urban residential areas

Phylogenetic analysis

Fifteen individuals tick samples from six host species were selected for the phylogenetic analysis. The partial 16S rDNA and COI gene sequences showed 97%–100% and 87%–94% similarities respectively to existing tick sequences in NCBI GenBank (Table 3). Neighbour-joining (NJ) tree was generated using both sequences of tick samples in this study and other reference sequences from the GenBank. NJ trees based on partial 16S rDNA and COI genes (Figures 1 and 2) showed the formation of different major clades of *Ixodes granulatus*, *Dermacentor* sp., *Amblyomma* sp., and separation from the outgroup (*Argas persicus*). For both 16S rDNA and COI trees, all *I. granulatus* ticks in this study formed a monophyletic clade separated from the *I. granulatus* ticks from China and Japan (bootstrap value = 100%). Pairwise sequence comparison of all the *I. granulatus* ticks in this study showed intraspecific variation of 0% to 0.03% and 0.02% to 0.25% for the partial 16S rDNA and COI sequences respectively. For *Dermacentor* sp. ticks, 283-SU002 and 289-RF005 were clustered with *Dermacentor atrosignatus* from Thailand and were separated from 275-SU002 and 365-RF001 in the NJ tree based of 16S rDNA (Figure 1, bootstrap value = 100%). Similar clustering of 283-SU002 and 289-RF005 was observed in the COI NJ tree (Figure 2), which was separated from 275-SU002 and 365-RF001 (bootstrap value = 68%). The samples here formed a separate clade from *Dermacentor silvarum* from China (Figure 2, bootstrap value = 100%).

Pairwise sequence comparison for 283-SU002 and 289-RF005 showed 0% and 0.09% dissimilarity for the partial 16S rDNA and COI sequences respectively. There were more dissimilar to 275-SU002 and 365-RF001 for both genes (0.6% for 16S rDNA and 0.16% to 1.7% for COI). The *Amblyomma* sp. tick in this study, 122-SU001, was separated from *Amblyomma testudinarium* from Japan and China in 16S rDNA and COI NJ trees respectively (Figure 1, bootstrap value = 88%, and Figure 2, bootstrap value = 99%).

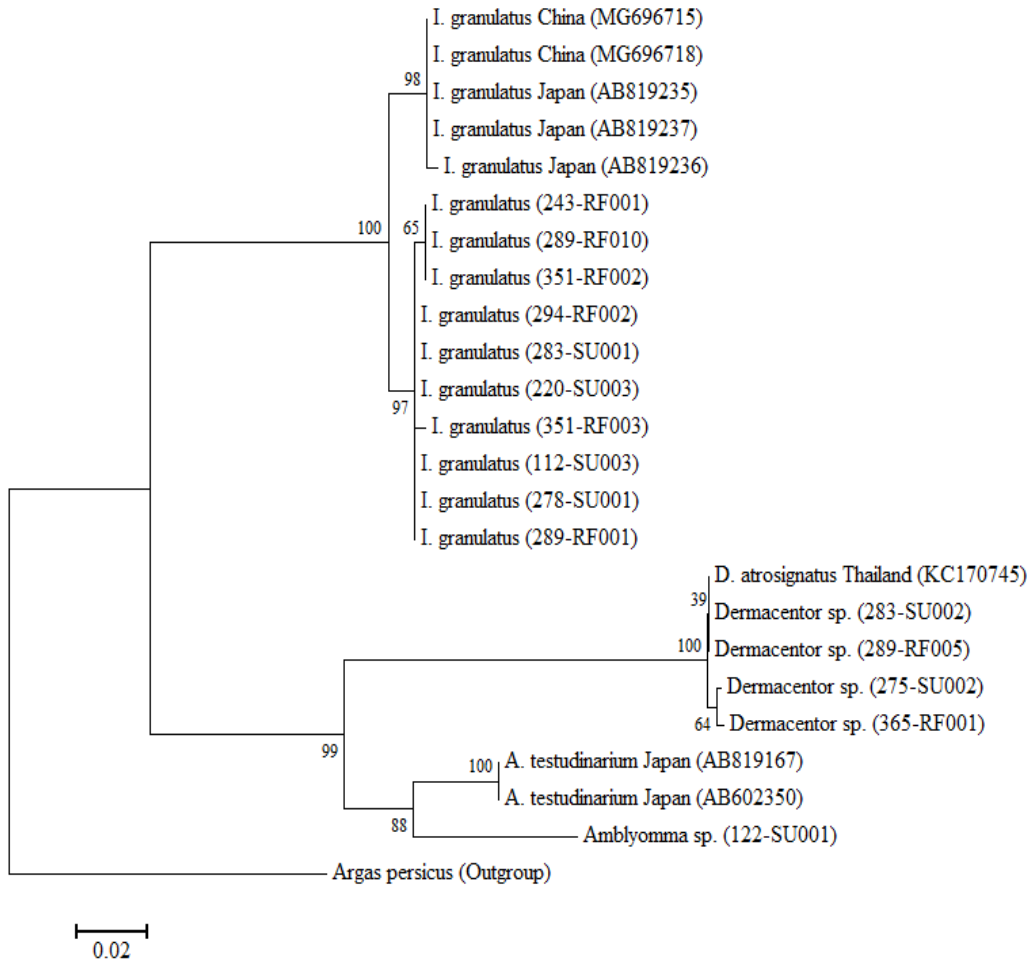

FIGURE 1. Phylogenetic relationships of 15 mitochondrial 16S rDNA genes of *Ixodes* sp., *Dermacentor* sp., and *Amblyomma* sp., rooted with the reference sequences (including 1 outgroup) available in the GenBank. The tree was constructed and analysed with the neighbour-joining method with 1000 bootstrap replications.

Prevalence and intensity of tick species in different study sites

From this study, small mammals captured were infested by three different tick species (Table 4) namely *Ixodes granulatus* with 79.40% (n=85), *Dermacentor* sp. with 18.60% (n=20), and *Amblyomma* sp. with 2% (n=2). The number of ticks per host ranged from 1 to 17. In RF, a total of 59 ticks were collected of which were *Ixodes granulatus* with 48.60% (n=52), and *Dermacentor* sp. with 6.54% (n=7) Meanwhile, in SU, a total of 48 ticks were collected which were *Ixodes granulatus* with 30.84% (n=33), *Dermacentor* sp. with 12.15% (n=13), and *Amblyomma* sp. with 1.87% (n=2).

The most common and abundant tick species in both areas was *Ixodes granulatus* (n=85). It was found in seven out of eight infested host species (*Maxomys whiteheadi, Maxomys rajah, Rattus rattus, Rattus tiomanicus, Sundamys muelleri, Leopoldamys sabanus* and *Tupaia glis*). There was a significant difference of *Ixodes granulatus* in different host species (one-way ANOVA, F=4.729, df=7, P=0.039). From the results, *Sundamys muelleri* harbours the highest infestation of *Ixodes granulatus* (n=62) compared to other tick species. Meanwhile, *Dermacentor* sp. was found on six host species (*Maxomys whiteheadi, Sundamys muelleri, Maxomys rajah, Sundasciurus tenuis, Tupaia glis,* and *Rattus rattus)* whereas *Amblyomma* sp. was found only on *Tupaia glis.* One-way ANOVA showed that there was no significant difference of *Dermacentor* sp. and *Amblyomma* sp. in different host species respectively (F=2.082, df=7, P>0.05) and (F=0.735, df=7, P>0.05). In addition, eight individuals of small mammals from three species (*Sundamys muelleri, Maxomys whiteheadi,* and *Maxomys rajah*) were found co-infested with *Ixodes granulatus* and *Dermacentor* sp.. In addition, all collected ticks found were adult females with 96%, (n=103) and only four males. These four males were found while mating with the females on the host.

TABLE 4. Host species, ticks load and a number of tick individuals according to species.

Study sites	Host species	Ticks Load	No. of tick individuals		
			Ixodes granulatus	*Dermacentor* sp.	*Amblyomma* sp.
RF	*Sundamys muelleri*	45	42	3	0
	Maxomys whiteheadi	2	1	1	0
	Rattus tiomanicus	5	5	0	0
	Rattus rattus	3	3	0	0
	Maxomys rajah	2	1	1	0
	Tupaia glis	1	0	1	0
	Sundasciurus tenuis	1	0	1	0
	Total	**59**	**52**	**7**	**0**
SU	*Sundamys muelleri*	30	20	10	0
	Maxomys whiteheadi	6	5	1	0
	Rattus rattus	1	0	1	0
	Rattus tiomanicus	1	1	0	0
	Maxomys rajah	2	2	0	0
	Tupaia glis	5	2	1	2
	Leopoldamys sabanus	3	3	0	0
	Total	**48**	**33**	**13**	**2**

RF: Recreational forests

SU: Semi-urban residential areas

Host sex and tick's infestation

From this study, the sex ratio of small mammals captured was almost similar (142 males/136 females). There is a significant association between host gender and tick's infestation (one-sample Chi-square test, χ^2= 4.903, df= 1, P= 0.027,). Males harboured almost twice number of individual ticks with 66.40%, (n= 71) compared to females with 33.60%, (n= 36).

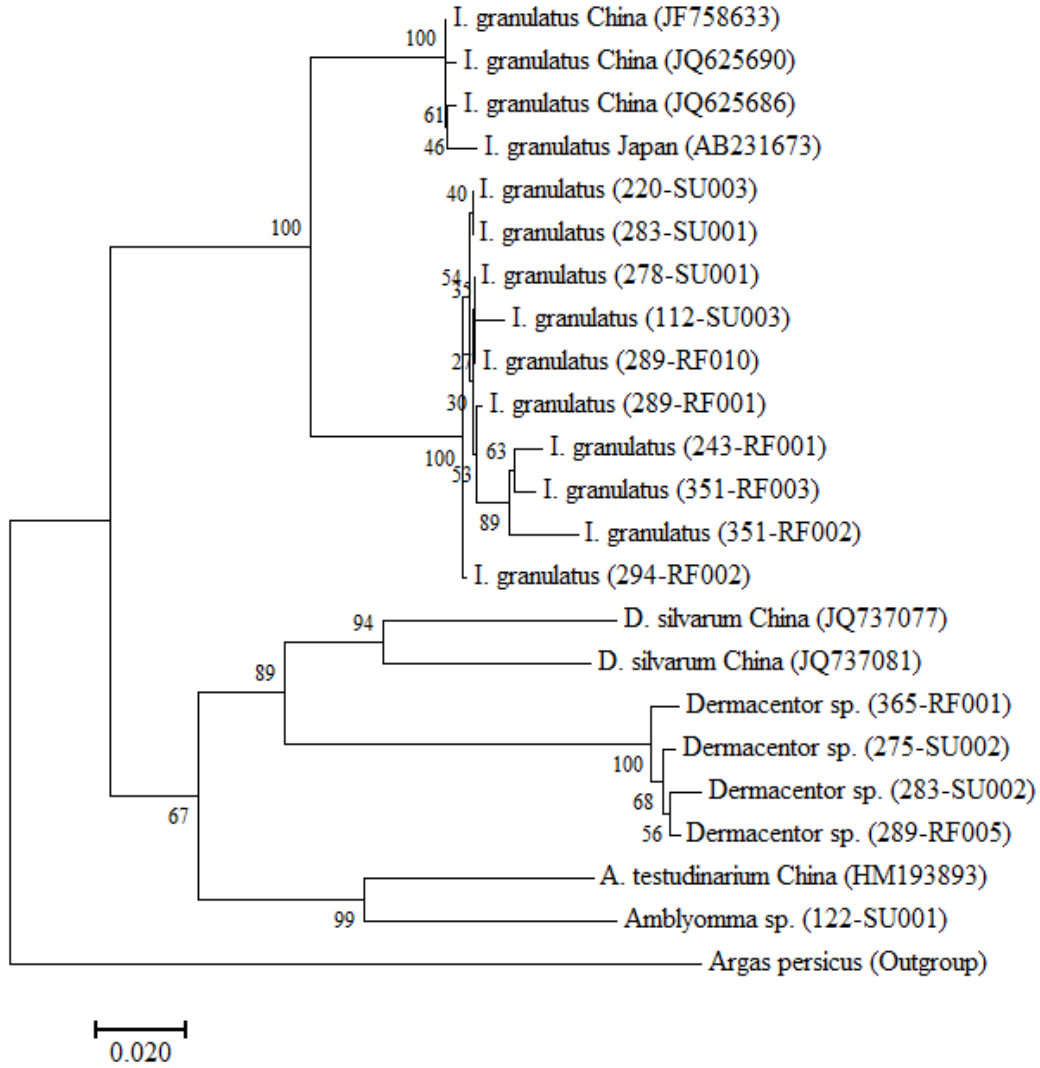

FIGURE 2. Phylogenetic relationships of 15 mitochondrial cytochrome oxidase subunit I (COI) genes of *Ixodes* sp., *Dermacentor* sp., and *Amblyomma* sp., rooted with the reference sequences (including 1 outgroup) available in the GenBank. The tree was constructed and analysed with the neighbour-joining method with 1000 bootstrap replications.

Discussion

This study reported the prevalence of ticks on small mammals captured in recreational forests and semi-urban residential area. Our findings showed that the small mammals trapped in the recreational forests are highly infested with ticks. Tick prevalence was almost two times higher in the recreational forests compared to the semi-urban areas. The forested environment is likely to provide the necessary biotic and abiotic requirements to support a high host density and the optimal microclimatic conditions to sustain the tick life cycle (Gray 1991; Gray 1998; Barandika *et al*. 2007). Most of the host species captured in recreational forests were forest species, which are known to host high abundance of ticks (Mihalca & Sandor 2013). The findings are consistent with a study by

Madinah *et al.* (2014), in which Scandentia (Tupaiidae) have lower ectoparasite loads as compared to Rodentia (Scuiridae and Muridae). The differences in the ectoparasite load may be explained by the differences in the behaviour, such as the irregular usage of nest by Scandentia, or biology, in which the fur of Scandentia provides less optimal microhabitat for ectoparasites (Shabrina & Rafaee 1993). Additionally, host-seeking behaviours in small mammals, including burrowing or nesting, could expose selected small mammals to more ectoparasites than others (Parola & Raoult 2001). We found that ticks were absent from six host species (*Rattus norvegicus, Maxomys surifer, Callosciurus notatus, Sundasciurus lowii, Lariscus insignis* and *Suncus murinus*). The absence of ticks on these host species sampled may be due to a very low infestation rate, or the ecology of the host species does not encourage tick infestation (Paramasvaran *et al*. 2009).

Similar to study by Paramasvaran *et al.* (2009), we found that S*undamys muelleri* was the most infested small mammal host species. This species was always found deep inside the forest forest edge, near streams and human modified landscapes (Payne *et al*. 2014). To date, there is still lack of information on the behaviour of this rodent species, especially its ranging and nesting patterns, although numerous studies have reported their wide distribution across Malaysia and the Southeast Asian region (Lynam & Billick 1999; Esselstyn *et al*. 2004; Paramasvaran *et al*. 2005; Charles & Ang 2010). Loong *et al.* (2018) has successfully cultured an opportunistic bacterial pathogen (*Paenibacillus lautus*) from ticks collected from this species. These opportunistic bacteria may be transmitted to humans and other host through tick bites and cause disease. Finding from this study is significant as it suggests that this species could sustain the ectoparasites within its home range and potentially spreading them to new locations. This in turn may result in the spread of potential ectoparasite-associated disease including tick-borne pathogens into new areas. This finding is similar to the study by Medlock *et al*. (2013), who found that the expansion of roe deer contributed to the spread of ticks into new geographical areas. Therefore, there is a necessity to further investigate the ranging behaviour, both in pristine forests and in disturbed areas due to anthropogenic activities, as well as to understand the potential role of *Sundamys muelleri* in sustaining and dispersing the ectoparasites it carries.

We also found that males of small mammal's host harboured a higher number of ticks compared to female. Males are likely to have bigger home range and travel further distances compared to female, increasing their chances of being exposed to tick infestation (Bantihun & Bekele 2015; Cull *et al*. 2017). In addition, our results showed that the majority of the ticks collected were adult females, whereby the males were found attached with the female ticks. Since male *Ixodes* ticks were not known to engorge as they typically do not feed on host (Durden *et al.* 2018), the likelihood of finding them on the host may be lower than female *Ixodes* ticks. Studies by Durden *et al.* (2018) also recorded higher number of females (123 individuals) than males (9 individuals) in which most of the recorded males had apparently been mating with females that were attached to the hosts.

Based on the morphological features, we were only able to identify one genus (*Ixodes*). However, the genetic identities of the ticks were further confirmed by using molecular approach using two different molecular markers. Sequence and phylogenetic analyses based on partial 16S rDNA and COI genes confirmed the presence of *I. granulatus* in this study. The *I. granulatus* in this study showed low intraspecific genetic variation for both partial 16S rDNA and COI sequences, consistent with previous findings on Malaysian *I. granulatus* (Ernieenor *et al*. 2016). However, the molecular marker sequences from the previous study was not publicly available, therefore we are unable to investigate the intraspecific variations between *I. granulatus* from this and the previous study. Separation of the *I. granulatus* in the phylogenetic trees appeared to be influenced by geographical origins, as ticks in our study formed a distinct clade from China and Japan specimens. Analyses of the molecular markers also enabled us to identify *Dermacentor* sp. and *Amblyomma* sp. ticks in this study. The partial 16S rDNA and COI of two of the *Dermacentor* sp. here appeared to

be very similar to the sequences of a *D. atrosignatus* from Thailand, suggesting the possibility of them being the same species. We were unable to identify the species of the other two *Dermacentor* sp., as well as the single *Amblyomma* sp. here, using the molecular markers due to the lack of reference sequences for tick species from this region. This implies there is a need to establish a database of molecular markers for the various tick species in the region for the benefit of research into ticks and tick-borne diseases here.

Ixodes granulatus was the most common tick species found in both study areas as this species was known to infest mammalian hosts such as rodents, and shrews (Nadchatram 2008; Chao *et al*. 2009; 2011; Madinah *et al*. 2011; 2013; Ernieenor *et al*. 2016). *I. granulatus* have been reported to host and possibly transmit a number of tick-borne pathogens, including *Rickettsia* and *Borrelia* (Kollars *et al*. 2001; Graves & Stenos 2003; Chao *et al*. 2010). The larvae of *I. granulatus* were also known to be human-biting, suggesting a potential risk of disease transmission to humans (Paperna 2006). It is currently unknown if the *I. granulatus* observed in the study areas are able to transmit any disease agents; further studies will be required to reveal any disease agents that may be vectored by ticks in these areas. Other adult tick species identified were *Dermacentor* sp. and *Amblyomma* sp. However, *Dermacentor* sp. and *Amblyomma* sp. are more likely to parasitize medium or large-sized mammals, such as wild boars, therefore they may not be commonly observed on rodents (Khoo *et al.* 2017).

The findings from this study suggest that visitors to the recreational forests and the residents of the semi-urban residential area are at risk of being exposed to the small mammals, which were not only potential reservoirs of *Leptospira* bacteria, but also ticks, and possibly other ectoparasites, that they carry. Therefore, there is need for control strategies to control the population of the small mammals, including the improvement hygiene of surrounding environment in order to prevent accumulation of rodents as pest, and ultimately to avoid contact with the small mammal hosts. For the recreational forests, protective measures may be recommended to visitors, including the use of insect repellants or protective clothing, to prevent the incident of tick bites and potential disease transmission. On the other hand, residents of the semi-urban area could be educated about the health risks of tick-borne diseases and encouraged to maintain high hygiene standards to reduce the introduction of wild rodents and the associated ticks into the household. Continuous monitoring of the host-parasite associations of the small mammals will be necessary not only to understand the ecology of the small mammals and ticks, but to reveal the potential risks of tick-borne disease transmission to humans sharing the same habitat with the animals.

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