

Detection of *Bartonella* sp. in ticks and their small mammal hosts in mangrove forests of Peninsular Malaysia

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Abstract. Bacteria of the genus *Bartonella* have been known as emerging zoonotic pathogens for several human diseases including cat scratch disease, Carrion's disease and trench fever. Numerous species of small mammals have been reported to play a role as a suitable reservoir to many pathogenic *Bartonella*. These infections are thought to be transmitted through blood-feeding arthropod vectors such as ticks, fleas and lice. The purpose of this study is to detect the presence of *Bartonella* species from tick samples collected from small mammals in mangrove forests of Peninsular Malaysia. Herein, 38 individual ticks and their small mammals host were evaluated for the presence of *Bartonella* DNA by conventional PCR targeting the 16S rRNA intergenic spacer region (ITS) and partial sequencing of 460 bp from this locus using *Bartonella* genus-specific primers. Two tick individuals from *Dermacentor auratus* and *Haemaphysalis hystricis* collected from *Rattus tiomanicus* (host), were PCR-positive for *Bartonella* DNA amplification. No *Bartonella* amplification was possible in other tick species (*Amblyomma* sp.). Phylogenetic analysis of ITS fragments demonstrated that the sequences from ticks were closely related to *Bartonella phoceensis*, a species that has been reported from black rats (*Rattus rattus*) in Australia. This is the first report of a *Bartonella* bacteria detected in ticks from small mammals in Malaysia. Further research should be warranted to investigate the transmission of *Bartonella* and the potential impact of this zoonotic pathogen in animals and humans as this mangrove ecosystem is significant for local economy and tourism.

INTRODUCTION

The genus *Bartonella* is categorized as Gram-negative bacteria, a slow growing and facultative intracellular parasite which can infect mainly mammalian hosts. *Bartonella* has been isolated from a variety of small mammals. Various blood-feeding arthropods have been implicated as vectors for *Bartonella* transmission between hosts and incidental transmission from reservoir hosts to humans (Fenollar *et al.*, 2005; Kosoy *et al.*, 2008; Kabeya *et al.*, 2010). Currently, a total of 35 *Bartonella* species and three subspecies are recognized in the Taxonomy Database of the National Center for Biotechnology Information (Mardosaitė-Busaitienė *et al.*, 2019). About 23 *Bartonella*

species or subspecies were acknowledged to infect humans and various animal species including domestic cat, dogs, horses, macaques, bats, cattle, and wild animals (Angelakis and Raoult 2014; Cheslock and Embers 2019). Meanwhile, three *Bartonella* species including *B. phoceensis*, *B. rochalimae*, and *B. cooper-splainsensis* particularly infect *Rattus* rats globally (Buffet *et al.*, 2013; Hayman *et al.*, 2013). Several of these infections have been associated with human disease such as *Bartonella bacilliformis* causes Carrion's disease and *Bartonella quintana* acts as the agent of trench fever (Berghlund *et al.*, 2010). The role of ticks as vectors of *Bartonella* is still disputed. Despite multiple reports of molecular detection of *Bartonella* in ticks, it

is still unclear if the bacteria detected in ticks is viable or if ticks act as competent vectors or if they simply acquire bacteria in bloodmeals but do not transmit the bacteria to new hosts. Experimental infection studies are still needed to confirm the vector competence of ticks.

Environmental conditions, plasticity of vector competence, and the occurrence of suitable reservoir hosts affect the prevalence of *Bartonella* species throughout the world (Dybing *et al.*, 2016). *Bartonella* bacteria have been detected in several wild and domesticated mammalian species, including rodents, carnivores, and ruminants (Saisongkorh *et al.*, 2009). High prevalence of *Bartonella* infection has been observed in several rodent species, such as *Rattus rattus* (Ellis *et al.*, 1999), *Rattus norvegicus* (Gundi *et al.*, 2004), *Maxomys* spp. (Tea *et al.*, 2004), *Mus* spp. (Jiyipong *et al.*, 2014), and others (Kosoy and Bai 2019). Nevertheless, we lack complete knowledge of the geographic distribution, host and vector associations, and pathogenicity of some *Bartonella* species.

Few reports are available of the prevalence of *Bartonella* species in Malaysia such as, Hou *et al.* (2018) have detected *Bartonella* spp. from a small flying fox (*Pteropus hypomelanus*) which was identified as *Bartonella* sp. KS013a and KS013b (two different strains) in Peninsular Malaysia. In addition, the DNA of *Bartonella henselae* and *Bartonella clarridgeiae* were detected in fleas from domestic dogs and cats (Mokhtar and Tay 2011), meanwhile *Bartonella bovis* was reported in *Haemaphysalis bispinosa* ticks collected from beef cattle in Peninsular Malaysia (Kho *et al.*, 2015). Up to date however, no reports of *Bartonella* available from ticks collected from small mammals. The objective of this study was to examine the presence of *Bartonella* DNA in ticks and their small mammal hosts in Peninsular Malaysia. This study could contribute to understanding the zoonotic origin of current bartonellosis cases in Malaysia, as well as informing the responsible agencies and communities of the risk of pathogen exposure.

Study areas

The study was carried out in mangroves forests located in three states of Peninsular Malaysia; Terengganu (Kg. Yakyah), Negeri Sembilan (Kg. Sungai Timun) and Perak (Kg. Dew) (Kampung, Kg. = village). These mangroves were situated inland from the coast and facing varying impacts from anthropogenic activities such as in the case of surface run-off pollution from adjacent oil palm plantation and domestic dumps into the river. More details on these anthropogenic activities and the impact on the ecosystem were described in Mohd-Taib *et al.* (2020). These study sites frequently receive visitors for eco-tourism activities, such as firefly-watching, boat riding, fishing, and birdwatching. In addition, these ecosystems still serve as important sea food product for local communities.

Field samplings

Field sampling was conducted from December 2017 until February 2018 for six days at each sampling site. A total of one hundred wired mesh cage traps (28 cm × 15 cm × 12.5 cm) were deployed in each study site. Banana, oil palm fruit and jackfruit were used as bait to lure the small mammals (Bernard *et al.*, 2004). Five stations were established along the river, which stretched as far as one kilometre from the jetty, and cage traps were deployed systematically at each station. Sampling stations were placed in higher elevation areas to minimize the risk of flooding due to tides or nearby rivers, keeping more than 300 m between traps in the riverbank. The traps were placed on the ground, on fallen logs, and tree stumps (Balete *et al.*, 2009; Rickart *et al.*, 2011). All traps were inspected twice a day at 9.00 am and 5.00 pm. Small mammal trapping and handling procedures for small mammals were approved by the animal research ethics committee at Universiti Kebangsaan Malaysia, Malaysia (FST/2020/FARAH SHAFAWATI/16-JAN./1080-FEB.-2020-FEB.-2022-NAR-CAT2).

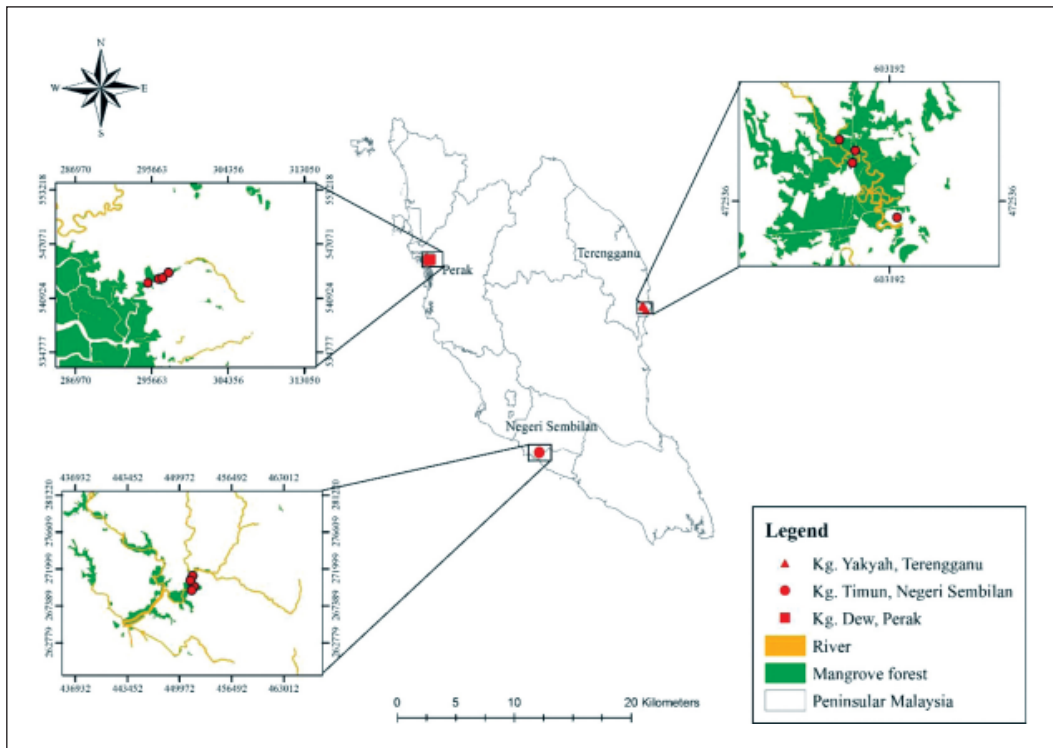


Figure 1. Location of mangrove areas at Kg. Dew, Perak; Kg. Yakyah, Terengganu; and Kg. Timun, Negeri Sembilan in Peninsular Malaysia.

Species identification of small mammals

Every small mammal individual captured was given the anaesthetic injection using Zoletil® 100 (Massolo *et al.*, 2003) with a volume between 0.1 mL and 0.3 mL according to the animal weight. After gaining consciousness, they were given the supplemental food and then released back to their captured sites. The morphological identification for each individual species was performed followed Francis (2008). Standard morphological measurements were recorded including length of tail, whole body, head, ears, and foot patch by using a Vernier calliper and ruler. Furthermore, other physical features such as hair color were noted to help in the identification process. A small cut from the tip of the tail (tissue sample) was taken and placed into 1.5 mL cryovial tube for molecular identification work in order to confirm the species. The tube was stored in a liquid nitrogen tank for transportation to the laboratory after collection in the field.

Collection and morphological identification of ticks

Ticks were collected using sterile stainless-steel tweezers from the skin, ears and fur of the rodents. Individual ticks from the same host were placed in a single micro vial tube, labelled and recorded in the datasheet. The vial tubes were then stored in liquid nitrogen before being processed in the laboratory. Each tick was surface sterilized with 70% ethanol solution prior to observation, followed by washing in sterile distilled water (Carpi *et al.*, 2011). Ticks were then observed under a stereo microscope (Motic SMZ-168 Stereo Zoom microscope) (Motic, Hong Kong) to examine the features and physical characteristics. Individual ticks were morphologically identified to the genus level classified based on the developmental stage and sex using published taxonomic keys (Yamaguti *et al.*, 1971; Baker 1999; Walker *et al.*, 2003), and molecularly identified to the species level by using specific primer.

DNA extraction and PCR amplification of small mammals and ticks

The ticks and tissue sample of hosts were subjected to molecular investigation, after the examination steps. DNA Extraction using HiYield Plus Genomic DNA Mini Kit (Real Biotech Corporation (RBC), Taiwan) was carried out according to the manufacturer's protocol. DNA of ticks and tails tissue of small mammals were extracted by adding 500 μL of phosphate buffer saline (PBS) to the sample. Then, 200 μL of QGT Buffer and 20 μL of Proteinase K was added and the samples were incubated after vortexing at 60°C overnight until the sample lysate became clear. The DNA extracted was stored in -20°C for further analysis.

PCR amplification for tick was performed with a pair of specific primer set designed by Black and Piesman (1994), namely 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') and 16S-1 (5'-CCGGTC TGAAC TCAGATCAAGT-3'). PCR reaction was conducted in a total volume of 35 μL containing 13 μL of 2 \times Taq PCR Master Mix (Lucigen, US), 10 μL of nuclease free water, 1 μL of 0.5 $\mu\text{mol/L}$ of each primer, and 5 μL of DNA template. The PCR was conducted using an Alpha Cyclor PCRmax machine (PCRmax, UK). The PCR amplification program for 16S rDNA gene was performed under the following condition: initial denaturation at 95°C (5 min), followed by 10 cycles of denaturation at 92°C (1 min), 48°C (1 min) and 72°C for (1.5 min). Next, continued with 32 cycles of 92°C (1 min), 54°C (35 sec), 72°C (1.5 min) and followed by final extension at 72°C (7 min).

Meanwhile, for DNA barcoding on small mammal species, the cytochrome c oxidase 1 (COI) gene was amplified by using primers of BatL5310 (5'-CCTACTC RGCCATTTTACCTATG-3') for the forward strand and R6036R (5'-ACTTCTGGGTG TCCAAAGAATCA-3') for the reverse strand (Robins *et al.*, 2007; Ivanova *et al.*, 2012). The PCR protocols used 35 cycles of denaturation, annealing, and elongation: 94°C (30 sec), 55°C (40 sec) and 72°C (90 sec). The purified PCR products were sequenced, and the final alignment contained 697 nucleotides for the COI gene.

In all cases, nuclease free water was used as a negative control to replace the DNA template. After that, the amplified products were visualized in 1.0% agarose gel (TAE buffer) electrophoresis and viewed under an ultraviolet trans-illuminator after staining with gel DNA stain (Florosafe stain). The DNA purification and further sequencing analysis were performed by MyTACG Bioscience Enterprise.

Bartonella ITS (16S-23S ribosomal RNA intergenic spacer) genus PCR amplification

Bartonella genus screening was carried out as previously described (Maggi and Breitschwerdt, 2005). PCR amplification of the *Bartonella* ITS region was performed in all 38 tick samples that were collected from 15 individual rodents, using oligonucleotides with the forward primer BA325s: 5'-CTTC AGATGATGATCCCAAGCCTTCTGGCG-3' and BA1100as: 5'-GAACCGACGACCCCC TGCTTGCAAAGCA-3' as the reverse primer. PCR amplification was performed in a 25 μL final volume reaction containing 12.5 μL of 2 \times Taq PCR Master Mix (Lucigen, US), 0.75 μL of 30 μM of each primer, 6 μL of nuclease free water, and 5 μL of DNA template from each sample. PCR negative control was prepared using nuclease free water. PCR was conducted using an Alpha Cyclor PCRmax machine (PCRmax, UK) under the following conditions: a single hotstart cycle at 95°C for 2 min followed by 55 cycles of denaturing at 94°C (15 sec). Next, annealing procedure was conducted at 66°C (15 sec) and followed by extension at 72°C (18 sec). Amplification was accomplished by an additional cycle at 72°C (1 min), and PCR products were analysed by 1.0% agarose gel electrophoresis and viewed under an ultraviolet trans-illuminator after staining with FloroSafe DNA stain (alternative stain). The DNA purification and further sequencing analysis were completed by MyTACG Bioscience Enterprise. PCR detection of *Bartonella* ITS was also performed on the host individuals, using the tissue samples.

Sequencing and BLAST analysis

The length of few sequences on small mammal tail samples, ticks and *Bartonella* bacteria were trimmed and edited manually to eliminate regions that were only available for some nucleotide sequences. The alignment of sequences was exported as FASTA format files by using MEGA (Molecular Evolutionary Genetic Analysis) software version 7 (Kumar *et al.*, 2016). The obtained results were then compared with other available sequences in the GenBank database using analysis of BLAST (<http://www.ncbi.nlm.gov/BLAST/>) for confirmation of ticks, small mammals and *Bartonella* species. Besides that, this tool revealed the prompt comparison of query sequences with database sequences leading to identification of species (Mitler *et al.*, 2010).

Phylogenetic analysis

The phylogenetic tree for all sequences were achieved by neighbor-joining (NJ) based on Kimura two-parameter model (K2) to conclude the relationships within and between small mammal and tick species. Node support across the phylogenetic tree was estimated using 1 000 bootstrap replicates. *Echinorex gymnura* (GenBank accession no. MK112014) was used as an outgroup for small mammals and *Escherichia coli* strain BzDS03 (JF961337) was used as an outgroup for *Bartonella* bacteria. An examination of the pairwise sequence comparison was generated using MEGA

software version 7. In addition, a total of 10 *Bartonella* sequences that available from GenBank were employed in the phylogenetic analysis as shown in Table 1.

RESULTS

A total of 94 small mammals belonging to six species (*Rattus tiomanicus*, *Rattus* R3, *Maxomys whiteheadi*, *Sundamys muelleri*, *Callosciurus notatus* and *Tupaia glis*), three family (Muridae, Sciuridae and Tupaiidae) and two orders were captured from three different mangrove sites. From that number, 15 individuals host were infested with ticks. Table 2 documented the list of representative small mammal host and the BLAST results from the GenBank. In general, the percent similarity between small mammal sequences and the closest match in NCBI GenBank was between 98% to 100%.

Phylogenetic relationships based on the alignment of CO1 gene were performed to analyse the phylogenetic status of the small mammal sequences in this study (Figure 2). This phylogenetic tree showed that four species from family Muridae were clustered together, which *R. tiomanicus* and *Rattus* R3 shared 100% bootstrap value. Meanwhile, *T. glis* and *C. notatus* were clustered separately with 0.25% of genetic distance value. In this analysis, *Echinorex gymnura* was used as an outgroup and was highly separated from another five major clades.

Table 1. DNA sequences obtained from GenBank employed in the phylogenetic analysis

No	Bacteria species	Locality	GenBank accession no.
1	<i>Bartonella henselae</i>	Brazil	KU291316
2	<i>Bartonella henselae</i>	Malaysia	KT318619
3	<i>Bartonella phoceensis</i>	Australia	KU170606
4	Uncultured <i>Bartonella</i> sp.	Australia	KU240396
5	Uncultured <i>Bartonella</i> sp.	Australia	KU240400
6	Uncultured <i>Bartonella</i> sp.	Australia	KU240402
7	Uncultured <i>Bartonella</i> sp.	Australia	KU240406
8	Uncultured <i>Bartonella</i> sp.	Australia	KU240410
9	Uncultured <i>Bartonella</i> sp.	Australia	KU240414
10	Uncultured <i>Bartonella</i> sp.	Australia	KU240416
11	<i>Bartonella bacilliformis</i>	Korea	JN810857
12	<i>Bartonella</i> sp.	Taiwan	FJ667566

Table 2. List of representative host species and BLAST results from the GenBank

ID code	Mangrove area	Host species (Identification)		Similarity with GenBank
		Morphology	Molecular	
PKY010	Kg. Yak Yah, Terengganu	<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
PKY012		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
PKY015		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
PKY016		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
PKY002		<i>Rattus rattus</i>	<i>Rattus</i> R3	99%
PKY009		<i>Rattus rattus</i>	<i>Rattus</i> R3	98%
PKY014		<i>Rattus rattus</i>	<i>Rattus</i> R3	98%
PKY004		<i>Maxomys whiteheadi</i>	<i>Maxomys whiteheadi</i>	99%
STM003	Kg. Sg. Timun, N. Sembilan	<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
STM026		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
STM032		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	99%
STM001		<i>Tupaia glis</i>	<i>Tupaia glis</i>	99%
STM014		<i>Tupaia glis</i>	<i>Tupaia glis</i>	99%
STM015		<i>Callosciurus notatus</i>	<i>Callosciurus notatus</i>	99%
STM020		<i>Callosciurus notatus</i>	<i>Callosciurus notatus</i>	99%
KDM016	Kg. Dew, Perak	<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	99%
KDM023		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	98%
KDM032		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	99%
KDM042		<i>Rattus</i> sp.	<i>Rattus tiomanicus</i>	99%
KDM006		<i>Sundamys muelleri</i>	<i>Sundamys muelleri</i>	99%
KDM036		<i>Sundamys muelleri</i>	<i>Sundamys muelleri</i>	99%
KDM037		<i>Sundamys muelleri</i>	<i>Sundamys muelleri</i>	98%
KDM038		<i>Sundamys muelleri</i>	<i>Sundamys muelleri</i>	99%
KDM004		<i>Tupaia glis</i>	<i>Tupaia glis</i>	99%
KDM012		<i>Tupaia glis</i>	<i>Tupaia glis</i>	100%

A total of 38 individuals of ticks (all female) were collected from 15 small mammals' individuals. These ticks were identified as *Ixodes granulatus* (n=2), *Amblyomma cordiferum* (n=26), *Dermacentor atrosignatus* (n=5), *Dermacentor auratus* (n=4) and *Haemaphysalis hystricis* (n=1) by a combination of morphological features and molecular analysis confirmed by BLAST. *Bartonella* ITS was detected in 2 of 38 ticks (5.26% prevalence) collected from the small mammal hosts. Both positive ticks were adult stage. These ticks were identified as *Dermacentor auratus* and *Haemaphysalis hystricis*, but other ticks species from various other hosts were not positive for this bacteria. Both positive ticks were collected from *Rattus tiomanicus*, where *D. auratus* were found in Kg. Yak Yah, whereas, *Haemaphysalis hystricis* were found in Kg. Sungai Timun. None of the ticks in Kg. Dew were found to be positive for *Bartonella*. Both *Rattus tiomanicus* host

individuals that produced positive ticks were also PCR-positive for *Bartonella* ITS from tissue samples.

To discover the most closely related homolog for the *Bartonella* species that may be existing in the tick samples, we performed sequence analysis of PCR amplicons obtained from two representative ticks and hosts sample and compared these sequences with the available reference sequences of *Bartonella* in the GenBank database. The ITS gene amplified from these tick specimens using the primers BA325s and BA1100as were found to be approximately 460 bp in length. The partial ITS sequences of *Bartonella* sp. were aligned to those of two tick species from the nucleotide database at NCBI.

Table 3 listed the four ITS sequences of bacterial *Bartonella* submitted to NCBI database. BLAST queries using sequences obtained from ticks and their hosts showed that both sequences were most closely

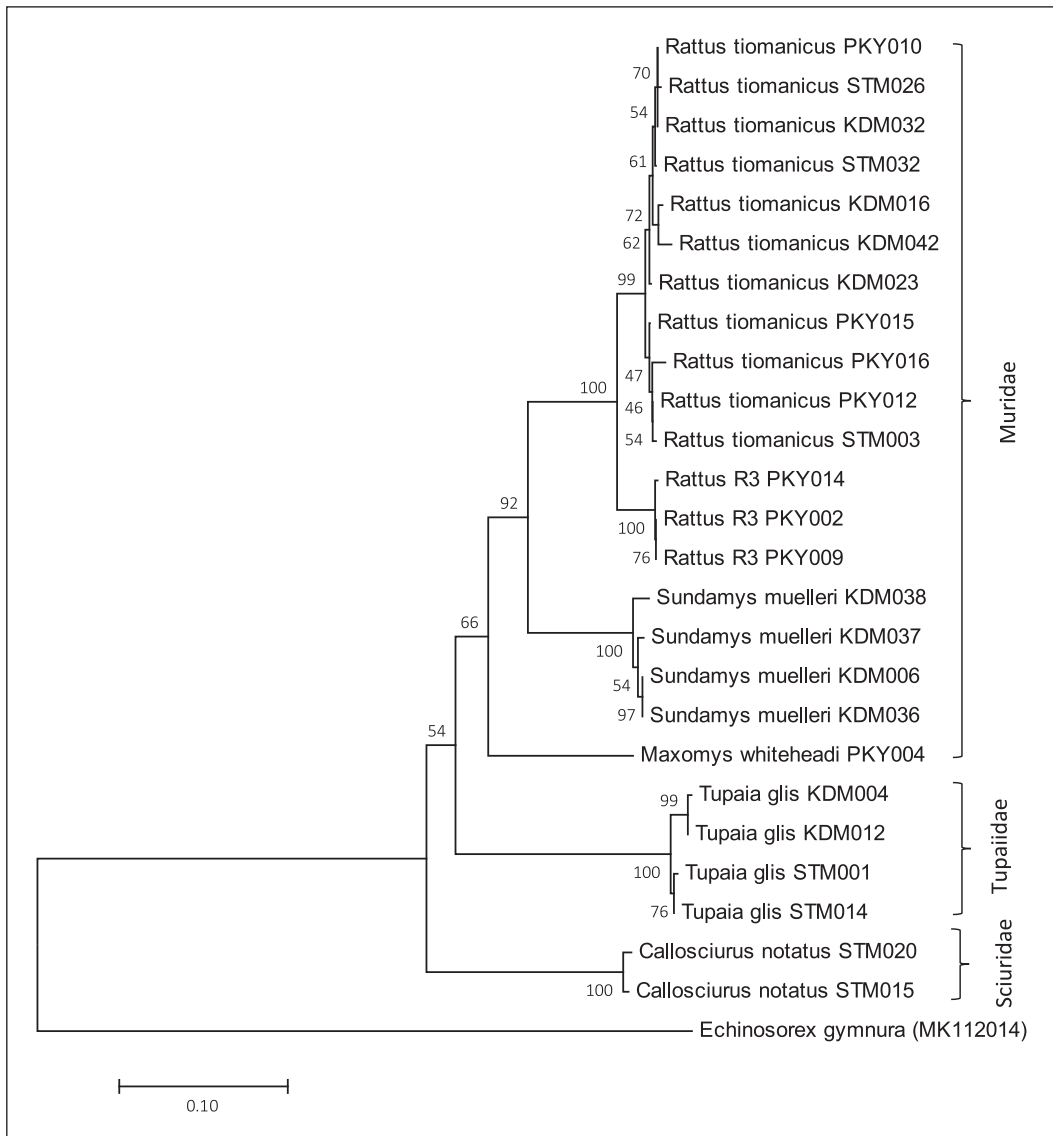


Figure 2. Phylogenetic relationship of small mammal's species based on COI gene sequences. The Neighbor-Joining tree was constructed based on Kimura two-parameter model (K2) to conclude the relationships within and between small mammal's species. *Echinorex gymnura* was used as an outgroup for this study.

Table 3. List of sequences submitted to NCBI database with GenBank accession numbers

ID code	Mangrove area	Tick species	Bacteria	Percent sequence identity	GenBank accession no.
PKY016-01	Kg. Yak Yah, Terengganu	<i>Dermacentor auratus</i>	<i>Bartonella</i> sp.	99.0%	MT792313
PKY016		<i>R. tiomanicus</i>	<i>Bartonella</i> sp.	98.0%	MT925967
STM016-01	Kg. Sg. Timun, N. Sembilan	<i>Haemaphysalis hystricis</i>	<i>Bartonella</i> sp.	97.8%	MT809005
STM016		<i>R. tiomanicus</i>	<i>Bartonella</i> sp.	97.9%	MT925968

related to *Bartonella* sp. 161210, a strain of *Bartonella phoceensis*, with 99% and 97% similarity.

Next, phylogenetic relationships based on the alignment of ITS sequences were performed to analyse the phylogenetic status of the *Bartonella* sequences in this study (Figure 3). From this result, both *Bartonella* sp. from ticks and their hosts in each study site were equivalent with each other. This phylogenetic tree showed that *Bartonella* sp. 16120 strain (PKY016-01) in tick and host sample (*Rattus tiomanicus* (PKY016)) were clustered together with *Bartonella phoceensis* and uncultured *Bartonella* sp. with a bootstrap value of 100%. From this study, *Bartonella* sp. 16120 strain (PKY016-01) was closely related to *Bartonella phoceensis* and uncultured *Bartonella* sp. whereas, there was slightly difference about 0.1% genetic distance with another *Bartonella* sp. 16120 strain (STM016-01). Besides that, PKY016-01 sample showed high percentage of sequence identity with *B. phoceensis* and uncultured *Bartonella* sp. While, for

Bartonella sp. 16120 strain (STM016-01) was slightly distant with other *Bartonella* sequence with only 0.1% distant. Moreover, most of *Bartonella* sequences (including STM016-01 and PKY016-01) were distantly grouped with *B. henselae* (KU291316 and KT318619), corresponding with a lower percentage identity compared on the DNA comparison which presented in Figure 3.

DISCUSSION

From this study, we discovered the presence of *Bartonella* species in ticks from *Dermacentor auratus* and *Haemaphysalis hystrix* at 5.26% prevalence, collected from *Rattus tiomanicus* in two mangrove sites of Peninsular Malaysia. To date, this is the first report of *Bartonella* sp. 16120 strain from ticks of small mammals in Malaysia, which closely related to *Bartonella phoceensis* documented in rodents from Australia and in ticks collected from cattle in Malaysia. Recently, *B. phoceensis* have

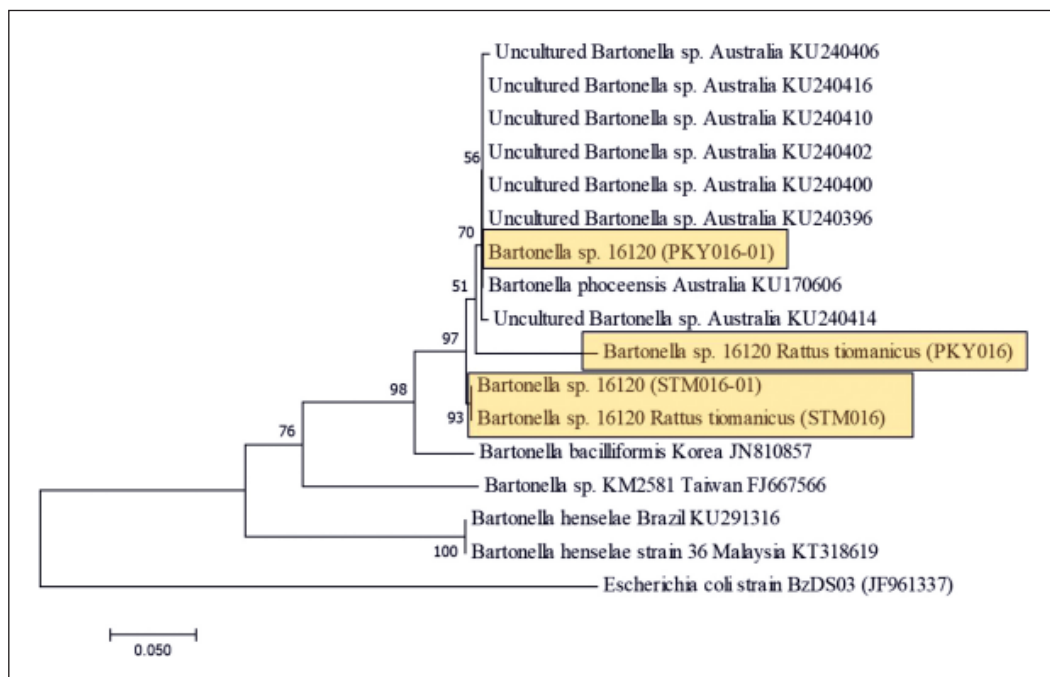


Figure 3. Phylogenetic relationship of *Bartonella* sp. with other bacteria species based on ITS (16S-23S rRNA) sequences. The Neighbor-Joining tree was constructed based on Kimura twoparameter model (K2) to conclude the relationships within and between *Bartonella* species. *Escherichia coli* strain BzDS03 was used as an outgroup for this study.

been reported firstly in Malaysia from synanthropic rodents (Low *et al.*, 2020). Previously, *B. phoceensis* also have been reported to be found in rodents in Asia country including Indonesia, Japan, Singapore, Taiwan, and Thailand (Billeter *et al.*, 2008, Tsai *et al.*, 2010). Hence, signifying that these genospecies may not only limited to Southeast Asia (Gundi *et al.*, 2004), but also Australasia. In addition, the PCR positive result also appeared in their small mammal host, which is might indicate that the hosts were carrying the same bacteria as the ticks which had acquired the bacteria from the host in a recent bloodmeal (Pichon *et al.*, 2005). However, from the phylogenetic analysis, *Bartonella henselae* which is a causative agent of cat scratch disease was distantly related to *Bartonella* sp. 16120 strain. Nonetheless, there was a slightly difference with *Bartonella bacilliformis* (Carrion's disease) reported from Korea.

Although, our study only used a partial of ITS gene for phylogenetic analysis, this gene has been revealed to be a reliable implement for distinguishing between *Bartonella* genotypes that closely related to *Bartonella* strains found in other geographical areas (La Scola *et al.*, 2003). Prevalence of *Bartonella* infections among ticks varies considerably in different parts of the world. For instance, it was documented in *Ixodes pacificus* and *I. scapularis* in the US, *I. ricinus* in the Netherlands and Italy, and *I. persulcatus* ticks in Western Siberia (Holmberg *et al.*, 2003; Kosoy *et al.*, 1997; Tea *et al.*, 2004). Another study reported that *Bartonella grahamii* strains detected from 10 rodent species, with different host specificity in geographic site; low host specificity in Europe and strict host specificity in North America and Asia (Inoue *et al.*, 2009). Kabeya *et al.* (2010) found identical *Bartonella* sp. in ticks from the same small mammal hosts, indicating that these bacteria could be originated from the host's blood.

Due to the location of the sampling areas which involved occupational activities, such as fishing, trekking, and logging, there is high potential of human contact with the infected tick vectors, as well as the small

mammal hosts itself. Therefore, implying the possibility of transmission of these bacteria to human (Regier *et al.*, 2016). Apart from that, tourism activities synonym to this area which include firefly and bird watching posed threat to the visitors. On another instance, the life-stages of ticks which include egg, larvae, nymph and adult more likely acquire different animal hosts (Kho *et al.*, 2015). According to Chang *et al.* (2001), ticks in larvae and nymph phases primarily prefer feed on small mammals and reptiles in which serve as a vital source of infection. Subsequently, the nymphal or adult ticks could then spread *Bartonella* sp. to large animals and probably directly to human through biting ticks. Besides, the small mammal hosts which recognized as preferred hosts of the adult ticks are the main factors of tick abundance in tick-permissive habitats (Gray *et al.*, 1992). In addition, these bacteria could also be transmitted by other arthropod ectoparasites like fleas, mites and lice as mentioned in Theonest *et al.* (2019).

Bartonella species have been recognized as significant sources of human febrile disease in several global scale but there still has been little study toward human health of bartonellosis (Halliday *et al.*, 2015). To date, vector-competence of some of arthropods vector has not been completed for *Bartonella* spp. transmission and are suspected in many more (Regier *et al.*, 2016). The discovery of *Bartonella* genotypes that are most similar to *B. phoceensis* and uncultured *Bartonella* sp. reference DNA sequences from small mammals trapped in Peninsular Malaysia infer great possibility of *Bartonella* infection upon unknown proportion of febrile illnesses in this region. This study also provided a little evidence regarding the prevalence of *Bartonella* species in ticks and their hosts from mangrove areas as well as eco-tourism areas in Malaysia.

Detection of *Bartonella* sp. in ticks species from small mammals in the mangroves forests imply that residents and visitors in that area are exposed to tick bites or even the adult stage of the ticks life cycle which inhabited around mangrove forest. We demonstrated that the existence of *Bartonella*

DNA in *D. auratus* and *H. hystricis* indicating these ticks should be added to the growing list of potential tick vectors to disclose their possible roles in zoonotic disease. However, there is a possibility of the pathogen to be transmitted to other ticks' species and other small mammals through blood-transmission. Subsequently, there is possibility of transmission to human which is in contact with mangrove habitat, regardless for occupational or tourism purposes. Thus, precautions need to be taken to avoid tick bites. Further examination is necessary to investigate the viable transmission of the *Bartonella* pathogens and the impact of this zoonotic infection in animals and human.

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