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Article

Description of ticks species from *Rattus tiomanicus* in Mangrove Forests through scanning electron microscopy

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Abstract

Ticks are ill-famed vectors of many pathogenic organisms which can cause various diseases and life-threatening illnesses to animals and humans. Each tick's species and its life stages have distinct morphological features that can permit them to be accurately identified. However, the use of conventional stereo microscopes limits the accuracy of species identification. The taxonomy of ticks, in general, is not much understood and existing information is based on sparse morphology data. Thus, this study aims to examine and describe the morphological characteristics of different species of ticks collected on rodents in mangrove forests using scanning electron microscopy (SEM). This method renders high-quality images of body parts of ticks. Five different morphospecies of ticks from one host species (*Rattus tiomanicus*) were examined under SEM, followed by the PCR technique using mitochondrial 16S rDNA gene for species validation. This study revealed that the ticks belong to five species: *Dermacentor auratus*, *Ixodes granulatus*, *Haemaphysalis hystricis*, *D. atrosignatus* and *Amblyomma cordiferum*. The combination of stereomicroscopic and SEM methods has improved our understanding of the morphological characteristics of different tick species, hence establishing up-to-date taxonomic keys for these species. Moreover, due to the lack of taxonomic keys on the immature stage of ticks, the SEM method is essential in characterising the morphological features of these stages in detail, subsequently helpful in revising the taxonomic keys for certain ticks species.

Keywords: Ectoparasites, SEM, morphology, *Rattus tiomanicus*, PCR, species validation

Introduction

Ectoparasites, such as ticks and mites, spend their adult stage on warm-blooded animals, thus, often found infesting terrestrial small mammals (rodents and scandents). There are 896 species of ticks identified throughout the world, belonging to three families: Ixodidae, Nuttalliellidae and Argasidae (Guglielmone *et al.* 2010). Ixodidae is the most prominent family, especially for the genus *Ixodes* Latreille 1795, with 243 species (Guzmán-Cornejo & Robbins 2010). The life-cycle of ticks comprises four developmental stages, including eggs, larvae (6 feets) (Coley 2015), nymph (8 feets) and adults (Fuente *et al.* 2008). Adult female ticks are able to detach and reattach to their hosts and continue feeding, while other immature stages are feeding just once during their lifespan (Barton *et al.* 1996). The larval stages are especially challenging to be identified based on morphological characters alone due to the lack of established taxonomic keys and descriptions (Beati & Keirans 2001). Understanding the detail of morphological features of the ticks could aid in establishing an up-to-date taxonomic description of the tick's species.

Scanning electron microscopy (SEM) has been widely used for steric observations of sample surfaces by scanning with an electron beam radiation with associated exposure to high vacuum pressure (Ishigaki *et al.* 2012). Similar to conventional microscopic observation, the SEM method produces pictures of the external characteristic of the specimens in detail (Aoki *et al.* 1982; Brahma *et al.* 2014). Since the 1970s, SEM has been widely utilised as a tool in observing and viewing small specimens like tick and mite because of its observation functioning on surface-fine structures in detail. Therefore, this method has significantly strengthened the identification process and balanced the information attained from stereo microscopic observation (Wergin *et al.* 2006). Recent advancement of SEM technology has been used to examine extracellular and intracellular surfaces (including cells and tissues) morphological structures of different stages of ticks (Abdel-Shafy *et al.* 2018).

Various studies of ectoparasites' identification collected from rodents have been examined in many countries worldwide, including Malaysia (Chulan *et al.* 2005; Paramasvaran *et al.* 2009; Madinah *et al.* 2011; Saraiva *et al.* 2012; Sponchiado *et al.* 2015; Ishak *et al.* 2018). These studies used conventional stereo microscopic morphological observation, as well as using molecular DNA marker to identify the taxonomic status of tick. Morphological identification is the initial step on species identification as it requires basic tools and apparatus unlike molecular approach but insufficient taxonomic keys is the main challenge. The barcoding method on the other hand provides much more valuable evidence about genetic differentiation and is one of the prospects to determine the intraspecific polymorphism of the nucleotide sequences (Ernieenor *et al.* 2016). However, these methods were also limited due to insufficient data from GenBank. This has called for a more advanced method than conventional stereomicroscopic, the SEM method, to better establish the identical description of tick species' external features. Hence, SEM observation has been proven to characterise the different tick stages with detailed concern given to the immature stage (Jin *et al.* 2016).

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. The information on tick's species infesting the small mammals is essential to establish base-line information on the diversity and ecology of ticks present in an area. Mangrove forest particularly was given less attention for tick's study, but a recent study by Mohd-Taib *et al*. (2021) has listed five species of ticks predominantly infesting one host species, *Rattus tiomanicus*. Thus, this study aimed to provide an overview of the morphological characterisation of different species of ticks collected from this rodent species in mangrove forests in Peninsular Malaysia. In addition, the ticks' species verification was also performed and analysed using molecular approach based on the 16S rDNA gene.

Materials and Methods

Sampling sites

This study was conducted at mangrove forests in three states in Peninsular Malaysia: Terengganu (Kampung Sungai Yakyah, KSY), Negeri Sembilan (Kg. Sungai Timun, KST) and Perak (Kg. Dew, KDP). The map of the study areas is illustrated in Figure 1. The samplings were carried out between December 2017 and February 2018, with seven days of trapping at each location. The samplings were conducted at five stations along 1 km from the jetty of each mangrove site, and each station was not more than 300 m from the riverbank. Sampling stations were placed in higher elevation parts to mitigate the risk of flooding due to tides and nearby rivers.

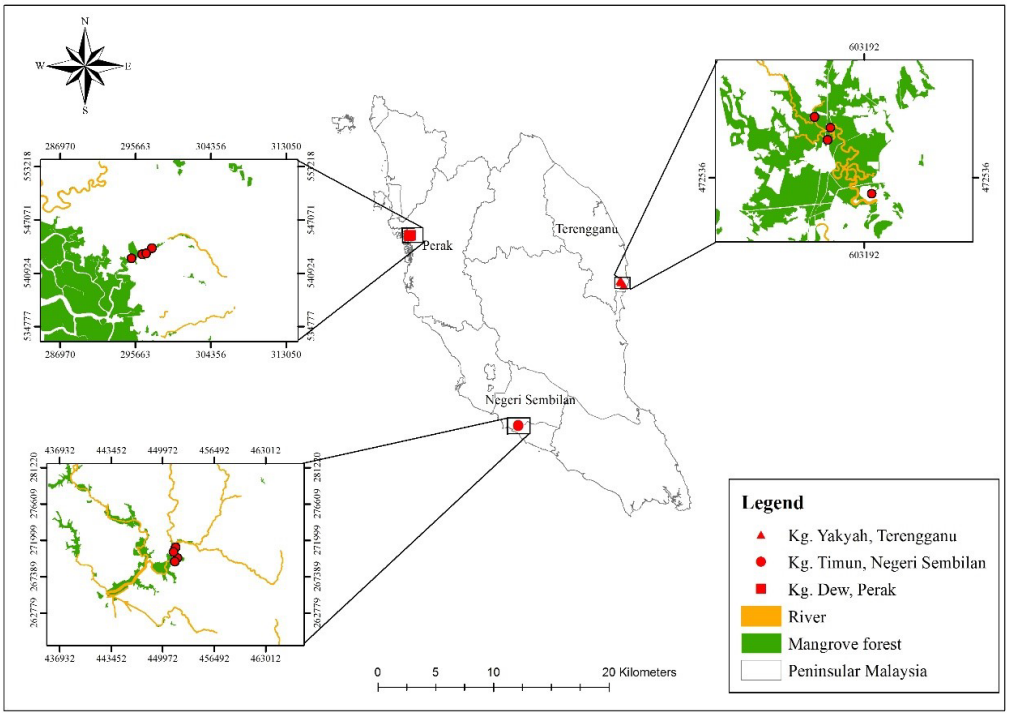

FIGURE 1. Map showing the geographical distribution of the three study sites of mangrove area (Kg. Dew, Perak, Kg. Yakyah, Terengganu, and Kg. Timun, Negeri Sembilan) in Peninsular Malaysia.

Rodent sampling

One hundred wire mesh cage traps (28 cm×15 cm×12.5 cm) were deployed in each study site along the river. A variety of baits, such as banana, oil palm fruit and jackfruit, were used (Bernard *et al.* 2004). The cage traps were randomly placed on tree stumps, fallen logs (Balete *et al.* 2009), and ground-level (Rickart *et al.* 2011). All traps were checked twice a day at 0900h and 1700h for six consecutive nights. Trapped animals were brought back to the research field station for samples collection. Morphological measurements of the animals were taken, and identification from physical appearance was based on the graphic illustrations and description by Francis (2008). Before handling, all rodents were anaesthetised with an intramuscular injection of Zoletil 100® with a volume between 0.1 mL and 0.3 mL according to the animal's weight, as described by Rivas *et al.* (2015). After gaining consciousness, they were released back to their original captured sites. Rodent-trapping and rodent-handling procedures have been approved by the animal research ethics committee of Universiti Kebangsaan Malaysia (FST/2020/FARAH SHAFAWATI/16-JAN./1080-FEB.-2020-FEB.-2022-NAR-CAT2).

Ticks collection

Each individual animal was examined carefully for ticks' infestation, and ticks were collected from different body parts, including ears, nose, around eyes and abdomen using a pair of fine forceps (Barker and Walker 2014). Once removed from the animal, the ticks were placed into individually-labelled 1.5 mL cryovial tubes. The tubes were then stored in a liquid nitrogen tank for transportation to the lab and stored at -40°C until further processing. Other information, namely collection date, locality and animal species, were also recorded.

Stereo microscopic and SEM observation

Each tick was surface-sterilized prior to observation by rinsing with 70% ethanol solution and then washed in sterile distilled water to eliminate and remove any debris from possible pollution and environment (Carpi *et al.* 2011). The ticks were preliminarily observed under a stereomicroscope (Motic SMZ-168 Stereo Zoom microscope) (Motic, Hong Kong) to examine the basic characteristics of tick samples to genera level and later in developmental stages (larvae, nymph, or adult) and gender. Morphological identification was based on the external features following taxonomic keys by Kohls (1957) and Walker *et al.* (2007).

Next, ticks were observed and photographed under the Hitachi TM-1000 Scanning Electron Microscope (Hitachi High-Technologies Corp., Tokyo, Japan). This application allows for stereoscopic morphological characterisation with a greater depth of focus and high resolution, which are not accessible with an optical microscope. The vacuum conditions were set at an accelerating voltage of 15 kV and the magnification of 20–10,000× (digital zoom, 2×, 4×). The observation of ticks was conducted at both sides gradually; dorsal and ventral sides were gently fixed with stainless steel tweezers on the conductive tape and positioned on a sample tub of SEM followed Ishigaki *et al.* (2012). All morphological surfaces were obtained, including the body, legs, capitulum, hypostome, anus, spiracular plate, claw, scutum, festoon, etc. The clear images were shown without any process of fixation and coating as observation was performed under vacuum pressure. The ticks were examined to species level based on their morphological characters using keys provided by Roberts (1970). All measurements (units: mm and μm) with their magnification scale are provided below the image.

DNA extraction of tick samples

The ticks were subjected to molecular investigation after the morphological examination as defined by Ishak *et al.* (2018). 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 was 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 following steps were performed following the manufacturer's protocol.

PCR amplification and DNA purification

Amplification of PCR was initially performed to amplify mitochondrial rDNA gene for the ticks' species with a pair of specific primer sets designed by Black and Piesman (1994), namely 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') and 16S-1 (5'-CCGGTCTGAACT CAGATCAAGT-3'). PCR reaction was conducted in a total volume of 35 μL containing 13 μL of 2×Taq PCR Master Mix (Lucigen, US), ten μL of nuclease-free water, one μL of 0.5 μmol/L of each primer, and five μL of DNA template. The PCR was conducted using an Alpha Cycler PCRmax machine (PCRmax, UK).

The PCR amplification program for 16S rDNA gene was performed under the following condition: initial denaturation at 95°C for 5 min, followed by ten cycles of denaturation at 92°C for 1 min, 48°C for 1 min and 72°C for 1.5 min. Next, continued with 32 cycles of 92°C for 1 min, 54°C for 35 sec, 72°C for 1.5 min and followed by a final extension at 72°C for 7 min. In all cases, nuclease-free water was used as a negative control to replace the DNA template. After that, the amplified products were visualised in 1.0% agarose gel (TAE buffer) electrophoresis and viewed under an ultraviolet trans-illuminator after staining with a gel DNA stain (Florosafe stain). The DNA purification and further sequencing analysis were performed by MyTACG Bioscience Enterprise.

Sequencing and BLAST analysis

The length of a few sequences was 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 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 BLAST analysis (http://www.ncbi.nlm.gov/BLAST/) for confirmation of ticks' species. Besides that, this tool revealed that the quick comparison of query sequences with database sequences led to species identification (Mitler *et al.* 2010). This approach detailed sequences' similarity relating to some requirements, such as maximum identical, expected value, maximum score and query coverage (Fassler & Cooper 2011).

Results

General morphology from stereo microscope

A total of 5 individual ticks, including larvae, nymph, adult and fully-engorged stages, were effectively examined from different individual hosts of *Rattus tiomanicus* (Table 1). From the stereo microscopic view, five different species of ticks were identified, namely, *Ixodes granulatus*, *Dermacentor* spp., *Haemaphysalis* sp. and *Amblyomma* sp. according to their appearances and external features. The images were visibly obtained without any conductive treatment and fixation process.

TABLE 1. List of localities of different individuals of *Rattus tiomanicus*, and the infesting ticks with life stages.

ID code (sample)	Locality	Tick species (morphology)	Stages
PKY002-01	Kg. Yak yah, Terengganu	*Ixodes* sp.	Adult (fully-engorged)
PKY016-01	Kg. Yak yah, Terengganu	*Dermacentor* sp.	Nymph
STM016-01	Sg. Timun, Negeri Sembilan	*Haemaphysalis* sp.	Adult (fully-engorged)
STM010-06	Sg. Timun, Negeri Sembilan	*Dermacentor* sp.	Larvae
KDM037-03	Kg. Dew, Perak	*Amblyomma* sp.	Larvae

TABLE 2. BLAST results against available sequences in the GenBank.

ID code	Tick species (morphology)	Tick species (molecular)	Similarity with GenBank	Accession Number
PKY002-01	*Ixodes* sp.	*Ixodes granulatus*	98%	U95885.1
PKY016-01	*Dermacentor* sp.	*Dermacentor auratus*	99%	KC170746.1
STM016-01	*Haemaphysalis* sp.	*Haemaphysalis hystricis*	95%	KC170733.1
STM010-06	*Dermacentor* sp.	*Dermacentor atrosignatus*	99%	KC170745.1
KDM037-03	*Amblyomma* sp.	*Amblyomma cordiferum*	98%	MK301096.1

Molecular identification

Molecular identification of ticks was accomplished in this study by targeting the mitochondrial 16S rDNA gene using the whole body of ticks. The amplified sequence on the 16S region corresponds to the average length of 460 bp, and the similarity with GenBank was 95–99% for each individual tick (Table 2). These sequences presented the highest similarity of about 99% to a mitochondrial 16S rDNA gene sequence from *Dermacentor* spp. collected in Terengganu and Negeri Sembilan through the BLAST search.

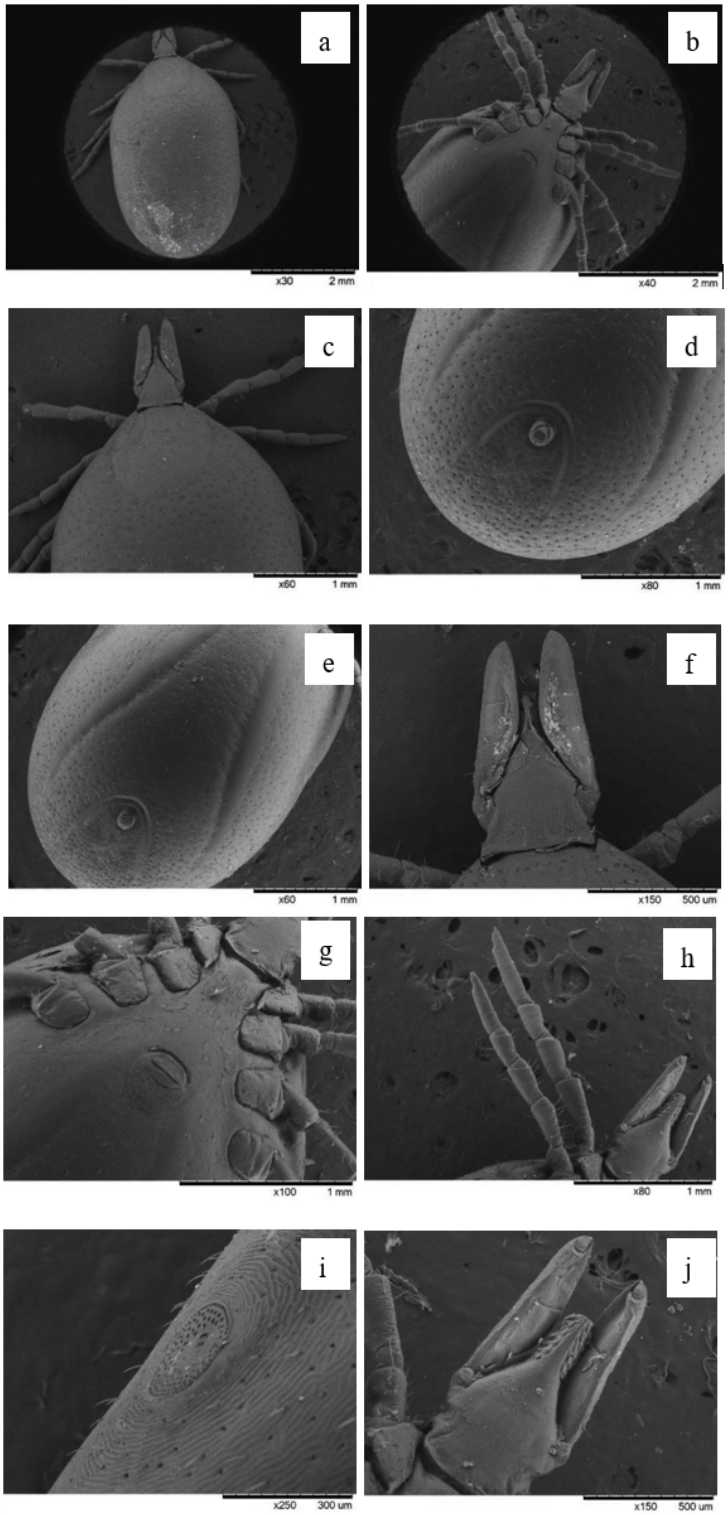

FIGURE 2. *Ixodes granulatus* (female): (a) dorsal view, (b) ventral view, (c) scutum, (d) anal groove (anterior to anus), (e) genital groove, (f) capitulum, (g) genital opening and coxae I-IV, (h) legs and pulvilli, (i) spiracular plate, (j) capitulum and hypostome (ventral).

Ticks observation under SEM

Ixodes granulatus Supino, 1897

Ultrastructural observations by SEM were applied to describe the morphological characteristics of adult (fully-engorged) *I. granulatus* ticks. Figure 2(a–j) illustrates the different body parts of *I. granulatus* (female) at different magnification. The dorsal view of this species shows a white teardrop body shape (Figure 2a). The ventral view of the anterior part is pointed at the mouthpart (Figure 2b). Scutum is very small and inornate (Figure 2c). The most distinct part of this species is the anal groove, which embraces the anterior anus as arch-like (Figure 2d and 2e). In the adult stages, the gnathosoma (mouthpart) are dorsally noticeable with long and narrow palpi (Figure 2f). Genital aperture is present, prominent and positioned parallel between coxa III and IV (Figure 2g). This species has four pairs of legs with a pale ring on each leg (Figure 2h). A pair of spiracular plates (stigmata) in sieve-like shape at both sites, below the coxa IV (Figure 2i). Hypostomes comprise tough-like projections and are distinct with a pair of 2+2 (Figure 2j).

Dermacentor auratus Supino, 1897

This species has a dorsal body surface with large punctuation from each hair and have long marginal groove and postero-median groove closely reaching the scutum (Figure 3a). The ventral view of the anterior part is pointed at the mouthpart and is round-shaped (Figure 3b). The scutum is triangular-shaped, and there is a narrow, distinct line through the middle of the scutum (Figure 3c). The enamel marking on palpi with lateral margin is slightly convexed (Figure 3d). The hypostome is short, lateral margins closely parallel and dentition with 3:3 (Figure 3e). Legs are brown, thick and ornate, with white markings on the dorsal surface (Figure 3f). Genital aperture is absent (nymph), and genital grooves nearly diverge broadly anterior to the anal opening (Figure 3g). The external spur of *D. auratus* is broad, shorter than other *Dermacentor* species, and there are two spurs on coxa I (Figure 3h). A spiracular plate pair is circular below coxa IV (Figure 3i). The anal opening is positioned opposite the spiracular plate (Figure 3j).

Dermacentor atrosignatus Neumann, 1906

There are no taxonomy keys recorded for the larvae stage of this species from the reference studies. The dorsal view of this species shows the elongated, oval body with marginal grooves (Figure 4a). The ventral view of the anterior part shows three pairs of legs (Figure 4b). Scutum is broadly pointed than long (Figure 4c). Mouthparts are the same length as the basis capitula, have a big gap between them, and the basis capituli have straight lateral margins (Figure 4d). The hypostome is short, the dental formula is 2:2 (Figure 4e). No genital area appears on the centre of the ventral side (Figure 4f). The external spur is large and only has one spur for each coxa (I-III) (Figure 4g). The anal plate is distinct and positioned posteriorly to the anus (Figure 4h). A genital groove is present beside and along with the anal plate (Figure 4i). This species has 11 festoons on the posterior of the dorsal surface (Figure 4j).

Haemaphysalis hystricis Supino, 1897

One of the significant morphological characteristics of *Haemaphysalis hystricis* is the existence of a blade-like projecting of the dorsal rearward on trochanter I (Figure 5c). The dorsal view of the external body part is oval-shaped and fully engorged (Figure 5a). The ornamentation assembly on the scutum is lacking, and the anal groove is distinct, which riggings the posterior anus, and the spiracular plate is circular-shaped but less visible through the illustration (Figure 5b). Their eyes on the anterior part are absent and has small scutum (Figure 5c). This species also has broad and short palps with the palp femur laterally prominent to the rectangular basis capituli (Figure 5d). Unfortunately, the image in Figure 5d was a bit vague. Next, the spur on the first pair of coxae is

bluntly pointed on the internal spur (Figure 5e). There are no pale rings on the legs that are uniform in colour (Figure 5f and 5g). On the posterodorsal spur, the palpal segment three overlaps the anterior ¼ of the segment, while the posteroventral margin of segment two with spur-like-angle (figure 5h).

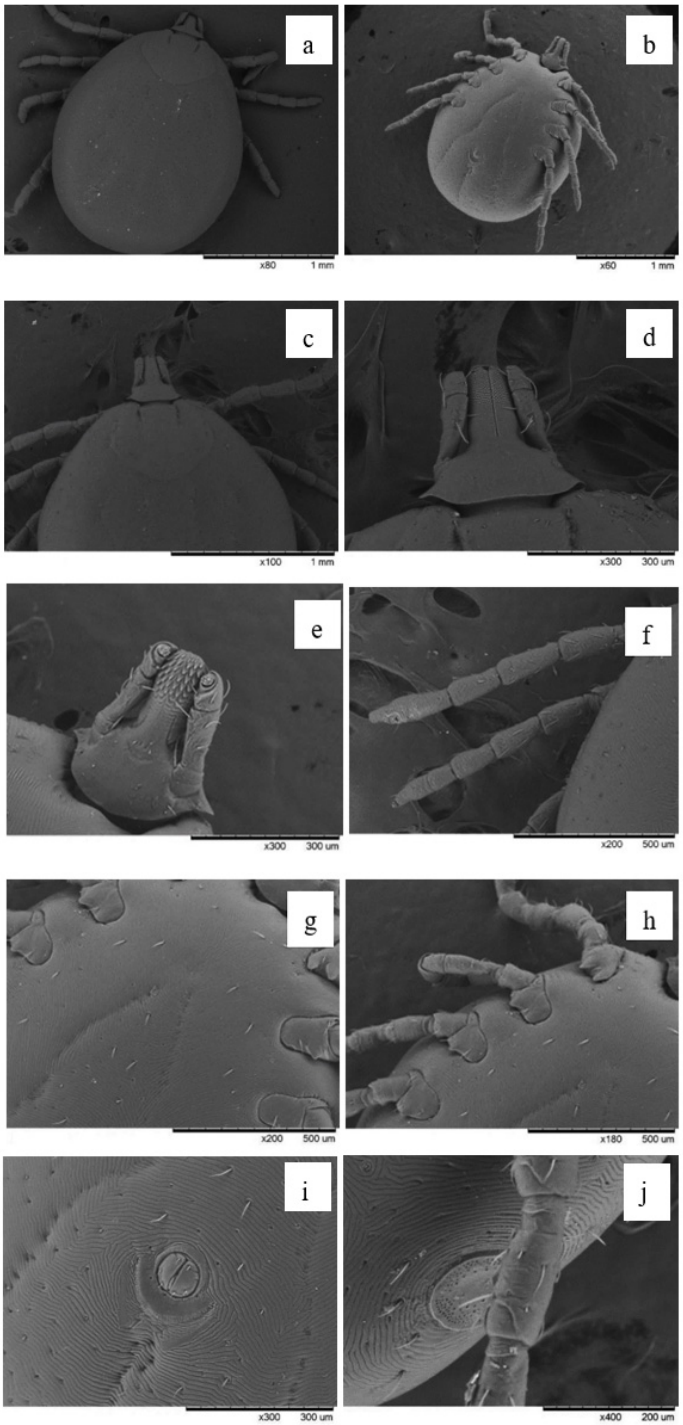

FIGURE 3. *Dermacentor auratus* (female): (a) dorsal view, (b) ventral view, (c) scutum, (d) capitulum, (e) ventral capitulum and hypostome, (f) legs and pulvilli, (g) genital groove (h) coxae I–IV, (i) anal groove (posterior to anus), (j) spiracular plate.

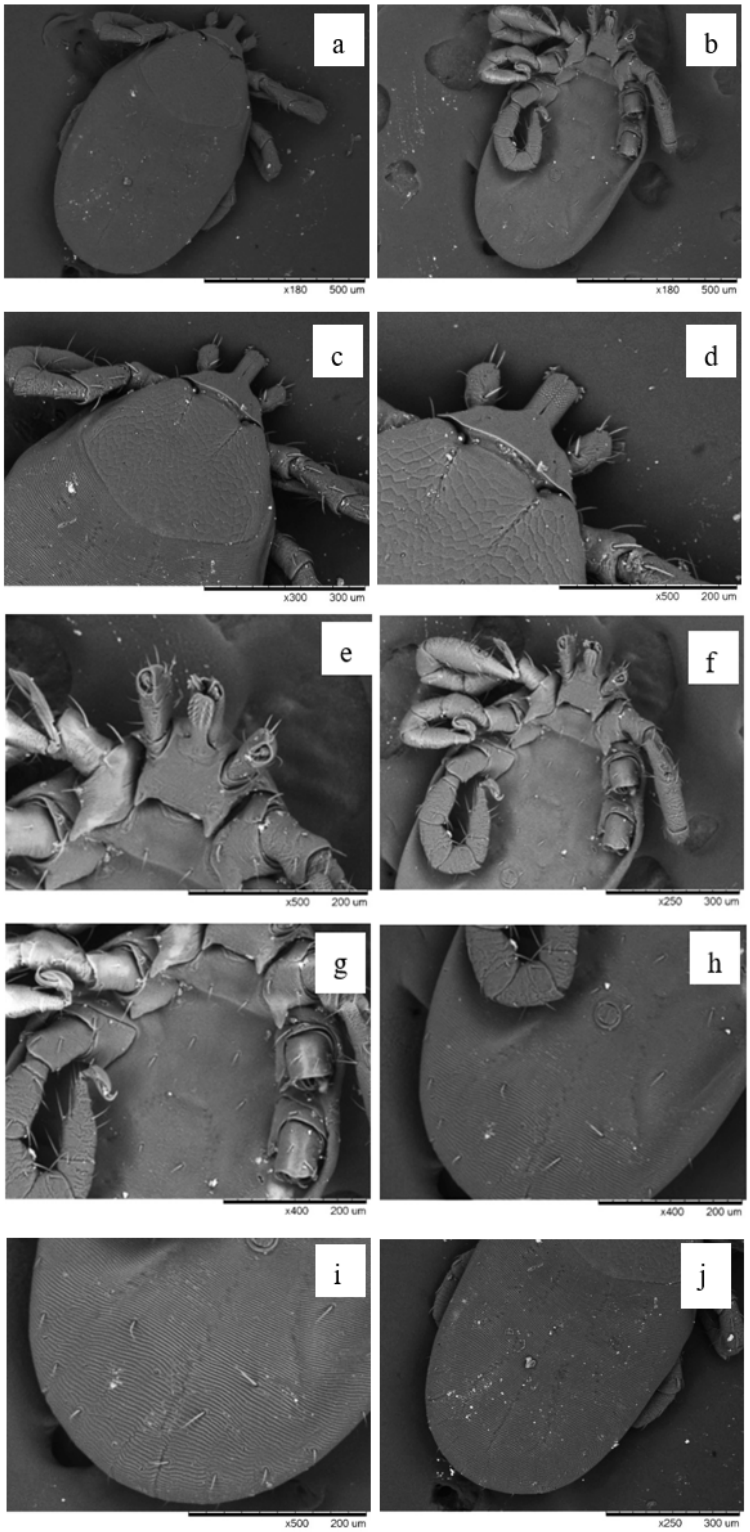

FIGURE 4. *Dermacentor atrosignatus* (female): (a) dorsal view, (b) ventral view, (c) scutum, (d) capitulum, (e) ventral capitulum and hypostome, (f) legs and pulvilli, (g) genital groove, (h) anal groove (posterior to anus), (i) festoon, (j) body structure (ventral).

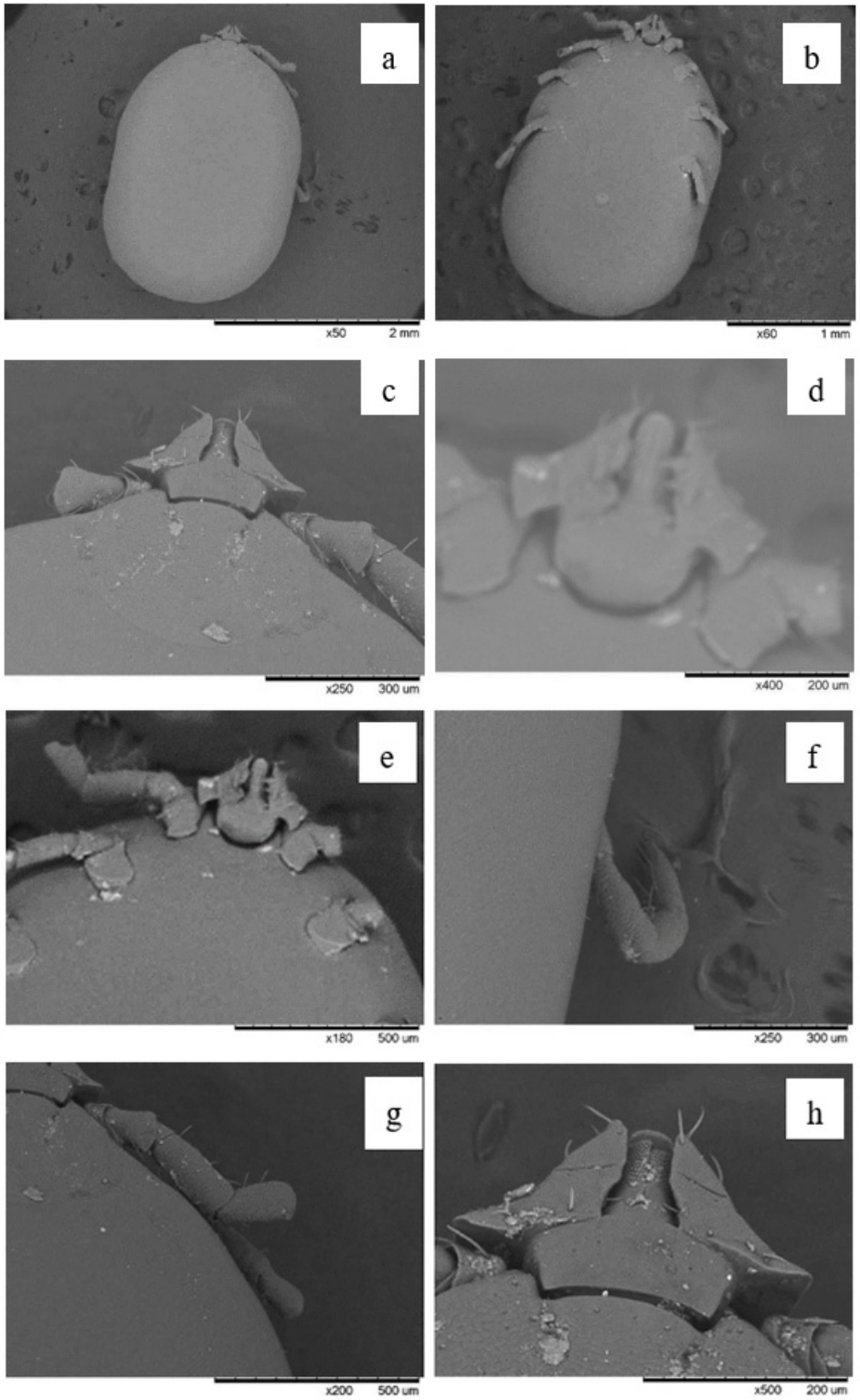

FIGURE 5. *Haemaphysalis hystricis* (female): (a) dorsal view, (b) ventral view, (c) scutum, (d) hypostome, (e) ventral capitulum, (f) legs and pulvilli, (g) legs (ventral), (h) capitulum.

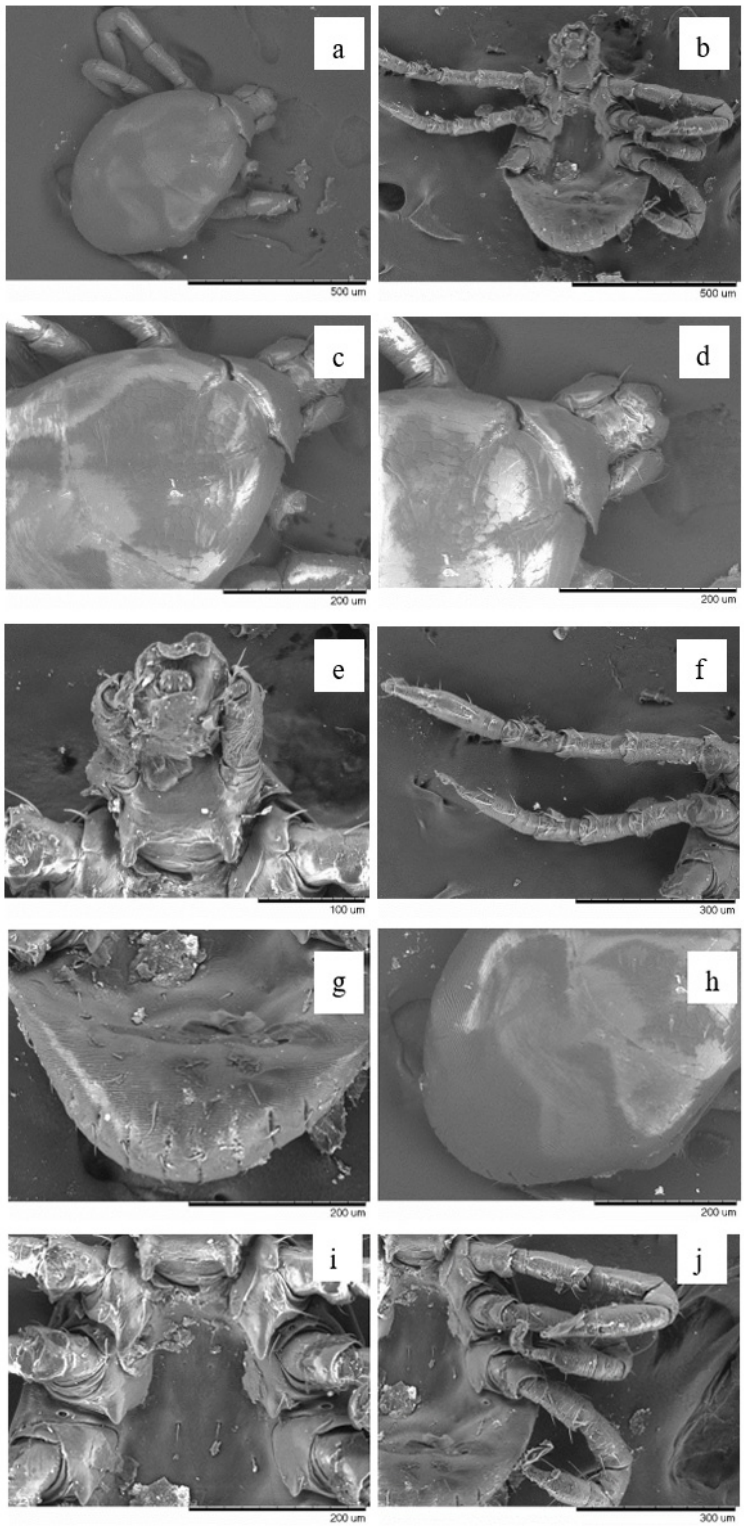

FIGURE 6. *Amblyomma cordiferum* (female): (a) dorsal view, (b) ventral view, (c) scutum, (d) capitulum, (e) ventral capitulum and hypostome, (f) legs and pulvilli, (g) festoon (h) body structure (dorsal), (i) coxa (I-III) and spurs, (j) 3 pair of legs (larvae stages).

Amblyomma cordiferum Neumann, 1899

The following descriptions of Amblyomma cordiferum are based on the morphological characteristics observed under the SEM. These images below show the immature stages of the tick (larvae), and there are several external bodies that are damaged as illustrated in Figure 6. The exterior body (dorsal) is oval-shaped (Figure 6a), and the ventral view of the anterior part has a damaged pierce at the mouthpart (Figure 6b). Scutum is inornate and almost half of the body size (Figure 6c). The basis of the capitulum is triangular and barely rounded (Figure 6d). The hypostome is invisible because there seems to be like an attachment on a rodent's skin (Figure 6e). The tarsus on the legs is long and uniform in colour (Figure 6f). Laterally, the festoons are relatively evident (Figure 6g). There is no marginal groove forming on the middle of the dorsal surface (Figure 6h). Coxa I with two triangular spurs are larger on the external spur, coxa II and III with single spur individually (Figure 6i). Three pairs of legs indicate larvae or immature tick stage (Figure 6j).

Table 3 presents the previous studies of tick species collected on *Rattus tiomanicus* at several localities in Peninsular Malaysia. The four genera of ticks found in the earlier studies, were also found in the current study, even though several tick individuals were not identified to species level, as shown in Table 3.

TABLE 3. Previous records of tick species infesting *Rattus tiomanicus* in Peninsular Malaysia.

ID Code	Tick species	Collection site	Reference
GSFR21-3	*Haemaphysalis* spp. *Ixodes granulatus* *Ixodes* spp.	Gunung Stong reserve forest, Kelantan	Mariana *et al*. 2005
SBN26-1 and SBN26-2	*Haemaphysalis hystricis*	Seremban, Negeri Sembilan	Ernieenor *et al*. 2017
SBN 01, SBN23-1 and SBN16-2	*Ixodes granulatus*	Kg Lambar, Labu	Ernieenor *et al*. 2013
SBN12-1, SBN28-1, SBN23-2 and SBN18-2	*Dermacentor* spp.		
SBN19	*Amblyomma* spp.		
BT07-03	*Ixodes granulatus*	Bukit Tinggi, Pahang	Ernieenor *et al*. 2016
289-RF002	*Ixodes granulatus*	Hulu Langat, Selangor	Ishak *et al*. 2018

Discussion

We reported five ticks' species collected from *Rattus tiomanicus* in three mangrove areas in Peninsular Malaysia. *R. tiomanicus* was commonly reported as a host for various ticks' species as found in our study, similar to Mariana *et al.* (2005), who found six adult ticks' species from this host species. *R. tiomanicus* is nocturnal, primarily arboreal and found in typical habitats (primary and secondary forest) and coastal forest, including Malaysia, Indonesia, Philippines and Thailand. From this study, only genus *Ixodes* was able to be identified up to species level, as *Ixodes granulatus* based on the external morphological characteristic using specific taxonomy keys and recent studies from Peninsular Malaysia (Ernieenor *et al.* 2016; Ishak *et al.* 2018). Apart from that, two individual ticks of the same genus (*Dermacentor*) and different life stages have been identified. The morphological and physical characteristics between these two *Dermacentor* species were obviously distinct, mainly based on their body shape and mouthpart (Nadchatram 2008). Additionally, the only adult stage was collected from *Haemaphysalis* sp., whereby larvae stage was collected from *Amblyomma* species. Nymph and larvae stages were quite difficult to accurately identify and distinguish based on the color

appearance and unavailability of taxonomic keys for further reference. All ticks collected from our study were female (sexual dimorphism), rendering to the smaller size of the scutum, unlike male which scutum entirely covered in dorsal part. The dorsal shield (scutum) in females is oval, finely granulated, and the length is longer than its width. The scutum of the female tick allows body engorgement and the existing porose area aided as olfactory organs (active during reproduction period) (Ernieenor *et al.* 2016).

I. granulatus species is the widespread tick species in Peninsular Malaysia and have been mostly documented in recent studies from rodents (Ishak *et al.* 2018). According to Ernieenor *et al.* (2016), the external features, including body shape (teardrop), the anal groove position (the most distinct genera) and the mouthpart structure, were the apparent features that described this tick species. Besides, the physical features and reference for this species have been extensively described from previous studies (Teng *et al.* 1991; Yamaguti *et al.* 1971). *I. granulatus* has been reported in various research, including Malaysia and known as exclusively Asian species limiting from Japan through Southeast Asia and westward to China and India (Nadchatram 2008). Furthermore, many studies have discovered this species extended many years in Peninsular Malaysia, and most of them are infesting on small mammals and rodents (Ernieenor *et al.* 2016; Madinah *et al.* 2011; Ishak *et al.* 2018). Their hosts range from birds and small to medium-size mammals, but rarely in humans (Hoogstraal *et al.* 1972; Tanskul *et al.* 1983; Lah *et al.* 2015). Hoogstraal and Wassef (1985) and Guglielmone *et al.* (2014) stated that this species mainly had been found in forests habitats from various hosts and possibly the most widespread member of its genus in continental Southeast Asia. From our study, *I. granulatus* (fully-engorged) has been collected from *R. tiomanicus* in Kg. Yak Yah, Terengganu mangrove forest, but not in the other two mangroves forest sites. This species was reported and known as a vector for several pathogens (Petney 1993). For instance, it was claimed as a vector of Langat Virus-like Russian Spring-Summer Encephalitis Complex (RSSE) (Smith 1956) and has associated Q fever and typhus cycle surrounding Peninsular Malaysia (Marchette 1965).

D. auratus (female stage) might be notable from those of *Dermacentor compactus* and *Dermacentor limbooliati* by the following types: the shape of scutum (round-shaped), genital aperture ascetically slight v-shaped, central patch-brown wide, has comparatively long alloscutum setae, broad and blunt internal spur on coxa I, reasonably narrow, long, and the external spur was pointed on coxa I and approximately triangular spur with tip-shaped. Adults of this species are similar to those of *D. compactus* and *D. limbooliati* which was previously misapprehended (Apanaskevich & Apanaskevich 2015). This species was widely distributed from Thailand, Vietnam, Burma, Laos, Sri Lanka, Bangladesh, Nepal, India, Peninsular Malaysia, and Indonesia. Nevertheless, it has not been documented from Philippines or Borneo (Hoogstraal *et al.* 1985). This adult tick commonly infests wild pigs (*Sus scrofa*) with infrequent histories from humans, domestic pigs, python (snake), and rhinoceros. Nymphs' stage was commonly found on small to medium mammals, rodents, wild hens, porcupines, and monkeys (Hoogstraal & Wassef 1985). Surprisingly, this species is the most extensively dispersed Asian *Dermacentor* species (Petney & Keirans 1996).

D. auratus and *D. atrosignatus* possibly coevolved with their primary hosts, *Sus scrofa,* while rodents act as intermediate hosts (Tanskul *et al.* 1983). *D. atrosignatus* reportedly widespread in Peninsular Malaysia, with the distributional range covering southward to Java and Sumatra, eastward to Busuanga, Borneo, Southern Philippines and Palawan. Apart from wild pigs (*S. scrofa* and *S. barbatus*), *D. atrosignatus* adults were also reported infesting other reptilian hosts, mammalian, and forest vegetation. Nevertheless, many records reported infestation of this species to humans rather than animals from several to countless larvae, nymphs, and adult ticks (Hoogstraal & Wassef 1985). The diagnosis of larvae and nymphs necessitate the validation because the immature stages of *D. atrosignatus* were incompletely identified as mentioned in Mariana *et al.* (2008). Therefore, ticks

from this genus are highly potential vectors to zoonotic diseases (Petney 1993). For instance, it has been reported as a vector of the Lanjan virus in Malaysia (Karabatsos 1985).

Compared to other studies, Muridae (*R. tiomanicus, Leopoldamys sabanus*, *Maxomys rajah* and *Sundamys muelleri*) is the leading host of *Haemaphysalis* ticks as reported by Ernieenor *et al.* (2017). Findings from previous studies have verified the identity of *Haemaphysalis* ticks primarily on the structure of its capitulum and mouthpart. This tick has a rectangular-shaped basis capitulum and the second palpal segment is expanded and prominent alongside the basis capitulum. Next, the second and third palpal segments anteriorly spill and, thus, the anterior of the capitulum to the basis capitulum appeared triangular structure. These palpal segments are significant for this species to be simply recognised through observation because of the special segments on its capitulum compared to other tick genus (Khoo *et al.* 2016). Ernieenor *et al.* (2017) reported the presence of *H. hystricis* from rodents (family Muridae, Sciuridae and Ptilocercidae) at several localities in Peninsular Malaysia. This species was regarded as obligate ectoparasite and pathogen vectors of mammals. Besides, this observation is reliable to support previous studies that described the richness of *Haemaphysalis* ticks and their occurrence in rodents and domestic animals throughout Southeast Asia (Burger *et al.* 2013). *Haemaphysalis* species prefers high rainfall and might be existing wherever the rodent hosts are present for the immature stages of ticks (Nosek 1971). *H. hystricis* primarily originates in Indonesia, Vietnam, Myanmar, Peninsular Malaysia, and Thailand, yet is present in Hong Kong, China, northwest India, Taiwan and Sri Lanka (Hoogstraal *et al.* 1964; Tanskul & Inlao 1989; Geevarghese & Mishra 2011; Ernieeror *et al.* 2017). Besides that, a variety of medium to large wild mammals, dogs and humans, are common hosts of the adult ticks, whereby barking deer and wild boar are hosts to both larvae and nymph (Khoo *et al.* 2016; Petney *et al.* 2019). Nymphs have also been collected from the black rat, *Rattus rattus*, squirrels and Asian palm civet (Hoogstraal *et al.* 1965a). However, this species was often misclaimed as *H. bispinosa*, *H. semermis*, *H. birmaniae* and *H. nadchatrami* (Hoogstraal *et al.* 1965b). Several *Haemaphysalis* tick are associated with numerous zoonotic diseases, such as anaplasmosis, rickettsial spotted fever, ehrlichiosis, and tick typhus (Khoo *et al.* 2016; Kang *et al.* 2016).

The previous studies showed that the genus *Amblyomma* embraces 130 species, and it's the third biggest genus of hard ticks (Guglielmore *et al.* 2010). Hosts for this species, however, were reported primarily on reptiles (particularly snakes) which parasitising reticulated pythons, *Malayopython reticulatus* in Malaysia (Tan *et al.* 2019) amphibians (Pandit *et al.* 2011) and mammals (Petney & Keirans 1996; Volzit & Keirans 2002). Other than *R. tiomanicus*, *Rattus rattus diardii*, *Niviventer rapit* and *L. sabanus* were also reported as hosts for this ticks' species (Pimsai *et al.* 2014). The *Ambylomma* specimen was morphologically identified based on a taxonomic key by Kohls (1957). The scutum of this species is rhomboid and almost covers half of the body. This approach is less applicable for damaged ticks and erroneous for close-related species due to incomplete current keys for larvae stages. *A. cordiferum* has been documented in Central and North Sulawesi, the island of Krakatau and the island of Banda in the Maluccas, but it is extensively dispersed in Southeast Asia together with Malaysia, Indonesian islands, Thailand, Taiwan, and Western Samoa (Lazell *et al.* 1991; Petney & Keirans 1994; Volzit & Keirans 2002). Nadchatram (1996) reported on the discovery of an unidentified virus from *A. cordiferum* found on *Python reticulatus* in Peninsular Malaysia. This tick species apparently feed on rodents in larvae or immature stages, as earlier reported in Barnard and Durden (2000). While in the adult stage, this tick prefers to feed on ophidian hosts, including *Ophiophagus hannah* (Ho & Ismail 1984). In addition, the most current report (survey of acarine ectoparasites in 2002–2009) was from Horsfield's fruit bat (*Cynopterus horsfieldii*), as previously mentioned by Ahamad *et al.* (2013). Members of the genus *Amblyomma* are acknowledged as vectors of several pathogens that can cause zoonotic diseases. However, there is still an absence of signs on the transmission of pathogens to humans.

Tick identification from both methods (observation and molecular) compliment the identification approaches necessary to validate the species. The SEM evidenced a beneficial means for examining the ticks morphologically and stereographically and distinguishing clearly on each part of the ticks. This study can aid in developing more revised taxonomic keys, especially in the immature stages. Besides that, the PCR sequencing methods using the 16S rDNA gene also provide a vital and effective tool for validating species of ticks. The findings of this study propose some information on the hosts, veterinary importance, and geographical dispersal of these ticks, included the molecular data. Further investigation of this tick species may be valuable to enhance the understanding of its biological necessities and distribution in Malaysia. Due to the role of small mammals as hosts for ectoparasites, specifically ticks, precaution measures must be taken to avoid possible infestation of ticks when visiting forested areas as they could be a vector of zoonotic diseases.

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