INTEGRATED PHENOTYPIC AND GENOTYPIC APPROACH FOR CHARACTERIZATION OF LOCAL QUESTING HARD TICK, *Dermacentor compactus* (ACARI: IXODIDAE)

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ABSTRACT

Identification of a tick species, *Dermacentor compactus* (Acari: Ixodidae) from Malaysia is still debatable in many aspects and remains poorly studied, especially on the immature stages. This study was conducted to characterize morphologically and molecularly of the *D. compactus* ticks collected from a forest reserve near the Forest Research Institute of Malaysia (FRIM). A total of three adults (two females and one male) of questing *D. compactus* ticks on lower vegetation or shrubs were collected by handpicking. Species identification was conducted based on specific illustrated taxonomic keys, and molecular characteristics of *D. compactus* was analyzed based on mitochondrial 16S rDNA gene (*mt-rrs*). A neighbor-joining tree was constructed to clarify the genetic variation of the *D. compactus*. Clustering analysis using *mt-rrs* gene revealed that the sequences of the *D. compactus* were formed in a monophyletic clade supported with 100% bootstrap value. Furthermore, a low intraspecific variation (1%) was observed amongst the species of *D. compactus*. Meanwhile, genetic distance of the *D. compactus* also supported that the species is genetically distinct from the other *Dermacentor* species with a high interspecific value (>13%). This study reported the first of 16S sequences of the *D. compactus* from Malaysia. These present results will contribute to the existing genotypic data of this species from Malaysia, thus merit further investigation as potential vector that related to any tick-borne diseases.

Keywords: Ticks, *Dermacentor compactus*, *mt-rrs*, phenotypic, genotypic

ABSTRAK

Pengenalpastian spesies sengkenit *Dermacentor compactus* (Acari: Ixodidae) dari Malaysia masih boleh diperdebatkan dalam pelbagai aspek dan masih kurang dikaji terutamanya pada

peringkat belum matang. Kajian ini dijalankan untuk mencirikan secara morfologi dan molekul sengkenit *D. compactus* yang dikumpulkan dari hutan simpan berhampiran Institut Penyelidikan Perhutanan Malaysia (FRIM). Sebanyak tiga individu dewasa (2 betina; 1 jantan) sengkenit *D. compactus* pada daun atau pokok renek telah dikutip menggunakan tangan. Pengecaman spesies dijalankan berdasarkan morfologi menggunakan kekunci taksonomi spesifik, di mana ciri molekul *D. compactus* dianalisis menggunakan gen mitokondria 16S rDNA (*mt-rrs*). Pohon *neighbor-joining* dibina untuk menerangkan variasi genetik dan pencirian di antara *D. compactus*. Analisis pengelompokan menggunakan gen *mt-rrs* menunjukkan bahawa jujukan *D. compactus* membentuk klad monofiletik dengan sokongan 100% nilai bootstrap. Tambahan lagi, variasi intraspesies yang rendah (1%) diperhatikan di antara spesies *D. compactus*. Sementara itu, jarak genetik *D. compactus* juga menyokong bahawa spesies ini berbeza secara genetik dari spesies *Dermacentor* lain dengan nilai interspesies yang tinggi (>13%). Kajian ini telah melaporkan jujukan gen 16S rDNA untuk *D. compactus* yang pertama di Malaysia. Hasil ini akan menyumbang kepada kewujudan data genotip sengkenit sedia ada dari Malaysia, oleh itu penyelidikan lanjut diperlukan untuk mengkaji potensi sebagai vektor yang berkaitan dengan penyakit bawaan sengkenit.

Kata kunci: Sengkenit, *Dermacentor compactus*, *mt-rrs*, fenotip, genotip

INTRODUCTION

Ticks are important vectors that carry numerous pathogenic and non-pathogenic microorganisms that cause diseases for animals and humans. A total of 720 species of ixodid ticks have been identified worldwide (Barker & Murrell 2004; Li et al. 2018). In Malaysia, there are 34 species of ticks belonging to the genera *Amblyomma, Ixodes*, *Haemaphysalis, Rhipicephalus* and *Dermacentor* (Ernieenor et al. 2017). However, the recent compilation of data showed an increase of 45 species (Madinah et al. 2021; Petney et al. 2019). Ticks of the genus *Dermacentor* Koch, 1844 are important vectors and reservoirs of bacilli, piroplasms, viruses and other pathogens in humans and livestocks (Sun & Xu 2013). Species taxonomy of the genus *Dermacentor* is still debatable in many aspects, and morphological identification of this genus is one of the most challenging tasks among the ixodid ticks (Apanaskevich & Apanaskevich 2015a). Immatures of *Dermacentor* extracted in a study of intra-aural ticks in Malaysia failed to be further identified up to the species level due to the unavailable of the taxonomic keys for the nymph and larval stages (Mariana et al. 2008a), and unavailable of comprehensive work on their systematic in most countries (Ernieenor et al. 2020; Perry 2014).

In Southeast Asia, six species of the genus *Dermacentor* were recorded namely *D. compactus, D. auratus, D. steini, D. atrosignatus, D. limbooliati,* and *D. tamokensis* (Apanaskevich & Apanaskevich 2015a, 2015b; Petney & Kierans 1996; Petney et al. 2019). Among these species, *Dermacentor compactus* Neumann, 1901 has been reported in China, Vietnam, Indonesia, Thailand and Malaysia (Sun & Xu 2013). Main hosts of the adults of *D. compactus* were reported as wild suids (Apanaskevich et al. 2020; Hoogstraal & Wassef 1984; Kolonin 1995), while the immature stages appeared on various species of squirrels and murid rodents (Apanaskevich 2016). However, the medical and veterinary significance of this species has not been adequately investigated. A literature review shows that *Dermacentor* spp. is one of the vectors for *Rickettsia* pathogen in Southeast Asia (Ishak et al. 2019). In Malaysia, *Dermacentor* was identified to be the most related genus (99.7%) for human otoacariasis cases, and *D. compactus* was reported as the second abundant species following *D. atrosignatus* (Mariana et al. 2008a). In that study, 82.4% of them were infested by the nymphal stage and it was quite challenging to differentiate the species within *Dermacentor* due to taxonomic

confusion caused by several overlapping species characters (Ernieenor et al. 2020; Petney & Kierans 1996). Since *D. compactus* feeds on humans, then it may share hosts with the other *Dermacentor* ticks. Thus, precise identification is necessary to avoid the possibility of the species co-infection.

Accurate identification of the specimens collected is highly important, as this is a crucial step in a chain of microbiological or epidemiological studies (Estrada-Pena et al. 2017). While there has been an increase in global awareness of ticks and tick-borne pathogens, there is still lacking adequate information on the identity of ticks in this country. In practice, species identification and differentiation of ticks have been based on morphological characters. These phenotypic approaches are economical and convenient, but substantially obscure due to limited acarology experts, inaccurate or misidentification of close-related species and difficulties due to variation caused by blood meal (Mangold et al. 1998; Lv et al. 2014a). Moreover, the morphological taxonomic keys for ticks covered only the species of medical interest, and hardly consider the larvae and nymphs (Estrada-Pena et al. 2017). Molecular tools have been applied for taxonomy studies of several tick species as an alternative to circumvent the constraints of morphological identification. Several species identification of ticks through gene sequencing such as ribosomal 12 subunit [12S rRNA] (Lv et al. 2014b), ribosomal 16 subunit [16S rRNA] (Li et al. 2017), the Cytochrome c oxidase subunit I [COI] (Ernieenor et al. 2017) and the second internal transcribed spacer [ITS2] (Soltan-Alinejad et al. 2020) were reported. The performance of 16S rDNA gene has been evaluated to identify hard and soft ticks (Black & Piesman 1994) while Takano et al. (2014) was successfully applied this molecular marker in their study for the identification of hard ticks in Japan. Few studies have found that the 16S rDNA gene is easier to align, variable enough to differentiate between species and has the most registered sequences in GenBank (Krakowetz et al. 2010; Norris et al. 1999; Takano et al. 2014).

No study has been conducted in Malaysia on the identification of local *D. compactus* ticks using a well-defined molecular approach. In the present study, we utilized *mt-rrs* gene for verifying its morphological status that could accurately distinguish *D. compactus* from other *Dermacentor* species. The genetic affiliation of the species was further analyzed by constructing a topology tree using phylogenetic analysis based on *mt-rrs* sequences. This molecular information regarding *Dermacentor* ticks found in this region will provide a better knowledge of the relationships between various *Dermacentor* species, as well as more informative data for tick systematic studies.

MATERIALS AND METHODS

Collection of Ticks and Morphological Identification

Questing ticks were collected from the Forest Research Institute of Malaysia (FRIM), 16 km northwest of Kuala Lumpur between September 2018 and May 2019. By implementing direct handpicking method, questing adult ticks on lower vegetations or shrubs with a height 0.2–1.2 m from the forest floor were collected along tourist nature trails. The study site was chosen primarily because it was a tourist attraction area with waterfall, and it was based on previous data showing a high number of wild boars as potential tick hosts in this habitat (Ho & Krishnasamy 1991; Shabrina 1990). The ticks were kept individually in specimen bottles and brought back alive to the laboratory of Acarology Unit, Institute for Medical Research (IMR). All tick specimens were preliminarily sorted according to sex, life stage and species level on the basis of their external morphological characteristics. Briefly, the ticks' external special characters were recorded using a stereomicroscope (Model Stemi DV4 Zeiss, Germany) with 40X magnification using specific illustrated taxonomic keys (Kohls 1957; Wassef & Hoogstraal

1983). Dorsal and ventral images of adult ticks were photographed using a digital camera for species identification. The ticks were preserved in 70% ethanol for further analysis.

DNA Extraction

Prior to DNA extraction, the ticks were washed with 70% ethanol and rinsed in sterile distilled water to remove microorganisms from their surface (Ernieenor et al. 2017, 2020). Total genomic DNA was extracted from their whole body using QIAamp DNeasy Tissue Kit (Qiagen, Hilden, Germany) with minor modifications. In brief, the ticks were first homogenized in microcentrifuge tube filled with 80 µl of sterile phosphate buffer saline (PBS) followed by adding 100 µl of animal tissue lysis (ATL) buffer. The homogenates were incubated at 56°C for 6 h after adding 20 µl Proteinase K for complete lyses. Further steps were performed by a DNeasy Tissue Kit, as instructed by the manufacturer's protocols. The DNA was stored at -20°C for further investigations in PCR.

PCR and Sequencing Analysis

The genotypic characterization of *D. compactus* was performed using PCR targeting the 16S rDNA gene (*mt-rrs*). These primers (*mt-rrs1* and *mt-rrs2*) previously described by Ushijima et al. (2003) were used to amplify ~460 bp mitochondrial 16S rDNA gene sequence from ticks. The PCR reactions were conducted in a final volume of 50 µl. Each PCR mixture contains 25 µl of Taq PCR Master Mix 2X (Qiagen, Germany), 2.5 µl of each primer at 0.5 µmol/L, 15 µl of water and 5 µl of genomic DNA. The PCR was carried out using an Eppendorf Master Cycler Personal machine (Eppendorf, Germany) under the following conditions: 95°C for 1 min followed by 35 cycles of denaturing at 95°C for 15s, annealing at 50°C for 15s and extension at 72°C for 30s. All PCR products were visualized through electrophoresis in 1% agarose gel stained with SeeNA II Nucleic Acid Stain DNA (Mbiotech, Korea). The positive PCR products were then purified using Wizard® SV Gel & PCR Clean-Up System (Promega, USA) depending on the kit procedures before being sequenced in both directions with the Applied Biosystems BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystem, USA).

Phylogenetic Analysis

The obtained sequencing chromatograms of *mt-rrs* gene sequences were manually analyzed using MEGA 7.0 software (Kumar et al. 2016) and sequence alignments were performed using Clustal-W multiple alignments of BioEdit (Hall 1999) to determine the similarity of characters between the sequences. In pairwise sequence comparisons, all sites with alignment gaps and missing data were deleted. In addition, 13 of 16S rDNA tick sequences from the genera of *Dermacentor, Ixodes, Amblyomma* and *Haemaphysalis* from GenBank were downloaded and aligned simultaneously for phylogenetic analysis of *D. compactus* (Table 1). After trimming the low-quality sequences at both ends, the resulting 16S rDNA gene sequences were compared with GenBank entries using Blast on the NCBI website (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*) for identification of the tick species and detection of sequence contamination. Phylogenetic relationships were analyzed through neighbor-joining (NJ) method and clade robustness was determined by bootstrap analysis with 1000 replicates as implemented in MEGA 7.0. Genetic distances were calculated to further determine the intraspecies and interspecies among individuals in the data set using Kimura 2-parameter (K2P) models in MEGA. In both analyses, a soft tick, *Carios capensis* (GenBank accession no. AB057537) sequence was selected as an outgroup for the 16S rDNA gene.

Table 1. The 16S rDNA gene sequences implemented in the dataset of *Dermacentor compactus* for phylogenetic and genetic distance analyses

Sample name	Country	GenBank accession no.
D. taiwanensis	Japan	AB819169
D. taiwanensis	Japan	AB89168
D. auratus	Thailand	KC170746
D. auratus Fujita90	Thailand	unpublished
D. auratus 0831t	Thailand	unpublished
D. nuttali	China	KU594274
D. albipictus	USA	AY676458
D. andersoni	USA	L34299
D. atrosignatus	Thailand	KC170745
Amblyomma geomydae	Japan	AB819161
Haemaphysalis hystricis	Malaysia	LT593122
Ixodes granulatus	Japan	AB819235
Carios capensis	Japan	AB057537

RESULTS

A total of three (two females and one male) adult ticks were included in this study. From the dorsal view, ticks from the genus *Dermacentor* were morphologically identified according to their distinctive rectangular-shaped of basis capituli and short palps. Furthermore, *Dermacentor* ticks also was recognized by the presence of flattened eyes at the edge of scutum, eleven festoons at the bottom and coxae I to IV that gradually increase in size (Figure 1a). On the idiosoma, a pair of respiratory spiracular plate or stigmata with pores was identified just posterior to the coxa IV in the nymphs and adults of *Dermacentor* (Figure 1b). In ixodid adult ticks, their mouthparts (gnathosoma) can be seen from the dorsal view. In this study, the adult tick was identified as *Dermacentor compactus* based on their unique and distinctive characters which is two-well separated internal (closest to body midline) and external (farthest from body midline) spurs at Coxa I (Figure 1c) as described by Wassef and Hoogstraal (1983).

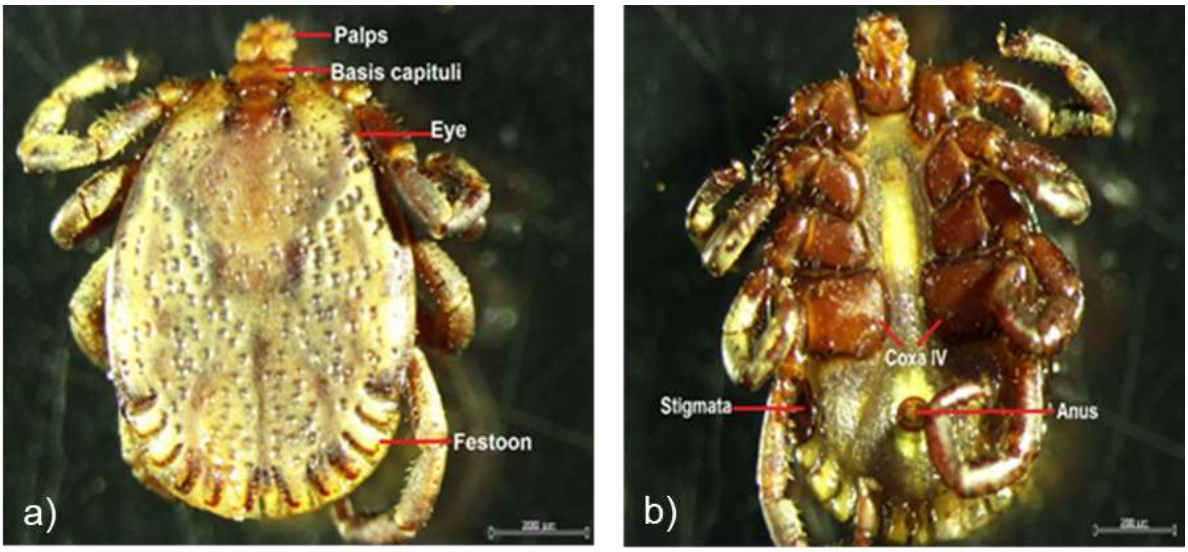

Figure 1. Habitus of *Dermacentor compactus* tick a) Dorsal view; b) Ventral view; c) Special character of the species

Female, ♀ [Based on 2 specimens (*D. compactus* B & C); Figure 2a, 2b]

Female *D. compactus* had a bigger size compared to male and measurement of adult females in the present study recorded a body length of approximately 8.0 mm with 4.2 mm breadth. Sexual dimorphism was verified by the presence of scutum of the female was finely granulated, broadly oval in shape and takes up only partial (1/3) of their dorsal surface to allow abdomen to enlarge when feeding and carrying egg (Figure 2a). The female *D. compactus* individuals were easily distinguished by its exceptionally small and narrow U-shaped genital aperture (Figure 2b).

Figure 2. Female habitus of *D. compactus* a) Dorsal view; b) Ventral view

Male, ♂ [Based on 1 specimen (*D. compactus* A); Figure 3a, 3b]
Body length from palp to scutum was approximately 6.2 mm and width 4.0 mm. Scutum was broadly oval with few punctuations and short of basis capituli. The coloration of dorsal integument as presented in Figure 3a; mostly brown color with a short pale yellowish at middle part of the body. The dorsal scutum was well developed and covers almost entire dorsal of their body. In this study, the adult male of *D. compactus* was differentiated from other *Dermacentor* species by the presence of only 2 short and sub-equal length spurs (rather than 3-6) on the coxa IV (Figure 3b). Furthermore, internal and external spurs on coxa I of the adults *D. compactus* are wide apart and they also have characteristic ornamentation pattern for each sex. In all *D. compactus* individual, coxae II - III consist of a relatively short and triangular shaped of spurs, whereas coxa IV enlarged in size.

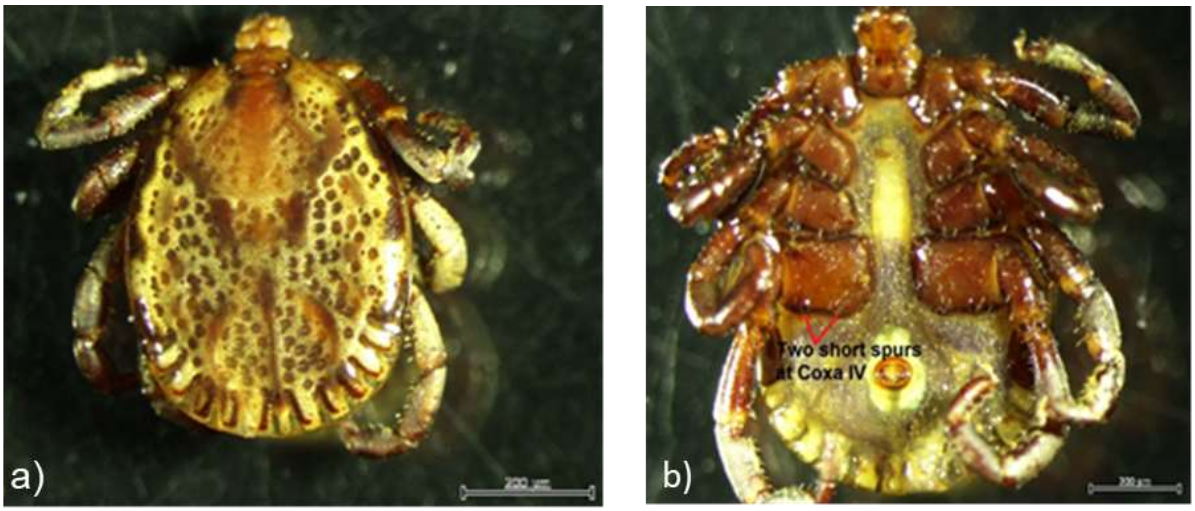

Figure 3. Male habitus of *D. compactus* a) Dorsal view; b) Ventral view

After successful amplification under optimal conditions, approximately 454 bp (including the primers) of PCR products were generated. BLAST comparison using 16S rDNA showed similarity between 88% and 87 % with the nearest species were *D. atrosignatus* and *D. taiwanensis* (GenBank accession numbers KC170745 and AB819169), respectively. In addition, our local sequences showed lowest similarity range from 83-86% with other *Dermacentor* species deposited in the online databases. Verification of the species morphology has been confirmed by an expert (acarologist), Dmitry Apanaskevich (pers. comm.). The 16S rDNA sequences of the *D. compactus* have been deposited in GenBank database under the accession number MK316359-MK316361.

A total of 428 bp data matrix were obtained from the multiple alignments of the 16S sequences. The mean nucleotide content of the 16S rDNA revealed that the adenine was the most abundant (41.2%), followed by thymine (35.7%), guanine (14.5%), and finally cytosine at merely 8.6%. These sequences indicated that of 428 bp of characters analyzed, 179 (41.8%) were variable sites and 240 (56.1%) were conserved sites, with 124 (29%) parsimony-informative characters.

The neighbor-joining tree presented monophyly of the local *D. compactus*. The formation of a significant grouping of this three local *D. compactus* (A, B and C) in independent monophyletic clade was obtained and supported by 100% bootstrap (Figure 4). Parallel to the

D. compactus clade, other *Dermacentor* species; *D. atrosignatus, D. auratus, D. nuttalli, D. albopictus* and *D. andersoni* formed in a clade, while the *D. taiwanensis* located at the basal part of the tree. The separation of the *D. compactus* ticks from the other species, supported with 99% bootstrap value. Pairwise distance analysis amongst local *D. compactus* showed the closest genetic distance with a value 0.01 (Table 2). However, a high level of genetic distance (0.13-0.15) was obtained between local *D. compactus* and other *Dermacentor* species such as *D. auratus* and *D. atrosignatus*.

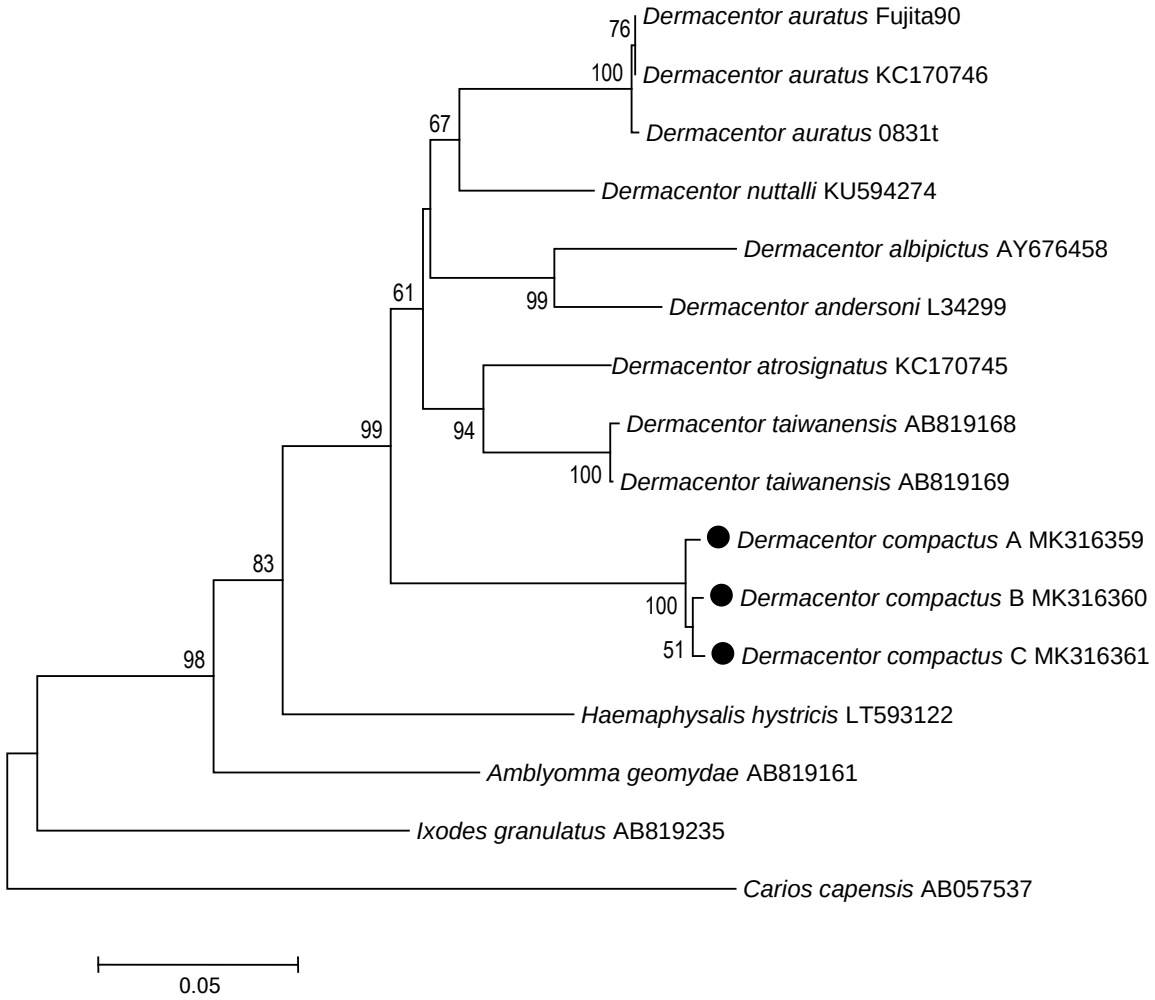

Figure 4. Neighbor-Joining tree for *Dermacentor compactus* using 16S rDNA sequences and generated using three sequences obtained in the present study (indicated as ●), and 13 representative sequences from GenBank. The numbers at the nodes represent the percentage of 1000 bootstrap replicates.

Table 2**.** Intra- and inter-species analysis of genetic distance values based on *mt-rrs* sequences amongst *D. compactus* and other *Dermacentor* species documented in GenBank

Species	***I. granulatus***	***A. geomydae***	***H. hystricis***	***D. auratus* Fujita90**	***D. auratus* 0831**	***D. auratus***	***D. atrosignatus***	***D. nuttalli***	***D. taiwanensis***	***D. taiwanensis***	***D. albipictus***	***D. andersoni***	***D. comp* A**	***D. comp* B**	***D. comp* C**
I. granulatus	-														
A. geomydae	0.21	-													
H. hystricis	0.21	0.17	-												
***D. auratus* Fujita90**	0.26	0.18	0.16	-											
***D. auratus* 0831t**	0.26	0.18	0.16	0.00	-										
D. auratus	0.26	0.18	0.16	0.00	0.00	-									
D. atrosignatus	0.23	0.16	0.15	0.10	0.10	0.10	-								
D. nuttalli	0.23	0.15	0.16	0.08	0.08	0.08	0.09	-							
D. taiwanensis	0.24	0.16	0.17	0.09	0.10	0.09	0.07	0.09	-						
D. taiwanensis	0.24	0.15	0.16	0.09	0.10	0.09	0.06	0.09	0.00	-					
D. albipictus	0.26	0.20	0.18	0.13	0.13	0.13	0.12	0.11	0.14	0.14	-				
D. andersoni	0.25	0.19	0.17	0.10	0.10	0.10	0.11	0.10	0.11	0.11	0.07	-			
***D. comp* A**	0.25	0.21	0.18	0.14	0.14	0.14	0.13	0.12	0.13	0.13	0.17	0.15	-		
***D. comp* B**	0.25	0.21	0.18	0.15	0.15	0.15	0.13	0.12	0.13	0.13	0.17	0.15	0.01	-	
***D. comp* C**	0.24	0.21	0.18	0.15	0.15	0.15	0.13	0.12	0.13	0.13	0.17	0.14	0.01	0.01	-

DISCUSSION

Accurate identification of ixodid ticks is a crucial step in the epidemiological studies, especially to establish the species distribution, determine their natural hosts, and to understand their life cycles in order to prevent the tick-borne diseases. *Dermacentor* is a newly evolved genus of ixodid ticks with 40 identified species worldwide (Perry 2014; Petney et al. 2007). Of these, six species from the genus *Dermacentor* have been identified morphologically including *D. compactus* and are widely distributed in Peninsular Malaysia (Petney et al. 2019). Despite their medical and veterinary importance (Apanaskevich 2016; Khoo et al. 2016; Mariana et al. 2008b) the systematics and phylogenetics of the *Dermacentor* species are poorly resolved.

In the present study, the tick specimens collected from the recreational forests in Selangor belong to the species *D. compactus*. The identification was conducted based on the morphological characters; widely separated short internal and external spurs of coxa I for both sexes as described by previous studies (Sun & Xu 2013; Wassef & Hoogstraal 1983). Studies by Apanaskevich (2016), Hoogstraal & Wassef (1984) and Mariana et al. (2011) have reported the occurrence and distribution of this species in Malaysia. Then, the nature lovers should be warned on the possibility of getting tick-bites from questing *D. compactus* ticks as the ticks present on the lower vegetation along the tourist trails (Kisomi et al. 2016). Visitors at FRIM should be reminded and advised to take personal precautions to avoid or reduce tick contact, as their existence is simply an indication of the possible risk. The presence of wild boars, *Sus scrofa* in this location was seen many times and discovered during the tick collection. Another evidence of their occurrence was the disturbed soil due to the digging activities for earthworms searching by the wild boars. The wild boars have been identified as the main host of *Dermacentor* ticks in Malaysia and the most common host of *Dermacentor* in tropical Asia (Hoogstraal & Wassef 1984; Mariana et al. 2008b; Petney & Kierans 1996). This finding was in accordance with previous studies that reported the habitats of ticks are associated with their host and local environment (Chae et al. 2017; Chong et al. 2013). It is also speculated that the wide distribution of tick species is likely due to the surrounding temperature and ecological factors such as the humid environment in the recreational forest and the presence of a river which may benefit tick vectors and their animal hosts' survival (Estrada-Pena et al. 2012; Mohd-Taib et al. 2018).

The mt-rrs1 and mt-rrs2 are universal DNA primers that extensively used in species identification and phylogenetic study due to their successful ability to amplify a ~450 bp region of the mitochondrial 16S rDNA gene from a diverse range of arthropods. Our findings show that using the 16S rDNA gene can sufficiently identify and discriminate *Dermacentor* tick species at the species level. Neighbor-joining tree topology also revealed a close grouping of local *D. compactus* with a 100% bootstrap value. The high bootstrap support of this node could be attributed to amino acid homoplasy in the 16S rDNA sequences (Krakowetz et al. 2010). On the other hand, the 16S rDNA gene offer several advantages as mitochondrial DNA including a shorter sequence length (<450 bp) and can be obtained in a single reaction. In addition, the sequence of this gene's fragment is the most commonly found in the GenBank (Takano et al. 2014) which is the return to the fact that mitochondrial genes are superior for molecular identification and phylogenetic studies of ticks due to their wide coverage as barcoding genes (Li et al. 2017).

In this study, the BLAST sequences were insufficient to produce maximum homology of the *D. compactus*. The discrepancies arise from the lack of *D. compactus* sequences in the international databases due to their restricted distribution to Southeast Asia. Furthermore, the

lack of genotypic studies contributes to their limited representation of genetic data in international databases. It is also recommended that a subset of samples from different localities in Peninsular Malaysia should be sequenced prior to the genetic diversity study of this tick species. Moreover, low percentage similarity value (87-88%) shown by the three individuals of *D. compactus* to the nearest species of *D. taiwanensis* and *D. atrosignatus* could be associated with the cryptic hybridization factor or geographical separations due to nucleotide substitutions (Rees et al. 2003). The present study also is the first to include *D. compactus* in a phylogenetic analysis of this genus worldwide. Based on the limited molecular data available, the neighbor joining tree presented a monophyletic clade of the *D. compactus* and with high bootstrap support and was congruent with morphological traits. Regarding the genetic distance, *D. compactus* ticks had a low intraspecific variation, but a high interspecific value compared to other species in the same genus. Thus, these observations have proven that the 16S rDNA gene can determine the genetic variation and taxonomic position of local *D. compactus* of Malaysia. The single-gene analysis used in this study may also merits further investigation in order to expand our understandings of the phylogenetic relationship of *D. compactus* as one of potential vector for tick-borne diseases in Malaysia. Additional research including the use of the multi-locus sequencing approach and exploit other mitochondrial DNA gene sequences will be required to verify this species genetic relationship.

Molecular data on *Dermacentor* ticks collected in this area will become a standard approach for tick taxonomy confirming morphological identification, as well as provide some data that may be used in their systematic (Apanaskevich & Apanaskevich 2015a, 2015b). The presented phylogenetic analyses in this study were the pioneer ones as there is no report of similar molecular taxonomy of *D. compactus* neither in Malaysia nor in the world. Although genotypic analysis, such as the *mt-rrs* used in this study, sometimes did not correlate with phenotypic-based analysis for phylogeny, we believe that the former could be a useful alternative to the latter for accurate identification of tick species (Lv et al. 2014b). Our findings also underscore the need for extensive distribution studies of *D. compactus* in recreational forests within this region.

CONCLUSION

In conclusion, this study present morphological data of adult male and female of *D. compactus* was supported by genotypic data using 16S rDNA gene sequence. The NJ tree showed a very clear grouping of the *D. compactus*, which supported with high bootstrap value. The recent study represents the first genetic characterization of the local adult of *D. compactus* tick from Malaysia. The present work also pointed out the existence of *D. compactus* in a recreational forest of Peninsular Malaysia and has greatly improved our confidence in the morphological identification of this tick species based on its morphological characters. It would be interesting to expand the study by using a large number of individual ticks at different life stages. Precise identification of the *D. compactus* ticks and the hosts will outline their geographical distribution in Malaysia, thus controlling potential tick-borne infection.

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