



Short communication

Amblyomma cordiferum Neumann, 1899 (Acari: Ixodidae) parasitizing reticulated pythons, *Malayopython reticulatus* (Schneider, 1801) (Reptilia: Pythonidae) in Peninsular Malaysia

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ABSTRACT

Two ticks were collected from a reticulated python (*Malayopython reticulatus*) caught in Tumpat District, Kelantan, Peninsular Malaysia. The ticks were first identified as *Amblyomma* sp. through morphological comparison with identification keys. Determination of the tick species was made through PCR and sequencing. However, BLAST analysis revealed 85–88% sequence nucleotide identity with *Amblyomma nitidum* and *Amblyomma geoemydae*. Additionally, the morphological features of the ticks collected in this study did not match either *A. nitidum* or *A. geoemydae*. Further examination of the ticks confirmed the species as *Amblyomma cordiferum*. This is the first record of *A. cordiferum* DNA sequence with morphological support of colour illustrations for adult *A. cordiferum*. This is also the most recent record of this host association in Peninsular Malaysia. Information from this report can serve as a reference for species identification using the described morphology or molecular sequences.

1. Introduction

The reticulated python, *Malayopython reticulatus* (Schneider, 1801) (Reptilia: Pythonidae), formerly known as *Python reticulatus*, is the longest nonvenomous snake (max length: 10 m) found in South Asia and Southeast Asia. Like other animals, this snake is prone to parasitism by ticks. In some circumstances, this infestation may cause physical obstruction, paralysis and skin conditions that may lead to secondary infection (Law, 2014; Catherine et al., 2017). *Amblyomma cordiferum* (Acari: Ixodidae) was first reported in Malaysia by Audy (1960) on *P. reticulatus*. Except for the current report, the most recent record of this species was from Horsfield's fruit bat (*Cynopterus horsfieldii*) from a survey of acarine ectoparasites in 2002–2009 (Ahmad et al., 2013). As vectors of various pathogens that affect both humans and animals, its importance in public health should not be neglected.

2. Materials and methods

In September 2018, a reticulated python was caught near a housing area in Tumpat District, Kelantan, Malaysia. Two ticks were observed

on the snake during physical examination, located at the proximal quarter of the snake. The ticks were collected and killed in 70% ethanol, then observed under a SZ61® zoom stereomicroscope (Olympus, Tokyo, Japan). The external features of the ticks were examined, and identification was done based on morphological characters described by Robinson et al. (1926) and Kohls (1957). All specimens were deposited in the Laboratory of Parasitology, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (accession number: T26).

Supportive confirmation through PCR identification was performed using genomic DNA extracted from the internal tissues of the ticks with a Genomic DNA Mini Kit (Geneaid, Taiwan). The mitochondrial 16S gene PCR assay used in this study was adopted from Lv et al. (2014). The primers used were 16S-F: 5'-TTA AAT TGC TGT RGT ATT-3' and 16S-R1: 5'-CCG GTC TGA ACT CAS AWC-3' (Lv et al., 2014). Sequence information of the ticks was compared with the sequences available in the GenBank of National Centre for Biotechnological Information (NCBI - www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) program. The representative sequence was then submitted to the GenBank database.

Prior to phylogenetic analysis, all the sequences were blasted

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Fig. 1. Close-up on the morphological features of *Amblyomma cordiferum* shows the elliptical eyes which are flat and located at the lateral margin of the scutum (A); hypostome (ventral view) with the length of approx. 1 mm, showing a dental formula 4/4 (B); Coxae I-II with 2 triangular spurs, external spur slightly larger than internal spur (C); anal groove situated posteriorly to the anus and 11 festoons can be observed (D).

against the GenBank database to search for *Amblyomma* species with a high similarity. A phylogenetic tree was constructed by using the Neighbor-joining statistical method with 1000 bootstrap replications and analyzed based on the Tamura 3-parameter (T92) model (Tamura, 1992). In this study, *Dermacentor reticulatus* and *Rhipicephalus appendiculatus* were used as outgroups. Experimental search for the tree was obtained automatically by applying the Neighbor-Joining algorithm. Evolutionary rate differences among sites were modeled using Gamma distribution (shape parameter = 1). All the evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

3. Results and discussion

Ticks collected were first identified as *Amblyomma* sp. based on their morphological characteristics. Measurements of these female ticks revealed a body length of approximately 10.0 mm (partially engorged) and 6.5 mm (unfed) respectively, with the scutum covering less than half of the body. The scutum is rhomboid and does not bear any dark brown ornamentation. Eyes are flat and elliptical, located at the lateral margins of the scutum (Fig. 1a). Palpi are long and parallel with a dental formula of 4/4 (Fig. 1b). Coxae I-II possess visible short, triangular internal spurs (arrows in Fig. 1c) that are absent on coxae III-IV; the internal spur on coxa III is reduced as a tubercle; all coxae possess distinct external spurs that are pointed and triangular (Fig. 1c). The genital aperture (Fig. 1c) is situated at a level between coxae II and III. Laterally, the spiracular plates (Fig. 1d) contain large maculae and are comma-shaped. The anal groove (Fig. 1d) is curved and situated posteriorly to the anus, and the posterior body margin bears 11 distinct festoons.

DNA extracted from the internal tissues of the tick yielded a PCR product of approximately 455 bp. BLAST analysis revealing only 85–88% sequence nucleotide identity with those sequences available from GenBank, which is *Amblyomma nitidum* and *Amblyomma geoemydae*. However, the tick specimens collected in this case are unlikely to be these two species based on their morphological features. *Amblyomma nitidum* is prominently different from *A. cordiferum* on its triangular scutum and single spur on coxae II to IV (Robinson et al., 1926). Besides, *A. nitidum* is host-specific to amphibious sea snakes of

the genus *Laticauda* that live and breed in isolation from other species of snakes (Hayashi and Masunaga, 2001; Nadchatram, 2006). *Amblyomma geoemydae*, which has commonly been reported from several snake species in Japan (Takahashi et al., 2012, 2017), was also not a morphological match as the characters of dorsal foveae, ornamented scutum and only coxa I with an internal spur, were not observed in our specimens (Robinson et al., 1926; Kohls, 1957; Yamaguti et al., 1971).

Both ticks were eventually identified as *A. cordiferum* based on their morphological features (Anastos, 1950; Voltzit and Keirans, 2002). All the morphological features matched with previous descriptions by Anastos (1950) and Norval et al. (2008). It was also noted that our specimen possesses a tubercle (reduced internal spur) on coxa III, as reported by Anastos (1950) and Voltzit and Keirans (2002). This feature differs from *A. cordiferum* found in Taiwan, where the internal spur was absent on coxa III, and this is believed to be a distinctive feature for this species in the locality (Norval et al., 2008).

DNA sequence or genome sequence of *A. cordiferum* has yet to be recorded in any of the databases available from GenBank. Therefore, a representative sequence was submitted to the GenBank database under accession number MK301096. Phylogenetic analysis revealed *A. cordiferum* to share the sub sister Clade I with *A. nitidum* with a low bootstrap value (39%); and to a larger sister Clade I, together with *A. geoemydae* with bootstrap value of 71% (Fig. 2). The division of this sister clade in this way may be due to the Asian originality of these three tick species, namely Malaysia, Thailand and Japan, respectively.

Reports of tick parasitism on wild snakes are relatively scarce. This might be due to the difficulties in snake sample collections and the scarcity of skilled personnel to handle snakes. Voltzit and Keirans (2003) stated that *A. cordiferum* is a rare tick species, which was corroborated by Norval et al. (2008) from the infrequent records of this tick on wild-caught snakes in Taiwan. Very little is known about the biology of this tick, but it was observed to exhibit parthenogenesis (Ho and Ismail, 1984). While members of the genus *Amblyomma* are known to be vectors of various pathogens that can cause diseases in both humans and animals, there is still a lack of evidence on the natural transmission of pathogens from *A. cordiferum* to humans. However, the increased international trade in live reptiles has contributed to the introduction of exotic ticks worldwide and to the transmission of tick-

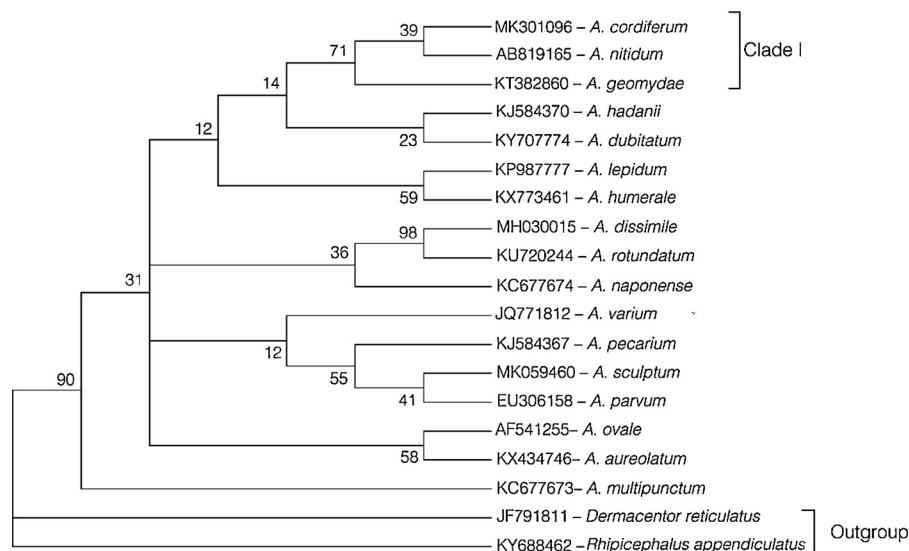


Fig. 2. Phylogenetic analysis of *Amblyomma cordiferum*.

borne pathogens (Karesh et al., 2005). The diversity of apparent suitable host species for *A. cordiferum* suggests that this species warrants more attention as a potential vector for infectious disease agents to other animals and even humans (Nadchatram, 2006).

4. Conclusion

We report the most recent record of *A. cordiferum* from *M. reticulatus* in Malaysia and provide first DNA sequences and colour figures of female *A. cordiferum* to facilitate identification of this tick species. Based on the current report, collection records of this tick species found on snakes in Malaysia has been updated. With regard to public health importance and zoonotic concern, further studies of this relatively rare tick species may increase our understanding of its ecological requirements and distribution in Malaysia.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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