# *CANDIDATUS* MIDICHLORIA SP IN A *RHIPICEPHALUS SANGUINEUS* S.L. NYMPHAL TICK COLLECTED FROM A CAT IN THAILAND

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**Abstract.** *Candidatus* Midichloria mitochondrii is a bacterial endosymbiont found in multiple tick species. It is released in tick saliva increasing the opportunity for it to spread to and cause disease in vertebrates, including humans. We report here finding *Candidatus* Midichloria sp in a *Rhipicephalus sanguineus* s.l. nymphal tick collected from a cat (*Felis catus*) in Surat Thani Province, southern Thailand. Tick was removed from a cat and identified to species level by molecular taxonomy. PCR and sequencing were conducted and confirmed the presence of *Candidatus* Midichloria sp. A phylogenetic tree was constructed to see the evolutionary relationship of this bacterium with other similar species. Phylogenetic analysis of the 16S rRNA of the isolated *Candidatus* Midichloria sp revealed it is closely related to *Candidatus* Midichloria sp found in a *Haemaphysalis wellingtoni* tick found on a chicken and is related to *Candidatus* Midichloria sp found in several tick genera. Our bacterium was from a branch different from that *Candidatus* Midichloria sp found in a *Rhiphicephalus sanguineus* tick reported from Israel. This is the first report of *Candidatus* Midichloria sp found in the nymphal stage of a *Rhipicephalus sanguineus* s.l. tick found on a cat. This is also the first report of *Candidatus* Midichloria mitochondrii in Thailand.

**Keywords:** *Candidatus* Midichloria sp, *Rhipicephalus sanguineus* s.l., tick, Thailand

## INTRODUCTION

Arthropod vectors can carry symbiotic organisms that may be potential pathogens. Symbiotic microorganisms of arthropod vectors are significant in uncovering the transmission of pathogens by bloodsucking vectors. *Candidatus* Midichloria mitochondrii (*Midichloria mitochondrii*) is a bacterial symbiont found in ticks (Beninati *et al*, 2004). It was previously called IricES1 and is found in the ovarian cells in the *Ixodes ricinus* tick, both in the cytoplasm and in mitochondria, where it resides in the intermembrane space (Beninati *et al*, 2004). It has been found in

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tick salivary glands raising the possibility it could potentially be infectious to vertebrates such as humans. Sassera *et al* (2006) proposed naming this bacteria '*Candidatus* Midichloria mitochondrii'. *Midichloria mitochondrii* belongs to the order Rickettsiales. Beninati *et al* (2009) found this bacterium in the *Ixodes holocyclus* tick but not in its mitochondria. Harrus *et al* (2011) detected it in *Rhipicephalus sanguineus* s.l. and *Hyalomma* spp ticks in Israel. It has also been found in *Amblyomma americanum* ticks in the eastern United States (Williams-Newkirk *et al*, 2012). Electron microscopy of *Rhipicephalus bursa* ticks showed it infects the mitochondria of ovarian cells (Epis *et al,* 2008).

Sassera *et al* (2008) used quantitative PCR to examine its growth and death and found its lifecycle corresponds to the phases of engorgement and moulting in *I. ricinus* ticks with a burst of growth after a blood meal. Bazzocchi *et al* (2013) suggested these bacteria may represent a novel group of vector-borne agents with the potential to infect mammalian hosts. Mukhacheva *et al* (2017) found *M. mitochondrii* is released in tick saliva, raising the possibility it could be infectious to vertebrates, including humans. Therefore, it is important to identify potential reservoirs of this bacterium. We report here finding *M. mitochondrii* in a *R. sanguineus* s.l. tick collected from a cat in Thailand.

## MATERIALS AND METHODS

### Tick collection, identification, PCR and DNA sequencing

In June 2014, a nymphal tick was collected from a cat (*Felis catus*) in Surat Thani Province, southern Thailand (Latitude: 9.104568, Longitude: 99.376796). The tick was removed from the cat and kept in 70% ethanol at 4°C before species identification (Kohls, 1957). The sampled tick was cleaned before DNA extraction with 70% ethanol, 10% sodium hypochlorite and sterile water of three replicates each. We conducted molecular taxonomy of the tick using a primer set consisting of the 16S + 1 and 16S − 1 to detect tick 16S mitochondrial DNA (16S mDNA) (Black and Piesman, 1994). Each PCR reaction was conducted using fd1-rp2 primers to detect bacteria as described previously (Weisburg *et al,* 1991). The PCR fragment of the sample positive for bacteria was then purified and sequenced.

### Phylogenetic analysis

The neighbor-joining (NJ) technique was used to identify phylogenetic relationships using MEGA5 (Tamura *et al*, 2011). Bootstrap values >50% were noted for the branches of 1,000 replicates (Fig 1).

## RESULTS

Tick collected from a cat in Surat Thani was identified as a *Rhipicephalus sanguineus* s.l. nymphal tick by molecular taxonomy using 16S mDNA gene. The 16S mDNA gene sequences of *Rhipicephalus. sanguineus* s.l. nymph was submitted to GenBank (GenBank: MF287371). In addition, sequences for *Candidatus* Midichloria sp in a *R. sanguineus* s.l. nymphal tick collected from a cat (*Felis catus*) was submitted to the GenBank database (GenBank: KY910125). The DNA sequencing result appeared to be closely related to the uncultured bacterium clone Hw124 16S ribosomal RNA gene, a partial sequence detected in the *Haemaphysalis wellingtoni* tick removed from a chicken in Thailand (GenBank: AF497583), with 98% identity (1053/1074 bp). It showed 95% identity (1025/1074 bp) to a *Candidatus* Midichloria mitochondrii partial 16S rRNA gene (GenBank: AJ566640), an endosymbiont of the tick *Ixodes ricinus*.

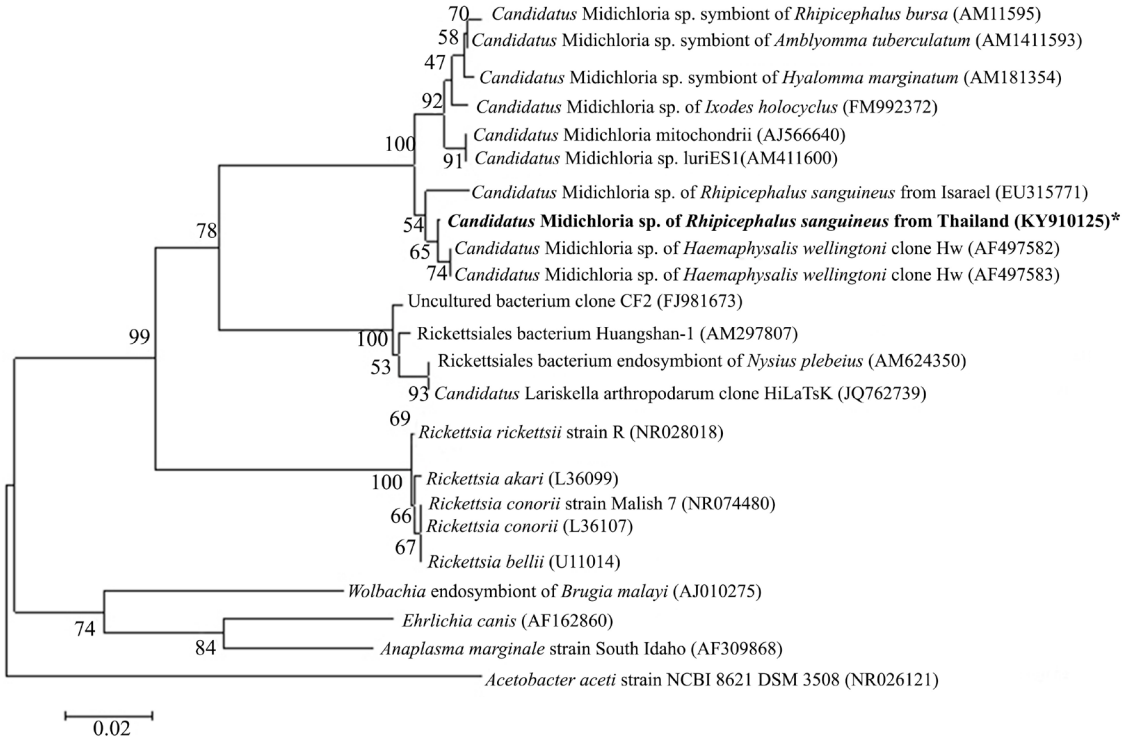


Fig 1–The phylogenetic tree constructed using the 16S rRNA gene of the studied *Candidatus* Midichloria sp obtained from the nymphal stage of a *Rhipicephalus sanguineus* s.l. tick collected from a cat in Thailand (asterisk). *Acetobactor aceti* was selected as an outgroup. The phylogenetic analysis was computed using the neighbor-joining method in MEGA5. Bootstrap tests of 1,000 pseudoreplicates are shown at the branch nodes.

Phylogenetic analysis with MEGA5 of *Candidatus* Midichloria sp 16S rRNA gene partial sequences showed this bacteria grouped with *Candidatus* Midichloria sp from *H. wellingtoni* from a chicken and was related to *Candidatus* Midichloria sp of *R. sanguineus* s.l. previously reported from Israel (GenBank: EU315771) (sister group). In addition, this bacterium was related to the *Candidatus* Midichloria sp from other tick genera (Fig 1).

## DISCUSSION

Mariconti *et al* (2012) provided evidence that *M. mitochondrii* has been found in tick salivary glands causing the possibility that *M. mitochondrii* is infectious to vertebrates. Cafiso *et al* (2016) highlighted the different prevalence levels and variable bacterial loads of this bacterium in different tick species which suggested different roles of *Midichloria* bacteria in different tick species. Our phylogenetic result showed *Candidatus* Midichloria sp in a *Rhipicephalus sanguineus* s.l. nymphal tick collected from a cat was related to the *Candidatus* Midichloria sp from other tick genera. Since these ticks are distantly related, this suggests '*M. mitochondrii*'

and its relatives may undergo horizontal transfer (Sassera *et al*, 2006). This is in agreement with the results of Duron *et al* (2017), that provided the evidence *M. mitochondrii* undergo occasional horizontal transfer events, as *Midichloria* strains of soft ticks appear to be, in a phylogenetic tree, scattered among those of hard ticks.

*Midichloria mitochondrii* was not found associated to *R. sanguineus* s.l. in the screening conducted by Epis *et al* (2008) (based on 11 females collected in Italy) or by Duron *et al* (2017) (although only 2 individuals were investigated collected in France, from dogs). It suggested that there may be a geographical variation in the association of *Midichloria* to some tick species, with a possible influence of the presence of *Midichloria* associated to other tick species found in sympatry, like *H. wellingtoni* in Thailand. Whether this *Candidatus* Midichloria sp found in *R. sanguineus* s.l. nymph in this study is an endosymbiont or is pathogenic for mammalian hosts, as well as its role in its tick vector needs further investigation.

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