Title

Diversity of Rickettsiales in the Microbiome of the Lone Star Tick, Amblyomma americanum

Running title

R. amblyommii and the lone star tick microbiome

Authors: Loganathan Ponnusamy\textsuperscript{a}, Antonio Gonzalez\textsuperscript{b}, Will Van Treuren\textsuperscript{b}, Sophie Weiss\textsuperscript{c}, Christian M Parobek\textsuperscript{d}, Jonathan J. Juliano\textsuperscript{d}, Rob Knight\textsuperscript{b,e}, R. Michael Roe\textsuperscript{a}, Charles S. Apperson\textsuperscript{a,f}, Steven R. Meshnick\textsuperscript{g,\#}

Affiliations

\textsuperscript{a}Department of Entomology, North Carolina State University, Raleigh, NC, 27695
\textsuperscript{b}Biofrontiers Institute, University of Colorado at Boulder, CO 80309
\textsuperscript{c}Chemical & Biological Engineering, University of Colorado at Boulder, C 80309
\textsuperscript{d}Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC
\textsuperscript{e}Howard Hughes Medical Institute, University of Colorado at Boulder, CO
\textsuperscript{f}Center for Comparative Molecular Medicine and Translational Research, North Carolina State University, Raleigh, NC; \textsuperscript{g}Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC.

L.P. and A.G. contributed equally to this manuscript

\#To whom correspondence should be addressed. 919-966-7414; meshnick@email.unc.edu
Abstract

Ticks are important vectors for many emerging pathogens. However, they are also infected with many symbionts and commensals, often competing for the same niches. In this paper, we characterize the microbiome of *Amblyomma americanum* (Acari: Ixodidae), the lone star tick, in order to better understand the evolutionary relationships between pathogens and nonpathogens. Multi-tag pyrosequencing of prokaryotic 16S ribosomal RNA genes (16S rRNA) was performed on 20 lone star ticks (including males, females and nymphs). Pyrosequencing of the *rickettsial Sca0* gene (also known as *ompA* or *RompA*) was performed on 6 ticks. Female ticks had less diverse microbiomes than males and nymphs, with greater population densities of *Rickettsiales*. The most common members of *Rickettsiales* were *Candidatus* Rickettsia amblyommii and *Candidatus* Midichloria mitochondrii. *R. amblyommii* was 2.6-fold more common in females than males and there was no sequence diversity in *Sca0* gene. These results are consistent with a predominantly vertical transmission pattern for *R. amblyommii*. 
Introduction

Tick-borne diseases are a growing public health problem in the US as well as globally (1, 12, 26). While the etiological agents of some diseases like Lyme disease and Babesiosis are well characterized, the causal agents for other diseases, such as Southern Tick Associated Rash Illness (STARI), have not been identified or well characterized (21). Identification of etiological agents in ticks is hindered by the complexity of its microbiome which contains many nonpathogenic microorganisms (2-5, 9, 17, 18, 23, 25, 37).

Ticks are commonly infected by intracellular bacteria of the order Rickettsiales. Some, like Candidatus Midichloria mitochondrii (14, 20, 25) and Wolbachia spp. (3, 5, 9, 28, 30) are not pathogenic to humans or other mammals. R. rickettsii, on the other hand, causes potentially fatal infections in humans (24). Additionally, there are some bacteria that appear to be associated with mild human infections, such as R. amblyommii and R. parkeri (5, 28). One obstacle to a better understanding of rickettsial disease, is the difficulty involved in diagnosis. Rickettsia organisms and DNA are extremely difficult to isolate from mammalian hosts. Furthermore, there is substantial serological cross-reactivity between rickettsial species (10). Thus, a better understanding of which Rickettsiales spp. are pathogens and which are not could lead to the development of better diagnostics.
Within arthropods, Rickettsiales species compete with each other. Rickettsiales species can be propagated vertically or horizontally. Vertical transmission occurs via infection of eggs and developing embryos. Vertically transmitted species attain a selective advantage by manipulating the arthropod’s reproductive outcomes, increasing the numbers of female offspring (reviewed in (29, 38)).

Horizontal transmission can be accomplished via infection of the intermediate blood-meal host. *R. rickettsii* could rely on horizontal transmission since it can cause severe infections and illness in mammalian hosts (24). *R. rickettsii*, in contrast, kills larval and nymphal ticks before they mature to reproducing adults; thus the *R. rickettsii* reservoir is not likely to be maintained by vertical transmission. Furthermore, the lethal effects of this pathogen on juvenile stages could prevent propagation of competing species which require vertical transmission (27). Since mammalian hosts, unlike ticks, are capable of an acquired antigen-specific immune response, one might expect to see signatures of balancing (diversifying) selection in pathogenic rickettsiae (40).

In order to better characterize the vectorial capacity and microbiome of the lone star tick, we performed 454 FLX-titanium amplicon pyrosequencing on bacterial 16S ribosomal RNA genes (16S rRNA) and the rickettsial Sca0 genes (also known as *ompA* or *RompA*) of *A. americanum* adults (male and female) and nymphs.
Material and methods

Tick Collection and Processing. Adult and nymphal lone star ticks were collected with a drag cloth from a single field near Siler City, Chatham County, NC, and preserved in 95% ethanol. In the laboratory, with the aid of a stereo microscope, ticks were sorted to species, sex and life stage. Sorted ticks were placed individually into 1.5-mL microcentrifuge tubes (USA Scientific, Ocala, FL) and then stored frozen at −80°C for subsequent extraction of genomic DNA.

DNA Extraction. Ticks were surface sterilized by rinsing for 1 min in each of the following solutions: 1% sodium hypochlorite; sterile phosphate buffered saline (PBS) [81mM Na2HPO4, 19mM NaH2PO4, 150mM NaCl, pH 7.4] and 70% ethanol. Finally, ticks were rinsed five times in sterile PBS for 1 min. DNA was extracted from ticks by a modification of a method described previously (35). Briefly, each tick was minced with a sterile blade and incubated at 37°C for 1 hour in 160 µL of lysis buffer 1 (TNE buffer [100 mM Tris; 0.2 M NaCl; 10 mM EDTA; pH 7.4] containing 20 µl of Lysozyme and 20 µl of Proteinase K). Subsequently, 200 µL of lysis buffer 2 (1% CTAB, 1.5 M NaCl; 0.5, Tris HCl, pH-8.0, 0.1M EDTA pH-8.0) was added with further incubation at 56°C for 1 hour. DNA was recovered through phenol/chloroform extraction and ethanol precipitation and the resulting DNA pellet was resuspended in 100 µl ultrapure water.

Subsequently, crude DNA was purified with the WIZARD DNA Cleanup System
(Promega, Madison, WI, USA) and quantified using a Nanodrop ND-1000. (NanoDrop Technologies, Montchanin, DE, USA).

**PCR amplification of tick mitochondrial 16S rRNA for Tick identification.** The quality of the prepared DNA was first assessed with primers (16S+1 and 16S−2) specific for tick mitochondrial 16S rRNA in a single-round PCR which yields a ~ 460-bp product (6). A 1.0-μl portion of extracted genomic tick DNA was amplified in a 25-μL reaction mixture containing 1X AmpliTaq Gold® 360 Master Mix (Life Technologies Corporation). Amplification was performed with a three-step program as follows: 10 min of denaturation at 94°C, followed by 30 cycles of 94°C for 60 s, 54°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 10 min. The PCR products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. The sequences of 16S rRNA were obtained using an ABI PRISM™ dye terminator cycle sequencing kit (PE Biosystem) and the primer 16S+1. The mitochondrial 16S rRNA sequences were compared to those in the GenBank database, using the Basic Local Alignment Search Tool (BLAST) and sequence homologies analysis.

**Amplification of Bacterial 16S rRNA and Sca0 Genes.** The hypervariable regions (V1–V3) of the bacterial 16S rRNA gene were amplified using genomic DNA from the 20 ticks. The primers contained 454 LifeSciences primers A and B (underlined), unique 10 bp barcodes (NNNNNNNNNN, Table S1) and the target-specific 27F and 534 R sequences (bold): (5′-
DNA amplifications were carried out in a final volume of 25 μL, using 2 μL of the extracted template DNA. The amplification mixture contained 1X AmpliTaq Gold® Master Mix (Applied Biosystems, Foster City, CA, USA). The PCR for each method was carried out in 25 μL reaction volumes in S1000 Thermal Cycler (BioRad, Hercules, CA, USA) with the following parameters: initial denaturation at 94°C for 10 min, followed by 30 cycles of 94°C for 40 s, 55°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 10 min. For the rickettsial Sca0 gene, we performed pyrosequencing using genomic DNA from two male (AaM20, AaM26), two female (AaF01, AaF04) and two nymphal (AaN45, AaN47) ticks. For Sca0, we used primers designed by Regnery et al. (34) to amplify the 5’ end (nucleotides 70-602) of the 190 kDa antigen with primer pair 190.70p and Rr190.602n.(34) to amplify the 5’ end (nucleotides 70-602) of the 190 kDa antigen with primer pair 190.70p and Rr190.602n. We amplified the PCR products for sequencing using reaction mixture as described above. Cycling parameters for the PCR were 94°C for 10 min followed by 30 cycles of 94°C for 45 s, 50C for 45 s, and 72°C for 1.50 min followed by a final 10-min extension step at 72°C.

**Gel Purification and Pyrosequencing.** Following PCR amplification, the presence of amplicons was confirmed by gel electrophoresis on a 1.5% agarose gel and staining with ethidium bromide. The band corresponding to bacterial 16S rRNA and Sca0 genes
was excised and DNA was purified using the QIAquick® Gel extraction kit (Qiagen, Inc., Valencia, CA). DNA in purified amplicons were quantified using Quant-iT PicoGreen kit (Invitrogen, Carlsbad, CA) and pooled in equimolar concentrations for pyrosequencing. Titanium method was carried out using the Titanium genomic kit at the Microbiome Core Facility in The School of Medicine, University of North Carolina at Chapel Hill, USA. For each tick, we performed three independent 16S PCR amplifications and 454 pyrosequencing runs, each time using two 1/8th plates. Thus, there were 6 datasets per tick.

**Bioinformatic analysis:** 16S sequence analysis was performed using the Quantitative Insights Into Microbial Ecology (QIIME) software package, version 1.7.0 (8). Each tick had three technical replicates which were analyzed as separate samples to test their reproducibility. Sequences were filtered based on length, with requirement that 200bp<sequence length<1000bp. Sequences were also filtered based on multiple quality metrics; if a sequence had more than 6 ambiguous bases and more than 6 homopolymers it was discarded and if the minimum average quality score for the sequence was below 25 it was discarded (as recommended by QIIME). Finally, no sequences with primer mismatches were accepted. To test the reproducibility between replicates we carried out 3 different OTU picking methods at 97% similarity: denoising (33) with chimeric sequence removal using Chimera Slayer (15), open reference picking using uclust (13) against Greengenes May 2013 (22) and usearch (13). To test the concordance between replicates we performed Procrustes analyses, and decided to use OTUs produced by the open-reference picking method (Table S2). In order to
To estimate the diversity richness of *Sca0* sequences, raw sequencing reads were first quality filtered and assembled against an *R. amblyommii Sca0* reference sequence using BWA-SW (19). Indexed alignments were then used as input for ShoRAH v0.6 (Short Reads Assembly into Haplotypes) (42), which performed error correction and haplotype reconstruction. The determined haplotypes were aligned and compared to one another and to all *R. amblyommii Sca0* sequences deposited in GenBank (queried August 13, 2013) to assess the diversity of this locus.
Results

Tick species confirmation

A ~ 460 bp sequence of the mitochondrial 16S rRNA gene was successfully amplified and sequenced for all 20 ticks. The sequences were 99 to 100% similar to that of *A. americanum*, confirming their taxonomic identification.

16S rRNA gene pyrosequencing results

A total of 156,948 quality sequences for 20 ticks were obtained with a minimum read length of 200 bp. The data were uploaded into QIIME. In order to assess replicability, each of the six 454 datasets for each tick was first analyzed individually. On visual inspection, there was good replicability between taxa on the genus level (Figure S1). Subsequently, the replicates for each tick were pooled and then analyzed. After rarefaction, analyses from 12 ticks (5 females, 4 males and 3 nymphs) passed quality control. Figure 1 shows the genus-level distribution of taxa highlighting some relevant taxa. The full distribution is given in Table S3 and has been uploaded onto the European Bioinformatics Institute (EBI) database (accession number ERP004063).

Alpha diversity

Since PCR efficiency can vary, alpha diversity was assessed using rarefaction curves. When males, female and nymph results were pooled rarefaction curves approached saturation (Fig. 2). Females were significantly less diverse than males or nymphs (p=.042 Chao1 estimator, p=.015 PD estimator, two-sample t test). Only 9 taxa (3
classifiable genera and 6 unclassified) represented ~70% of the population (Table 1). The numbers of observed taxa sampled per tick varied by up to 6-fold (Figure S2).

Three of the most common genera found were *Rickettsia*, *Midichloria* and *Ehrlichia*, all members of the order *Rickettsiales* (Fig. 1), representing 53% (31%-75%, median, interquartile range) of the reads. *Rickettsiales* represented a significantly higher proportion of reads in the 5 females (75%, 65-77) than in the 7 males and nymphs (45%, 0.4-53) (p=0.028, Mann-Whitney U test). The population fraction of the genus *Rickettsia* was 2.6-times higher in females than in males (Table 1). Thus, females had the lowest overall bacterial diversity but the highest population density of *Rickettsiales*.

A previous analysis of the *A. americanum* microbiome found an abundance of *Coxiella* (11). *Coxiella* were present in 10 of the 12 ticks in this study, but only represented a small percentage of the population (0.25%, 0.175 - 0.525) (Supplemental Table S3).

Two genera, which were common in the study by Clay et al, *Massilia* and *Duganella*, were not found in the current study. *Methylobacterium* (1.35%, 0.8 -1.8) and *Sphingomonas* (5.2%, 1.2 - 7.6) were about as common in this study as in the previous study and were found in every tick (Supplemental Table S3).

**Beta diversity**

Of the 237 genera, 93 (39%) were shared by females, males and nymphs (Figure S2). An additional 35 genera (15%) were shared by males and nymphs, but not females. Females shared 8 genera with males alone (3%) and 9 genera with nymphs alone (4%).
Only 7 genera (2.9%) were unique to females, whereas 37 (15.6%) and 48 (20.3%) were unique to males and nymphs, respectively. The high frequency of shared taxa, and low frequency of taxa unique to females, is consistent with transovarial and transstadial transmission.

Beta diversity (genetic relatedness) was calculated by unweighted UniFrac. The PCoA plot is shown in Figure 3. The 4 male ticks are spread out on graphs of both PC1 v PC2 and PC1 vs PC3. There is some possible clustering of nymphs and females, while the males appear to be highly diverse.

**Rickettsiales 16S rRNA sequence analysis**

The *Rickettsiales* OTU sequences were then analyzed in greater detail (Table 2). There were 8 OTUs found >40 times, representing 95% of all OTUs identified as members of the order *Rickettsiales*. OTU332714, representing 54% of all *Rickettsiales* reads, was found to be 100% identical to sequences published for *R. amblyommii*. OTU 31 was 99% identical to *R. amblyommii* and constituted 8% of reads. Two other OTUs, OTU15617 and OTU2108, were 97% identical to *R. amblyommii* as well *R. massiliae*. Two other OTUs, OTU38 and OTU7478, had 100% identity with *Candidatus* Midichloria mitochondrii, and comprised 26% of reads. OTU13580 was 94% identical to *M. mitochondrii* but only constituted 1% of reads. OTU2465 (1%) was 100% identical to *E. chaffeensis*.

**Rickettsial Sca0 sequence analysis**
In order to better understand the rickettsial diversity, Sca0 was deep-sequenced. More than $2.5 \times 10^4$ reads of 400 bp length or greater were obtained from 6 ticks (2 males, 2 females and 2 nymphs). On average we used 5400 reads per tick (range: 4523-6386) to construct haplotypes. After alignment and haplotype prediction, each tick contained a single haplotype of the Sca0 gene, which was identical for all six ticks (GenBank accession number KF609546). These sequences were then compared to 77 R. amblyommii Sca0 sequences previously deposited in GenBank. The predicted haplotype of Sca0 from our six ticks was identical to 15 previously reported Sca0 sequences from Missouri (Genbank accessions EU544293-94, no reference), Tennessee (EU544295, no reference) and Maryland (EF450685-96, (36) and had only rare differences with the rest. Thus, there is a surprising absence of diversity in this gene despite the fact that its product is highly immunogenic (31, 32).
In this study, we characterized the microbiome of *A. americanum*, the most common tick in the US Southeast. 16S ribosomal gene sequence analysis revealed the presence of genus *Rickettsia* in 11 of 12 of the ticks. OTUs within the genus *Rickettsia* represented 35% of the bacterial population. As expected, most rickettsiae were identical to *R. amblyommii*. Also common were *M. mitochondrii* which represented 13% of the population and were found in 5 of 12 ticks. This is consistent with the previous observations of Williams-Newkirk et al, (41). *M. mitochondrii* is a member of a novel *Rickettsiales* family, *Midichloriaceae*; it has a flagellum and lives in the mitochondrial vacuole of ticks and many other eukaryotes (reviewed in (25)).

The 237 genera identified in this study represent a larger number of genera than identified in previous tick microbiome studies. The cattle tick, *Rhipicephalus microplus*, contained 121 bacterial genera, with the OTUs largely representing nonpathogenic enteric bacteria (3). 108 bacterial genera were found in *Ixodes ricinus* adults from northern Italy (9). Thirty genera were reported from adult *Dermacentor variabilis* and *Ixodes scapularis* ticks (16). Thirty genera were reported from adult *Dermacentor variabilis* and *Ixodes scapularis* ticks (16). These differences were probably due to variations in the sequencing and analytical methods used.

In a previous study of the microbiome of *A. americanum* from Indiana, the most abundant genus (*ca. 40% of all sequences*) was *Coxiella*, while *Rickettsia* represented
only 5% of all sequences (11). In our study, in contrast, *Rickettsia* were much more common than *Coxiella*. This finding suggests that the composition of the microbiome of *A. americanum* is influenced by its geographic distribution. The possibility of competition between *Coxiella* and *Rickettsia* should be further investigated.

The absence of diversity among *Sca0* sequences is surprising, since the 16S sequence analyses suggest that there are several minor OTUs belonging to the genus *Rickettsia* which are not completely identical with *R. amblyommii* (Table 2). One explanation is that the *Sca0* region sequenced in this and other papers is a conserved region rather than one exposed to immunological selective pressure. Further research on the diversity of other *Sca0* domains is needed.

One important unanswered question is whether *R. amblyommii* could be a pathogen with a vertebrate reservoir. Two findings from this study suggest that this is not the case. First, *Rickettsia* is 2.6 times as common in female ticks as in males. A similar increase in the population density of *Rickettsia* in females was seen in a previous lone star tick microbiome study (11). Such an adaptation would be advantageous to an organism that relies on vertical (transovarial) transmission rather than horizontal transmission. Second, there is no genetic diversity in the *Sca0* gene, either within or between tick isolates that we sequenced. This is not the pattern that would be expected for a horizontally transmitted pathogen which requires infection of an intermediate vertebrate host. An infection in a vertebrate host with the ability to generate antigen-specific (acquired) immunity, should lead to balancing (diversifying) selection of the
Sca0 gene (40). Such diversifying selection is apparent, for example, in the *Borrelia burgdorferi* *ospC* gene, which varies in sequence by up to 20% (7, 39). Thus, if *R. amblyommii* is a pathogen, the ticks themselves may serve both as vector and reservoir.

**Conclusion**

*R. amblyommii* appears to be exceptionally well adapted to survival in lone star ticks. There are few or no other species of *Rickettsia* other than *R. amblyommii* present in the ticks studied. The predilection of this organism for female ticks combined with the absence of evidence for balancing selection suggests that this organism is predominantly transmitted transovarially. This implies that the ticks themselves could serve as a reservoir for infection. The absence of other *Rickettsia* species may be the result of competition, but further studies are needed to confirm this.

**Acknowledgments**

We thank Natasha Butz, Andrea Azcarate-Peril and the UNC Microbiome Core Facility for performing 454 sequencing. We also thank Dr Bharath Prithiviraj for helping with initial analyses. The project was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Award Number UL1TR000083 and by the NCSU Center for Comparative Molecular Medicine and Translational Research through award number 2011-2611.
**Figure Legends**

**Figure 1.** Relative abundance of five bacterial taxa in male, female, and nymph *A. americanum*. Full distribution of taxa shown in Supplementary Table S3.

**Figure 2.** Rarefaction curves of observed species in male, female and nymph ticks. Error bars represent standard error.

**Figure 3.** PCoA using unweighted UniFrac of the microbial composition of female, male and nymph ticks.
References


10. CDC 2012, posting date. Other Spotted Fever Group Rickettsial Infections. [Online.]


Midichloriaceae” fam. nov. (Rickettsiales), an ecologically widespread clade of intracellular alphaproteobacteria. Appl Environ Microbiol 79:3241-3248.


Observed species vs. Number of reads

- Female
- Male
- Nymph

Number of reads:
0 200 400 600 800 1000

Observed species:
0 100 200 300 400 500

On June 27, 2017 by guest
Table 1. Female/Male occurrence ratios of bacterial taxa representing ≥2% of total population

<table>
<thead>
<tr>
<th>Bacterial taxa</th>
<th>Population fraction</th>
<th>F/M ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales</td>
<td>0.35</td>
<td>2.61</td>
</tr>
<tr>
<td>f__Rickettsiaceae;g__Rickettsia; (R. amblyommii) *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales</td>
<td>0.13</td>
<td>1.17</td>
</tr>
<tr>
<td>(M. mitochondrii)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;</td>
<td>0.06</td>
<td>0.43</td>
</tr>
<tr>
<td>f__Sphingomonadaceae;g__Sphingomonas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;</td>
<td>0.05</td>
<td>0.67</td>
</tr>
<tr>
<td>f__Sphingomonadaceae;Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales; Other;Other</td>
<td>0.03</td>
<td>0.37</td>
</tr>
<tr>
<td>p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Acidobacteriaceae</td>
<td>0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;Other;Other;Other</td>
<td>0.02</td>
<td>1.39</td>
</tr>
<tr>
<td>p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales</td>
<td>0.02</td>
<td>0.32</td>
</tr>
<tr>
<td>f__Acidobacteriaceae;Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>g__Bradyrhizobium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*as identified by QIIME (p: phylum; c: class; o: order; g: genus.) R. amblyommii and M. mitochondrii identified by subsequent BLAST search (see Table 2).
Table 2. BLAST identification of most common OTUs in the order *Rickettsiales*

<table>
<thead>
<tr>
<th>OTU</th>
<th>#ticks</th>
<th>Occurrences</th>
<th>Top BLAST hit(s)</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>332714</td>
<td>10</td>
<td>3223 (56%)</td>
<td><em>R. amblyommii</em>, NR_074471.1, CP003334.1</td>
<td>100%</td>
</tr>
<tr>
<td>38</td>
<td>5</td>
<td>915 (16%)</td>
<td><em>Midichloria mitochondrii</em>, NR_074492.1, JQ031634.1</td>
<td>100%</td>
</tr>
<tr>
<td>7472</td>
<td>2</td>
<td>582 (10%)</td>
<td><em>Midichloria mitochondrii</em>, NR_074492.1, JQ031634.1</td>
<td>100%</td>
</tr>
<tr>
<td>31</td>
<td>9</td>
<td>487 (8%)</td>
<td><em>R. amblyommii</em>, NR_074471.1, CP003334.1</td>
<td>99%</td>
</tr>
<tr>
<td>15617</td>
<td>9</td>
<td>100 (2%)</td>
<td><em>R. amblyommii</em>, NR_074471.1, <em>R. massiliae</em>, CP003319.1</td>
<td>97%</td>
</tr>
<tr>
<td>2108</td>
<td>8</td>
<td>77 (1%)</td>
<td><em>R. amblyommii</em>, NR_074471.1, <em>R. massiliae</em>, CP003319.1</td>
<td>97%</td>
</tr>
<tr>
<td>13580</td>
<td>4</td>
<td>56 (1%)</td>
<td><em>Midichloria mitochondrii</em>, NR_074492.1, JQ031634.1</td>
<td>94%</td>
</tr>
<tr>
<td>2465</td>
<td>1</td>
<td>44 (1%)</td>
<td><em>E. chaffeensis</em>, NR_074500.1, AF416764.1</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Also 95% identity with both *R. amblyommii*, NR_074471.1, and *R. rhipicephali*, NR_074473.1*