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Rickettsia amblyommatis sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central and South America

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Abstract

In 1973, investigators isolated a rickettsial organism, designated strain WB-8-2^T, from an adult *Amblyomma americanum* tick collected at Land Between the Lakes National Recreation Area, TN, USA. This organism is now recognized as highly prevalent in *A. americanum,* as well as several other *Amblyomma* species found throughout the Western hemisphere. It has been suggested that cross-reactivity to WB-8-2^T and similar strains contributes to the increasing number of spotted fever cases reported in the USA. In 1995, investigators provided preliminary evidence that this strain, as well as another strain from Missouri, represented a distinct taxonomic unit within the genus *Rickettsia* by evaluating sequences of the 16S rRNA and 17 kDa protein genes. However, the bacterium was never formally named, despite the use of the designation '*Rickettsia amblyommii*' and later '*Candidatus* Rickettsia amblyommii', for more than 20 years in the scientific literature. Herein, we provide additional molecular evidence to identify strain WB-8-2^T as a representative strain of a unique rickettsial species and present a formal description for the species, with the proposed name modified to *Rickettsia amblyommatis* sp. nov. to conform to the International Code of Nomenclature of Prokaryotes. We also establish a pure culture of strain WB-8-2^T and designate it as the type strain for the species. The type strain is WB-8-2^T (=CRIRC RAM004^T=CSURP2882^T).

In 1973, investigators isolated a novel spotted fever group (SFG) *Rickettsia* strain from an adult *Amblyomma americanum* tick collected from vegetation in Land Between the Lakes National Recreation Area, TN, USA (Burgdorfer *et al*., 1974, 1981). This strain, designated WB-8-2^T, was subsequently identified in 16 % of *A. americanum* from SC, USA, in 11 % of the *A. americanum* ticks from TN, USA and in 41 % of ticks from AR, USA. It was determined that WB-8-2^T was a member of SFG of the genus *Rickettsia* and distinguishable

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The GenBank accession numbers for *Rickettsia amblyommatis* Ac/Pa are KX151486 (*gltA*), KX151487 (*sca5*) and KX151488 (*sca4*).
Two supplementary figures are available with the online Supplementary Material.

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from other *Rickettsia* species by using mouse serotyping and SDS-PAGE protein analyses (Burgdorfer *et al.*, 1981). Two decades later, Stothard (1995) further characterized this and another strain, also isolated from *A. americanum* (MO 85-1084), by using newly developed molecular tools. Although the *rrs* sequence was similar to other spotted fever group rickettsiae (SFGR), analysis of the 17 kDa antigen gene indicated that WB-8-2^T and MO 85-1084 were distinct from other named species of the genus and that these strains represented a novel *Rickettsia* species (Stothard, 1995). In her dissertation, Stothard described the bacterium as '*Rickettsia amblyommii*', Unfortunately, this name was never validly published. Nonetheless, Stothard and Fuerst used this name in their pioneering phylogenetic studies of this organism, and the designation '*Rickettsia amblyommii*' (Stothard & Fuerst, 1995) has since been used regularly in scientific literature. Herein, we confirm previous findings that this organism is a distinct taxonomic entity; however, we propose the novel species name *amblyommatis* to conform to the rules of the International Code of Nomenclature of Prokaryotes. As this bacterial species is named for the tick genus *Amblyomma* (Greek third declension), the species epithet must be in the Latin third declension; thus, we propose the name *Rickettsia amblyommatis* sp. nov. (Oren *et al.*, 2016).

'*Rickettsia amblyommatis*' occurs in several tick species of the genus *Amblyomma* throughout the Western hemisphere, but it is most commonly detected in *A. americanum*, with rates of infection that often exceed 40 % of questing adult ticks (Table 1). Both transstadial and transovarial transmissions occur in *A. americanum* ticks, and filial infection rates range between 30 and 100 % depending on the level of rickettsiae in ovarial tissues (Burgdorfer *et al.*, 1981). Other tick species in which '*Rickettsia amblyommatis*' has been detected include *Amblyomma longirostre* in Brazil (Labruna *et al.*, 2004), *Amblyomma neumannii* and *Amblyomma hadanii* in Argentina (Labruna *et al.*, 2007; Mastropaolo *et al.*, 2016), *Amblyomma cajennense* in Mexico, Costa Rica and Colombia (Faccini-Martínez *et al.*, 2016; Hun *et al.*, 2011; Medina *et al.*, 2007), *Amblyomma mixtum* and *Haemaphysalis juxtakochi* in Panama (Castro *et al.*, 2015), *Amblyomma coelebs* in French Guyana (Parola *et al.*, 2007) and *Dermacentor variabilis* in the USA (Moncayo *et al.*, 2010). In this context, '*Rickettsia amblyommatis*' likely represents the most prevalent and widely distributed SFG rickettsial species in the Americas.

Strains of this organism can be cultivated in chicken fibro-blasts, primary embryonated chicken eggs, Vero cells, ISE6 tick cells and AAE2 tick cells (Burgdorfer *et al.*, 1981; Carmichael, 2008; Stothard, 1995; Stothard & Fuerst, 1995). In their initial studies, Burgdorfer *et al.* found that WB-8-2^T was non-pathogenic to guinea pigs (*Cavia porcellus*), but the strain produced mild and transient infections in the tunica vaginalis of male meadow voles (*Microtus pennsylvanicus*) when inoculated with densely infected cell culture suspensions. They concluded that the organism was not likely pathogenic to humans due to its inability to cause disease in guinea pigs and the lack of epidemiological evidence of fever, rash or headache in humans from areas with high prevalence of WB-8-2^T and high population densities of *A. americanum* (Burgdorfer *et al.*, 1981). Nonetheless, some serological evidence suggests that humans develop a robust immune response to this organism (Apperson *et al.*, 2008; Medina *et al.*, 2007) and it may be associated with disease manifestations in some patients (Delisle *et al.*, 2016). Repeated exposure to infected ticks can induce very high serological titres in dogs, and there is recent molecular evidence that

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this organism can infect dogs (Barrett *et al.*, 2014). Although Burgdorfer *et al.* (1981) did not observe complement-fixing antibody development in inoculated guinea pigs and voles, Blanton *et al.* (2014) demonstrated that guinea pigs developed very high titres by indirect immunofluorescence assay, despite no obvious clinical signs of infection. Recently, investigators demonstrated that an isolate from Costa Rica causes fever and pathological signs of disease in guinea pigs and that '*Rickettsia amblyommatis*' DNA can be detected in guinea pig testicles up to 2 days after I.P inoculation (Rivas *et al.*, 2015).

Strains of '*Rickettsia amblyommatis*' may also play a role in the ecology and epidemiology of other pathogenic SFGR. *A. americanum* is a potential vector of at least two confirmed rickettsial pathogens, *Rickettsia rickettsii* and *Rickettsia parkeri* (Berrada *et al.*, 2011; Cohen *et al.*, 2009; Parker *et al.*, 1933, 1943), yet in nature the prevalence of these bacteria in *A. americanum* is extremely low (Berrada *et al.*, 2011; Cohen *et al.*, 2009; Gaines *et al.*, 2014; Wright *et al.*, 2015) In this context, it is possible that the observed high rates of '*Rickettsia amblyommatis*' infection could inhibit the transovarial transmission of other SFGR, including *R. rickettsii* and *R. parkeri* (Macaluso *et al.*, 2002). Infection with '*Rickettsia amblyommatis*' in *A. americanum* has been shown to inhibit, but not completely block, the acquisition of *R. parkeri* during experimental co-feeding with infected ticks (Wright *et al.*, 2015). Infection with '*Rickettsia amblyommatis*' may also provide some level of protection against subsequent infection with *R. rickettsii* in guinea pigs (Blanton *et al.*, 2014; Rivas *et al.*, 2015).

A americanum is a common human-biting tick and the high prevalence of '*Rickettsia amblyommatis*' in this tick may complicate the diagnosis and surveillance of other SFG rickettsial infections in humans. Most cases of Rocky Mountain spotted fever (RMSF) are diagnosed using serological assays, and it has been suggested that the cross-reactivity of antibodies to strains like WB-8-2^T against *R. rickettsii* antigens may help explain the large numbers of so-called 'mild RMSF' cases in areas where *A. americanum* is prevalent (Apperson *et al.*, 2008; Delisle *et al.*, 2016; Openshaw *et al.*, 2010; Parola *et al.*, 2013; Stromdahl *et al.*, 2008). Multiple serological assessments of military personnel involved in training exercises in *A. americanum*-infested habitats demonstrated high rates of seroconversion to antigens of SFG rickettsial species among individuals who had developed asymptomatic or relatively mild illnesses not characteristic of RMSF (McCall *et al.*, 2001; Sanchez *et al.*, 1992; Yevich *et al.*, 1995). Recently, a correlation between the presence of *A. americanum* in a geographical area and decreased hospitalization rates of RMSF (i.e., less severe or mild RMSF cases) in that same area was identified (Dahlgren *et al.*, 2016). These data suggest the influence of a less pathogenic SFG rickettsial species on human disease reports.

The high prevalence of WB-8-2^T and other strains in ticks poses a problem for investigators using molecular assays to assess rickettsial levels in tick populations. Cohen *et al.* (2009) found low levels of dually infected ticks; however, these individual ticks contained much higher bacterial loads of '*Rickettsia amblyommatis*' than *R. rickettsii*, and common PCR and sequencing techniques only revealed the presence of the more abundant '*Rickettsia amblyommatis*' (Cohen *et al.*, 2009). It was only after the PCR amplicons were cloned and sequenced that *R. rickettsii*-specific sequences were identified. Berrada *et al.* (2011) also

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relied on cloning to reveal the presence of *R. rickettsii*-specific sequences in extracts of '*Rickettsia amblyommatis*'-infected *A. americanum* ticks from KS, USA (Berrada *et al.*, 2011).

Molecular testing using the Universal Mycoplasma Detection Kit (ATCC) revealed the presence of a contaminating *Mycoplasma* species in the available stocks of strain WB-8-2^T. To obtain a *Mycoplasma*-free stock of strain WB-8-2^T for deposition and distribution, 50 μg ml^{-1} lincomycin was added to the medium and cell cultures were exposed to the antibiotic-supplemented medium for 6 weeks (Ogawa *et al.*, 2013). Cultures were shown to be negative for infection with *Mycoplasma* species at 4, 5 and 6 weeks by using the PCR assay (data not shown), after which time the antibiotic was removed from the medium. Cultures were evaluated again after additional 4 weeks of antibiotic-free growth and showed no evidence of re-infection by *Mycoplasma* species.

After isolation from tick haemolymph into primary chicken fibroblasts, strain WB-8-2^T forms plaques of 1.7–2.0 mm in diameter. Passage into embryonated chicken eggs results in embryo death in 5–7 days (Burgdorfer *et al.*, 1981). The organisms stain a pink colour with Giménez stain and appear round to oval in shape, often arranged in pairs (Fig. 1a). Immunofluorescence of the organisms in similar shape and arrangement may be seen by using mouse polyclonal conjugate made to *R. rickettsii.* All tick tissues may be infected, with ovary and Malpighian tubules being more heavily infected (Burgdorfer *et al.*, 1981).

In this study, five geographically unique isolates (Table 2) were propagated in Vero E6 cells grown in Minimal Essential Media (Gibco) supplemented with 0.1 mM Minimal Essential Media non-essential amino acids (Gibco), 10 mM HEPES buffer (Gibco), 2 mM L-glutamine (Gibco), 10 mM sodium pyruvate (Gibco) and 5 % (w/v) heat-inactivated foetal bovine serum (Atlas Biologicals) at 32 °C with 5 % (v/v) CO_2. When observed under bright-field microscopy, WB-8-2^T appeared as small bacilli found free-living in the host cell cytoplasm (Fig. 1a). To prepare *Rickettsia*-infected cells for electron microscopy, an infected monolayer was washed in 0.1 M phosphate buffer, pH 7.3, and fixed in buffered 2.5 % (w/v) glutaraldehyde for 5 min at 4 °C. The monolayer was gently removed from the flask using a cell scrapper, and the cells were centrifuged at 1000 ***g*** for 5 min at 4 °C. The remaining glutaraldehyde was removed, and the cell pellet was covered with 0.1 M phosphate buffer and stored at 4 °C. The cells were post-fixed in 1 % (w/v) buffered osmium tetroxide, stained in 4 % (w/v) uranyl acetate, dehydrated through a graded series of alcohols and acetone and embedded in a mixture of Epon substitute and Araldite. Thin sections were stained with 4 % (w/v) uranyl acetate and Reynold's lead citrate.

Electron microscopy of WB-8-2^T revealed a Gram-negative, bacillary morphology consistent with other members of the genus *Rickettsia*. A comparison of 45 individual bacterial cells revealed an average length of 0.832 μm (median, 0.798 μm) and an average width of 0.427 μm (median, 0.416 μm). These dimensions are compatible with the observations of Burgdorfer *et al.* (1981) when examining infected tick tissues (Burgdorfer *et al.*, 1981). Strain WB-8-2^T has a cell wall with inner and outer membranes separated by a peri-plasmic space (white arrow, Fig. 1b). The cell wall is surrounded by a translucent area

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consistent with an outer slime layer (black arrow, Fig. 1b) (Silverman, 1991) which is in agreement with the original description of WB-8-2^T (Burgdorfer *et al.*, 1981).

Traditionally, DNA–DNA hybridization techniques have been used to define bacterial species, with a 70 % relatedness cutoff generally used to differentiate species (Wayne *et al.*, 1987). However, DNA sequences are highly conserved between different rickettsial species, and if the traditional 70 % relatedness criteria were applied to the genus, many defined species would be consolidated into a single species (Myers & Wisseman, 1981). In 2003, investigators proposed a molecular scheme for the classification of rickettsial species to maintain the established species structure of the genus (Fournier *et al.*, 2003). This scheme utilizes a multi-locus sequence typing (MLST) approach based on the sequences of five rickettsial genes; *rrs*, *gltA*, *sca0* (*ompA*), *sca5* (*ompB*) and *sca4* (Gene D). PCR amplification and DNA sequencing were performed on WB-8-2^T and three other strains (Table 2). All reactions were performed as described previously (Fournier *et al.*, 2003) with the exception of the *gltA* PCR, which used an annealing temperature of 55 °C. PCR amplicons were sequenced using a BigDye Terminator V3.1 kit and an ABI 3130xl genetic analyser (Applied Biosystems, Carlsbad, CA, USA). Sequences were assembled using Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA), and MEGA 6.05 (Tamura *et al.*, 2013) was used to create alignments so that the individual sequences from the four sequenced isolates could be compared along with that of the available sequenced full genome of strain GAT-30V (GenBank accession number NC_017028.1).

According to the proposed MLST classification scheme, for an isolate to be confirmed as a new rickettsial species, it should have no more than one locus with an identity equal to or greater than 99.8, 99.9, 98.8, 99.2 and 99.3 % identities to an established rickettsial species for *rrs*, *gltA*, *sca0*, *sca5* and *sca4*, respectively (Fournier *et al.*, 2003). When the sequences for WB-8-2^T are compared to the sequences of other rickettsial type strains, *Rickettsia raoultii* strain Khabarovsk^T often is the most similar, with 99.3, 99.1, 97.4 and 97.3 % identities for *rrs*, *gltA*, *sca0* and *sca5*, respectively. The WB-8-2^T gene sequence for *sca4* is most similar to *Rickettsia japonica* YH^T, with 97.6 % identity. All five of the WB-8-2^T loci sequence comparisons fall below the cutoffs suggested by the MLST classification scheme, confirming that WB-8-2^T represents a novel SFG rickettsial species.

To better assess the genetic relationship of strain WB-8-2^T compared to recognized SFG *Rickettsia* species, a phylogenetic analysis was performed using the concatenated sequences of the five loci. Homologous sequences were obtained from National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/) for validated species of SFGR and for *Rickettsia felis*, a member of the transitional group rickettsiae (Table 3) (Gillespie *et al.*, 2008), which was included to be used as an outgroup. The nucleotide sequences were concatenated and aligned using MEGA 6.05 (Tamura *et al.*, 2013) in the following order: *rrs*, *gltA*, *sca0*, *sca5* and *sca4*; gaps were removed from the alignment using the simple indel coding method (Ogden & Rosenberg, 2007). The MEGA 6.05 software package was also used to infer the evolutionary history. The neighbour-joining phylogenetic analysis agrees with the MLST analysis and clearly shows that strain WB-8-2^T is a distinct member of the genus *Rickettsia* (Fig. 2). Analyses utilizing the maximum-parsimony and maximum-

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likelihood methods are in agreement with the neighbour-joining analysis (Figs S1 and S2, available in the online Supplementary Material).

Sequencing of the five loci was completed for strains and compared to the genome sequence for strain GAT-3OV in GenBank (accession number NC_017028.1) in order to identify genetic variation among these isolates. The sequences for all five isolates were 100 % identical to each other in the regions of *rrs* and *sca0* that were compared. Four of the five sequences were 100 % identical to *gltA*, with the exception of strain Ac/Pa, which contains one single-nucleotide polymorphism in the 1048 bp examined. When the *sca5* sequences were compared, again strain Ac/Pa was the only strain to exhibit variation, with four single-nucleotide polymorphisms identified across the 4845 bp examined. Strain Ac/Pa was also the only strain to have sequence variation in the 2941 bp examined in *sca4*, with Ac/Pa containing six single-nucleotide polymorphisms compared to the other four strains. Thus, very little genetic variation was observed in the loci examined, with the five isolates maintaining 99.9 % or higher sequence identity in each locus.

The results of phylogenetic, phenotypic, and biologic features of strain WB-8-2^T indicate that this is a novel species. Additional strains belong to this species, and this taxon can be distinguished from previously described species of the genus *Rickettsia*. The original species name, '*Rickettsia amblyommii*', was proposed in a dissertation by Diane Stothard (Stothard, 1995) and has been accepted by many authors since then. However, this name does not conform to the International Code of Nomenclature of Prokaryotes; thus, we propose strain WB-8-2^T as the type strain for the species *Rickettsia amblyommatis* sp. nov.

Description of Rickettsia amblyommatis sp. nov

Rickettsia amblyommatis (am.bly.om'ma.tis. N.L. gen. neut. n. *amblyommatis* of *Amblyomma*, the genus of hard ticks from which the type strain was isolated).

In Vero E6 cells, these Gram-negative bacilli have an average length of 0.832 μm and an average width of 0.427 μm. In ticks, the round- or oval-shaped organisms are often in pairs. This intracellular organism infects and replicates in eukaryotic cells where it is found free in the cytoplasm. In nature, a variety of tick species from North, Central and South America are infected with '*Rickettsia amblyommatis*' including *A. americanum* Linneaus (1758), *A. longirostre* Koch (1844), *A. neumannii* Ribga (1902), *A. cajennense* Fabricius (1787) and *A. coelebs* Neumann (1899). Other tick genera are infected occasionally including the species *Dermacentor* and *Haemaphysalis*. The infection occurs in all tick tissues, but ovaries and Malpighian tubules are more intensely infected. This organism is cultivated in several established cell lines including Vero cells. Antigens of '*Rickettsia amblyommatis*' react with polyclonal antisera raised to other SFG *Rickettsia* species; however, microimmunofluorescence assay with specific mouse antisera shows no cross-reaction with other SFG rickettsial serotypes. Although the pathogenic potential of this organism remains unclear for humans, experimentally infected guinea pigs and dogs mount a robust serological response by IFA assay when infected with '*Rickettsia amblyommatis*'. The WB-8-2^T DNA sequences analysed in regions of the *rrs*, *sca0*, *sca5*, *gltA* and *sca4* genes are 100 % identical to those publicly available for strain GAT-3OV. The complete genome

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sequence of '*Rickettsia amblyommatis*' strain GAT-3OV is available in GenBank (accession number NC_017028) and consists of one circular chromosome (1 407 796 bp; 32.4 mol% G+C) and three circular plasmids containing 31 974, 18 263 and 22 851 bp, respectively.

The type strain, WB-8-2^{T}(=CRIRC RAM004^{T}=CSUR-P2882^{T}), was isolated from an adult *A. americanum* tick collected from vegetation at Land Between the Lakes National Recreation Area, Stewart County, TN, USA, in 1973.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MLST	multi-locus sequence typing
RMSF	Rocky Mountain spotted fever
SFG	spotted fever group
SFGR	spotted fever group rickettsiae

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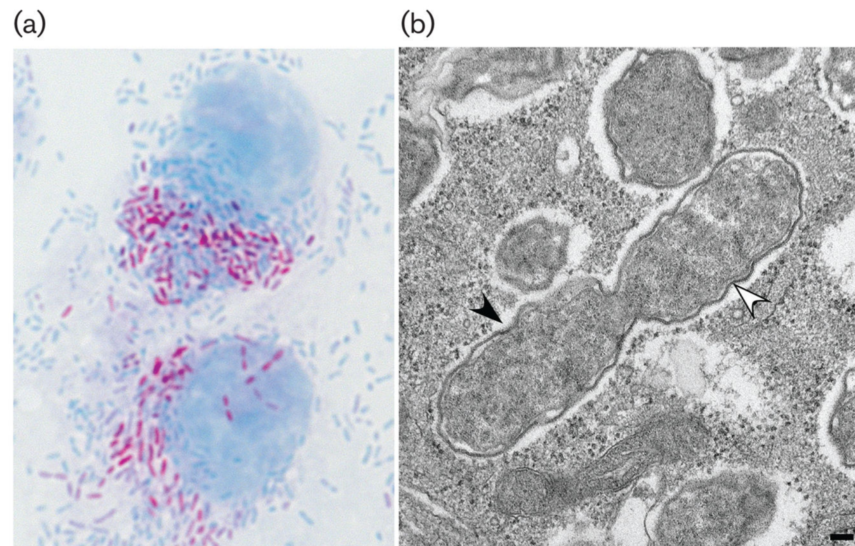

Fig. 1.

Light and electron microscopical appearance of '*Rickettsia amblyommatis*' in Vero E6 cells. (a) Giménez stain of '*Rickettsia amblyommatis*' in Vero E6 cells. (b) Transmission electron microscopy image of '*Rickettsia amblyommatis*' infecting Vero E6 cells. White arrow, inner and outer membranes separated by a periplasmic space; black arrow, outer slime layer.

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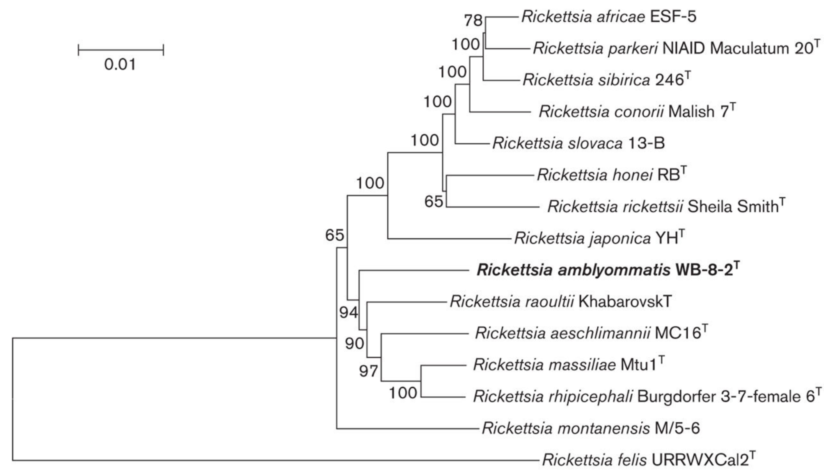

Fig. 2.

Phylogenetic relationship of '*Rickettsia amblyommatis*' to other SFGR using five concatenated sequences. The evolutionary history was inferred using the neighbour-joining method while the evolutionary distances were computed using the maximum-composite-likelihood method. A total of 10 640 positions were included in the analysis. The scale bar is in units of the number of base substitutions per site. Bootstrap values for 1000 replicates are displayed next to the branches.

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Table 1

Frequency of '*Rickettsia amblyommatis*' infection in *A. americanum* ticks in the USA

State	No. of ticks tested (% positive for '*Rickettsia amblyommatis*')	Reference
AR	463 (41.9 %)	Burgdorfer *et al.* (1981)
AR	653 (37 %)	Trout Fryxell *et al.* (2015)
FL	151 (37.1 %)	Mixson *et al.* (2006)
FL	1479 (57.1 %)	Sayler *et al.* (2014)
GA	704 (44.7 %)	Mixson *et al.* (2006)
IA	19 (57.9 %)	Mixson *et al.* (2006)
MD	502 (64.1%)	Zhang *et al.* (2012)
MO	74 (2.7 %)	Hermance *et al.* (2014)
NC	391 (55.2 %)	Mixson *et al.* (2006)
NC	3695 (56.4 %)	Smith *et al.* (2010)
NJ	121 (6.6 %)	Mixson *et al.* (2006)
NY	475 (41.7 %)	Mixson *et al.* (2006)
OH	21 (38 %)	Kelly *et al.* (2005)
OH	308 (30.2 %)	Fitak *et al.* (2014)
OK	60 (10 %)	Mixson *et al.* (2006)
RI	38 (47.4 %)	Mixson *et al.* (2006)
SC	545 (11.7 %)	Burgdorfer *et al.* (1981)
SC	79 (45.6 %)	Mixson *et al.* (2006)
TN	96 (16.6 %)	Burgdorfer *et al.* (1981)
TN	655 (39.5 %)	Moncayo *et al.* (2010)

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Table 2

Isolates characterized as *Rickettsia amblyommatis* sp. nov.

Strain	Source	Geographic origin	CRIRC isolate number	Reference
WB-8-2^T	*A. americanum*	Land Between the Lakes National Recreation Area, TN, USA	RAM004^T	Burgdorfer *et al.* (1974)
GAT-3OV	*A. americanum*	Stockbridge, GA, USA	RAM007	Unpublished
Darkwater	*A. americanum*	Sopchoppy, FL, USA	RAM003	Baldridge *et al.* (2010)
Line Creek	*A. americanum*	Peachtree City, GA, USA	RAM005	This study
Ac/Pa	*A. cajennense*	Panama	RAM002	Baldridge *et al.* (2010)

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Table 3

GenBank accession numbers of rickettsial gene sequences used in the phylogenetic analysis

Strain species and strain	*rrs*	*gltA*	*sca0*	*sca5*	*sca4*
R. aeschlimannii MC16^T	U74757	U59722	U43800	AF123705	AF163006
R. africae ESF-5	l36098	U59733	U43790	AF123706	AF151724
R. conorii Malish 7^T	AF541999	U59730	U43806	AF123721	AF163008
R. felis URRWXCal2^T	L28944	AF210692	AF210694	AF210695	AF196973
R. honei RB^T	U17645	AF018074	AF018075	AF123711	AF163004
R. japonica YH^T	NR_074459	AP011533	AP011533	AP011533	AP011533
R. massiliae Mtu1^T	L36214	U59719	U43799	AF123714	AF163003
R. montanensis M/5–6	L36215	U74756	U43801	AF123716	AF163002
R. parkeri NIAID Maculatum 20^T	L36673	U59732	U43802	AF123717	AF155059
R. rhipicephali Burgdorfer 3–7-female 6^T	L36216	U59721	U43803	AF123719	AF155053
R. rickettsii Sheila Smith^T	NR_102941	CP000848	CP000848	CP000848	CP000848
R. sibirica 246^T	L36218	U59734	U43807	AF123722	AF155057
R. slovaca 13-B	L36224	U59725	U43808	AF123723	AF155054
R. raoultii Khabarovsk^T	DQ365810	DQ365804	DQ365801	DQ365798	DQ365808