



Spotted fever group *Rickettsia* and *Borrelia* sp. cooccurrence in *Amblyomma sculptum* in the Midwest region of Brazil

Leandro de Oliveira Souza Higa¹ · Bárbara Guimarães Csordas² · Marcos Valério Garcia² · Leandra Marla Oshiro² · Pâmella Oliveira Duarte¹ · Jacqueline Cavalcante Barros³ · Renato Andreotti³

Received: 30 September 2019 / Accepted: 16 June 2020 / Published online: 22 June 2020
© Springer Nature Switzerland AG 2020

Abstract

More than 70 tick species are found in Brazil, distributed over five genera and including main vectors of infectious disease agents affecting both animals and humans. The genus *Amblyomma* is the most relevant for public health in Brazil, wherein *Amblyomma aureolatum*, *Amblyomma ovale* and *Amblyomma sculptum* have been incriminated as vectors of *Rickettsia* and *Borrelia* pathogens. The objective of this study was to investigate the presence of *Rickettsia* spp. and *Borrelia* spp. in ticks in the Brazilian mid-western savannah. DNA extraction, PCR for *Borrelia* spp. (*flgE* gene) and *Rickettsia* spp. (*ompA* and *gltA* genes) and subsequent sequencing were performed. A total of 1875 ticks were collected and identified as *A. sculptum* except for two *Amblyomma coelebs* ticks. Molecular evidence for *Borrelia* spp. and *Rickettsia parkeri* was found in *A. sculptum*. This is the first molecular evidence for *R. parkeri* in *A. sculptum* ticks in the Midwest region and *Borrelia* spp. circulating in a tick of the *Amblyomma* genus in Brazil.

Keywords Tick-borne disease · Pathogen · Savannah · Ixodidae

Introduction

Along with mosquitoes, ticks are considered an important source of pathogens for humans, which places them at the forefront of disease transmission and public health (Colwell et al. 2011). Due to advances in diagnostic methods as well as the effects of climate change

✉ Renato Andreotti
renato.andreotti@embrapa.br

¹ Pós-Graduação em Doenças Infecciosas e Parasitárias-Faculdade de Medicina, UFMS – Univ. Federal de Mato Grosso do Sul, Laboratório de Biologia do carrapato, Embrapa Gado de Corte, Campo Grande, MS, Brazil

² Laboratório de Biologia do Carrapato, Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Corte, Bolsista FUNDAPAM, Campo Grande, MS, Brazil

³ Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Corte, Campo Grande, MS, Brazil

caused by societal progress, the number of reports of arthropod-borne diseases in humans has increased (Beugnet and Chalvet-Monfray 2013).

With more than 900 described species worldwide, ticks are obligatory blood-sucking ectoparasites that can transmit various types of disease-causing agents, such as viruses, protozoa, and bacteria during the feeding process (Jongejan and Uilenberg 2004). A tick-transmitted disease of great importance in Americas are the rickettsioses caused by obligatory intracellular gram-negative bacteria belonging to the genus *Rickettsia*. First described in the USA in 1899, the disease has been found in Canada, Mexico, Panama, Costa Rica, Argentina, Colombia, and Brazil (Dantas-Torres 2007). The main species of medical importance in Brazil is *Rickettsia rickettsii*, a bacterium that causes Rocky Mountain fever (or Brazilian macular fever). This disease is associated with clinical complications due to its nonspecific symptoms (myalgia, headache, fever) as well as more serious symptoms (neurological disorders, renal failure, respiratory difficulty, jaundice), with maculopapular rash as a pathognomonic sign (Angerami et al. 2009). Currently the main vectors of *R. rickettsii* are *Amblyomma cajennense* (and *A. sculptum*) and *A. aureolatum* (Labruna 2009; Ogrezewalska et al. 2012). Other *Rickettsia* bacteria belonging to the spotted fever group may be reported in other tick species as well (Almeida et al. 2013; Matias et al. 2015).

Lyme disease, another disease related to Ixodidae ticks, is mainly transmitted by ticks belonging to the genus *Ixodes* and is considered to be most common in the Northern Hemisphere (Jaenson 1991; Mead 2015; Durand et al. 2017). Lyme disease is a multisystem inflammatory disease caused by spirochete bacteria belonging to the *Borrelia burgdorferi* sensu lato complex and manifests as classic symptoms such as the presence of erythema migrans in the skin initially and joint, neurological, and cardiac complications at later stages (Sanchez 2015).

The *B. burgdorferi* s.l. complex consists of 18 genospecies recognized in North America, Europe, and Asia, with *Borrelia afzelli*, *Borrelia garinii*, and *B. burgdorferi* being the most pathogenic in humans (Mead 2015). Studies conducted in Brazil using protein-based serology reported some inconsistencies in the diagnostic results for *B. burgdorferi*. These facts, together with the absence of bacterial isolation from tissues or body fluids, have shown that until now, Lyme disease has never been confirmed in Brazil (Oliveira et al. 2018).

However, clinical manifestation similar to Lyme disease has been reported in Brazil, including erythema migrans (Mantovani et al. 2007). According to the authors, there is a possibility that a new tick-borne disease related to a spirochete belonging to the *Borrelia* genus may exist, known as Lyme disease-like syndrome or Lyme Imitator Syndrome.

As *A. cajennense* complex (*A. cajennense* and *A. sculptum*) was speculated in the literature as possible participant in the transmission of Brazilian borreliosis (Dantas-Torres 2008; Yoshinari et al. 2010) and this tick is known as the main transmitters of rickettsial diseases, the objective of the present study was to investigate *Rickettsia* spp. and *Borrelia* spp. by molecular evidence in *A. sculptum* from the Midwest region of Brazil.

Materials and methods

Study site

Tick collection was carried out between December 2017 and August 2018 in the Terenos Municipality in the state of Mato Grosso do Sul, Brazil. The municipality is situated in the

west-central region of the state (22 km from the capital Campo Grande), with a population of ca. 17.146, a size of ca. 2.844 km², and an average elevation of 408 m above sea level (<https://www.cidade-brasil.com.br/municipio-terenos.html>). The ticks were collected in a forest fragment belonging to the Cerrado biome located in the rural area of the municipality (−20.405580, −55.014690). The entire area covered in the study is surrounded by cattle-breeding pastures, and the presence of wild animals has been reported (e.g., *Myrmecophaga tridactyla* and *Tapirus terrestres*).

Tick collection

Free-living ticks were collected using CO₂ traps, as described by Oliveira et al. (2000), and transported alive to the Embrapa Cattle Tick biology laboratory where they were identified according to Barros-Battesti et al. (2006).

DNA extraction

DNA was extracted individually from adult ticks using the acid guanidinium thiocyanate–phenol–chloroform extraction protocol (Sangioni et al. 2005), and the samples were quantified by spectrophotometry (NanoDrop ND-1000 Uniscience) and subsequently subjected to polymerase chain reaction (PCR). The CS-78 and CS-323 oligonucleotides were used to amplify a 401-bp fragment of the citrate synthase gene (*gltA*) in species of the genus *Rickettsia* (Labruna et al. 2004). The PCR assay was standardized to a final volume of 25 µl, and the DNA concentration used was between 50 and 150 ng/µl, with A₂₆₀ nm/A₂₈₀ nm ≥ 1.8. Positive samples were subjected to another round of PCR using oligonucleotides Rr190.70p and Rr190.602n, which amplify a 530-bp fragment of the *ompA* gene (protein 190 kDa) only for *Rickettsia* species belonging to the Rocky Mountain spotted fever group (Regnery et al. 1991). All samples were also tested for the presence of *B. burgdorferi* using the oligonucleotides *flgE*-F and *flgE*-R, which target the *Borrelia* flagellar hook, amplifying a 262-bp fragment with adaptations (Sal et al. 2008). The PCR-amplified *ompA* and *flgE* products were visualized on 1.5% agarose gels stained with ethidium bromide (EtBr) and purified using a PureLink Quick Gel Extraction Kit (Invitrogen). The products were subsequently cloned and sequenced using the Sanger method (Sanger et al. 1977) with an ABI 3130 Genetic Analyzer (Applied Biosystems). The consensus sequence was obtained using BioEdit software (Hall 1999) and compared with data available in GenBank. A BLASTn search was performed for sequence identity (Altschul et al. 1990), and phylogenetic analyses were conducted using MEGA v.7.0 software (Kumar et al. 2016).

Sequence alignment and phylogenetic tree construction

The *ompA* (GenBank: MK231013) and *flgE* (GenBank: MK231014) sequences were aligned with those in GenBank using BLASTn, and a database was constructed that contained all similar sequences obtained from the analysis. The MEGA v.6.0 program (Tamura et al. 2013) was applied to align the sequences.

Bayesian phylogenetic analysis was performed using the MrBayes v.3.2.6 program (Ronquist and Huelsenbeck 2003). For the data set used in this study, approximately 10⁷ generations were found to be sufficient for generating topologies; plots were prepared using the FigTree v.1.4.2 program (Tree Bio 2016). All analyses for *ompA* and *flgE* were initiated

with random starting trees and run for 10^6 generations, with sampling every 1000 generations. To determine the stationarity of the Markov chain, the log-likelihood scores of sample points were plotted against the generation time. The first 25% of samples was discarded as burn-in for each data set, and the remaining samples were retained for generating consensus trees. Each sample included a tree topology that incorporates branch length and substitution model parameter values. These topologies were used to generate a 50% majority rule consensus tree, with the percentage of sample recovering any particular clade representing the posterior probability of a clade ($1 = 100\%$). No manual editing of the trees was performed. *Rickettsia australis* (GenBank: AF149108) and *Treponema pallidum* (GenBank: CP021113) were used as outgroups in the phylogenetic analyses.

Results

A total of 1875 ticks (1873 *A. sculptum* and 2 *A. coelebs*) were collected from the environment during the study period, as shown in Table 1. Of these, 144 ticks (including the *A. coelebs* specimens) were subjected to PCR to detect *Rickettsia* and *Borrelia* spp. genetic material. An adult *A. sculptum* tick presented molecular evidence of *Rickettsia* spp. based on *ompA* as the target region for PCR. Another individual of the same species presented molecular evidence of *Borrelia* sp. DNA, which was confirmed by PCR targeting the *flgE* gene. No molecular evidence for any of the aforementioned bacteria was found for the *A. coelebs* specimens.

Both pathogens were sequenced for identity confirmation, and BLASTn analysis of the amplified fragment revealed 100% identity with the Atlantic Forest strain of *Rickettsia parkeri* (*ompA* gene, 530 bp; GenBank: MF536975), 98.5% with *B. burgdorferi* B31 (GenBank AE000783.1) and 99.6% with *B. burgdorferi* LS2 (GenBank: KY073268.1) (*flgE* gene, 262 bp). A phylogenetic tree was generated using the *ompA* sequences obtained in this study and GenBank MK231013 sequences (Fig. 1). The same procedure was performed for the *B. burgdorferi* sequence (GenBank: MK231014), with another phylogenetic tree generated (Fig. 2).

Discussion

Among the tick species collected in the present study, *A. sculptum* was the most prevalent as well as the most abundant in the environment. *Amblyomma sculptum* exhibits a wide distribution in the environment, a wide range of hosts, and a high affinity for humans (Parola and Raoult 2001). Thus, this species is commonly related to cases of parasitism in humans in South America (Guglielmone et al. 2006) and *A. sculptum* is known to be the main transmitter of *R. rickettsi* (Labruna 2009).

Table 1 Number of adult female (F) and male (M) ticks collected on various dates in the Terenos municipality, MS, Brazil

Collection	11/16/2017		02/23/2018		05/03/2018		08/28/2018	
	F	M	F	M	F	M	F	M
<i>Amblyomma sculptum</i>	200	194	441	337	198	265	150	90
<i>Amblyomma coelebs</i>	0	0	1	1	0	0	0	0

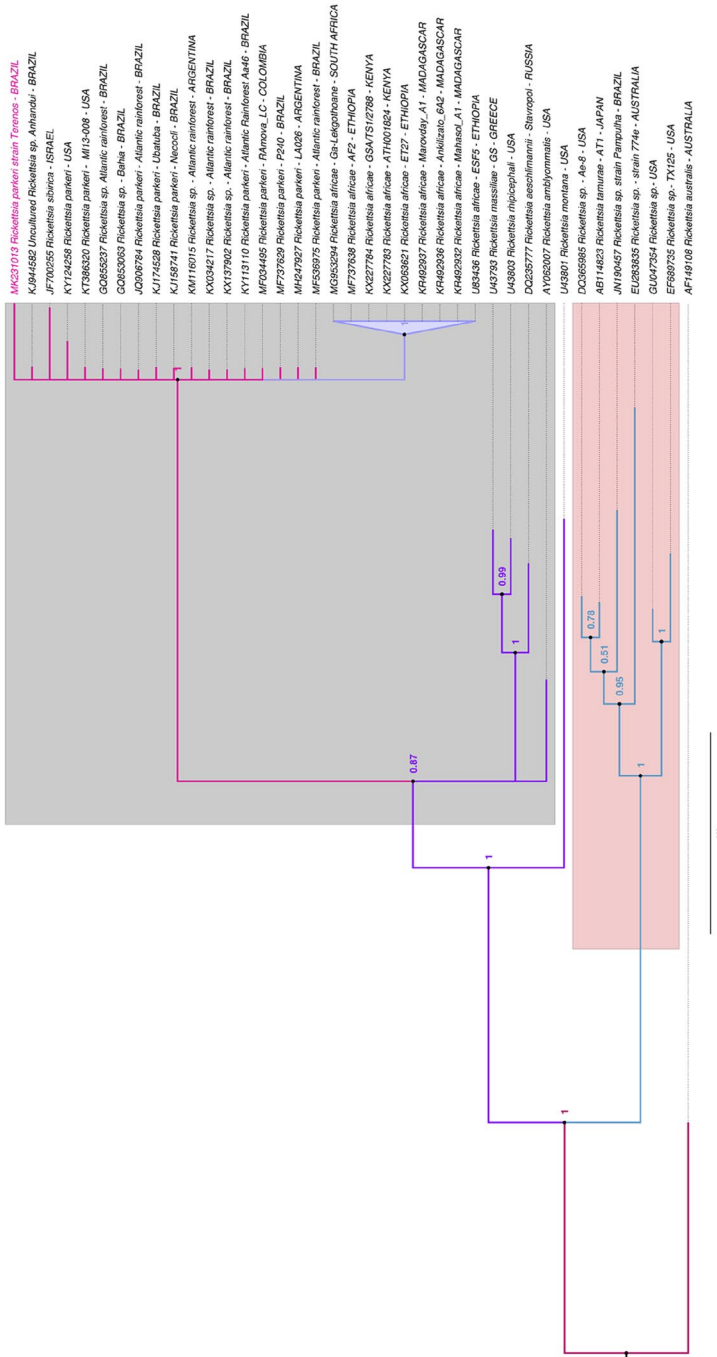


Fig. 1 Phylogenetic tree of *Rickettsia parkeri* (GenBank: MK231014). Evolutionary history was based on the Bayesian inference tree with probability scores for the *ompA* gene. The scale bar indicates 0.02 changes per nucleotide position. The sample sequences obtained in this study are shown in pink. (Color figure online)

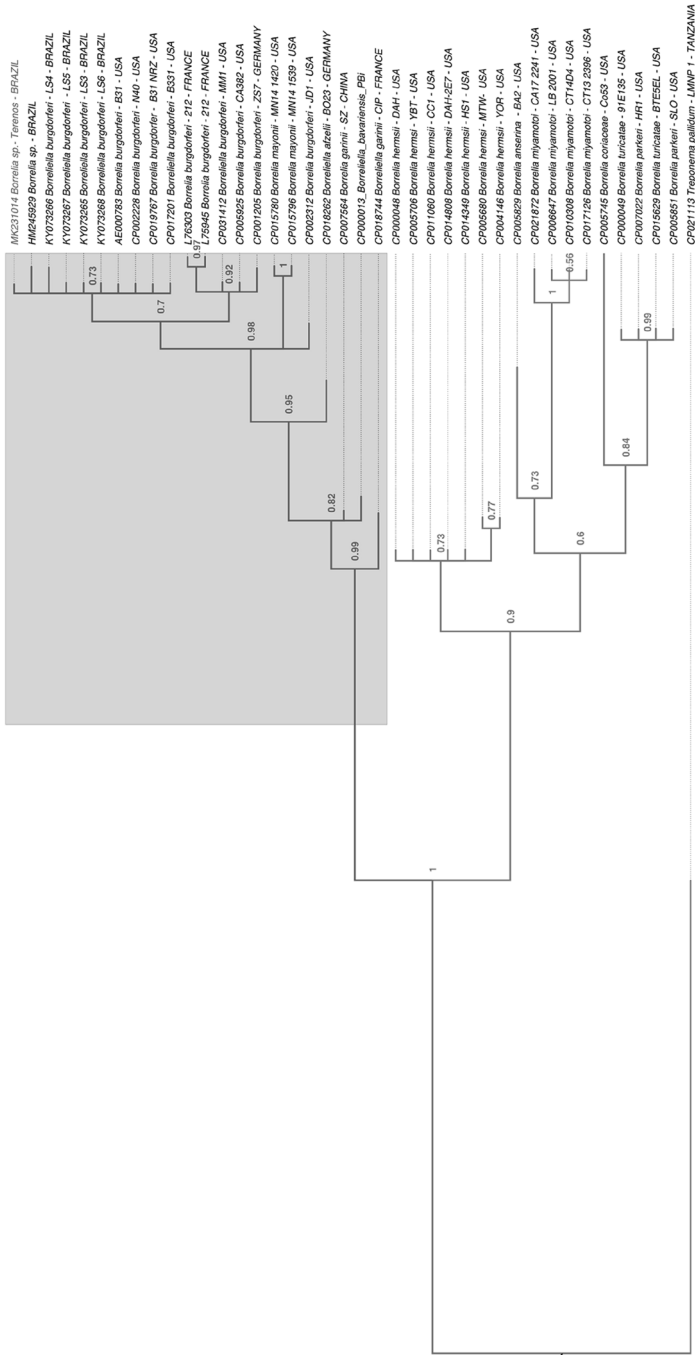


Fig. 2 Phylogenetic tree of *Borrelia* spp. (GenBank: 231013). Evolutionary history was based on the Bayesian inference tree with probability scores for the *flgE* gene. The scale bar indicates 0.03 changes per nucleotide position. The sample sequences obtained in this study are shown in blue. (Color figure online)

As well as *R. rickettsii*, *R. parkeri* belongs to the Rocky Mountain spotted fever bacterial group and is recognized as the etiological agent of rickettsial disease in Brazil (Spolidorio et al. 2010). Compared with other rickettsial diseases, spotted fever associated with *R. parkeri* presents milder symptoms, primarily fever, eschar, lymphadenopathy, rash and lethal cases have not been reported (Silveira et al. 2007).

In Brazil, *A. ovale* is considered one of the main vectors of *R. parkeri* (Sabatini et al. 2010), beside *A. tigrinum* and *A. triste* (Faccini-Martínez et al. 2018). According to the genotypes pre-established in the literature, various phylogenetic groups exist (Nieri-Bastos et al. 2018) as do various ticks related to *R. parkeri* (Table 2). It is important to state that, according to the literature and Table 1, the Atlantic Forest strain is related to *A. ovale* and may be the main etiological agent of the disease (Spolidorio et al. 2010; Nieri-Bastos et al. 2018).

In this work, molecular evidence of a 100% identity *R. parkeri* Atlantic Forest strain was obtained from a free-living *A. sculptum* adult tick. As shown in Table 2, most reports of *R. parkeri* related to ticks are described in the South (41.9%) and Southeast (29%) regions, corroborating with other available data in literature (Spolidorio et al. 2010; Krawczak et al. 2016).

The first time that *R. parkeri* was found in *A. sculptum* was in Minas Gerais state (Szabó et al. 2019). According to the authors, the relationship between *R. parkeri* and *A. sculptum* remains unclear. Simultaneous parasitism with *A. nodosum* was recorded, however, with no DNA amplification in this tick. In our study a free-living female tick was found with *R. parkeri* but, until an experiment with infected and non-infected *A. sculptum* is performed, we can only suggest that transstadial transmission may occur.

In addition to molecular evidence of the *Rickettsia* described above, another *A. sculptum* adult tick showed DNA amplification for a borrelial gene (*flgE*). Whereas Lyme borreliosis has a wide distribution in the Northern Hemisphere, which is linked to the presence of its main vector *Ixodes* spp. (Gray 1998), a similar disease is present in Brazil and the corresponding vector has remained unclear (Dantas-Torres 2008), which has inspired new studies, as shown in Table 3.

According to Table 3, we can highlight the importance of serological diagnoses for both humans and animals in epidemiological investigations of the disease in Brazil. Indeed, serological investigations can identify potential reservoirs and suggest areas of infection risk. Most diagnoses for humans are also achieved through serological tests and clinical evaluations as reported in Table 3 and in agreement with the studies by Yoshinari et al. (2010) and Mantovani et al. (2012). However, it is important to state that serological cross-reactions may occur (Magnarelli et al. 1987) and these findings do not state that classical Lyme disease exists in Brazil (de Oliveira et al. 2018).

In a study conducted in the Brazilian Pampas region, the presence of bacterial DNA belonging to *B. burgdorferi* s.l. was found in larvae and nymphs of *Ixodes longiscutatus* using the *flaB* gene in nested PCR (Dall'Agnol et al. 2017). Despite belonging to the genus of ticks classified as main vectors for this disease in the Northern Hemisphere, this ectoparasite is not very relevant to the direct transmission of TBD to humans in Brazil, and it mainly participates in the maintenance and circulation of the pathogen in wild animals (de la Fuente et al. 2008; Dall'Agnol et al. 2017).

In the state of Mato Grosso do Sul, a molecular investigation utilizing the *flgE* gene revealed evidence for *Borrelia* sp. in the tick *Rhipicephalus microplus* (Rezende et al. 2016). However, this tick is closely related to livestock-related losses, and humans are considered sporadic hosts (Guglielmone et al. 2006; Kaur et al. 2019).

Table 2 Tick species described in the literature as carrying *Rickettsia parkeri* or its various strains

Ticks	<i>Rickettsia parkeri</i>	Location	References
<i>Amblyomma ovale</i>	Atlantic forest strain	Itoupava, SC	Barbieri et al. (2014)
		Environmental reserve, SP	Luz et al. (2016)
		Rio Grande do Sul	Voizzoni et al. (2016)
<i>Amblyomma aureolatum</i>		Poconé, MT	Witter et al. (2016)
		Ibiraçu, ES	Acosta et al. (2018)
		Águas Mornas, Blumenau, Urussanga, SC	Medeiros et al. (2011)
		Environmental reserve, SP	Sabatini et al. (2010)
	<i>R. parkeri</i> -like	Itoupava, SC	Dall'Agnol et al. (2018)
	<i>R. parkeri</i> s.s. clone RS	Santana do Livramento and Triunfo, RS	Dall'Agnol et al. (2018)
	Atlantic forest strain	Blumenau, SC	Medeiros et al. (2011)
	<i>R. parkeri</i> s.s. clone RS	Santana do Livramento and Triunfo, RS	Dall'Agnol et al. (2018)
	<i>Rickettsia</i> sp. AF strain	Rosário do Sul, RS	Week et al. (2017)
	<i>R. parkeri</i> -like	Environmental reserve Paraíba	Lugarini et al. (2015)
<i>Amblyomma tigrinum</i>	NOD strain	Perdizes and Uberlândia, MG; Bebedouro, SP; Anastácio, MS	Szabó et al. (2019)
		Cuiabá, MT	Witter et al. (2016)
		Pantanal and Cerrado, MT	Ramos et al. (2015)
		Pantanal, MT	Melo et al. (2015)
		Paulicéia, SP	Silveira et al. (2007)
		Água Clara, MS	Nieri-Bastos et al. (2013)
		Ivinhema, MS	Ogrzewalska et al. (2013)
		Juiz de Fora, MG	Zeringota et al. (2017)
		Paraná	Pacheco et al. (2012)
		Paraná	Pacheco et al. (2012)
<i>Amblyomma triste</i>	Pantanal strain At46		
	Strain At24		
<i>Amblyomma calcaratum</i>	<i>R. parkeri</i>		
<i>Amblyomma parkeri</i>	NOD strain		
	ApPR strain		
<i>Amblyomma longirostre</i>	NOD strain		

Table 2 (continued)

Ticks	<i>Rickettsia parkeri</i>	Location	References
<i>Amblyomma dubitatum</i>	<i>R. parkeri</i> clone RS Cooperi strain	Toropi and Quevedos, RS Pedreira, SP	Weck et al. (2017) Labruna et al. (2004)
<i>Rhipicephalus sanguineus</i>	Atlantic forest strain	Blumenau, SC Environmental reserve, SP	Medeiros et al. (2011) Sabatini et al. (2010)
<i>Amblyomma sculptum</i>	<i>Rickettsia</i> sp. AF strain NOD strain Atlantic forest strain	Rosário do Sul, RS Sponsorship, MG Terenos, MS	Weck et al. (2017) Szabó et al. (2019) Present study

Table 3 Records of the detection of borreliosis in Brazil

Location	Pathogen	Vector/host	Method	References
North and Northeast	<i>B. burgdorferi</i> s.l.	Domestic dog	Serology	Pacheco et al. (2016)
	<i>B. burgdorferi</i> s.l.	Human	Clinical, serology	Rodrigues et al. (2007)
	<i>B. burgdorferi</i>	Horse	Serology	Galo et al. (2009)
	<i>B. burgdorferi</i> s.l.	Human	Serology, microscopy	Talhari et al. (2010)
	<i>Borrelia</i> sp.	Human	Clinical, serology	Carranza-Tamayo et al. (2012)
Southeast	<i>Borrelia</i> sp.	Human	Clinical, serology	Azulay et al. (1991)
	<i>B. burgdorferi</i>	Human	Clinical, serology	Yoshinari et al. (2003)
	<i>Borrelia</i> sp.	Rodents, tick	Serology	Abel et al. (2000)
	<i>Borrelia</i> sp.	Domestic dog	Serology	Alves et al. (2004)
South	<i>B. burgdorferi</i>	Human	Serology	Gouveia et al. (2010)
	<i>B. burgdorferi</i>	Dog, horse, and human	Serology	Spolidorio et al. (2010)
	<i>B. burgdorferi</i>	Wild and domestic mammals	Serology	Montandon et al. (2014)
	<i>Borrelia</i> sp.	Human	PCR	Mantovani et al. (2012)
	<i>B. burgdorferi</i> s.l.	Domestic dog	Serology	Gonçalves et al. (2015)
South	<i>B. burgdorferi</i> s.s.	<i>Dermacentor nitens</i>	PCR	Gonçalves et al. (2013)
	<i>B. burgdorferi</i>	Domestic dog, horse, and human	Serology	Nascimento et al. (2016)
	<i>B. burgdorferi</i> s.l.	<i>Ixodes longiscittatus</i>	PCR	Dall'Agnol et al. (2017)
	<i>B. burgdorferi</i>	Horse	Serology	Socoloski et al. (2018)
	<i>B. burgdorferi</i> s.l.	Human	Serology, PCR	Lopes et al. (2017)
Midwest	<i>B. burgdorferi</i> s.l.	Human and <i>Rhipicephalus microplus</i>	PCR	Rezende et al. (2016)
	<i>B. burgdorferi</i>	<i>Amblyomma sculptum</i>	PCR	Current study

It is important to note that all reports cited above represent ticks with no direct relevance to human parasitism. Based on epidemiological investigations, the most accepted hypothesis of Brazilian Lyme-like disease transmission to humans in Brazil is via *A. sculptum*, previously known as *A. cajennense* (Dantas-Torres 2008; Beati et al. 2013; Nava et al. 2014). According to Gray (1998), ticks belonging to the genus *Ixodes* possess certain characteristics that cause them to be the main vectors of the disease, such as a heteroxenous biological cycle, parasitization of mainly birds and small and medium-sized mammals in immature stages, and distinct seasonality in the search for hosts with regulation via the diapause mechanism. These ecological and physiological requirements are mostly present in *A. sculptum*, except for specific differences due to different location. Moreover, *A. sculptum* accounted for 99.9% of the specimens collected in our study, and this high availability, along with its anthropophilic characteristics, supports its potential as a vector.

In addition to molecular evidence for the bacterium itself, sequencing of the DNA obtained in this study using *flgE* primers enabled a phylogenetic analysis (Fig. 1). The phylogenetic analyses of the *flgE* fragment grouped our sample into the *B. burgdorferi* clade with other sequences from the USA and Brazil. It is noteworthy that the identity found with Brazilian strains was 99.4%, differing from the sequences found in the USA (98.5%), corroborating with studies by Mantovani et al. (2012).

Conclusions

This study reports for the first time molecular evidence of the Atlantic Forest strain of *R. parkeri* in *A. sculptum* ticks in the Midwest region of Brazil. Moreover, genetic material from *Borrelia* spp. was detected (by PCR) for the first time in a tick of the genus *Amblyomma* in Brazil. Our data emphasize the need for further studies related to *A. sculptum* competence as a vector of the two agents described.

Acknowledgements The authors thank the Program for Technological Development in Tools for Health-PDTISFIOCRUZ for the use of its facilities. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES) Finance Code 001, Fundação de Apoio à Pesquisa Agropecuária e Ambiental (FUNDAPAM/MS), Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT/MS) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

References

- Abel IS, Marzagao G, Yoshinari NH, Schumaker TT (2000) *Borrelia*-like spirochetes recovered from ticks and small mammals collected in the Atlantic Forest Reserve, Cotia county, State of Sao Paulo, Brazil. Mem Inst Oswaldo Cruz 95:621–624
- Acosta I, Luz HR, Faccini-Martinez AA, Munoz-Leal S, Cerutti C, Labruna MB (2018) First molecular detection of *Rickettsia* sp. strain Atlantic rainforest in *Amblyomma ovale* ticks from Espirito Santo state Brazil. Rev Bras Parasitol Vet 27:420–422

- Almeida RFC, Garcia MV, Cunha RC, Matias J, Labruna MB, Andreotti R (2013) The first report of *Rickettsia* spp. In *Amblyomma nodosum* in the State of Mato Grosso do Sul. Brazil. Ticks Tick Borne Dis 4:156–159
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
- Alves AL, Madureira RC, da Silva RA, Corrêa FN, Botteon RCCM (2004) Frequência de anticorpos contra *Borrelia burgdorferi* em cães na região metropolitana. Pesq Vet Bras 24:203–206
- Angerami RN, Nunes EM, Nascimento EM, Freitas AR, Kemp B, Feltrin AF et al (2009) Clusters of Brazilian spotted fever in Sao Paulo State, southeastern Brazil. A review of official reports and the scientific literature. Clin Microbiol Infect 15(Suppl 2):202–204
- Azulay RD, Azulay-Abulafia L, Sodre CT, Azulay DR, Azulay MM (1991) Lyme disease in Rio de Janeiro, Brazil. Int J Dermatol 30:569–571
- Barbieri AR, Filho JM, Nieri-Bastos FA, Souza JC Jr, Szabo MP, Labruna MB (2014) Epidemiology of *Rickettsia* sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. Ticks Tick Borne Dis 5:848–853
- Barros-Battesti DM, Arzua M, Bechara GH (2006) Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies, 1st edn. Butantan, São Paulo, p 223
- Beati L, Nava S, Burkman EJ, Barros-Battesti DM, Labruna MB, Guglielmone AA et al (2013) *Amblyomma cajennense* (Fabricius, 1787) (*Acari: Ixodidae*), the Cayenne tick: phylogeography and evidence for allopatric speciation. BMC Evol Biol 13:267
- Beugnet F, Chalvet-Monfray K (2013) Impact of climate change in the epidemiology of vector-borne diseases in domestic carnivores. Comp Immunol Microbiol Infect Dis 36:559–566
- Carranza-Tamayo CO, da Costa JNG, Bastos WM (2012) Lyme disease in the state of Tocantins, Brazil: report of the first cases. Braz J Infect Dis 16:586–589
- Colwell DD, Dantas-Torres F, Otranto D (2011) Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet Parasitol 182:14–21
- Dall'Agnol B, Michel T, Weck B, Souza UA, Webster A, Leal BF et al (2017) *Borrelia burgdorferi* sensu lato in *Ixodes longiscutatus* ticks from Brazilian Pampa. Ticks Tick Borne Dis 8:928–932
- Dall'Agnol B, Souza UA, Weck B, Trigo TC, Jardim MMA, Costa FB et al (2018) *Rickettsia parkeri* in free-ranging wild canids from Brazilian Pampa. Transbound Emerg Dis 65:224–230
- Dantas-Torres F (2007) Rocky mountain spotted fever. Lancet Infect Dis 7:724–732
- Dantas-Torres F (2008) Canine vector-borne diseases in Brazil. Parasit Vectors 1:25
- de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE (2008) Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci 13:6938–6946
- de Oliveira SV, dos Santos JP, Aver G, Corrêa LLC, Vizzoni VF, Gazeta GS (2018) Serological evidence of lyme disease and possible introduction of *Borrelia* along migratory bird routes in Brazil. Vector Biol J 3:1
- Durand J, Herrmann C, Genne D, Sarr A, Gern L, Voordouw MJ (2017) Multistrain infections with lyme borreliosis pathogens in the tick vector. Appl Environ Microbiol 83:1–14
- Faccini-Martínez AA, de Oliveira SV, Cerutti Junior C, Labruna MB (2018) Febre Maculosa por *Rickettsia parkeri* no Brasil: condutas de vigilância epidemiológica, diagnóstico e tratamento. J Health Biol Sci 6:299–312
- Galo KR, Fonseca AH, Madureira RC, Neto JDB (2009) Frequência de anticorpos homólogos anti-*Borrelia burgdorferi* em equinos na mesorregião metropolitana de Belém, Estado do Pará. Pesq Vet Bras 29:229–232
- Gonçalves DD, Carreira T, Nunes M, Benitez A, Lopes-Mori FM, Vidotto O et al (2013) First record of *Borrelia burgdorferi* B31 strain in *Dermacentor nitens* ticks in the Northern Region of Parana (Brazil). Braz J Microbiol 44:883–887
- Gonçalves D, Moura RA, Dreer M, Nascimento DDAG, Rodrigues GV, Caetano I et al (2015) First record of *Borrelia burgdorferi* sensu lato antibodies in stray dogs in the Northwest Region of Parana State, Brazil. Semin Cienc Agrar 36:2641–2648
- Gouveia EA, Alves MF, Mantovani E, Oyafuso LK, Bonoldi VL, Yoshinari NH (2010) Profile of patients with Baggio-Yoshinari Syndrome admitted at "Instituto de Infectologia Emilio Ribas". Rev Inst Med Trop Sao Paulo 52:297–303
- Gray JS (1998) Review the ecology of ticks transmitting lyme borreliosis. Exp Appl Acarol 22:249–258
- Guglielmone AA, Beati L, Barros-Battesti DM, Labruna MB, Nava S, Venzal JM et al (2006) Ticks (*Ixodidae*) on humans in South America. Exp Appl Acarol 40:83–100
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
- Jaenson TGT (1991) The epidemiology of Lyme Borreliosis. Parasitol Today 7:39–45

- Jongejan F, Uilenberg G (2004) The global importance of ticks. *Parasitology* 129(Suppl):S3–14
- Kaur N, Prasher P, Kumar K, Dhingra S (2019) *Rhipicephalus (Boophilus) microplus (Arachnida: Ixodidae)* larvae infestation of human eyelids. A rare case. *Acarologia* 59:21–25
- Krawczak FS, Muñoz-Leal S, Guztzakzy AC, Oliveira SV, Santos FC, Angerami RN et al (2016) *Rickettsia* sp. Strain Atlantic rainforest infection in a patient from a spotted fever-endemic area in Southern Brazil. *Am J Trop Med Hyg* 95:551–553
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Labruna MB (2009) Ecology of *Rickettsia* in South America. *Ann N Y Acad Sci* 1166:156–166
- Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A et al (2004) *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. *J Clin Microbiol* 42:90–98
- Lopes FA, Rezende J, Silva D, Alves FCG, Oliveira CE, Costa IPD (2017) Molecular evidence of *Borrelia burgdorferi* sensu lato in patients in Brazilian central-western region. *Rev Bras Reumatol Engl Ed* 57:641–645
- Lugarini C, Martins TF, Ogrzewalska M, de Vasconcelos NC, Ellis VA, de Oliveira JB et al (2015) Rickettsial agents in avian ixodid ticks in northeast Brazil. *Ticks Tick Borne Dis* 6:364–375
- Luz HR, McIntosh D, Furusawa GP, Flausino W, Rozental T, Lemos ER et al (2016) Infection of *Amblyomma ovale* with *Rickettsia* species Atlantic rainforest in Serra do Mar, Sao Paulo State, Brazil. *Ticks Tick Borne Dis* 7:1265–1267
- Magnarelli LA, Anderson JF, Johnson RC (1987) Cross-Reactivity in serological tests for Lyme Disease and other spirochetal infections. *J Infect Dis* 156:183–188
- Mantovani E, Costa IP, Gauditano G, Bonoldi VLN, Higuchi ML, Yoshinari NH (2007) Description of Lyme disease-like syndrome in Brazil. Is it a new tick borne disease or Lyme disease variation? *Braz J Med Biol Res* 40:443–456
- Mantovani E, Marangoni RG, Gauditano G, Bonoldi V, Yoshinari NH (2012) Amplification of the flgE gene provides evidence for the existence of a Brazilian borreliosis. *Rev Inst Med trop Sao Paulo* 54:153–157
- Matias J, Garcia MV, Cunha RC, Aguirre AAR, Barros JC, Csordas BG, Andreotti R (2015) Spotted fever group *Rickettsia* in *Amblyomma dubitatum* tick from the urban area of Campo Grande, Mato Grosso do Sul, Brazil. *Ticks Tick Borne Dis* 6:107–110
- Mead PS (2015) Epidemiology of Lyme disease. *Infect Dis Clin North Am* 29:187–210
- Medeiros AP, Souza AP, Moura AB, Lavina MS, Bellato V, Sartor AA et al (2011) Spotted fever group *Rickettsia* infecting ticks (*Acari: Ixodidae*) in the state of Santa Catarina, Brazil. *Mem Inst Oswaldo Cruz* 106:926–930
- Melo AL, Alves AS, Nieri-Bastos FA, Martins TF, Witter R, Pacheco TA et al (2015) *Rickettsia parkeri* infecting free-living *Amblyomma triste* ticks in the Brazilian Pantanal. *Ticks Tick Borne Dis* 6:237–241
- Montandon C, Yoshinari NH, Milagres BS, Barcelos R, Gomes G, Moreira H et al (2014) Evidence of *Borrelia* in wild and domestic mammals from the state of Minas Gerais, Brazil. *Rev Bras Parasitol Vet* 23:287–290
- Nascimento DA, Vieira RF, Vieira TS, Toledo RD, Tamekuni K, Santos NJ et al (2016) Serosurvey of *Borrelia* in dogs, horses, and humans exposed to ticks in a rural settlement of southern Brazil. *Rev Bras Parasitol Vet* 25:418–422
- Nava S, Beati L, Labruna MB, Caceres AG, Mangold AJ, Guglielmo AA (2014) Reassessment of the taxonomic status of *Amblyomma cajennense* with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum*, and *Amblyomma sculptum* (Ixodida: Ixodidae). *Ticks Tick Borne Dis* 5:252–276
- Nieri-Bastos FA, Szabó MPJ, Pacheco RC, Soares JF, Morase-Filho J, Dias RA et al (2013) Comparative evaluation of infected and noninfected *Amblyomma triste* ticks with *Rickettsia parkeri*, the agent of an emerging Rickettsiosis in the New World. *Biomed Res Int* 2013:1–6
- Nieri-Bastos FA, Marcili A, De Sousa R, Paddock CD, Labruna MB (2018) Phylogenetic evidence for the existence of multiple strains of *Rickettsia parkeri* in the new world. *Appl Environ Microbiol*. <https://doi.org/10.1128/aem.02872-17>
- Ogrzewalska M, Saraiva DC, Moraes-Filho J, Martins TF, Costa FB, Pinter A et al (2012) Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. *Ticks Tick Borne Dis* 4:145–147
- Ogrzewalska M, Martins T, Capek M, Literak I, Labruna MB (2013) A *Rickettsia parkeri*-like agent infecting *Amblyomma calcaratum* nymphs from wild birds in Mato Grosso do Sul, Brazil. *Ticks Tick Borne Dis* 4:145–147

- Oliveira PR, Borges LM, Lopes CM, Leite RC (2000) Population dynamics of the free-living stages of *Amblyomma cajennense* (Fabricius, 1787) (*Acari: Ixodidae*) on pastures of Pedro Leopoldo, Minas Gerais State, Brazil. *Vet Parasitol* 92:295–301
- Pacheco RC, Arzua M, Nieri-Bastos FA, Moraes-Filho J, Marcili A, Richtzenhain LJ et al (2012) Rickettsial infection in ticks (*Acari: Ixodidae*) collected on birds in southern Brazil. *J Med Entomol* 49:710–716
- Pacheco A, Bahia M, Cordeiro MD, Prado WS, Scofield A, Góes-Cavalcante, et al (2016) Frequência de anticorpos contra *Toxoplasma gondii* e *Borrelia* spp. em cães domiciliados no município de Castanhal, estado do Pará. *Braz J Vet Med* 38:119–123
- Parola P, Raoult D (2001) Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin Infect Dis* 32:897–928
- Ramos DG, Melo AL, Martins TF, Ada SA, Tdos AP, Pinto LB et al (2015) Rickettsial infection in ticks from wild birds from Cerrado and the Pantanal region of Mato Grosso, midwestern Brazil. *Ticks Tick Borne Dis* 6:836–842
- Regnery RL, Spruill CL, Plikaytis BD (1991) Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* 173:1576–1589
- Rezende J, Lopes F, Alves F, Alves G, Bruno AR, Moreno SE et al (2016) Detection of *Borrelia burgdorferi* sensu lato in Mato Grosso Do Sul Brazil. *JSM Trop Med Res* 1:1003
- Rodrigues BD, Meireles VMB, Braz MN (2007) Borreliose de lyme simile—relato de caso. *Rev Para Med* 21:63–67
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Sabatini GS, Pinter A, Nieri-Bastos FA, Marcili A, Labruna MB (2010) Survey of ticks (*Acari: Ixodidae*) and their *Rickettsia* in an Atlantic rain forest reserve in the State of Sao Paulo, Brazil. *J Med Entomol* 47:913–916
- Sal MS, Li C, Motalab MA, Shibata S, Aizawa S, Charon NW (2008) *Borrelia burgdorferi* uniquely regulates its motility genes and has an intricate flagellar hook-basal body structure. *J Bacteriol* 190:1912–1921
- Sanchez JL (2015) Clinical manifestations and treatment of lyme disease. *Clin Lab Med* 35:765–778
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463–5467
- Sangioni LA, Horta MC, Vianna MC, Gennari SM, Soares RM, Galvao MA et al (2005) Rickettsial infection in animals and Brazilian spotted fever endemicity. *Emerg Infect Dis* 11:265–270
- Silveira I, Pacheco RC, Szabo MP, Ramos HG, Labruna MB (2007) *Rickettsia parkeri* in Brazil. *Emerg Infect Dis* 13:1111–1133
- Socoloski SNG, de Castro BG, Cordeiro MD, da Fonseca AH, Cepeda MB, Nicolino RR et al (2018) Epidemiological investigation of *Borrelia burgdorferi* in horses in the municipality of Sinop-MT, Brazil. *Trop Anim Health Prod* 50:831–836
- Spolidorio MG, Labruna MB, Machado RZ, Moraes-Filho J, Zago AM, Donatele DM et al (2010) Survey for tick-borne zoonoses in the state of Espírito Santo, southeastern Brazil. *Am J Trop Med Hyg* 83:201–206
- Szabó MPJ, Pascoal JO, Martins MM, Ramos VDN, Osava CF, Santos ALQ et al (2019) Ticks and *Rickettsia* on anteaters from southeast and central-west Brazil. *Ticks Tick Borne Dis* 10:540–545
- Talhari S, Santos MNDS, Talhari C, Ferreira LCDL, Silva RM Jr, Zelger B et al (2010) *Borrelia burgdorferi* "sensu lato" in Brazil: occurrence confirmed by immunohistochemistry and focus floating microscopy. *Acta Trop* 115:200–204
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Tree Bio (2016) FigTree. <https://tree.bio.ed.ac.uk/>. Accessed 2 Jan 2018
- Voizzoni VF, Silva AB, Cardoso KM, dos Santos FB, Stenzel B, Amorim M et al (2016) Genetic identification of *Rickettsia* sp. strain Atlantic rainforest in an endemic area of a mild spotted fever in Rio Grande do Sul state Southern Brazil. *Acta Trop* 162:142–145
- Weck B, Dall'Agnol B, Souza U, Webster A, Stenzel B, Klafke G et al (2017) *Rickettsia parkeri* in *Amblyomma dubitatum* ticks in a spotted fever focus from the Brazilian Pampa. *Acta Trop* 171:182–185
- Witter R, Martins TF, Campos AK, Melo AL, Correa SH, Morgado TO et al (2016) Rickettsial infection in ticks (*Acari: Ixodidae*) of wild animals in midwestern Brazil. *Ticks Tick Borne Dis* 7:415–423
- Yoshinari NH, Abrao MG, Bonoldi VL, Soares CO, Madruga CR, Scofield A et al (2003) Coexistence of antibodies to tick-borne agents of babesiosis and lyme borreliosis in patients from Cotia county, State of Sao Paulo, Brazil. *Mem Inst Oswaldo Cruz* 98:311–318

- Yoshinari NH, Mantovani E, Bonoldi VL, Marangoni RG, Gauditano G (2010) Brazilian lyme-like disease or Baggio-Yoshinari syndrome: exotic and emerging Brazilian tick-borne zoonosis. *Rev Assoc Med Bras* 56:363–369
- Zeringota V, Maturano R, Luz HR, Senra TOS, Daemon E, Faccini JLH et al (2017) Molecular detection of *Rickettsia rhipicephali* and other spotted fever group *Rickettsia* species in *Amblyomma* ticks infesting wild birds in the state of Minas Gerais, Brazil. *Ticks Tick Borne Dis* 8:81–89

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.