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Original article

Phenology of *Amblyomma sculptum* in a degraded area of Atlantic rainforest in north-eastern Brazil

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ABSTRACT

Amblyomma sculptum is the principal vector of *Rickettsia rickettsii*, the main agent of spotted fever rickettsiosis in Brazil. Little information is available regarding the population dynamics of this tick in some regions, including north-eastern Brazil, where cases of spotted fever rickettsioses are increasingly reported. Herein, we studied the phenology of *A. sculptum* in a rural area in north-eastern Brazil. Ticks were collected from the environment, using dry ice-baited traps, monthly for two consecutive years. In total, 1500 ticks were collected: 94 females (6.3%), 74 males (4.9%), 468 nymphs (31.2%), and 864 larvae (57.6%). All nymphs and females were identified as *A. sculptum*. Males were tentatively identified as *A. sculptum* and larvae as *Amblyomma* spp. Ticks were more numerous during spring and summer, followed by autumn and winter. Peaks of larvae and nymphs were recorded during summer and spring, respectively, whereas adults were more frequently collected in spring. A total of 380 ticks were tested by PCR for the *gltA* gene of *Rickettsia* spp., but none of them were positive. While our results revealed a seasonal pattern for *A. sculptum* in north-eastern Brazil that is distinct from the seasonal pattern in south-eastern Brazil, we caution that the observed pattern could have been biased by the relatively low number of ticks collected. Finally, the absence of *Rickettsia*-infected ticks does not rule out the possibility that rickettsial organisms are circulating in the study area and further long-term studies are warranted.

1. Introduction

Spotted fever rickettsioses are endemic in Brazil, where *Rickettsia rickettsii* and *Rickettsia parkeri* have been recognized as disease agents in humans (Labruna et al., 2014; Nieri-Bastos et al., 2018). From 2007 to 2015, 17,117 suspected cases of spotted fever rickettsioses were reported, of which 1245 were confirmed across 12 states (Oliveira et al., 2016). While most of the cases are concentrated in south-eastern Brazil, spotted fever rickettsioses are increasingly reported in the north-eastern region (Oliveira, 2016). For instance, several cases of a milder spotted fever rickettsiosis have been documented in the Maciço de Baturité region of Ceará state (Moerbeck et al., 2016; Oliveira, 2016). Cases of spotted fever rickettsioses in this focus have been associated with *R. parkeri* (Atlantic rainforest strain) (Moerbeck et al., 2016; Oliveira, 2016) and a recent study reported the isolation of a *R. rickettsii*-like strain from *Rhipicephalus sanguineus* sensu lato collected from dogs in the Maciço de Baturité region (Silva et al., 2017). More recently, a fatal case diagnosed as "spotted fever rickettsiosis" was documented in Pernambuco state (Oliveira et al., 2018). While this case cannot be confirmed as spotted fever rickettsiosis (i.e., no serology performed and PCR positivity only for *gltA* gene), it reinforces the need for further studies in north-eastern Brazil.

Vectors of spotted fever rickettsiae are present in Pernambuco. For instance, *Rickettsia amblyommatis* has been reported in *Amblyomma auricularium* and *Amblyomma pseudoconcolor* in different areas of Pernambuco (Saraiva et al., 2013; Silva et al., 2018). In addition, *Amblyomma sculptum* (reported as "*Amblyomma cajennense*"), the main vector of *R. rickettsii* in Brazil, was reported in Amaraji, Gravatá, and Sairé (Dantas-Torres, 2009).

While *A. sculptum* is known to occur in Pernambuco and other states in north-eastern Brazil (Martins et al., 2016), there is virtually no information about the phenology of this tick in this region. In fact, information about the seasonal dynamics of *A. sculptum* derives from studies conducted in south-eastern Brazil (Oliveira et al., 2000; Szabó et al., 2007; Guedes and Leite, 2008; Labruna et al., 2002; Veronez et al., 2010; Barbieri et al., 2019) and may not necessarily apply to other regions. In this context, we studied the phenology of *A. sculptum* in Amaraji, where the tick was found for the first time in Pernambuco

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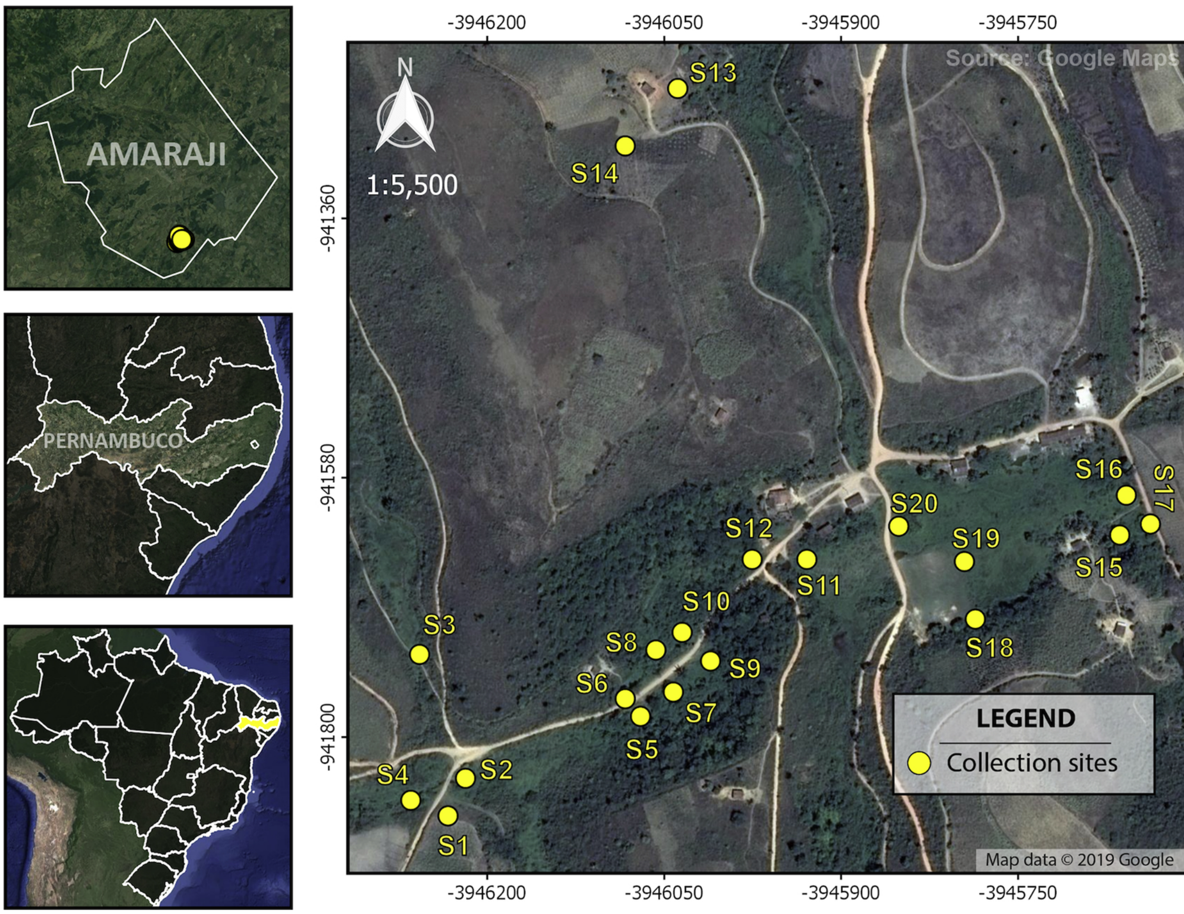

Fig. 1. Location of the collection sites in Amaraji, Pernambuco, Brazil. Maps created using QGIS 2.18.28 (QGIS Development Team, 2019), with the QuickMapServices plugin and shapefiles from Google Maps.

(Dantas-Torres, 2009). We also assessed the presence of *Rickettsia* spp. DNA in these ticks.

2. Material and methods

This study was conducted in a rural area in Amaraji (08°22′58″ S, 35°27′10″ W, altitude: 289 m), located 96 km from Recife (the capital city). Amaraji is located in the southern part of the Atlantic rainforest region of Pernambuco and its climate is hot tropical and humid (*Am*, in Köppen climate classification), with annual average temperature of 23.1 °C and annual average precipitation of 1805 mm. With a land area of 234.78 km^2, Amaraji is home to a human population of around 22,600. Most of the original vegetation coverage has been replaced by croplands and grazing areas, but degraded forest fragments are still present. Small rodents and marsupials are abundant in both forest fragments and croplands (Brandão-Filho et al., 2003). Horses and dogs are known hosts of *A. sculptum* in these areas (Dantas-Torres, 2009).

Ticks were collected monthly, from January 2015 to December 2016, in 20 pre-selected, georeferenced collection sites (S1-S20), covering an area of approximately 170 m^2 (Fig. 1). Geographical coordinates of each collection site were recorded using a Garmin eTrex Venture HC GPS (Garmin International, Olathe, KS, USA). Sites chosen for tick collection were represented by grasslands near forest fragments, plantations, pastures and rural properties. Collection sites were also located near small lakes and approximately 2.5 km from the Amaraji river (Fig. 2). Briefly, a 1 m^2 piece of white flannel, with double-sided tape surrounding its edges and a plastic pot containing 500 g of pelletized dry ice (CO_2 source), was placed on the ground vegetation in each collection site. Collections were carried out in the afternoon and CO_2 traps were left in the environment for 2 h. Overall, 19–20 traps were used monthly (i.e., 20 traps during the first 15 months, and 19 traps in the last nine months), totalling 471 traps installed and 942 trapping hours.

Ticks found on the traps were collected and placed in labelled vials containing 70% ethanol. Adults and nymphs were identified to species level using morphological keys and species descriptions (Aragão and Fonseca, 1961; Onofrio et al., 2006; Martins et al., 2010; Nava et al., 2014). While males of *A. cajennense* and *A. sculptum* cannot be morphologically distinguished with certainty, *A. sculptum* is the only species belonging to the *A. cajennense* group present in the Atlantic rainforest biome (Martins et al., 2016). Furthermore, male ticks from Amaraji were already genetically characterized as *A. sculptum* (Martins et al., 2016). Males collected in this study were therefore tentatively assigned to *A. sculptum.* Larvae were identified to genus level. Voucher specimens (two males, two females and 10 nymphs) were deposited in the tick collection “Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva” of the University of São Paulo (accession number CNC-3647).

Ticks were subjected to DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA), following the manufacturer’s instructions. DNA from adults was extracted individually, whereas DNA from nymphs was extracted from pools containing 2–10 specimens. Pools were formed by nymphs collected in the same CO_2 trap and on the same day. Extracted DNA samples were subjected to PCR for the detection of *Rickettsia* spp. DNA, using primers CS-78 (forward) and CS-323 (reverse), targeting a 401 bp fragment of the citrate synthase (*gltA*) gene, which is present in all species of the genus *Rickettsia* (Labruna et al., 2004). The final reaction volume (25 μl) contained 6.5 μl of DNA-free water, 12.5 μl of GoTaq Colourless Master

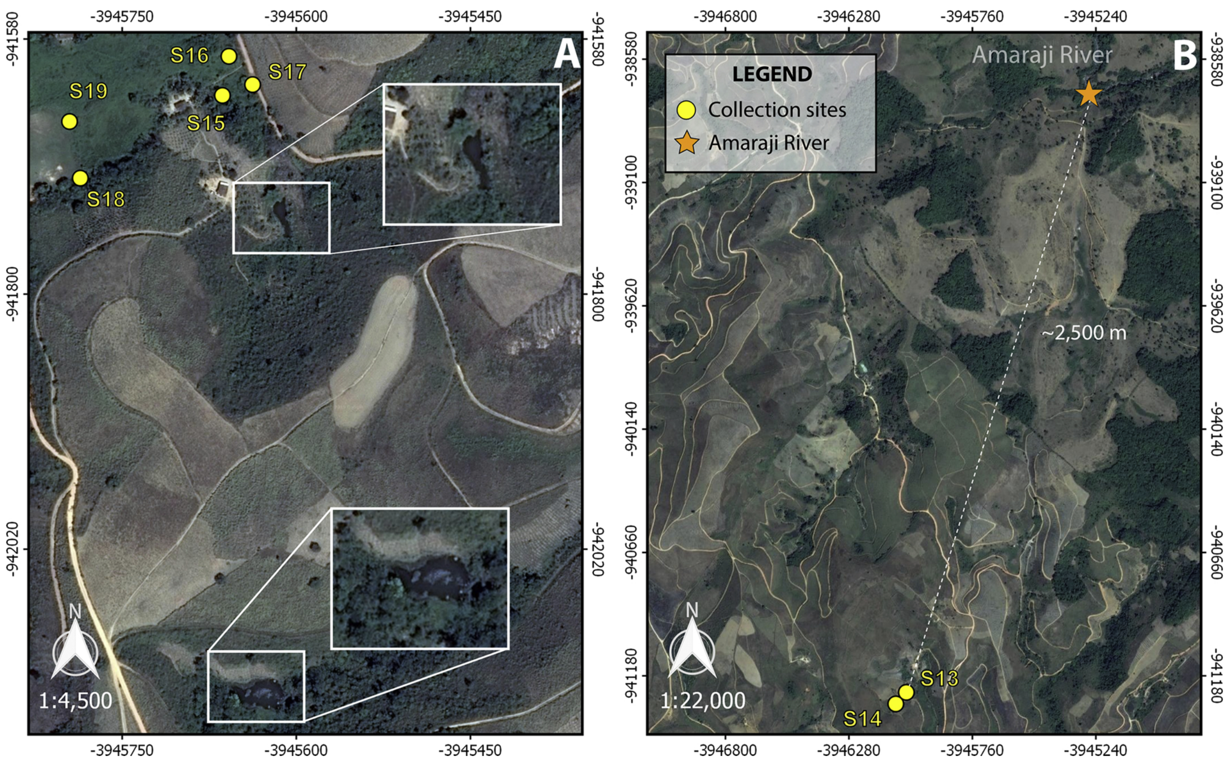

Fig. 2. Location of small lakes (highlighted in rectangles) (A) and of the Amaraji river (orange star) (B) near some of the tick collection sites (S13 to S18), Amaraji, Pernambuco, Brazil. Maps created using QGIS 2.18.28 (QGIS Development Team, 2019), with the QuickMapServices plugin and shapefiles from Google Maps.

Mix (Promega, Southampton, UK), 2 µl of each primer (at the concentration of 25 pmol/µl) and 2 µl of template DNA. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, 40 cycles at 95 °C for 15 s, 58 °C for 30 s and 72 °C for 30 s, and final extension at 72 °C for 7 min. Each PCR run included a negative (DNA-free water) and a positive control (DNA from a *Rickettsia*-positive tick). PCR products were analysed by electrophoresis and visualized in a 1.5% agarose gel stained with ethidium bromide (stock concentration 10 mg/ml) under a UV transilluminator.

Meteorological data were obtained from the National Institute of Meteorology (INMET), using information recorded from the meteorological station number 82900 (68 km away from the study area). Frequencies (%) were calculated for the developmental stages (larvae, nymphs, and adults) and sex (male and female) of the ticks collected. Normality of data was assessed using the Lilliefors test. Monthly numbers of ticks, according to collection site and developmental stage, were compared using Kruskal-Wallis H-test and Dunn's post-hoc test. The level of significance was set at $P \leq 0.05$. Statistical analyses were performed using BioEstat software, v5.3 (Mamirauá Institute of Sustainable Development, Tefé, AM, Brazil). For logistic reasons, only 15 trapping sessions were conducted in S6, as compared to 24 in all other sites. For this reason, this site was excluded from data analysis.

3. Results

In total, 1500 ticks were collected: 94 females (6.3%), 74 males (4.9%), 468 nymphs (31.2%), and 864 larvae (57.6%). Females, males and nymphs were all identified as *A. sculptum* and larvae as *Amblyomma* spp. On one occasion, one male and one female (both dead) of *Dermacentor nitens* were found under one of the traps (S10), which was placed near a horse. These two ticks were not accounted for in this study.

Ticks were present in all collection sites (Supplemental Table S1), except in site S6 where no single tick was collected during the first 15 months of the study when this site was assessed. Ticks were present during all months of the study, from January 2015 to December 2016. Their overall numbers did not vary significantly according to collection site, except between S12 and S16 (Kruskal-Wallis H-test: $H = 31.0$, $df = 18$, $P = 0.03$; Dunn's post-hoc test, $P < 0.05$). Moreover, ticks were not collected consistently in individual sites throughout the study period, with ticks absent for several consecutive months in some sites. In particular, ticks were present in 10–16 out of 24 months in ten sites (i.e., S2, S3, S7, S8, S9, S10, S11, S14, S16 and S17). In the remaining sites (S1, S4, S5, S12, S13, S15, S18, S19 and S20), ticks were present in 4–9 out of 24 months. The spatial distribution of ticks was significantly aggregated across collection sites ($\chi^2 = 82.28$, $df = 18$; $P < 0.0001$). Indeed, 64% of the ticks were collected in just six sites, namely, S14 (19.7%), S18 (15.3%), S10 (8.6%), S9 (8%), S8 (6.7%) and S17 (5.7%), with two sites (S14 and S18) concentrating 35% of the collections.

Ticks were present during all months of the year, both in 2015 and 2016 (Fig. 3), but the monthly total numbers of larvae, nymphs and adults (Supplemental Table S2) did not vary significantly (Kruskal-Wallis H-test: $H = 3.1613$, $df = 2$, $P = 0.2058$). Integrating data gathered from both years, the highest numbers of larvae and nymphs were recorded during summer and spring, respectively, whereas adults were most frequent in spring.

In total, 380 ticks (88 females and 65 males tested individually, and 227 nymphs tested in pools) were tested by PCR, but none of them were positive for *Rickettsia* spp. DNA.

4. Discussion

Amblyomma sculptum was the only *Amblyomma* species found in this study. This tick species is mainly associated with the Cerrado biome (a vast tropical savannah ecoregion of Brazil) and is generally absent from preserved areas of the Atlantic rainforest biome (Szabó et al., 2007; Veronez et al., 2010; Martins et al., 2016). The high humidity of rainforests seems to be deleterious for *A. sculptum* (Labruna, 2018), but forest degradation may alter the microclimate and the microenvironment, thus favouring the presence of this tick (Szabó et al., 2009).

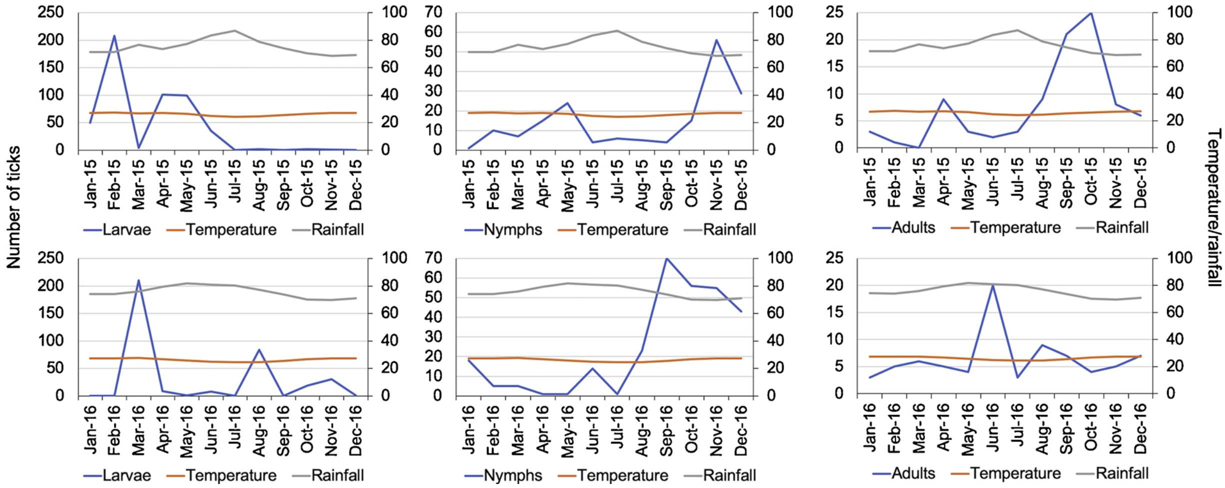

Fig. 3. Monthly numbers of *Amblyomma* spp. larvae and *A. sculptum* nymphs and adults collected in Amaraji, Pernambuco, Brazil, from January 2015 to December 2016, in relation to monthly average temperature and rainfall.

While the rural area investigated herein is characterized by the presence of croplands and grazing areas with degraded forest patches, the low abundance of relevant hosts (e.g., capybaras) may partly explain the low abundance of *A. sculptum*. As a perspective, it would be valuable to investigate the presence and seasonality of *A. sculptum* in other areas of Pernambuco, where the presence of this tick has been ascertained (Dantas-Torres, 2009).

Considering all developmental stages and both years of study, ticks were more numerous during spring and summer. The peaks observed in spring and summer were associated with nymphs and larvae, respectively, whereas adults peaked in spring. This seasonal pattern is different from that reported for *A. sculptum* in south-eastern Brazil, where a one-year generation pattern is characterized by larvae predominating in autumn, nymphs in winter, and adults during spring and summer (Oliveira et al., 2000; Labruna et al., 2002; Szabó et al., 2007; Barbieri et al., 2019). These differences in tick seasonality in the north-eastern (closer to the equator) and south-eastern regions could be related to the distinct seasonal weather patterns observed in these regions (e.g., dry period concentrated in winter and summer in the south-eastern and in north-eastern regions, respectively).

While the nymphal peak in spring, before the larval peak in summer, suggest a different seasonal pattern for north-eastern Brazil, our data relied on relatively low tick numbers, which could have biased or masked the "real" peaks of *A. sculptum* in the study area. In fact, a relatively low abundance of *A. sculptum* adults was observed during the whole study period, even though their numbers tended to be higher during spring, winter and autumn, as compared to summer. In south-eastern Brazil, *A. sculptum* adults predominate during spring and summer (Oliveira et al., 2000; Labruna et al., 2002; Szabó et al., 2007; Barbieri et al., 2019), which partly agrees with our results.

The collections of *A. sculptum* adults were apparently inhibited by monthly rainfall. In fact, adults were also more abundant during hotter months, which is in line with a laboratory study showing a greater capacity of this species to survive under higher saturation deficit conditions as compared to *A. cajennense* sensu stricto (Labruna, 2018). Further research with more numerous tick samples are needed for a better understanding of the relationship between *A. sculptum* phenology and meteorological variables in north-eastern Brazil.

None of the 380 ticks tested by PCR for the *gltA* gene of *Rickettsia* spp. was positive. This is in line with most studies conducted in Brazil, where *A. sculptum* ticks are usually all negative or only a very small proportion of them are positive for rickettsial organisms (Labruna et al., 2004; Guedes et al., 2005; Estrada et al., 2006; Vianna et al., 2008; Guedes et al., 2011; Pacheco et al., 2011; Toledo et al., 2011; Barros Lopes et al., 2014; Krawczak et al., 2014; Brites-Neto et al., 2015; Bitencourth et al., 2017; Machado et al., 2018; Sousa et al., 2018; Szabó et al., 2019). Although a relatively infrequent finding, different species of *Rickettsia* have been reported in *A. sculptum* in Brazil, including *R. rickettsii*, *R. bellii*, *R. amblyommatis*, *R. parkeri* (strain NOD), *R. felis* and '*Candidatus* Rickettsia andeanae' (Guedes et al., 2005; Krawczak et al., 2014; Bitencourth et al., 2017; Barbieri et al., 2019; Machado et al., 2018; Sousa et al., 2018; Szabó et al., 2019). Interestingly, the finding of *R. rickettsii*-positive *A. sculptum* ticks is rare (Guedes et al., 2005, 2011; Krawczak et al., 2014; Labruna et al., 2017), even if tick is considered the most important vector of this bacterium in Brazil (Szabó et al., 2013). Recent studies have ascertained the importance of capybaras for the occurrence of *R. rickettsii*-spotted fever transmitted by *A. sculptum* in Brazil (Polo et al., 2017, 2018). Perhaps, the apparent absence of *R. rickettsii* in the studied area may be explained by absence or low density of these amplifying hosts. Indeed, in spite of the year-long presence of *A. sculptum*, capybaras are not abundant in this area according to locals, probably as a result of years of illegal hunting and habitat degradation.

In conclusion, this study revealed a distinct seasonal pattern for *A. sculptum* in north-eastern Brazil, as compared to data gathered from studies conducted in south-eastern Brazil; however, such distinct pattern could have been biased or masked by the relatively low tick numbers that were collected. Finally, the absence of *Rickettsia*-infected *A. sculptum* ticks does not rule out the possibility that rickettsial organisms are circulating in the study area. Further long-term studies are warranted to confirm (or rule out) this possibility.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.07.007.

References

Aragão, H.B., Fonseca, F., 1961. Notas de ixodologia. VIII. Lista e chave para representantes da fauna ixodológica brasileira. Mem. Inst. Oswaldo Cruz 59, 115–129.

Barbieri, A.R.M., Szabó, M.P.J., Costa, F.B., Martins, T.F., Soares, H.S., Pascoli, G., Torga, K., Saraiva, D.G., Ramos, V.N., Osava, C., Gerardi, M., Dias, R.A., Moraes Jr, E.A., Ferreira, F., Castro, M.B., Labruna, M.B., 2019. Species richness and seasonal dynamics of ticks with notes on rickettsial infection in a Natural Park of the Cerrado biome in Brazil. Ticks Tick. Borne Dis. 10, 442–453.

Barros Lopes, L., Guterres, A., Rozental, T., Carvalho de Oliveira, R., Mares-Guia, M.A., Fernandes, J., Figueredo, J.F., Anschau, I., de Jesus, S., Almeida, A.B., Cristina da Silva, V., Gomes de Melo Via, A.V., Bonvicino, C.R., D'Andrea, P.S., Barreira, J.D., Sampaio de Lemos, E.R., 2014. *Rickettsia bellii*, *Rickettsia amblyommii*, and Laguna Negra hantavirus in an Indian reserve in the Brazilian Amazon. Parasit. Vectors 7, 191.

Bitencourth, K., Amorim, M., Oliveira, S.V., Caetano, R.L., Voloch, C.M., Gazêta, G.S., 2017. *Amblyomma sculptum*: genetic diversity and rickettsias in the Brazilian Cerrado biome. Med. Vet. Entomol. 31, 427–437.

Brandão-Filho, S.P., Brito, M.E., Carvalho, F.G., Ishikawa, E.A., Cupolillo, E., Floeter-Winter, L., Shaw, J.J., 2003. Wild and synanthropic hosts of *Leishmania* (*Viannia*) *braziliensis* in the endemic cutaneous leishmaniasis locality of Amaraji, Pernambuco State, Brazil. Trans. R. Soc. Trop. Med. Hyg. 97, 291–296.

Brites-Neto, J., Brasil, J., Roncato Duarte, K.M., 2015. Epidemiological surveillance of capybaras and ticks on warning area for Brazilian spotted fever. Vet. World 8, 1143–1149.

Dantas-Torres, F., 2009. Ticks on domestic animals in Pernambuco, Northeastern Brazil. Rev. Bras. Parasitol. Vet. 18, 22–28.

Estrada, D.A., Schumaker, T.T., Souza, C.E., Rodrigues Neto, E.J., Linhares, A.X., 2006. Rickettsiae detection in *Amblyomma*ticks (Acari: Ixodidae) collected in the urban area of Campinas City, SP. Rev. Soc. Bras. Med. Trop. 39, 68–71.

Guedes, E., Leite, R.C., 2008. Seasonal dynamics of the free-living stages of *Amblyomma cajennense* and *Amblyomma dubitatum*(Acari: Ixodidae) in an endemic area for spotted fever in Coronel Pacheco region, Minas Gerais State. Rev. Bras. Parasitol. Vet. 17 (Suppl. 1), 78–82.

Guedes, E., Leite, R.C., Pacheco, R.C., Silveira, I., Labruna, M.B., 2011. *Rickettsia* species infecting *Amblyomma* ticks from an area endemic for Brazilian spotted fever in Brazil. Rev. Bras. Parasitol. Vet. 20, 308–311.

Guedes, E., Leite, R.C., Prata, M.C., Pacheco, R.C., Walker, D.H., Labruna, M.B., 2005. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem. Inst. Oswaldo Cruz 100, 841–845.

Krawczak, F.S., Nieri-Bastos, F.A., Nunes, F.P., Soares, J.F., Moraes-Filho, J., Labruna, M.B., 2014. Rickettsial infection in *Amblyomma cajennense* ticks and capybaras (*Hydrochoerus hydrochaeris*) in a Brazilian spotted fever-endemic area. Parasit. Vectors 7, 7.

Labruna, M.B., Santos, F.C., Ogrzewalska, M., Nascimento, E.M., Colombo, S., Marcili, A., Angerami, R.N., 2014. Genetic identification of rickettsial isolates from fatal cases of Brazilian spotted fever and comparison with *Rickettsia rickettsii* isolates from the American continents. J. Clin. Microbiol. 52, 3788–3791.

Labruna, M.B., Whitworth, T., Bouyer, D.H., McBride, J., Camargo, L.M., Camargo, E.P., Popov, V., Walker, D.H., 2004. *Rickettsia bellii* and *Rickettsia amblyommii* in *Amblyomma*ticks from the State of Rondônia, Western Amazon, Brazil. J. Med. Entomol. 41, 1073–1081.

Labruna, M., Krawczak, F., Gerardi, M., Binder, L., Barbieri, A., Paz, G.F., Rodrigues, D.S., Araújo, R.N., Bernardes, M.L., Leite, R.C., 2017. Isolation of *Rickettsia rickettsii* from the tick *Amblyomma sculptum* from a Brazilian spotted fever-endemic area in the Pampulha Lake region, southeastern Brazil. Vet. Parasitol. Reg. Stud. Rep. 8, 82–85.

Labruna, M.B., 2018. Comparative survival of the engorged stages of *Amblyomma cajennense* sensu stricto and *Amblyomma sculptum* under different laboratory conditions. Ticks Tick. Borne Dis. 9, 996–1001.

Labruna, M.B., Kasai, N., Ferreira, F., Faccini, J.L., Gennari, S.M., 2002. Seasonal dynamics of ticks (Acari: Ixodidae) on horses in the state of São Paulo, Brazil. Vet. Parasitol. 105, 65–77.

Machado, I.B., Bitencourth, K., Cardoso, K.M., Oliveira, S.V., Santalucia, M., Marques, S.F.F., Amorim, M., Gazêta, G.S., 2018. Diversity of rickettsiae and potential vectors of spotted fever in an area of epidemiological interest in the Cerrado biome, midwestern Brazil. Med. Vet. Entomol. 32, 481–489.

Martins, T.F., Barbieri, A.R., Costa, F.B., Terassini, F.A., Camargo, L.M., Peterka, C.R., Pacheco, R.C., Dias, R.A., Nunes, P.H., Marcili, A., Scofield, A., Campos, A.K., Horta, M.C., Guilloux, A.G., Benatti, H.R., Ramirez, D.G., Barros-Battesti, D.M., Labruna, M.B., 2016. Geographical distribution of *Amblyomma cajennense* (sensu lato) ticks (Parasitiformes: Ixodidae) in Brazil, with description of the nymph of *A. cajennense* (sensu stricto). Parasit. Vectors 9, 186.

Martins, T.F., Onofrio, V.C., Barros-Battesti, D.M., Labruna, M.B., 2010. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. Ticks Tick. Borne Dis. 1, 75–99.

Moerbeck, L., Vizzoni, V.F., Machado-Ferreira, E., Cavalcante, R.C., Oliveira, S.V., Soares, C.A., Amorim, M., Gazêta, G.S., 2016. *Rickettsia* (Rickettsiales: Rickettsiaceae) vector biodiversity in high altitude Atlantic forest fragments within a semiarid climate: a new endemic area of spotted-fever in Brazil. J. Med. Entomol. 53, 1458–1466.

Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 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, F.A., Marcili, A., De Sousa, R., Paddock, C.D., Labruna, M.B., 2018. Phylogenetic Evidence for the existence of multiple strains of *Rickettsia parkeri* in the New World. Appl. Environ. Microbiol. 84, e02872–17.

Oliveira, P.R., Borges, L.M., Lopes, C.M., Leite, R.C., 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. 2000, 295–301.

Oliveira, S.V., Costa, R.M.F., Ferreira, G., Pereira, S.V.C., Amorim, M., Monteiro, M.F.M., Alves, L.C., Gazeta, G.S., 2018. Fatal case of spotted fever in a patient from Northeastern Brazil. Rev. Inst. Med. Trop. Sao Paulo 60, e21.

Oliveira, S.V., Guimarães, J.N., Reckziegel, G.C., Neves, B.M., Araújo-Vilges, K.M., Fonseca, L.X., Pinna, F.V., Pereira, S.V., Caldas, E.P., Gazeta, G.S., Gurgel-Gonçalves, R., 2016. An update on the epidemiological situation of spotted fever in Brazil. J. Venom. Anim. Toxins Incl. Trop. Dis. 22, 22.

Oliveira, S.V., 2016. Tick-borne spotted fever in the northeast of Brazil: the series of cases a new endemic area. Rev. Med. UFC. 56, 8–9.

Onofrio, V.C., Labruna, M.B., Pinter, A., Giacomin, F.G., Barros-Battesti, D.M., 2006. Comentários e chaves para as espécies do gênero Amblyomma. In: Barros-Battesti, D.M., Arzua, M., Bechara, G.H. (Eds.), Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para a identificação de espécies. Vox/ICTTD-3/Butantan, São Paulo, pp. 53–113.

Pacheco, R.C., Moraes-Filho, J., Guedes, E., Silveira, I., Richtzenhain, L.J., Leite, R.C., Labruna, M.B., 2011. Rickettsial infections of dogs, horses and ticks in Juiz de Fora, southeastern Brazil, and isolation of *Rickettsia rickettsii* from *Rhipicephalus sanguineus* ticks. Med. Vet. Entomol. 25, 148–155.

Polo, G., Mera Acosta, C., Labruna, M.B., Ferreira, F., 2017. Transmission dynamics and control of Rickettsia rickettsii in populations of *Hydrochoerus hydrochaeris* and *Amblyomma sculptum*. PLoS Negl. Trop. Dis. 11, e0005613.

Polo, G., Mera Acosta, C., Labruna, M.B., Ferreira, F., Brockmann, D., 2018. Hosts mobility and spatial spread of Rickettsia rickettsii. PLoS Comput. Biol. 14, e1006636.

QGIS Development Team, 2019. QGIS Geographic Information System. Open Source Geospatial Foundation Project. (Accessed 18 March 2019). https://www.qgis.org/en/site/.

Saraiva, D.G., Nieri-Bastos, F.A., Horta, M.C., Soares, H.S., Nicola, P.A., Pereira, L.C., Labruna, M.B., 2013. *Rickettsia amblyommii* infecting *Amblyomma auricularium* ticks in Pernambuco, northeastern Brazil: isolation, transovarial transmission, and transstadial perpetuation. Vector Borne Zoonotic Dis. 13, 615–618.

Silva, A.B., Cardoso, K.M., de Oliveira, S.V., Costa, R.M.F., Oliveira, G., Amorim, M., Alves, L.C., Monteiro, M.F.M., Gazeta, G.S., 2018. *Rickettsia amblyommatis* infecting *Amblyomma pseudoconcolor* in area of new focus of spotted fever in northeast Brazil. Acta Trop. 182, 305–308.

Silva, A.B., Duarte, M.M., da Costa Cavalcante, R., de Oliveira, S.V., Vizzoni, V.F., de Lima Duré, A.Í., de Melo Iani, F.C., Machado-Ferreira, E., Gazêta, G.S., 2017. *Rickettsia rickettsii* infecting *Rhipicephalus sanguineus* sensu lato (Latreille 1806), in high altitude Atlantic forest fragments, Ceara State, Brazil. Acta Trop. 173, 30–33.

Sousa, K.C.M., Herrera, H.M., Rocha, F.L., Costa, F.B., Martins, T.F., Labruna, M.B., Machado, R.Z., André, M.R., 2018. *Rickettsia* spp. among wild mammals and their respective ectoparasites in Pantanal wetland, Brazil. Ticks Tick. Borne Dis. 9, 10–17.

Szabó, M.P., Castro, M.B., Ramos, H.G., Garcia, M.V., Castagnolli, K.C., Pinter, A., Veronez, V.A., Magalhães, G.M., Duarte, J.M., Labruna, M.B., 2007. Species diversity and seasonality of free-living ticks (Acari: Ixodidae) in the natural habitat of wild Marsh deer (*Blastocerus dichotomus*) in Southeastern Brazil. Vet. Parasitol. 143, 147–154.

Szabó, M.P., Labruna, M.B., Garcia, M.V., Pinter, A., Castagnolli, K.C., Pacheco, R.C., Castro, M.B., Veronez, V.A., Magalhães, G.M., Vogliotti, A., Duarte, J.M., 2009. Ecological aspects of the free-living ticks (Acari: Ixodidae) on animal trails within Atlantic rainforest in south-eastern Brazil. Ann. Trop. Med. Parasitol. 103, 57–72.

Szabó, M.P., Pinter, A., Labruna, M.B., 2013. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front. Cell. Infect. Microbiol. 3, 27.

Szabó, M.P.J., Pascoal, J.O., Martins, M.M., Ramos, V.D.N., Osava, C.F., Santos, A.L.Q., Yokosawa, J., Rezende, L.M., Tolesano-Pascoli, G.V., Torga, K., de Castro, M.B., Suzin, A., Barbieri, A.R.M., Werther, K., Silva, J.M.M., Labruna, M.B., 2019. Ticks and *Rickettsia* on anteaters from Southeast and Central-West Brazil. Ticks Tick. Borne Dis. 10, 540–545.

Toledo, R.S., Tamekuni, K., Filho, M.F., Haydu, V.B., Barbieri, A.R., Hiltel, A.C., Pacheco, R.C., Labruna, M.B., Dumler, J.S., Vidotto, O., 2011. Infection by spotted fever rickettsiae in people, dogs, horses and ticks in Londrina, Parana State, Brazil. Zoonoses Public Health 58, 416–423.

Veronez, V.A., Freitas, B.Z., Olegário, M.M., Carvalho, W.M., Pascoli, G.V., Thorga, K., Garcia, M.V., Szabó, M.P., 2010. Ticks (Acari: Ixodidae) within various phytophysiognomies of a Cerrado reserve in Uberlândia, Minas Gerais, Brazil. Exp. Appl. Acarol. 50, 169–179.

Vianna, M.C., Horta, M.C., Sangioni, L.A., Cortez, A., Soares, R.M., Mafra, C.L., Galvão, M.A., Labruna, M.B., Gennari, S.M., 2008. Rickettsial spotted fever in capoeirão village, Itabira, Minas Gerais, Brazil. Rev. Inst. Med. Trop. Sao Paulo 50, 297–301.