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Original article

Species richness and seasonal dynamics of ticks with notes on rickettsial infection in a Natural Park of the Cerrado biome in Brazil

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ABSTRACT

This study evaluated the species richness and seasonal dynamics of ticks and rickettsial agents infecting ticks in the largest natural Reserve of the Cerrado biome of Brazil, the Grande Sertão Veredas National Park. During 2012–2014, a total of 9531 host-seeking ticks were collected by dry ice traps and dragging, whereas 1563 ticks were collected from small mammals, and 1186 ticks from domestic animals. Overall, the following 12 tick species were identified: *Amblyomma auricularium, Amblyomma dubitatum, Amblyomma naponense, Amblyomma ovale, Amblyomma parvum, Amblyomma sculptum, Amblyomma tigrinum, Amblyomma triste, Dermacentor nitens, Rhipicephalus microplus*, *Rhipicephalus sanguineus* sensu lato, and *Ornithodoros mimon*. The three most abundant tick species, *A. sculptum, A. parvum,* and *A. triste,* are likely to develop one generation per year, with adults predominating between spring and autumn, and immature ticks during autumn-winter. Small mammals seem to be important hosts for immature stages of *A. parvum,* and *A. triste,* but not for *A. sculptum.* Molecular analyses revealed the presence of the human pathogen *Rickettsia parkeri* in 10% of the *A. triste* ticks, whereas two agents of unknown pathogenicity, *Rickettsia bellii* and '*Candidatus* Rickettsia andeanae' were found in 7 and 5%, respectively, of the *A. parvum* ticks. A fourth rickettsial agent, *Rickettsia amblyommatis,* was found in a single *A. sculptum* tick. Several Vero cell-established isolates of *R. parkeri* and *R. bellii* were obtained from *A. triste* and *A. parvum,* respectively. Serological analyses of small mammals suggest that they have been infected by *R. parkeri* and *R. bellii,* possibly via natural infestations by *A. triste* and *A. parvum,* respectively. Because the Park has suffered low anthropic alterations, our results should provide baseline data that shall be used for future comparisons with other Cerrado areas with higher degree of anthropic changes.

1. Introduction

There are 73 tick species with established populations in Brazil, where they feed on a variety of mammals, birds, reptiles and/or amphibians (Barros-Battesti et al., 2006; Labruna et al., 2016; Michel et al., 2017; Muñoz-Leal et al., 2017, 2018a,b). Many of these tick species are known to parasitize humans (Guglielmone et al., 2006), to whom they are vectors of spotted fever group (SFG) rickettsiae (Szabó et al., 2013a). Until 2004, *Rickettsia rickettsii,* the etiological agent of Brazilian spotted fever, was the only known tick-borne *Rickettsia* species in Brazil (Labruna, 2009). During the last 15 years, eight additional tick-borne *Rickettsia* species were reported in Brazil, namely the SFG agents *Rickettsia parkeri, Rickettsia amblyommatis, Rickettsia rhipicephali, 'Candidatus* Rickettsia andeanae', *Rickettsia* sp. strain Colombianensi, *Rickettsia* sp. strain Pampulha, and the basal group agents *Rickettsia bellii* and *Rickettsia monteiroi* (Almeida et al., 2011; Labruna et al., 2011;

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Nieri-Bastos et al., 2014; 2018; Karpathy et al., 2016; Luz et al., 2018). Among these, only *R. parkeri* has been shown to be pathogenic to humans (Nieri-Bastos et al., 2018), in addition to *R. rickettsii.*

The Cerrado, a tropical savannah biome, is considered one of the 35 hotspots of global biodiversity, that is, a region with endemic species richness and a great threat to the integrity of vegetation, particularly by human activities (Cincotta et al., 2000; Sloan et al., 2014). The Cerrado extends over a large continuous area in central Brazil and occurs also in discontinuous areas to the south and north of the country. The Cerrado has an approximate extension of 2,036,448 Km^2 occupying a total area of ≈24% of the Brazilian territory, losing in size only to the Amazon forest (http:/www.ibge.gov.br). The Cerrado has flat to gently undulating relief, with about 50% of its area in altitudes between 300 and 600 m above the sea level; only 5.5% go beyond 900 m. Currently, only 2.2% of the Cerrado extension are legally protected areas; therefore, this biome is more threatened than the Amazon forest, losing its territory to agriculture, pasture and urban expansion (Marris, 2005).

The present study evaluated the species richness and seasonal dynamics of ticks and possible rickettsial agents infecting these ticks in the largest natural Reserve of the Cerrado biome of Brazil, the Grande Sertão Veredas (GSV) National Park. Because this Park has suffered low anthropic alterations, our results should provide baseline data that shall be used for future comparisons with other Cerrado areas with higher degree of anthropic changes.

2. Materials and methods

2.1. Ethical statement

This study has been approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine of the University of São Paulo (protocol 2718/2012). Capture of native fauna was authorized by the Brazilian Ministry of the Environment (permit SISBIO No. 25554-3).

2.2. Study site

The Grande Sertão Veredas (GSV) National Park occupies an area of 230,853.42 ha in the Brazilian states of Minas Gerais and Bahia (Fig. 1). The Park has a rich fauna of large-sized mammals, which include the felids *Panthera onca* (jaguar) and *Puma concolor* (cougar), the canid *Chrysocyon brachyurus* (maned wolf), the deer *Blastocerus dichotomus* (marsh deer) and *Ozotoceros bezoarticus* (Pampas deer), the peccaries *Tayassu pecari* (white-lipped peccary) and *Pecari tajacu* (collared peccary), in addition to *Tapirus terrestris* (South American tapir), *Myrmecophaga tridactyla* (giant anteater), *Priodontes maximus* (giant armadillo), and *Hydrochoerus hydrochaeris* (capybaras) (Brasil, 2003). In addition, dozens of species of medium (Carnivora, armadillos) and small mammals (rodents, marsupials, bats) have been reported in the Park (Brasil, 2003). The weather in the Park, as in a typical savannah, is characterized by a hot and rainy summer, and a cool and dry winter. Annual mean temperature is around 23 °C with maximum of 37–40 °C and minimal of 16–19 °C, whereas annual rainfall averages 1400 mm (Brasil, 2003).

Four common Cerrado phytophysiognomies of the Park are: (i) Carrasco, (ii) Cerradão, (iii) Gallery forest, and (iv) Vereda. Carrasco (Fig. 2A) has a typical savannah vegetation with many thorny trees 3–4 m tall, interposed by arbustive plants; the soil is only sparsely covered by grasses, has a relatively thin, many times discontinuous leaf litter (< 3 cm), and does not get flooded. Cerradão (Fig. 2D) is a dense savanna, also called as xeromorphic forest; trees are 8–15 m tall, providing a 50–90% canopy cover. Gallery forest is a dense forest with trees up to 20–30 m tall that provide 80–100% canopy cover; the soil tends to be highly humid throughout the year due to a thick leaf litter (5–10 cm) and seasonal flooding in some parts. Vereda (Fig. 2B) is an extensive stream surrounded by a flood-adapted vegetation, where the moriche palm or "buriti" (*Mauritia flexuosa*) is most of the times the tallest tree (12–25 m tall). The soil, extensively covered by grasses and bushes, is saturated throughout the year; seasonal flooding is common. Among the above four phytophysiognomies, Carrasco is the driest, followed by Cerradão. Cerradão is a transition area between Carrasco and Gallery forest; therefore, it has a less shadowed ground and thinner leaf litter when compared to Gallery forest. Gallery forest and Vereda are the most humid; however, while the humidity in the Gallery forest is primarily related to its 80–100% canopy cover, the humidity of Vereda is related to its year-round saturated soil. A typical landscape of the Park is the Vereda and Carrasco 'side-by-side', separated by an open-field grass (Fig. 2C).

From May 2012 to February 2014 we did eight field expeditions at ≈3 month-intervals to the Park; therefore, each expedition

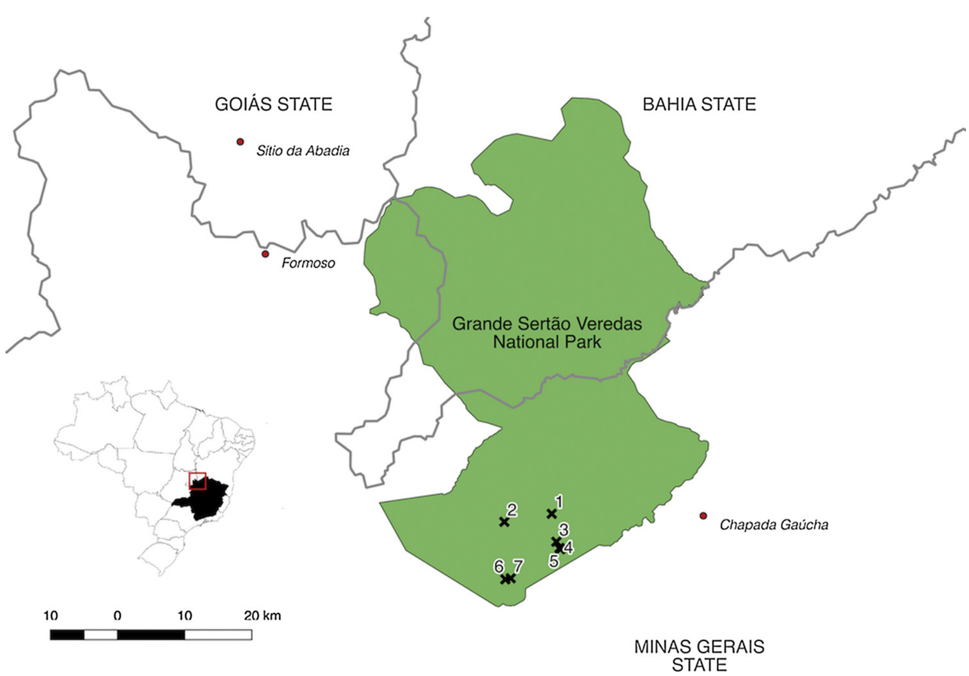

Fig. 1. Map of the Grande Sertão Veredas National Park (green area), indicating the seven areas sampled in the present study, all located within Minas Gerais state. 1-Carrasco; 2-Vereda-A; 3-Vereda-B; 4-Cerradão; 5-Gallery forest; 6-Farm-A; 7-Farm-B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

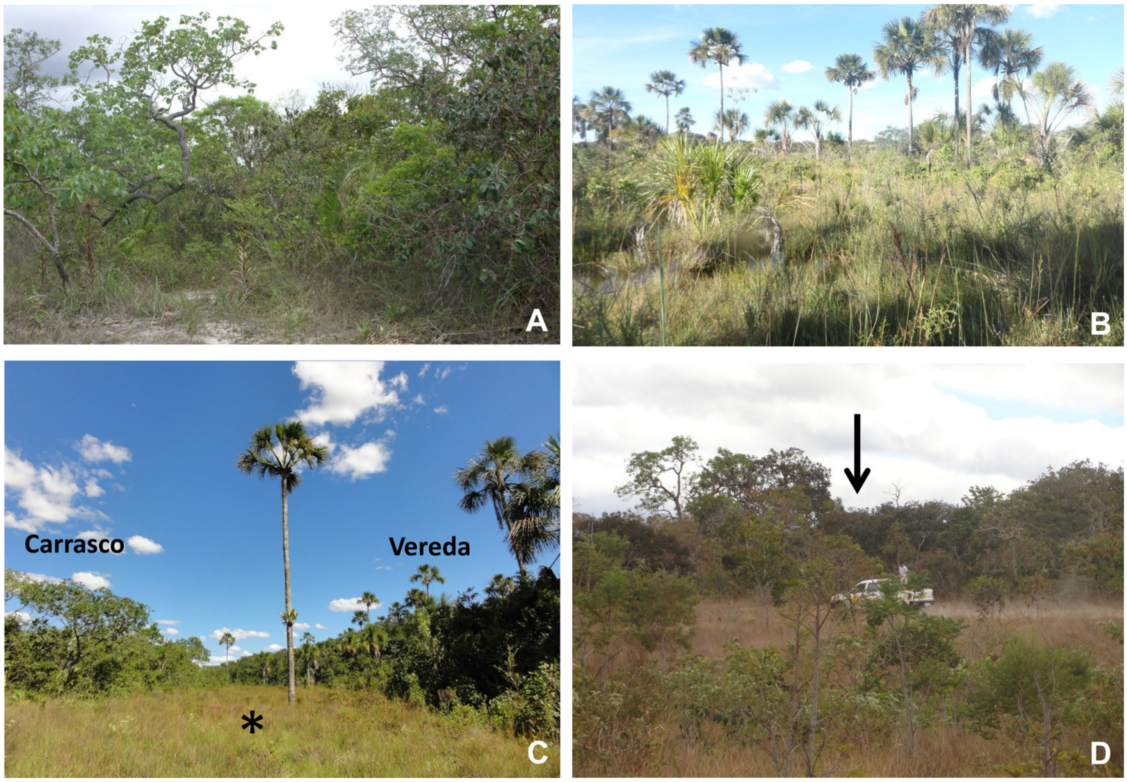

Fig. 2. Typical phytophysionomies of the Grande Sertão Veredas National Park. A. Carrasco. B. Vereda. C. Carrasco and Vereda side-by-side, separated by an open field grass (asterisk). D. Cerradão in the background (indicated by arrow).

Table 1
Areas of the Grande Sertão Veredas National Park that were sampled during 8 field expeditions (1 to 8) at 3-month intervals from May 2012 to February 2014.

Area	Coordinates (S;W)	Eleva- tion (m)	Type of sampling in field expeditions 1 to 8[a]			
			Host-questing ticks		Small mammals	Domestic animals
			Dry ice	dragging		
Carrasco	15°18'00"; 45°49'11"	781	1 to 8	1 to 8	1 to 8	
Vereda-A	15°18'39"; 45°53'08"	729	1 to 8	1 to 8	1 to 8	
Vereda-B	15°20'15"; 45°48'48"	786	1 to 8	1 to 8	1 to 8	
Cerradão	15°20'45"; 45°48'32"	794		1 to 8		
Gallery forest	15°20'51"; 45°48'28"	792		3 to 8		
Farm-A	15°23'16"; 45°53'03"	776				3 to 8
Farm-B	15°23'11"; 45°36'17"	772				3 to 8

[a] Each of the field expeditions 1–8 corresponded to a year season, as follows: 1-autumn (May 2012); 2-winter (July-August 2012); 3-spring (November 2012); 4-summer (February 2013); 5-autumn (May 2013); 6-winter (August-September 2013); 7-spring (November 2013); 8-summer (February 2014).

corresponded to a year season (autumn, winter, spring or summer) of each of the two 12-month sampling periods. Five natural areas uninhabited by domestic animals were sampled in the Park (area name corresponds to its phytophysiognomy): (i) Carrasco; (ii) Vereda-A; (iii) Vereda-B; (iv) Cerradão; (v) Gallery forest (Table 1). In addition, we also sampled domestic animals in two small farms (Farm-1 and Farm-2) located in the southern limit of the Park (Fig. 1).

2.3. Host-questing ticks

In each of the eight field expeditions, host-questing ticks were collected by dry ice traps following Szabó et al. (2009), and dragging following Terassini et al. (2010), in three areas (Carrasco, Vereda-A, Vereda-B). For this purpose, a total of 30 dry ice traps were set at 10 m intervals in each area, while a distance of ≈600 m was sampled by dragging in each area. Additionally, host-questing ticks were collected by dragging in a fourth area (Cerradão) during the eight field expeditions, and in a fifth area (Gallery forest) from the 3rd to the 8th field expeditions (Table 1). Collected larvae and nymphs were immediately placed in absolute ethanol, whereas adult ticks were taken alive to the laboratory.

2.4. Small mammals

Wild small mammals were sampled in three areas (Carrasco, Vereda-A, Vereda-B) during the eight field expeditions (Table 1). In each area during each field expedition, we used 60 live-traps (23 × 9 x 9.5 cm) set at 10–20 m apart from each other, during 4 consecutive nights, giving a sample effort of 1920 trap-nights per area through the study. Traps were baited with pineapple, sausage, and peanut butter. Trapped animals were anaesthetized with intramuscular ketamine (50 mg/kg) and xylazine (20 mg/kg), and carefully examined for ticks, which were collected and placed in absolute ethanol. Blood samples were collected by venipuncture from the caudal vein (marsupials) or submandibular vein (rodents), or through the intracardiac route when not possible from veins. Blood samples were centrifuged for serum separation, which was kept frozen until serological analysis in the laboratory. Mammal taxonomic identifications followed Bonvicino et al. (2008). A few specimens of each mammal species were euthanized and their carcasses were sent to the Zoology Museum of the Pontifícia Universidade Católica in Belo Horizonte, Minas Gerais state, for confirming identification and deposit of voucher specimens.

2.5. Domestic animals

During expeditions 3–8, we examined cattle, horses, and dogs for the presence of ticks in two small farms (Farm-A and Farm-B) located in the periphery of the Park (Table 1, Fig. 1). All animals were reared unrestrained and had free access to some areas of the Park, where cattle and horses used to graze mainly on native grasses, since the formation of cultivated grasses was prohibited by Park official regulations. Collected ticks were immediately placed in absolute ethanol and taken to the laboratory.

2.6. Tick taxonomic identification

Adult and nymphal ticks were morphologically identified to species level following Barros-Battesti et al. (2006) and Martins et al. (2010). A portion of the larvae of the genus *Amblyomma* was identified to species level by molecular analysis. For this purpose, individual larvae were submitted to DNA extraction by the guanidine isothiocyanate phenol technique (Sangioni et al., 2005) and tested by polymerase chain reaction (PCR) with primers 5′-CCG GTC TGA ACT CAG ATC AAG T-3′ and 5′-GCT CAA TGA TTT TTT AAA TTG CTG T-3′, which amplify a ≈460-bp of the tick mitochondrial 16S rRNA gene, as previously described (Mangold et al., 1998). PCR products were purified and sequenced in an automatic sequencer (model ABI 3500 Genetic Analyzer; Applied Biosystems/Thermo Fisher Scientific, Foster City, CA) according to the manufacturer's protocol. The generated sequences were submitted to BLAST analysis (www.ncbi.nlm.nih.gov/blast) to infer the closest similarities available in GenBank.

2.7. Rickettsial infection in ticks

We attempted to isolate viable rickettsiae in Vero cell culture from adult ticks that arrived alive at the laboratory. For this purpose, each tick was submitted to the hemolymph test with Gimenez staining, as previously described (Labruna et al., 2007a). Ticks showing *Rickettsia*-like organisms within their hemocytes were selected to be processed by the shell-vial technique, as previously described (Labruna et al., 2004a). Briefly, cultures of Vero cells were inoculated with tick-body homogenates, centrifuged, and incubated at 28 °C. The percentage of Vero cells infected with rickettsiae was monitored by the use of Giménez staining of cells scraped from each inoculated monolayer. After the establishment of each isolate in the laboratory (i.e. at least 3 cell passages, with the prevalence of infected cells exceeding 90%), rickettsial DNA was extracted from the infected cells and tested in a battery of different PCR protocols, with primers CS-78 and CS-323 targeting a 401-bp fragment of the rickettsial *gltA* gene (Labruna et al., 2004a), primers 17k-5 and 17k-3 targeting a ≈530-bp fragment of the rickettsial *htrA* gene (Labruna et al., 2004a), primers Rr190.70 F and Rr190.701R targeting a ≈620-bp fragment of the rickettsial *ompA* gene (Roux et al., 1996), and primers 120.M59 and 120–807 targeting a ≈820-bp fragment of the rickettsial *ompB* gene (Roux and Raoult, 2000). PCR products were purified and sequenced in an automatic sequencer (model ABI 3500 Genetic Analyzer; Applied Biosystems/Thermo Fisher Scientific, Foster City, CA) according to the manufacturer's protocol. The generated sequences were submitted to BLAST analysis (www.ncbi.nlm.nih.gov/blast) to infer the closest similarities available in GenBank.

Random samples of the remaining ticks were submitted to DNA extraction by the guanidine isothiocyanate phenol technique (Sangioni et al., 2005). Adult ticks were processed individually, while nymphs were processed individually or in pools of 2–3 ticks from the same individual host. Extracted DNA samples were tested by the PCR protocols mentioned above for the rickettsial *gltA* and *ompA* genes. PCR products were sequenced as described above, and the resultant sequences were compared with GenBank data by BLAST analysis.

2.8. Serological analysis of small mammals

Wild small mammal (marsupials and rodents) sera were tested by the indirect immunofluorescence assay (IFA) using crude antigens of 6 *Rickettsia* species from Brazil (*R. rickettsii* strain Taiaçu, *R. parkeri* strain At24, *R. amblyommatis* strain Ac37, *R. rhipicephali* strain HJ5, *R. bellii* strain Mogi, and *R. felis* strain Pedreira), as previously described (Labruna et al., 2007b; Szabó et al., 2013b). Slides were incubated with fluorescein isothiocyanate-labelled sheep anti-opossum IgG (CCZ, São Paulo, Brazil) for sera from marsupials, goat anti-rat IgG (Sigma, St Louis, MO, USA) for sera from Cricetidae rodents, and goat anti-guinea pig IgG (Sigma, St Louis, MO, USA) for sera from Caviomorpha rodents. In each slide, a serum previously shown to be non-reactive (negative control) and a known reactive serum (positive control) were tested at the 1:64 dilution. These sera derived from the studies of Horta et al. (2009) and Krawczak et al. (2016). Samples that reacted at the screening dilution (1:64) were then titrated using two-fold serial dilutions to determine the IgG endpoint titer reacting with each of the 6 *Rickettsia* antigens. An endpoint titer at least 4-fold higher for a *Rickettsia* species than those observed for all other *Rickettsia* species was considered probably homologous to the first *Rickettsia* species or to a very closely related species (Labruna et al., 2007b; Szabó et al., 2013b).

2.9. Statistical analyses

The Chi-square test was used to compare the proportions of free-living ticks collected from different areas and different seasons, and the proportions of infested small mammals among areas and seasons. Values were considered statistically different when $P < 0.05$. All analyses were performed using the program Minitab® Release 17.

3. Results

3.1. Host-seeking ticks

A total of 3672 ticks (1982 adults, 980 nymphs, 710 larvae) were collected by dry ice traps in three areas of the study, namely Carrasco, Vereda-A, and Vereda-B. These ticks were identified into four species, as follows: *Amblyomma sculptum* (745 males, 780 females, 854 nymphs, 9 larvae), *Amblyomma parvum* (97 males, 125 females, 115 nymphs), *Amblyomma triste* (108 males, 123 females, 2 nymphs), and *Amblyomma dubitatum* (1 male, 3 females, 9 nymphs). The 9 *A. sculptum* larvae were identified by molecular methods (Table 2), whereas the remaining 701 larvae were retained as *Amblyomma* spp. Among the three most abundant tick species, *A. sculptum* was collected at similar numbers from the 3 areas, *A. parvum* was collected mostly from Carrasco, and *A. triste* was collected only in Vereda-A and Vereda-B (Fig. 3A). The few *A. dubitatum* ticks were all collected in Vereda-B. The proportions of each tick species among the three areas differed significantly in some cases ($P < 0.05$) with a higher proportion of *A. parvum* collected in Carrasco and that of *A. triste* in the Veredas A and B, in contrast to an even distribution of *A. sculptum* throughout the sampled areas.

A total of 5859 ticks (719 adults, 1217 nymphs, 3923 larvae) were collected by dragging onto five areas of the study, namely Carrasco, Vereda-A, Vereda-B, Cerradão, and Gallery forest. These ticks were identified into four species, as follows: *A. sculptum* (323 males, 254

Table 2
Larval ticks identified by molecular methods through generating a partial sequence of the tick mitochondrial 16S rRNA gene.

No. larvae	Study area	Origin	Results of BLAST analysis	No. haplotypes[a]
1	Carrasco	Dry ice	100% *A. sculptum* (MG523424)	1 (MK059459)
12	Vereda-A	Dry ice, dragging	99-100% *A. sculptum* (MG523424)	2 (MK059459, MK059460)
5	Vereda-B	Dry ice, dragging	99-100% *A. sculptum* (MG523424)	2 (MK059459, MK059460)
10	Cerradão	Dragging	99-100% *A. sculptum* (MG523424)	2 (MK059459, MK059460)
4	Gallery forest	Dragging	99-100% *A. sculptum* (MG523424)	2 (MK059459, MK059460)
17	Carrasco	*Didelphis albiventris*	100% *O. mimon* (KC677676)	1 (MK059461)
1	Vereda-A	*Gracilinanus agilis*	100% *O. mimon* (KC677676)	1 (MK059461)
5	Vereda-A	*Monodelphis domestica, Thrichomys apereoides, Dasyprocta azarae*	99-100% *A. auricularium* (KR869156)	2 (MK059462, MK059463)
4	Carrasco	*D. albiventris, M. domestica, Galea spixii*	99% *A. parvum* (EU306158)	2 (MK059464, MK059465)
17	Vereda-A	*M. domestica, T. apereoides*	99% *A. parvum* (EU306158)	4 (MK059464 - MK059467)
5	Vereda-B	*D. albiventris, Cerradomys marinhus*	99% *A. parvum* (EU306158)	2 (MK059464, MK059468)
41	Vereda-A	*T. apereoides, M. domestica, Oxymycterus delator, G. agilis, Calomys tener, C. marinhus*	99-100% *A. triste* (KU284991)	2 (MK059469, MK059470)
21	Vereda-B	*O. delator, Rhipidomys* sp., *Holochilus sciureus*	98-100% *A. triste* (KU284991)	4 (MK059469, MK059471 - MK059473)
4	Vereda-B	*D. albiventris*	100% *A. sculptum* (MG523424)	1 (MK059459)

[a] haplotypes have been submitted to GenBank (accession numbers in parentheses).

females, 1198 nymphs, 23 larvae), *A. parvum* (30 males, 31 females, 16 nymphs), *A. triste* (42 males, 39 females, 2 nymphs), and *A. dubitatum* (1 nymph). The 23 *A. sculptum* larvae were identified by molecular methods (Table 2), whereas the remaining 3900 larvae were retained as *Amblyomma* spp. Among the three most abundant tick species, *A. sculptum* was collected at highest numbers from the Cerradão (34% of the ticks) and Gallery forest (25%), followed by Vereda-A (19%), Vereda-B (14%), and Carrasco (8%). *Amblyomma parvum* was collected

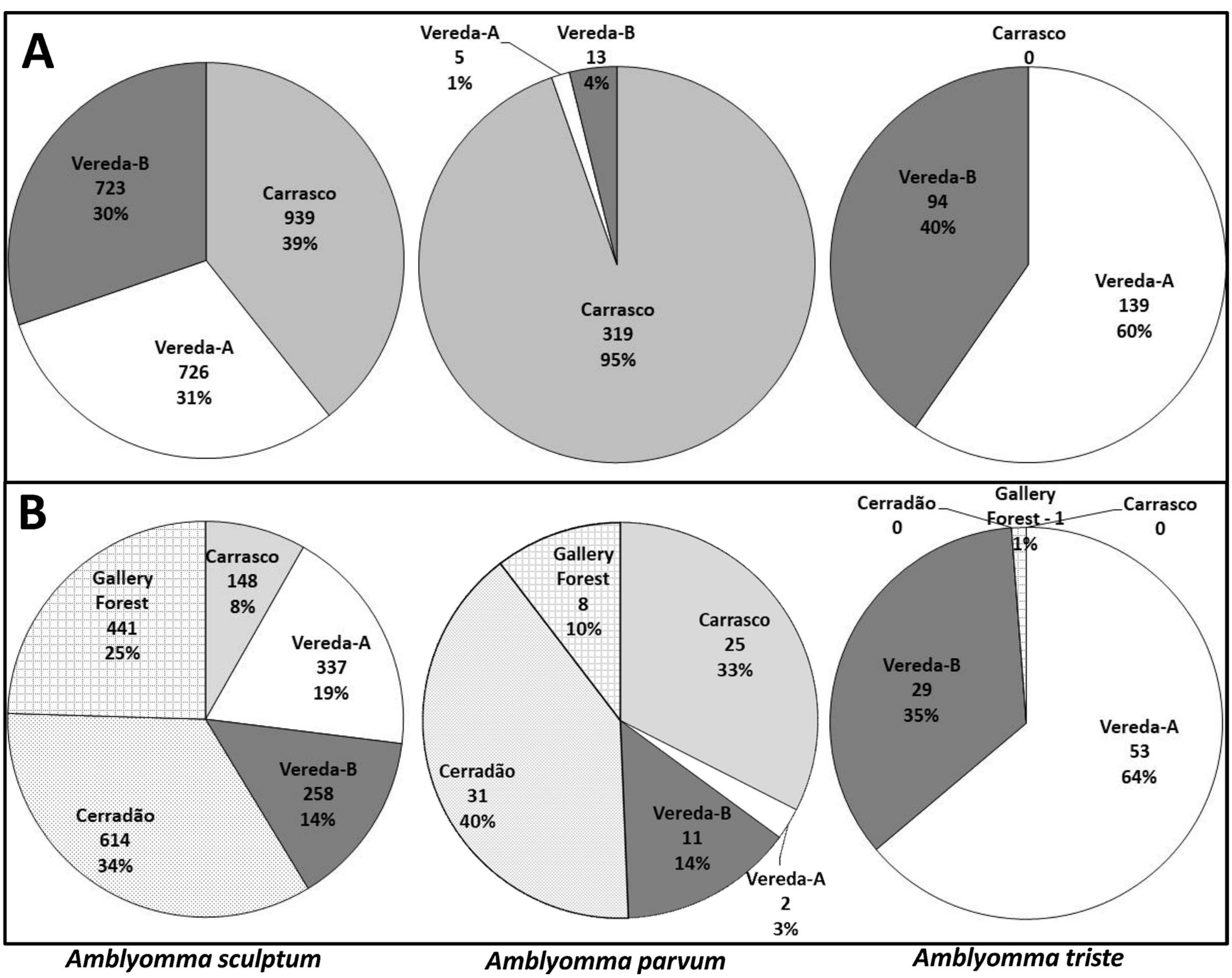

Fig. 3. Proportions of *Amblyomma sculptum*, *Amblyomma parvum,* and *Amblyomma triste* that were captured by dry ice traps in three areas (Carrasco, Vereda-A, and Vereda-B), and by dragging in five areas (Carrasco, Vereda-A, Vereda-B, Cerradão, Gallery Forest) in the Grande Sertão Veredas National Park from May 2012 to February 2014.

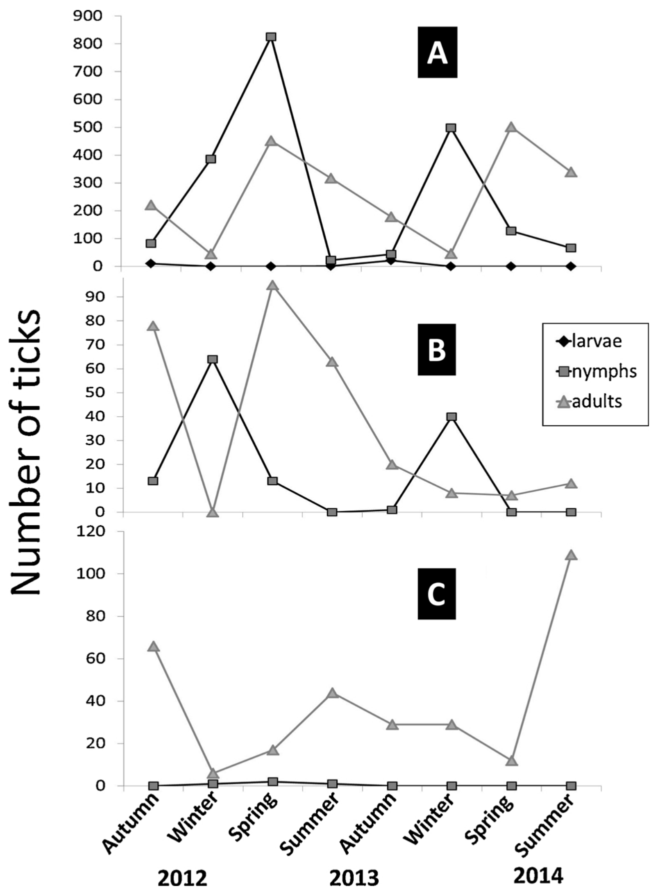

Fig. 4. Seasonal distribution of *Amblyomma sculptum* (A), *Amblyomma parvum* (B) and *Amblyomma triste* (C) free-living ticks in the Grande Sertão Veredas National Park from May 2012 to February 2014.

mostly from Cerradão (40% of the ticks) and Carrasco (33%), and then at lower numbers from Vereda-B (14%), Gallery forest (10%), and Vereda-A (3%). Almost 100% of the *A. triste* were collected from the Veredas (64% in Vereda-A and 35% in Vereda-B); only 1% was collected in Gallery forest, and none at Cerradão or Carrasco (Fig. 3B). The single *A. dubitatum* tick was collected in Vereda-B. The proportions of each tick species, in each of the five areas, differed significantly ($P < 0.05$), with higher proportions of *A. sculptum* collected in Cerradão and Gallery Forest, that of *A. parvum* in Cerradão and Carrasco, and that of *A. triste* in the Veredas A and B.

For seasonal analysis of the three most abundant tick species (*A. sculptum, A. parvum,* and *A. triste*), we pooled the data of ticks collected in all 5 areas by dry ice traps and dragging. For *A. sculptum* (Fig. 4A), nymphs peaked in the winter/spring seasons, whereas adults peaked during spring/summer seasons. The few host-seeking larvae that could be identified as *A. sculptum* were collected in the autumn season of each of the two years. For *A. parvum,* nymphs peaked in the winter season of each year, and adults during the first autumn season, and then in the following spring-summer seasons (Fig. 4B). For *A. triste,* adults peaked in the first autumn season, and then in each of the two summer seasons; the few *A. triste* nymphs were collected in the winter-spring-summer seasons of the first year (Fig. 4C). Regarding the 4601 *Amblyomma* spp. larvae that could not be identified to species level, 56.5% were collected during autumn seasons, 23.9% during winter, 17.4% during spring, and only 2.1% of the larvae were collected during summer seasons. The proportions of different tick stages of each tick species were significantly different between seasons ($P < 0.05$), validating the observed peaks of each stage.

3.2. Small mammals and ticks

A total of 75 small mammals of 13 species were captured throughout the study, giving an overall capture-success of 1.3% (75/ 5,760 × 100). Considering the three areas separately, the highest capture-success was in Vereda-A (2.1%), and the lowest in Carrasco (0.4%); whereas capture-success in Vereda-B was of 1.4%. The 75 small

Table 3
Ticks collected from small mammals at three areas [Carrasco (C); Vereda-A (V-A); Vereda-B (V-B)] of the Grande Sertão Veredas National Park from May 2012 to February 2014.

Host species	A (n)[a]	Number of ticks (number infested animals)[b]													
		Apa		Atr		Aau		Asc		Ati	Adu	Ana	Omi	Asp	Isp
		N	L	N	L	N	L	N	L	N	N	N	L	L	L
Didelphis albiventris	C (2)	12 (2)	2 (2)			2 (1)		1 (1)					90 (2)	39 (2)	
	V-B (1)	2 (1)	4 (1)					1 (1)	4 (1)					144 (1)	
Gracilinanus agilis	C (2)	2 (1)													
	V-A (2)	5 (1)			1 (1)	1 (1)							3 (1)		
Thylamys karimii	C (2)		1 (1)												
Monodelphis domestica	V-A (1)		8 (1)		2 (1)		1 (1)							5 (1)	
Thrichomys apereoides	V-A (12)	30 (5)	9 (3)	3 (2)	9 (5)	14 (6)	1 (1)							560 (7)	
	V-B (10)			13 (6)										14 (2)	
Galea spixii	C (1)	63 (1)	1 (1)			5 (1)				4 (1)				51 (1)	
Calomys tener	V-A (2)				3 (1)									1 (1)	
	V-B (2)													6 (1)	
Cerradomys marinhus	V-A (2)				1 (1)									12 (1)	
	V-B (2)		1 (1)											3 (1)	
Holochilus sciureus	V-B (3)				1 (1)			1 (1)						1 (2)	
Oligoryzomys sp.	V-A (1)													5 (1)	1 (1)
	V-B (1)														
Oxymycterus delator	V-A (19)			39 (6)	25 (5)	1 (1)					1 (1)			119 (7)	
	V-B (3)			4 (1)	19 (3)									185 (3)	
Rhipidomys sp.	V-B (6)			2 (1)	1 (1)										
Dasyprocta azarae	V-B (1)						3 (1)	1 (1)				1 (1)		19 (1)	
TOTAL	C + V-A + V-B (75)	114 (11)	26 (10)	61 (16)	62 (19)	23 (10)	5 (3)	4 (4)	4 (1)	4 (1)	1 (1)	1 (1)	93 (3)	1167 (32)	1 (1)

[a] A (n): area (number of captured animals).n): area (number of captured animals).

[b] Apa: *Amblyomma parvum*; Atr: *Amblyomma triste*; Aau: *Amblyomma auricularium*; Asc: *Amblyomma sculptum*; Ati: *Amblyomma tigrinum*; Adu: *Amblyomma dubitatum*; Ana: *Amblyomma naponense*; Omi: *Ornithodoros mimon*; Asp: *Amblyomma* spp.; Isp: *Ixodes* sp.; N: nymphs; L: larvae.

mammals consisted of 10 marsupials of the family Didelphidae [*Didelphis albiventris* (3 specimens), *Gracilinanus agilis* (4), *Monodelphis domestica* (1), *Thylamys karimii* (2)], and 65 rodents of the families Echimyidae [*Thrichomys apereoides* (22 specimens)], Caviidae [*Galea spixii* (1)], Cricetidae [*Calomys tener* (4), *Cerradomys marinhus* (4), *Holochilus sciureus* (3), *Oligoryzomys* sp. (2), *Oxymycterus delator* (22), *Rhipidomys* sp. (6)] and Dasyproctidae [*Dasyprocta azarae* (1)] (Table 3).

Among the 75 animals, 46 (61%) were infested by a total of 1563 ticks, which were all immature stages (208 nymphs, 1355 larvae). The overall mean number of ticks per infested host was 34.0 (range: 1–207). Nymphs were collected from 29 animals, giving a mean number of 7.2 nymphs/host (range: 1–72). Larvae were collected from 36 animals, giving a mean number of 37.2 larvae/host (range: 1–203). Ticks collected from small mammals were identified into the following 8 species in decreasing order of abundance: *A. parvum* (114 nymphs, 26 larvae), *A. triste* (61 nymphs, 62 larvae), *Ornithodoros mimon* (93 larvae), *Amblyomma auricularium* (23 nymphs, 5 larvae), *A. sculptum* (4 nymphs, 4 larvae), *Amblyomma tigrinum* (4 nymphs), *A. dubitatum* (1 nymph), and *Amblyomma naponense* (1 nymph). The above *Amblyomma* spp. larvae were identified to species level by molecular methods, whereas morphological identification of *O. mimon* larvae was confirmed by molecular methods in 18 individuals (Table 2). In addition, 1164 *Amblyomma* spp. larvae and 1 *Ixodes* sp. larva could not be identified to species level by morphological or molecular analyses and were retained to the genus level (Table 3).

Amblyomma parvum and *A. triste* were the two most prevalent and abundant tick species on small mammals (Table 3); therefore, their infestation patterns were analyzed separately in time and space during the study. Among the 140 immature specimens of *A. parvum*, 74% (103 specimens) were collected from rodents of the families Echimyidae (*T. apereoides*) and Caviidae (*G. spixii*). The remaining 37 *A. parvum* specimens (26%) were collected mostly from marsupials (*D. albiventris*, *G. agilis*, *T. karimii, M. domestica*); only a single specimen was collected from a Cricetidae rodent, namely *C. marinhus* (Table 3). Among the 123 immature specimens of *A. triste*, 77% (95 specimens) were collected from Cricetidae rodents, especially the species *O. delator*; 25 out of the remaining 28 *A. triste* specimens were collected from the Echimyidae rodent *T. apereoides*, while only 3 specimens were collected from marsupials (*M. domestica* and *G. agilis*) (Table 3). *Amblyomma parvum*-infested hosts were captured in the 3 sampled areas (Carrasco, Vereda-A, and Vereda-B), although the majority of the ticks (58%) were collected from 5 animals in the Carrasco. *Amblyomma triste*-infested hosts were captured only in Vereda-A and Vereda-B. The proportions of *A. parvum* or *A. triste* ticks collected from small mammals were significantly different ($P < 0.05$) between the three areas, corroborating the predominance of *A. parvum* in Carrasco and *A. triste* in Veredas A and B.

Because of the relatively small number of small mammals collected in this study, data were pooled per season. Grouping the two years, 27 (36%) small mammals were captured during autumn, 21 (28%) in winter, 16 (21%) in spring, and 11 (15%) in summer. Prevalence of tick infestation per season was 67% (18/27) in autumn, 71% (15/21) in winter, 50% (8/16) in spring, and 45% (5/11) in summer. Among the total of 208 nymphal specimens collected from small mammals, 147 (71%) were collected in the winter, 32 (15%) in the autumn, and only 16 (8%) and 13 (6%) in the summer and spring, respectively. Among the total of 1358 larval specimens collected from small mammals, 932 (69%) were collected in the autumn, 396 (29%) in the winter, and only 23 (1.5%) and 7 (0.5%) in the spring and summer, respectively. Considering only *A. parvum* and *A. triste,* larvae of both species showed the highest peak in the autumn, whereas nymphs peaked in the winter (Fig. 5). While the proportions of infested small mammals were not significantly different ($P > 0.05$) among the four seasons, the number of larvae and nymphs of *A. parvum* and *A. triste* were significantly different ($P < 0.05$) among seasons, validating the observed peaks of each stage.

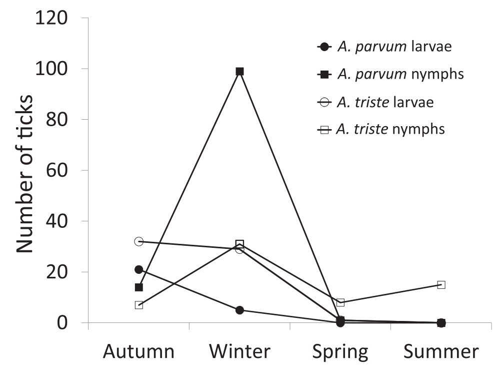

Fig. 5. Seasonal distribution of *Amblyomma parvum* and *Amblyomma triste* immature ticks on small mammals in the Grande Sertão Veredas National Park from May 2012 to February 2014.

Table 4
Ticks collected from unrestrained domestic animals in two farms (Farm-A and Farm-B) in the Grande Sertão Veredas National Park from November 2012 to February 2014.

Tick species and stage	Number of ticks according to hosts and farms (number of animal examinations)				
	Dogs		Horses		Cattle
	Farm-A (18)	Farm-B (18)	Farm-A (10)	Farm-B (10)	Farm-A (12)
Amblyomma ovale					
Females	1				
Amblyomma parvum					
Males	4	11	1		1
Females	10	25	1		2
Amblyomma sculptum					
Males	6	17	64	61	70
Females	1	8	121	70	13
Nymphs	74	57	1	50	2
Amblyomma tigrinum					
Males	3	2		1	1
Females	5	6		2	
Amblyomma triste					
Males				2	
Females				2	
Amblyomma spp.					
Larvae	59	18	111	18	
Dermacentor nitens					
Males			24	17	
Females			16	17	
Nymphs			9	8	
Rhipicephalus microplus					
Males	1			6	29
Females			9	6	85
Nymphs					40
Larvae		1			
Rhipicephalus sanguineus s.l.					
Males	2	5			
Females	6	4			

3.3. Ticks from domestic animals

Ticks collected from domestic animals in Farm-A and Farm-B during field expeditions 3 to 8 are shown in Table 4. Because many times the same individual animal was repeatedly examined among different field expeditions, we refer to as "number of animal examinations" instead of "number of examined animals". All tick specimens found on dogs were collected, while only a random sample was collected from cattle and horses during a 5-minute examination per individual host. A total of 1186 tick specimens were collected, represented by eight species

(Table 4). Overall, domestic dogs were found infested by 6 tick species, namely *Amblyomma ovale, A. parvum, A. sculptum, A. tigrinum, Rhipicephalus microplus*, and *Rhipicephalus sanguineus* sensu lato; horses were infested by 6 tick species, *A. parvum, A. sculptum, A. tigrinum, A. triste, Dermacentor nitens* and *R. microplus*; and cattle were infested by 4 tick species, *A. parvum, A. sculptum, A. tigrinum,* and *R. microplus.*

Voucher tick specimens collected in the present study have been deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) of the University of São Paulo, under the accession numbers CNC 3841-3873.

3.4. Rickettsiae in ticks

A total of 381 free-living adult ticks (33 *A. sculptum,* 210 *A. triste,* 138 *A. parvum*) that arrived alive at the laboratory were processed by the hemolymph test. *Rickettsia*-like organisms were visualized in the hemolymph of 63 (30%) *A. triste,* 42 (30%) *A. parvum,* and none (0%) of the *A. sculptum* ticks. Among the hemolymph test-positive ticks, 12 *A. triste* and 8 *A. parvum* were submitted to the shell vial technique, resulting in 5 rickettsial isolates from *A. triste* and 4 from *A. parvum.* These 9 isolates were established in Vero cells for several passages; frozen stocks have been deposited at the rickettsial collection of our laboratory. All five isolates from *A. triste* were identified as *R. parkeri* sensu stricto, since DNA extracted from the 3rd Vero cell passage yielded *gltA* (350-bp), *htrA* (483-bp), *ompA* (559-bp), and *ompB* (772-bp) sequences 100% identical to *R. parkeri* strain Portsmouth (GenBank accession number CP003341). The four isolates from *A. parvum* were identified as *R. bellii,* as their 3rd Vero cell passage-DNA samples yielded *gltA* (350-bp) and *htrA* (495-bp) sequences 100% identical to *R. bellii* strain Ac25 (AY362702, AY362703). No *ompA* or *ompB* fragment was amplified by PCR on the *R. bellii* isolates.

A total of 400 vegetation-collected adult ticks (200 *A. sculptum,* 100 *A. triste,* and 100 *A. parvum*) were tested individually for the presence of rickettsial DNA by molecular methods. Rickettsiae were detected in 1 *A. sculptum* (*Rickettsia amblyommatis*), 10 *A. triste* (*R. parkeri*), and 12 *A. parvum* (*R. bellii* or '*Candidatus* Rickettsia andeanae') (Table 5). Among ticks collected from small mammals, we tested 32 nymphs; rickettsial DNA were detected in 2 *A. triste* (*R. parkeri*) and 1 pool of *A. parvum* (*R. bellii*) (Table 5). The *R. parkeri gltA* and *ompA,* and *R. bellii gltA* partial sequences generated from these ticks were 100% identical to the conspecific isolate sequences mentioned above. The *R. amblyommatis gltA* (350-bp) and *ompA* (556-bp) partial sequences were 100% identical to *R. amblyommatis* strain Ac37 (CP012420), whereas the '*Candidatus* R. andeanae' *gltA* (350-bp) and *ompA* (567-bp) partial sequences were 100% identical to '*Candidatus* R. andeanae' strain Ap (KF030931, KF030932)

3.5. Serology of small rodents

Serum samples were obtained from 64 mammals, and tested by IFA against 6 rickettsial antigens (Table 6). Overall, *R. parkeri* was the rickettsial agent displaying the highest number of seroreactive animals (28%), followed by *R. amblyommatis* (17%), *R. rickettsii, R. rhipicephali* and *R. bellii* (14% each), and then by *R. felis* (only 2%). Highest endpoint titers were 2048 for *R. parkeri*, 1024 for *R. rhipicephali,* 512 for *R. rickettsii, R. amblyommatis* and *R. bellii,* and 64 for *R. felis.* In all mammal species, the proportion of seroreactive animals was equal or higher to *R. parkeri* than to the other 5 *Rickettsia* species. The only exceptions were *T. apereoides* and *G. spixii,* in which the highest proportion of seroreactive animals was for *R. bellii*. Every animal that reacted to *R. amblyommatis*, *R. rickettsii, R. rhipicephali* or *R. felis* also reacted to *R. parkeri,* with endpoint titers generally higher for the later species. On the other hand, five animals (4 *T. apereoides* and 1 *G. spixii*) reacted to *R. bellii* without reacting to any other rickettsial antigen. Four rodents (1 *C. tener,* 1 *H. sciureus,* 1 *O. delator*, 1 *T. apereoides*) had endpoint titers to *R. parkeri* at least 4-fold higher than those observed for the other five *Rickettsia* species, indicating a possible homologous reaction to *R. parkeri* or closely related species. Using the same criterion, three *T. apereoides* were exposed to *R. bellii* or a closely related species.

4. Discussion

A total of 12 tick species were identified in this study. Indeed, the presence of these ticks can be linked to the presence in the Park of their respective major host species for the tick adult stage. This assumption relies on the fact that the tick adult stage is more host-specific than larvae and nymphs (Nava and Guglielmone, 2013), and its reproductive fitness depends on its successful feeding; therefore, the tick adult stage' hosts are determinant on the establishment of a tick species in a given area. For example, tapirs and peccaries are major hosts of *A. sculptum* (Aragão, 1936; Martins et al., 2011, 2016), as well as marsh deer for *A. triste* (Szabó et al., 2003), Carnivora, deer, and possibly tapirs for *A. parvum* (Labruna et al., 2005; Nava et al., 2008a; Labruna and Guglielmone, 2009), armadillos for *A. auricularium* (Aragão, 1936), maned wolf and other Carnivora for *A. tigrinum* (Labruna et al., 2005; Martins et al., 2011), capybara for *A. dubitatum* (Nava et al., 2010), and peccaries for *A. naponense* (Labruna et al., 2010). All these wild vertebrate hosts are known to occur in the Park (Brasil, 2003), and were occasionally visualized during our field work (data not shown); therefore, they are indeed linked to the presence of the above tick species in the Park. In fact, the CNC tick collection contains the following unpublished records of adult ticks collected from medium- to large-sized mammals in the Grande Sertão Veredas National Park between 2007 and 2013: *A. sculptum*, *A. parvum* and *A. triste* from a tapir (CNC-3853); *A. parvum* from three cougars (CNC-1411, 1436, 1955), two maned wolves (CNC-1413, 1455), two crab-eating foxes *Cerdocyon thous* (CNC-1437, 3866), and one ocelot *Leopardus pardalis* (CNC-1412); *A. tigrinum* from two maned wolves (CNC-1413, 1455), one cougar (CNC-1955), and one crab-eating fox (1437).

Immature stages of some tick species show host preferences very

Table 5
Rickettsial infection in ticks collected from hosts and vegetation in the Grande Sertão Veredas National Park, Brazil, from May 2012 to February 2014.

Tick species	Stage	Source	No. ticks tested by PCR	No. infected ticks (%)	*Rickettsia* species (no. ticks)
Amblyomma triste	Adult	vegetation	100	10 (10)	*R. parkeri* (13)
	Nymph	*Thrichomys apereoides*	10	1 (10) [a]	*R. parkeri* (1)
	Nymph	*Oxymycterus delator*	4	1 (25)	*R. parkeri* (1)
A. sculptum	Adult	vegetation	200	1 (0.5)	*R. amblyommatis* (1)
	Nymph	*Didelphis albiventris*	1	0	_
	Nymph	*Dasyprocta azarae*	1	0	_
A. naponense	Nymph	*D. azarae*	1	0	_
A. parvum	Adult	vegetation	100	12	*R. bellii* (7), *Ca.* R. andeanae (5)
	Nymph	*D. albiventris*	6	0	_
	Nymph	*T. apereoides*	9	1 (11.1) [a]	*R. bellii* (1)

[a] % value refers to minimal infection rate, since ticks were tested in pools of 2–3 ticks, each pool from a different individual host.

Table 6
Seroreactivity to six *Rickettsia* species of small mammals from the Grande Sertão Veredas National Park, Brazil, from May 2012 to February 2014.

Mammal species (No. tested)	No. seroreactive animals to each of the *Rickettsia* species (% seroreactivity for each animal species)						No. animals with determined homologous reaction (PAIHR in parentheses)[a]
	R. rickettsii	*R. parkeri*	*R.amblyommatis*	*R. rhipicephali*	*R. bellii*	*R. felis*	
MARSUPIALS							
Didelphis albiventris (3)	1 (33)	1 (33)	1 (33)	0	0	0	0
Gracilinanus agilis (4)	0	0	0	0	0	0	
Monodelphis domestica (1)	0	0	0	0	0	0	0
Thylamys karimii (2)	0	0	0	0	0	0	0
RODENTS							
Cerradomys marinhus (3)	0	0	0	0	0	0	0
Calomys tener (4)	0	2 (50)	0	0	1 (25)	0	1 (*R. parkeri*)
Dasyprocta azarae (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0
Galea spixii (1)	0	0	0	0	1 (100)	0	0
Holochilus sciureus (2)	1 (50)	1 (50)	1 (50)	1 (50)	0	0	1 (*R. parkeri*)
Oxymycterus delator (19)	7 (37)	10 (53)	8 (42)	5 (26)	2 (11)	1 (5)	1 (*R. parkeri*)
Oligoryzomys sp. (2)	0	0	0	0	0	0	0
Rhipidomys sp. (4)	0	1 (25)	0	1 (25)	1 (25)	0	0
Thrichomys apereoides (18)	0	2 (11)	0	1 (6)	5 (28)	0	1 (*R. parkeri*), 3 (*R. bellii*)
Total (64)	9 (14)	18 (28)	11 (17)	9 (14)	11 (14)	1 (2)	

[a] A homologous reaction was determined when an endpoint titer to a *Rickettsia* species was at least 4-fold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species (or a very closely related species) involved in the highest endpoint titer was considered the possible antigen involved in a homologous reaction (PAIHR).

distinct from adult ticks, as for example, *A. parvum, A. triste,* and *A. auricularium*; larvae and nymphs of these three species have been reported mostly on small mammals (Horta et al., 2011; Nava et al., 2008b; Colombo et al., 2018). These reports were corroborated in the present study, in which the most abundant ixodid ticks on small mammals were immature stages of *A. parvum, A. triste,* and *A. auricularium.* Interestingly, larvae of an argasid tick, *O. mimon,* was the third most abundant tick species on small mammals, as shown by a total of 93 specimens collected from 3 arboreal marsupials. While *O. mimon* is considered a typical parasite of bats, there have been many reports on arboreal marsupials, possibly due to habitat sharing with bats (Labruna et al., 2014; Sponchiado et al., 2015). Finally, it is noteworthy that very few immature ticks of *A. sculptum* were collected from small mammals, although this tick was far the most abundant tick species in the Park. This contrasting finding reinforces previous studies that have proposed that *A. sculptum* immature stages have preferences for medium- to large-sized hosts (Labruna et al., 2002a; Ramos et al., 2017).

Free-living ticks were collected in four phytophysiognomies, Carrasco, Cerradão, Gallery forest, and Vereda, which represent the overall landscape of the GSV Park (Brasil, 2003). Our results showed a marked association of *A. triste* with the Vereda, where nearly 100% of the *A. triste* adult ticks were collected (Fig. 3). In addition, all *A. triste*-infested rodents were also collected in the Vereda. The principal host of *A. triste* adults is the marsh deer, a large mammal that typically lives in wetlands or swampy habitats like the Vereda phytophysiognomy (Piovezan et al., 2010). Among the 8 rodent species that were found infested by *A. triste* immature ticks, one single species, *O. delator,* harbored 71% (87/123) of the ticks (Table 3), indicating this rodent species as a major mammal host for *A. triste* immatures at the GSV National Park. Interestingly, rodents of the genus *Oxymycterus* are also strictly associated with swampy habitats (Emmons and Feer, 1997). Previous studies in Argentina (Nava et al., 2011), Uruguay (Venzal et al., 2008), and another Cerrado area of Brazil (Szabó et al., 2007a) also reported the association of free-living adults of *A. triste* with swampy or marshy areas; in the studies of the first two countries, small rodents (including *Oxymycterus* spp.) were also found to be a major hosts for *A. triste* immature ticks.

Most of the free-living *A. parvum* ticks were collected in Carrasco and Cerradão (Fig. 3), which were the two driest phytophysiognomies sampled in this study. In addition, 58% (81/140) of the *A. parvum* immature ticks collected from small rodents were from animals trapped in Carrasco, where a single specimen of the Caviidae *G. spixii* was infested by 63 larvae and 1 nymph (Table 3). The Echimyidae rodent *T. apereoides* had the second highest abundance of *A. parvum* ticks. While Table 3 shows that all *A. parvum*-infested *T. apereoides* were from Vereda-A, it is noteworthy that none of these animals were trapped in the typical swampy area of this phytophysiognomy; they were actually trapped in the open field grass area that separated Carrasco from Vereda in the Vereda-A area (data not shown), just like it is illustrated in Fig. 2C. In fact, it is well known that *T. apereoides* is a rodent species typically associated with xeric or non-floodable habitats (Bonvicino et al., 2008); therefore, we would not expect to trap this rodent inside the typical Vereda phytophysiognomy. Interestingly, while we found some *T. apereoides* to be infested by either *A. triste* (a typical Vereda tick) or *A. parvum* (a typical Carrasco tick) (Table 3), all these rodents were trapped in this open field grass that separates Carrasco and Vereda side-by-side (data not shown). These results are corroborated by the study of Szabó et al. (2007b), who reported a greater abundance of *A. parvum* in drier phytophysiognomies of the Cerrado in central-western Brazil.

Amblyomma sculptum was far the most abundant tick species in the GSV national Park. The number of free-living specimens of *A. sculptum* outnumbered *A. parvum* and *A. triste* in all four phytophysiognomies (Fig. 3), indicating a high plasticity of *A. sculptum* to different types of environment. At the same time, the highest numbers of *A. sculptum* ticks were collected in the Cerradão and Gallery forest, which differ from the other phytophysiognomies by having a denser canopy cover and thicker leaf litter. Indeed, such attributes are important to provide optimal microclimate conditions for the free-living developmental stages of *A. sculptum,* which might be intermediate between Carrasco (too dry) and Vereda (too humid, swampy soil). In fact, a previous study in another natural area of Cerrado reported a significantly greater abundance of *A. sculptum* in forest patches than in the marsh areas associated with this forest (Szabó et al., 2007a). These observations are corroborated by a laboratory study that showed that all free-living developmental phases of *A. sculptum* (molting, oviposition and egg incubation) displayed highest success when exposed to high relative humidity (78 to 100%) without water immersion (Labruna, 2018). Altogether, these results indicate that in the GSV National Park, *A. triste* is strictly associated with Veredas, whereas *A. parvum* is associated with more xeric phytophysiognomies, such as Carrasco and Cerradão. On the other hand, *A. sculptum* has an ubiquitous distribution with a tendency for greater population densities in areas with denser canopy cover. In each of these areas, suitable hosts for each tick species are available, guaranteeing the establishment of tick populations.

Our seasonal observations of free-living *A. sculptum* ticks indicate that this tick species develops one generation per year, with adults predominating in spring-summer, larvae in autumn, and nymphs in winter-spring (Fig. 4A). This one-year generation pattern is in agreement with several previous studies in southeastern Brazil (Oliveira et al., 2000; Labruna et al., 2002b; Szabó et al., 2007a) and northern Argentina (Tarragona et al., 2018), and is known to be primarily controlled by larval behavioral diapause (Labruna et al., 2003), which in turn, is regulated by photoperiod and temperature (Cabrera and Labruna, 2009). Our results also suggest that *A. parvum* develops a similar one-year generation pattern, since its free-living adults peaked in spring-summer of at least one year, and nymphs in the winter of both years (Fig. 4B). Additionally, our results from small mammals showed *A. parvum* larvae peaking in autumn, and nymphs in winter (Fig. 5). This pattern has been reported for *A. parvum* populations in northern Argentina (Nava et al., 2008b), indicating that *A. parvum* and *A. sculptum* have similar seasonal dynamics in Argentina and Brazil. Free-living adults of *A. triste* peaked in summer-autumn (Fig. 4C), whereas larvae and nymphs collected from small mammals peaked in autumn and winter, respectively (Fig. 5). These results suggest an one-year generation pattern similar to that observed for *A. sculptum* and *A. parvum.* In another area of Cerrado, Szabó et al. (2007a) observed highest peaks of free-living *A. triste* adults in early autumn, somewhat similar to the present study. In southern latitudes of Uruguay and Argentina (temperate climate), *A. triste* also develops one generation per year; however, with adults mostly active during late winter and spring, and larvae and nymphs during summer and autumn (Venzal et al., 2008; Nava et al., 2011). This condition was speculated to be controlled by behavioral diapause of adult ticks during winter, in order to avoid exposure of engorged females or eggs to the low winter temperatures (Nava et al., 2011). Indeed, this condition is very different from the tropical Cerrado, whereas adults seem to be active throughout the year, albeit with highest peaks in autumn-summer.

Our findings of *R. parkeri* infecting *A. triste,* and '*Candidatus* R. andeanae' infecting *A. parvum* have been previously reported in different parts of South America, including areas of the Cerrado biome (Nieri-Bastos et al., 2013, 2014; Costa et al., 2017). We also found one (0.5%) out of 200 *A. sculptum* adult ticks to be infected by *R. amblyommatis.* While many previous studies have demonstrated that *R. amblyommatis* is not associated to *A. sculptum* ticks (Sangioni et al., 2005; Vianna et al., 2008; Pacheco et al., 2009; Costa et al., 2017; Machado et al., 2018), at least two studies have reported this rickettsial agent infecting *A. sculptum* (Alves et al., 2014; Nunes et al., 2015); however, always at very low infection rates ($\leq 2\%$), similarly to the present study. On the other hand, *R. amblyommatis*-infection rates on other tick species (e.g., *Amblyomma cajennense* sensu stricto, *Amblyomma coelebs, A. auricularium*) have been usually > 20-50% (Labruna et al., 2004b; Saraiva et al., 2013; Soares et al., 2015; Costa et al., 2017). A fourth rickettsial agent, *R. bellii,* was surprisingly found at higher infection rate (7%) than '*Candidatus* R. andeanae' (5% infection rate) in *A. parvum* free-living ticks. This finding not only constitutes the first report of *R. bellii* infecting *A. parvum,* but also contrasts to previous studies that reported '*Candidatus* R. andeanae' as the sole rickettsial agent infecting *A. parvum* ticks, usually at 20–70% infection rates (Pacheco et al., 2007; Nieri-Bastos et al., 2014; Costa et al., 2017). Altogether our findings indicate that the high tick species richness within the park is paralleled by a high *Rickettsia* species richness.

Results of the serological analyses of small mammals are somewhat corroborated by our results of ticks infesting these animals, and the rickettsial agents that were found in these ticks. On one hand, highest endpoint titers to *R. parkeri* were detected in small rodent species that constituted hosts for *A. triste* immature stages, i.e., *C. tener, H. sciureus, O. delator,* and *T. apereoides* (Tables 3,6). On the other hand, highest endpoint titers to *R. bellii* were detected in *T. apereoides,* which was a main host for *A. parvum* immature stages. These findings provide evidence that these rodent species have been exposed to *R. parkeri* or *R. bellii* via infected ticks in the GSV National Park. While the vector competence of *A. triste* for *R. parkeri* has been demonstrated in the laboratory (Nieri-Bastos et al., 2013), our results highlight the potential of *A. parvum* to transmit *R. bellii,* a condition yet to be convincingly demonstrated. Even though the *A. parvum* population of this study was also infected by '*Candidatus* R. andeanae', we consider minimal the interference of this agent in our serological agents, since one study demonstrated that '*Candidatus* R. andeanae' is not efficiently transmitted to the host skin during tick feeding (Grasperge et al., 2014). Finally, we also exclude a significant interference of *R. amblyommatis* in our serological results because this agent was found only at a very low infection rate in *A. sculptum,* which was not an important tick infesting small mammals.

Domestic animals from two farms in the southern limit of the Park were shown to be exposed to a variety of tick species (Table 4), which include 3 exotic species (*R. microplus, D. nitens,* and *R. sanguineus* s.l.) that were introduced in southeastern Brazil with domestic animals during the last few centuries (Aragão, 1936; Aragão and Fonseca, 1953). However, our acarological surveys in domestic animal-free areas of the Park failed to collect any of these exotic ticks, suggesting that they have been restricted to domestic animals' habitats. On the other hand, the presence of native tick species on domestic animals indicate that these animals, and potentially humans in direct contact with these animals, are exposed to the rickettsial organisms that were detected in these ticks, which includes *R. parkeri,* the etiological agent of a spotted fever illness in humans (Nieri-Bastos et al., 2013).

5. Conclusions

We have found a species richness of ticks and tick-borne rickettsiae in a Natural Park of the Cerrado biome that has suffered low anthropic alterations in Brazil. The three most abundant tick species, *A. sculptum, A. parvum,* and *A. triste,* are likely to develop one generation per year, with adults predominating between spring and autumn, and immature ticks during autumn-winter. Small mammals seem to be important hosts for immature stages of *A. parvum,* and *A. triste,* but not for *A. sculptum.* The Vereda phytophysiognomy was shown to sustain a natural cycle of *A. triste* that involves a variety of small rodents as hosts for larva and nymphs, and possibly marsh deer for adult ticks. Noteworthy, 10% of these *A. triste* ticks were found infected by the human pathogen *R. parkeri.* This infection rate is within the 7–20% rates that have been reported for other *A. triste* populations in Brazil, Uruguay and Argentina, including areas with massive anthropic alterations and endemicity for spotted fever in humans (Venzal et al., 2004; Silveira et al., 2007; Nava et al., 2008c; Monje et al., 2014). Drier phytophysiognomies, such as Carrasco and Cerradão, were the major habitats for *A. parvum,* which seem to use a number of marsupial and rodent species as hosts for larvae and nymphs, and possibly several large wild mammals for adult ticks. This *A. parvum* population was shown to harbor two *Rickettsia* species, *R. bellii* (reported for the first time in this tick species) and '*Candidatus* R. andeanae'; this last rickettsia was found in much lower infection rates than usually reported in other Cerrado areas (Nieri-Bastos et al., 2014; Costa et al., 2017). Serological analyses of small mammals suggest that they have been infected by *R. parkeri* and *R. bellii,* possibly via natural infestations by *A. triste* and *A. parvum,* respectively. *Amblyomma sculptum* was far the most abundant tick in the Park, although higher populations were found in phytophysiognomies with denser canopy cover, namely Gallery forest and Cerradão. Domestic animals living in some isolated parts of the Park have been exposed to native ticks, and at the same time, have maintained exotic ticks such as *R. microplus, D. nitens,* and *R. sanguineus* s.l.; however, there is no evidence that these exotic ticks have become established among wildlife without habitat sharing with domestic animals.

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