Molecular characterization of bacterial communities of two neotropical tick species (*Amblyomma aureolatum* and *Ornithodoros brasiliensis*) using rDNA 16S sequencing

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- Brazil

ABSTRACT

Ticks are one of the main vectors of pathogens for humans and animals worldwide. However, they harbor non-pathogenic microorganisms that are important for their survival, facilitating both their nutrition and immunity. We investigated the bacterial communities associated with two neotropical tick species of human and veterinary potential health importance from Brazil: *Amblyomma aureolatum* and *Ornithodoros brasiliensis*. In *A. aureolatum* (adult ticks collected from wild canids from Southern Brazil), the predominant bacterial phyla were Proteobacteria (98.68%), Tenericutes (0.70%), Bacteroidetes (0.14%), Actinobacteria (0.13%), and Acidobacteria (0.05%). The predominant genera were *Francisella* (97.01%), *Spiroplasma* (0.70%), *Wolbachia* (0.51%), *Candidatus Midichloria* (0.25%), and *Alkanindiges* (0.13%). The predominant phyla in *O. brasiliensis* (adults, fed and unfed nymphs collected at the environment from Southern Brazil) were Proteobacteria (90.27%), Actinobacteria (7.38%), Firmicutes (0.77%), Bacteroidetes (0.44%), and Planctomycetes (0.44%). The predominant bacterial genera were *Costiella* (87.71%), *Nocardiooides* (1.73%), *Saccharopolyspora* (0.54%), *Marmoricola* (0.42%), and *Staphyloccoccus* (0.40%). Considering the genera with potential importance for human and animal health which can be transmitted by ticks, *Costiella* sp. was found in all stages of *O. brasiliensis*, *Francisella* sp. in all stages of *A. aureolatum* and in unfed nymphs of *O. brasiliensis*, and *Rickettsia* sp. in females of *A. aureolatum* from Banhado dos Pacheos (BP) in Viamão municipality, Brazil, and in females and unfed nymphs of *O. brasiliensis*. These results deepen our understanding of the tick-microbiota relationship in Ixodidae and Argasidae, driving new studies with the focus on the manipulation of tick microbiota to prevent outbreaks of tick-borne diseases in South America.

1. INTRODUCTION

The microbiome is a major factor in mammalian physiology (Rosshart et al., 2017). The microbiota of mammals is associated with obesity (Mulders et al., 2018), diabetes (Inoue et al., 2017), cancer (Dietel et al., 2018), viral infections (Dietel et al., 2018), multiple sclerosis (Dietel et al., 2018), hypertension, chronic kidney disease (Sircana et al., 2019), and other metabolic, nervous, cardiovascular, and immune diseases. These associations have been strengthened by several lines of evidence that link the effect of the microbiota to the physiology of mammals (mainly humans). However, all metazoans have partnered with a small or large consortium of microbes to enhance their health and survival, which is also valid for ticks (Narasimhan and Fikrig, 2015).

Endosymbioses are interactions in which a smaller partner (the endosymbiont) lives inside the cell of a larger individual (the host). Examples of this relationship include the origin of mitochondria in eukaryotic cells (Dietel et al., 2018), and the Costiella mutualist symbiont, which is essential to the development of *Rhipicephalus microplus* (Guizzo et al., 2017). Ticks are among the main vectors of diseases for...
humans and animals, but little is known about the interactions of these arthropods with their microbiota (Bonnet et al., 2017). It is well known that the microbiota plays a vital role in the physiology of its host, either by preventing infections by pathogens or by the synthesis of compounds necessary for the survival of the host organism (Bonnet et al., 2017; Gerhart et al., 2016). In addition to endosymbionts, pathogens of medical and veterinary importance, such as species of the genera Anaplasma, Borrelia, Coxiella, Ehrlichia, Francisella, and Rickettsia, are present in ticks (Greay et al., 2018). Furthermore, arthropod microbiota could influence the presence and transmission of mammalian pathogens. For instance, commensal and symbiotic bacteria can inhibit infection by Plasmodium spp., Trypanosoma spp. and the Dengue virus in Anopheles spp., Glossina spp., and Aedes aegypti, respectively (Weiss and Aksy, 2011). In this sense, recently, several studies have addressed the microbiome of ticks of medical importance, such as Ixodes rricinus (Hernandez-Jarquin et al., 2018), Ixodes scapularis (Clow et al., 2018; Narasimhan et al., 2014), Ixodes pacificus (Kwan et al., 2017), Dermacentor andersoni (Clayton et al., 2015; Gall et al., 2016), Amblyomma americanum (Ponsumay et al., 2014; Trout, Fryxell and DeBruyn, 2016), Amblyomma maculatum (Budachetti et al., 2014), and Haemaphysalis longicornis (Nakao et al., 2013; Zhang et al., 2019; Zhuang et al., 2018).

Despite the abundance of tick species and the impact of tick-borne diseases in South America, to date, there have been few research studies on the microbiome of South American native ticks. In recent years, microbiome studies of Neotropical ticks have become available, such as for Amblyomma cajennense sensu stricto and Amblyomma sculptum. In these studies, the genera Coxiella, Francisella, and Rickettsia are consistently present (Binetruy et al., 2019; Binetruy et al., 2020). It is important to note that the genera Coxiella, Francisella, and Rickettsia contain both endosymbiont and pathogen species. Particularly, the genus Coxiella harbors a pathogen species, the Q fever agent Coxiella burnetii, and a plethora of closely related Coxiella endosymbionts. A similar pattern can be observed in Francisella and Rickettsia species (Binetruy et al., 2019, 2020).

Brazil has a tick fauna composed of 70 species, of which 47 belong to the Ixodidae and 23 to the Argasidae (Dantas-Torres et al., 2019). Considering the Ixodidae, the Amblyomma aureolatum tick has been recognized as a vector of Spotted Fever Group (SFG) rickettsiae (Dal’Agnol et al., 2018; Saraiva et al., 2014) and the protozoan Rangelia vitalii (Soares et al., 2018, 2014). On the other hand, among the Argasidae ticks, Ornithodoros brasiliensis is associated with a toxicosis syndrome in humans and animals (Reck et al., 2013, 2011), and has been linked to the transmission of a Borrelia species (Davis, 1952). Therefore, the aim of this work was to investigate the bacterial communities associated with two neotropical tick species of human and veterinary health importance from Brazil: A. aureolatum and O. brasiliensis.

2. MATERIALS AND METHODS

2.1. Ticks

From December 2014 to December 2016, A. aureolatum (Acari: Ixodidae) adult ticks were collected from their natural host, free-ranging crab-eating fox (Cerdocyon thous). Wild canids were captured using Tomahawk live-traps in two areas of environmental preservation, in the Pampa biome in the Rio Grande do Sul (RS) state, southern Brazil: Banhado dos Pachecos (BP) in Viamão municipality, and APA Ibirapitã, in Santana do Livramento municipality.

Specimens of O. brasiliensis (Acari: Argasidae) (fed adults, fed and unfed nymphs) were collected in July 2016 in their natural environment from the Atlantic Rainforest biome in the Jaquirana municipality, Southern Brazil highlands, RS. Ticks were manually collected by sifting the soil. Vertebrate hosts of O. brasiliensis include dogs, armadillos, and possibly skunks, besides humans (Reck et al., 2011; Reck et al., 2013).

After the ticks were collected, they were immediately taken to the laboratory and washed thrice in 70% ethanol, followed by a final wash using sterile ultrapure water to remove debris and to disinfect the surface. Ticks were identified up to species level by dichotomous keys (Barros-Battesti et al., 2006, 2012). Samples of A. aureolatum adults were discriminated by gender (males and females), whereas O. brasiliensis specimens were classified as fed adult males, fed adult females, and fed or unfed nymphs.

2.2. DNA extraction, library preparation, and sequencing

The DNA extraction procedures were performed in a biosafety cabinet to ensure sample protection from environmental contaminants and the protection of researchers from potential infectious pathogens. Genomic DNA was extracted from each individual whole tick using the PureLink Genomic DNA MiniKit (Invitrogen, Carlsbad, CA, USA), including treatment with 50 μl lysozyme (20 mg/mL) (Sigma-Aldrich, Dorset, UK) at 30 min/37 °C for peptidoglycan disruption. In all DNA extraction procedures, a negative control (ultrapure water) was included. Ticks were grouped by host (for A. aureolatum), sex, stage/instar, and locality, as indicated in Table 1.

To synthesize the sequencing libraries, the V4 region of the bacterial 16S rRNA gene was amplified according to Kozich and coworkers (2013) and the 16S metagenomic sequencing library preparation guide (Illuminina Inc.) (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf?ga=2.236812035.389663062.162237564.-255547950.1622237564). In all PCR reactions, a negative control (ultrapure water) was included. Sequencing was performed on a MiSeq (Illumina) platform using a 500-cycle v2 kit generating 250-bp paired-end reads. A drawback of our study is that we are not able to include a mock control for library sequencing due to the high cost for the inclusion of additional samples.

2.3. Bioinformatics analysis

Raw FASTQ files were used as input for the Mothur MiSeq SOP (Kozich et al., 2013), and data analysis was performed in the statistical language R. The operational taxonomic units (OTU) generated were compared with the ribosomal RNA database Silva (https://www.arb-silva.de/).

3. RESULTS

The mean number of raw reads obtained per sample before and after quality control was 313,515.2 (100,548 to 765,590) and 276,052.3 (91,728 to 665,380), respectively (Table 2).

In A. aureolatum, the predominant bacterial phyla were Proteobacteria (98.68%), Tenericutes (0.70%), Bacteroidetes (0.14%), Actinobacteria (0.13%), and Acidobacteria (0.05%). The predominant genera were Francisella (97.01%), Spiroplasma (0.70%), Wolbachia (0.51%), Candidatus Midichloria (0.25%), and Alkanindiges (0.13%), as shown in Fig. 1. Francisella was the predominant genus in all samples of A. aureolatum. In ticks from BP, Spiroplasma sp. was found in all samples, whilst in ticks from APA Ibirapitã, it was found only in one female and in a small proportion. On the other hand, Wolbachia sp. was found only in samples from BP, at a larger proportion in males than in females. Candidatus Midichloria sp. was also found only in ticks from BP. Alkanindiges sp. was found only in males and Morganella sp. only in females from BP. Rickettsiella sp. was found only in male ticks from BP. A greater amount of unclassified Proteobacteria was found in APA Ibirapitã than in BP. Mycobacterium sp. and Burkholderia sp. were also present in a larger amount in BP-collected ticks.

The predominant phyla in O. brasiliensis were Proteobacteria (90.27%), Actinobacteria (7.38%), Firmicutes (0.77%), Bacteroidetes (0.44%), and Planctomycetes (0.22%). The predominant bacterial genera were Coxiella (87.71%), Nocardioides (1.73%), Saccharopolyspora (0.54%), Marmoricola (0.42%), and Staphyloccoccus (0.40%), as shown in
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Table 1
Data about origin of the tick pool used in the DNA metabar-coding analysis.

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Biome</th>
<th>latitude</th>
<th>longitude</th>
<th>Tick species</th>
<th>NCBI taxon id of the tick</th>
<th>Library name</th>
<th>Life stage</th>
<th>Sex</th>
<th>Number of ticks</th>
</tr>
</thead>
<tbody>
<tr>
<td>APA Ibirapuítica</td>
<td>Pampa</td>
<td>30° 20'32.5&quot;S</td>
<td>55° 41'09.7&quot;W</td>
<td>Amblyomma aureolatum</td>
<td>187,763</td>
<td>B</td>
<td>Adult</td>
<td>Male</td>
<td>5</td>
</tr>
<tr>
<td>APA Ibirapuítica</td>
<td>Pampa</td>
<td>30° 20'32.5&quot;S</td>
<td>55° 41'09.7&quot;W</td>
<td>Amblyomma aureolatum</td>
<td>187,763</td>
<td>C</td>
<td>Adult</td>
<td>Female</td>
<td>5</td>
</tr>
<tr>
<td>APA Ibirapuítica</td>
<td>Pampa</td>
<td>30° 20'32.5&quot;S</td>
<td>55° 41'09.7&quot;W</td>
<td>Amblyomma aureolatum</td>
<td>187,763</td>
<td>D</td>
<td>Adult</td>
<td>Female</td>
<td>5</td>
</tr>
<tr>
<td>Banhado dos Pachecos</td>
<td>Jaquirana</td>
<td>30° 05'37.9&quot;S</td>
<td>50° 51'01.5&quot;W</td>
<td>Amblyomma aureolatum</td>
<td>187,763</td>
<td>E</td>
<td>Adult</td>
<td>Male</td>
<td>5</td>
</tr>
<tr>
<td>Banhado dos Pachecos</td>
<td>Jaquirana</td>
<td>30° 05'37.9&quot;S</td>
<td>50° 51'01.5&quot;W</td>
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<td>F</td>
<td>Adult</td>
<td>Male</td>
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<td>30° 05'37.9&quot;S</td>
<td>50° 51'01.5&quot;W</td>
<td>Amblyomma aureolatum</td>
<td>187,763</td>
<td>G</td>
<td>Adult</td>
<td>Female</td>
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<tr>
<td>Banhado dos Pachecos</td>
<td>Jaquirana</td>
<td>30° 05'37.9&quot;S</td>
<td>50° 51'01.5&quot;W</td>
<td>Amblyomma aureolatum</td>
<td>187,763</td>
<td>H</td>
<td>Adult</td>
<td>Female</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2
Data on the metagenomic sequencing process, and analysis of bacterial diversity based on the different OTUs.

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Tick species</th>
<th>Library name</th>
<th>Life stage</th>
<th>Sex</th>
<th>Number of raw reads</th>
<th>Number of reads after quality control</th>
<th>Shannon index</th>
<th>Simpson index</th>
<th>inv/Simpson index</th>
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</thead>
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<td>APA Ibirapuítica</td>
<td>Amblyomma aureolatum</td>
<td>B</td>
<td>Adult</td>
<td>Male</td>
<td>294,424</td>
<td>277,238</td>
<td>0.097180283</td>
<td>0.019209686</td>
<td>1.019585882</td>
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<tr>
<td>APA Ibirapuítica</td>
<td>Amblyomma aureolatum</td>
<td>C</td>
<td>Adult</td>
<td>Female</td>
<td>346,018</td>
<td>327,598</td>
<td>0.055127549</td>
<td>0.013924067</td>
<td>1.014120674</td>
</tr>
<tr>
<td>APA Ibirapuítica</td>
<td>Amblyomma aureolatum</td>
<td>D</td>
<td>Adult</td>
<td>Female</td>
<td>100,548</td>
<td>91,728</td>
<td>0.080087477</td>
<td>0.024767181</td>
<td>1.025396194</td>
</tr>
<tr>
<td>RVS Banhado dos Pachecos</td>
<td>Amblyomma aureolatum</td>
<td>E</td>
<td>Adult</td>
<td>Male</td>
<td>318,006</td>
<td>298,018</td>
<td>0.752314061</td>
<td>0.28020152</td>
<td>1.389274852</td>
</tr>
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<td>RVS Banhado dos Pachecos</td>
<td>Amblyomma aureolatum</td>
<td>F</td>
<td>Adult</td>
<td>Male</td>
<td>312,700</td>
<td>285,202</td>
<td>1.597470598</td>
<td>0.561248189</td>
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<tr>
<td>RVS Banhado dos Pachecos</td>
<td>Amblyomma aureolatum</td>
<td>G</td>
<td>Adult</td>
<td>Female</td>
<td>144,174</td>
<td>131,514</td>
<td>0.11195518</td>
<td>0.02694269</td>
<td>1.027688625</td>
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<td>H</td>
<td>Adult</td>
<td>Female</td>
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<tr>
<td>Jaquirana</td>
<td>Ornithodoros brasiliensis</td>
<td>J</td>
<td>Adult</td>
<td>Male</td>
<td>148,164</td>
<td>119,234</td>
<td>1.116713161</td>
<td>0.267420311</td>
<td>3.65030238</td>
</tr>
<tr>
<td>Jaquirana</td>
<td>Ornithodoros brasiliensis</td>
<td>K</td>
<td>Adult</td>
<td>Female</td>
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<td>323,768</td>
<td>1.125283115</td>
<td>0.25330364</td>
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<tr>
<td>Jaquirana</td>
<td>Ornithodoros brasiliensis</td>
<td>L</td>
<td>Adult</td>
<td>Female</td>
<td>429,368</td>
<td>376,904</td>
<td>1.046095937</td>
<td>0.239096409</td>
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<tr>
<td>Jaquirana</td>
<td>Ornithodoros brasiliensis</td>
<td>M</td>
<td>Fed nymph</td>
<td>Immature</td>
<td>381,608</td>
<td>319,306</td>
<td>1.58776802</td>
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<td>1.646172909</td>
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<td>N</td>
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<td>Immature</td>
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<td>Ornithodoros brasiliensis</td>
<td>P</td>
<td>Unfed nymph</td>
<td>Immature</td>
<td>346,760</td>
<td>294,182</td>
<td>0.611494688</td>
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<td>Unfed nymph</td>
<td>Immature</td>
<td>765,590</td>
<td>665,380</td>
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<td>Jaquirana</td>
<td>Ornithodoros brasiliensis</td>
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<td>Unfed nymph</td>
<td>Immature</td>
<td>273,130</td>
<td>222,450</td>
<td>0.750464292</td>
<td>0.176728503</td>
<td>1.21463965</td>
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</table>

Fig. 2. In the immature stages (nymphs), Staphylococcus sp. and Escherichia-Shigella were present in greater numbers compared to adults. Unclassified Gammaproteobacteria were found in greater amounts in unfed nymphs, whilst the genera Rickettsiella, Nitrosospira, Rubrobacter, Planctomyces, and Acidibacter were present in greater quantity in adults (males and females). Bacterial diversity seemed to be higher in the A. aureolatum samples collected in BP compared to those from APA Ibirapuítica, and there was no
evident difference between males and females (Fig. 3). Since O. brasiliensis ticks were collected in only one location, diversity evaluation only allowed a comparison among different stage, with a greater diversity in fed nymphs (Fig. 3).

Considering the genera with potential importance for human and animal health that can be transmitted by ticks, Coxiella sp. was found in all stages of O. brasiliensis, Francisella sp. in all stages of A. aureolatum and unfed nymphs of O. brasiliensis, and Rickettsia sp. in females of A. aureolatum from BP and females and unfed nymphs of O. brasiliensis.
4. DISCUSSION

Ticks are one of the main vectors of pathogens for humans and animals worldwide. However, they harbor non-pathogenic microorganisms that are important for their survival, helping in both their nutrition and immunity. Ticks depend on bacteria, such as Coxiella-like and Francisella-like endosymbionts, that probably provide nutrients that are lacking in their diet. In addition to endosymbionts, ticks may also be present and transmit pathogens to mammals, including C. burnetii and Francisella tularensis. However, the evolutionary relationship between endosymbiotic and pathogenic species of the same genus is puzzling (Duron et al., 2018). Analyzing the genome of Coxiella-like endosymbionts (CLE) of Rhipicephalus sanguineus sensu lato and Rhipicephalus turanicus, Tsemzentzi et al. (2018) observed that both genomes encode numerous pseudogenes, consistent with an ongoing genome reduction process. Similarly, Francisella-like endosymbionts (FLEs) contain pseudogenized versions of virulence genes present in F. tularensis, indicating that the common ancestor of FLEs and mammalian pathogens were equipped to function as a pathogen (Gerhart et al., 2018). In silico flux balance metabolic analysis (FBA) revealed the excess production of L-proline for CLE genomes, indicating a possible proline transport from Coxiella to the tick. Additionally, CLE genomes encode multiple copies of the proline/betaine transporter proP gene (Tsemzentzi et al., 2018). Moreover, FLE genomes contain intact pathways for the synthesis of several B vitamins and cofactors lacking in vertebrate blood (Gerhart et al., 2018). This possible symbiotic mechanism of B vitamin provisioning by FLE has formally been demonstrated by Duron et al. (2018) through genomic and microbiome studies.

The active search for new microorganisms harbored in ticks should ideally be coordinated to prevent new cases of diseases, since tick-associated microorganisms of unknown pathogenicity may be identified first, and then an association with human or animal diseases could be found (Varela-Stokes et al., 2017). For instance, Rickettsia parkeri was recovered from Amblyomma maculatum in Texas approximately 60 years prior to the index case in humans, which was only reported in 2004. In addition, the spirochete Borrelia miyamotoi, first identified in Ixodes persulcatus in Japan, was considered as a non-pathogenic microorganism until the first human cases were reported only 15 years later (Varela-Stokes et al., 2017).

In A. aureolatum ticks, the principal genus found was Francisella. This corroborates the results from Pavanella and coworkers (2020), who found a dominance of the genus Francisella in the A. aureolatum midgut microbiota. Budachetri and coworkers (2014) and Varela-Stokes and coworkers (2018) also reported Francisella as the main genus in A. maculatum (another Amblyomma species restricted to the Americas). Additionally, the presence of Francisella sp. has been reported in high abundance in Dermacentor species, including Dermacentor variabilis, Dermacentor andersoni, Dermacentor hunteri, Dermacentor nitens, Dermacentor occidentalis, and Dermacentor albipictus (Budachetri et al., 2014; Niebylski et al., 1997; Scoles, 2004; Sun et al., 2000). On the other hand, the most abundant genera in Amblyomma variegatum (an Afrotropical tick species) were Clavibacter (13.4%) in males and Borrelia (8.6%) in females (Nakao et al., 2013). In Amblyomma testudinarium (an Oriental-Paleartic tick species) nymphs, the predominant genus was Pseudalteromonas (17.2%) (Nakao et al., 2013). In Amblyomma americanum (a Neartic tick species), Coxiella was found in a greater amount in nymphs (26%), whereas Bradyrhizobium and Phenylobacterium were predominant in adults (28 to 45%) (Menchaca et al., 2013). However, Ponnusamy and coworkers (2014) observed that three of the most dominant genera found in A. americanum were Rickettsia, “Candidatus Midichloria mitochondrii”, and Ehrlichia, all members of the order Rickettsiales, representing 53% (median; interquartile range, 31 to 75%) of the reads. Trout Fryxell and DeBruyn (2016) found that the most dominant OTUs were highly variable among species of A. americanum, but the most abundant ones (> 1% relative abundance) across all species were Flavobacterium (24.4 ± 13.3%), an unclassified Gammaproteobacteria (2.22 ± 12.4%), Rickettsia (9.1 ± 14.5%), Sphingomonas (4.6 ± 3.6%), Singulisphaera (1.91 ± 1.81), Hymenobacter (1.95 ± 3.00), and Bacillus (1.86 ± 11.7%). In Amblyomma tuberculatum (a Neartic tick species), the main genera found were Rickettsia (55.8%) and Francisella (35.2%) (Budachetri et al., 2016). Budachetri and coworkers (2017) determined the microbiomes of Amblyomma longirostre, Amblyomma nodosum, Amblyomma maculatum, and Haemaphysalis juxtakochi collected from migratory bird species and found that the most prevalent genera observed, with abundance levels above 1%, were Lactococcus, Raoultella, Wolbachia, Francisella, Propionibacterium, Ewingella, Elizabetthkingia, Rickettsia, Massilia, and Methyllobacterium.

Besides Francisella, the main genera found in A. aureolatum were Spiroplasma, Wolbachia, Candidatus Midichloria, and Alkanindiges.

Fig. 3. The different indexes of alpha diversity of bacterial microbiomes of Amblyomma aureolatum and Ornithodoros brasiliensis. Panel. (A) Bacterial diversity by Shannon Index. (B) Bacterial diversity by Simpson Index. The horizontal lines show the mean of the replicates, and the symbols (circles, squares, triangles, diamonds) represent each sample analyzed. Abbreviations: APA, APA do Ibirapuãtã; BP, Banhado dos Pachecos.
Spiroplasma is common in arthropods (for a comprehensive review, see Bonnet et al., 2017 and O. Duron et al., 2017) and, although their effect in ticks is unknown, these bacteria showed a male-killer effect in diverse insect species (Engelstädter and Hurst, 2009). The genus Wolbachia is highly common in arthropods and is also present in ticks (Bonnet et al., 2017). The effect of this genus on ticks is largely unknown, but it is responsible for reproductive alterations in many arthropods (Engelstädter and Hurst, 2009). It is a facultative mutualist (defensive symbiosis) in mosquitoes (Brownlie and Johnson, 2009; Hamilton and Peruñán, 2013) and an obligate symbiont in bed bugs (Hosokawa et al., 2010; Nikoh et al., 2014). At least in the case of I. ricinus, it has been demonstrated that the detection of Wolbachia sp. was due to contamination by a hymenopteran parasitoid (Plantard et al., 2012). In several ticks, Candidatus Midichloria sp. resides in high numbers in female reproductive tissues. It lives inside tick mitochondria, but its effects on tick physiology and metabolism remain unknown (Buyssse and Duron, 2018; Epis et al., 2013). Nevertheless, it has recently been suggested that Candidatus Midichloria sp. is an obligate nutritional symbiont providing B vitamins (O. Duron et al., 2017) since its genome encodes pathways for the synthesis of major B vitamins and cofactors (Buyssse and Duron, 2018; Sassera et al., 2011). Male I. holocyclus ticks also appear to inherit and harbor Candidatus Midichloria sp.; however, I. ricinus males fail to establish stable bacterial populations. In addition, Candidatus Midichloria sp. is found in I. ricinus salivary glands, from where it is introduced to vertebrate hosts, including humans, during feeding. However, the consequences of Midichloria infection in vertebrate hosts, if any, are unknown (Gofton et al., 2015; Mariconti et al., 2012; Serra et al., 2018). Alkanindiges sp. is not commonly found in high proportions in ticks, although R. microplus eggs have been described to present a relative abundance of 0.2% (Andreotti et al., 2011). It seems that Alkanindiges sp. are found in oilfield soils (Bogan et al., 2003; Chang et al., 2011; Fuentes et al., 2016; Sun et al., 2015; Zheng et al., 2018), patients with parotid abscesses (Woo et al., 2005) and with, bone and joint infections (Fenollar et al., 2006), patients with asthma (Fazlollahi et al., 2018), activated sludge systems (Klein et al., 2007), tonsils of healthy pigs (Lowe et al., 2012), lettuce (Erlacher et al., 2014; Rastogi et al., 2012), and drinking water wells (Krawczyk and Lucders, 2014).

To the best of our knowledge, there are only few studies on the microbiome characterization of Argasidae. Ticks of this family could exhibit characteristics remarkably different from those of Ixodidae, for instance, fast feeding (minutes) and a long life (some species may live for several years). They can also induce severe injuries directly associated with the tick bite (tick toxicosis), may have several nymphal stages, and females do not die after oviposition (Ramirez et al., 2016; Reck et al., 2013). In Ornithodoros tholozani collected from buffaloes from Pakistan, the main bacterial genera found were Ralstonia (40.0%), Staphylococcus (22.8%), Enterococcus (13.9%), Saccharomonospora (4.5%), and Bacillus (4.3%) (Karim et al., 2017). The most predominant genus found in O. brasiliensis was Coxella. This bacterium was also the main symbiont of Rhipicephalus spp. (Bernasconi et al., 2002; Guizzo et al., 2017). Guizzo and coworkers (2017) showed that, in Rhipicephalus microplus, 99 and 98.3% of bacteria present in eggs and larvae, respectively, were Coxiiella sp. The levels of Coxiiella sp. were affected in fully engorged females injected with tetracycline; the development of larva with reduced levels of the Coxiiella sp. was arrested at the metanymph stage. In Argasidae, the endosymbiont Coxiiella sp. was found in Caront capensis (Reeves, 2005), Argas monolakensis (Reeves, 2008), Ornithodoros rostratus (Andreotti et al., 2012), Ornithodoros moubata (Al-Deeb et al., 2016), and in at least 10 other soft tick species, including O. brasiliensis (O. Duron et al., 2017).

Besides Coxiiella sp., the main genera found in O. brasiliensis were Nocardioides, Saccharopolyspora, Marmoricola, and Staphylococcus. Nocardioides was a symbiont of ants and was involved in ant-plant mutualisms (Hansbrow et al., 2015; Reyes and Cafaro, 2015); it was also isolated from the water flea Daphnia cucullata (Crustacea: Cladocera) (Toth et al., 2008). Saccharopolyspora was also found associated with ants (Reyes and Cafaro, 2015) and was isolated from the gut of a termite (Speculitermes sp.) (Sinna et al., 2011). Members of the genus Saccharopolyspora are a potentially rich source of natural products, but only erythromycin, produced by Saccharopolyspora erythraea, and spinosad, an insecticide based on chemical compounds found in the bacterial species Saccharopolyspora spinosa, are currently commercially available (Prabhu et al., 2011; Sinna et al., 2011). The genus Marmoricola was also found in spiders from Japan (Iwai et al., 2009) and ants from the USA (Ishak et al., 2011). Andreotti and coworkers (2011) showed that the genus Staphylococcus was relatively abundant in males (32%) and in the eggs (18%) of R. microplus ticks, but not in adult females (0.7%).

In our study, among the genera with potential importance for human and animal health that can be transmitted by ticks, the presence of Coxiiella, Francisellia, and Rickettsia is noteworthy. We found Rickettsia sp. in A. aureolatum, which corroborates the studies regarding its importance as a vector of spotted fever in Brazil (Dall’Agnol et al., 2016; Saraiva et al., 2014). We also found Rickettsia sp. in O. brasiliensis. Bacteria of the genus Rickettsia has never been reported in O. brasiliensis and have only recently been found in ticks in Brazil (Lafri et al., 2015; Milhano et al., 2014; Moreira-Soto et al., 2017; Pader et al., 2012; Sánchez-Montes et al., 2016; Socolovschi et al., 2012; Tahir et al., 2016). Therefore, tick-transmitted rickettsioses may be a neglected subject that may have an impact on public health, considering the amount of human cases of parasitism by Argasidae, mainly by O. brasiliensis (Martins et al., 2011; Reck et al., 2013). Further studies may clarify the potential roles of Rickettsia spp. in the syndrome associated with the O. brasiliensis bite.

Finally, it was not possible to detect Borrelia sp. in O. brasiliensis. Several species of Ornithodoros (such as Ornithodoros hermsi, Ornithodoros sonrai, Ornithodoros turicata, Ornithodoros erraticus, Ornithodoros moubata, and Ornithodoros rudis), especially those presenting a public health impact, have been associated with the transmission of Borrelia sp., belonging to the relapsing fever group (Boyle et al., 2014; Lopez et al., 2011; McCoy et al., 2010; Muñoz-Leal et al., 2018; Schwahn et al., 2012; Trape et al., 2013). Conversely, Davis (1952) reported the isolation of Borrelia sp. in samples of O. brasiliensis. This difference corroborates the results found in this study, as we were able to describe significant differences in the bacteriome composition within individuals of a single species and found that the factors sex, developmental stage, mammal host, and environment may influence it. Additionally, these data show the importance of investigating pathogen presence, specifically since bacterial genera present in small proportions in a sample may not appear in the analysis of rRNA 16S gene metabarcoding (Frey et al., 2014). Abundant bacterial endosymbionts limit the effectiveness of next-generation 16S bacterial community profiling in arthropods by masking less abundant bacteria, including pathogens (Gofton et al., 2015). In addition, the samples of O. brasiliensis were collected only from one place. The presence of Borrelia sp. in samples from different locations and using techniques with a higher sensitivity could help to clarify this issue (Boyle et al., 2014; Muñoz-Leal et al., 2018).

In A. aureolatum, bacterial diversity was higher in samples collected in BP compared to those from APA Ibirapuá. The BP is a wildlife conservation refuge surrounded by small farms, with stray dogs carrying ticks in and out. The APA Ibirapuá has an extremely low human population and animal densities. These differences in the bacterial species diversity of fed nymphs, most likely because the former enters into contact with the host’s microbiota during the blood meal. Males and females also had a lower diversity compared to fed nymphs; this difference may be due to the fact that the adult ticks were analyzed individually and the fed nymphs in pools of five individuals. There may also be variation among specimens.

In this study, we determined the bacteriomes of two tick species native to Brazil. Furthermore, we were able to compare the
Aureolatum} bacterial diversity from specimens of different ecological characteristics. These results facilitate our understanding of the tick-microbiota relationship in Ixodidae and Argasidae and may drive new studies with a focus on the manipulation of tick microbiota to prevent outbreaks of tick-borne diseases in South America.

**Authors’ contributions**

BD did most of the experiments, collaborated in the study design, analyzed data, and wrote the manuscript. JR and CASF co-supervised the project, conceived/designed the study, and wrote the paper. PAR, UAS and RLD participated in the field collections and did some experiments. AW and UAS collaborated in some experiments and data analysis. FQM performed the DNA sequencing and contributed to the study design. JAM contributed with data analysis. All authors read and approved the final version of the paper.

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**Ethics approval**

The study protocol was approved by the Committee for Animal Care and Experimentation of IPVDF (CEUA/IPVDF 28/2014) and by the Brazilian Ministry of Environment (SISBIO 47,357-3).

**Consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and material**

Data available on request from the authors.

**Code availability**

Not applicable.

**Declaration of Competing Interest**

The authors declare that they have no conflict of interest.

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