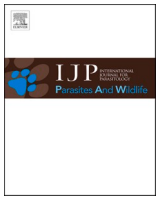
International Journal for Parasitology: Parasites and Wildlife 24 (2024) 100943

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw



Bacterial community of ticks (Acari: Ixodidae) and mammals from Arauca, Colombian Orinoquia

Paula A. Ossa-López [a,b], Héctor E. Ramírez-Chaves [b,c], María Elena Álvarez López [d], Gabriel Jaime Castaño Villa [e], Fredy A. Rivera-Páez [b,*]

[a] Doctorado en Ciencias, Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Calle 65 No. 26-10, 170004, Manizales, Caldas, Colombia
[b] Grupo de Investigación en Genética, Biodiversidad y Manejo de Ecosistemas (GEBIOME), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Calle 65 No. 26-10, 170004, Manizales, Caldas, Colombia
[c] Centro de Museos, Museo de Historia Natural, Universidad de Caldas, Calle 58 No. 21-50, 170004, Manizales, Caldas, Colombia
[d] Grupo de Investigación en Genética, Biodiversidad y Manejo de Ecosistemas (GEBIOME), Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Universidad de Caldas, Calle 65 No. 26-10, 170004, Manizales, Caldas, Colombia
[e] Grupo de Investigación en Genética, Biodiversidad y Manejo de Ecosistemas (GEBIOME), Departamento de Desarrollo Rural y Recursos Naturales, Facultad de Ciencias Agropecuarias, Universidad de Caldas, Calle 65 No. 26-10, 170004, Manizales, Caldas, Colombia

ARTICLE INFO

Keywords:
Blood
Hard ticks
Mammals
Microbiome
Pathogens

ABSTRACT

Ticks are obligate hematophagous ectoparasites of vertebrates and are relevant worldwide due to the number of bacterial and other pathogens they can transmit. To date, the knowledge about the microorganisms that ticks harbor and transmit to their hosts is incipient. In this study, 24 samples of mammals belonging to four taxonomic orders and ticks of the genera *Amblyomma* and *Rhipicephalus* from the Orinoco region of Colombia were analyzed to described and compare the bacterial microbiome. Genetic extraction was performed, and the V3–V4 region of the *16S* rRNA gene was amplified by PCR. Libraries were created, and those samples with adequate quality indices were sequenced using Illumina MiSeq technology. Bacterial taxonomic assignment analyses were conducted through Amplicon Sequence Variants (ASVs) and Operational Taxonomic Units (OTUs). The results correspond to 16 samples that passed the quality filters, with 3218 OTUs (415 families). Although a considerable number of unknown bacteria was found, Enterobacteriaceae, Beijerinckiaceae, Moraxellaceae, and Burkholderiaceae are the most prevalent families, and the presence of the genera *Coxiella*, *Escherichia-Shigella*, *Enterobacter*, which can harbor pathogenic species was confirmed. In individuals of *Amblyomma mixtum* found actively feeding on *Hydrochoerus hydrochaeris*, bacteria of the genera *Escherichia-Shigella* and *Enterobacter* were documented. Similarly, *Rhipicephalus microplus* found actively feeding on *Odocoileus virginianus cariacou* shared *Escherichia-Shigella*. *Ralstonia* was shared among the blood samples of *H. hydrochaeris*, while *Anaplasma* and *Eubacterium* were shared in blood and liver samples of *O. v. cariacou*. Shared bacteria between *A. mixtum* and *R. microplus* included *Bacillus*, *Coxiella*, and *Escherichia-Shigella*. The results highlight the need of additional studies in other natural regions of Colombia and other American countries where tick-borne diseases have been detected. Likewise, the recorded data are the first at the level of bacterial communities in ticks of the family Ixodidae and provide valuable knowledge for the understanding host-tick and pathogen interactions.

1. Introduction

Ticks (Acari: Ixodida) are vectors of a wide variety of tick-borne pathogens (TBPs) causing several tick-borne diseases (TBD) in humans and other vertebrates worldwide (Sonenshine and Roe, 2014; Gondard et al., 2017; Maqbool et al., 2022). The family Ixodidae includes ticks recognized as vectors of bacteria, helminths, protozoa, and viruses that can cause different conditions in humans (e.g., human anaplasmosis, Lyme borreliosis, and tick-borne encephalitis), in domestic animals (e.g., babesiosis and ehrlichiosis), and in wildlife (e.g., rickettsiosis) (Baneth, 2014; Efstratiou et al., 2021; Michalski et al., 2021; Bezerra-Santos et al., 2022; Velásquez-Guarín et al., 2024). In addition to pathogens, the set of microbial biotas associated with the tick is defined as the "microbiome" and includes other commensal and endosymbiotic microorganisms that

* Corresponding author.
E-mail address: fredy.rivera@ucaldas.edu.co (F.A. Rivera-Páez).

https://doi.org/10.1016/j.ijppaw.2024.100943
Received 28 February 2024; Received in revised form 8 May 2024; Accepted 9 May 2024
Available online 9 May 2024

ticks harbor and can be vertically inherited (Greay et al., 2018; Wu-Chuang et al., 2021; Grandi et al., 2023). Little is known about the role that microorganisms (including bacteria), plays in ticks, they might contribute to the arthropods' fitness by providing nutrients ticks cannot synthesize, such as B group vitamins, among others (Duron et al., 2015, 2018; Brenner et al., 2021; Zhong et al., 2021).

The tick microbiota also seems to play a role in the establishment, maintenance of TBPs infection and transmission, most likely through interaction with the tick's immune system (Mateos-Hernández et al., 2021; Narasimhan et al., 2021; Grandi et al., 2023). Considering this, the control of tick populations or the associated TBPs could be achieved by manipulating the tick microbiota or particular endosymbionts (Mateos-Hernández et al., 2021; Narasimhan et al., 2021). In Ixodidae, the most extensively studied bacterial communities are those associated with: *Amblyomma americanum*, *Amblyomma maculatum*, *Amblyomma ovale*, *Amblyomma scalpturatum*, *Amblyomma tuberculatum*, *Dermacentor andersoni*, *Dermacentor silvarum*, *Ixodes ricinus*, and *Rhipicephalus microplus*; these studies have enabled the characterization of pathogenic bacterial species belonging to the genera *Anaplasma*, *Borrelia*, *Ehrlichia*, and *Rickettsia* (Andreotti et al., 2011; Menchaca et al., 2013; Budachetri et al., 2014, 2016; Wu-Chuang et al., 2021; Guizzo et al., 2022a; Rojas-Jaimes et al., 2022). However, the evaluations of the dynamics and complexity of the microbiomes associated with the tick diet are still insufficient, and technical limitations might underestimate bacteria with low abundances (Swei and Kwan, 2017; Ross et al., 2018; Guizzo et al., 2020; Narasimhan et al., 2021). Traditionally, research have focused on the occurrence of a single TBP and, in some cases, co-infections (Couper and Swei, 2018; Mateos-Hernández et al., 2021; Grandi et al., 2023; Sarani et al., 2024). Similarly, and despite being part of the tick, host, and pathogen triangle, few studies have highlighted the bacterial communities present in ticks and their wild hosts (Egan et al., 2021; Sarani et al., 2024).

In Neotropical and ecological complex countries such as Colombia, the presence of 57 tick species (42 Ixodidae and 15 Argasidae) has been confirmed (Rivera-Páez et al., 2018a; Labruna et al., 2020; Ortíz-Giraldo et al., 2021; Saracho-Bottero et al., 2020, 2021; Guglielmone et al., 2021, 2023). For the Orinoquia region, one of the six natural regions of Colombia that is shared with Venezuela and belongs to the Orinoco River watershed, the presence of ticks such as *Amblyomma mixtum*, *Amblyomma triste* (Ixodidae), and *Ornithodoros hasei* (Acari: Argasidae) that are confirmed or potential vectors of pathogens has been confirmed (Rivera-Páez et al., 2016; Ossa-López et al., 2022, 2023). Similarly, molecular evidence of rickettsial DNA and the detection of antibodies provided strong evidence for the Orinoquia region to be considered the third endemic region for Rocky Mountain spotted fever (RMSF) group rickettsiosis (Miranda et al., 2011; Riveros-Pinilla et al., 2015; Gómez-Quintero et al., 2017; Rivera-Páez et al., 2018b; Cardona-Romero et al., 2022; Velásquez-Guarín et al., 2024). A metagenomic analysis conducted on the soft tick *O. hasei* (Acari: Argasidae) and bats of the species *Cynomops planirostris*, *Molossus pretiosus*, and *Noctilio albiventris* captured in the Orinoquia of Colombia (Carvajal-Agudelo et al., 2022), detected species related to potentially pathogenic genera *Borrelia* sp., *Bartonella tamiae*, *Ehrlichia* sp., and *Rickettsia*-like endosymbiont in both ticks and bats.

In this context, the Orinoquia region of Colombia presents conditions that warrant study to contribute information that enhances the understanding of the interactions between ticks-hosts and pathogens. Thus, the aim of the present research was to characterize the bacterial microbiomes present in hard ticks within Ixodidae and in wild and domestic mammals from the municipality of Arauca, Department of Arauca, Orinoquia region of Colombia.

2. Materials and methods

2.1. Study area

The field work was performed in November (end of the rainy season and beginning of the dry season to maximize capture numbers) of two years (2018 and 2021), in the "veredas" El Socorro (~06°46′, 70°43′W; 134 m), and Las Plumas (~06°36′ N, 70°29′ W, 110–125 m), Municipality of Arauca, Department of Arauca, Colombia, situated in the lowlands of the Orinoquia region of Colombia (Fig. 1). The region has a unimodal rainfall regime with a rainy season from April to November and a dry season from November to February (Buriticá-Mejía, 2016).

2.2. Collection of samples and identification of specimens

Sherman traps, Tomahawk traps, and mist nets were used to capture wild mammals using standard protocols (Voss and Emmons, 1996; Proulx, 2022). The work with domestic mammals was made possible thanks to the Unidad Administrativa Especial de Salud de Arauca, and the landowners and the general community of the localities. Some wild mammals were collected and euthanized following animal care recommendations (Sikes, 2016; Institutional Animal Care and Use Committee – IACUC, 2018). Additionally, blood samples were collected (Table 1) by puncture in the brachial vein (in bats), as well as through a small cut at the tip of the tail (in *Sus domesticus*). The identification of the collected mammals was based on taxonomic keys (e.g., Gardner, 2008; Patton et al., 2015), and all samples and specimens collected were deposited in the mammals and ectoparasites collections of the Museo de Historia Natural of the Universidad de Caldas (MHN-UCa). Manual search for ectoparasites was conducted on all mammals, and hard ticks were removed from hosts with the aid of entomological forceps, then placed alive in cryogenic tubes for subsequent morphological identification using a Zeiss DV4 stereomicroscope (Barros-Battesti et al., 2006; Nava et al., 2017; Guglielmone et al., 2021), and molecular confirmation was carried out through the amplification of two partial mitochondrial *16S* rRNA and *cox1* genes. An approximately 460 bp fragment of the *16S* rRNA gene was obtained using the primers 16S + 1 5′-CCGGTCTGAACTCAGATCAAGT-3' (Norris et al., 1996) and 16S - 1 5′-GCTCAATGATTTTTTAAATTGCTGT -3' (Mangold et al., 1998). The Cytochrome *c* oxidase subunit 1 (*cox1*) gene was amplified using the primers LCO1490 (F) 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198 (R) 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ which amplify an approximate 700 bp fragment (Folmer et al., 1994). The amplicons were sequenced using the Sanger technique at Macrogen (South Korea). We evaluated and edited the sequences with Geneious Prime® 2023.2.1 (Kearse et al., 2012). We also performed the confirmation of tick species using BLASTN in the National Center for Biotechnology Information (NCBI). The obtained sequences were deposited in the GenBank of the NCBI.

2.3. Sample preparation

Female hard ticks collected actively feeding on hosts were selected (Table 2). They were washed with 5% sodium hypochlorite for 5 min, followed by 5 washes of 5 min each in deionized distilled water, following a modified protocol from Kemp and Smith (2005) and Hoffmann et al. (2020). Some *Amblyomma* ticks (n = 3) were stored in a 2 mL Eppendorf tube in DNA/RNA Shield™ Reagent (Zymo Research) as they originated from the same host. Other ticks were dissected (*Amblyomma*: n = 9; *Rhipicephalus*: n = 5) in sterile phosphate-buffered saline (PBS) at 1% with the assistance of a Zeiss DV4 stereomicroscope to separate the midguts (m), ovaries (o) with oviduct (od), and salivary glands (sg) (Camargo-Mathias, 2013, 2018; Grandi et al., 2023). Subsequently, the organs were separately washed in a 1% PBS solution and pooled by host and each of the organs to be stored in 0.2 mL Eppendorf tubes with DNA/RNA Shield™ Reagent (Grandi et al., 2023). The blood collected

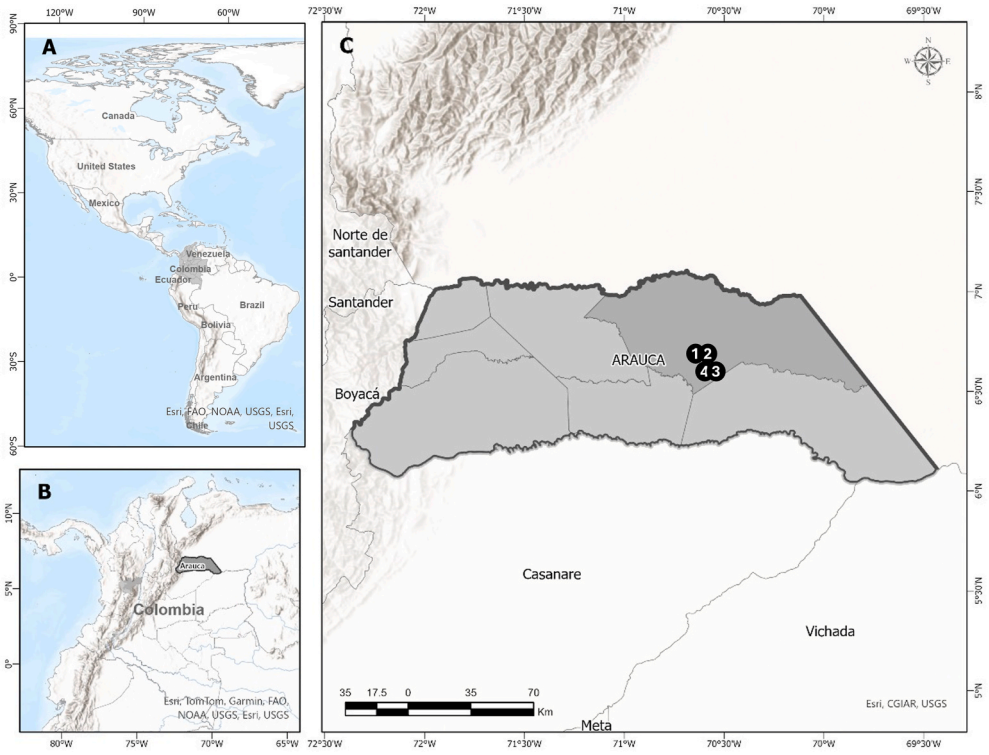


Fig. 1. Study area. (**A**) American Continent, (**B**) Colombia, (**C**) Department of Arauca, Colombia. Sampled localities in the municipalities of Arauca. (1) Vereda El Socorro, Finca Los Trompillos. (2) Vereda El Socorro, Finca Palmira. (3) Vereda Las Plumas, El Caño. (4) Vereda Las Plumas, Los Iguanitos.

Table 1
Collection information of sampled mammals, Municipality Arauca, Department of Arauca, Orinoquia region, Colombia.

Locality	Collection date	Coordinates	Elevation (m)	Mammal species (host)	Museum code (host)	Sample/Metagenome ID
Vereda El Socorro, Finca Los Trompillos	Nov. 1, 2021	06°46′46.4″ N, 70°43′00″ W	135	*Eptesicus orinocensis*	MHN-UCa-M 3691	blood/**MH1** liver/**MH2**
Vereda El Socorro, Finca Los Trompillos	Nov. 1, 2021	06°46′46.3″ N, 70°42′59.2″ W	135	*Desmodus rotundus*	MHN-UCa-M 3682	blood/MH3 liver/MH4
Vereda El Socorro, Finca Los Trompillos	Nov. 3, 2021	06°47′21.1″ N, 70°42′44.7″ W	130	[a] *Didelphis marsupialis*	MHN-UCa-M 3674	blood/MH5 liver/MH6
Vereda El Socorro, Finca Los Trompillos	Nov. 4, 2021	06°47′17.9″ N, 70°42′40.9″ W	127	*Marmosa robinsoni*	MHN-UCa-M 3676	blood/MH7 liver/MH8
Vereda El Socorro, Finca Los Trompillos	Nov. 5, 2021	06°47′20.1″ N, 70°42′46.5″ W	125	*Oecomys speciosus*	MHN-UCa-M 3678	blood/**MH9** liver/MH10
Vereda El Socorro, Finca Palmira	Nov. 5, 2021	06°46′47″ N, 70°42′59.8″ W	121	*Hydrochoerus hydrochaeris*	MHN-UCa-M 3733	blood/MH11 liver/MH12
Vereda El Socorro, Finca Los Trompillos	Nov. 7, 2021	06°46′47.5″ N, 70°43′02.3″ W	130	*Odocoileus v. cariacou*	MHN-UCa-M 3734	blood/MH14 liver/MH15
Vereda Las Plumas, El Caño	Nov. 12, 2018	6°36′18″ N; 70°31′51″ W	120	*H. hydrochaeris*	–	blood/MH19
Vereda El Socorro, Finca Los Trompillos	Nov. 3, 2021	06°46′46.3″ N, 70°42′59.2″ W	130	*Bos taurus*	–	tick collection only
Vereda Las Plumas, Los Iguanitos	Nov. 12, 2018	06°36′39.90″ N, 70°31′51.20″ W	121	*Sus domesticus*	MHN-UCa-M 2077	blood/MH24

Bold codes: Low quality in library construction.
[a] Synanthropic mammals.

from the mammals was deposited in 5 mL heparinized tubes mixed in a 1:9 ratio, with DNA/RNA Shield reagent (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions.

Liver samples from euthanized wild mammals were individually placed in sterile Petri dishes and washed with 1% PBS (Farbehi et al., 2021). Longitudinal or transverse cuts were made on each liver sample, excess blood was removed with sterile WypAll towels, and then stored in DNA/RNA Shield™ Reagent. All samples were stored at 4 °C and upon arrival at the Laboratory of Molecular Biology of the Universidad de Caldas, they were stored at −80 °C until molecular processing. All materials used in organ manipulation (forceps, scissors, Petri dishes, WypAll towels) were washed with povidone-iodine and distilled water and sterilized in a portable disinfection box (UV sterilization box 99% Obecilc I-lmh200317).

2.4. DNA extraction and sequencing

DNA from the samples (organs and blood) of ticks and/or mammals, as well as whole ticks, was obtained using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research), according to the specific instructions that involve the maceration of all samples through bead beating (30 min), and specifically for blood, 750 μL of sampled blood (blood + RNA/

Table 2
Collection information of hard ticks (females) parasitizing mammals in the Municipality of Arauca, Department of Arauca, Orinoquia region, Colombia.

Mammal species (host)	Museum code (host)	Tick species (ectoparasite)	No. Ticks (pool)	Sample type	Metagenome ID
H. hydrochaeris	MHN-UCa-M 3733	*A. mixtum*	3	whole ticks	MH13
Odocoileus v. cariacou	MHN-UCa-M 3734	*R. microplus*	5	salivary glands	MH16
				midguts	**MH17**
				ovaries	MH18
B. taurus	–	*A. mixtum*	6	salivary glands	**MH20**
				midguts	**MH21**
				ovaries	**MH22**
S. domesticus	–	*A. mixtum*	3	whole ticks	**MH23**

Bold codes: Low quality in library construction.

DNA shield) were used to the whole process according to the specific instructions. Maceration processes were carried out with a Beadbug homogenizer (Benchmark, US) at 4.000 rpm. The DNA was quantified by fluorometry on a Quantus Fluorometer™ (Promega®) using the QuantiFluor® dsDNA System (Promega®), meeting the concentration parameters (>0.1 ng/μL). Samples were sent for sequencing to amplify the V3–V4 region of the *16S* gene bacterial rRNA. Amplicon library construction was done using Macrogen's (Macrogen Inc., South Korea) default primers (Bakt_341F: CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011). This includes quality control to verify the viability of the sequencing process, *16S* gene library preparation and sequenced with 300-bp paired-end chemistry on a MiSeq instrument (Illumina, 2013). The raw data are available under the BioProject ID PRJNA1080232.

2.5. Bioinformatics analysis

Microbiome analysis on the resulting data was performed using Quantitative Insights Into Microbial Ecology 2 Software - QIIME 2.0 version 2023.2 (Bolyen et al., 2019). The quality was checked using fastq (Phred33 applied for quality control), and cutadapt (v.2.6) (Martin, 2011), to remove reads contaminated with adapter sequences, ambiguous bases (N bases), and low complexity. Denoising (quality filtering, trimming, paired-end sequence merging and chimera) and dereplication in Amplicon Sequence Variants (ASVs) filtering were performed with the DADA2 (Callahan et al., 2016; Bokulich et al., 2013). ASVs from DADA2 were assigned taxonomically with classify-sklearn with a Naive Bayes supervised learning algorithm using the trained SILVA 16S rRNA gene database version 138 (Quast et al., 2013; Yilmaz et al., 2014; Bokulich et al., 2018) and sample taxonomic composition, and structure was visualised using QIIME 2 bar plot. Extract-reads from the SILVA database to create a classifier (classifier_silva_138) specifically for the V3–V4 region of the *16S* rRNA gene. Operational Taxonomic Units (OTUs) were generated from the ASVs in the previous step using vsearch cluster-features-de-novo (percent identity of 97%), followed by a qiime taxa collapse, level 6 (genus), and the individual Relative Frequency was obtained (Rognes et al., 2016). Organellar 16S rRNA sequences, i.e., from mitochondria and chloroplasts, were eliminated. A non-parametric Kruskal–Wallis test and the Goods coverage index were calculated using the q2-diversity plugin's core-metrics-phylogenetic action for the OTUs, this action is another QIIME 2 pipeline (Estaki et al., 2020). Finally, Venn diagrams were generated to compare the OTUs composing the blood and liver samples by species, ticks with their host, and among ticks. Additionally, all OTUs from all samples were included for inter-sample comparison to determine prevalence by family (at least one shared OTU between two samples), the analyses were performed using the web tool InteractiVenn (Heberle et al., 2015).

3. Results

A total of 10 individuals from nine mammal species including artiodactyls (Artiodactyla: Bovidae: *Bos taurus*; Cervidae: *Odocoileus virginianus cariacou*; and Suidae: *Sus domesticus*), bats (Chiroptera: Phyllostomidae: *Desmodus rotundus*; Vespertilionidae: *Eptesicus orinocensis*), opossums (Didelphimorphia: Didelphidae: *Didelphis marsupialis* and *Marmosa robinsoni*), and rodents (Rodentia: Caviidae: *Hydrochoerus hydrochaeris*; and Cricetidae: *Oecomys speciosus*) were analyzed (Table 1). A total of 17 female hard ticks *Amblyomma mixtum* and *Rhipicephalus microplus* were found parasitizing the examined mammals (Table 2). The GenBank accession numbers of the DNA sequences obtained from the ticks analyzed in this study are: PP627073, PP627079–PP627081 for the mitochondrial 16S rRNA gene, and PP590342 for the *cox1* gene.

Library construction was completed for 24 samples (MH1-MH24) corresponding to nine blood samples, seven liver samples, and eight tick samples (Tables 1 and 2). Library QC reported eight samples with low quantity and unexpected size libraries that have failed, which were excluded from the study (Tables 1 and 2). In total 3,302,190 (paired-end) sequencing reads were obtained from the 16 samples; 570,608 reads from tick samples and 2,731,582 reads from mammalian blood and liver samples. After trimming the demultiplexed reads, the mean number of reads for each sample was 103,193.44 (forward and reverse reads). The minimum number of reads in a sample was 77,945, corresponding to the liver sample of the common vampire bat, *D. rotundus*, and the maximum was 143,377 for the liver sample of the Robinson's mouse opossum, *M. robinsoni*. In the random sampling of 10,000 out of 1,651,095 sequences without replacement, the minimum sequence length identified during subsampling was 282 bases (median = 284 nt) for forward reads, and 234 bases (median = 280 nt) for reverse reads. A total of 3970 ASVs were obtained with a total frequency of 648,328, represented in 3218 OTUs. After taxa collapse, 746 OTUs remained at the genus level.

The Relative Frequency analysis showed that the bacterial communities are mainly characterized by the following families: Anaplasmataceae (in the liver of the white-tailed deer *O. v. cariacou*), Clostridiaceae (in the liver of the common opossum, *D. marsupialis*), Coxiellaceae (in *A. mixtum*; and salivary glands and ovaries of *R. microplus*), Enterococcaceae (in the liver of *D. rotundus*), Moraxellaceae (in the liver of *M. robinsoni*), Mycoplasmaceae (in the blood of the domestic pig, *S. domesticus* and the capybara, *H. hydrochaeris* - MH19), Prevotellaceae (in the blood of *O. v. cariacou*), Pseudomonadaceae (in the blood of *D. marsupialis*), Rhizobiaceae (in the blood of *D. rotundus*), Rhodobacteraceae (in the blood of *M. robinsoni*), and Streptococcaceae (in the blood of *H. hydrochaeris* - MH11) (Fig. 2A; Table 3; Supplementary material 1). In the liver sample of the arboreal rice rat, *Oecomys speciosus*, only the class Gammaproteobacteria could be identified, and in most samples, percentages of bacteria that could not be taxonomically determined were found (Fig. 2A; Table 3; Supplementary material 1).

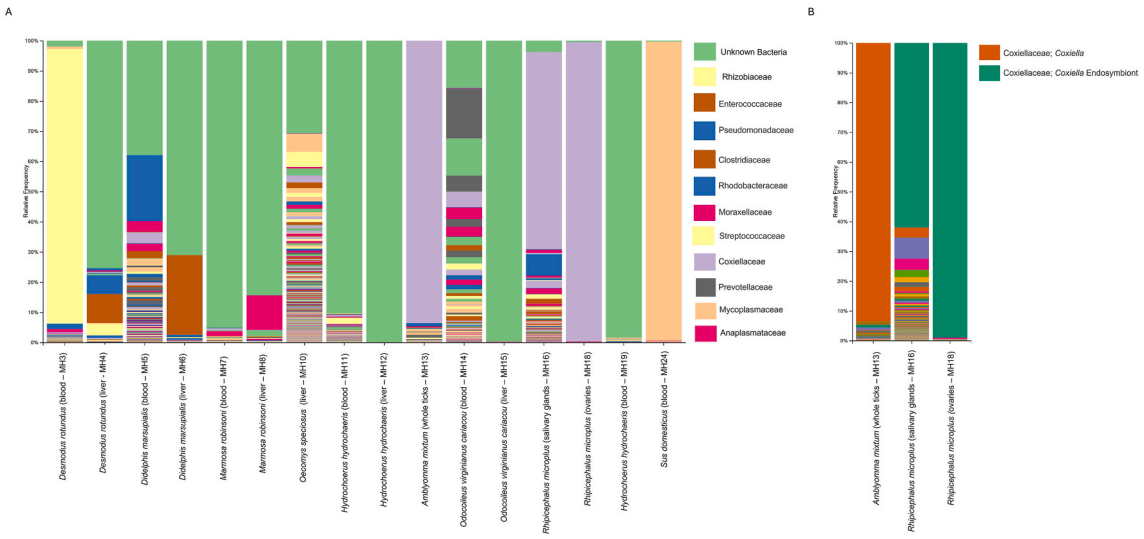


Fig. 2. Relative frequency of OTUs. The bars represent the bacterial communities and the representative families are indicated. **(A)** OTUs in blood and liver of mammals and ticks (16 samples); bacterial taxa were grouped up to the genus level (6 levels). **(B)** Relative frequency of OTUs in ticks (3 samples: MH13; MH16 and MH18). Bacterial taxa were grouped at the species level (7 levels). Complete relative frequencies in supplementary material 2.

Table 3
Major percentages of the resuts of the relative frequency of the studied bacterial communities of hard ticks (females) and mammals in the Municipality of Arauca, Department of Arauca, Orinoquia region, Colombia. The frequency (reads – sequences) values correspond to retained 609,266 (100.00 %) features in 15 (100.00 %) samples.

Species	Sample/Metagenome ID	Frequency (reads - sequences)	Relative Frequency
			Family/Genus with percentage
D. rotundus	blood/MH3	43,550	Rhizobiaceae/*Bartonella* (91.02)
	liver/MH4	12,040	Unknown Bacteria (75.31); Enterococcaceae/*Enterococcus* (9.61)
D. marsupialis	blood/MH5	62,107	Unknown Bacteria (37.92); Pseudomonadaceae/*Pseudomonas* (21.9)
	liver/MH6	37,752	Unknown Bacteria (71.04); Clostridiaceae/*Sarcina* (26.42)
H. hydrochaeris	blood/MH11	54,149	Unknown Bacteria (90.44); Streptococcaceae/*Streptococcus* (2.02)
	liver/MH12	45,331	Unknown Bacteria (99.96)
	blood/MH19	38,729	Unknow Bacteria (98.4); Mycoplasmaceae/*Mycoplasma* (0.31)
M. robinsoni	blood/MH7	22,353	Unknown Bacteria (95.14); Rhodobacteraceae/*Rubellimicrobium* (1.64)
	liver/MH8	60,209	Unknown Bacteria (84.34); Moraxellaceae/*Acinetobacter* (11.41)
Odocoileus v. cariacou	blood/MH14	28,039	Prevotellaceae/*Prevotella* (16.52)
	liver/MH15	31,480	Unknown Bacteria (99.76); Moraxellaceae/*Acinetobacter* (0.032); Anaplasmataceae/*Anaplasma* (0.102)
O. speciosus	liver/MH10	45,385	Unknown Bacteria (30.66); [a]Gammaproteobacteria (6.04)
S. domesticus	blood/MH24	41,516	Mycoplasmaceae/*Mycoplasma* (99.06)
A. mixtum	whole ticks/MH13	45,067	Coxiellaceae/*Coxiella* (93.6)
R. microplus	salivary glands/MH16	29,018	Coxiellaceae/*Coxiella* (65.35)
	ovaries/MH18	51,603	Coxiellaceae/*Coxiella* (99.01)

[a] Taxonomic classification at Class level.

Regarding the family Coxiellaceae, it was determined that the species *A. mixtum* has a frequency of 93.6% for *Coxiella*, and the samples of salivary glands and ovaries of *R. microplus* contain frequencies of 62% and 99.02%, respectively, for *Coxiella* Endosymbiont (Fig. 2B; Supplementary material 2). Venn diagrams allow visualizing the percentage of shared bacterial OTUs at the genus or species level among the blood and liver samples of each mammal species and tick (Fig. 3; Table 4). All samples obtained a value $\geq$ 97% for the Goods coverage index, indicating that only 3% of OTUs are probably not covered during sequencing, except for the liver sample of *O. speciosus* (MH10) with a value of 83% (Fig. 4). The results of the Kruskal–Wallis test for the different phylogenetic lineages and taxonomic richness did not show significant differences (p-value = 0.38 and 0.35, respectively).

Finally, the prevalence of bacterial OTUs represented at the family level (at least one shared OTU between two samples) were obtained as follows: 62.5% for Enterobacteriaceae; 50% for Beijerinckiaceae and Moraxellaceae; 43.75% for Burkholderiaceae; 37.5% for Bacillaceae and Propionibacteriaceae; 31.25% for Bacteroidaceae, Butyricicoccaceae, Comamonadaceae, and Streptococcaceae; 25% for Caulobacteraceae, Pseudomonadaceae, Lachnospiraceae, Rhizobiaceae, and Xanthobacteraceae; 18.75% for Corynebacteriaceae, Coxiellaceae, Peptostreptococcaceae, and Planococcaceae; 12.5% for the families Anaplasmataceae, Azospirillaceae, Clostridiaceae, Dermabacteraceae, Gastranaerophilales, Intrasporangiaceae, Lactobacillaceae, Micrococcaceae, Mycobacteriaceae, Nocardiaceae, Nocardioidaceae, Oscillospiraceae, Peptostreptococcaceae, Prevotellaceae, Rickettsiaceae, Sphingomonadaceae, Staphylococcaceae, and Weeksellaceae (Table 5). Two OTUs were identified up to the class level (Clostridia and Thermoleophilia), and 43 OTUs up to the Bacteria domain level. The highest prevalence among mammals were observed for the families Beijerinckiaceae, Burkholderiaceae, Enterobacteriaceae, Moraxellaceae, and Propionibacteriaceae; these also exhibited the highest prevalence for families shared between ticks and mammals.

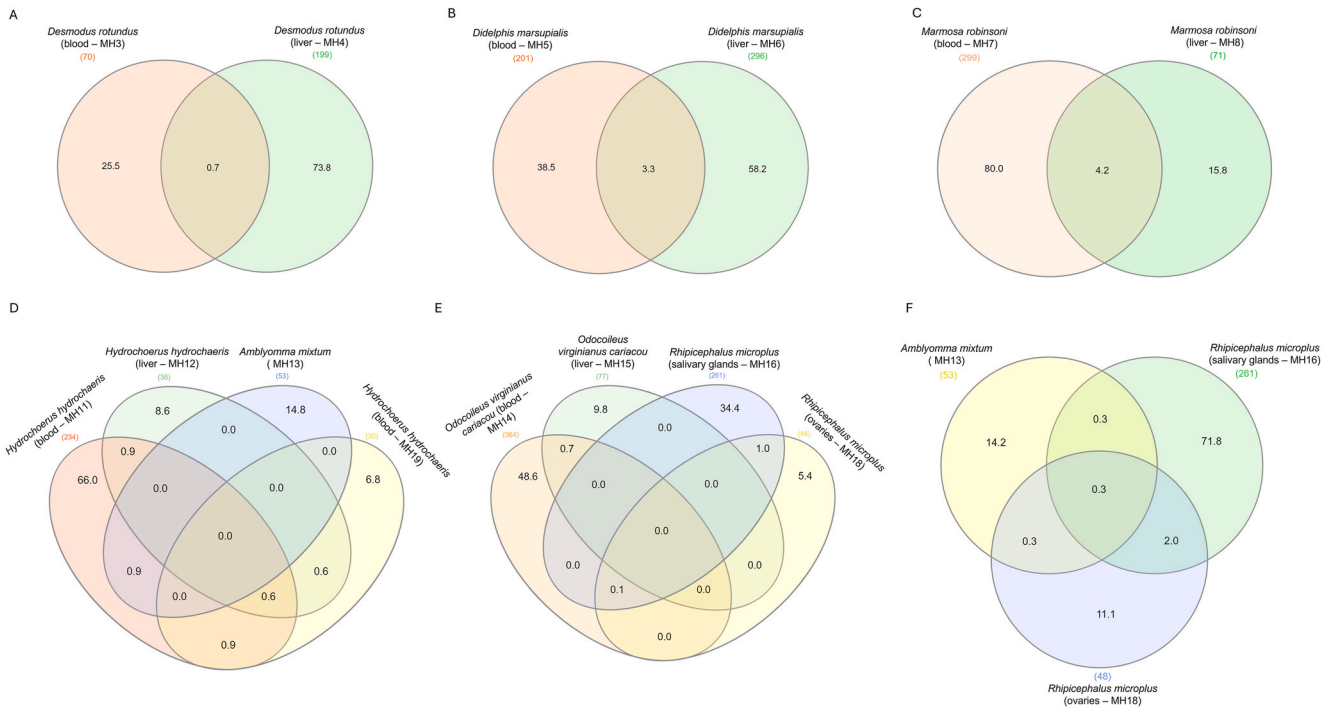


Fig. 3. Operational taxonomic units (OTUs) shared at the genus and species level for mammals and ticks. Percentage values are found in the Venn diagrams. **(A)** Samples of *Desmodus rotundus*. **(B)** Samples of *Didelphis marsupialis*. **(C)** Samples of *Marmosa robinsoni*. **(D)** Samples of *Hydrochoerus. hydrochaeris* and *Amblyomma mixtum*. **(E)** Samples of *Odocoileus virginianus cariacou* and *Rhipicephalus microplus*. **(F)** Samples of *Amblyomma mixtum* and *Riphicephalus microplus*.

4. Discussion

The results obtained of the ticks' microbiome are consistent with previous studies in which bacteria of the family Coxiellaceae, encompassing *Coxiella burnetii* and *Coxiella*-like endosymbionts (CLEs), have been detected (Wu-Chuang et al., 2021; Guizzo et al., 2022b). *Coxiella burnetii* causes Q fever in humans and coxiellosis in animals, this distinction between *C. burnetii* and the *Coxiella*-like endosymbionts (CLEs) in ticks is problematic besides the latter are non-infectious to vertebrate hosts but are necessary for the health of these arthropods (Duron et al., 2015; Brenner et al., 2021). The presence of bacteria within the families Bacillaceae, Burkholderiaceae, and Enterobacteriaceae in ticks is consistent with reported in the microbial diversity associated with the tick *Hyalomma dromedarii* (Alreshidi et al., 2020). These families contain species within *Bacillus*, *Enterobacter*, *Shigella*, and *Escherichia*, which can be either harmless or pathogenic to arthropods and mammals, including humans (Turnbull, 1996; Fukushima et al., 2002; Khalaf et al., 2018; Alphonse and Odendall, 2023). Regarding Intrasporangiaceae, this family contains saprophytic bacteria associated with the hematophagous mite *Dermanyssus gallinae* (Valiente Moro et al., 2009). Within this family, the genus *Ornithinimicrobium* has been reported in *Amblyomma* tick species, and the feces of birds and bats (Lee et al., 2021; Huang et al., 2023).

The prevalence of bacteria in ticks is similar to that in mammals, with the addition of the families Beijerinckiaceae and Moraxellaceae, which include genera such as *Acinetobacter*, *Bosea*, *Methylobacterium-Methylorubrum* reported for the first time related with tick-borne diseases (TBDs) by Portillo et al. (2019). Some of these genera (*Acinetobacter, Bosea, Methylobacterium*) and others (e.g., *Bradyrhizobium, Brevundimonas, Burkholderia, Chryseobacterium, Comamonas, Devosia, Erwinia, Flavobacterium*) were primarily associated with the tick cuticle or the environment (Portillo et al., 2019). However, Hernández-Jarguín et al. (2018), Binetruy et al. (2019), and Lejal et al. (2021), also detected bacterial genera commonly found in the environment as part of the microbiota of the organs of the tick *I. ricinus* previously cleaned with bleach. *Enhydrobacter* (Moraxellaceae) was reported by Adegoke et al. (2022) as the only genus represented exclusively in eggs of *A. maculatum*, and in our study, it was reported in the salivary glands.

The relative frequency of the genus *Bartonella* (Rhizobiaceae) observed in mammals aligns with the reports of this bacteria associated with the common vampire bat, *D. rotundus*, in the Brazilian Atlantic Forest biome (Ferreira et al., 2018). Other bacteria genera such as *Acinetobacter* (Moraxellaceae), *Anaplasma* (Anaplasmataceae), *Enterococcus* (Enterococcaceae), *Prevotella* (Prevotellaceae), *Pseudomonas* (Pseudomonadaceae), *Rubellimicrobium* (Rhodobacteraceae), and *Sarcina* (Clostridiaceae) contains zoonotic pathogens that infect a wide range of wild and domestic mammal species (Tate et al., 2013; Battilani et al., 2017; Chen et al., 2020; Nowakiewicz et al., 2021; Qi et al., 2022; dos Santos Costa et al., 2023; Makovska et al., 2023).

Furthermore, several species of *Prevotella* and *Sarcina* have been associated with pathologies primarily related to the digestive system of different animals, including humans, macaques, livestock, rodents, and insects (Chen et al., 2017, 2020; Dumitru et al., 2020; O'Hara et al., 2020; Owens et al., 2021; Betancur-Murillo et al., 2023; Makovska et al., 2023). Our study included two rodent species (*H. hydrochaeris* and *O. speciosus*), which are recognized reservoirs and vectors of zoonoses (at least 60 diseases; Taylor et al., 2008; Dahmana et al., 2020). The functional role played by rodents in the amplification, transmission, and spread of pathogens, including *Streptococcus* (Streptococcaceae) and *Pseudomonas* (class Gammaproteobacteria), is underestimated (Ayyal et al., 2019; Dahmana et al., 2020; Jahan et al., 2021), and new studies should be performed. Furthermore, in the domestic pig, *S. domesticus*, the genus *Mycoplasma* (Mycoplasmaceae) which is of porcine importance was detected. Within the genus, *Mycoplasma suis* stands out as the etiological agent of porcine hemoplasmosis, which is part of the group of hemotropic mycoplasmas (Ritzmann et al., 2009).

The results of the bacterial communities of the ticks analyzed in this study align with the findings of Wu-Chuang et al. (2021) and several other studies that suggest that bacterial diversity in tick microbiomes is not as high as initially thought. Studies on microbial diversity in *Ixodes pacificus*, *I. scapularis*, *I. ricinus*, *R. microplus* and *Dermacentor* spp., have shown that bacterial communities are dominated by a few core species,

Table 4
Relationships of Operational Taxonomic Units (OTUs) shared at the genus level, between mammalian blood, liver samples, and tick samples.

Species	Sample/ Metagenome ID	Shared bacterial OTUs
		Family/Genus (N°. OTUs)
D. rotundus	blood/MH3 liver/MH4	Enterobacteriaceae/*Escherichia-Shigella* (1 OTU); Unknown Bacteria (1 OTU)
D. marsupialis	blood/MH5 liver/MH6	Caulobacteraceae/*Brevundimonas* (1 OTU); Butyricicoccaceae/Genus unknown (1 OTU); Unknown Bacteria (14 OTUs)
M. robinsoni	blood/MH7 liver/MH8	Streptococcaceae/*Streptococcus* (1 OTU); Unknown Bacteria (15 OTUs)
H. hydrochaeris	blood/MH11 liver/MH12	Unknown Bacteria (3 OTUs)
H. hydrochaeris *A. mixtum*	blood/MH11 whole ticks/ MH13	Enterobacteriaceae/*Escherichia-Shigella* (1 OTU); Enterobacteriaceae/*Enterobacter* (1 OTU); Moraxellaceae/Genus unknown (1 OTU);
H. hydrochaeris	blood/MH11 blood/MH19	Burkholderiaceae/*Ralstonia* (1 OTU); Xanthobacteraceae/*Bradyrhizobium* (1 OTU); Unknown Bacteria (1 OTU)
	liver/MH12 blood/MH19	Unknown Bacteria (2 OTUs)
H. hydrochaeris	blood/MH11 liver/MH12 blood/MH19	Unknown Bacteria (2 OTUs)
Odocoileus v. cariacou	blood/MH14	Enterobacteriaceae/*Escherichia-Shigella* (1 OTU)
R. microplus	salivary glands/ MH16 ovaries/MH18	
Odocoileus v. cariacou	blood/MH14 liver/MH15	Anaplasmataceae/*Anaplasma* (1 OTU); Oscillospiraceae/Genus unknown (1 OTU); Lachnospiraceae/*Eubacterium* (1 OTU); Unknown Bacteria (1 OTU)
R. microplus	salivary glands/ MH16 ovaries/MH18	Coxiellaceae/*Coxiella* (1 OTU); Enterobacteriaceae/*Escherichia-Shigella* (1 OTU); Intrasporangiaceae/ *Ornithinimicrobium* (1 OTU); Burkholderiaceae/*Ralstonia* (1 OTU); Unknown Bacteria (3 OTUs)
A. mixtum	whole ticks/ MH13	Coxiellaceae/*Coxiella* (1 OTU)
R. microplus	salivary glands/ MH16	
A. mixtum	whole ticks/ MH13	Bacillaceae/*Bacillus* (1 OTU)
R. microplus	ovaries/MH18	
A. mixtum	whole ticks/ MH13	Enterobacteriaceae/*Escherichia-Shigella* (1 OTU)
R. microplus	salivary glands/ MH16 ovaries/MH18	

likely endosymbionts (Ross et al., 2018; Chicana et al., 2019; Couper et al., 2019; Guizzo et al., 2020). Genomes of tick-transmitted intracellular pathogens such as *Anaplasma, Borrelia, Coxiella*, *Ehrlichia* and *Rickettsia* lack interbacterial effector immunity genes involved in bacteria-bacteria interactions (Ross et al., 2018). O'Keeffe et al. (2020) proposed that the negative selection of the effector genes may be explained by low selective pressure on interbacterial competition pathways mediated by a poor microbiota. However, host microbiota can also facilitate pathogen infections and microbiome-pathogen interactions go well beyond protein-mediated interactions (Stevens et al., 2021). Several variables including poor health, the application of antibiotics, or infection by invading pathogens might cause a loss of the host microbiota diversity or disruption to the environment allowing the expansion of harmful microbes (Stevens et al., 2021). The microbiota and the relation between invading pathogens are complex and need more research.

The present research, along with other studies conducted on tick-borne pathogens (TPB), have also detected a considerable range of non-pathogenic microorganisms in almost all tick species examined so far (Alreshidi et al., 2020; Bonnet et al., 2017; Portillo et al., 2019). These microorganisms include maternally inherited intracellular symbionts, that they could strongly influence the biology of the tick hosts, as well as the dynamics of infection by tick-borne pathogens (TBP) in mammals (Bonnet et al., 2017). Thus, the sequences of unknown bacteria detected in our study may correspond to taxa that have not been previously described or are phylogenetically distant from the genomes deposited in public databases, representing another level of uncharacterized diversity (Lagier et al., 2012; Thomas and Segata, 2019). According to Rinke et al. (2013) and Thomas and Segata (2019) at the deepest level of hidden diversity there are those members of the community that are not captured at all by the experiment named the undetected unknowns. These might include crucial taxa with low abundances, whose genetic material is below the level of detection and is not sampled by sequencing techniques.

5. Conclusion

The results show the relative frequencies of bacterial OTUs corresponding to the family or genus level. The results reveal a considerable number of unknown bacteria, and circulation of pathogenic and non-pathogenic bacteria between mammals and ticks. Additionally, the results are the first data at the level of bacterial microbiome of different wild and domestic mammalian hosts of ticks of the family Ixodidae in the Colombian Orinoquia region.

6. Ethics declarations

Sample collection was conducted under the framework permit granted by the National Environmental Licensing Authority (ANLA) to

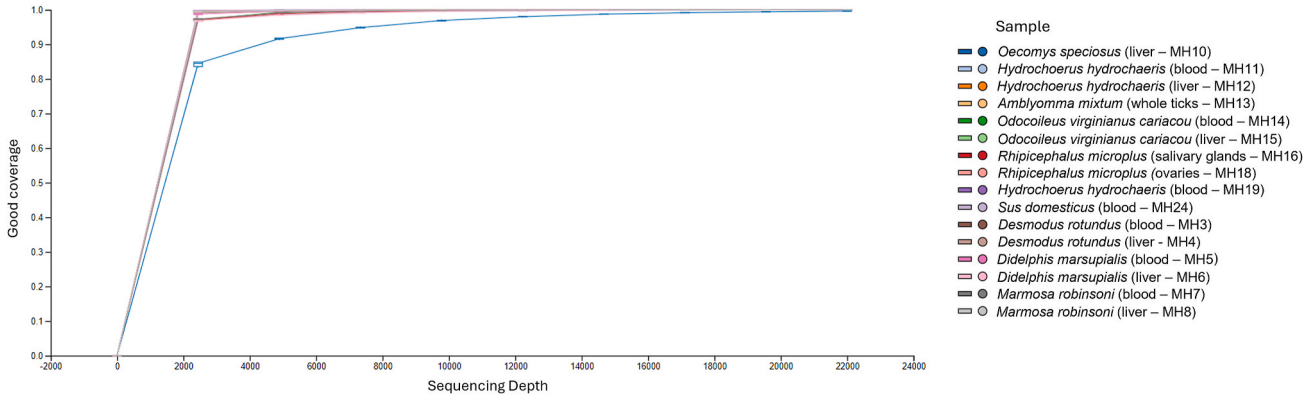


Fig. 4. Goods coverage index for each sample using the OTUs. Retained 330,000 (49.90%) features in 15 (93.75%) samples at the specified sampling depth (22,000).

Table 5
Prevalence of bacterial OTUs (97% sequence identity; OTUs found in at least two samples) for samples (n = 16) of mammals and ticks from Arauca, Department of Arauca, Orinoquia region, Colombia.

Bacteria		Samples															
		D. rotundus		*D. marsupialis*		*M. robinsoni*		*O. speciosus*	*H. hydrochaeris*		*A. mixtum*	*Odocoileus v. cariacou*		*R. microplus*		*H. hydrochaeris*	*S. domesticus*
Family	**Genus**	Blood	Liver	Blood	Liver	Blood	Liver	Liver	Blood	Liver	whole ticks	Blood	Liver	Salivary glands	Ovaries	Blood	Blood
Enterobacteriaceae	*Enterobacter*								X		X						
	Escherichia-Shigella	X	X		X		X		X		X	X		X	X		
	Unknown		X	X			X							X			
Beijerinckiaceae	*Bosea*							X			X						
	Methylobacterium-Methylorubrum	X		XX					X								X
	Unknown				X									X			
Moraxellaceae	*Enhydrobacter*	X		X			X							X			
	Acinetobacter	X		X			X				X	X		X			X
	Unknown								X		X						
Burkholderiaceae	*Ralstonia*								X					X	X	X	X
	Paraburkholderia	X		X													
Bacillaceae	*Bacillus*			XX					X	X	X			X	X		
Propionibacteriaceae	*Cutibacterium*		X	X			X							X		X	X
Bacteroidaceae	*Bacteroides*	X	X	X						X				X			
Butyricicoccaceae	Unknown	X		X	X	X								X			
Comamonadaceae	*Aquabacterium*	X		XX		X			X					X			
	Curvibacter	X		X													
Streptococcaceae	*Streptococcus*					X	X		X						X		
	Lactococcus			X			X										
Caulobacteraceae	*Brevundimonas*			XX	X				X		X						
Pseudomonadaceae	*Pseudomonas*	XX	X	XX		X											
Lachnospiraceae	*Eubacterium*											X	X				
	Lachnoclostridium			X		X											
Rhizobiaceae	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	X		X				X						X			
Xanthobacteraceae	*Bradyrhizobium*			X					X			X				X	
Corynebacteriaceae	*Lawsonella*			X		X								X			
Coxiellaceae	*Coxiella*										X			X			
	Coxiella Endosymbiont													X	X		
Peptostreptococcaceae	*Romboutsia*	X		X										X			
Planococcaceae	*Kurthia*			X			X							X			
Anaplasmataceae	*Anaplasma*											X	X				
Azospirillaceae	*Nitrospirillum*	X		X													
Clostridiaceae	*Clostridium*			X										X			
Dermabacteraceae	*Brachybacterium*	X									X						
Gastranaerophilales	*Gastranaerophilales*	X		X													
Intrasporangiaceae	*Ornithinimicrobium*													X	X		
Lactobacillaceae	*Lactobacillus*							X			X						
Micrococcaceae	*Micrococcus*	X				X											
Mycobacteriaceae	*Mycobacterium*			X							X						
Nocardiaceae	*Rhodococcus*			X										X			
Nocardioidaceae	*Nocardioides*	X												X			
Oscillospiraceae	Unknown											X	X				
Peptostreptococcaceae	*Finegoldia*			X					X								
Prevotellaceae	*Prevotella*										XX	XX					
Rickettsiaceae	*Rickettsia*	X								X							
Sphingomonadaceae	*Novosphingobium*							X	X								
Staphylococcaceae	*Staphylococcus*	X												X			
Weeksellaceae	*Chryseobacterium*	X							X								

X represents the presence of OTUs in the sample.
XX represent different OTUs in the same sample.

the Universidad de Caldas as stipulated in Resolution N° 02497 of December 31, 2018, updated by the resolution N° 000026 of January 9, 2024. Additionally, no species registered in the red list of threatened species of Colombian consigned in the resolution N° 1912 of 2017, updated by the resolution N° 0126 of February 6, 2024 were collected. Wild mammal capture and collection were conducted with the approval of the Comité de Bioética de la Facultad de Ciencias Exactas y Naturales of the Universidad de Caldas (June 2, 2017 and September 20, 2019). All samples and specimens collected were deposited in the mammals and ectoparasites collections of the Museo de Historia Natural of the Universidad de Caldas (MHN-UCa).

Funding

This project was funded by the Vicerrectoría de Investigaciones y Posgrados - Universidad de Caldas - project "Morfología interna y marcadores moleculares en garrapatas (Acari: Ixodidae): una aproximación a las interacciones con pequeños mamíferos y sus patógenos" [code 0318322]. Program "Relación, distribución, taxonomía de especies de garrapatas asociadas a mamíferos silvestres en zonas endémicas de rickettsiosis en Colombia. Un acercamiento a la comprensión de la relación vectores patógenos-reservorios", granted by the Ministerio De Ciencia, Tecnología e Innovación - Minciencias (Code: 120385270267 and CTO 80740- 200–2021) – project "Garrapatas asociadas a mamíferos silvestres en el departamento de Caldas: Diversidad, detección de patógenos y distribución (Code:71717)". Ministerio de Ciencia, Tecnología e innovación of Colombia - Minciencias for funding the PhD in Science-Biology of Paula Andrea Ossa López "Convocatoria del Fondo de Ciencia, Tecnología e Innovación del Sistema General de Regalías para la conformación de una lista de proyectos elegibles para ser viabilizados, priorizados y aprobados por el OCAD dentro del Programa de Becas de Excelencia cohorte 1–2019". Part of this work was funded by Award "For Women in Science (2022)" conducted in collaboration with L'Oréal, Ministerio de Ciencia Tecnología e Innovación of Colombia - Minciencias, ICETEX and La Comisión Nacional de Cooperación con la UNESCO.

CRediT authorship contribution statement

Paula A. Ossa-López: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. **Héctor E. Ramírez-Chaves:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **María Elena Álvarez López:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Gabriel Jaime Castaño Villa:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Fredy A. Rivera-Páez:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors disclose that no substantial portion of the study has been published or is under consideration for publication elsewhere and that its submission for publication has been approved by all the authors. All authors approve the submission of this article to *International Journal for Parasitology: Parasites and Wildlife*. The authors have no conflicts of interest to declare.

Acknowledgements

We thank the Universidad de Caldas, Colombia; Unidad Administrativa Especial de Salud de Arauca, Colombia. Award "For Women in Science (2022)" conducted in collaboration with L'Oréal; Ministerio de Ciencia Tecnología e Innovación of Colombia - Minciencias; ICETEX, Colombia; and la Comisión Nacional de Cooperación con la UNESCO, France. Thanks to Lorys Mancilla Agrono and Lizeth Fernanda Banguero Micolta, members of the GEBIOME research group.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.100943.

References

Adegoke, A., Kumar, D., Budachetri, K., Karim, S., 2022. Hematophagy and tick-borne Rickettsial pathogen shape the microbial community structure and predicted functions within the tick vector, *Amblyomma maculatum*. Front. Cell. Infect. Microbiol. 12, 1037387.

Alphonse, N., Odendall, C., 2023. Animal models of shigellosis: a historical overview. Curr. Opin. Immunol. 85, 102399 https://doi.org/10.1016/j.coi.2023.102399.

Alreshidi, M.M., Veettil, V.N., Noumi, E., Campo, R.D., Snoussi, M., 2020. Description of microbial diversity associated with ticks *Hyalomma dromedarii* (Acari: Ixodidae) isolated from camels in Hail region (Saudi Arabia) using massive sequencing of 16S rDNA. Bioinformation 16 (8), 602–610. https://doi.org/10.6026/97320630016602.

Andreotti, R., de Leon, A.A.P., Dowd, S.E., Guerrero, F.D., Bendele, K.G., Scoles, G.A., 2011. Assessment of bacterial diversity in the cattle tick *Rhipicephalus (Boophilus) microplus* through tag-encoded pyrosequencing. BMC Microbiol. 11, 6. https://doi.org/10.1186/1471-2180-11-6.

Ayyal, N.M., Abbas, Z.A., Karim, A.J., Abbas, Z.M., Al-Salihi, K.A., Khalaf, J.M., Mahmood, D.D., Mohammed, E.A., Jumaa, R.S., Abdul-Majeed, D.I., 2019. Bacterial isolation from internal organs of rats (*Rattus rattus*) captured in Baghdad city of Iraq. Vet. World 12 (1), 119–125. https://doi.org/10.14202/vetworld.2019.119-125.

Baneth, G., 2014. Tick-borne infections of animals and humans: a common ground. Int. J. Parasitol. 44 (9), 591–596. https://doi.org/10.1016/j.ijpara.2014.03.011.

Barros-Battesti, D.M., Arzua, M., Bechara, G.H., 2006. Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies. Instituto Butantan, São Paulo, p. 239.

Battilani, M., De Arcangeli, S., Balboni, A., Dondi, F., 2017. Genetic diversity and molecular epidemiology of *Anaplasma*. Infect. Genet. Evol. 49, 195–211. https://doi.org/10.1016/j.meegid.2017.01.021.

Betancur-Murillo, C.L., Aguilar-Marín, S.B., Jovel, J., 2023. *Prevotella*: a key player in ruminal metabolism. Microorganisms 11, 1. https://doi.org/10.3390/microorganisms11010001.

Bezerra-Santos, M.A., de Macedo, L.O., Nguyen, V.L., Manoj, R.R., Laidoudi, Y., Latrofa, M.S., Beugnet, F., Otranto, D., 2022. *Cercopithifilaria* spp. in ticks of companion animals from Asia: new putative hosts and vectors. Ticks Tick-borne Dis. 13 (4), 101957 https://doi.org/10.1016/j.ttbdis.2022.101957.

Binetruy, F., Dupraz, M., Buysse, M., Duron, O., 2019. Surface sterilization methods impact measures of internal microbial diversity in ticks. Parasites Vectors 12 (1), 268. https://doi.org/10.1186/s13071-019-3517-5.

Bonnet, S.I., Binetruy, F., Hernández-Jarguín, A.M., Duron, O., 2017. The tick microbiome: why non-pathogenic microorganisms matter in tick biology and pathogen transmission. Front. Cell. Infect. Microbiol. 7, 236. https://doi.org/10.3389/fcimb.2017.00236.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90. https://doi.org/10.1186/s40168-018-0470-z.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10 (1), 57–59. https://doi.org/10.1038/nmeth.2276.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Brenner, A.E., Muñoz-Leal, S., Sachan, M., Labruna, M.B., Raghavan, R., 2021. *Coxiella burnetii* and related tick endosymbionts evolved from pathogenic ancestors. Genome Biol. Evol. 13 (7), evab108. https://doi:10.1093/gbe/evab108.

Budachetri, K., Browning, R.E., Adamson, S.W., Dowd, S.E., Chao, C.-C., Ching, W.-M., Karim, S., 2014. An insight into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). J. Med. Entomol. 51, 119–129. https://doi.org/10.1603/ME12223.

Budachetri, K., Gaillard, D., Williams, J., Mukherjee, N., Karim, S., 2016. A snapshot of the microbiome of *Amblyomma tuberculatum* ticks infesting the gopher tortoise, an endangered species. Ticks Tick Borne Dis. 7, 1225–1229. https://doi.org/10.1016/j.ttbdis.2016.07.010.

Buriticá-Mejía, N., 2016. Sabanas Inundables de la Orinoquía Colombiana Documento Resumen. In: Research Institute of Biological Resources Alexander von Humboldt. Repositorio Institucional de Documentación Científica, Bogotá, p. 19.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi:10.1038/nmeth.3869.

Camargo-Mathias, M.I., 2013. Guia básico de morfologia interna de carrapatos ixodídeos. Editora unesp. Ciências Biológicas, p. 124.

Camargo-Mathias, M.I., 2018. Inside ticks. Morphophysiology, toxicology and therapeutic perspectives. Editora unesp, Ciências Biológicas 198.

Cardona-Romero, M., Martínez-Sánchez, E.T., Alvarez-Londoño, J., Pérez-Cárdenas, J.E., Ossa-López, P.A., Castaño-Villa, G.J., Binder, L.C., Faccini-Martínez, Á.A., Rivera-Páez, F.A., 2022. Seroprevalence and detection of *Rickettsia* spp. in wild birds of Arauca, Orinoquia region, Colombia. Vet. Parasitol. 30, 100720 https://doi.org/10.1016/j.vprsr.2022.100720.

Carvajal-Agudelo, J.D., Ramírez-Chaves, H.E., Ossa-López, P.A., Rivera-Páez, F.A., 2022. Bacteria related to tick-borne pathogen assemblages in *Ornithodoros* cf. *hasei (*Acari: Argasidae) and blood of the wild mammal hosts in the Orinoquia region, Colombia. Exp. Appl. Acarol. 87 (2–3), 253–271. https://doi.org/10.1007/s10493-022-00724-9.

Chen, T., Li, Y., Liang, J., Li, Y., Huang, Z., 2020. Gut microbiota of provisioned and wild rhesus macaques (*Macaca mulatta*) living in a limestone forest in southwest Guangxi, China. Microbiology 9 (3), e981. https://doi.org/10.1002/mbo3.981.

Chen, T., Long, W., Zhang, C., Liu, S., Zhao, L., Hamaker, B.R., 2017. Fiber-utilizing capacity varies in *Prevotella*- versus bacteroides-dominated gut microbiota. Sci. Rep. 7, 2594. https://doi.org/10.1038/s41598-017-02995-4.

Chicana, B., Couper, L.I., Kwan, J.Y., Tahiraj, E., Swei, A., 2019. Comparative microbiome profiles of sympatric tick species from the far-western United States. Insects 10, 353. https://doi.org/10.3390/insects10100353.

Couper, L., Swei, A., 2018. Tick microbiome characterization by Next-Generation 16S rRNA amplicon sequencing. J. Vis. Exp. 25 (138), 58239 https://doi.org/10.3791/58239.

Couper, L.I., Kwan, J.Y., Ma, J., Swei, A., 2019. Drivers and patterns of microbial community assembly in a Lyme disease vector. Ecol. Evol. 9, 7768–7779. https://doi.org/10.1002/ece3.5361.

Dahmana, H., Granjon, L., Diagne, C., Davoust, B., Fenollar, F., Mediannikov, O., 2020. Rodents as hosts of pathogens and related zoonotic disease risk. Pathogens 10 (3), 202. https://doi.org/10.3390/pathogens9030202, 9.

dos Santos Costa, J., dos Santos, P.B., de Souza, A.T.H.I., Morgado, T.O., Cândido, S.L., Silva, T.R.D., Nakazato, L., Dutra, V., 2023. KPC-2-producing *Pseudomonas aeruginosa* isolated from wild animals in Brazil. Braz. J. Microbiol. 54, 3307–3313. https://doi.org/10.1007/s42770-023-01143-7.

Dumitru, A., Aliuș, C., Nica, A.E., Antoniac, I., Gheorghiță, D., Grădinaru, S., 2020. Fatal outcome of gastric perforation due to infection with *Sarcina* spp. A case report. IDCases 19, e00711. https://doi.org/10.1016/j.idcr.2020.e00711.

Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., Loire, E., Ménard, C., Bouchez, O., Vavre, F., Vial, L., 2018. Tick bacteria mutualism depends on B vitamin synthesis pathways. Curr. Biol. 28, 1896–902e5. https://doi.org/10.1016/j.cub.2018.04.038.

Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.K., Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. PLoS Pathog. 11, e1004892 https://doi.org/10.1371/journal.ppat.1004892.

Efstratiou, A., Karanis, G., Karanis, P., 2021. Tick-borne pathogens and diseases in Greece. Microorganisms 9 (8), 1732. https://doi.org/10.3390/microorganisms9081732.

Egan, S.L., Taylor, C.L., Banks, P.B., Northover, A.S., Ahlstrom, L.A., Ryan, U.M., Irwin, P.J., Oskam, C.L., 2021. The bacterial biome of ticks and their wildlife hosts at the urban-wildland interface. Microb. Genom. 7 (12), 000730 https://doi.org/10.1099/mgen.0.000730.

Estaki, M., Jiang, L., Bokulich, N.A., McDonald, D., González, A., Kosciolek, T., Martino, C., Zhu, Q., Birmingham, A., Vázquez-Baeza, Y., Dillon, M.R., Bolyen, E., Caporaso, J.G., Knight, R., 2020. QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data. Curr Protoc Bioinformatics 70 (1), e100. https://doi.org/10.1002/cpbi.100.

Farbehi, N., Janbandhu, V., Nordon, R.E., Harvey, R.P., 2021. FACS enrichment of total interstitial cells and fibroblasts from adult mouse ventricles. Bio-protocol 11, e4028. https://doi.org/10.21769/BioProtoc.4028.

Ferreira, M.S., Guterres, A., Rozental, T., Novaes, R.L.M., Vilar, E.M., Oliveira, R.C., Fernandes, J., Forneas, D., Junior, A.A., Brandão, M.L., Cordeiro, J.L.P., del Valle Alvarez, M.R., Althoff, S.L., Moratelli, R., Cordeiro-Estrela, P., Silva, R.C.D., Lemos, E.R.S., 2018. *Coxiella* and *Bartonella* spp. in bats (Chiroptera) captured in the Brazilian atlantic forest biome. BMC Vet. Res. 14, 279. https://doi.org/10.1186/s12917-018-1603-0.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Fukushima, M., Kakinuma, K., Kawaguchi, R., 2002. Phylogenetic analysis of *Salmonella*, Shigella, and *Escherichia coli* strains on the basis of the gyrB gene sequence. J. Clin. Microbiol. 40 (8), 2779–2785. https://doi.org/10.1128/JCM.40.8.2779-2785.2002.

Gardner, A.L. (Ed.), 2008. Marsupials, Xenarthrans, Shrews, and Bats. Mammals of South America, 1. University of Chicago Press, Chicago, IL, USA, p. 690.

Gómez-Quintero, C.H., Faccini-Martínez, Á.A., Botero-García, C.A., Lozano, M., Sánchez-Lerma, L., Miranda, J., Mattar, S., Hidalgo, M., 2017. Probable case of spotted fever group rickettsial infection in a new suspected endemic area, Colombia. J. Infect. Public. Heal. 10, 353–356. https://doi.org/10.1016/j.jiph.2016.08.012.

Gondard, M., Cabezas-Cruz, A., Charles, R.A., Vayssier-Taussat, M., Albina, E., Moutailler, S., 2017. Ticks and tick-borne pathogens of the caribbean: current understanding and future directions for more comprehensive surveillance. Front. Cell. Infect. Microbiol. 29 (7), 490. https://doi.org/10.3389/fcimb.2017.00490.

Grandi, G., Chiappa, G., Ullman, K., Lindgren, P.E., Olivieri, E., Sassera, D., Östlund, E., Omazic, A., Perissinotto, D., Söderlund, R., 2023. Characterization of the bacterial microbiome of Swedish ticks through 16S rRNA amplicon sequencing of whole ticks and of individual tick organs. Parasites Vectors 30 (1), 39. https://doi.org/10.1186/s13071-022-05638-4, 16.

Greay, T.L., Gofton, A.W., Paparini, A., Ryan, U.M., Oskam, C.L., Irwin, P.J., 2018. Recent insights into the tick microbiome gained through next-generation sequencing. Parasites Vectors 11, 12. https://doi.org/10.1186/s13071-017-2550-5.

Guglielmone, A.A., Nava, S., Robbins, R.G., 2021. Neotropical Hard Ticks (Acari: Ixodida: Ixodidae). Springer International Publishing, p. 486.

Guglielmone, A.A., Nava, S., Robbins, R.G., 2023. Geographic distribution of the hard ticks (Acari: Ixodida: Ixodidae) of the world by countries and territories. Zootaxa 5251 (1), 1–274. https://doi.org/10.11646/ZOOTAXA.5251.1.1.

Guizzo, M.G., Dolezelikova, K., Neupane, S., Frantova, H., Hrbatova, A., Pafco, B., Fiorotti, J., Kopacek, P., Zurek, L., 2022a. Characterization and manipulation of the bacterial community in the midgut of *Ixodes ricinus*. Parasites Vectors 15 (1), 248. https://doi.org/10.1186/s13071-022-05362-z.

Guizzo, M.G., Neupane, S., Kucera, M., Perner, J., Frantová, H., da Silva Vaz, I., de Oliveira, P.L., Kopacek, P., Zurek, L., 2020. Poor unstable midgut microbiome of hard ticks contrasts with abundant and stable monospecifc microbiome in ovaries. Front. Cell. Infect. Microbiol. 10, 211. https://doi.org/10.3389/fcimb.2020.00211.

Guizzo, M.G., Tirloni, L., Gonzalez, S.A., Farber, M.D., Braz, G., Parizi, L.F., Dedavid, E., Silva, L.A., da Silva Vaz, I.Jr., Oliveira, P.L., 2022b. *Coxiella* Endosymbiont of *Rhipicephalus microplus* Modulates Tick Physiology with a major impact in blood feeding capacity. Front. Microbiol. 13, 868575 https://doi.org/10.3389/fmicb.2022.868575.

Heberle, H., Meirelles, G.V., da Silva, F.R., Telles, G.P., 2015. Minghim, R. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. BMC Bioinf. 16, 169. https://10.1186/s12859-015-0611-3.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. Nature Publishing Group 5, 1571–1579. https://doi.org/10.1038/ismej.2011.41.

Hernández-Jarguín, A., Díaz-Sánchez, S., Villar, M., de la Fuente, J., 2018. Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus*. Ticks Tick Borne Dis. 9, 1241–1251.

Hoffmann, A., Fingerle, V., Noll, M., 2020. Analysis of tick surface decontamination methods. Microorganisms 8, 987. https://doi.org/10.3390/microorganisms8070987.

Huang, Y., Zhang, S., Tao, Y., Yang, J., Lu, S., Jin, D., Pu, J., Luo, W., Zheng, H., Liu, L., Jiang, J.F., Xu, J., 2023. Morphological and genomic characteristics of two novel actinomycetes, *Ornithinimicrobium sufpigmenti* sp. nov. and *Ornithinimicrobium faecis* sp. nov. isolated from bat faeces (*Rousettus leschenaultia* and *Taphozous perforates*). Front. Cell. Infect. Microbiol. 13, 1093407 https://doi.org/10.3389/fcimb.2023.1093407.

Illumina, 2013. 16S metagenomic sequencing library preparation. Rev. B. 28.

Institutional Animal Care and Use Committee – IACUC., 2018. Guidelines and Policies. Available online: https://ursa.research.gsu.edu/files/2016/02/IACUC-Policies-and-Procedures.pdf. (Accessed 22 August 2018).

Jahan, N.A., Lindsey, L.L., Larsen, P.A., 2021. The role of peridomestic rodents as reservoirs for zoonotic foodborne pathogens. Vector Borne Zoonotic Dis. 21 (3), 133–148. https://10.1089/vbz.2020.2640.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28 (12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Kemp, B.M., Smith, D.G., 2005. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci. Int. 154 (1), 53–61. https://doi.org/10.1016/j.forsciint.2004.11.017.

Khalaf, J.M., Mohammed, I.A., Karim, A.J., 2018. The epidemiology of tick in transmission of Enterobacteriaceae bacteria in buffaloes in Marshes of the south of Iraq. Vet. World 11 (12), 1677–1681. https://doi.org/10.14202/vetworld.2018.1677-1681.

Labruna, M.B., Onofrio, V.C., Barros-Battesti, D.M., Gianizella, S.L., Venzal, J.M., Guglielmone, A.A., 2020. Synonymy of *Ixodes aragaoi* with *Ixodes fuscipes*, and reinstatement of *Ixodes spinosus* (Acari: Ixodidae). Ticks Tick Borne Dis 11 (2), 101349. https://doi.org/10.1016/j.ttbdis.2019.101349.

Lagier, J.C., Armougom, F., Million, M., Hugon, P., Pagnier, I., Robert, C., Bittar, F., Fournous, G., Gimenez, G., Maraninchi, M., Trape, J.F., Koonin, E.V., La Scola, B., Raoult, D., 2012. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin. Microbiol. Infect. 18, 1185–1193. https://doi.org/10.1111/1469-0691.12023.

Lee, S.Y., Sung, H., Kim, P.S., Kim, H.S., Lee, J.Y., Lee, J.Y., Jeong, Y.S., Tak, E.J., Han, J. E., Hyun, D.W., Bae, J.W., 2021. Description of *Ornithinimicrobium ciconiae* sp. nov., and *Ornithinimicrobium avium* sp. nov., isolated from the faeces of the endangered and near-threatened birds. J. Microbiol. 59 (11), 978–987. https://doi.org/10.1007/s12275-021-1323-1.

Lejal, E., Chiquet, J., Aubert, J., Robin, S., Estrada-Peña, A., Rue, O., Midoux, C., Mariadassou, M., Bailly, X., Cougoul, A., Gasqui, P., Cosson, J.F., Chalvet-Monfray, K., Vayssier-Taussat, M., Pollet, T., 2021. Temporal patterns in *Ixodes*

ricinus microbial communities: an insight into tick-borne microbe interactions. Microbiome 9, 1–20. https://doi.org/10.1186/s40168-021-01051-8.

Makovska, M., Killer, J., Modrackova, N., Ingribelli, E., Amin, A., Vlkova, E., Bolechova, P., Neuzil-Bunesova, V., 2023. Species and strain variability among *Sarcina* isolates from diverse mammalian hosts. Animals 13 (9), 1529. https://doi.org/10.3390/ani13091529.

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). Parasitol. Res. 84, 478–484. https://doi.org/10.1007/s004360050433.

Maqbool, M., Sajid, M.S., Saqib, M., Anjum, F.R., Tayyab, M.H., Rizwan, H.M., Rashid, M.I., Rashid, I., Iqbal, A., Siddique, R.M., Shamim, A., Hassan, M.A., Atif, F. A., Razzaq, A., Zeeshan, M., Hussain, K., Nisar, R.H.A., Tanveer, A., Younas, S., Kamran, K., Rahman, S.U., 2022. Potential mechanisms of transmission of Tick-borne viruses at the virus-tick interface. Front. Microbiol. 13, 846884 https://doi.org/10.3389/fmicb.2022.846884.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J17, 10–12. https://doi.org/10.14806/ej.17.1.200.

Mateos-Hernández, L., Obregón, D., Wu-Chuang, A., Maye, J., Bornères, J., Versillé, N., de la Fuente, J., Díaz-Sánchez, S., Bermúdez-Humarán, L.G., Torres-Maravilla, E., Estrada-Peña, A., Hodžić, A., Šimo, L., Cabezas-Cruz, A., 2021. Anti-microbiota vaccines modulate the tick microbiome in a taxon-specifc manner. Front. Immunol. 12, 704621 https://doi.org/10.3389/fimmu.2021.704621.

Menchaca, A.C., Visi, D.K., Strey, O.F., Teel, P.D., Kalinowski, K., Allen, M.S., Williamson, P.C., 2013. Preliminary assessment of microbiome changes following blood-feeding and survivorship in the *Amblyomma americanum* nymph-to-adult transition using semiconductor sequencing. PLoS One 8 (6), e67129. https://doi.org/10.1371/journal.pone.0067129.

Michalski, M.M., Kubiak, K., Szczotko, M., Dmitryjuk, M., 2021. Tick-borne pathogens in ticks collected from wild ungulates in north-eastern Poland. Pathogens 10 (5), 587. https://10.3390/pathogens10050587.

Miranda, J.L., Sanchez, l., Amaya, K., Mattar, S., 2011. Primera prueba serológica de *Rickettsia* sp. del grupo de la febre manchada en el departamento del Meta. Biomedica 31, 105.

Narasimhan, S., Swei, A., Abouneameh, S., Pal, U., Pedra, J.H.F., Fikrig, E., 2021. Grappling with the tick microbiome. Trends Parasitol. 37, 722–733. https://doi.org/10.1016/j.pt.2021.04.004.

Nava, S., Venzal, J.M.M., González-Acuña, D.G., Martins, T.F.F., Guglielmone, A.A., 2017. Ticks of the southern cone of America: diagnosis, distribution, and hosts with taxonomy. Ecology and Sanitary Importance, first ed. Elsevier, London, San Diego, p. 348.

Norris, D.E., Klompen, J.S.H., Keirans, J.E., Black, W.C., 1996. Population genetics of *Ixodes scapularis* (Acari: Ixodidae) based on mitocondrial 16S and 12S genes. J. Med. Entomol. 33, 78–89. https://doi.org/10.1093/jmedent/33.1.78.

Nowakiewicz, A., Zięba, P., Gnat, S., Osińska, M., Łagowski, D., Kosior-Korzecka, U., Puzio, I., Król, J., 2021. Analysis of the occurrence and molecular characteristics of drug-resistant strains of *Enterococcus faecalis* isolated from the gastrointestinal tract of insectivorous bat species in Poland: a possible essential impact on the spread of drug resistance? Environ. Pollut. 269, 116099.

O'Hara, E., Neves, A.L.A., Song, Y., Guan, L.L., 2020. The role of the gut microbiome in cattle production and health: driver or passenger? Annu. Rev. Anim. Biosci. 8, 199–220. https://doi.org/10.1146/annurev-animal-021419-083952.

O'Keeffe, K.R., Oppler, Z.J., Brisson, D., 2020. Evolutionary ecology of Lyme *Borrelia*. Infect. Genet. Evol. 85, 104570 https://doi.org/10.1016/j.meegid.2020.104570.

Ortíz-Giraldo, M., Tobón-Escobar, W.D., Velásquez-Guarín, D., Usma-Marín, M.F., Ossa-López, P.A., Ramírez-Chaves, H.E., Carvajal-Agudelo, J.D., Rivera-Páez, F.A., 2021. Ticks (Acari: ixodoidea) associated with mammals in Colombia: a historical review, molecular species confirmation, and establishment of new relationships. Parasitol. Res. 120 (2), 383–394. https://doi.org/10.1007/s00436-020-06989-6.

Ossa-López, P.A., Mancilla-Agrono, L.Y., Micolta, L.F.B., Ramírez-Chaves, H.E., Agudelo, J.D.C., Muñoz-Leal, S., Labruna, M.B., Lloyd, V., Rivera-Páez, F.A., 2023. Morphological and molecular confirmation of *Ornithodoros hasei* (schulze, 1935) (Acari: Argasidae) in Colombia. Ticks Tick Borne Dis 14 (3), 102142. https://10.1016/j.ttbdis.2023.102142.

Ossa-López, P.A., Robayo-Sánchez, L.N., Uribe, J.E., Ramírez-Hernández, A., Ramírez-Chaves, H.E., Cortés-Vecino, J.A., Rivera-Páez, F.A., 2022. Extension of the distribution of *Amblyomma triste* Koch, 1844: morphological and molecular confirmation of morphotype I in Colombia. Ticks Tick Borne Dis 13 (3), 101923. https://10.1016/j.ttbdis.2022.101923.

Owens, L.A., Colitti, B., Hirji, I., Pizarro, A., Jaffe, J.E., Moittié, S., Bishop-Lilly, K.A., Estrella, L.A., Voegtly, L.J., Kuhn, J.H., Suen, G., Deblois, C.L., Dunn, C.D., Juan-Sallés, C., Goldberg, T.L., 2021. A *Sarcina* bacterium linked to lethal disease in sanctuary chimpanzees in Sierra Leone. Nat. Commun. 12, 763. https://doi.org/10.1038/s41467-021-21012-x.

Rodents. In: Patton, J.L., Pardiñas, U.F.J., D'elía, G. (Eds.), 2015. Mammals of South America, 2. The University of Chicago Press, Chicago, IL, USA, p. 1336.

Portillo, A., Palomar, A.M., de Toro, M., Santibáñez, S., Santibáñez, P., Oteo, J.A., 2019. Exploring the bacteriome in anthropophilic ticks: to investigate the vectors for diagnosis. PLoS One 14 (3), e0213384. https://doi.org/10.1371/journal.pone.0213384.

Proulx, G., 2022. Mammal Trapping Wildlife Management, Animal Welfare and International Standards. Alpha Wildlife Publications, p. 298.

Qi, Y., Ai, L., Zhu, C., Lu, Y., Lv, R., Mao, Y., Lu, N., Tan, W., 2022. Co-Existence of multiple *Anaplasma* species and Variants in ticks feeding on hedgehogs or cattle poses potential threats of anaplasmosis to humans and livestock in eastern China. Front. Microbiol. 13, 913650. https://10.3389/fmicb.2022.913650.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Opens external link in new window. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.

Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.F., Darling, A., Malfatti, S., Swan, B.K., Gies, E.A., Dodsworth, J.A., Hedlund, B.P., Tsiamis, G., Sievert, S.M., Liu, W.T., Eisen, J.A., Hallam, S.J., Kyrpides, N.C., Stepanauskas, R., Rubin, E.M., et al., 2013. Insights into the phylogeny and coding potential of microbial dark matter. Nature 499, 431–437. https://doi.org/10.1038/nature12352.

Ritzmann, M., Grimm, J., Heinritzi, K., Hoelzle, K., Hoelzle, L.E., 2009. Prevalence of Mycoplasma suis in slaughter pigs, with correlation of PCR results to hematological findings. Vet. Microbiol. 133 (1–2), 84–91. https://10.1016/j.vetmic.2008.06.015.

Rivera-Páez, F.A., Labruna, M.B., Martins, T.F., Perez, J.E., Castaño-Villa, G.J., Ossa-López, P.A., Gil, C.A., Sampieri, B.R., Aricapa-Giraldo, H.J., Camargo-Mathias, M.I., 2018a. Contributions to the knowledge of hard ticks (Acari: Ixodidae) in Colombia. Ticks Tick Borne Dis 9, 57–66. https://doi.org/10.1016/j.ttbdis.2017.10.008.

Rivera-Páez, F.A., Labruna, M.B., Martins, T.F., Sampieri, B.R., Camargo-Mathias, M.I., 2016. *Amblyomma mixtum* Koch, 1844 (Acari: Ixodidae): first record confirmation in Colombia using morphological and molecular analyses. Ticks Tick Borne Dis. 7 (5), 842–848. https://doi.org/10.1016/j.ttbdis.2016.03.020.

Rivera-Páez, F.A., Martins, T.F., Ossa-López, P.A., Sampieri, B.R., Camargo-Mathias, M.I., 2018b. Detection of *Rickettsia* spp. in ticks (Acari: Ixodidae) of domestic animals in Colombia. Ticks Tick Borne Dis 9, 819–823. https://doi.org/10.1016/j.ttbdis.2018.03.006.

Riveros-Pinilla, D.A., Acevedo, G.L., Londono, A.F., Gongora, A., 2015. Antibodies against spotted fever group *Rickettsia* sp., in horses of the Colombian Orinoquia. Revista MVZ 20, 5004–5013. https://doi.org/10.21897/rmvz.14.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics.". PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.

Rojas-Jaimes, J., Lindo-Seminario, D., Correa-Núñez, G., Diringer, B., 2022. Characterization of the bacterial microbiome of *Amblyomma scalpturatum* and *Amblyomma ovale* collected from *Tapirus terrestris* and *Amblyomma sabanerae* collected from *Chelonoidis denticulata*, Madre de Dios- Peru. BMC Microbiol. 22, 305. https://doi.org/10.1186/s12866-022-02717-5.

Ross, B.D., Hayes, B., Radey, M.C., Lee, X., Josek, T., Bjork, J., Neitzel, D., Paskewitz, S., Chou, S., Mougous, J.D., 2018. *Ixodes scapularis* does not harbor a stable midgut microbiome. ISME J. 12, 2596–2607. https://doi.org/10.1038/s41396-018-0161-6.

Saracho-Bottero, M.N., Beati, L., Venzal, J.M., Guardia, L., Thompson, C.S., Mangold, A. J., Guglielmone, A.A., Nava, S., 2021. *Ixodes silvanus* n. sp. (Acari: Ixodidae), a new member of the subgenus *Trichotoixodes* Reznik, 1961, from northwestern Argentina. Ticks Tick Borne Dis 12 (1), 101572. https://doi.org/10.1016/j.ttbdis.2020.101572.

Saracho-Bottero, M.N., Venzal, J.M., Tarragona, E.L., Thompson, C.S., Mangold, A.J., Beati, L., Guglielmone, A.A., Nava, S., 2020. The *Ixodes ricinus* complex (Acari: Ixodidae) in the Southern Cone of America: *Ixodes parairicinus, Ixodes aragaoi* and *Ixodes* cf. *I. affinis*. Parasitol. Res. 119, 43–54. https://doi.org/10.1007/s00436-01906470-z.

Sarani, S., Enferadi, A., Hasani, S.J., Sarani, M.Y., Rahnama, M., Sarani, F., 2024. Identification of zoonotic pathogenic bacteria from blood and ticks obtained from hares and long-eared hedgehogs (*Hemiechinus megalofis*) in eastern Iran. Comp. Immunol. Microbiol. Infect. 104, 102097 https://doi.org/10.1016/j.cimid.2023.102097.

Sikes, R.S., 2016. The animal care and use committee of the American society of mammalogists. Guidelines of the American society of mammalogists for the use of wild mammals in research and education. J. Mammal. 97, 663–688. https://doi.org/10.1093/jmammal/gyw078.

Sonenshine, D.E., Roe, R.M., 2014. Biology of Ticks. Oxford University Press, Oxford, p. 560.

Stevens, E.J., Bates, K.A., King, K.C., 2021. Host microbiota can facilitate pathogen infection. PLoS Pathog. 17, e1009514 https://doi.org/10.1371/journal.ppat.1009514.

Swei, A., Kwan, J.Y., 2017. Tick microbiome and pathogen acquisition altered by host blood meal. ISME J. 11, 813–816. https://doi.org/10.1038/ismej.2016.152.

Tate, C.M., Howerth, E.W., Mead, D.G., Dugan, V.G., Luttrell, M.P., Sahora, A.I., Munderloh, U.G., Davidson, W.R., Yabsley, M.J., 2013. *Anaplasma odocoilei* sp. nov. (family Anaplasmataceae) from white-tailed deer (*Odocoileus virginianus*). Ticks Tick Borne Dis 4 (1–2), 110–119. https://10.1016/j.ttbdis.2012.09.005.

Taylor, P.J., Arntzen, L., Hayter, M., Iles, M., Frean, J., Belmain, S., 2008. Understanding and managing sanitary risks due to rodent zoonoses in an African city: beyond the Boston Model. Integr. Zool. 3, 38–50. https://doi.org/10.1111/j.1749-4877.2008.00072.x.

Thomas, A.M., Segata, N., 2019. Multiple levels of the unknown in microbiome research. BMC Biol. 17, 48. https://doi.org/10.1186/s12915-019-0667-z.

Turnbull, P.C.B., 1996. Bacillus. In: Baron, S. (Ed.), Medical Microbiology, fourth ed. University of Texas Medical Branch at Galveston, p. 326.

Valiente Moro, C., Thioulouse, J., Chauve, C., Normand, P., Zenner, L., 2009. Bacterial taxa associated with the hematophagous mite *Dermanyssus gallinae* detected by 16S rRNA PCR amplification and TTGE fingerprinting. Res. J. Microbiol. 160 (1), 63–70. https://doi.org/10.1016/j.resmic.2008.10.006.

Velásquez-Guarín, D., Pérez Cárdenas, J.E., Serpa, M.C.A., Labruna, M.B., Faccini-Martínez, A.A., Rivera-Páez, F.A., Ramírez-Chaves, H.E., 2024. *Rickettsia* spp. seroprevalence in wild mammals from Arauca, Orinoquia region of Colombia. Mastozool. Neotrop. 31 (1), e0963 https://doi.org/10.31687/saremMN.24.31.01.10.e0963.

Voss, R.S., Emmons, L.H., 1996. Mammalian diversity in Neotropical lowland rainforests: a preliminary assessment. Bull. Am. Mus. Nat. Hist. 230, 1–115.

Wu-Chuang, A., Hodžić, A., Mateos-Hernández, L., Estrada-Peña, A., Obregon, D., Cabezas-Cruz, A., 2021. Current debates and advances in tick microbiome research. Curr Res Parasitol Vector Borne Dis 1, 100036. https://doi.org/10.1016/j.crpvbd.2021.100036.

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "All-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, D643–D648. https://doi.org/10.1093/nar/gkt1209.

Zhong, Z., Zhong, T., Peng, Y., Zhou, X., Wang, Z., Tang, H., Wang, J., 2021. Symbiont-regulated serotonin biosynthesis modulates tick feeding activity. Cell Host Microbe 29, 154557e4. https://doi.org/10.1016/j.chom.2021.08.011.