Characterization of the bacterial microbiome of *Rhipicephalus (Boophilus) microplus* collected from *Pecari tajacu* “Sajino” Madre de Dios, Peru

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Ticks are arthropods that can host and transmit pathogens to wild animals, domestic animals, and even humans. The bacterial microbiome of adult (males and females) and nymph *Rhipicephalus microplus* ticks collected from a collared peccary, *Pecari tajacu*, captured in the rural area of Botijón Village in the Amazon region of Madre de Dios, Peru, was evaluated using metagenomics. The Chao1 and Shannon–Weaver analyses indicated greater bacterial richness and diversity in female ticks (GARH; 375–4.15) and nymph ticks (GARN; 332–4.75) compared to that in male ticks (GARM; 215–3.20). Taxonomic analyses identified 185 operational taxonomic units representing 147 bacterial genera. Of the 25 most prevalent genera, *Salmonella* (17.5%) and *Vibrio* (15.0%) showed the highest relative abundance followed by several other potentially pathogenic genera, such as *Paracoccus* (7.8%), *Staphylococcus* (6.8%), *Pseudomonas* (6.6%), *Corynebacterium* (5.0%), *Cloacibacterium* (3.6%), and *Acinetobacter* (2.5%). In total, 19.7% of the detected genera are shared by GARH, GARM, and GARN, and they can be considered as the core microbiome of *R. microplus*. To the best of our knowledge, this study is the first to characterize the microbiome of ticks collected from *P. tajacu* and to report the presence of *Salmonella* and *Vibrio* in *R. microplus*. The pathogenic potential and the role of these bacteria in the physiology of *R. microplus* should be further investigated due to the possible implications for public health and animal health in populations neighboring the habitat of *P. tajacu*.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| GARH | Female tick |
| GARM | Male tick |
| GARN | Nymph tick |
| m.a.s.l. | Meters above sea level |
| PCR | Polymerase chain reaction |
| SW | Shannon–Weaver |
| OTU | Operational taxonomic units |
| PGM | Ion personal genome machine system |
| NGS | Next-generation sequencing |
| RNA | Ribosomal RNA |
| WGS | World geodetic system |
| SW | Shannon–Weaver |
| NCBI | National Center for Biotechnology Information |
| pb | Base pairs |

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Ticks are arthropods that can host a range of pathogens of other organisms and are one of the main vectors for vector-borne diseases. Babesia sp. and Rickettsia sp. are pathogens frequently transmitted by ticks, whose detection and identification have been facilitated by molecular methods, particularly by the emergence of next-generation sequencing (NGS) techniques. NGS techniques allow for (i) the precise characterization of the composition of complex microbiomes independent of the traditional culture techniques, (ii) the identification of pathogens, opportunist, probiotics, or commensals for the arthropod and/or host, and (iii) the calculation and comparison of the diversity and richness of microbiomes. Although commensal and symbiotic bacteria have been identified by metagenomic studies in ticks, these studies have focused on the microbiome with pathogenic potential from the veterinary and human perspective. The microbiome biology in ticks still remains generally unexplored and neglected, and whether the microbiome has a neutral, harmful, or beneficial effect on the arthropod with regard to nutritional processes, adaptation, development, reproduction, or defense in adverse environments needs to be determined. Furthermore, previous studies on Ixodes pavlovskyi have described Rickettsia, Anaplasma, Ehrlichia, and Borrelia burgdorferi as well as their impact on the vector and susceptible hosts. Another study on Dermacentor occidentalis has identified an emerging pathogenic bacterium in humans called Rickettsia philipii as well as two new bunyaviruses. The microbiome of Rhipicephalus (Boophilus) microplus has been characterized in cattle by pyrosequencing techniques, while the pathogens Anaplasm, Bartonella, Borrelia, Ehrlichia, Franciscella, and Rickettsia have been identified in ticks of the genera Amblyomma, Ixodes, and Haemaphysalis sp. Metagenomics has also been used to identify other infectious agents in Rhipicephalus sp., such as viruses, particularly nairoviruses that cause important diseases in humans.

This study aims to analyze the bacterial microbiome in R. microplus collected from wild Pecari tajacu using metagenomics.

Results

Ticks collected from P. tajacu. Taxonomic identification indicated that all the collected ticks in Madre de Dios (Fig. 1) belong to R. microplus.

Statistical values and diversity in the R. microplus microbiome. Microbiome analysis using the 16S-515F/16S-806R primers and amplicon sequencing on Ion Torrent PGM (Ion Personal Genome Machine System, THERMO FISHER SCIENTIFIC) generated a total of 117,192 raw reads (39,604 average) from the three analyzed samples (Table 1). After rigorous data curation, 55,805 high-quality sequences were retained with an average of 20,462 sequences per sample and an average length of 150 bp. The maximum number of filtered sequences (26,549) was obtained from the female tick sample, which exceeded those found in male
and nymph samples by 164.7% and 204.8%, respectively18. These sequences were assigned to 1075 total unique sequences corresponding to 185 abundant (<0.005%) OTUs based on a >97% identity cutoff for bacterial 16S rRNA genes18. At the individual sample level, the microbiome from nymphs surpassed that from females and males (221, 195, and 148 OTUs, respectively). Those OTUs were mainly identified as prokaryotes (99.89%) and to a lesser extent as unknown sequences (0.11%). At the taxonomic level, a total of 147 genera distributed in 99 families, 59 orders, 30 classes, and 12 phyla were detected.

The SW index reflects the specific diversity of each sample, whose value increases as the number of different OTUs increases. In this study, the microbiome obtained from nymph tick samples showed a higher SW index than the female and male microbiomes. On the other hand, Chao1, the index that evaluates specific richness, showed that the number of expected OTUs decreased from 375 in GARH to 332 in GARN and 215 in GARM after the standardization of the sample size to 14,000 sequences. Statistical analyses of variance of the SW and Chao1 indexes in the GARH, GARM, and GARN samples showed significant differences (P < 0.01)19–21.

| Codes | Total Genera |
|-------|--------------|
| GARH GARM GARN | 29 (19.7%) |
| GARH GARM GARN | 5 (3.4%) |
| GARH GARM GARN | 14 (9.5%) |
| GARH GARM GARN | 2 (1.4%) |
| GARH GARM GARN | 39 (26.5%) |
| GARH GARM GARN | 21 (14.3%) |
| GARH GARM GARN | 37 (25.2%) |

**Table 1.** Statistical summary of the microbiota from *Rhipicephalus microplus*. *Significant differences (P < 0.01).*

**Table 2.** Composition of the core microbiome according to *R. microplus* sex and stage.

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**Composition of the core and shared and individual microbiome from *R. microplus***. The comparative analysis of the composition of the microbiota from GARH, GARM, and GARN revealed that 19.7% out of the 147 genera found in *R. microplus* were common to the three groups. This shared community is considered as the core microbiota (Table 2). The percentages showed a decreasing proportionality in GARH, GARN, and GARM in relation to the non-shared bacterial genera. A higher percentage of shared microbiota was observed between GARH and GARN (9.5%) compared to that between GARH and GARM (3.4%) and between GARM and GARN (1.4%).

**Microbiota according to *R. microplus* sex and stage.** Regarding the abundance of bacterial genera in *R. microplus*, *Salmonella* was the most abundant genus in GARM, while *Vibrio* was the most abundant genus in...
GARH and GARN, and Paracoccus was the second most abundant genus in GARH. On average, these were the most abundant genera in *R. microplus*, 17.5%, 15%, and 7.8%, respectively (Fig. 2).

**Discussion**

The richness and diversity indexes revealed that the microbiota present in GARH and GARN exhibit greater bacterial genera diversity and richness than the microbiota in GARM. This is in agreement with previous studies on *R. microplus* that were collected from cattle9. Previous studies in male and female ticks of *Ixodes ovatus*, *I. persulcatus*, and *Amblyomma variegatum* have shown differentiated microbiome profiles both at the taxonomic and functional levels between sexes of the same tick species22.

A metagenomic study showed that the microbiome profile in ticks is related to metabolic processes and that their resilience and adaptability to the environment is related to their sex22. In addition, geographical location, temperature, humidity, species, sex, anatomical location, and type of diet have been shown to affect the microbiome of ticks23–28. In our study, although ticks were of the same species and were collected from the same host, significant differences were found in bacterial diversity and richness related to the sex and developmental stage of ticks.

Among the 147 different genera identified, the core microbiome that included the majority of the most prevalent genera stood out. Several of the identified genera within the core microbiome are known to be human pathogens (i.e., *Salmonella*, *Vibrio*, *Paracoccus*, *Staphylococcus*, *Pseudomonas*, *Corynebacterium*, *Cloacibacterium*, and *Acinetobacter*). In addition, a greater bacterial microbiome was shared between nymph and female ticks [14 (9.5%) compared to that shared between male and female ticks [5 (3.4%)]. We suggest that these differences have a behavioral origin. Thus, female and nymph ticks are more prone to remain on the same host, whose microbiota impact on the tick gut microbiome, while male ticks frequently change hosts22. This hypothesis is supported by studies on other genera that reported higher relative abundance and alpha diversity in female ticks than in male ticks22. Additionally, it is necessary to consider that the role of nuclei bacterial genera and the species included in these may present different roles as pathogens or symbionts depending on whether they are found in the arthropod or in the vertebrate that hosts the arthropod.

The most prevalent genus among the three groups of ticks was identified as *Salmonella*, whose members cause gastrointestinal tract infection and dysentery and can lead to serious clinical conditions, especially in children29. The genus *Vibrio*, the second in abundance (15.6%), represents a finding of great interest as, to the best of our knowledge, this is the first study showing its presence in *R. microplus*. The genus *Vibrio* is a common commensal of aquatic arthropods and has a remarkable capacity for adaptation to the environment30,31. Its presence evinces the adaptation of this genus to the gastrointestinal system of *R. microplus*, which inhabits a jungle ecosystem.
Many Vibrio are opportunistic pathogens of both arthropods and humans. Therefore, studying the virulence of the identified species is essential. Paracoccus, the third most abundant genus (6.97%), is a coccobacillary bacterium that is typically present in a wide range of ecosystems. Staphylococcus, with a prevalence of 6.63%, is mainly related to infections in soft tissues and has been previously reported in the gut of R. microplus and with a high prevalence in female Amblyomma variegatum. Pseudomonas showed an abundance of 5.87% in R. microplus. In previous studies, the presence of this bacterial genus in R. microplus and in male Amblyomma variegatum with a high prevalence has been reported. Pseudomonas has been suggested to be involved in the infection of soft tissues, including the tissues of the respiratory system. The presence of Corynebacterium, with an abundance of 5.87%, is important because some Corynebacterium species produce the diphtheria toxin or can cause osteomyelitis. In addition, this genus has been previously identified in eggs and male adults of R. microplus. Clouacibacterium, with a prevalence of 2.93% in R. microplus, are gram-negative bacteria that proliferate in aqueous environments with high content of organic matter. Acinetobacter, with an abundance of 2.53%, has been reported in a metagenomic study in I. persulcatus, I. pavlovskyi, and Dermacentor reticulatus. Sphingomonas, the ninth most abundant genus (2.47%), includes non-fermenting and strictly aerobic gram-negative bacteria. Some species, such as S. paucimobilis and S. wittichii, can cause infections in immunocompromised patients.

In contrast to the bacterial microbiome relevant to human health identified in our study, a previous study on bacterial diversity in R. microplus collected from cattle identified Ehrlichia sp., Coxiella sp., and Bartonella sp. This indicates that the bacterial microbiome would also depend on the host parasitized by the ticks. Some bacteria, such as Leptospira interrogans, Mycobacterium, Salmonella, Clostridium, and Pasteurella, and tick genera, such as Haemaphysalis, Dermacentor, and Amblyoma, have been identified in the genus Pecari and R. microplus, a tick that mainly parasitizes cattle, was found in P. tajacu (sajino). P. tajacu was possibly tick infected due to the proximity of Botijón Village, where livestock farming is practiced. This highlights that ticks can infect cattle, P. tajacu, and humans, with the potential risks of pathogen transmission that this implies.

Regarding the role of bacteria in ticks, note that nonpathogenic microorganisms present in ticks could cause infections in humans and other animals. For example, ecological studies have shown that Rickettsia, Francisella, and Coxiella, which are considered vertebrate pathogens, can change their pathogenic role and have a mutualistic and symbiotic relationship with ticks. Therefore, studying the interaction between the bacterial microbiota and ticks is of utmost importance for the control of pathogens and the development of the arthropod. Coxiella sp. infects at least two-thirds of the ticks and is important for the survival of Amblyomma americanum and Rhipicephalus sp. Nonetheless, it has not been found in our study. Coxiella sp. and Francisella sp. are linked to the synthesis of vitamins necessary for the survival of ticks. Likewise, other symbiotic bacteria, such as Francisella, Rickettsia, and Rickettsiella, have been reported, with Rickettsia sp. and Coxiella sp. having become strict endosymbionts. According to previous studies, the endosymbiotic bacteria of a species of tick vary depending on the ecology and the number of ticks studied, for example, although in the case of Coxiella it was previously described R. microplus collected from cattle, previous studies that have demonstrated that the infection rates by Coxiella in R. microplus ticks are highly variable. A 2016 study evaluated R. microplus from Brazil, and found that only approximately 37% of the samples contained Coxiella. In 2015 a study evaluated Coxiella in many species of ticks without finding the bacteria, one of the species of ticks evaluated was 3 R. microplus samples from Benin (west Africa) and did not find any Coxiella. Therefore, the importance of our study is the finding of the new microbiome of R. microplus collected from Pecari tajacu.

The small number of ticks was justified by the fact that R. microplus ticks are not very common on the wild host Pecari tajacu; therefore, we could not collect a larger sample of ticks. On the other hand, we found interesting to test these ticks because We wanted to search for the microbiota of an exotic tick (R. microplus) infesting a mammal species native to the Amazonia (Pecari tajacu). Again, even though our sample was small, we have to highlight the interesting results We have found from these ticks.

Among the limitations of our study is the bacterial microbiome found in 5 females, 5 males and 2 nymphs of ticks collected from P. tajacu, which implies a bacterial microbiome representative of a specific circumstance and ecology. Therefore, studies with a greater number of samples could show a greater diversity of species and different percentages of bacterial abundance.

Conclusion
In this study, we found a high bacterial diversity in female, male, and nymph R. microplus collected from P. tajacu. The greatest bacterial diversity and richness was found in females and nymph ticks compared to male ticks. The most frequent bacterial genera were Salmonella, Vibrio, and Paracoccus. This is the first bacterial metagenomic study performed in R. microplus collected from P. tajacu in the Peruvian jungle, and the presence of Vibrio is highlighted. This study lays the foundations for future studies on the importance of the role of the identified bacteria on arthropods and animal and human health.

Material and methods
Ethical aspects. This study was approved by the Oficina de Salud Pública y Medio Ambiente del Consejo Regional de Madre de Dios (Office of Public Health and Environment of the Regional Council Madre de Dios), Peru. Laboratory procedures for bacterial identification were conducted in accordance with the international guidelines for the use of animals in research and the standards of the Comité de Cuidado y Uso de Animales del Área de Investigación en Salud de la Junta del Consejo Regional de Madre de Dios (Animal Care and Use Committee of the Health Research Area of the Madre de Dios Regional Council Board). The study was carried out in compliance with the ARRIVE guidelines.
**Geographic location.** The study was conducted in the outskirts of Botijón Village (12° 07' 12.95" S, 69° 04' 31.47" W; WGS 84, 267 m a.s.l.), Tambopata province, Madre de Dios region, Peru (Fig. 1). The collection site corresponds to a forest area where hunting of wild animals is allowed. The average annual rainfall in the study area is 1600 mm, and the average annual temperature is 25 °C. The area is in the tropical wet forest zone. During sample collection, the weather was hot and humid.

**Sample collection.** A wild male of *P. tajacu (sajino)* was captured in Botijón Village in June 2012. The ticks from its abdominal region were collected 3 h after its sacrifice using forceps and were individually placed in 2 ml cryovials containing 96% ethyl alcohol. Cryovials were labeled with an identification code for the sampling site and the animal from which the sample was collected. Five male ticks, five female ticks, and two nymph ticks were identified. On sterile plates ticks were washed for 15 min in a solution 0.9% isotonic sterile sodium chloride saline followed by 15 min in a solution of 96% ethanol to remove surface contaminants. Excess solution was absorbed and ticks were air-dried prior to manipulation under sterile conditions. Each tick was individually cut in half lengthwise using sterile scalpels number 15.

**Taxonomic classification.** Ticks were identified using taxonomic keys at the Laboratorio de Entomología del Instituto Nacional de Salud del Perú en Lima (Entomology Laboratory of the National Institute of Health of Peru in Lima).

**DNA extraction.** Total DNA extraction from ticks was performed using Gentra Puregene Tissue kits (QIAGEN, Halden-Germany) according to the manufacturer’s instructions from pools for each tick sex and stage, i.e., GARH (females), GARM (males), and GARN (nymphs) pools.

**Metagenomics.** To study the bacterial diversity and richness in the microbiota from *Rhipicephalus microplus*, the presence and quality of the extracted DNA was verified by PCR amplification of the 16S rRNA gene using the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG-3') and 1492R (5' GGYTACCTTGTGACGACTT-3') that generate a product of about 1500 base pairs (bp). All reactions were performed in 25 μl (total volume) mixtures containing 2.5 μl 10x buffer, 2.5 μl 25 mM MgCl₂, 0.6 μl 10 mM dNTPs, and 2 U of Taq DNA polymerase (THERMO SCIENTIFIC). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, hybridization at 55 °C for 45 s, elongation at 72 °C for 1 min, and a final elongation at 72 °C for 10 min. The PCR products were visualized by electrophoresis on a 1.5% agarose gel. Total DNA extractions were analyzed by spectrophotometry (NANODROP EPPENDORF), and the samples with sufficient quality and quantity were shipped to MR DNA (Shallowater, TX, USA) and sequenced on the PGM platform (Ion Personal Genome Machine System, THERMO FISHER SCIENTIFIC). Metagenomic analysis was performed on the PCR amplification products of the V4 hypervariable region of the 16S rRNA gene using the S15F/806R primers.

**Analysis and processing of metagenomic data.** The sequences generated by Ion Torrent were analyzed by QIIME v1.9.115, where the initial sequences were processed based on filtering of barcodes ≤ 6 bp, Q25 quality scores, 150 bp sequence length, and chimera detection using usearch6116,17. High-quality sequences were classified taxonomically using the High Quality Ribosomal RNA Databases "SILVA" v132 database (https://www.arb-silva.de/). Likewise, unrepresentative OTUs ≤ 0.005% were filtered during analysis18.

Lastly, the final OTUs were processed to analyze the Shannon–Weaver (SW) alpha diversity index, Chao1 richness index, beta diversity (venn and heatmap), and taxonomic abundance (barplot) of the microbial communities using the phyloseq and ampvis packages with the statistical program RStudio version 3.2.3.16,19,20. Sequences shorter than 250 bp were removed. The obtained OTUs were then taxonomically classified using BLASTn and compared with a curated database derived from Greengenes, RPDII, and NCBI (www.ncbi.nlm.nih.gov). The sequences were registered in Metagenomics Analysis Server "MG-RAST" ID: mgp95793; available at https://www.mg-raft.org/linkin.cgi?project=mgp95793.

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**References**

1. Bonnet, S. L., Binetruy, F., Hernández-Jarguin, A. M. & Duron, O. 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.2017.00226 (2017).
2. Burgdorfer, W., Hayes, S. & Mavros, A. Non-pathogenic rickettsiae in *Dermacentor andersoni*: A limiting factor for the distribution of *Rickettsia rickettsii*. In *Rickettsia and Rickettsial Disease* (eds Burgdorfer, A. A. & Anacker, R. L.) 585–594 (Academic, 1981).
3. Chauvin, A., Moreau, E., Bonnet, S., Plantard, O. & Malandrin, L. Babesia and its hosts: Adaptation to long-lasting interactions as a way to achieve efficient transmission. *Virol. Res.* 40, 37. https://doi.org/10.1016/S0168-1343(97)00090-2 (2009).
4. Ravi, A. et al. Metagenomic profiling of ticks: Identification of novel rickettsial genomes and detection of tick-borne canine parvovirus. *PLoS Negl. Trop. Dis.* 13(1), 1–19 (2019).
5. Grey, T. L. et al. Recent insights into the tick microbiome gained through next-generation sequencing. *Parasites Vectors* 11(1), 1–14 (2018).
6. Ravi, V. et al. Detection and genetic characterization of a wide range of infectious agents in *Ixodes pavlovskyi* ticks in Western Siberia, Russia. *Parasites Vectors* 10(1), 1–24 (2017).
7. Filippova, N. A. Asexual Ticks of the Subfamily Ixodinae (Publishing House Nauka, 1977).
8. Bouquet, J. et al. Metagenomic-based surveillance of pacific coast tick dermacentor occidentalis identifies two novel bunyaviruses and an emerging human Rickettsial pathogen. Sci. Rep. 7(1), 1–10. https://doi.org/10.1038/s41598-017-12047-6 (2017).
9. Andreotti, R. et al. Assessment of bacterial diversity in the cattle tick Rhipicephalus (Boophilus) microplus through tag-encoded pyrosequencing. BMC Microbiol. 11(6), 1–11 (2011).
10. Nakaok, R. et al. A novel approach, based on BLStom (batch learning self-organizing maps), to the microbiome analysis of ticks. ISME J. 7(5), 1003–1015. https://doi.org/10.1038/ismej.2013.171 (2013).
11. Xia, H. et al. Metagenomic profile of the viral communities in Rhipicephalus spp. ticks from Yunnan, China. PLoS ONE 10(3), 1–16. https://doi.org/10.1371/journal.pone.0121609 (2015).
12. Barros-Battesti, D., Arzuza, M. & Bechara, H. Carrapato de Importância Medico-Veterinaria da Região Neotropical: Um Guia Ilustrado para Identificação de Espécies (Ticks of Medical-Veterinary Importance in the Neotropical Region: An Illustrated Guide for Species Identification), 8th edition (Butantan Publication, 2006).
13. QIAIEN. Genta, Puregene (QIAIEN GROUP), 2007–2010 (accessed 9 June 2017); https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/genta-puregene-tissue-kit/orderinginformation.
14. Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. & Knight, R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16), 2194–2200 (2011).
15. Gising, A. et al. Changes in 16S RNA gene microbial community profiling by concentration of prokaryotic DNA. J. Microbiol. Methods 119, 239242 (2015).
16. Sokulich, N. A. et al. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10(1), 57–59 (2013).
17. Anderssen, K. S., Kirkegaard, R. H., Karst, S. M. & Albertsen, M. ampvis2: An R package to analyse and visualise 16S RNA amplicon data. BioRxiv. https://doi.org/10.1101/295357 (2018).
18. McMurdie, P. J. & Holmes, S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8(4), e1–11 (2013).
19. DelSantis, T. Z. et al. Greenenges, a chimeria-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72(7), 5069–5072 (2006).
20. Obrégón, D., Bard, E., Abrial, D., Estrada-Peña, A. & Cabezas-Cruz, A. Sex-specific linkages between taxonomic and functional profiles of tick gut microbiomes. Front. Cell. Infect. Microbiol. 9, 298. https://doi.org/10.3389/fcimb.2019.00298 (2019).
21. Qiu, Y., Nakaok, R., Ohnuma, A., Kawamori, F. & Sugimoto, C. Microbial population analysis of the salivary glands of ticks: a possible strategy for the surveillance of bacterial pathogens. PLoS ONE 9(3), e103961 (2014).
22. Van Treuren, W. et al. Variation in the microbiota of Ixodes ticks with regard to geography, species, and sex. Appl. Environ. Microbiol. 81, 6200–6209 (2015).
23. Carp, G. et al. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. PLoS ONE 6(10), e25604 (2011).
24. Zhang, X.-C., Yang, Z.-N., Lu, B., Ma, X.-F. & Zhang, X.-C. The composition and transmission of microbiome in hard tick, Ixodes persulcatus, during blood meal. Ticks Tick Borne Dis. 5, 864–870 (2014).
25. Menchaca, A. C. et al. Preliminary assessment of microbiome changes following blood-feeding and survivorship in the Amblyomma americanum nymph-to-adult transition using semiconductor sequencing. PLoS ONE 8, 1–10 (2013).
26. Clayton, K. A., Gall, C. A., Mason, K. L., Scoles, G. A. & Brayton, K. A. The characterization and manipulation of the bacterial microbiome of the Rocky Mountain wood tick, Dermacentor andersoni. Parasites Vectors 8, 1–5 (2018).
27. Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A. & Parry, C. M. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin. Microbiol. Rev. 1, 901–937. https://doi.org/10.1128/CMBR.00022-15 (2015).
28. Jessor, K. J. & Noble, R. T. Vibrbo ecology in the Neuse River Estuary, North Carolina, characterized by next-generation amplicon sequencing of the gene encoding heat shock protein 60 (Hsp60). Appl. Environ. Microbiol. 84, 1–21. https://doi.org/10.1128/AEM.00333-18 (2018).
29. Payne, S. M., Mey, A. R. & Wyckoff, E. E. Vibrbo iron transport: Evolutionary adaptation to life in multiple environments. Microbiol. Mol. Biol. Rev. 80, 69–90. https://doi.org/10.1128/MMBR.00046-16 (2016).
30. Boyd, E. F. et al. Post genomic analysis of the evolutionary history and innovations of the family Vibrionaceae. Microbiol. Spectr. 3(5), 1–43. https://doi.org/10.1128/microbiolspec.VE-0009-2014 (2015).
31. Maj, A. et al. Plasmids of carotensoid-producing Paracoccus spp. (Alphaproteobacteria)—Structure, diversity and evolution. PLoS ONE 8(11), 1–27. https://doi.org/10.1371/journal.pone.0080258 (2013).
32. Patric, L. P. J. & Rathinavelan, T. The sanguary arm of Klebsiella species. Front. Cell. Infect. Microbiol. 9, 1–23. https://doi.org/10.3389/fcimb.2019.00367 (2019).
33. Folkesson, A. et al. Adaptation of Pseudomonas aeruginosa to the cystic fibrosis airway: An evolutionary perspective. Nat. Rev. Microbiol. 17, 841–851. https://doi.org/10.1038/nrmicro2907 (2019).
34. Wong, I. S. J. et al. Corynebacterium acicolens-associated pelvic osteomyelitis. J. Clin. Microbiol. 48(2), 654–655 (2010).
35. Gay, N. R., Fleming, E. & Oh, J. Draft genome sequence of Cloacibacterium normanense NRS-1 isolated from municipal wastewater. Genome Announc. 4(6), 1–2. https://doi.org/10.1128/genomeA.01397-16 (2016).
36. Kurlishikov, A. et al. Comparative metagenomic profiling of symbiotic bacterial communities associated with ixodes persulcatus, ixodes pavlovskyi and dermacentor reticulatus ticks. PLoS ONE 10(7), 1–13 (2015).
37. Martínez, M. A. Retrato microbiológico. J. Microbiol. Immunol. Infect. 44(1), 289–295 (2011).
38. Moreno-Feroro, S. K. & Van-Der-Meer, J. R. Genome-wide analysis of Sphingomonas wittichii RW1 behaviour during inoculation and growth in contaminated sand. ISME J. 9(1), 150–165 (2015).
39. Giron, S. Diversidad bacteriana de la garrapata Rhipicephalus (Boophilus) microplus en el ganado bovino del estado de Tamaulipas (Bacterial diversity of Rhipicephalus (Boophilus) microplus tick in cattle of the state of Tamaulipas). (2015). [Thesis] Thesis to obtain the title of Master of Science in Genomic Biotechnology viable (accessed 14 October 2019); https://tesis.ipn.mx/handle/123456789/24552.
40. Jimenez, M., Gasper, M., Carmona, M. & Terio, K. Suidae and Tayassuasidae. Pathol. Wildl. Zoo Anim. 1, 207–228 (2018).
41. Sutherland-Smith, M. Suidae and Tayassuasidae (Wild Pigs, Peccaries). Fowler’s Zoo Wild Anim. Med. 1(6), 588–584 (2018).
42. Bermúdez, S., Meyer, N., Moreno, R. & Artavia, A. NOTAS SOBRE Pecari tajacu (L., Y Tayassu peccari (LINK, 1795) (ARTIODACT- YLA: TAYASSUIDAE) COMO HOSPEDADRES DE GARRAPATAS DURAS (ACARI: IXODIDAE) EN PANAMÁ. Tznocenciæa 20(1), 61–70 (2008).
43. Rodríguez-Vivas, R. L., Quiñones, A. F. & Fragoso, S. H. Epidemiología y control de la garrapata Boophilus en México (Epidemiology and control of Boophilus tick in Mexico). In Enfermedades de Importancia Económica en Producción Animal (Diseases of Economic Importance in Animal Production) (ed. Rodríguez-Vivas, R. L.) 571–592 (McGraw-Hill-UADY, 2005).

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https://doi.org/10.1038/s41598-021-86177-3
46. Duron, O. et al. Evolutionary changes in symbiont community structure in ticks. *Mol. Ecol.* **26**, 2905–2921. https://doi.org/10.1111/mec.14094 (2017).

47. Zhong, J., Jasinskas, A. & Barbour, A. G. Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. *PLoS ONE* **2**, 1–7. https://doi.org/10.1371/journal.pone.0000405 (2007).

48. Gottlieb, Y., Lalzar, I. & Klasson, L. Distinctive genome reduction rates revealed by genomic analyses of two Coxiella-like endosymbionts in ticks. *Genome Biol. Evol.* **7**, 1779–1796. https://doi.org/10.1111/gbe.13425 (2015).

49. Gerhart, J. G., Moses, A. S. & Raghavan, R. A. Francisella-like endosymbiont in the Gulf Coast tick evolved from a mammalian pathogen. *Sci. Rep.* **6**, 1–6. https://doi.org/10.1038/srep33670 (2016).

50. Sjödin, A. et al. Genome characterisation of the genus Francisella reveals insight into similar evolutionary paths in pathogens of mammals and fish. *BMC Genomics* **13**, 1–13. https://doi.org/10.1186/1471-2164-13-268 (2012).

51. Machado-Ferreira, E. et al. Coxiella symbionts are widespread into hard ticks. *Parasitol. Res.* **115**(12), 4691–4699. https://doi.org/10.1007/s00436-016-5230-z (2016).

52. Duron, O. The IS1111 insertion sequence used for detection of *Coxiella burnetii* is widespread in Coxiella-like endosymbionts of ticks. *FEMS Microbiol. Lett.* **362**(17), 1–8. https://doi.org/10.1093/femsle/fnv132 (2015).

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Author contributions
J.R.J. designed, performed the field work, and wrote and approved the final version of the article. G.C.N. performed the field work and wrote and approved the final version of the article. D.L.S. performed the analysis and bioinformatics study and approved the final version of the article. B.D. designed the molecular study and wrote and approved the final version of the article.

Competing interests
The authors declare no competing interests.

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