Bacterial Microbiota Analysis Demonstrates That Tick Can Acquire Bacteria From Habitat and Host Blood Meal

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Research

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Abstract

Exploring the bacterial microbiota is imperative to tick control since it has an important role in tick physiology and vector capacity. The life cycle of ticks consists of parasitic and non-parasitic stages, with a diversity of habitats and host blood meals. Whether and how these factors, such as tick developmental stages, tick organs, habitats and host blood meals affect tick bacterial microbiota is poorly elucidated. In the present study, we investigated the bacterial microbiotas of hard tick *Haemaphysalis longicornis*, their blood meals and habitats using *16S* rRNA high-throughput sequencing. The bacterial richness and diversity in ticks varied depending on the tick developmental stage, feeding status and the tick organs. Results showed that fed ticks present a higher bacterial richness suggesting that ticks may acquire bacteria from blood meals. The significant overlap of the bacteriota of fed ticks and the host blood also support this possibility. Another possibility is that blood meals can stimulate the proliferation of certain bacteria. However, most shared bacteria cannot transmit throughout the tick life cycle, as they were not present in tick eggs. The most shared bacteria between ticks and habitats are genus of *Staphylococcus*, *Pseudomonus*, *Enterobacter*, *Acinetobacer* and *Stenotrophomonas*, some of them are also present in tick organ, suggesting that these environmental bacteria cannot be completely washed away and can be acquired by ticks. As tick reproductive organ, ovary showed the lowest bacterial richness and diversity compared to other organs. The predominant proportion of *Coxiella* in fed females and ovary further demonstrated that this genus is required for *H. longicornis* reproduction system. These findings further reveal that the bacterial composition of ticks is influenced by a variety of factors and will help in subsequent studies of the function of these bacteria.

Introduction

Ticks are obligate blood-feeding ectoparasites, threatening the livestock industry and public health through direct bite and pathogen transmission [1]. In many areas, distributions of ticks and tick-borne diseasesare increasing owing to climate and land-use changes [2, 3]. Ticks also carry various non-pathogenic microorganisms, which received much attention in recent years since it has an important role in tick physiology and vector capaciaty [4, 5]. Among them, C*oxiella*-like endosymbiontis widely distributed and is essential to many tick species as a B vitamin provider [6, 7]. A similar nutritional symbiosis is found in *Francisella*-like endosymbiont and its tick host, *Ornithodorosmoubata* [8]. In *Dermacentor andersoni*, pathogen susceptibility is influenced by tick bacterial microbiota [9]. Overall, tick microbiota's biological roles cannot be ignored in view of tick ecology and evolution.

The life cycle of ticks consists of parasitic and non-parasitic stages, in the parasitic stage, ticks require host blood meals to complete their development, while in the non-parasitic stage, ticks live in a variety of habitats [10]. The bacterial-microbial composition of the tick is inevitably influenced by host and environmental factors. Lines of studies on tick bacterial microbiota suggest that various factors, including tick species [11], tick life stage [12, 13], tick sex [12, 13], tick organ [12] and tick feeding status [14] could shape the bacterial community. Tick bacterial microbiota also vary depending on the sampling season [15], the sampling site [16], the rearing condition [17], and the presence of a pathogen [13].

Obregónet et al. [18] demonstrated that the diverse array of microbiota might give ticks competitive merit in the environment. Experimental studies found that tick gut microbiota can directly mediate tick-borne pathogen colonization and influence their early survival [19]. These findings reveal the complex associations between bacterial microbiotas and their tick hosts and provide new insights into the control of ticks and tick-borne disease. In contrast, bacterial microbiota in the blood meal and habitat of ticks are less well studied, and it is worth investigating whether they are a source of bacteria in the tick and how they affect the microbial composition of the tick.

The hard tick *Haemaphysalis longicornis* is widely distributed in East Asia, Australia, and New Zealand, and carries diverse pathogens that cause diseases in human and wild animals [20–23]. *H. longicornis* is an important vector of the tick species,and have invaded the United States of America [24]. The phylogenetic analysis found that *H. longicornis* population lack clear geographic structuring, which suggests that this species is selected for dispersion rather than local competitiveness [25]. *H. longicornis* is “three-host tick”, and feed on a variety of mammalian species. Each developmental stage (larva, nymph, and adult) except for the egg feeds on a host, and then drops off for development or reproduction in the natural environment [26]. There were several studies on *H. longicornis* bacterial microbiota. For example, Liu et al. [27] found three bacterial symbionts in field-collected *H. longicornis* by diagnostic PCR, and the bacterial microbiotas in *H. longicornis* concerning developmental stages, sex and feeding status were investigated using high-throughput sequencing [28, 29]. However, there are still some concerns to be solved, such as the variation of bacterial microbiota throughout the whole tick life cycle and between tick organs, and whether ticks acquire bacteria from blood meals and habitats.

In this study, the bacterial microbiotas of ticks at different life stages, rabbit blood, tick organs and tick habitat were surveyed using *16S* rRNA high-throughput deep sequencing.

Materials And Methods

Tick collection and sample preparation

In May 2018, the questing ticks of *H. longicornis* were initially collected from vegetation using dragging in the Xiaowutai Natural Reserve Area (N39.97, E115.4) of Hebei Province, the north of China. Ticks were fed on rabbits as previously described [30] and maintained in an environmental incubator (26 ± 1°C, 85 ± 5% relative humity (R.H.), 6-h:18-h L:D photoperiod) (Percival Scientific, Inc., Lowa, USA). Before performing this experiment, these ticks have been reared for four generations in the laboratory. Figure 1 illustrates the samples used for bacterial microbiota analyses. Throughout the life cycle of the tick, samples of freshly laid eggs (Egg 1d, 3 sample pools), ten-day-old eggs (Egg 10d, 3 sample pools), freshly hatched larva (Larva, 3 sample pools), engorged larva (Fed larva, 3 invidual samples), freshly emerged nymph (Nymph, 3 invidual samples), engorged nymph (Fed nymph, 3 invidual samples), freshly emerged adult female (Female, 3 invidual samples), engorged female (Fed female, 5 invidual samples), freshly emerged adult male (Male, 3 invidual samples) and engorged male (Fed male, 1invidual sample) were collected. After the alcohol wipe, the venous blood were collected from the rabbits’s ear using a syringe, and the blood

samples were divided into two groups (blood and bitten blood) according to whether the rabbit was bitten by ticks. Four tick organs, salivary gland (Hereinafter referred to as SG), midgut (Hereinafter reffererd to as MG), malpighian tubule (Hereinafter reffered to as MT), and ovary (Hereinafter reffered to as OV) were dissected from engorged females. The ticks were first cleaned using alcohol, dissected in PBS buffer to obtain individual organs and washed sequentially in PBS, alcohol and sterile water. After washing the tick surface with sterile water, the water was permeabilised to obtain a tick surface sample. Ticks were reared in centrifuge tubes, after the tick was removed, the remaining tick skin and excreta were rinsed with sterile water and the water was permeabilised to obtain a tick habitat sample. Detail information of these samples can be found in Table 1 and Fig. 1.

Table 1
Alpha diversity of observed OTUs in the bacterial microbiotas of blood, environmental, tick and tick organ samples

Sample type	Sample name (N)	Sobs index (± SD)	Shannon index (± SD)
Blood samples	Blood (3)	1763.33 (± 166.86) A	5.87(± 0.23) A
	Bitten blood (3)	1842.33 (± 145.82) A	5.33 (± 0.29) A
Environmental samples	Tick surface (3)	848.67 (± 154.5) A	2.8 (± 0.04) A
	Tick habitat (3)	660.67 (± 57.98) A	2.17 (± 0.07) B
Tick Samples	Egg 1d (3)	136.33 (± 62.7) A	1.97 (± 0.21) BC
	Egg 10d (3)	107.67 (± 33.47) A	1.83 (± 0.25) BC
	Larva (3)	148.33 (± 8.22) A	2.16 (± 0.14) BC
	Fed larva(3)	599 (± 231.34) BC	4.19 (± 0.2) A
	Nymph(3)	228.67 (± 25.69) B	2.38 (± 0.11) B
	Fed nymph(3)	235 (± 52.73) B	1.36 (± 0.42) C
	Female(3)	431.33 (± 18.55) B	1.46 (± 0.25) C
	Male(3)	235.33 (± 47.93) B	2.45(± 0.24) B
	Fed female(6)	908.67 (± 93.84) C	2.05(± 0.31) BC
	Fed male(1)	254	3.39
Tick organ samples	OV(5)	880(± 111.23) A	1.26(± 0.36) B
	MG(3)	1070.33(± 473.75) A	4.11(± 0.91) A
	MT(3)	1111.33(± 342.79) A	3.81(± 1.18) A
	SG(3)	925(± 124.04) A	4.54(± 0.43) A

N, Number of samples. Alpha diversity differences are compared between samples within the same type, and different letters indicate significant differences ($P < 0.05$).

Dna Extraction

Before DNA extraction, tick samples were surface-sterilized in three washes of 70% ethanol, followed by one wash of sterile, nuclease-free, deionized water to avoid contamination from the environment. DNA extraction was performed using a QIAamp Fast DNA Stool Mini Kit (Qiagen, California, USA). The concentration and quality of DNA were measured using Nanodrop 2000 (Thermo Scientific, USA) and 1%

gel electrophoresis detection, respectively. The results showed that the extracted DNA is capable of being used for subsequent experiments.

16S rRNA PCR amplification and sequencing

The barcode-indexed primers (338F: 5'-ACTCCTACGGGAGGCAGCAG-3'and 806R: 5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 region of the bacterial *16S* ribosomal RNA (rRNA) gene, using TransStart®Fastpfu DNA Polymerase (TransGen, Beijing, China). PCRs were performed on GeneAmp® 9700PCR instrument (AppliedBiosystems, USA). As a negative control, sterile water was used as a DNA template for PCR. This primer set, resulting in a 401- to 440 bp PCR products. Amplicons were then purified using AxyPrep DNA GelExtraction Kit (Axygen Biosciences, USA) and were quantified using QuantiFluor-ST (Promega, USA). The purified amplicons were pooled in equimolar concentrations, and paired-end sequencing was performed on the Illumina MiSeqPE300 platform (Illumina, lnc.) (Shanghai Majorbio Bio-pharm Technology Co., Ltd, China) using standard protocols.

Data analysis

The data were analyzed on the free online platform of the Majorbio I-Sanger Cloud Platform (https://cloud.majorbio.com/). MiSeqsequence data were merged and filtered using the Trimmomatic software as previously described [31]. Quality-filtered merged reads were aligned to the Silva database [32] and screened for chimeras using the Uchime algorithm [33]. Sequences with 97% similarity were then grouped into operational taxonomic units (OTUs) using the OptiClust clustering algorithm [34]. The OTU table was processed in Qiime (MACQIIMEver.1.9.0) [35]. OTUs were taxonomically assigned using the RDP Classifier ver. 2.2.0 [36] against the Greengenes *16S rRNA* database ver. 13.5.0 with 70% confidence [37], and out relative abundances were summarized across taxonomic levels from the phylum level to the species level.

Sufficient sequencing depth was determined based on rarefaction curves for the observed number of OTUs from all samples. The number of sequences was normalizated based on the minimum sequence number, and these sequences were used for subsequent analyses. The bacterial composition of each sample was visualized as a bar figure. Two alpha diversity indices, Observed Species and Shannon were calculated to measure bacterial community richness and diversity between groups, respectively using Mothur ver. 1.30.1 [38]. The student's-testand one-way analysis of variance (ANOVA) were used to test whether the two indices are significantly different in the pair-wise comparisons or not. Beta diversity wasexamined using weighted and unweighted UniFrac analysis [39] to compareand to plot the differences among samples using principal coordinate analysis (PCoA). Analysis of similarities (ANOSIM) was also used, with 999 permutations based on the Bray-Curtis index, to determine the percent variation of bacterial composition explained by sample type.

Results

Miseq sequencing data

There were no PCR amplification bands in the negative control, while the total of 57 samples were successfully sequenced (Table 1), resulting in 3,017,655 trimmed reads, and the number of reads per sample ranged from 30,242 to 87, 211 (Table S1, Additional files). Rarefaction curves of the Shannon index of all samples can reach a plateau, suggesting sequencing coverage was sufficient and can be used in subsequent analyses (Fig. S1, Additional files). This result was also supported by an adequate coverage (Good's coverage from 99.7% to 100%) estimate for each sample.

OTU and alpha diversity analyses

In total, 1,283 distinct OTUs were observed in these samples (Table S2, Additional files). Fed male had only one sample, which was excluded from alpha diversity comparison. Fed female had the highest bacterial richness, followed by the fed larva and female, and the difference between fed female and fed larva was not significant (ANOVA F=2.759, df = 8, P=0.177). By comparison, the bacterial richness in other samples was significantly lower (ANOVA F=8.76, df =24, P<0.05) (Table 1). The bacterial richness in bitten blood was higher than that in blood, without significant difference (t = 1.697, df = 3, P= 0.188) (Table 1). Four organs of fed female had similar levels of bacterial richness (ANOVA F = 0.3228, df = 13, P = 0.809) (Table 1). Shannon index was used to evaluate the bacterial diversity in these samples. Fed larva and fed nymph had the highest and lowest bacterial diversity, respectively. Five samples (Egg 1d, Egg 10d, Larva, Nymph, and Male) had intermediate levels of bacterial diversity, and their diversity was significantly different from the above two samples (ANOVA F = 14.45, df = 36, P<0.0001). Female had relatively lower diversity compared to the five samples (Table 1). The bacterial diversity did not differ between blood and bitten blood (t = 3.158, df = 3, P= 0.051) (Table 1). In the fed female, the lowest bacterial diversity was observed in OV, and the difference between it and SG and MG was significant (ANOVA F = 5.302, df = 3, 8, P = 0.026) (Table 1).

Bacterial microbiota composition

The bacterial microbiota was further assigned to 28 phyla, 67 classes, 165 orders, 274 families, 545 genera, and 836 species. At the phylum level, four phyla (Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes) had high relative abundances in all samples. Bacterial microbiota communities varied along with tick development. The relative abundances of Proteobacteria, Firmicutes, and Actinobacteria displayed a decrease or an increase with tick development, while Bacteroidetes was mostly detected in fed ticks. Blood and bitten blood had similar bacterial communities at the phylum level. The relative abundance of bacteria differed between tick surface and tick habitat samples. Additionally, four organs from fed female had distinct bacterial communities. It is worth noting that some phyla with low relative abundance were generally present in the blood, fed tick, and organ samples (Fig. 2A, Table S2, Additional files).

At the genus level, *Staphylococcus* was present in all samples, and in tick samples, its relative abundance firstly increased and then decreased along with tick development. Fed nymph had the highest relative abundance of *Staphylococcus*. Also, this genus showed higher relative abundance in fed ones than unfed ones in immature ticks, while a contrast trend was observed in adult ticks. *Staphylococcus*

also had high relative abundance in environmental samples and bitten blood. Egg and larva had a higher relative abundance of *Pseudomonas* than other life stages; blood meals seemed to decrease its abundance. The relative abundance of *Brevibacterium* increased along with tick development with the exception of fed ticks, in which abundances were very low. *Brevibacterium* was also detected from environmental samples. In addition, the relative abundance of *Kocuria* was high in environmental samples, and this genus was also present in almost all tick samples. In the fed female, *Coxiella* was the dominant bacterium, and its proportion reached to 77.29%. *Alcaligenes* and *Corynebacterium* were mainly present in immature ticks, including egg, larva, and nymph. Many genera, such as *Faecalibacterium*, *Bacteroides*, *Kosakonia*, *Parabacteroides*, *Bifidobacterium*, *Agathobacter*, *Alistipes*, *Lachnospira,* and *Blautia*, were solely present or had higher abundances in fed male. Similarly, high abundances of *Enterococcus*, *Olsenella* and *Prevotella* were detected in fed larva. Bacterial microbiota communities did not differ between blood and bitten blood at the genus level. Bacterial microbiota communities also varied across tick organs, and bacterial abundances differed between them. As a prominent bacterium in the fed female, *Coxiella* was present in four organs and showed abundant in the OV and the MT (Fig. 2B).

In total of 28 OTUs were shared by fed ticks and blood samples (Fig. 3A), suggesting that ticks can acquire bacteria from blood meals, and few shared bacteria can occupy and transmit through tick development as they were present in tick eggs (Fig. 2B, Table S2, Additional files). The most shared OTUs between tick and environmental samples are genera of *Staphylococcus*, *Pseudomonas*, *Brevibacterium*, *Enterobacter*, *Acinetobacer* and *Stenotrophomonas* (Fig. 3B), some of them are also present in tick organ, suggesting that these environmental bacteria cannot be completely washed away and can be acquired by ticks.

The variation of bacterial communities in blood, tick, environmental and organ samples were further evaluated by the PCoA and ANOSIM analyses. The unweighted UniFrac PCoA (which does not account for abundance data) explained 16.02% (PC1) and 10.1% (PC2) of the variation among blood, tick and environmental samples, and blood samples clustered together and were clearly segregated from other samples, however, environmental samples clustered with tick samples. Tick samples were separated according to their developmental stages (Fig. 4A). In addition, the ANOSIM result (R=0.6106, *P*=0.001) also showed significant differences in bacterial composition between samples. The weighted UniFrac PCoA and ANOSIM (R=0.5863, *P*=0.001) obtained a similar result considering the abundance of bacteria (Fig. 4B). For organ samples, bacterial composition showed significant differences between organs when bacterial abundance was considered in the weighted UniFrac PCoA, which explained 52.77% (PC1) and 22.01% (PC2) of the variation (Fig. 4C, R=0.655, *P*=0.002). However, no distinct clustering was observed for each organin unweighted UniFrac PCoA, explaining 18.61% (PC1) and 13.41% (PC2) of the variation (Fig. 4D, R=0.1637, *P*=0.097).

Discussion

In recent years, most studies have focused on the diversity of microbes in ticks and their impact on the biology of tick hosts and the transmission of pathogens, these studies provided a basis for subsequent

tick control efforts through the manipulation of microbiota [40–42]. As an important vector pest, *H. longicornis* carries and transmits various pathogens, including *Borrelia burgdorferi*, *Theileria* spp., *Coxiella burnetti*, *Babesia* spp., *Anaplasma phagocytophilum*, *Ehrlichia* spp., *Bartonella* spp., spotted-fever group rickettsiae, and virus [23]. In early microbiota studies of *H. longicornis* that was collected from Mengyin County of Shandong Province, four pathogens (*Ehrlichia* sp., *Borrelia* sp., *Anaplasma* sp., and *Bartonella* sp.) were identified [28, 29], however, *H. longicornis* in this study was free of pathogens, suggesting the presence of pathogens was influenced by tick strains that were collected from different locations. There were also differences in the microbial composition of *H. longicornis* in this study and between the Mengyin populations, further confirming the effect of sampling location on the microbiota, and the underlying reason is that different populations of ticks have different habitats and host blood meals.

Bacterial richness and diversity varied across the life stages of *H. longicornis*, which is line with previous findings [12, 13, 43]. However, there was no noticeable trend in whether the richness and diversity of bacteria increase or decrease along with tick development. Kwan et al. [13] reported a decreased pattern of the bacterial richness from the larval stage to the adult stage in *Ixodes pacificus*. Fed larva of *H. longicornis* had the highest bacterial richness and diversity, while egg of *H. longicornis* displayed the highest bacterial diversity in another study, without considering fed ticks [29]. Ticks have complex life cycles and the period of off-host is long, therefore the microbiota inhabiting in ticks could be shaped by the environment, and through interactions with blood meals, these influences have been identified in many studies on tick microbiota [13–17]. In this study, blood meals can increase bacterial richness in *H. longicornis*, as some bacteria that were initially inhabiting in blood will enter ticks through blood-feeding, and this result is consistent with previous study [28]. The presence of shared bacteria between fed ticks and blood further supported this possibility. Another possibility is that blood meals can stimulate the proliferation of certain bacteria, for example, *Coxiella* has a high abundance in fed female ticks. However, the effect of blood meals on tick microbial richness varied between tick species, as the study by Kueneman et al. [43] found that host blood source does not explain variation in tick microbial richness.

The bacterial diversity in fed nymph was lower than the unfed nymph, which was due to the extremely high abundance of *Staphylococcus* in the fed nymph. The bacterial richness in female ticks was higher than that in male ticks, but the bacterial diversity of female ticks was lower than that of male ticks. A previous study also highlighted that higher bacterial diversity was present in male *H. longicornis* [28], and differences in bacterial diversity between the sex have also been observed in other tick species [12, 13, 44], although the underlying reason was unclear.

Compared to whole ticks, the bacterial microbiota in tick organs is relatively poorly studied [45]. In the present study, the bacterial microbiotas in four organs of *H. longicornis* were investigated and found similar levels of bacterial richness between organs, however, the bacterial diversity of the ovary was much lower than that of other organs owing to the genus of *Coxiella* is predominate in ovary. A similar result was found in *Ixodes ricinus*, whose ovary had a very low bacterial diversity and a very high and stable bacterial abundance with the dominant endosymbiont, *Midichloria* sp. [45].

The bacterial community showed changes during the development of *H. longicornis*, as Proteobacteria was the most abundant phylum in egg, larva, and fed female, but the dominant phyla changed to Firmicutes and Actinobacteria in nymph and adult ticks, respectively. These findings differed from another study of *H. longicornis*, of which Proteobacteria was always the dominant phylum from egg to adult stage [29]. Additionally, our PCoA results showed that the bacterial community at the genus level was distinct according to the tick development stages. Bacterial communities of egg, larva, and fed female displayed clustered in the weighted UniFrac PCoA, suggesting that the initial bacterial community of the tick was obtained from the mother. Thereafter, the bacterial community appears to change as bacteria were acquired or lost during tick development because each stage of the tick life cycle has different survival strategies and physiological processes. Changes in the composition of these bacteria can reveal the influence of the environment in shaping tick microbiota and explore the distinct bacteria that are necessary for the tick life cycle. The phylum of Bacteroidetes was mostly present in fed ticks, and showed high abundance in the blood. Additionally, some phyla with low relative abundance were generally present in blood and fed ticks. These findings further indicated that ticks could directly acquire bacteria from blood meals. However, most acquired bacteria might be lost with tick development as they cannot be detected in tick eggs. The reason for the lost of the acquired bacteria may be that their entry stimulates the immune system of the tick and is thus removed, or they can not survive in a changed environment [41, 42].

Notably, we detected the bacteria from Burkholderiaceae family in ticks and rabbit blood. These bacteria had large proportions in rabbit blood, fed ticks, and midgut, which suggest that ticks might acquire this bacterium from blood meals. Coenye et al. [46] highlighted that many members of Burkholderiaceae are pathogens to humans and animals, inidcating further studies on its pathogenic role in *H. longicornis* are required.

Many soil or environmental bacterial genera such as *Staphylococcus*, *Brevibacterium*, *Pseudomonas*, *Acinetobacter*, *Corynebacterium*, and *Ralstonia* were observed in tick, blood and environmental samples, suggesting that these bacteria might be acquired from their environments and maintained through the tick life cycle. Other tick studies also found their presence, and there has been ongoing debate as to whether these shared bacteria are environmeantal contaminants or whether they consititute an important part of the intrinsic tick microbiota [12, 19, 41, 47–49]. For example, Lejal et al. [50] used multiple controls in tick microbiota analysis and found that approximately 50.9% of the amplified sequences were considered contaminants. In these above studies, ticks were generally washed with alcohol, while a study by Binetruy et al. [51] confirmed that the surface sterilization method is not effective in removing tick cuticular bacteria. The presence of these bacteria may be due to inadequate sterilization or they may indeed have entered the tick from a blood meal or habitat, and microbiota survey in tick midgut supported for the bacterial acquisition hypothesis [12, 52].

Both analyses of bacterial community and PCoA showed that the bacterial composition of the ovary was obviously different from that of other organs. *Coxiella* is the most abundant bacterial genus in the ovary, and its presence can inhibit other bacteria. In previous study by Liu et al. [27], *Coxiella* was transmitted

stably in the laboratory-reared *H. longicornis*, while two other symbionts (*Arsenophonus* and *Rickettsia*) that present in field-collected ticks were lost from the ticks in laboratory.In this study, *Arsenophonus* and *Rickettsia* were not observed, further suggesting that they are unstable transmitted in *H. longicornis*. The stable transmission and predominant proportion of *Coxiella* make it as an obligate symbiont and is essential for the reproduction of *H. longicornis* [53]. *Coxiella*'s essential role in tick reproduction was also found in other tick species [54–56]. Further studies indicated that *Coxiella* provides the host with essential nutrients that are lacking in the blood [57, 58]. In the life cycle, *H. longicornis* will begin ovarian development and oviposition after engorgement, and in this process, *Coxiella* rapidly proliferates to meet the reproductive needs of ticks. The relatively low abundance of *Coxiella* at other stages of *H. longicornis* is partly due to the lack of proliferation of the bacterium and partly to the presence of relatively high abundance environmental bacteria, which resulted in the bacterium not being fully amplified in the analysis. Another interesting finding was that *Coxiella* was also present with high abundance in malpighian tubule, although its role in malpighian tubule is not yet clear. We also found the presence of *Coxiella* in environmental samples and whether they are excluded from the body via malpighian tubule needs to be further investigated.

Conclusions

The present study systematically explored the bacterial microbiome in ticks and their habitats and blood meals, and found that bacterial composition varies with tick developmental stage and with different organs of the tick. The presence of shared bacteria between ticks and habitats and blood meals suggests that ticks acquire bacteria from the environment. These findings have important implications for revealing the relationship between the tick and bacterial microbiota and for tick control.

Abbreviations

16S rRNA
16S ribosomal RNA; PCR:polymerase chain reaction; OTU:operational taxonomic unit; ANOVA:one-way analysis of variance; PCoA:principal coordinate analysis; ANOSIM:analysis of similarities.

Declarations

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Ethics approval and consent to participate

The animal study complied with the guidelines for ethical review of experimental animal welfare GB/T 35892-2018 and were approved by the Experimental Animal Ethics Committee of Hebei Normal University.

Consent for publication

Not applicable.

Availability of data and materials

The raw data are available on the NCBI Sequence Read Archive (SRA) archive under the accession number PRJNA661974.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SSL,XYZ, JZL and YKZ developed and wrote the manuscript. XJZ and KLC collected the samples. AM provided critical reviews and suggestions for the content. All authors read and approved the final manuscript.

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Figures

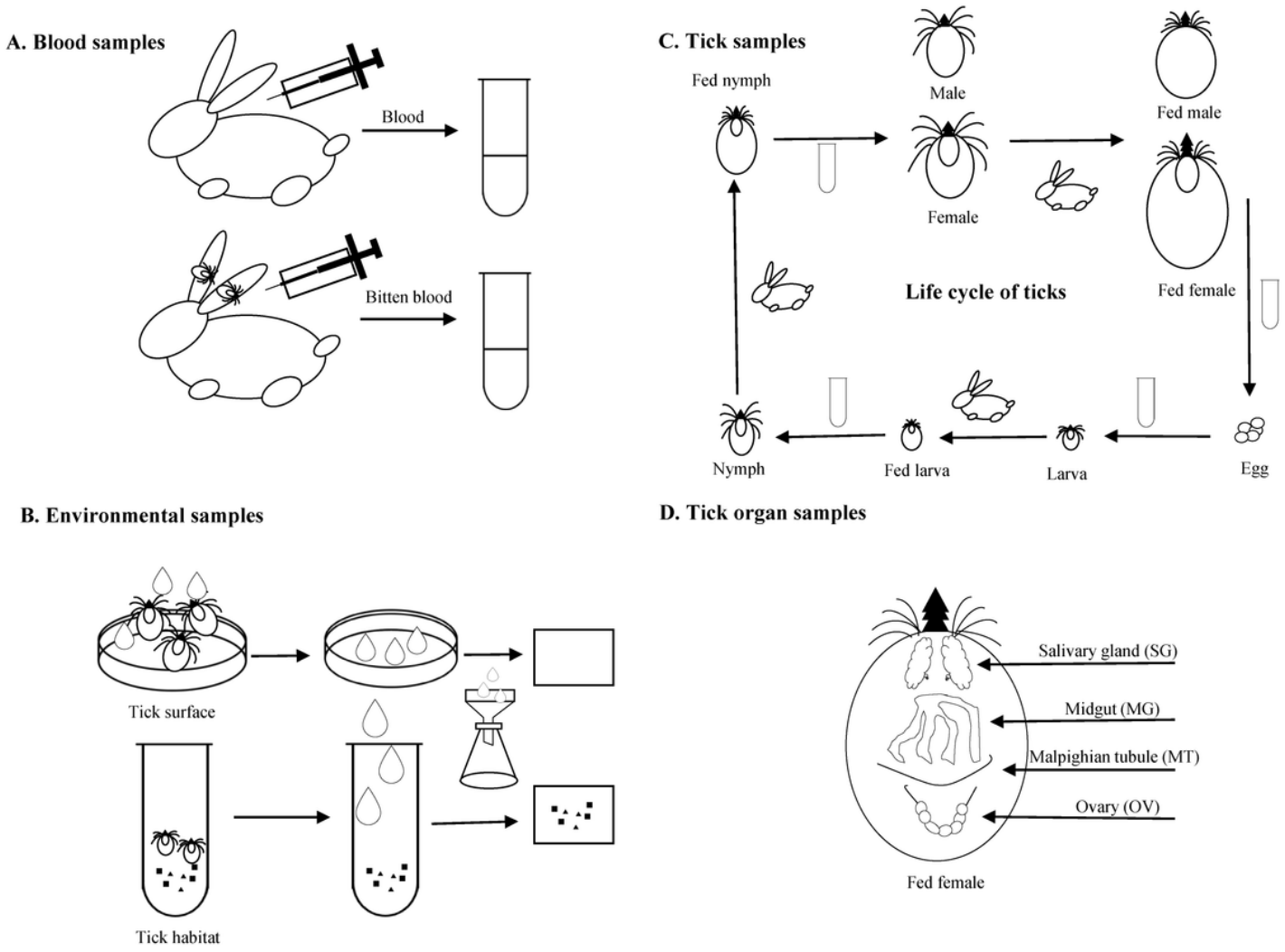

Figure 1

Information on the samples used for bacterial microbiota analysis in this study. A Blood samples. B Tick samples. C Environmental samples. D Tick organ samples.

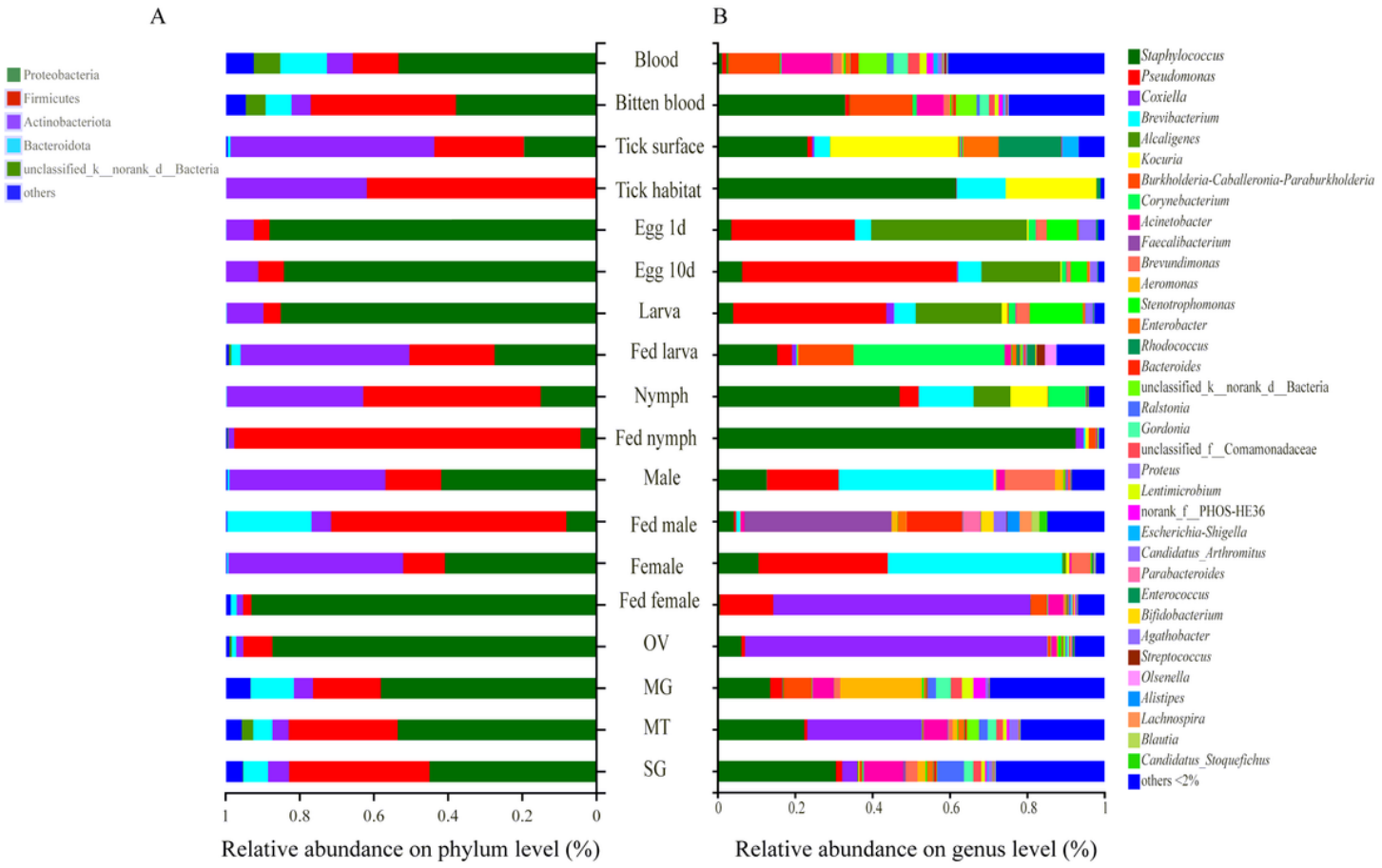

Figure 2

Relative abundance of the bacterial microbiotain tick, tick organ, rabbit blood and environmental samples. A Phylum level, B Genus level.

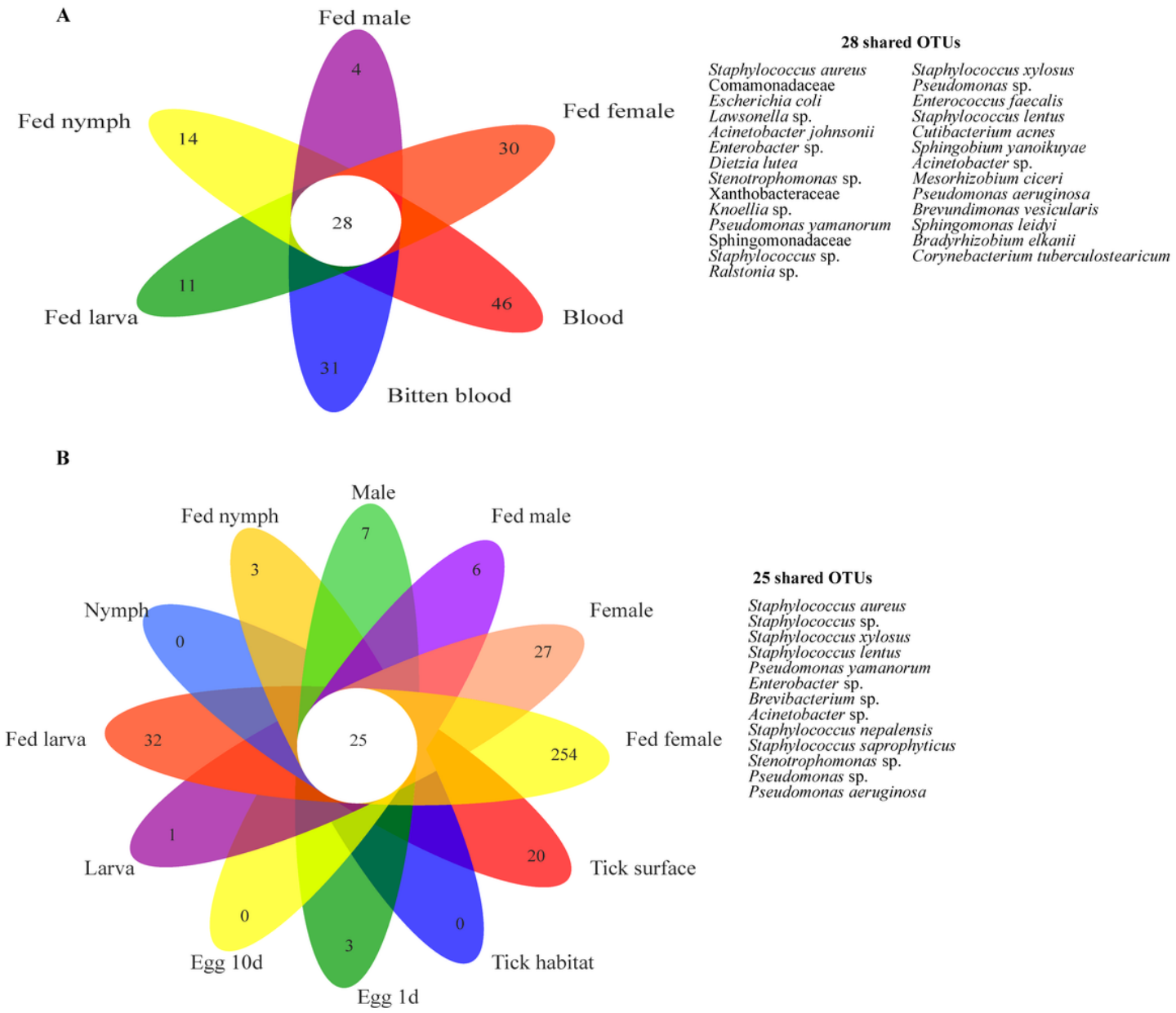

Figure 3

Venn diagramsof unique and shared OTU in different samples, information on the shared OTU is shown on the right. A Fed tick and blood samples. B Tick and environmental samples.

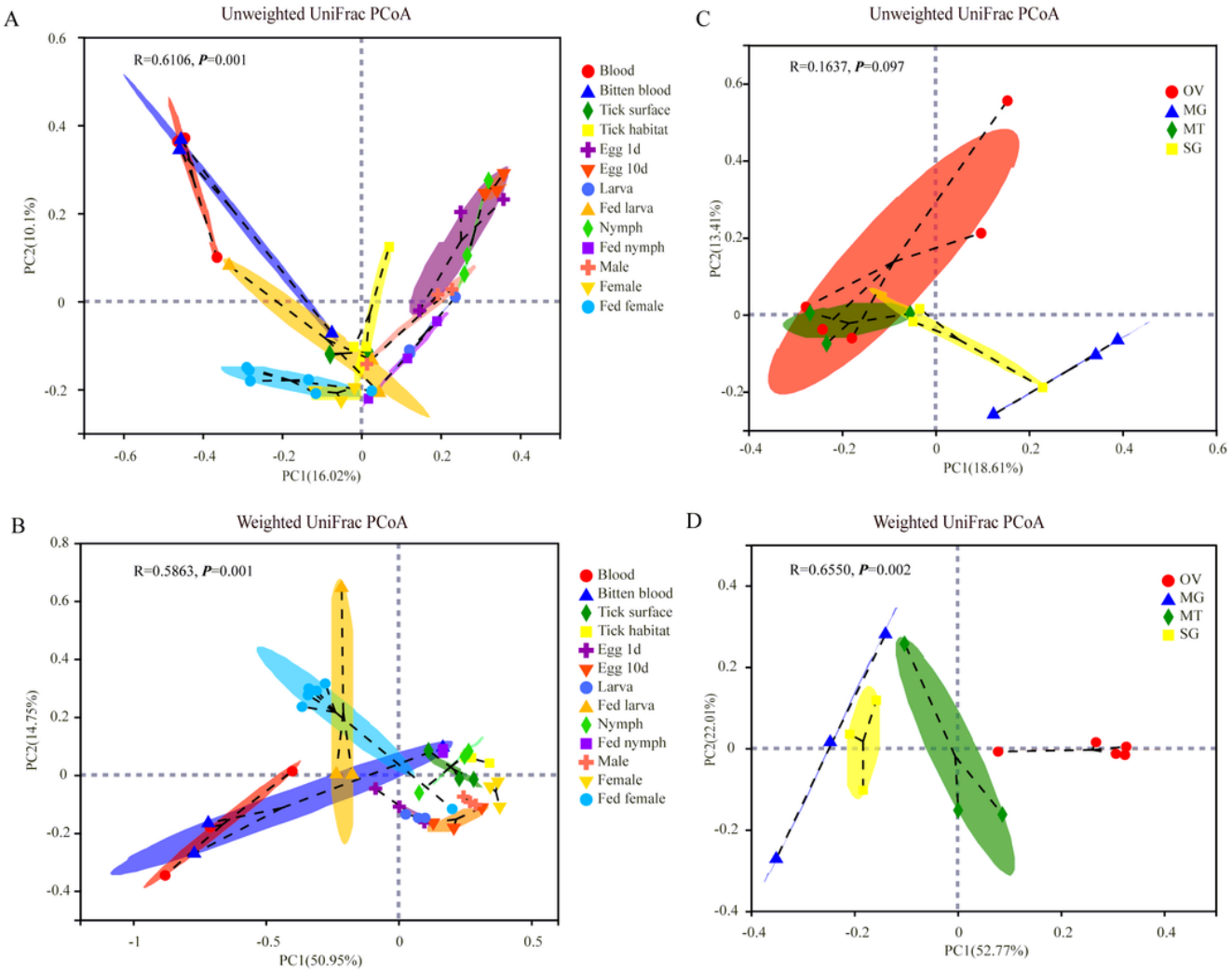

Figure 4

PCoA results of the bacterial microbiotas intick, tick organ, rabbit blood and environmental samples. PCoA plots of weighted UniFrac distances (A) and unweighted UniFrac distances (B) for tick, blood and environmental samples. PCoA plots of weighted UniFrac distances (C) and unweighted UniFracdistances (D) for tick organ samples.

Supplementary Files

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