High-throughput sequencing to detect the bacterial microbiome in Ixodes granulatus in China

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Abstract

Background

Ixodes granulatus is of significant research value as the primary vector of Lyme disease in Asia, yet the bacterial community structure and diversity carried by these ticks have not been reported so far. Part of bacteria in ticks can affect tick development and the transmission of tick-borne pathogens. With the emergence of new pathogens, it is necessary to identify the bacterial microbiome carried by *I. granulatus*.

Methods

Ixodes granulatus were collected from rodent animals in the Binchuan, China. The bacterial DNA was extracted from non-engorged adult female *I. granulatus*. Sequencing of the V3-V4 regions of the 16S rRNA genes was performed using the Illumina NovaSeq sequencing platform. Initial data was assembled in FLASH, QIIME, and UCHIME algorithm. Uparse and MUSCLE software were used to annotate and analyze the effective data.

Result

The predominant phyla in all samples were Firmicutes, Proteobacteria, Actinobacteria, and Spirochaetota. The major genera were *Spiroplasma*, *Staphylococcus*, *Candidatus-Lariskella*, *Corynebacterium*, *Ralstonia*, *Borreliella* (*Borrelia*), *Vibrio*, *Bacillus*, and *Listeria*. *Staphylococcus xylosus*, *Ralstonia pickettii*, *Corynebacterium mastitidis*, *Bryopsis hypnoides*, and *Vibrio metschnikovii* were dominant bacterial species. The results demonstrated that there were discrepancies in bacterial microbiome richness and diversity among different individual ticks of the same species, and found a large number of pathogenic bacteria and opportunistic pathogens, yet *Coxiella* and *Rickettsia* were not detected.

Conclusion

The results presented here speculate that *Spiroplasma* is the endosymbiont of *I. granulatus* and competitively inhibits *Borrelia*. Our study suggested that Binchuan is at risk of Lyme disease transmission and needs to be taken seriously. These findings may serve fundamental knowledge for developing novel strategies to control ticks and their transmission of diseases.

Background

Ticks are second only to mosquitoes as vectors for the transmission of various pathogens. And as obligate blood-sucking ectoparasites, they cause the threat to human and animal health wherever they live, while actually all human tick-borne diseases are zoonoses, such as Lyme disease, *Babesiosis*, Q-fever, tick-borne encephalitis, etc [1, 2]. A variety of ticks and tick-borne diseases are prevalent in China, and a tick may carry either a specific single pathogen or multiple pathogens [3, 4]. *Ixodes granulatus* is widely distributed in many provinces in China (Yunnan, Taiwan, Hubei, Zhejiang, Fujian) and other Southeast Asian countries (Malaysia, Laos, Thailand, Korea, Japan) [5–8]. *I. granulatus*, a parasitic tick common discovery of small mammals, attacks the overwhelming majority of rodent animals, for instance *Apodemus agrarius*, *Apodmus chevrieri*, *Rattus tanezumi*, *Rattus norvegicus*, *Niviventer fulvescens*, *Niviventer niviventer*, *Eothenomys melanogaster*, etc [9]; and they main regions live in mountain forest and plain grassland.

Ixodes granulatus, as a three-host tick, isn't moulting on the host and change hosts three times during the whole development stage from egg to adult, so they spread more widely and the tick-borne pathogens spread more quickly [1]. Its hosts are widely distributed and most of their home ranges are often in close contact with humans and domestic animals. *I. granulatus* is an important potential vector for the transmission of zoonotic diseases, which can carry a number of pathogens including *Borrelia*, *Ehrlichia*, *Babesia* and spotted fever group *Rickettsia* [10–13]. The ticks are capable of biting humans and animals in the three stages (larvae, nymph, adult) and transmitting pathogens, which cause an enzoonotic cycle between *I. granulatus* ticks and rodent hosts in the natural foci. As countries increasingly trade and travel more frequently, some pathogens are prone to cross over. The study reported that Lyme disease *Borrelia* species in northeastern China are similar to the *Borrelia* species isolate from Russia and Japan [14]. Lyme disease spirochetes, widely distributed in the Americas, Europe and parts of Asia, are the essential tick-borne pathogen and can cause serious damage to human body when it infects people [15]. In China, Lyme disease is the second most common tick-borne disease, carried by approximately 13 tick species [3]. At present, the hard ticks of *I. persuleatus* and *I. granulatus* have been considered to the main vector for the transmission of *Borrelia* spp. spirochetes in the North and South China, respectively [16–19].

In general, most microorganisms are accurately identified by culture-dependent approach. However, some microbes with complex and harsh culture conditions are difficult to be obtained by culturing. With the fast and high-efficiency development of sequencing technology, major breakthroughs have been made in the field of microbiology, including the timely and rapid discovery of emerging and re-emerging pathogens. Previous studies have used Next-generation sequencing describing the bacterial diversity within wildlife ticks and identifying the three novel *Anaplasmataceae* species [20]. The bacterial community of the three tick genera were found a 14.3% infection rate with pathogenic bacteria by metagenomics analysis [21]. At present, related bacterial community structure and diversity in the whole tick, four stages, midgut and salivary gland of *I. granulatus* ticks have not yet been reported.

In the present study, we performed paired-end sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene using the Illumina NovaSeq sequencing platform. To investigate the abundance and diversities of bacteria communities in twelve adult female *I. granulatus* collected from rodent animals in the county of Binchuan in Yunnan province, China. Furthermore, the possible pathogens carried by this tick were identified to establish a strategy for preventing and controlling the ticks and tick-borne diseases in this region.

Methods

Tick collection and DNA extraction

Forty-five tick samples were collected from the body surfaces of rodent animals in Binchuan, Dali City, Yunnan Province (25º83'N, 100º57'E), China, in July 2021. All ticks were immediately preserved in 95% ethanol and stored at − 80 ◦C for further processing. The collected ticks were identified as *I. granulatus* based upon the key morphological characteristics [22, 23]. A total of 21 *I. granulatus* were classified. The 12 non-engorged adult female *I. granulatus* were used for this study.

In order to remove debris and disinfect the environment, the body surface of ticks was washed thrice in 75% ethanol for 5 min followed by sterile deionized water. Total genome DNA was extracted using the modifed cetrimonium bromide (CTAB) method. DNA concentration and purity was monitored on 1% agarose gels. DNA was diluted to l ug/μL using sterile water on the basis of concentration. All of the extraction procedures were conducted in a biosafety cabinet to ensure the samples protection from environmental contamination.

Library preparation and sequencing

16S rRNA genes of distinct the V3 and V4 regions were amplified used specific primer (forward primer 5 -CCTAYGGGRBGCASCAG-3 and reverse primer 5 -GGACTACNNGGGTATCTAAT-3) with the barcode. All PCR reactions were carried out with 15 μl of Phusion® High-Fidelity PCR Master Mix (New England Biolabs) and 10 ng of template DNA, and 0.2 μM of forward and reverse primers. Thermal cycling consisted of initial denaturation at 98 ℃ for 1 min, followed by 30 cycles of denaturation at 98 ℃ for 10 s, annealing at 50 ℃ for 30 s, and elongation at 72 ℃ for 30 s, and a final elongation at 72 ℃ for 5 min [24]. The same volume of 1× loading buffer (contained SYB green) was mixed with the PCR products and operated electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios. Then, the mixture PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The quality library was analyzed using the Agilent Bioanalyzer 2100 system and assessed on the Qubit@2.0 Fluorometer (Thermo Scientific). At last, the library was prepared and sequenced on an Illumina NovaSeq platform of the Personalbio Biotechnology Co., Ltd. (Shanghai, China), and 250 bp paired-end reads were obtained.

Data analysis

Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH (VI.2.7,http://ccb.jhu.edu/software/FLASH/) [25], a very fast and accurate analysis tool, which was designed to merge paired-end reads when at least some of the reads overlap the read generated from the opposite end of the same DNA fragment, and the splicing sequences were called raw tags. Quality filtering on the raw tags was performed under specific filtering conditions to obtain the high-quality clean tag [26] according to the QIIME(V1.9.1, http://qiime.org/scripts/split libraries fastq.html) [27] quality controlled process. The tags were compared with the reference database(Silva database, https://www.arb-silva.de/) using the UCHIME algorithm (UCHIME Algorithm,http://www.drive5.com/usearch/manual/uchime_algo.html) [28] to detect chimera sequences and then remove chimeric sequences [29], obtain effective Tags.

All effective tags were clustered by Uparse software (Uparse v7.0.1001, http://www.drive5.com/uparse/) [30]. Afterwards, operational taxonomic units (OTUs) were sorted as sequences with 97% identity. The OTUs sequences were annotated and analyzed by the Mothur method and SSUrRNA database of SILVA138.1 (http://www.arb-silva.de/) [31, 32] (with a threshold of 0.8-1), to obtain species composition of each sample at each taxonomic levels. Next, multiple sequence alignment of OTUs were performed using MUSCLE (Version 3.8.31, http://www.drive5.com/muscle/) [33] software, and finally the least amount of data in the sample was used as the standard for homogenization. Alpha diversity and Beta diversity were calculated using QIIME (Version 1.9.1) and visualized in R software (Version 2.15.3).

Result

Species annotation

Three group 12 samples were used for sequencing (I.G.1 - I.G.12) in Table 1. A total of 954,137 tags were generated by Illumina NovaSeq platform. After the quality filtering and chimera removal, 895,074 effective tags were obtained for subsequent analysis, and 69,822 – 83,632 tags were got per sample. In order to study the bacterial community structure in *Ixodes granulatus*, the effective data was clustered with Operational Taxonomic Units (OTUs) with 97% identity, and then the sequences of OTUs were annotated by species. There were 17 OTUs shared by all the samples, and 452, 989, 234, 307, 108, 1,813, 604, 155, 804, 1,340, 620 and 315 OTUs were unique to I.G.1 - I.G.12, respectively (Fig. 1). The species annotation of bacteria were classified into Two kingdoms, 34 phyla, 76 classes, 149 orders, 203 families, 302 genera, and 130 species.

Table 1
Data quality control for 16S V3-V4 regions of each sample

Sample[a]	Raw Tags[b]	Clean Tags[c]	AvgLen(nt)[d]	Q20[e]	Q30[f]	GC[g](%)	Effective[h](%)
I.G.1	78,984	73,805	416	97.68	92.99	47.18	90.79
I.G.2	80,648	73,991	398	98.03	93.85	48.89	88.62
I.G.3	81,852	75,766	422	97.85	93.42	47.21	90.21
I.G.4	80,274	76,780	422	97.67	92.97	46.98	95.01
I.G.5	74,934	71,952	405	97.42	92.55	47.16	89.21
I.G.6	79,865	73,901	383	97.61	93.07	50.64	80.84
I.G.7	77,173	70,772	415	97.72	93.12	49.05	89.33
I.G.8	86,570	83,632	419	97.81	93.34	47.06	88.42
I.G.9	78,851	73,475	407	97.75	93.19	48.17	91.13
I.G.10	74,300	69,822	395	97.55	92.81	48.37	78.95
I.G.11	82,314	75,452	414	97.88	93.50	47.77	87.71
I.G.12	78,372	75,726	421	97.82	93.29	46.71	95.62

[a] Sample name

[b] The tags obtained the computer

[c] Effective data

[d] The average length of Effective data

[e] The percentage of Effective Data with base quality values > 20 (sequencing error rate < 1%)

[f] The percentage of Effective Data with base quality values > 30 (sequencing error rate < 0.1%)

[g] GC base content in Effective Data

[h] Percentage of Clean Tags and Raw Tags

Relative abundance of bacterial

The most abundant phyla were Firmicutes (relative abundance 63.6–96.2%), Proteobacteria (0.5–26.9%), Actinobacteria (0.1–4.3%), Spirochaetota (0.1–3.7%), which accounted for more than 92% of total micropopulation and were present in the twelve samples. And the other less abundant phyla in the 10 top included Cyanobacteria (11/12), Chloroflexi (11/12), Bacteroidota (12/12), Acidobacteriota (9/12) (Fig. 2). Firmicutes was the highest relative abundance and the widest variation range in all sample, which was the predominant phyla of *I. granulatus*. Archaea were detected at the phylum level, which were Euryarchaeota (5/12), Crenarchaeota (9/12), Nanoarchaeota (1/12) respectively, whereas their relative abundance was less than 0.1% (Additional file: Table S1).

At the genus level, the most represented bacterial taxa were *Spiroplasma* (relative abundance 75.8%), *Staphylococcus* (10.1%), *Candidatus-Lariskella* (2.6%), *Corynebacterium* (0.5%), *Ralstonia* (0.3%), *Borreliella**Borrelia* (0.5%), *Vibrio* (0.3%), *Bacillus* (0.2%), and *Listeria* (0.1%) in Fig. 3. Seventeen genera were present in all 12 samples and shown in Additional file: Table S2. These were *Spiroplasma*, *Staphylococcus*, *Corynebacterium*, *Borreliella*, *Vibrio*, *Sphingomonas*, *Shigella*, *Lactobacillus*, *Halomonas*, *Faecalibacterium*, *Romboutsia*, *Methyloversatilis*, *Bacteroides*, *Pseudomonas*, *Blautia*, *Ruminococcus*, and *Roseburia*. Numerous bacterial genera were found in the present study, some of which were associated with the soil or environment and the pathogenic to humans or animals. *Coxiella* and *Rickettsia* were not detected in all samples.

In total, 130 bacterial species were annotated in the all samples. The relative abundance of the seven common species and the other species were shown in Table 2 and Additional file: Table S3, respectively. Of these, *Staphylococcus xylosus*, *Ralstonia pickettii*, *Corynebacterium mastitidis*, *Bryopsis hypnoides*, *Vibrio metschnikovii*, *Sphingomonas faeni*, *Lactobacillus fermentum*, *Enterococcus faecalis*, *Escherichia coli*, and *Halomonas hydrothermalis* were dominant bacterial species. The seven species were present in each of the 12 samples, namely, *Staphylococcus xylosus*, *Sphingomonas faeni*, *Escherichia coli*, *Halomonas hydrothermalis*, *Faecalibacterium prausnitzii*, *Romboutsia ilealis*, and *Ruminococcus* sp. N15.MGS-57.

Table 2
Relative abundance of the seven common bacterial species in the 12 samples of *Ixodes granulatus*

Bacterial species	Relative abundance									
	I.G.1	I.G.2	I.G.3	I.G.4	I.G.5	I.G.6	I.G.7	I.G.8	I.G.9	I.G.10
Staphylococcus xylosus	0.0138503	0.0226641	0.0186048	0.0108622	0.0000188	0.0348042	0.3990453	0.0001128	0.0428287	0.01505
Sphingomonas faeni	0.0001879	0.0001315	0.0002067	0.0000940	0.0054499	0.0006953	0.0000940	0.0097346	0.0000940	0.00020
Escherichia coli	0.0001503	0.0008269	0.0026310	0.0016913	0.0003383	0.0001315	0.0003383	0.0004510	0.0037961	0.00381
Halomonas hydrothermalis	0.0000564	0.0000564	0.0000376	0.0000376	0.0018417	0.0000376	0.0000376	0.0038713	0.0000188	0.00007
Faecalibacterium prausnitzii	0.0004698	0.0026498	0.0023303	0.0009021	0.0001315	0.0012215	0.0005638	0.0001128	0.0008645	0.00088
Romboutsia ilealis	0.0005826	0.0009021	0.0003571	0.0000188	0.0000752	0.0009584	0.0011652	0.0001128	0.0000188	0.00242
Ruminococcus sp. N15.MGS-57	0.0001691	0.0001503	0.0000376	0.0003195	0.0000564	0.0001691	0.0006014	0.0000752	0.0000940	0.00007

Cluster analysis of relative abundance

The abundance information of the predominant 35 genera for 12 samples were exhibited in the heat map (Fig. 4). According to the abundance information in each sample, clustering was carried out from the species level, which was convenient to discover which species in which samples clustered more or less. The results showed that the bacterial community of the *I. granulatus* in different individuals were different. *Hoeflea* and *Marivita* were highly abundant in I.G.1; *Streptococcus*, *Candidatus-Lariskella*, *Agathobacter*, and *Subdoligranulum* were highly abundant in I.G.2; *Lactobacillus* was highly abundant in I.G.3; *Dubosiella* and *Borreliella* were highly abundant in I.G.5; *Prevotella-9* was highly abundant in I.G.6; *Halomonas*, *Sphingomonas*, and *Vibrio* were highly abundant in I.G.8; *Bacillus*, *Limosilactobacillus*, *Salmonella*, *Corynebacterium*, *Listeria*, and *Enterococcus* were highly abundant in I.G.9; *Holdemanella* and *Ralstonia* were highly abundant in I.G.10; *Alistipes* was highly abundant in I.G.11. Moreover, the abundance of *Spiroplasma* was lowest in I.G.6; *Staphylococcus* was higher in I.G.6 and I.G.7; *Bacteroides* was higher in I.G.7 and I.G.11; *Escherichia-Shigella* was higher in I.G.9, I.G.10, and I.G.11; *Klebsiella* was higher in I.G.3 and I.G.9.

Alpha diversity analysis

The gradual flattening of the rarefaction curves in each sample indicated sufficient sequencing depth for subsequent analysis (Fig. 5). The alpha diversity indices, including the Chao1, shannon and Good's coverage indices, were used to assess the bacterial richness and diversity. The Good's coverage for each sample was more than 99%, indicating that the current 16SrDNA sequencing volume in this study was sufficient to satisfy the bacterial diversity of *I. granulatus*. The Shannon diversity index and Chao1 richness index were significantly different in the different individuals, and indicated that the I.G.6 had the uppermost bacterial diversity and richness, while the I.G.12 and I.G.5 appeared the lowest diversity and richness, respectively. In addition, the results exhibited exceedingly unever in Simpson evenness index among the samples (Table 3).

Table 3
Alpha diversity indices of the 12 samples of *Ixodes granulatus*

Sample	Shannon	Simpson	Chao1	ACE	Number of OTUs	Good's coverage
I.G.1	1.221	0.210	954.851	1024.962	806	0.995
I.G.2	2.625	0.599	1623.600	1710.895	1429	0.992
I.G.3	1.372	0.254	638.922	713.342	564	0.997
I.G.4	0.779	0.128	705.710	788.454	580	0.996
I.G.5	1.518	0.445	310.400	334.777	257	0.998
I.G.6	3.261	0.676	2461.262	2492.665	2252	0.990
I.G.7	1.862	0.565	1003.926	1063.816	882	0.995
I.G.8	0.951	0.198	388.281	443.524	320	0.998
I.G.9	2.074	0.425	1186.188	1215.587	1022	0.994
I.G.10	2.154	0.359	1822.191	1854.511	1666	0.992
I.G.11	1.742	0.280	1281.864	1350.809	1096	0.994
I.G.12	0.671	0.107	638.922	698.701	560	0.997

Community difference analysis

In order to research the similarity between different individuals, Unweighted Pair-group Method with Arithmetic Mean (UPGMA) was carried out to build a cluster tree of samples (Fig. 6a). The Principal Coordinates Analysis (PCoA) based on Unweighted Unifrac also showed 15.0% (PC1) and 13.0% (PC2) of the similarity and clustering separation among twelve samples of *Ixodes granulatus* (Fig. 6b). There were some differences in bacterial community between different individuals of the same species. Moreover, the bacterial communities of the I.G.5 and I.G.8 were similar, while the community structure was significantly different with that of the remaining 10 samples.

Discussion

As three-host tick, *Ixodes granulatus* change hosts three times throughout their development and therefore acquire many different bacteria form the environment and hosts or even from other co-hosts vectors [1, 13]. Similarly, ticks can be infected with pathogens they never transmitted and then as new vectors for those pathogens that assist the indirect transmission of infected vecters to new vectors [34, 35]. Rodent animals, with a great variety ectoparasites, are widely distributed and intimate contact with human. *I. granulatus* is a type of tick that parasitize the rodent animals and thus was able to become a new vector for numerous unknown pathogens. To the best of our knowledge, no study has reported bacterial microbiome structure and diversity of the whole *I. granulatus* ticks. Accordingly, it is imperative to study the bacterial microbiome composition and diversity of *I. granulatus* to make up for the lack of research data and further control tick-borne diseases.

To our knowledge, this study is the first to investigate the bacterial microbiome of the female adult *I. granulatus* ticks by high-throughput sequencing technology. In the present study, the bacterial microbiome in the *I. granulatus* was 16S rRNA genes of distinct the V3 and V4 regions by Illumina NovaSeq platform. The result found that six bacterial phyla, 17 genera and seven species existed in all of the 12 samples. The data proved that the uniformly dominant phyla of Firmicutes, Proteobacteria, and Actinobacteria, were in agreement with previous studies [36–41]. Nevertheless, there are some discrepancies with other previous study in which Proteobacteria was the predominant phylum with high relative abundance in all samples. Firmicutes with higher relative abundant was the dominant bacterial phylum due to the large number of *spiroplasma* and *staphylococcus* present in all samples.

Through the analysis of the diversity of bacterial microbiome carried by *I. granulatus*, it was found that there were plentiful pathogenic bacteria and opportunistic pathogens, such as some species of *Staphylococcus*, *Corynebacterium*, *Sphingomonas*, *Enterococcus*, *Shigella*, *Salmonella*, *Bacteroides*, *Klebsiella*, *Pseudomonas*, and *Acinetobacter*, which can cause disease in humans and animals under certain conditions. *Mycoplasma* was detected in only one set of samples. However, most genera were prevalent in soils and the environment, including *Bacillus*, *Marivita*, *Streptococcus*, *Streptomyces*, *Brevibacterium*, *Micrococcus*, *Arthrobacter*, *Gordonia*, and *Rhodococcus*, albeit in low relative abundance. Prior studies indicated that ticks could obtain some of their bacteria from the environmental soil system [42–45]. Furthermore, *Lactobacillus*, *Faecalibacterium*, *Romboutsia*, *Blautia*, *Ruminococcus*, and *Roseburia* were common in the intestinal core bacterial genera all detected in 12 samples. This study confirmed the existence of Archaea based on 16S rRNA high-throughput sequencing, nonetheless, the relative abundance was low, mainly *Methanobacterium*, *Methanobrevibacter*, and Nitrososphaeraceae.

Tick, as a momentous vector of multiple pathogens, the bacterial community is usually composed of pathogenic and non-pathogenic microorganisms. Specific bacterial microbiome plays essential roles in tick reproductive, vector competence, and pathogen transmission [46–49]. One previous study reduced vector competence in *Dermacentor andersoni* by the microbiome manipulation, demonstrating that the relationship of endosymbionts and pathogens had different interactions [50]. Therefore, manipulation of the tick microbiome can provide a new idea for biocontrol of tick-borne diseases in the future [51]. Until now, the relationship between tick microbiome and pathogen interactions and the factors that determine bacterial community variations are inadequacy understood. Current possible factors include: tick life stage, species, and sex [52–55], geographical origin [56, 57], environment [58–61], host blood meal [62–64], and engorgement status [65, 66], have been reported in previous studies. The above-mentioned driving factors ultimately lead to variation in tick microbiome, and there are certain differences in the composition and diversity of the bacterial community between the tick individuals. In the present study, the dominant bacterial genera in the twelve samples were consistent, yet there were significant differences in their relative abundance and bacterial microbiome diversity.

Spiroplasma is a maternally inherited endosymbiont described from many arthropods, including a variety of ticks [67]. The *Spiroplasma* genus establish commensal, mutualistic and pathogenic relationships with arthropods and plants [68, 69]. Thus far, *Spiroplasma* as a male-killing phenotype is discovered in arthropod hosts like Drosophila [70]. Nevertheless, *Spiroplasma* is commonly considered as insect symbiotes [71, 72], and its functional role in ticks is still unclear. In fact, some findings indicate that *Spiroplasma* can act as a defense symbiote in ticks and have a defensive response mechanism against of pathogens [73, 74]. Mutual tick symbionts include *Coxiella*, *Francisella*, *Candidatus Midichloria*, *Rickettsia*, *Spiroplasma*, *Wolbachia*, and *Arsenophonus* [75], and there are co-infection and interaction between symbionts [76, 77]. In this study, *Spiroplasma* was detected in *I. granulatus* with a high relative abundance, suggesting it may be an endosymbiot of *I. granulatus*. The high relative abundance of *Spiroplasma* may had inhibited the survival of other tick symbionts, while few other symbionts were detected in *I. granulatus* ticks. Consequently, it's necessary to further investigation the interaction between *Spiroplasma* and tick host and other bacteria.

Borrelia (*Borreliella*) causes human Lyme disease, which circulates between ticks and their hosts through horizontal transmission [78, 79]. *Borrelia* is transmitted among rodent animals and mammals by hard ticks of the genus Ixodes, mainly *Ixodes scapularis*, *I. ricinus*, *I. pacificus*, *I. persulcatus*, and *I. granulatus* [10, 80, 81]. Previous studies have shown that *Borrelia* may be impressionable inhibition by competitor bacteria, and there may be a potential antagonistic interaction between *Borrelia* and endosymbiont *Rickettsia* [82, 83]. There is little information about the interactions between *Borrelia* and other bacteria. Whereas, only Rickettsiales was detected, not *Rickettsia* during this study, and its interaction could not be proved. In the present study, *Borrelia* was detected in each samples with low relative abundance. However, due to the defects of experimental methods and techniques, the specific species of *Borrelia* could not be identified. We hypothesized that *Borrelia* might be competitively inhibition by *Spiroplasma* bacteria. The results suggested a risk of Lyme disease transmission in this area. From the point of view of competitor bacteria to prevent pathogen transmission in the future.

Conclusion

This is the first bacterial community and diversity study for individual *Ixodes granulatus* ticks from rodent animals in Yunnan province,China, using the high-throughput sequencing method. *Staphylococcus*, *Sphingomonas*, and *Pseudomonas* were potential opportunistic pathogenic of *I. granulatus*. We found a low abundance of *Borrelia* but that still harbored in each sample. *I. granulatus* may be a major vector of the Lyme disease pathogen *Borrelia* in the Binchuan region of China, and suggested a possible risk of *Borrelia* transmission in the region. Accordingly, we should enhance the prevention of *I. granulatus* and Lyme disease in this area. We speculate that *Spiroplasma* is the central endosymbiont of *I. granulatus*, while its mechanism of action on ticks remains unclear and needs further investigation. And *Borrelia* may be competitively inhibition by *Spiroplasma*. These findings provide fresh knowledge on the symbiont of ticks and interactions of bacteria. We can think from a novel direction and formulate more effective tick-borne disease prevention and control strategies.

Declarations

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Availability of data and materials

The raw Illumina sequencing data generated in this study are available from NCBI Sequence Read Archive, BioProject no. PRJNA916290 with BioSamples accession nos. SRR22923315, SRR22923316, SRR22923314, SRR22923313, SRR22923312, SRR22923309, SRR22923310, SRR22923311, SRR22923308, SRR22923307, SRR22923306, SRR22923305.

Authors' contributions

BC and XY conceived and designed the study. DDJ and XYL conducted data gathering. BC and YFL performed the experiments. XW and TGR analyzed the data. BC drafted and wrote the manuscript. GPY and LG help with the results discussion. XY revised the manuscript. All authors read and provided critical viewpoint, and approved the final manuscript.

Ethics approval and consent to participate

Rodent animals capture protocols and procedures were approved by the animal ethics

committees at Dali University. The approval ID is MECDU-202106.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Figures

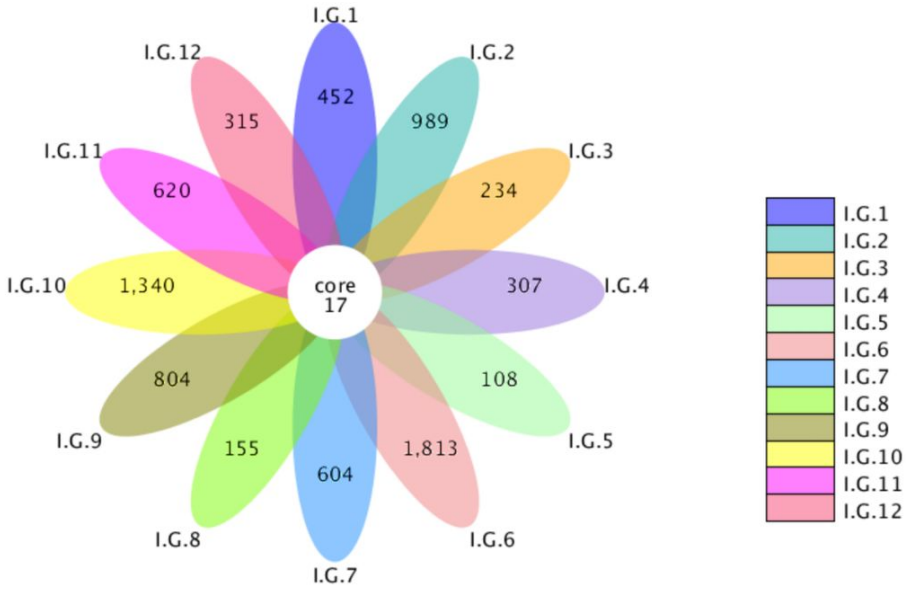

Figure 1

Venn diagram of the OTUs distribution of each sample

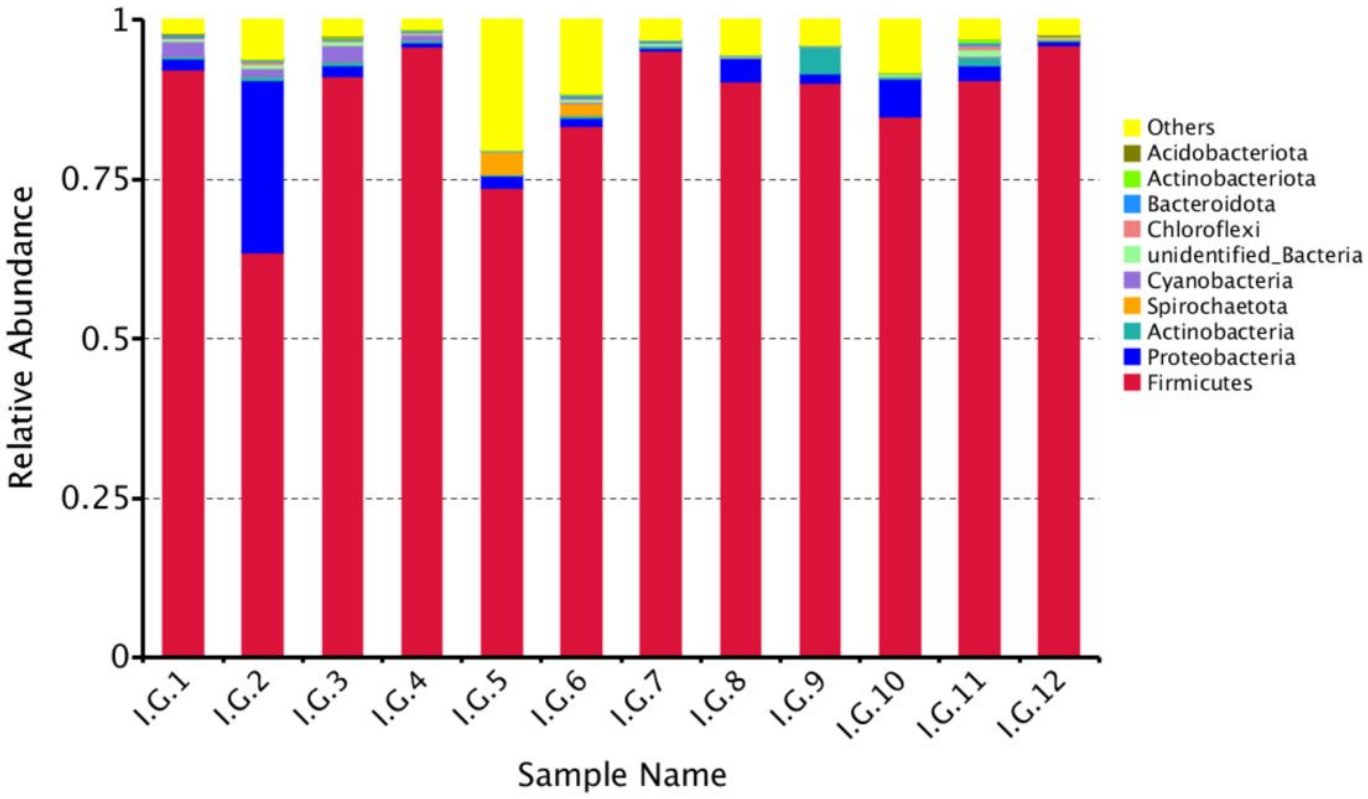

Figure 2

Bacteria relative abundance of the 10 most abundant phyla in the 12 samples of *Ixodes granulatus*

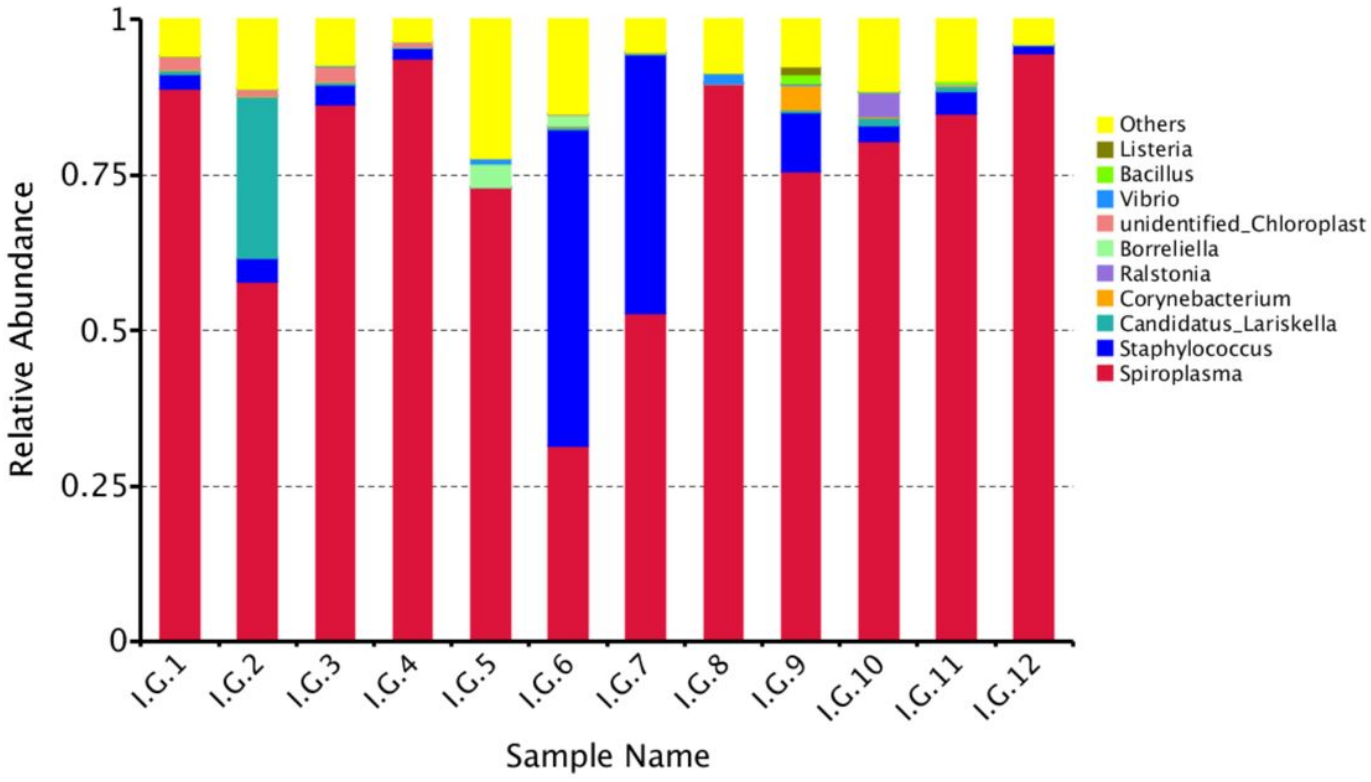

Figure 3

Bacteria relative abundance of the 10 most abundant genera in the 12 samples of *Ixodes granulatus*

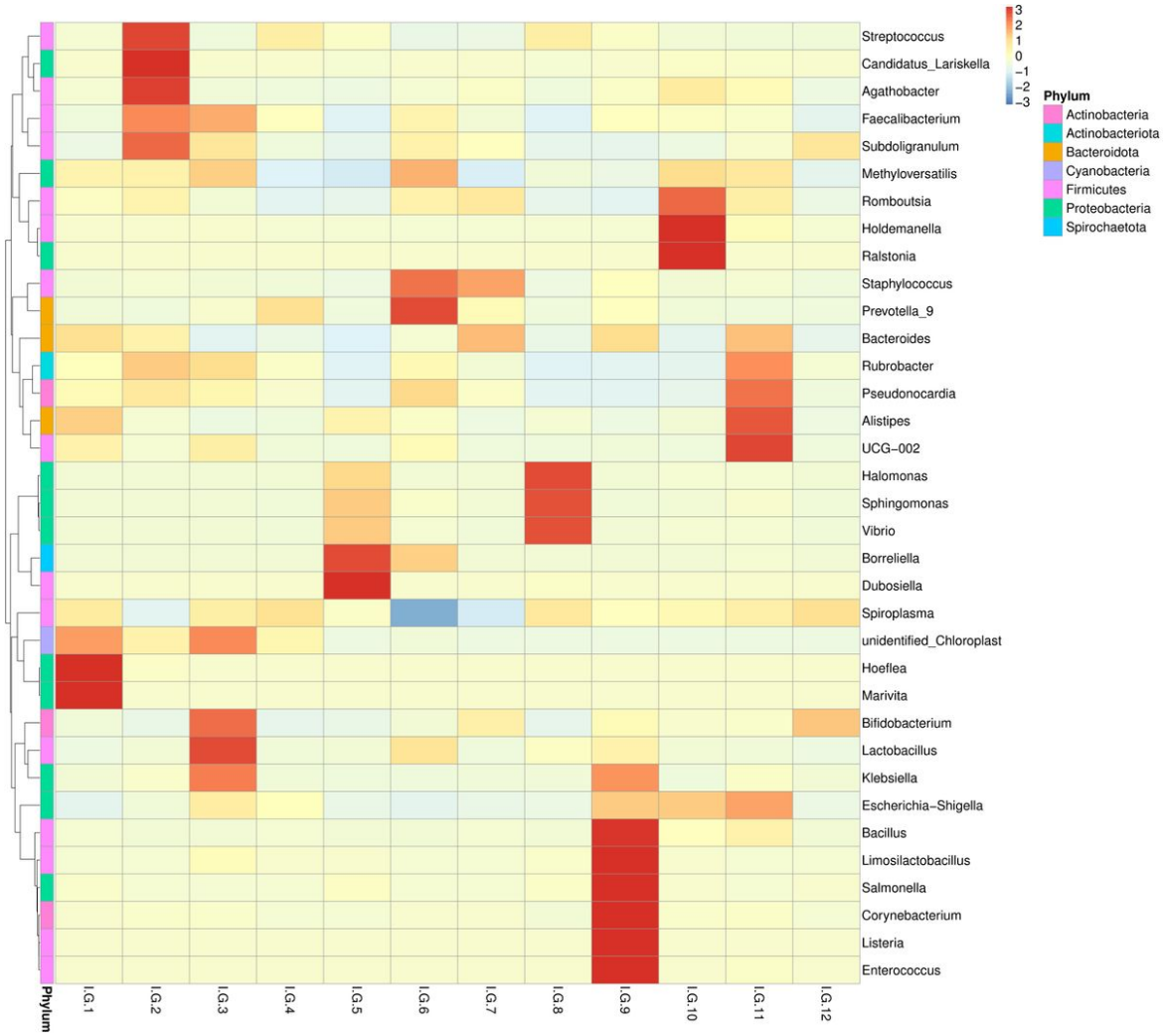

Figure 4

Heat map of the dominant 35 genera among the 12 samples of *Ixodes granulatus*

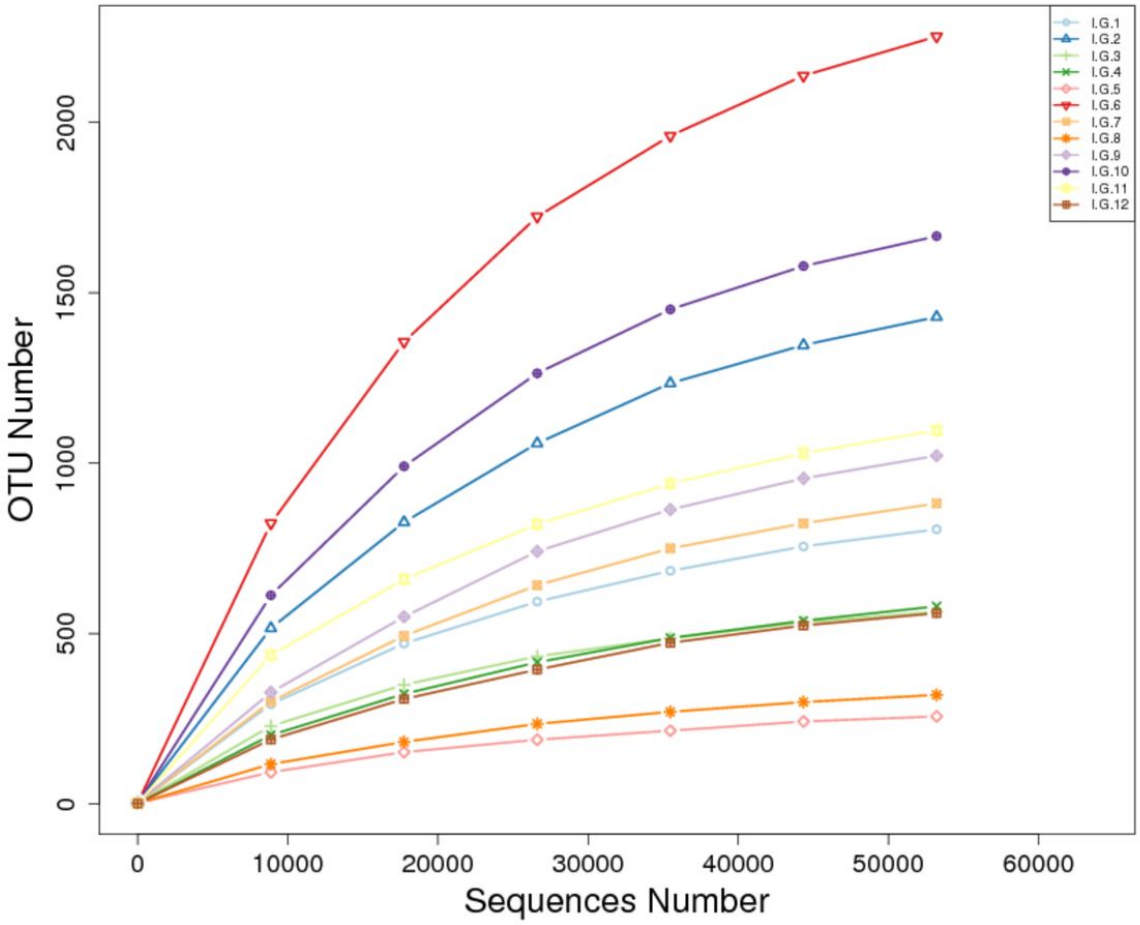

Figure 5

Rarefaction curve of the 12 samples of *Ixodes granulatus*

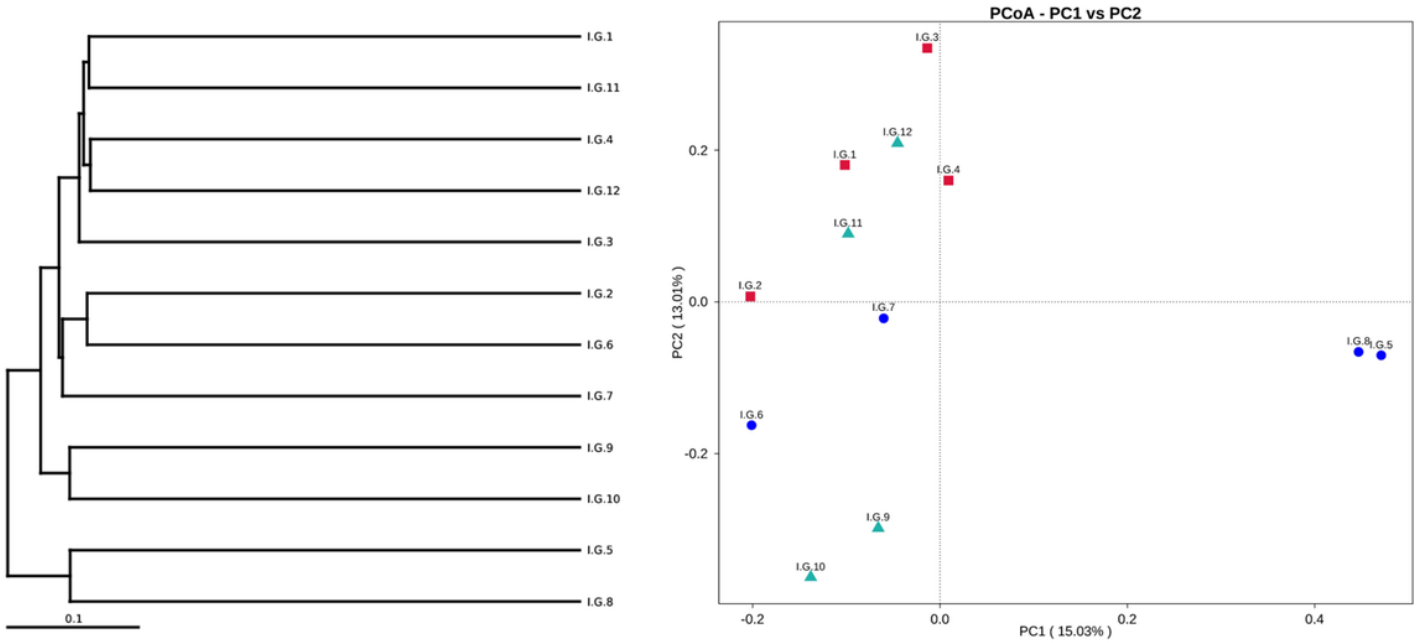

Figure 6

Community difference analysis of *Ixodes granulatus*. (a) UPGMA cluster analysis. (b) Principal coordinate analysis (PCoA) analysis based on Unweighted Unifrac

Supplementary Files

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