Identification and Diversity of Bacterial Communities Associated with the Venom Glands of the Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)

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Abstract

Background Ant-microbe interactions that naturally occur in the venom microenvironment remain mostly unknown. To best of our knowledge, no research exists that shows the identity and diversity of bacterial communities in the fires ants, and what adaptive advantages that venom-associated microbes might offer to their hosts or that hosts might provide to venom-associated bacteria. This study assessed the diversity and identity of bacteria associated with the venom glands of *Solenopsis invicta* and compared this community with the bacterial communities of two other stinging ants, *Solenopsis geminata* and *Diacamma rugosum*.

Results The major phylum associated with the venom glands of *S. invicta* (workers, alates, and queens) and *S. geminata* (workers) was Proteobacteria, while Firmicutes were abundant in the venom glands of *D. rugosum* (workers). Tenericutes were also more abundant in the venom glands of *S. invicta* queens than in those of workers and alates. The relative abundance of Spiroplasma in *S. geminata* was significantly higher than those in *D. rugosum* and *S. invicta*. A microbial diversity analysis of venom glands of worker ants of Solenopsis species showed that the relative abundances of *Bacillus* and *Lactococcus* were higher in Guangxi *S. invicta* workers than in workers collected from Guangzhou. However, the abundance of *Lactococcus* in workers of *D. rugosum* was higher than those in workers of *S. geminata* from Guangxi and *S. invicta* collected from Guangzhou.

Conclusions This study provides the first insight into the microbiota in the venom glands of *S. invicta*, *S. geminata*, and *D. rugosum*, which might contribute to a more comprehensive view of the role of bacteria in the synthesis or degradation of active venom components in the host. We hypothesized that the differences in bacterial communities of three ant species could be due to the local adaptation of insects along with the coevolution of bacteria and hosts.

Background

Ants (Family: Formicidae) are the most abundant group of venomous organisms that dominate terrestrial environment [1]. Some ant species have a true sting apparatus associated with their venom glands and sting hundreds of thousands of people each year around the globe [2–4]. One of the best-known species in this family is the red imported fire ant (*Solenopsis invicta*), which is an aggressive invasive insect spread around the world via ship cargo and the leading cause of pain-producing pharmacological activities in humans [5, 6]. The venom gland apparatus of fire ants typically consists of paired venom secreting tubules that converge into a single convoluted gland, which in turn empties into a sac-like reservoir that leads to the sting [7, 8]. Fire ants produce venom in the poison gland that is stored in a venom sac and directly injected into prey or victims through the stinger [8]. Venoms of these ants contain chemicals that cause intense pain and serve as an effective deterrent against predators or are used to kill prey. Venoms of the majority of stinging ants are predominately composed of proteinaceous mixtures; however, fire ant venoms mainly consist of alkaloids (> 95.0%) with only a minor proteinaceous component (0.1-1.0%) [3, 9]. The venom of fire ants exhibits a diversity of bioactivities, including paralytic,

cytolytic, hemolytic, allergenic, proinflammatory, insecticidal, antimicrobial, and nontoxic functions such as roles in chemical communication involving trail and sex pheromones, deterrents, and aggregators [5, 6, 9, 10]. Reactions of human beings to the sting of red imported fire ants vary from a burning sensation to severe anaphylactic shock and even death [11]. Because of pronounced allergenic reactions, the venom of *S. invicta* has been the subject of numerous investigations into its extraction, chemical composition, and bioactivities against other organisms [6, 12].

Ants and other insects host resident bacterial communities that influence their many physiological, metabolic, and immune processes. Symbiotic bacteria are present in various organs of their hosts and might promote the growth of their hosts by providing some necessary nutrients, protect against natural enemies, and even improve the host ability to adapt to new environments [13–18]. Several microorganisms have evolved to live in one of the most hostile environments, the venom glands, of many organisms. They are common and viable in the venoms of both vertebrates and invertebrates [19]. Microbial fauna associated with invertebrate venom glands has been studied previously [20–24]. However, the ant-microbe interactions that naturally occur in the venom microenvironment remain mostly unknown. As far as we are aware, no studies have attempted to examine the diversity of bacteria in venom glands of fire ants (such as *S. invicta*). This investigation analyzed microbial diversity in the venom glands of *Solenopsis invicta* Buren, *Solenopsis geminata* (Fabricius), and *Diacamma rugosum* Le Guillou using high-throughput sequencing. The composition and diversity of bacterial communities in the venom gland of *S. invicta* were compared with those of bacterial communities of *S. geminata* and *D. rugosum*.

Results

Bacterial Communities Associated with Venom Glands in Different Ant Species and Different Castes of S. invicta

A total of 2,334,172 reads were obtained after filtering the sequencing data, averaging 129,676 reads per sample, and 1,453 OTUs were identified from all samples. A sample-based species dilution curve showed that the sequencing depth was sufficient to cover all bacterial species and reflect the richness level, which also ensured that the sample sequencing data in each group were reasonable (Figure S1). The alpha diversity, including the Shannon diversity index, was analyzed to assess the species diversity of each individual sample. As shown in Figure S2, there was no significant difference between the flora diversities of samples (one-way ANOVA $F_{5,12}$=0.786, df = 5, p = 0.579, Figure S2). Microbial composition analysis of each group showed that the numbers of phyla in the different samples were not the same: 21 in DrW, 18 in GZSiQ, 30 in GZSiG, 18 in GZSiW, 18 in GXSiW, and 20 in GXSgW. Proteobacteria (mean ± SE) were the dominant bacteria in GXSiW (49.85 ± 13.88%), GXSgW (85.65 ± 14.77%), GZSiG (74.35 ± 35.74%), GXSiQ (51.12 ± 32.76%), and GZSiW (98.08 ± 1.23%) but less abundant in DrW (35.27 ± 6.62%). Firmicutes were dominant in DrW (46.67 ± 22.92%) compared to GXSiW (22.30 ± 22.30%), GXSgW (7.61 ± 8.15%), and GZSiW (11.70 ± 14.71%). Tenericutes (19.81 ± 34.21%) occupied a secondary position (Fig. 1) in the Guangzhou red fire ant queens.

At the genus level, higher percentages of *Pseudomonas* were associated with venom glands of DrW (11.56 ± 9.59%), followed by GXSgW (11.44 ± 11.11%) and GZSiW (0.97 ± 0.66%). *Pseudomonas* associated with the glands of GZSiQ (5.29 ± 1.64%) and GZSiG (2.23 ± 0.69%) was the second and third most abundant genus, respectively. *Mesoplasma* was most abundant in the venom glands of GXSiW (19.80 ± 34.20%) and GZSiQ (20.82 ± 36.02%). The percentage of *Streptococcus* associated with GZSiG (4.48 ± 7.42%) was higher than that associated with other ants. *Exiguobacterium* was most associated with the glands of GXSiW (16.03 ± 24.90%), GXSgW (5.87 ± 7.14%), and GZSiW (0.35 ± 0.07%) ants. The percentage of the genus *Proteus* was significantly higher in the glands of DrW (8.56 ± 14.80) than those of all other groups of ants (Table 1).

Table 1
Relative abundances (mean ± SE) of the most common bacterial genera associated with the venom glands of *Solenopsis invicta, Solenopsis geminata* and *Diacamma rugosum*

Taxon	DrW (%)	GXSiW (%)	GXSgW (%)	GZSiG (%)	GZSiQ (%)	GZSiW (%)
Mesoplasma	0.06 ± 0.03	19.8 ± 34.2	0.75 ± 0.98	0.03 ± 0.01	20.83 ± 36.03	0.04 ± 0.03
Pseudomonas	11.56 ± 9.59	8.04 ± 3.48	11.45 ± 11.11	2.23 ± 0.69	5.29 ± 1.64	0.97 ± 0.66
Exiguobacterium	1.2 ± 0.91	16.03 ± 24.91	5.87 ± 7.14	2.09 ± 1.59	1.48 ± 1.22	0.35 ± 0.07
Acinetobacter	2.87 ± 2.76	5.19 ± 6.01	0.42 ± 0.38	1.83 ± 2.14	1.75 ± 1.82	0.14 ± 0.05
Proteus	8.56 ± 14.8	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0	0.01 ± 0.01	0.02 ± 0
Streptococcus	0.51 ± 0.85	0.44 ± 0.52	0.01 ± 0	4.48 ± 7.42	2.22 ± 2.31	0.01 ± 0
Bacillus	0.01 ± 0.01	0.61 ± 0.19	0.03 ± 0.04	0.88 ± 1.05	2.44 ± 4.18	0.06 ± 0.07
Enterococcus	0.5 ± 0.83	2.85 ± 4.15	0.01 ± 0	0.08 ± 0.13	0	0
Rothia	0.01 ± 0	0.09 ± 0.15	0.01 ± 0	1.58 ± 2.73	1 ± 1.15	0.01 ± 0
Stenotrophomonas	1.83 ± 2.23	0.23 ± 0.22	0.58 ± 0.95	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0

For each sample, the abundance of each taxon was compared to total abundance of the microbial community members. DrW: *D. rugosum* workers, GXSgW: Guangxi *S. geminata* workers from, GZSiW: Guangzhou *S. invicta* workers, GXSiW: Guangxi *S. invicta* workers, GZSiG: Guangzhou *S. invicta* alates, GZSiQ: Guangzhou *S. invicta* queens.

Beta Diversity Analysis inside Venom Glands of Different Ant Species or Castes of S. invicta

PCoA was used to further compare species diversity differences in bacterial communities associated with the ant venom glands. PCoA for workers of the three ant species, GZSiW, GXSgW, and DrW, showed significant differences in the microbial community diversity of the three ant species (PERMANOVA F = 5.822, df = 2, p = 0.002, Fig. 2A). PCoA performed on the venom gland-associated bacterial communities of workers ants of *S. invicta* collected from different locations (GXSiW and GZSiW) (Fig. 2B) also showed significant differences in diversity. There were considerable diversity differences in bacterial communities present in the venom glands of castes (workers, alates, and queens) of *S. invicta* collected from Guangzhou (PERMANOVA F = 1.190, df = 2, p = 0.033), as depicted in the PCoA graph (Fig. 2C).

Relative Abundances in Bacterial Communities Associated with Venom Glands of Three Ant Species and Different Castes of S. invicta

The PERMANOVA test was carried out on the bacterial communities of the venom glands of GZSiW, GZSiG and GZSiQ. ANOVA of bacterial populations with relative abundances greater than 1% in at least one replicate showed that the relative abundance of *Pseudomonas* in the venom glands of *S. invicta* queens (GZSiQ) was significantly higher than those of workers (GZSiW) and alates (GZSiG) of this ant species (ANOVA F = 12.280, df = 2, p = 0.008, Fig. 3A). The relative abundance of *Spiroplasma* in GXSgW was significantly higher than those in DrW and GZSiW (Kruskal-Wallis H = 6.006, df = 2, p = 0.05, Fig. 3B), while the relative abundance of *Lactococcus* associated with the venom gland of DrW was considerably higher than those in GXSgW and GZSiW (Kruskal-Wallis H = 6.489, df = 2, p = 0.039, Fig. 3C).

Relative Abundances in Bacterial Communities of S. invicta **Workers Collected from Different Locations**

Comparing the whole bacterial communities in the venom gland of *S. invicta* workers collected from two populations (GZSiW and GXSiW) showed differences between GZSiW and GXSiW. A PCoA map (Fig. 2B) showed that the relative abundances of *Bacillus* and *Lactococcus* in the venom glands of GXSiW were significantly higher than those of GZSiW (t-test, t_1 = 4.689, df_1 = 4, p_1 = 0.009; t_2 = 5.462, df_2 = 4, p_2 = 0.005, Fig. 4A, B).

Discussions

Host-microbe relationships that naturally occur in the venom of ants and other arthropods are not very well known [19]. Culture-dependent and culture-independent studies on the microbial communities in venom microenvironments reveal the presence of archaea, algae, bacteria, fungi, protozoa, and viruses [19]. However, the existence of microbes associated with the venom or venom glands of ants has remained unknown until this study. The current study examined the diversity of microbial bacteria related to the venom glands of *S. invicta* and compared it with microbial communities of two other stinging ant species. The major phylum associated with the venom glands of *S. invicta* (workers, alates, queens) and *S. geminata* (workers) was *Proteobacteria*, while *Firmicutes* were abundant in the venom glands of *D. rugosum* (workers). *Tenericutes* were also more abundant in the venom glands of *S. invicta* queens compared to those of workers and alates. *Proteobacteria* are well-known cuticular and gut microbiomes

of ants and are both harmful and beneficial [28]. *Firmicutes* are commonly present in insect guts and supply nutrients for healthy growth but are primarily influenced by the host diet [29]. *Exiguobacterium* was most prevalent among *Tenericutes*, which has also been reported in the guts of other insects [30]. *Pseudomonas* was more common in queen venom than in worker and alate venoms. *Pseudomonas* has been previously reported in the larvae, pupae, and guts of adult *S. invicta* workers [31]. However, its role as a commensal bacterium in *S. invicta* has not been reported in the current literature. *Pseudomonas* species are commonly found as part of the healthy flora in the oral cavity and intestinal tracts of venomous reptiles. These can produce toxins and can kill insects and other organisms by affecting the gut epithelium [32, 33]. The relative abundance of *Spiroplasma* in *S. geminata* was significantly higher than those in *D. rugosum* and *S. invicta*. Previously, *Spiroplasma* was found to be abundant in *S. geminata* colonies and less abundant in *S. invicta* [34]. The role of *Spiroplasma* has been studied in *Drosophila*, ladybugs, and butterflies, and *Spiroplasma* bacteria are considered commensal, mutualistic or pathogenic and have been reported as male-killing bacteria in *Drosophila* [35, 36]. *Spiroplasma* injected into insects establishes vertical transmission and can kill insects [37–40]. Studies have also shown that *Spiroplasma* infections enhance host viability and resistance to parasitic natural enemies in insects [41–43]. A novel symbiosis has also been identified between Myrmica ants and the facultative bacterial symbiont *Spiroplasma* [44]. The present analysis of the microbial diversity in the venom glands of workers ants of *Solenopsis* species shows that the relative abundances of *Bacillus* and *Lactococcus* were higher in GXSiW than in GZSiW. However, the abundance of *Lactococcus* in DrW was higher than those in GXSgW and GZSiW. *Bacillus* is a common commensal bacterium in insects and commonly found in other invertebrates and has been reported in *S. invicta* queens, larvae, and pupae [31, 45]. Some *Bacillus* species are also insect pathogens and often used to control agricultural pests [46, 47]. Several species of *Bacillus* secrete antibiotics against various fungal pathogens and nematodes of plants [48–50]. Studies have shown that *Bacillus* can promote the growth of termites by interacting with fungi, e.g., *Bacillus-Termitomyces* binding may be beneficial for the breakdown of lignin [51]in the gut of termites. However, some Bacillus species inhibit potentially antagonistic fungi in colonies of higher termites [52]. *Bacillus* species have also been found to be associated with the plant ant *Pseudomyrmex ferrugineus* [53]. *Lactococcus* has been previously reported in the brood of *S. invicta* [34]. It is a fermenting bacterium known to produce lactic acid from sugars and antibacterial substances and may serve an essential role in the digestive system of ant larvae [34], but its functions in the venom gland of ants have not been reported.

Beta diversity analysis showed significant differences in the microbes among the workers of three species and in different castes of *S. invicta*. Similarly, differences were also observed in bacterial communities of *S. invicta* collected from separate locations. The differences in the bacterial communities between different species and different regions may be related to dietary structure, environment, and other factors. Host specificity and host phylogeny could be a determining factor in the distribution of bacterial communities in these associations. For example, the microbial diversity of spiny ants in various areas varied, and some bacteria were unique to a particular area [54]. The role of microorganisms in the ant venom gland in ant hosts has not been reported in the literature and may be related to the local

adaptation of insects. However, the genetic architecture of the venom gland, functional characteristics, and compositional variations of venom in three ant species could be other reasons. Our research focused on the diversity of bacterial communities in the venom glands, but we were not sure about the functions of these bacteria in the venom gland. It is well known that venom of fire ants has a few proteins, including allergens, phospholipases, and neurotoxins [5]. We speculated that these bacterial communities might affect the components of the venom. Correlating microbial community profiles with functional characteristics of venom would deepen our insight into the mechanisms driving venom variation [19]. Therefore, in the future, we need to focus on the specific functions of these microorganisms in the venom gland and how microbes colonize and thrive there.

Conclusions

The current study examined the diversity of microbial bacteria associated with the venom glands of *S. invicta* and compared it with those of microbial communities of two other stinging ant species. The major phylum associated with the venom glands of *S. invicta* (workers, alates, and queens) and *S. geminata* (workers) was *Proteobacteria*, while *Firmicutes* were abundant in the venom glands of *D. rugosum* (workers). *Tenericutes* were also more abundant in the venom glands of *S. invicta* queens than in workers and alates. This is the first evidence that ant venoms and venom glands host diverse bacterial communities. These results challenge perceptions on the sterility of fire ant venom.

Materials And Methods

Ants

Colonies of *S. invicta* were collected from two provinces in China. One location was the campus of South China Agricultural University (Guangzhou, Guangdong, China), which had workers (GZSiW), alates (GZSiG), and queens (GZSiQ). Colonies of *S. invicta* collected from Guangxi Province consisted of workers (GXSiW) only. Workers of *D. rugosum* (DrW) were obtained from Yunnan Province (China), and we received workers of *S. geminata* (GXSgW) from Guangxi Province (China). *Solenopsis invicta* (red imported fire ant) and *S. geminata* (tropical fire ant) belong to the subfamily Myrmicinae and produce defensive venom that causes severe systemic reactions in the victim with a high incidence. On the other hand, *D. rugosum* belongs to the subfamily Ponerinae and occasionally induces little or no pain in its envenomated prey.

For each ant species, three colonies were collected from each site. Colonies were maintained separately in 25 L plastic boxes painted with a mixture of talc powder and ethanol to prevent ants from escaping [25]. Ants were fed sugar-water solution (10% w/w) and frozen locusts (*Locusta migratoria* Linnaeus, 1758) under laboratory conditions (24 ± 2°C, 75% relative humidity (RH), and 14:10 h L:D).

Extraction of Venom Glands

The removal of venom glands was completed within two weeks after the collection of ants by following the method described by [26]. Briefly, 30 workers, five alates, and three queens from each of the *S. invicta* colonies, 30 workers from each of the *S. geminata* colonies, and five workers from each of the *D. rugosum* colonies were randomly sampled and dissected under the microscope in a laminar flow hood. For dissection, ants were placed on sterile petri dishes, and the last two dorsal abdominal sclerites of ants were torn to pull the venom pouch free of the abdomen. After the cuticle was removed from the gasters and separated from the stinger, the poison gland and its reservoir were pulled and collected with a pair of microdissecting forceps. The dissected venom sacs were immediately transferred to a 1.5 mL centrifuge tube containing 50 µl of GA Buffer (TIANamp Micro DNA Kit for DNA extraction), and the tweezers were sterilized every time to ensure that all extract came from the ants.

DNA Extraction

Total venom apparatus genomic DNA was extracted using a TIANamp Micro DNA Kit (Tiangen Biotech (Beijing) Co., Ltd) according to the manufacturer's protocols. The TIANamp Bacteria Genomic DNA Kit is based on silica membrane technology and a special buffer system for extracting gDNA from a wide range of Gram-negative and Gram-positive bacteria [27]. DNA samples were stored at -20°C and then used for PCR.

PCR Amplification and Illumina HiSeq 2500 Sequencing

For microbial diversity analysis, the V3-V4 region of the 16S rRNA gene was amplified using PCR with 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GGACTACHVGGGTATCTAAT-3') primers. PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 27 cycles of denaturation at 98°C for 10 s, annealing at 62°C for 30 s, and extension at 68°C for 30 s and a final extension at 68°C for 10 min. PCRs were performed in triplicate (50 µL mixtures) containing 5 µL of 10X KOD buffer, 5 µL of 2.5 mM dNTPs, 1.5 µL of each primer (5 µM), 1 µL of KOD polymerase, and 100 ng of template DNA. Amplicons were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor-ST (Promega, U.S.). Each set of experiments included negative controls with sterile distilled water instead of template DNA. No amplified products were found in the negative controls.

Purified amplicons were pooled in equimolar concentrations and paired end sequenced (2 × 250) on an Illumina platform according to standard protocols (Illumina, San Diego, CA, USA).

Bioinformatics and Statistical Analysis

Raw reads containing > 10% unknown nucleotides (N) or > 80% of bases with quality (Q-value) > 20 were removed by using FASTP (https://github.com/OpenGene/fastp). Paired-end clean reads were merged as raw tags using FLASH (v 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%. Noisy sequences of raw tags were filtered by the QIIME (V1.9.1) pipeline under specific filtering conditions to obtain high-quality clean tags. Clean tags were searched against the reference database

(http://drive5.com/uchime/uchime_download.html) to perform reference-based chimera checking using the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). All chimeric tags were removed, and the remaining tags were subjected to further analysis. The effective tags were clustered into operational taxonomic units (OTUs) of ≥ 97% similarity using the UPARSE pipeline. The tag sequence with the highest abundance was selected as a representative sequence within each cluster. Between groups, Venn analysis was performed in R (version 3.4.1) to identify unique and common OTUs. The representative sequences were associated with organisms by a naive Bayesian model using the RDP classifier (Version 2.2) based on the SILVA database (https://www.arb-silva.de/). Shannon indices were calculated in QIIME using the default parameters. OTU rarefaction and rank abundance curves were plotted in QIIME. Unweighted Uni Frac distance matrices generated by QIIME were used to calculate the beta diversity and were visualized with principal coordinates analysis (PCoA). Multivariate analyses were performed to compare groups by a PERMANOVA with weighted UniFrac, as depicted in PCoA.

To determine the bacterial taxa that most likely explained differences between sites, we used nonparametric tests (Mann-Whitney test) and one-way ANOVA. Tukey's honestly significant difference (HSD) test was used to compare Shannon indices between groups in SPSS at the 5% level of significance.

List Of Abbreviations

OUT: Operational Taxonomic Unit, GZSiW: workers of *Solenopsis invicta* collected from Guangzhou, GZSiG: alates of *Solenopsis invicta* collected from Guangzhou, GZSiQ queens of *Solenopsis invicta* collected from Guangzhou, GXSiW: Workers of *Solenopsis invicta* collected from Guangxi Province, DrW, workers of *Diacamma rugosum* collected from Yunnan Province, GXSgW, workers of *Solenopsis geminata* collected from Guangxi Province, gDNA: genomic DNA, PCR: Polymerase chain reaction, PCoA: Principal Coordinates Analysis

Declarations

AVAILABILITY OF DATA AND MATERIAL

The sequence reads generated during this study have been submitted to SRA - NCBI under the accession number PRJNA597571. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher. Rest of data is included within the article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Sampling of all specimens occurred on either private land or at research sites

and permission was not required for collection. Otherwise, this is not applicable.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: YX; analyzed the data: FY, SC and BH; contributed reagents/materials/analysis tools: JY, DN and YX; wrote the paper: FY, JY, BH and YX. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

All authors declare that the submitted work was not carried out in the presence of any personal, professional or financial relationships that could potentially be construed as a conflict of interest.

CONSENT FOR PUBLICATION

Not applicable

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Figures

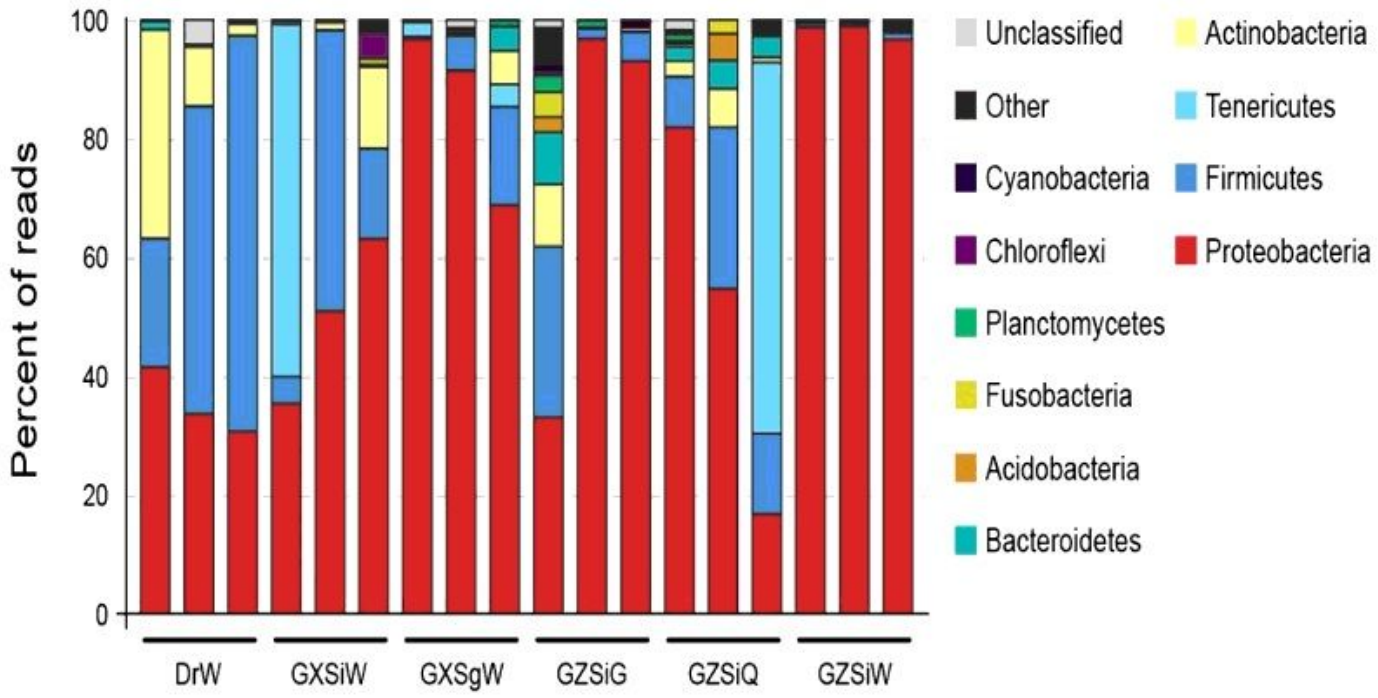

Figure 1

Taxonomic profiles of the bacterial communities associated with the venom glands of Solenopsis invicta, Solenopsis geminata, and Diacamma rugosum at the phylum level. Only phyla making up at least 2% of the total obtained sequences are shown. DrW: D. rugosum workers, GXSgW: Guangxi S. geminata workers from, GZSiW: Guangzhou S. invicta workers, GXSiW: Guangxi S. invicta workers, GZSiG: Guangzhou S. invicta alates, GZSiQ: Guangzhou S. invicta queens.

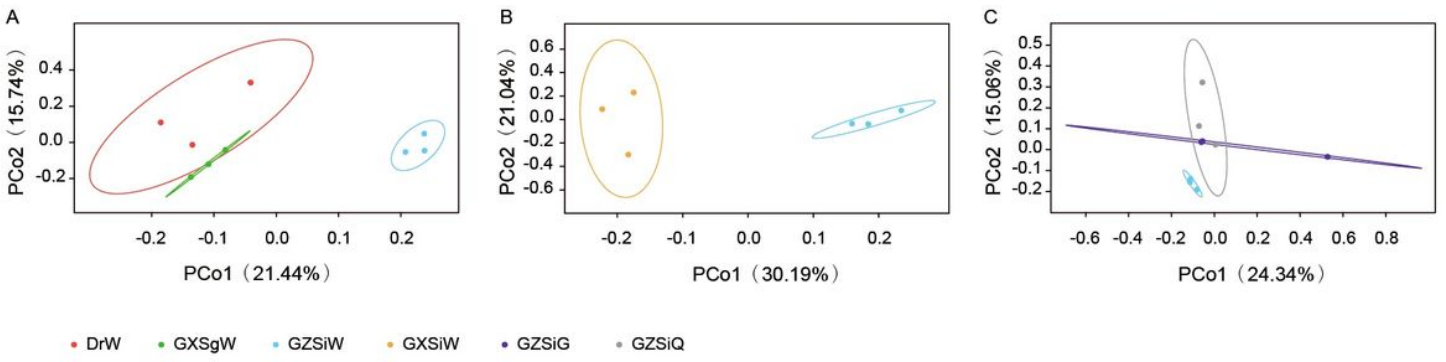

Figure 2

Principal coordinates analysis (PCoA) of the venom gland-associated gut microbiota based on weighted UniFrac distances. Unweighted UniFrac distances among the three ant species DrW, GXSgW, and GZSiW (A). Unweighted UniFrac distance between S. invicta workers (GXSiW and GZSiW) collected from different locations (B). Unweighted UniFrac distance among different castes of S. invicta (GZSiW, GZSiG, GZSiQ) (C). DrW: D. rugosum workers, GXSgW: Guangxi S. geminata workers from, GZSiW: Guangzhou S. invicta workers, GXSiW: Guangxi S. invicta workers, GZSiG: Guangzhou S. invicta alates, GZSiQ: Guangzhou S. invicta queens.

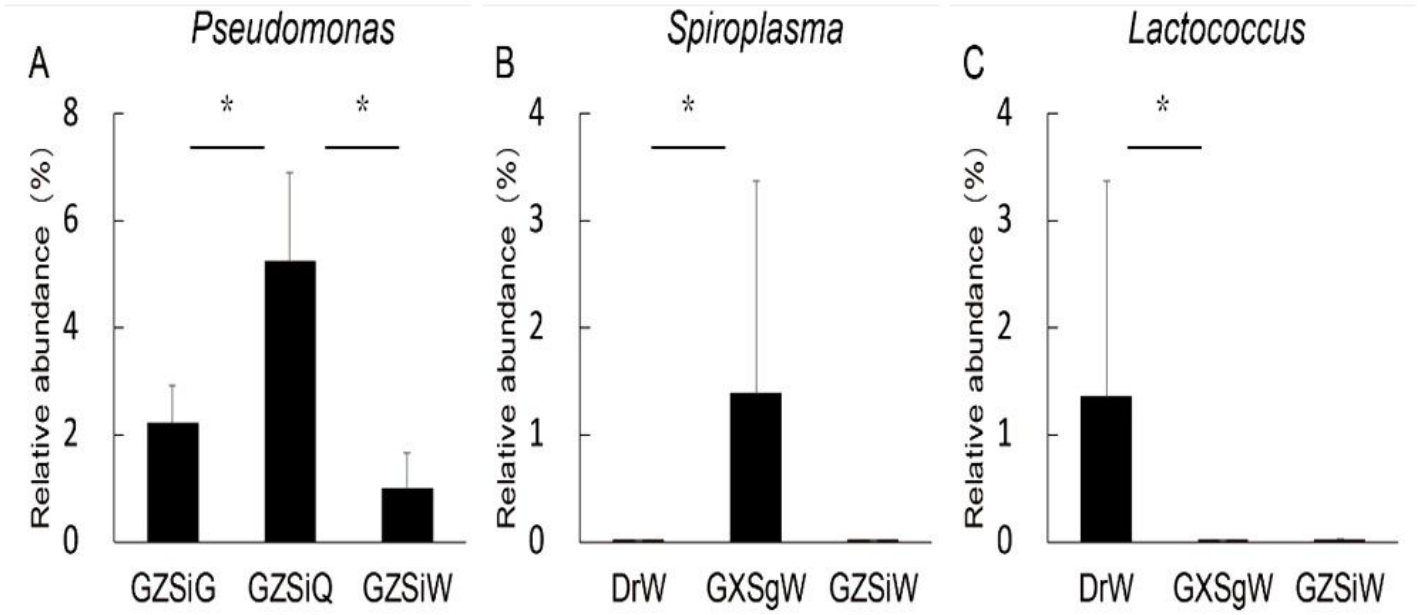

Figure 3

Abundances of key bacterial taxa (mean ±SE) in the microbial communities associated with the venom glands in different ant species or different castes of Solenopsis invicta. Pseudomonas associated with the venom gland of S. invicta workers, alates, and queens (A). Spiroplasma associated with the venom gland of workers of three species (B). Lactococcus associated with the venom gland of workers of three

species (C). DrW: D. rugosum workers, GXSgW: Guangxi S. geminata workers from, GZSiW: Guangzhou S. invicta workers, GXSiW: Guangxi S. invicta workers, GZSiG: Guangzhou S. invicta alates, GZSiQ: Guangzhou S. invicta queens. Error bars show standard errors, and "*" indicates a significant difference in relative abundance at $p<0.05$.

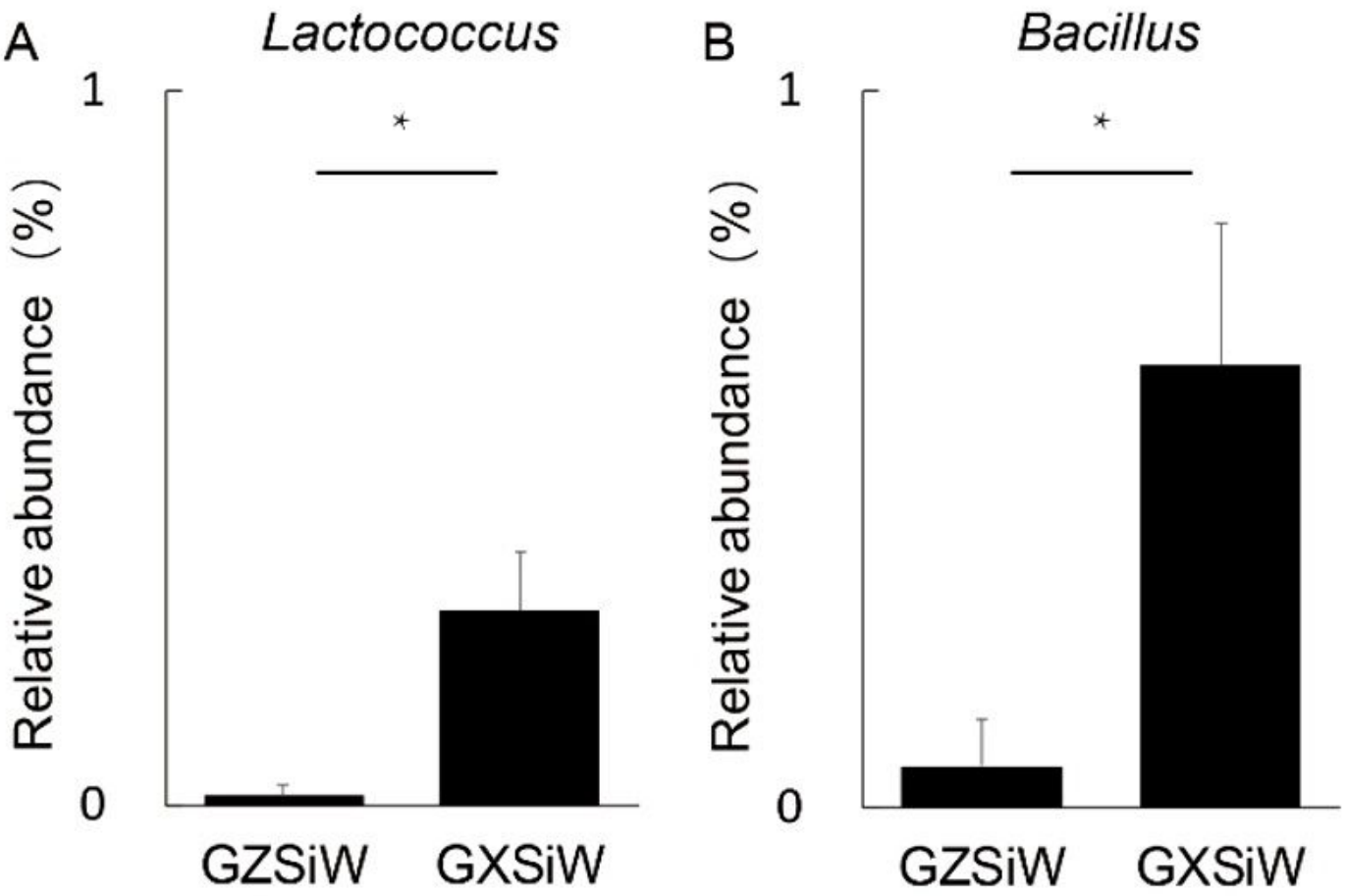

Figure 4

Abundances of key bacterial taxa (Mean ±SE) in the microbial community associated with venom glands of Solenopsis invicta workers collected from different locations in P.R. China. GZSiW: Guangzhou S. invicta workers, GXSiW: Guangxi S. invicta workers. Error bars show the standard errors, and "*" indicates a significant difference in relative abundance at $p<0.05$.

Supplementary Files

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