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Identification and diversity analysis of bacteria in the venom glands of the fire ant, *Solenopsis invicta*, and two other ants (Hymenoptera: Formicidae)

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Abstract: 【Aim】 The objective of this study is to assess the diversity of bacteria in the venom glands of the red imported fire ant, *Solenopsis invicta*, and to compare the bacterial communities in the venom glands with those of other two stinging ants, *Solenopsis geminata* and *Diacamma rugosum*. 【Methods】 16S rRNA V3-V4 regions of bacterial community of venom glands in *S. invicta* workers, alates and queens, *S. geminata* workers and *D. rugosum* workers were sequenced by using the Illumine Hiseq 2500 platform. Then, bioinformatic analysis was performed based on sequencing data. 【Results】 Proteobacteria were dominant in the venom glands of *S. invicta* workers, alates, and queens and *S. geminata* workers, while Firmicutes were abundant in the venom glands of *D. rugosum* workers. Tenericutes were more abundant in the venom glands of *S. invicta* queens than in the venom glands of workers and alates. The relative abundance of *Pseudomonas* in *S. invicta* queens was significantly higher than that in *S. invicta* alates and workers from Guangzhou. The relative abundance of *Spiroplasma* in the venom glands of *S. geminata* workers was significantly higher than that in *D. rugosum* workers. Microbial diversity analysis of venom glands of worker ants of *Solenopsis* species showed that the relative abundance of *Bacillus* and *Lactococcus* in *S. invicta* workers collected from Guangxi was significantly higher than that in *S. invicta* workers collected from Guangzhou. However, the relative abundance of *Lactococcus* in *D. rugosum* workers was significantly higher than that in workers of *S. geminata* from Guangxi. 【Conclusion】 The bacterial composition and diversity are different among the workers of three ant species, and among the three castes in red imported fire ant.

Key words: *Solenopsis invicta*; *Solenopsis geminata*; *Diacamma rugosum*; species diversity; abundance; microorganism; venom gland

1 INTRODUCTION

Ants are the most abundant group of venomous organisms that dominate terrestrial environments (Casewell *et al.*, 2013). Some ant species have a true sting apparatus associated with their venom glands and sting hundreds of thousands of people each year around the world (Postma, 2009; Coleman and Wall, 2015; Golden, 2017). One of the best-known species in this family is the red imported fire ant *Solenopsis invicta*, which is an aggressive invasive insect spreading around the world via ship cargo and the leading cause of pain-producing pharmacological activities in humans (dos Santos Pinto *et al.*, 2012; Fox, 2014). 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 (Fox *et al.*, 2010; Torres *et al.*, 2013). Fire ants produce venom in the poison gland that is stored in a venom sac and directly injected into prey or victims through the sting (Torres *et al.*, 2013). 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

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mainly consist of alkaloids (>95.0%) with only a minor proteinaceous component (0.1% – 1.0%) (Touchard *et al.*, 2016; Golden, 2017). 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 (dos Santos Pinto *et al.*, 2012; Fox, 2014; Touchard *et al.*, 2016; Fox *et al.*, 2018). Reactions of human beings to the sting of red imported fire ants vary from a burning sensation to severe anaphylactic shock and even death (Xu *et al.*, 2012). 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 (Li *et al.*, 2012; Fox, 2014; Yu *et al.*, 2014).

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 (Douglas, 1998; Chen *et al.*, 2000; Oliver *et al.*, 2003; Russell and Moran, 2006; Cheng *et al.*, 2017, 2019). 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 (Ul-Hasan *et al.*, 2019). Microbial fauna associated with invertebrate venom glands has been studied previously (Webb and Summers, 1990; Monteiro *et al.*, 2002; Gaver-Wainwright *et al.*, 2011; Simmonds *et al.*, 2016; Debat, 2017). 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 *S. invicta* using high-throughput sequencing. For the comparison, we selected a closely related invasive fire ant *S. geminata* that produces defensive venom and causes severe systemic reactions in the victim, and non-invasive queen-less generalist predator *Diacamma rugosum* that occasionally induces little or no pain in its envenomated prey (Blum, 1992; Hoffman, 2010). The composition and diversity of bacterial communities in the venom glands of *S. invicta* were compared with those of bacterial communities in the venom glands of *S. geminata* and *D. rugosum* aiming at finding the difference of bacterial communities in the above three species and ascertaining the special bacterium in each species.

2 MATERIALS AND METHODS

2.1 Test ants

Colonies of *S. invicta* workers (GZSiW), alates (GZSiG) and queens (GZSiQ) were collected from South China Agricultural University (Guangzhou), and *S. invicta* workers (GXSiW) only were collected from Guangxi Province. Workers of *D. rugosum* (YNDrW) were collected from Yunnan Province. Workers of *S. geminata* (GXSgW) were collected from Guangxi Province. 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 (Ning *et al.*, 2019). Ants were fed with sugar-water solution (10% w/w) and frozen locusts (*Locusta migratoria*) under laboratory conditions (24 ± 2℃, 75% RH, and 14L: 10D photoperiod).

2.2 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 Chen *et al.* (2009). Briefly, 30 workers, five alates, and three queens from each of *S. invicta* colonies, 30 workers from each of *S. geminata* colonies, and five workers from each of *D. rugosum* colonies were randomly sampled and dissected under the microscope in a laminar flow hood. Three colonies were sampled for each species from each site as replicates. 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 venom 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.

2.3 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 DNA from a wide range of Gram-negative and Gram-positive bacteria (Yan *et al.*, 2017). DNA samples were stored at −20℃ and then used for PCR.

2.4 PCR amplification and Illumina HiSeq 2500 sequencing

For microbial diversity analysis, the V3-V4 region of the 16S rRNA gene of bacteria was amplified using PCR with primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAT-3′). PCRs were performed in triplicate (50 μL mixtures) containing 5 μL of 10 × KOD buffer, 5 μL of 2.5 mmol/L dNTPs, 1.5 μL of each primer (5 μmol/L), 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, USA) according to the manufacturer's instructions and quantified using QuantiFluor-ST (Promega, USA). PCR conditions were as follows: initial denaturation at 95℃ for 2 min, followed by 27 cycles of denaturation at 98℃ for 10 s, annealing at 62℃ for 30 s, and extension at 68℃ for 30 s and a final extension at 68℃ for 10 min. 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 HiSeq 2500 platform according to standard protocols (Illumina, San Diego, CA, USA).

2.5 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. 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 Unifrac distance matrix 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 (Kruskal-Wallis test), one-way ANOVA and *t*-test. Tukey's honestly significant difference (HSD) test was used to compare Shannon indices between groups in SPSS at the 5% level of significance. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

3 RESULTS

3.1 Bacterial communities in the venom glands in *S. invicta* and other two stinging ants

The sequence reads generated during this study have been submitted to SRA-NCBI under the accession number PRJNA597571. From all samples 1 453 bacterial OTUs were identified. A sample-based species dilution curve showed that the sequencing depth was sufficient to cover all bacterial species and reflected the richness level, which also ensured that the sample sequencing data in each group were reasonable. The alpha diversity analysis showed that there was no significant difference between the flora diversities of samples (one-way ANOVA, $F_{5,12} = 0.786$, $df = 5$, $P = 0.579$). Microbial composition at the phylum level showed that there were 21 phyla in YNDrW, 18 phyla in GZSiQ, 30 phyla in GZSiG, 18 phyla in GZSiW, 18 phyla in GXSiW, and 20 phyla in GXSgW. Proteobacteria were dominant in GXSiW (49.85% ± 13.88%), GXSgW (85.65% ± 14.77%), GZSiG (74.35% ± 35.74%), GZSiQ (51.12% ± 32.76%), and GZSiW (98.08% ± 1.23%) but less abundant in YNDrW (35.27% ± 5.62%). Firmicutes were

dominant in YNDrW (46.67% ± 22.92%) compared to those in GXSiW (22.30% ± 22.30%), GXSgW (7.61% ± 8.15%), and GZSiW (11.70% ± 14.71%). Tenericutes (19.81% ± 34.21%) occupied the second position (Fig. 1) in the Guangzhou red fire ant queens.

At the genus level, the relative abundance of *Pseudomonas* was the highest in the venom glands of YNDrW (11.56% ± 9.61%), followed by that in the venom glands of GXSgW (11.44% ± 11.13%). *Pseudomonas* in the GXSiW (8.03% ± 3.45%) presented a high level but was not the most abundant genus in GXSiW. Moreover, *Pseudomonas* was the second most abundant genus in the venom glands of GZSiQ (5.28% ± 1.63%) and GZSiG (2.23% ± 0.70%). *Mesoplasma* was the most abundant genus in the venom glands of GXSiW (19.79% ± 34.17%) and GZSiQ (20.85% ± 36.07%). The relative abundance of *Streptococcus* in GZSiG (4.47% ± 7.41%) was higher than that in others. *Exiguobacterium* was abundant in the venom glands of GXSiW (16.06% ± 24.96%), GXSgW (5.88% ± 7.13%), and GZSiG (2.08% ± 1.59%). The relative abundance of *Proteus* in the venom glands of YNDrW (8.54% ± 14.75%) was significantly higher than that in the venom glands of all others (Table 1).

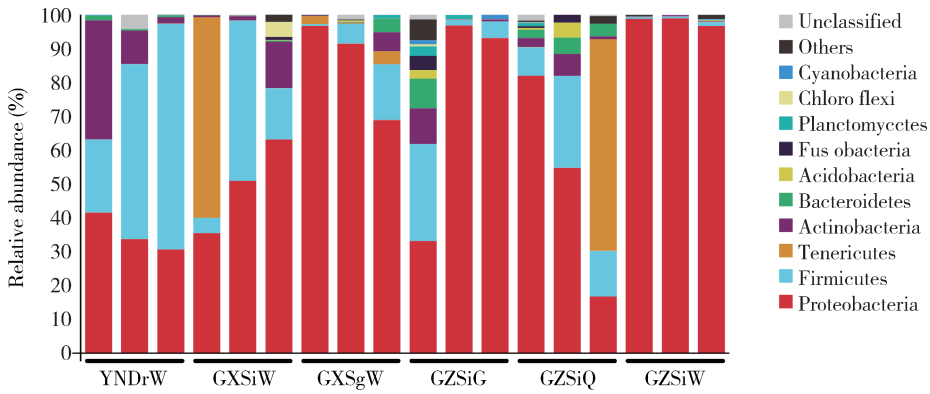

Fig. 1 Taxonomic profiles of the bacterial communities in the venom glands of *Solenopsis invicta*, *Solenopsis geminata*, and *Diacamma rugosum* at the phylum level

Three columns for each species from each site represent three replicates. Only phyla making up at least 2% of the total obtained sequences were shown. YNDrW: *D. rugosum* workers from Yunnan; GXSgW: *S. geminata* workers from Guangxi; GZSiW: *S. invicta* workers from Guangzhou; GXSiW: *S. invicta* workers from Guangxi; GZSiG: *S. invicta* alates from Guangzhou; GZSiQ: *S. invicta* queens from Guangzhou. The same below.

Table 1 Relative abundance (%) (mean ± *SD*) of the most common bacterial genera in the venom glands of *Solenopsis invicta*, *Solenopsis geminata* and *Diacamma rugosum*

Taxa	YNDrW	GXSiW	GXSgW	GZSiG	GZSiQ	GZSiW
Mesoplasma	0.06 ± 0.03	19.79 ± 34.17	0.75 ± 0.98	0.03 ± 0.01	20.85 ± 36.07	0.05 ± 0.03
Pseudomonas	11.56 ± 9.61	8.03 ± 3.45	11.44 ± 11.13	2.23 ± 0.70	5.28 ± 1.63	0.97 ± 0.66
Exiguobacterium	1.19 ± 0.92	16.06 ± 24.96	5.88 ± 7.13	2.08 ± 1.59	1.47 ± 1.22	0.36 ± 0.07
Acinetobacter	2.86 ± 2.75	5.20 ± 6.01	0.42 ± 0.38	1.82 ± 2.13	1.76 ± 1.82	0.14 ± 0.04
Proteus	8.54 ± 14.75	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0	0.01 ± 0.01	0.02 ± 0
Streptococcus	0.51 ± 0.86	0.45 ± 0.52	0.01 ± 0	4.47 ± 7.41	2.22 ± 2.31	0.01 ± 0
Bacillus	0.01 ± 0.01	0.62 ± 0.20	0.03 ± 0.04	0.88 ± 1.05	2.44 ± 4.19	0.06 ± 0.07
Enterococcus	0.50 ± 0.84	2.84 ± 4.15	0.01 ± 0	0.08 ± 0.13	0	0
Rothia	0.01 ± 0	0.09 ± 0.15	0.01 ± 0	1.59 ± 2.74	1.00 ± 1.16	0.01 ± 0
Stenotrophomonas	1.83 ± 2.22	0.24 ± 0.22	0.58 ± 0.95	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0

For each sample, the relative abundance of each taxon was compared to the total abundance of the microbial community members.

3.2 Beta diversity of bacteria in the venom glands of *S. invicta* and other two stinging ants

PCoA was used to further compare differences in the species diversity of bacterial communities in the venom glands. There were significant differences in the diversity of microbial communities among GZSiW, GXSgW, and YNDrW (PERMANOVA, $F = 5.822$, $df = 2$, $P = 0.002$) (Fig. 2: A) and in workers of *S. invicta* collected from different locations (GXSiW and GZSiW) (Fig. 2: B). There were considerable differences in the diversity of bacterial communities in the venom glands of castes GZSiW, GZSiG and GZSiQ of *S. invicta* (PERMANOVA, $F = 1.190$, $df = 2$, $P = 0.033$) (Fig. 2: C).

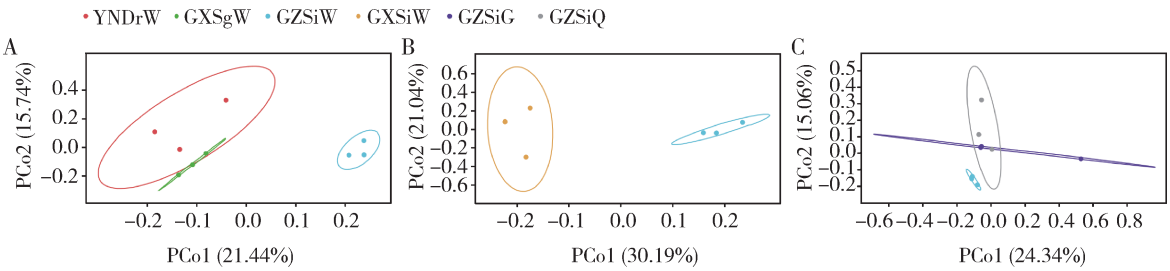

Fig. 2　Principal coordinates analysis (PCoA) of the diversity of bacteria in the venom glands of *Solenopsis invicta*, *Solenopsis geminata* and *Diacamma rugosum* based on weighted UniFrac distances

A: Unweighted UniFrac distances among YNDrW, GXSgW, and GZSiW; B: Unweighted UniFrac distance between GXSiW and GZSiW; C: Unweighted UniFrac distance among different castes of *S. invicta* (GZSiW, GZSiG, GZSiQ).

3.3　Relative abundance of key bacterial genera in the venom glands of *S. invicta* and other two stinging ants

The relative abundance of *Pseudomonas* in the venom glands of GZSiQ was significantly higher than that of GZSiW and GZSiG of *S. invicta* (one-way ANOVA, $F = 12.280$, $df = 2$, $P = 0.008$) (Fig. 3: A). The relative abundance of *Spiroplasma* in GXSgW was significantly higher than that in YNDrW (Kruskal-Wallis, $H = 6.006$, $df = 2$, $P = 0.05$) (Fig. 3: B), while that of *Lactococcus* in the venom glands of YNDrW was significantly higher than that in GXSgW (Kruskal-Wallis, $H = 6.489$, $df = 2$, $P = 0.0390$) (Fig. 3: C).

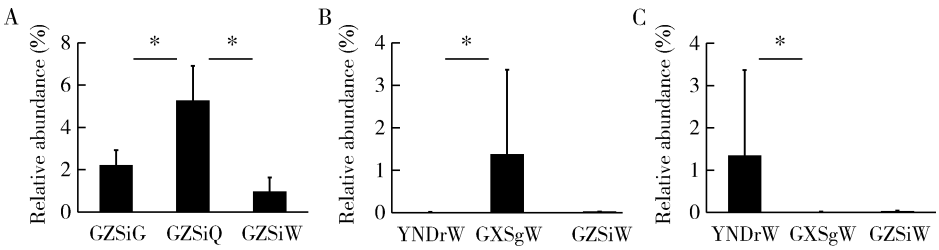

Fig. 3　Relative abundance of key bacterial genera *Pseudomonas* (A), *Spiroplasma* (B) and *Lactococcus* (C) in the venom glands of *Solenopsis invicta*, *Solenopsis geminata* and *Diacamma rugosum*.

Data in the figure are mean ± *SD*. Asterisk above bars indicates significant difference in the relative abundance ($P < 0.05$, one-way ANOVA for Fig. A, and Kruskal-Wallis test for Figs. B and C).

3.4　Relative abundance of bacterial genera in the venom glands of *S. invicta* workers collected from different locations

Comparison of the whole bacterial communities in the venom glands of GZSiW and GXSiW showed differences between GZSiW and GXSiW (Fig. 2: B). Further analysis showed that the relative abundance of *Lactococcus* and *Bacillus* in the venom glands of GXSiW was significantly higher than that in the venom glands 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. 4: A, B).

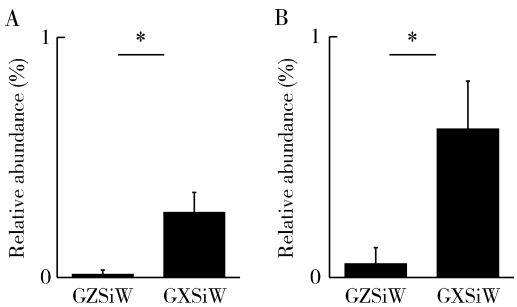

Fig. 4　Relative abundance of key bacterial genera *Lactococcus* (A) and *Bacillus* (B) in the venom glands of *Solenopsis invicta* workers collected from different locations

Data in the figure are mean ± *SD*. Asterisk above bars indicates significant difference in relative abundance ($P < 0.05$, t-test).

4　DISCUSSION

In this study we examined the diversity of bacteria in the venom glands of *S. invicta* compared with those of two other stinging ant species. The major bacteria phylum in 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 than in the venom glands of workers and alates. Geographic location, host species and stage of the host significantly influence the composition and abundance of bacterial communities associated with insects (Engel and Moran, 2013; Liu *et al.*, 2018; Koskinioti *et al.*, 2019). Our results showed significant differences in the abundance of bacterial communities in workers,

alates and queens of ant species. However, geographic location has limited effect on bacterial community at the phylum level and we observed Proteobacteria as a major phylum in venom glands of workers of *S. invicta* collected from Guangzhou and Guangxi. Furthermore, the dominant bacteria at the phylum level in venom glands of *D. rugosum* was not common in those of *S. invicta* or *S. geminate*, which may be attributed to host-dependent differences of insect-symbiotic bacteria. Proteobacteria are well-known cuticular and gut microbiomes of ants and are both harmful and beneficial (Seipke *et al.*, 2013). Firmicutes are commonly present in insect guts and supply nutrients for healthy growth but are primarily influenced by the host diet (Moreau and Rubin, 2017). *Exiguobacterium* was most prevalent among Tenericutes, which has also been reported in the guts of other insects (Rani *et al.*, 2009). *Pseudomonas* was more common in queen venom than in worker and alate venoms (Table 1). *Pseudomonas* has been previously reported in the larvae, pupae, and guts of adult *S. invicta* workers (Lee *et al.*, 2008). 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 (Flury *et al.*, 2016; Glare and O′Callaghan, 2019). The relative abundance of *Spiroplasma* in *S. geminata* was significantly higher than that in *D. rugosum* (Fig. 3: B). Previously, *Spiroplasma* was found to be abundant in *S. geminata* colonies and less abundant in *S. invicta* (Ishak *et al.*, 2011). The role of *Spiroplasma* has been studied in *Drosophila*, ladybugs, and butterflies. *Spiroplasma* bacteria are considered commensal, mutualistic or pathogenic and have been reported as male-killing bacteria in *Drosophila* (Hurst and Majerus 1993; Hurst *et al.*, 2003a). *Spiroplasma* injected into insects establishes vertical transmission and can kill insects (Williamson and Poulson, 1979; Hurst *et al.*, 2003b; Anbutsu and Fukatsu 2010, 2011). Studies have also shown that *Spiroplasma* infections enhance host viability and resistance to parasitic natural enemies (Moya-Raygoza *et al.*, 2007; Jaenike *et al.*, 2010; Xie *et al.*, 2010) in insects. A novel symbiosis has also been identified between *Myrmica* ants and the facultative bacterial symbiont *Spiroplasma* (Ballinger *et al.*, 2018). The present analysis of the microbial diversity in the venom glands of worker ants of *Solenopsis* species shows that the relative abundance of *Bacillus* and *Lactococcus* was higher in GXSiW than in GZSiW (Fig. 4). However, the abundance of *Lactococcus* in YNDrW was higher than that in GXSgW (Fig. 3: C). *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 (Lee *et al.*, 2008; Tufts and Bextine, 2009). Some *Bacillus* species are also insect pathogens and often used to control agricultural pests (Wenzel *et al.*, 2002; Ertürk and Demirbağ, 2006). Several species of *Bacillus* secrete antibiotics against various fungal pathogens and nematodes of plants (Chaurasia *et al.*, 2005; Swain *et al.*, 2008; González-Teube *et al.*, 2014). 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 (Mathew *et al.*, 2012) in the gut of termites. However, some *Bacillus* species inhibit potentially antagonistic fungi in colonies of higher termites (Um *et al.*, 2013). *Bacillus* species have also been found to be associated with the plant ant *Pseudomyrmex ferrugineus* (Eilmus and Heil, 2009). *Lactococcus* has been previously reported in the brood of *S. invicta* (Ishak *et al.*, 2011). 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 (Ishak *et al.*, 2011), but its functions in the venom glands 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 (Engel and Moran, 2013; Liu *et al.*, 2018; Koskinioti *et al.*, 2019). 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 (Ramalho *et al.*, 2017). The role of microorganisms in the ant venom glands 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 venom gland, functional characteristics, and compositional variations of venom in three ant species could be other reasons.

This study focused on the diversity of bacterial communities in the venom glands, but we are not sure about the functions of these bacteria in the venom glands. It is well known that venom of fire ants has a few proteins, including allergens, phospholipases, and neurotoxins (dos Santos Pinto *et al.*, 2012). 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 (Ul-Hasan *et al.*, 2019). Therefore, in the future, we need to focus on the specific functions of these microorganisms in the venom glands and how microbes colonize and thrive there.

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.

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红火蚁及其他两种蚂蚁毒腺细菌鉴定与多样性分析

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摘要：【目的】本研究旨在分析红火蚁 *Solenopsis invicta* 毒腺细菌群落多样性，并与热带火蚁 *Solenopsis geminata* 和聚纹双刺猛蚁 *Diacamma rugosum* 比较毒腺细菌群落差异。【方法】采用 Illumine Hiseq 2500 测序平台对红火蚁（工蚁、有翅蚁和蚁后）、热带火蚁（工蚁）及聚纹双刺猛蚁（工蚁）毒腺细菌群落 16S rRNA 基因 V3-V4 区测序，基于测序数据进行生物信息学分析。【结果】变形菌门（Proteobacteria）在红火蚁工蚁、有翅蚁和蚁后以及热带火蚁工蚁毒腺中占优势，而厚壁菌门（Firmicutes）在聚纹双刺猛蚁工蚁毒腺中占优势。与红火蚁工蚁和有翅蚁相比，柔壁菌门（Tenericutes）在红火蚁蚁后毒腺中更丰富。广州红火蚁蚁后毒腺中假单胞杆菌 *Pseudomonas* 相对丰度显著高于其在有翅蚁及工蚁中的。螺原体 *Spiroplasma* 相对丰度在热带火蚁毒腺中显著高于在聚纹双刺猛蚁工蚁毒腺中的。毒腺中细菌多样性分析发现，芽孢杆菌属 *Bacillus* 和乳酸杆菌属 *Lactobacillus* 在广西红火蚁工蚁中的相对丰度显著高于在广州红火蚁工蚁中的。然而，乳酸杆菌属细菌在聚纹双刺猛蚁工蚁毒腺中的相对丰度显著高于广西的热带火蚁工蚁毒腺中的。【结论】毒腺细菌组成和多样性在 3 种不同种类蚂蚁工蚁和红火蚁不同品级中存在差异。

关键词：红火蚁；热带火蚁；聚纹双刺猛蚁；物种多样性；丰度；微生物；毒腺

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