A peer-reviewed version of this preprint was published in PeerJ on 15 January 2020.

View the peer-reviewed version (peerj.com/articles/8411), which is the preferred citable publication unless you specifically need to cite this preprint.

Guo C, Peng X, Zheng X, Wang X, Wang R, Huang Z, Yang Z. 2020. Comparison of bacterial diversity and abundance between sexes of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) from China. PeerJ 8:e8411 https://doi.org/10.7717/peerj.8411

Comparison of the bacterial abundance and diversity in the *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) between both sexes

Chunhui Guo [1], **Xin Peng** [1], **Xialin Zheng** [2], **Xiaoyun Wang** [2], **Ruirui Wang** [1], **Zongyou Huang** [2], **Zhende Yang** [Corresp. 3]

[1] College of Forestry, Guangxi University, Nanning, China

[2] Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, College of Agriculture, Guangxi University, Nanning, China

[3] College of Forestry/Guangxi Key Laboratory of Forest Ecology and Conservation, Guangxi University, Nanning, China

Corresponding Author: Zhende Yang
Email address: dzyang68@126.com

Background. Insects harbor a myriad of microorganisms, many of which can affect the sex ratio and manipulate the reproduction of the host. *Leptocybe invasa* is an invasive pest that causes serious damage to eucalyptus plantations, and both female-biased sex ratios and thelytokous parthenogenesis in *L. invasa* contribute to the rapid invasion and fast growth of the population. However, the interior bacterial composition and abundance of *L. invasa* and the differences between both sexes remain unclear.

Results. The Illumina MiSeq platform was used to compare the composition of the bacterial community in adult females and males by sequencing with variation in the V3-V4 region of the 16S ribosomal DNA gene. The results showed that 1320 operational taxonomic units (OTUs) were obtained in total. These OTUs were annotated into 24 phyla, 71 classes, 130 orders, 245 families and 501 genera. At the genus level, the dominant bacteria in females and males was *Rickettsia* and *Rhizobium*, respectively.

Conclusion. The bacteria living in *L. invasa* adult females and males had high diversity. There were differences in the bacterial community in *L. invasa* between both sexes, and the bacterial diversity in male adults was more abundant than that in female adults. This study presents a comprehensive comparison of bacterial communities living in *L. invasa* between sexes, which plays a significant role in reproductive strategy, sex regulation and the invasive mechanism of *L. invasa* and provides a basis for follow-up studies on the coevolution and interaction between *L. invasa* and its predominant bacteria.

Comparison of the bacterial abundance and diversity in the *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) between both sexes

Chunhui Guo[1], Xin Peng[1], Xialin Zheng[2], Xiaoyun Wang[2], Ruirui Wang[1], Zongyou Huang[2], Zhende Yang[1, 3]

[1] College of Forestry, Guangxi University, Nanning, Guangxi Zhuang Autonomous Region, P. R. China

[2] Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, College of Agriculture, Guangxi University, Guangxi Zhuang Autonomous Region, P. R. China

[3] Guangxi Key Laboratory of Forest Ecology and Conservation, Forestry College, Guangxi University, Guangxi Zhuang Autonomous Region, P. R. China

Corresponding Author:

Zhende Yang [1, 2]

Daxuedong Street, Nanning, Guangxi Zhuang Autonomous Region, 530004, P. R. China

E-mail address: dzyang68@126.com

Comparison of the bacterial abundance and diversity in the *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) between both sexes

Chunhui Guo[1], Xin Peng[1], Xialin Zheng[2], Xiaoyun Wang[2], Ruirui Wang[1], Zongyou Huang[2], Zhende Yang[1, 3]

[1]College of Forestry, Guangxi University, Nanning, Guangxi Zhuang Autonomous Region, P. R. China

[2]Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, College of Agriculture, Guangxi University, Guangxi Zhuang Autonomous Region, P. R. China

[3]Guangxi Key Laboratory of Forest Ecology and Conservation, Forestry College, Guangxi University, Guangxi Zhuang Autonomous Region, P. R. China

Corresponding Author:

Zhende Yang[1, 2]

Daxuedong Street, Nanning, Guangxi Zhuang Autonomous Region, 530004, P. R. China

E-mail address: dzyang68@126.com

Abstract

Background. Insects harbor a myriad of microorganisms, many of which can affect the sex ratio and manipulate the reproduction of the host. Leptocybe invasa is an invasive pest that causes serious damage to eucalyptus plantations, and both female-biased sex ratios and thelytokous parthenogenesis in *L. invasa* contribute to the rapid invasion and fast growth of the population. However, the interior bacterial composition and abundance of *L. invasa* and the differences between both sexes remain unclear.

Results. The Illumina MiSeq platform was used to compare the composition of the bacterial community in adult females and males by sequencing with variation in the V3-V4 region of the 16S ribosomal DNA gene. The results showed that 1320 operational taxonomic units (OTUs) were obtained in total. These OTUs were annotated into 24 phyla, 71 classes, 130 orders, 245 families and 501 genera. At the genus level, the dominant bacteria in females and males was *Rickettsia* and *Rhizobium*, respectively.

Conclusion. The bacteria living in *L. invasa* adult females and males had high diversity. There were differences in the bacterial community in *L. invasa* between both sexes, and the bacterial diversity in male adults was more abundant than that in female adults. This study presents a comprehensive comparison of bacterial communities living in *L. invasa* between sexes, which plays a significant role in reproductive strategy, sex regulation and the invasive mechanism of *L. invasa* and provides a basis for follow-up studies on the coevolution and interaction between *L. invasa* and its predominant bacteria.

Introduction

There are numerous microorganisms living in insects, including bacteria, fungi, yeast and viruses, that play a vital role in the growth and reproduction of host insects (*Dillon & Dillon, 2004; Doğanlar, 2005; Crotti et al., 2012; Frago et al., 2012; Engel & Moran, 2013; Hammer & Bowers., 2015*). In the course of long-term coevolution, microorganisms have a close relationship with host insects, which may have an effect on reproduction, survival, community interaction, and the ability to resist predators and vectors (*Oliver et al., 2003, 2010; Moran, 2007; Clark et al., 2008; Moran et al., 2008; Moya et al., 2008*). Bacterial diversity and function have been well studied in some insects. For instance, the bacteria in termites focus on *Bacteroidetes, Firmicutes* and *Actinobacteria* and could assist their hosts in breaking down lignocellulose and promoting the nitrogen cycle (*Warnecke et al., 2007; Brune, 2014).* The bacteria in *Aphis gossypii* improve the resistance and adaptation of the host (*Łukasik et al., 2013a, b*).

In addition, previous investigations have shown that sex is an important factor affecting bacterial diversity. For example, due to different attacking behaviors, the overall diversity and richness of bacterial communities associated with female *Dendroctonus valens* are relatively higher than those associated with male beetles (*Xu et al., 2016*). The bacterial composition of mosquitoes was also affected by the different sexes (*Minard et al., 2013; Zouache et al., 2011*). Different anatomies and life histories of male and female flies could provide differential opportunities for bacterial colonization (*Tang et al., 2012*).

The blue gum chalcid *Leptocybe invasa* Fisher & LaSalle (Hymenoptera: Eulophidae: Tetrastichinae) is a cosmopolitan pest that damages many *Eucalyptus* species (*Mendel et al., 2004; Le et al., 2018*). *L. invasa*, originated in Australia, was first recorded in 2000 and has been discovered in 45 countries of Asia, Europe, Africa, Oceania and America thus far (*Le et al., 2018; Zheng et al., 2014*). Every delicate twig, vein and petiole of Eucalyptus trees may provide a spawning ground for this pest, and galls ultimately lead to the stunted growth of the trees, causing great losses in local eucalyptus plantations (*Mendel et al., 2004; Zheng et al., 2014; Huang et al., 2018*).

Until now, few studies have reported on the overall interior bacteria of *L. invasa,* which is an invasive and gall insect. Only a few studies have reported their interior bacteria completely. *Wang et al. (2018)* cultured 11 strains in female adults of *L. invasa* in winter using traditional methods and classified them into 3 phyla (*Firmicutes, Actinobacteria, Proteobacteria*), 3 classes

(*Bacilli, Actinobacteria, Gammaproteobacteria*) and 4 orders (*Bacillales, Micrococcales, Lactobacillales, Enterobacterales*) that were related to growth, development, nutrition metabolism and immunity. *Nugnes et al. (2015)* researched the bacteria living in adults among different populations through denaturing gradient gel electrophoresis (DGGE) analysis and found that *Rickettsia* occurred in the reproductive tissues of female *L. invasa*, resulting in the speculation of a relationship with its thelytokous parthenogenesis. *L. invasa* harbors a myriad of bacteria (*Wang et al., 2018; Nugnes et al., 2015*), and bacterial differences between sexes have a large effect on insects. Therefore, the overall interior bacterial composition and abundance of *L. invasa* and the differences between both sexes are important to study.

In this study, the interior bacteria in female and male adults of *L. invasa* were sequenced by 16S rDNA from the V3-V4 region to shed light on the interior bacterial composition. Adult females and males were also compared to address sexual differences in the interior bacteria. These results would provide valuable bacterial pool of *L. invasa* and would further contribute to understanding their productive strategies and invasion mechanisms.

Materials & Methods

Insect sampling

L. invasa female and male adults were captured from *Eucalyptus* plantations located at the Teaching and Experiment Base of Forestry College, Guangxi University (108°17′ E, 22°51′ N), Nanning City, Guangxi Zhuang Autonomous Region. The host plant in this survey was DH201-2 (*Eucalyptus grandis* × *E. tereticornis*) (Myrtales: Myrtaceae).

Total DNA extraction

Both sexes adults of *L. invasa* newly emerged into 12 h were fasted for 6 h, and each sex included 50 adults. Then both samples sterilized externally with 75% ethanol for 2-5 min, and rinsed third times in sterilized water to remove microbes on the surface. Total bacterial DNA of each samples were extracted using the Power Soil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's instructions. DNA quality and quantity were assessed by the ratios of 260 nm/280 nm and 260 nm/230 nm. Then the qualified DNA was stored at -80 ℃ until further processing.

PCR amplification and cloning of bacterial 16S rDNA gene

The V3-V4 hypervariable region of the bacterial 16S rRNA gene was performed using bacteria-universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-

GACTACHVGGGTWTCTAAT-3'). The PCR reactions were carried out in a 50 μL solution containing 10 μL 10 × buffer, 0.2 μL Q5 High-Fidelity DNA Polymerase, 10 μL High GC Enhancer, 1 μL dNTP, 10 μM of each forward and reverse primer, 60 ng genome DNA and up to 50 μL with dd H_2O. The amplifications were performed in an ABI Applied Biosystems 9902 thermal cycler with an initial denaturation step at 95 ℃ for 5 min, followed 35 cycles of annealing and extending (each cycle occurred at 95 ℃ for 1 min, followed by 50 ℃ for 1 min and an extension step at 72 ℃ for 1 min) and the final extension at 72 ℃ for 7 min. The PCR products were checked by electrophoresis on an agarose gel (1.8% agarose, 1 × TBE) followed by staining with ethidium bromide and visualization under ultraviolet light. The PCR products from the first step PCR were purified through VAHTSTM DNA Clean Beads. A second round PCR was then performed in a 40 μL reaction which contained 20 μL 2 × Phμsion HF MM, 8 μL ddH_2O, 10 μM of each forward and reverse primer and 10 μL PCR products from the first step. The second PCR was run under the following conditions: an initial denaturation at 98 ℃ for 30s, followed by 10 cycles at 98 ℃ for 10 s, 65 ℃ for 30 s and 72 ℃ for 30 s, with a final extension at 72 ℃ for 5 min. Finally, all PCR products were quantified and pooled together by Quant-iT™ dsDNA HS Reagent. High-throughput sequencing analysis of bacterial rRNA genes was performed on the purified, pooled sample using the Illumina Hiseq 2500 platform at Biomarker Technologies Co., Ltd, Beijing, China.

Bioinformatics and statistical analysis

After sequencing, PE Reads obtained from HiSeq sequencing were merged by overlapping to obtain raw tags. To obtain clean tags, the raw tags were denoised, sorted and separated using Trimmomatic (version 0.33). The remaining sequences were filtered for redundancy, and all unique sequences for each sample were then clustered into operational taxonomic units (OTUs) at similarities of 97%. Low-abundance OTUs were identified and eliminated using UCHIME v4.2. The taxonomic notes of the OTUs were conducted in the Silva reference database. Species abundance tables were generated by QIIME, and community structures in every taxon category were plotted by R software. The relative abundances of the bacteria were determined as percentages. The relatively high abundances at the genus level were selected to construct the phylogenetic tree.

Alpha diversity based on Chao1 richness and ACE richness estimators, as well as Simpson and Shannon diversity indices, was evaluated using the Mothur v.1.11.0 program. Among them,

Chao1 and ACE measured species richness in the samples, Shannon reflected community diversity, Simpson reflected the concentration degree of dominant species in the community, and coverage index reflected whether the sequencing results represented the real situation of microorganisms in the samples. A higher Chao1, ACE and Shannon index and a lower Simpson index indicates that the species in a sample are more abundant. A higher coverage indicates a higher probability of a detected species and a lower probability of an undetected species.

Results

Sequencing and Classification

A total of 533266 raw tags (370680 from males and 162586 from females) were obtained from *L. invasa,* and 476235 effect tags (328833 from males and 147402 from females) were generated (Table S1), which were classified into different OTUs based on the identity level at 97%. Among the 476235 effect tags, a total of 1320 OTUs were obtained; of these 1320 OTUs, 154 OTUs were common to both sexes, and there were 38 and 1128 specific OTUs belonging to female and male adults, respectively (Fig 1).

Analysis of Alpha Diversity

Alpha diversity was estimated by five indices: Chao1, Shannon, Simpson, ACE and coverage. The results in Table 1 show that the bacteria in *L. invasa* adults were diverse between both sexes. Among them, the Chao1 (229.50 vs 1282.00) and ACE estimators (212.84 vs 1282.28) were lower in the females than in the males. Good agreement was also observed between Simpson and Shannon indices. The Shannon index (0.59 vs 6.13) was lower in the females than in the males, while the Simpson index (0.85 vs 0.01) was higher in the female wasps than in the male wasps, indicating that the diversity of the bacterial community in males was higher than that in females. The coverage was near 100% for both males and females, illustrating a higher probability of bacteria that were detected and a lower probability of bacteria that were undetected.

The Analysis of Community Composition and Species Abundance

The bacterial community composition and species abundance in both sexes of *L. invasa* were analyzed (abundance more than 0.1%) based on the results of the OTUs (Table 2, Fig 2). At the phylum level, a total of 24 phyla were detected and classified in the samples. *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, Saccharibacteria, Fusobacteria, Acidobacteria* and *Chloroflexi* were the dominant bacteria annotated in females, and of them, *Proteobacteria* was the highest, accounting for 95.63% of the total. Males not only had the same

bacteria as females but also had *Gemmatimonadetes, Nitrospirae, Spirochaetae* and *Tenericutes*. Among them, Proteobacteria was also the dominant bacteria in males, with an abundance of 34.99%, and *Firmicutes* was the subdominant bacteria, accounting for 33.06% of the total. At the class level, 71 classes were annotated, including *Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria, Clostridia, Bacteroidia, Bacilli, Fusobacteria, Actinobacteria, Deltaproteobacteria, Sphingobacteria, Erysipelotrichia, Gemmatimonadetes, Spirochaetes, Flavobacteria, Acidimicrobia, Solibacteres, Negativicutes,* and *Epsilonproteobacteria*. *Alphaproteobacteria* were the dominant bacteria in females, with an abundance of 94.45%. The dominant and subdominant bacteria in males were *Clostridia* (abundance was 22.95%) and *Alphaproteobacteria* (abundance was 16.28%), respectively. There were 130 orders detected and classified, including *Rickettsiales, Clostridiales, Bacteroidales, Rhizobiales, Lactobacillales,* and *Fusobacteriales*, and among them, 40 orders were common to both sexes. The difference was that *Rickettsiales* had the highest abundance in females, accounting for 93.72%, but *Clostridiales*, *Bacteroidales* and *Rhizobiales* were the most abundant in males, accounting for 22.90%, 13.16% and 10.59%, respectively. At the family level, 245 families were detected and classified. The dominant bacteria were *Rickettsiaceae* in females, with an abundance of 93.67% in total, and the dominant and subdominant bacteria in males were *Ruminococcaceae* and *Lachnospiraceae* with abundances of 10.43% and 8.65%, respectively. At the genus level, 501 genera were classified, including *Rickettsia*, *Rhizobium*, *Fusobacterium*, and *Sphingomonas*. *Rickettsia* (an abundance of 93.67%) and *Rhizobium* (an abundance of 5.73%) were the dominant bacteria in females and males, respectively. In addition, it was noteworthy that the abundance of *Rickettsia* was less than 1% in males (Table 3). The phylogenetic relationship of bacteria in both sexes of *L. invasa* is shown in Fig 3.

Discussion

Insects harbor various bacteria, some of which influence the reproduction of host insects over a long period of coevolution (*Dillon & Dillon, 2004; Frago et al., 2012*). Indeed, bacteria that manipulate the sex rate and reproduction of *L. invasa* could exist (*Nugnes et al., 2015*). Previous studies have suggested that the reproductive mode of *L. invasa* is mainly thelytokous parthenogenesis, but male adults have also been found in Turkey (*Doğanlar, 2005*), China (*Zheng et al., 2014*) and India (*Kumari et al., 2010*). The sex ratio (male: female) was 1: 2.1-1: 5.5 in some areas and up to 1: 23.2-1: 195 in others (*Doğanlar, 2005; Zheng et al., 2014, 2018*).

Undoubtedly, the rapid spread and fast growth of populations of *L. invasa* are closely related to the female-biased sex ratios and thelytokous (*Zheng et al., 2014*). Therefore, comparing the bacterial communities and various sexes in this paper may be very important for the reproductive strategies and biocontrol of *L. invasa*.

Differences in the bacteria between female and male adults

This research revealed that the bacteria harbored in *L. invasa* have high diversity. The microorganisms found in the female adults were classified into 10 phyla, 26 classes, 44 orders, 76 families, and 122 genera, and those in the male adults were classified into 24 phyla, 69 classes, 127 orders, 238 families, and 487 genera (Table 2). The diversity of the bacterial community in males was higher than that in females, which also appeared in the Alpha diversity analysis (Table 1). Furthermore, the bacterial phylotypes and their relative abundances differed significantly between male and female wasps of *L. invasa*. The abundance of *Proteobacteria* varied at the phylum level, although *Proteobacteria* was the dominant bacteria in both sexes. The dominant bacteria in both sexes of *L. invasa* were dissimilar at other levels. In females, the dominant bacteria were *Alphaproteobacteria, Rickettsiales, Rickettsiaceae* and *Rickettsia*, while *Clostridia, Clostridiales, Ruminococcaceae* and *Rhizobium* were the dominant bacteria in males (Fig 2). In addition, sequences of *Gemmatimonadetes, Spirochaetae, Sphingobacteria, Rhizobiaceae*, *Chitinophagaceae*, *Xanthomonas* and *Vibrio* were detected in males. The variation of bacterial communities between males and females may be partly explained by the different physiological structure between the two sexes of *L. invasa,* namely, that the female wasps have ovaries, which harbor an abundance of *Rickettsia*, and occupy different bacterial niches than the males (*Nugnes et al., 2015*). Another possibility is that insects could also launch innate and systematic immune responses to cope with the colonization of microbes (*Leulier & Royet, 2009*), and females have stronger immune systems than males (*Kurtz et al., 2000*).

Comparison of the bacteria with other insects

The bacterial community analysis at the phyla level demonstrated that *Proteobacteria* was the most dominant group in female and male wasps, and *Firmicutes, Bacteroidetes, Actinobacteria* and *Fusobacteria* were also annotated. Previous studies revealed that *Proteobacteria* were dominant in many insects, such as *Bactrocera tau* (*Prabhakar et al., 2012*), *Lutzomyia* sand fly (*Sant'Anna et al., 2012*), *Schistocerca gregaria* (*Dillon et al., 2010*) and *Anopheles stephensi* (*Rani et al., 2009*). Moreover, the major bacteria were also *Proteobacteria* in *Bactrocera minax*

(*Wang et al., 2004*), ground beetles (*Jonathan et al., 2007*), *Helicoverpa armigera* larvae (*Priya et al., 2012*) and *Holotrichia parallela* larvae (*Huang et al., 2013*). Furthermore, *Proteobacteria* or *Firmicutes* were the dominant bacteria in *Plutella xylostella* larvae (*Xia et al., 2013*), *Aedes albopictus* and *A. aegypti* (*Zouache et al., 2011*). In contrast, *Firmicutes* and *Bacteroidetes* were the major bacteria phyla detected in the guts of termites (*Xiang et al., 2012*) and bees (*Mohr & Tebbe, 2006*).

Functional prediction of dominant bacteria

Several of the bacteria detected in this study are commonly described in insects at the genus level, and some have been found in Hymenoptera, such as honeybees (*Mohr & Tebbe, 2006*) and termites (*Xiang et al., 2012*). Intriguingly, two genera, *Staphylococcus* and *Escherichia*, were known to contain cultivable species (*Wang et al., 2018*). Gloverin and lysozyme gene expression was upregulated when silkworm larvae were fed *Escherichia* and *Staphylococcus*, indicating that the two bacteria are closely related to the immune signaling pathway of the silkworm (*Douglas, 2015*). We hypothesized that *Escherichia* and *Staphylococcus* may also be involved in the immunoreaction of *L. invasa*. Functions have been suggested for some of the other bacterial genera detected in this study. The *Enterobacteriaceae* that are associated with insects help with digestion, the detoxification of toxic substances, resistance to pathogens and enhance the adaptability of the host (*Anand et al., 2010*). Adding *Enterobacter* in feed could extend the life span of Mediterranean flies (*Behar et al., 2005, 2008*). Similarly, *Enterobacteriaceae* (*Hongoh & Ishikawa, 2000*) and *Acinetobacter* (*Broderick et al., 2004*) could facilitate carbon-nitrogen metabolism and accelerate the growth and development of host insects, e.g., the *Acinetobacter* belonging to termites have a nitrogen-transforming function according to Warnecke's *(2007)* research. *Enterobacteriaceae* and *Acinetobacter* have significant effects on the growth of *L. invasa*, and carbon, nitrogen and other elements play a very important role in nutrition as essential amino acids rely on these elements to build central carbon skeletons. Some bacteria associated with immunization were also discovered in *L. invasa*, such as *Lactobacillus*. *Lactobacillus* had some positive effects on insect resistance (*Xia et al., 2013*). In addition, *Bacillales* were also detected in this study and may be insect pathogens, such as *Bacillus thuringiensis* and *B. cereus* (*Broderick et al., 2004; Raymond et al., 2010; Song et al., 2014*). In contrast, some *Bacillus* in termites might be involved in the degradation of cellulose and hemicellulose (*Konig, 2006*). In this study, *Bacillales* were detected in both genders, and their

specific functions need further study. Nevertheless, *Acinetobacter* was detected in *L. invasa,* and previous research showed that *Acinetobacter* produces an antiviral compound that inhibits a tobacco mosaic virus (*Lee et al., 2009*). Moreover, members of *Bacteroidetes* are specialized in the degradation of complex organic matter, including lignocellulosic compounds (*Yuki et al., 2015*). *Bacteroidetes* are also involved in the decomposition and metabolism of polysaccharides (*Xu et al., 2003; Sonnenburg et al., 2010*), which are beneficial to the absorption and digestion of the host (*Liu et al., 2011*). In addition, the *Bacteroidetes* also include some *Azotobacter*, such as *Azobacteroides pseudotrichonympha*, which could provide a host with amino acids for nutrition (*Doda et al., 2009; Desai & Brune, 2012*). *Bacteroidetes* related to degradation and fermentation of phytomass could influence the nutrient absorption of *L. invasa*, but further studies are needed. Many other groups of bacteria with undefined functions were detected in *L. invasa* for the first time in this study. A better knowledge of the bacteria associated with *L. invasa* will allow researchers to investigate their role in host biology.

A sequence similarity search revealed that *Rhizobium* was the dominant bacterium in male adults (Fig 2, Table 3). *Rhizobium* produces a variety of enzymes with cellulose- and pectin-hydrolyzing activities that can hydrolyze the glycoside skeleton of the plant cell wall and play a very important role in the symbiosis between *Rhizobium* and leguminous plants (*Robledo et al., 2008; Huang et al., 2018*). *Rhizobium* is an endosymbiont detected in the gut of some phytophagous insects and can help the host synthesize nitrogen-containing substances that are lacking in food (*Russell et al., 2009*).

Rickettsia (an abundance of 93.67%) was the dominant bacteria present in female adults, while less than 1% was present in males (Fig 2, Table 3). *Rickettsia* is a maternally inherited intracellular bacterium in a wide range of arthropods and is capable of controlling populations by reproductive manipulations, such as parthenogenesis inducing (PI) (*Hagimori et al., 2006; Adachi-Hagimori et al., 2008; Giorgini et al., 2010*) and male killing (Lawson *et al*., 2001; Schulenburg *et al*., 2001; Majerus & Maherus, 2010). Moreover, *Rickettsia* affects the fitness in the host and avoids adverse environmental conditions (*Oliver et al., 2003; Sakurai et al., 2005; Chiel et al., 2009; Himler et al., 2011; Brumin et al., 2011*). For instance, preadult development of *Bemisia tabaci* B-biotype was faster with *Rickettsia* infection than without (*Chiel et al., 2009*). Compared with uninfected whiteflies, *Himler et al. (2011)* found that *Rickettsia*-carrying whiteflies produced more offspring, developed faster, had a higher rate of survival to adulthood,

and produced a higher proportion of daughters. *Nugnes et al. (2015)* found that *Rickettsia* is located in reproductive tissues in females and passed to the next generation through vertical transmission; thus, a possible reason for thelytokous parthenogenesis in *L. invasa*. The female *L. invasa* is dominant and plays an important role in invasion and colonization (*Zheng et al., 2014*). The results of the current investigation could explain why the sex ratio in wasps is female-biased and support the hypothesis that *Rickettsia* can induce thelytokous parthenogenesis in *L. invasa*. However, both explanations need further testing. In this research, a low level of *Rickettsia* was present in males. A previous investigation suggested that *Rickettsia* could pass to the offspring by vertical transmission (*Nugnes et al. 2015*), and a threshold density of *Rickettsia* bacteria in eggs is required to trigger the development of female embryos (*Giorgini et al., 2010*). Although no evidence has shown that the *Rickettsia* living in *L. invasa* can be transmitted horizontally (*Gualtieri et al., 2017*), we cannot rule out the possibility that male-*Rickettsia* is obtained through horizontal transmission in some way. Removing *Rickettsia* by feeding antibiotics could produce more male offspring. *Giorgini et al. (2010)* found that *Rickettsia*-infected *Pnigalio soemius* only generate female progeny, and after 24 h, when the *Rickettsia* were removed by 20 mg/mL rifampin, adults produced almost all male offspring. *Hagimori et al. (2006)* declared that *Rickettsia* was related to the thelytokous parthenogenesis of *Neochrysocharis formosa*, a dominant parasite of leaf miner, and after removing *Rickettsia* from the adults by feeding tetracycline, female offspring without *Rickettsia* were present. Therefore, future studies should clarify whether *Rickettsia* is involved in the reproductive manipulation of *L. invasa* through feeding with antibiotics.

Conclusions

The results in this study characterize the bacterial diversity and differences between both sexes in *L. invasa* by high-throughput sequencing, suggesting that the interior bacterial community was abundant and that the majority of these species remained uncultivated. Moreover, the males harbored a more diverse bacterial community than the females, and the bacterial communities of *L. invasa* varied between the two sexes. These results enrich the information of microbial information of *L. invasa*, help research the reproductive strategy, sex control and invasive mechanism, and lay the foundation for further studies on the excavation and utilization of microbes for the biological control of *L. invasa*.

Acknowledgements

The authors thank Prof. Yongqiang He for sharing his knowledge of bacteria, the State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, and the menbers of the Guangxi Key Laboratory of Forest Ecology and Conservation.

References

Adachi-Hagimori T, Miura K, Stouthamer R. 2008. A new cytogenetic mechanism for bacterial endosymbiont-induced parthenogenesis. *Proceedings of the Royal Society B-Biological Sciences* **275(1652):** 2667-2673 DOI 10.1098/rspb.2008.0792.

Anand AA, Vennison SJ, Sankar SG, Prabhu, DIG, Vasan PT, Raghuraman T, Geoffrey CJ, Vendan SE. 2010. Isolation and characterization of bacteria from the gut of Bombyx mori that degrade cellulose, xylan, pectin and starch and their impact on digestion. *Journal of Insect Science* **10:** 1-20 DOI 10.1673/031.010.10701.

Behar A, Yuval B, Jurkevitch E. 2005. Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly *Ceratitis capitata*. *Molecular Ecology* **14(9):** 2637-2643 DOI 10.1111/j.1365-294X.2005.02615.x.

Behar A, Yuval B, Jurkevitch E. 2008. Community structure of the Mediterranean fruit fly microbiota: seasonal and spatial sources of variation. *Israel Journal of Ecology & Evolution* **54(2):** 181-191 DOI 10.1080/15659801.2008.10639612.

Briones-Roblero CI, Rodriguez-Diaz R, Santiago-Cruz JA, Zuniga G, Rivera-Orduna FN. 2017. Degradation capacities of bacteria and yeasts isolated from the gut of *Dendroctonus rhizophagus* (Curculionidae: Scolytinae). *Folia Microbiologica* 1-9 DOI 10.1007/s12223-016-0469-4.

Broderick NA, Raffa KF, Goodman RM, Handelsman J. 2004. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Applied Environmental Microbiology* **70(1):** 293-300 DOI 10.1128/AEM.70.1.293-300.2004.

Brune A. 2014. Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology* **12(3):** 168-180 DOI 10.1038/nrmicro3182.

Brumin M, Kontsedalov S, Ghanim M. 2011. *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. *Insect Science* **18(1):** 57-66 DOI 10.1111/j.1744-7917.2010.01396.x.

Chiel E, Inbar M, Mozes-Daube N, White JA, Hunter MS, Zchori-Fein E. 2009. Assessments of fitness effects by the facultative symbiont, *Rickettsia*, in the sweetpotato whitefly (Hemiptera: Aleyrodidae). *Annals of the Entomological Society of America* **102(3):** 413-418 DOI 10.1603/008.102.0309.

Clark ME, Bailey-Jourdain C, Ferree PM, England SJ, Sullivan W, Windsor DM, Werren JH. 2008. *Wolbachia* modification of sperm does not always require residence within developing sperm. *Heredity* **101(5):** 420-428 DOI 10.1038/hdy.2008.71.

Crotti E, Balloi A, Hamdi C, Sansonno L, Marzorati M, Gonella E, Favia G, Cherif A, Bandi C, Alma A, Daffonchio D, 2012. Microbial symbionts: a resource for the management of insect-related problems. *Microbial Biotechnology* **5(3):** 307-317 DOI 10.1111/j.1751-7915.2011.00312.x.

Desai MS, Brune A. 2012. *Bacteroidales* ectosymbionts of gut flagellates shape the nitrogen-fixing community in dry-wood termites. *ISME Journal* **6(7):** 1302-1313 DOI 10.1038/ismej.2011.194.

Dillon RJ, Dillon VM. 2004. The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology* **49:** 71-92 DOI 10.1146/annurev.ento.49.061802.123416.

Dillon RJ, Webster G, Weightman AJ, Charnley AK. 2010. Diversity of gut microbiota increases with aging and starvation in the desert locust. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* **97(1):** 69-77 DOI 10.1007/s10482-009-9389-5.

Doğanlar O. 2005. Occurrence of *Leptocybe invasa* Fisher & La Salle, 2004 (Hymenoptera: Chalcidoidea: Eulophidae) on Eucalyptus camaldulensis in Turkey, with description of the male sex. *Zoology in the Middle East* **35:** 112-114 DOI 10.1080/09397140.2005.10638116.

Douglas AE. 2015. Multiorganismal Insects: Diversity and Function of Resident Microorganisms. *Annual Review of Entomology* **60:** 17-34 DOI 10.1146/annurev-ento-010814-020822.

Engel P, Moran NA. 2013. The gut microbiota of insects-diversity in structure and function. *FEMS Microbiology Reviews* **37(5):** 699-735 DOI 10.1111/1574-6976.12025.

Frago E, Dicke M, Godfray HCJ. 2012. Insect symbionts as hidden players in insect-plant interactions. *Trends in Ecology & Evolution* 27(12): 705-711 DOI 10.1016/j.tree.2012.08.013.

Giorgini M, Bernardo U, Monti MM, Nappo AG, Gebiola M. 2010. *Rickettsia* symbionts cause parthenogenetic reproduction in the parasitoid wasp *Pnigalio soemius* (Hymenoptera: Eulophidae). *Applied and Environmental Microbiology* 76(8): 2589-2599 DOI 10.1128/AEM.03154-09.

401 **Gualtieri L, Nugnes F, Nappo AG, Gebiola M, Bernardo U. 2017.** Life inside a gall:
402 closeness does not favour horizontal transmission of *Rickettsia* between a gall wasp and its
403 parasitoid. *FEMS Microbiology Ecology* **93(7):** fix087 DOI 10.1093/femsec/fix087.
404 **Hagimori T, Abe Y, Date S, Miura K. 2006.** The first finding of a *Rickettsia* bacterium
405 associated with parthenogenesis induction among insects. *Current Microbiology* **52(2):** 97-
406 101 DOI 10.1007/s00284-005-0092-0.
407 **Hammer TJ, Bowers MD. 2015.** Gut microbes may facilitate insect herbivory of chemically
408 defended plants. *Oecologia* **179(1):** 1-14 DOI 10.1007/s00442-015-3327-1.
409 **Himler AG, Adachi-Hagimori T, Bergen JE, Kozuch A, Kelly SE, Tabashnik BE, Chiel E,**
410 **Duckworth VE, Dennehy TJ, Zchori-Fein E, Hunter MS. 2011.** Rapid spread of a
411 bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias.
412 *Science* **332(6026):** 254-256 DOI 10.1126/science.1199410.
413 **Hongoh Y, Ishikawa H. 2000.** Evolutionary studies on uricases of fungal endosymbionts of
414 aphids and planthoppers. *Journal of Molecular Evolution* **51(3):** 265-277 DOI
415 10.1007/s002390010088.
416 **Huang S, Zhang H. 2013.** The impact of environmental heterogeneity and life stage on the
417 hindgut microbiota of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). *PLoS ONE*
418 **8(2):** e57169 DOI 10.1371/journal.pone.0057169.
419 **Huang ZY, Li J, Lu W, Zheng XL, Yang ZD. 2018.** Parasitoids of the eucalyptus gall wasp
420 *Leptocybe* spp.: a global review. *Environmental Science and Pollution Research* **25(30):**
421 29983-29995 DOI 10.1007/s11356-018-3073-0.
422 **Jonathan G, Lundgren R, Michael L, Joanne CS. 2007.** Bacterial communities within
423 digestive tracts of ground beetles (Coleoptera: Carabidae). *Annals of the Entomological*
424 *Society of America* **100(2):** 275-282 DOI 10.1603/0013-8746(2007)100[275:
425 BCWDTO]2.0.CO;2.
426 **Konig H. 2006.** *Bacillus* species in the intestine of termites and other soil invertebrates. *Journal*
427 *of Applied Microbiology* **101:** 620–627 DOI 10.1111/j.1365-2672.2006.02914.x.
428 **Kumari KN, Kulkarni H, Vastrad AS, Goud KB. 2010.** Biology of eucalyptus gall wasp,
429 *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae). *Karnataka Journal of*
430 *Agricultural Sciences* **23:** 211-212.
431 **Kurtz J, Wiesner A, Gotz P, Sauer KP. 2000.** Gender differences and individual variation in

the immune system of the scorpionfly *Panorpa vulgaris* (Insecta: Mecoptera). *Development and Comparative Immunology* **24(1):** 1-12 DOI 10.1016/S0145-305X(99)00057-9.

Lawson ET, Mousseau TA, Klaper R, Hunter MD, Werren JH. 2001. *Rickettsia* associated with male-killing in a buprestid beetle. *Heredity* **86(4):** 497-505 DOI 10.1046/j.1365-2540.2001.00848.x.

Le NH, Nahrung HF, Griffiths M, Lawson SA. 2018. Invasive *Leptocybe* spp. and their natural enemies: Global movement of an insect fauna on eucalypts. *Biological Control* **125:** 7-14 DOI 10.1016/j.biocontrol.2018.06.004.

Lee JS, Lee KC, Kim KK, Hwang IC, Jang C, Kim NG, Yeo WH, Kim BS, Yu YM, Ahn JS. 2009. *Acinetobacter antiviralis* sp. nov., from Tobacco plant roots. *Journal of Microbiology and Biotechnology* **19(3):** 250-256 DOI 10.4014/jmb.0901.083.

Leulier F, Royet J. 2009. Maintaining immune homeostasis in fly gut. *Nature Immunology* **10(9):** 936-938 DOI 10.1038/ni0909-936.

Liu N, Yan X, Zhang ML, Xie L, Wang QA, Huang YP, Zhou XG, Wang SY, Zhou ZH. 2011. Microbiome of fungus-growing termites: a new reservoir for lignocellulase genes. *Applied and Environmental Microbiology* **77(1):** 48-56 DOI 10.1128/AEM.01521-10.

Łukasik P, Guo H, Van Asch M, Ferrari J, Godfray HCJ. 2013a. Protection against a fungal pathogen conferred by the aphid facultative endosymbionts *Rickettsia* and *Spiroplasma* is expressed in multiple host genotypes and species and is not influenced by co-infection with another symbiont. *Journal of Evolutionary Biology* 26(12): 2654-2661.

Łukasik, P, Van Asch M, Guo HF, Ferrari J, Godfray HCJ. 2013b. Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters* **16(2):** 214-218 DOI 10.1111/ele.12031.

Majerus TMO, Majerus MEN. 2010. Discovery and identification of a male-killing agent in the Japanese ladybird *Propylea japonica* (Coleoptera: Coccinellidae). *BMC Evolution Biology* **10:** 37 DOI 10.1186/1471-2148-10-37.

Mendel Z, Protasov A, Fisher N, La Salle J. 2004. Taxonomy and biology of *Leptocybe invasa* gen. & sp. n. (Hymenoptera: Eulophidae), an invasive gall inducer on *Eucalyptus*. *Australian Journal of Entomology* **43:** 101-113 DOI 10.1111/j.1440-6055.2003.00393.x.

Minard G, Mavingui P, Moro CV. 2013. Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasites & Vectors* **6:** 146 DOI 10.1186/1756-3305-6-146.

Mohr KI, Tebbe CC. 2006. Diversity and phylotype consistency of bacteria in the guts of three bee species (*Apoidea*) at an oilseed rape field. Environmental Microbiology **8(2):** 258-272 DOI 10.1111/j.1462-2920.2005.00893.x.

Moran NA. 2007. Symbiosis as an adaptive process and source of phenotypic complexity. *Proceedings of the National Academy of Sciences of the United States of America* **104:** 8627-8633 DOI 10.1073/pnas.0611659104.

Moran NA, McCutcheon JP, Nakabachi A. 2008. Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics* **42:** 165-190 DOI 10.1146/annurev.genet.41.110306.130119.

Moran NA. 2016. Insights into the roles of bacterial symbionts within flagellates of termite guts. *Environmental Microbiology Reports* **8(5):** 559-559 DOI 10.1111/1758-2229.12471.

Moya A, Pereto J, Gil R, Latorre A. 2008. Learning how to live together: genomic insights into prokaryote–animal symbioses. *Nature Review of Genetic*s **9(3):** 218-229 DOI 10.1038/nrg2319.

Noda S, Hongoh Y, Sato T, Ohkuma M. 2009. Complex coevolutionary history of symbiotic Bacteroidades bacteria of various protist in the gut of termites. *BMC Evolutionary Biology* **9:** 1-12 DOI: 10.1186/1471-2148-9-158.

Nugnes F, Gebiola M, Monti MM, Gualtieri L, Giorgini M, Wang JG, Bernardo U. 2015. Genetic diversity of the invasive gall wasp *Leptocybe invasa* (Hymenoptera: Eulophidae) and of its *Rickettsia* endosymbiont, and associated sex-ratio differences. *PLoS One* **10(5):** e0124660 DOI 10.1371/journal.pone.0124660.

Oliver KM, Russell JA, Moran NA, Hunter MS. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America* **100(4):** 1803-1807 DOI 10.1073/pnas.0335320100.

Oliver KM, Degnan PH, Burke GR, Moran NA. 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology* **55:** 247-266 DOI 10.1146/annurev-ento-112408-085305.

Prabhakar CS, Sood P, Kanwar SS, Sharma PN, Kumar A, Mehta PK. 2012. Isolation and characterization of gut bacteria of fruit fly, *Bactrocera tau* (Walker). *Phytoparasitica* **41(2):** 193-201 DOI 10.1007/s12600-012-0278-5.

Priya NG, Ojha A, Kajla MK, Raj A, Rajagopal R. 2012. Host Plant Induced Variation in Gut

Bacteria of *Helicoverpa armigera*. *PLoS one* **7(1):** e30768 DOI 10.1371/journal.pone.0030768.

Rani A, Sharma A, Rajagopal R, Adak T, Bhatnagar RK. 2009. Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected *Anopheles stephensi*-an Asian malarial vector. *BMC Microbiology* **9:** 1471-2081 DOI 10.1186/1471-2180-9-96.

Raymond B, Johnston PR, Nielsen LC, Lereclus D, Crickmore N, Lereclus D, Crickmore N. 2010. Bacillus thuringiensis: an impotent pathogen? *Trends in Microbiology* **18(5):** 189-194 DOI 10.1016/j.tim.2010.02.006.

Robledo M, Jimenez-Zurdo JI, Velazquez E, Trujillo ME, Zurdo-Pineiro JL, Ramirez-Bahena MH, Ramos B, Diaz-Minguez JM, Dazzo F, Martinez-Molina E, Mateos PF. 2008. *Rhizobium* cellulase CelC2 is essential for primary symbiotic infection of legume host roots. *Proceedings of the National Academy of Sciences of the United States of America* **105(19):** 7064-7069 DOI 10.1073/pnas.0802547105.

Russell JA, Moreau CS, Goldman-Huertas B, Fujiwara M, Lohman DJ, Pierce NE. 2009. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. P *Proceedings of the National Academy of Sciences of the United States of America* **106(50):** 21236-21241 DOI 10.1073/pnas.0907926106.

Sakurai M, Koga R, Tsuchida T, Meng XY, Fukatsu T. 2005. *Rickettsia* symbiont in the pea aphid *Acyrthosiphon pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. *Applied and Environmental Microbiology* **71(7):** 4069-4075 DOI 10.1128/AEM.71.7.4069-4075.2005.

Sant'Anna MRV, Darby AC, Brazil RP, Montoya, LJ, Dillon VM, Bates PA, Dillon RJ. 2012. Investigation of the bacterial communities associated with females of *Lutzomyia* Sand fly species from South America. *PLoS One* **7(8):** e42531 DOI: 10.1371/journal.pone.0042531.

Schulenburg JHGV, Habig M, Sloggett JJ, Webberley KM, Bertrand D, Hurst GDD, Majerus MEN. 2001. Incidence of male-killing *Rickettsia* spp. (α-Proteobacteria) in the ten-spot ladybird beetle *Adalia decempunctata* L. (Coleoptera: Coccinellidae). *Applied Environmental Microbiology* **67(1):** 270-277 DOI 10.1128/AEM.67.1.270-277.2001.

Song F, Peng Q, Brillard J, Lereclus D, LeRoux CN. 2014. An insect gut environment reveals

the induction of a new sugar-phosphate sensor system in *Bacillus cereus*. *Gut Microbes* **5(1):** 58-63 DOI 10.4161/gmic.27902.

Sonnenburg ED, Zheng H, Joglekar P, Higginbottom SK, Firbank SJ, Bolam DN, Sonnenburg JL. 2010. Specificity of polysaccharide use in intestinal Bacteroides species determines diet-induced microbiota alterations. *Cell* **141(7):** 1241-1252 DOI 10.1016/j.cell.2010.05.005.

Tang X, Adler PH, Vogel H, Ping LY. 2012. Gender-specific bacterial composition of black flies (Diptera: Simuliidae). *FEMS Microbiology Ecology* **80(3):** 659-670 DOI 10.1111/j.1574-6941.2012.01335.x.

Wang AL, Yao ZC, Zheng WW, Zhang HY. 2014. Bacterial Communities in the gut and reproductive organs of *Bactrocera minax (Diptera: Tephritidae)* based on 454 Pyrosequencing. *PLoS ONE* **9(9):** e106988 DOI 10.1371/journal.pone.0106988.

Wang RR, Hu Y, Yang ZD, Guo CH, Zhu LH, Zheng XL, Yu SZ. 2018. Isolation, identification and diversity of culturable bacteria in female adults of *Leptocybe invasa* Fisher & La Salle. *Journal of Southern Agriculture* **49(12):** 2432-2439. (in Chinese with English abstract)

Warnecke F, Luginbuhl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, McHardy AC, Djordjevic G, Aboushadi N, Sorek R, Tringe SG, Podar M, Martin HG, Kunin V, Dalevi D, Madejska J, Kirton E, Platt D, Szeto E, Salamov A, Barry K, Mikhailova N, Kyrpides NC, Matson EG, Ottesen EA, Zhang X, Hernandez M, Murillo C, Acosta LG, Rigoutsos I, Tamayo G, Green BD, Chang C, Rubin EM, Mathur EJ, Robertson DE, Hugenholtz P, Leadbetter JR. 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* **450(7169):** 560-565 DOI 10.1038/nature06269.

Xia XF, Zheng DD, Zhong HZ, Qin BC, Gurr GM, Vasseur L, Lin HL, Bai JL, He WY, You MS. 2013. DNA sequencing reveals the midgut microbiota of diamondback moth, *Plutella xylostella* (L.) and a possible relationship with insecticide resistance. *PLoS ONE* **8(7):** e68852 DOI 10.1371/journal.pone.0068852.

Xiang H, Xie L, Zhang J, Long YH, Liu N, Huang YP, Wang Q. 2012. Intracolonial difference in gut bacterial community between worker and soldier castes of *Coptotermes formosanus*. *Insect Science* **19(1):** 86-95 DOI 10.1111/j.1744-7917.2011.01435.x.

Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, Gordon JI. 2003. A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. *Science* **299(5615):** 2074-2076 DOI 10.1126/science.1080029.

Xu LT, Lu M, Xu DD, Chen L, Sun JH. 2016. Sexual variation of bacterial microbiota of *Dendroctonus valens* guts and frass in relation to verbenone production. *Journal of Insect Physiology* **95:** 110-117 DOI 10.1016/j.jinsphys.2016.09.014.

Yuki M, Kuwahara H, Shintani M, Izawa K, Sato T, Starns, D, Hongoh Y, Ohkuma M. 2015. Dominant ectosymbiotic bacteria of cellulolytic protists in the termite gut also have the potential to digest lignocellulose. *Environmental Microbiology* **17(12):** 4942-4953 DOI 10.1111/1462-2920.12945.

Zheng XL, Li J, Yang ZD, Xian ZH, Wei JG, Lei CL, Wang XP, Lu W. 2014. A review of invasive biology, prevalence and management of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). *African Entomology* **22(1):** 68-79 DOI 10.4001/003.022.0133.

Zheng XL, Huang ZY, Li J, Yang ZD, Yang XH, Lu W. 2018. Reproductive Biology of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae). *Neotropical Entomology* **47(1):** 19-25 DOI 10.1007/s13744-017-0502-6.

Zouache K, Raharimalala FN, Raquin V, Tran-Van V, Raveloson LHR, Ravelonandro P, Mavingui P. 2011. Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. *FEMS Microbiology Ecology* **75(3):** 377-389 DOI 10.1111/j.1574-6941.2010.01012.x.

Figure 1

Venn diagram of OTU distribution in *Leptocybe invasa* female and male adults.

Numbers within compartments indicate OTU counts of according to mathematical sets.

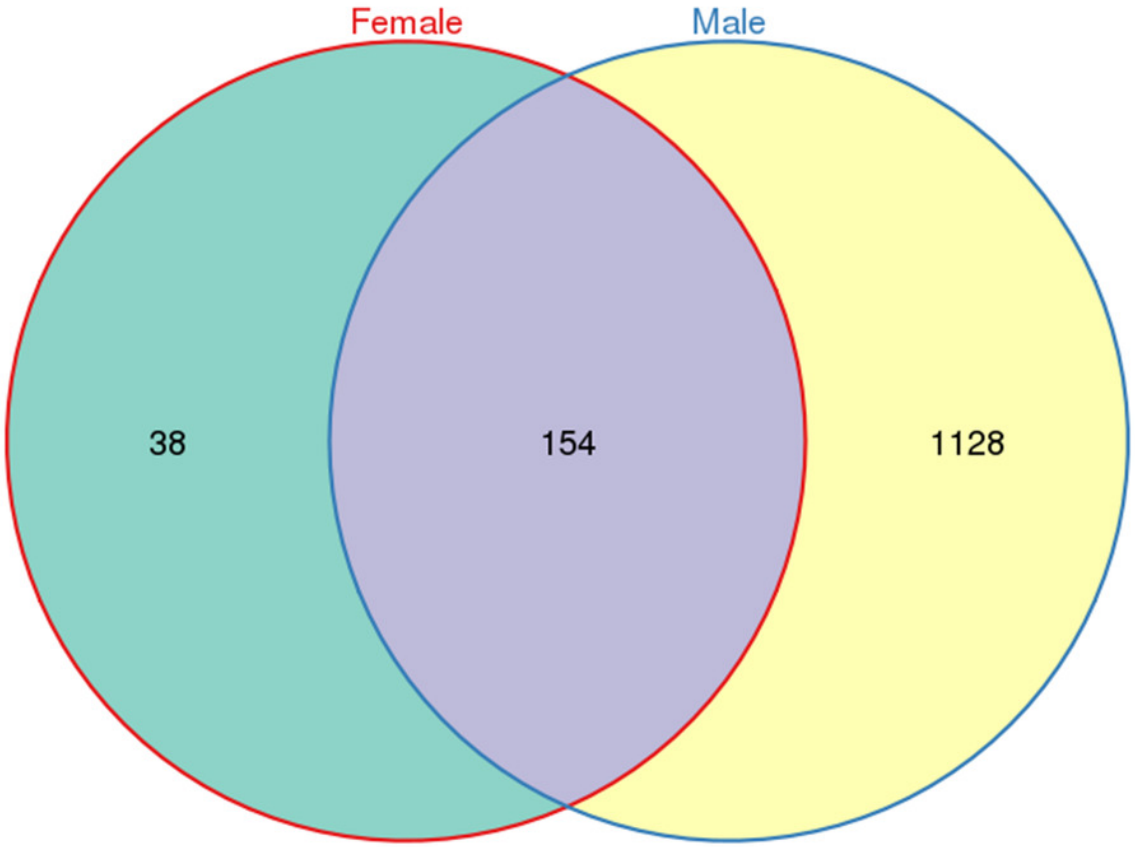

Figure 2

Relative abundance of top 10 bacteria at the levels of phylum (A), class (B), order (C), family (D) and genus (E) in female and male adults of *Leptocybe invasa*.

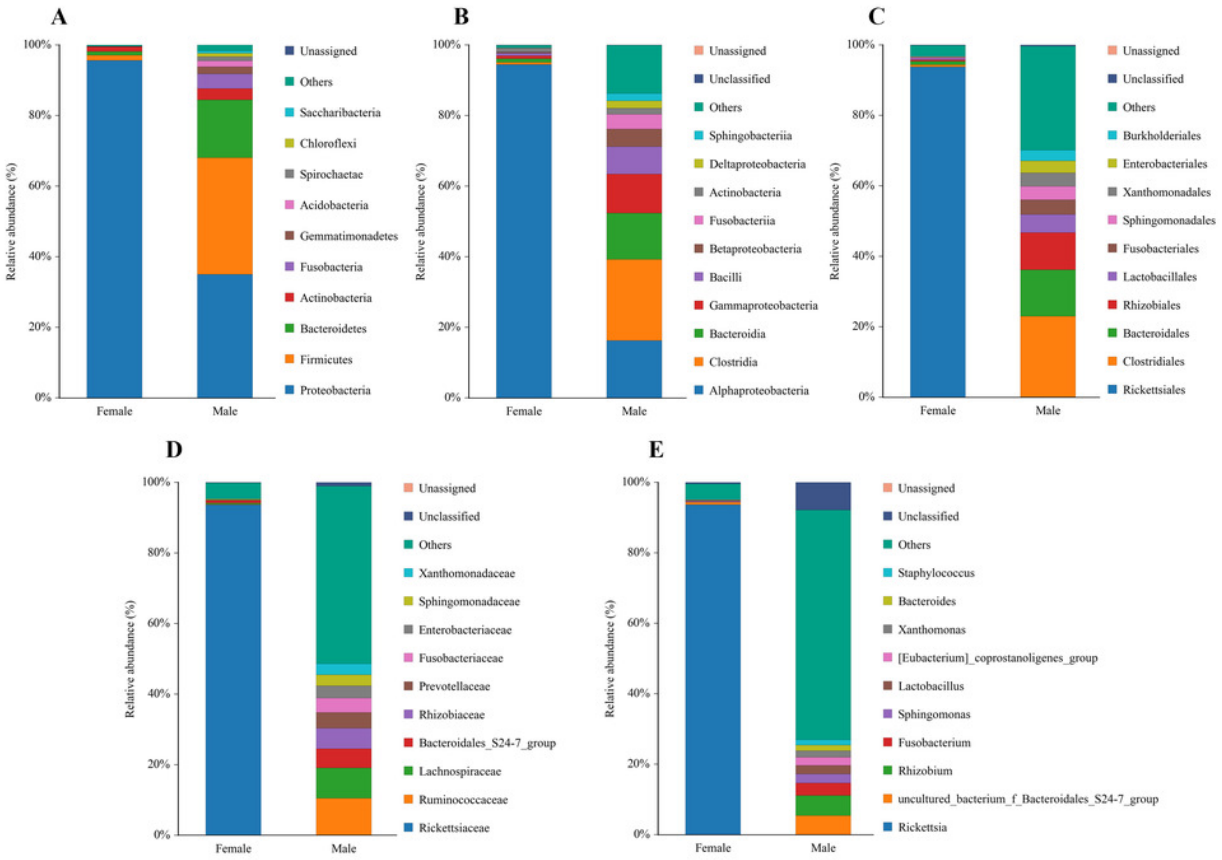

Figure 3

Phylogenetic tree of the bacteria in female and male adults of *Leptocybe invasa* at the genus level (top 100).

The genus of the same phylum was shown with the same color.

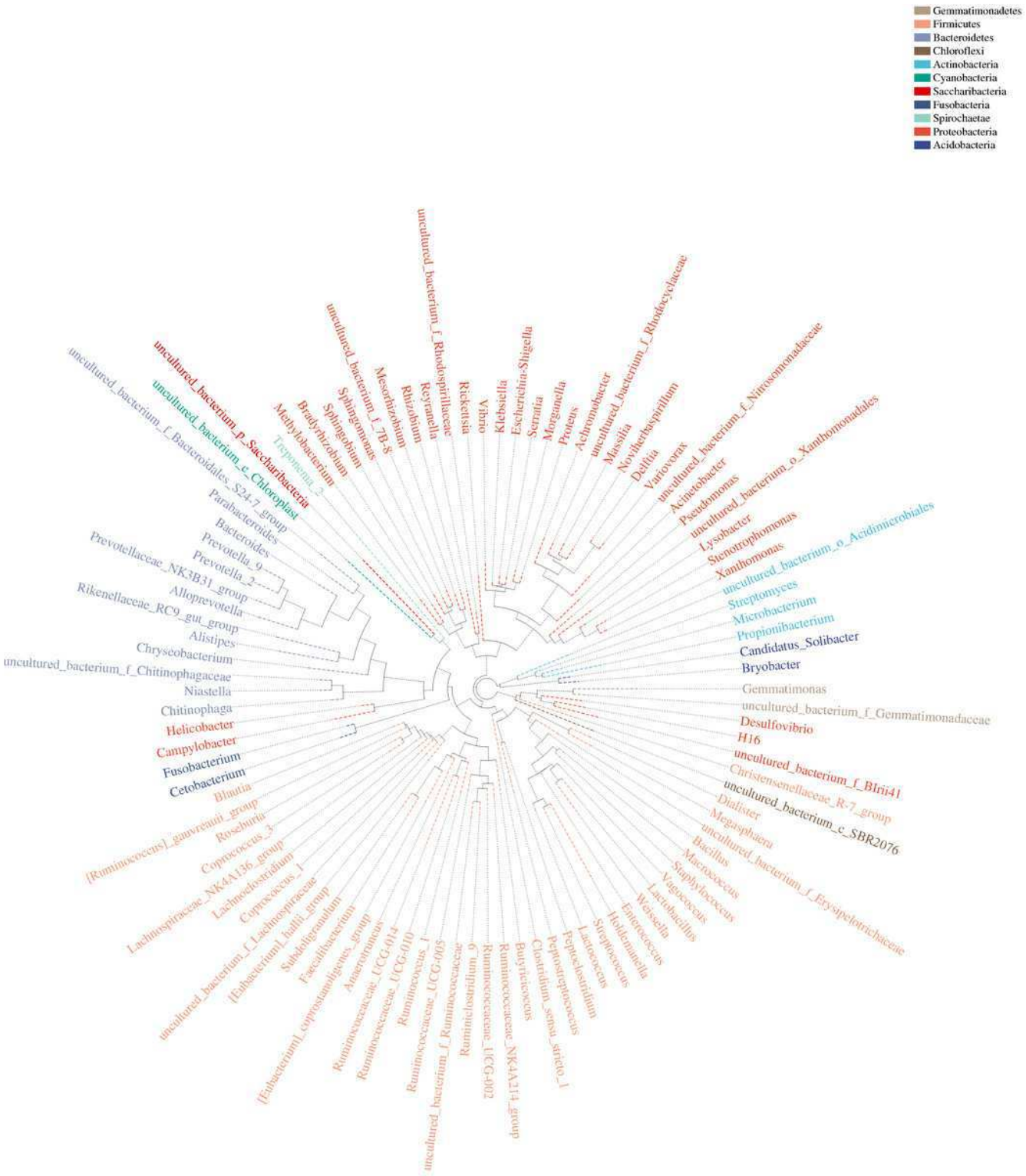
Gemmatimonadetes
Firmicutes
Bacteroidetes
Chloroflexi
Actinobacteria
Cyanobacteria
Saccharibacteria
Fusobacteria
Spirochaetae
Proteobacteria
Acidobacteria
uncultured_bacterium_f_Rhodospirillaceae
Rickettsia
Vibrio
Klebsiella
Escherichia-Shigella
Serratia
Morganella
Proteus
Achromobacter
uncultured_bacterium_f_Rhodocyclaceae
Massilia
Novherbaspirillum
Delftia
Variovorax
uncultured_bacterium_f_Nitrosomonadaceae
Acinetobacter
Pseudomonas
uncultured_bacterium_o_Xanthomonadales
Lysobacter
Stenotrophomonas
Xanthomonas
uncultured_bacterium_o_Acidimicrobiales
Streptomyces
Microbacterium
Propionibacterium
Candidatus_Solibacter
Bryobacter
Gemmatimonas
uncultured_bacterium_f_Gemmatimonadaceae
Desulfovibrio
H16
uncultured_bacterium_f_BIrii41
Christensenellaceae_R-7_group
uncultured_bacterium_c_SBR2076
Dialister
Megasphaera
uncultured_bacterium_f_Erysipelotrichaceae
Bacillus
Macrococcus
Staphylococcus
Vagococcus
Lactobacillus
Weissella
Enterococcus
Holdemanella
Streptococcus
Lactococcus
Peptostreptococcus
Clostridium_sensu_stricto_1
Butyricicoccus
Ruminococcaceae_NK4A214_group
Ruminococcaceae_UCG-002
Romboutsia
uncultured_bacterium_f_Ruminococcaceae
Ruminococcaceae_UCG-005
Ruminococcus_1
Ruminococcaceae_UCG-010
Ruminococcaceae_UCG-014
Anaerotruncus
[Eubacterium]_coprostanoligenes_group
Subdoligranulum
Faecalibacterium
[Eubacterium]_hallii_group
uncultured_bacterium_f_Lachnospiraceae
Coprococcus_1
Lachnoclostridium
Coprococcus_2
Lachnospiraceae_NK4A136_group
Roseburia
[Ruminococcus]_gnavus_group
Blautia
Cetobacterium
Fusobacterium
Campylobacter
Helicobacter
Chitinophaga
Niastella
uncultured_bacterium_f_Chitinophagaceae
Chryseobacterium
Alistipes
Rikenellaceae_RC9_gut_group
Alloprevotella
Prevotellaceae_NK3B31_group
Prevotella_2
Prevotella_9
Bacteroides
Parabacteroides
uncultured_bacterium_f_Bacteroidales_S24-7_group
uncultured_bacterium_c_Chloroplast
uncultured_bacterium_p_Saccharibacteria
Treponema_2
Methylobacterium
Bradyrhizobium
Sphingobium
Sphingomonas
uncultured_bacterium_f_7B-8
Mesorhizobium
Rhizobium
Reyranella

Table 1(on next page)

Statistics of alpha diversity indices of the bacteria in female and male adults of *Leptocybe invasa.*

1

Sample	ACE	Chao1	Simpson	Shannon	Coverage
Female	212.84	229.50	0.85	0.59	1.00
Male	1282.28	1282.00	0.01	6.13	1.00

2

Table 2(on next page)

Basic composition of the bacterial colonies in female and male adults of *Leptocybe invasa*.

1

Sample	Phylum	Class	Order	Family	Genus
Female	10	26	44	76	122
Male	24	69	127	238	487
Female-specific	0	2	3	7	14
Male-specific	14	45	86	169	379
Sex-in common	10	24	41	69	108
Total	24	71	130	245	501

2

3

Table 3(on next page)

Relative abundance of dominate bacteria at the levels of genus in female and male adults of *Leptocybe invasa.*

1

Genus	Female (%)	Male (%)
Rickettsia	93.67	0.04
uncultured_bacterium_f_*Bacteroidales*_S24-7_group	0.71	5.37
Lactobacillus	0.31	2.38
Sphingomonas	0.25	2.62
Bacteroides	0.11	1.65
Fusobacterium	0.04	3.49
[Eubacterium]_*coprostanoligenes*_group	0	2.34
Rhizobium	0	5.73
Unknown	0	0.01
Xanthomonas	0	1.83
Others	4.48	66.68
Unclassified	0.44	7.86

2