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

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, China
² Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, College of Agriculture, Guangxi University, Nanning, Guangxi, China
³ Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning, Guangxi, 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 the thelytokous parthenogenesis, low temperature resistance, protection in galls, generation overlap and small body of *L. invasa* contribute to its rapid invasion and population growth. However, the endosymbiotic bacterial composition, abundance and sex differences of *L. invasa* remain unclear. Therefore, this research aimed to identify the bacterial communities in *L. invasa* adults and compare them between the sexes of *L. invasa* lineage B.

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

**Conclusion.** The endosymbiotic bacteria of *L. invasa* females and males were highly diverse. There were differences in the bacterial community of *L. invasa* between sexes, and the bacterial diversity in male specimens was greater than that in female specimens. This study presents a comprehensive comparison of bacterial communities in *L. invasa* and these data will provide an overall view of the bacterial community in both sexes of *L. invasa* with special attention on sex-related bacteria.
Comparison of bacterial diversity and abundance between sexes of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) from China

Chunhui Guo¹, Xin Peng¹, Xialin Zheng², Xiaoyun Wang², Ruirui Wang¹, Zongyou Huang², Zhende Yang¹, ³

¹ College of Forestry, Guangxi University, Nanning, Guangxi, China
² Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, College of Agriculture, Guangxi University, Nanning, Guangxi, China
³ Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning, Guangxi, China

Corresponding Author:
Zhende Yang¹, ³
Daxuedong Street, Nanning, Guangxi, 530004, China
E-mail address: dzyang68@126.com
Comparison of bacterial diversity and abundance between sexes of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) from China

Chunhui Guo¹, Xin Peng¹, Xialin Zheng², Xiaoyun Wang², Ruirui Wang¹, Zongyou Huang², Zhende Yang¹,³

¹College of Forestry, Guangxi University, Nanning, Guangxi, China
²Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, College of Agriculture, Guangxi University, Nanning, Guangxi, China
³Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning, Guangxi, China

Corresponding Author:
Zhende Yang¹,³

Daxuedong Street, Nanning, Guangxi, 530004, 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 the thelytokous parthenogenesis, low temperature resistance, protection in galls, generation overlap and small body of L. invasa contribute to its rapid invasion and population growth. However, the endosymbiotic bacterial composition, abundance and sex differences of L. invasa remain unclear. Therefore, this research aimed to identify the bacterial communities in L. invasa adults and compare them between the sexes of L. invasa lineage B.

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

Conclusion. The endosymbiotic bacteria of L. invasa females and males were highly diverse. There were differences in the bacterial community of L. invasa between sexes, and the bacterial diversity in male specimens was greater than that in female specimens. This study presents a comprehensive comparison of bacterial communities in L. invasa and these data will provide an overall view of the bacterial community in both sexes of L. invasa with special attention on sex-related 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). Over the course of long-term coevolution, microorganisms develop a close relationship with host insects, which may have an effect on the reproduction, survival, community interactions, and the ability to resist predators and vectors of the hosts (Oliver et al., 2003, 2010; Moran, 2007; Clark et al., 2008; Moran et al., 2008; Moya et al., 2008). In light of the significant functions of the microorganisms, they have received much attention from the international academic community. In some insects, the diversity and function of endosymbiotic bacteria have been well studied. For instance, the bacteria in termites are mainly Bacteroidetes, Firmicutes and Actinobacteria and can assist their hosts in breaking down lignocellulose and promoting the nitrogen cycle (Warnecke et al., 2007; Brune, 2014). The bacteria in Aphis gossypii improve its resistance and adaptation (Łukasik et al., 2013a, b). In recent years, manipulating endosymbionts for pest control has raised wide concern, and its theory and methods have been applied successfully to some extent. Introduction of antimalarial endosymbionts into the mid gut of host pests could inhibit the breeding of plasmodia and in turn reduce the efficiency of mosquito transmission of malaria (Wang et al., 2012). Mixed application of antibiotics and insecticides effectively reduced the quantity of endosymbionts in Nilaparvata lugens while improving the control effect of insecticides (Shentu et al., 2016). Based on research on the related incompatible insect technique (IIT), researchers used the maternally inherited endosymbiotic bacterium Wolbachia for sterilization, which had good effects on eliminating the fecundity of mosquitoes (Zheng et al., 2019). Obviously, it is necessary to clarify the bacterial composition and diversity in insects, which are the bases of manipulating endosymbionts for pest control. In addition, previous investigations have shown that sex is an important factor affecting bacterial diversity. For example, due to different attack behaviors, the overall diversity and richness of bacterial communities associated with female Dendroctonus valens are higher than those associated with males of this beetle species (Xu et al., 2016). The bacterial composition of mosquitoes was also affected by sex (Minard et al., 2013; Zouache et al., 2011). Different anatomies and life histories between 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*, originating in Australia, was first recorded in 2000 and has since been discovered in 45 countries of Asia, Europe, Africa, Oceania and America (*Le et al., 2018; Zheng et al., 2014a*). A new study demonstrated that an increasing number of areas will become suitable for *L. invasa* due to climate warming (*Huang et al., 2019*). Every delicate twig, vein and petiole of eucalyptus trees may provide a spawning ground for this pest, and galls ultimately lead to stunted growth of the trees, causing great losses in local eucalyptus plantations (*Mendel et al., 2004; Zheng et al., 2014a; Huang et al., 2018*). DNA barcode data indicated that *L. invasa* includes two genetically separate lineages (lineages A and B). Researchers considered the Italian, Argentinean and Tunisian populations to belong to lineage A and the Chinese population to belong to lineage B (*Le et al., 2018; Dittrich-Schröder et al., 2018*). The absence of natural enemies, presence of large amounts of suitable host plants, small size, protection in galls, strong resistance to low temperature and thelytokous parthenogenesis of *L. invasa* caused its rapid invasion and growth of in China (*Zheng et al., 2014a*). As a result, it has become one of the most difficult pests to control (*Zheng et al., 2014a; Huang et al., 2018; Le et al., 2018*). It is important in terms of theory and application to study the endosymbiotic bacterial diversity of *L. invasa* and then control the wasps by using these endosymbiotic bacteria.

To date, few studies have reported on the overall endosymbiotic bacteria of *L. invasa*, which is an invasive gall-inducing insect. Only a few studies have comprehensively examined the endosymbiotic bacteria in this species. *Wang et al. (2018)* cultured 11 strains from female adults of *L. invasa* in winter using traditional methods and classified them into 3 phyla (*Firmicutes, Actinobacteria*, and *Proteobacteria*), 3 classes (*Bacilli, Actinobacteria*, and *Gammaproteobacteria*) and 4 orders (*Bacillales, Micrococcales, Lactobacillales*, and *Enterobacterales*) that were related to growth, development, nutrition metabolism and immunity. *Nugnes et al. (2015)* researched the bacteria living in adults among different populations via denaturing gradient gel electrophoresis (DGGE) analysis and found that *Rickettsia* occurred in the reproductive tissues of female *L. invasa*, suggesting a relationship with its thelytokous parthenogenesis. *L. invasa* harbors a myriad of bacteria, and bacterial differences between sexes have strong effects on insects, such as effects on reproductive regulation (*Wang et al., 2018; Nugnes et al., 2015*). Therefore, the overall endosymbiotic bacterial composition and abundance...
of *L. invasa* and the differences between sexes are important to study. In this study, the endosymbiotic bacteria in female and male adults of *L. invasa* were indentified by 16S rRNA sequencing of the V3-V4 region to shed light on their internal bacterial compositions. The females and males were also compared to address sexual differences in the endosymbionts. These results will provide a valuable bacterial pool for *L. invasa* and will further contribute to understanding its reproductive strategies and invasion mechanisms.

**Materials & Methods**

**Insect sampling**

Branches of DH 201-2 (*Eucalyptus grandis* × *E. tereticornis*) (Myrtales: Myrtaceae) harboring galls of *L. invasa* were removed from the Teaching and Experiment Base of Forestry College, Guangxi University (108°17′ E, 22°51′ N), Nanning city, Guangxi Zhuang Autonomous Region from July to August 2018. The branches were placed in a plastic bottle filled with water to retain freshness and transferred into a sealed net cage (40 cm × 40 cm × 80 cm) at room temperature to keep the adults from escaping. The water in the plastic bottle was renewed daily until the emergence of *L. invasa* adults. Sexes were identified by morphological observation (Zheng et al., 2014b).

**DNA extraction**

Fifty adults of each sex of *L. invasa* newly emerged within 12 h were fasted for 6 h. Then, both samples were sterilized externally with 75% ethanol for 2-5 min and rinsed 3 times with sterilized water to remove microbes on the surface. The total bacterial DNA of each sample was extracted using a Power Soil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer’s instructions. The quality and quantity of DNA were assessed by the ratios of 260 nm/280 nm and 260 nm/230 nm. Then, the qualified DNA was stored at -80 °C for further processing. The DNA of each individual was extracted by using a Chelex-100 and proteinase K-based method (Gebiola et al., 2009).

**PCR amplification and cloning of the bacterial 16S rRNA gene**

Amplification of the V3-V4 hypervariable region of the bacterial 16S rRNA gene was performed by using the universal bacterial primers 338F (5'-ACTCCTACGGGAGGCAGC-3') and 806R (5'- GACTACHVGGGTWTCTAAT-3'). PCRs were carried out in 50 μL solutions containing 10 μL of 10× buffer, 0.2 μL of Q5 High-Fidelity DNA Polymerase, 10 μL of High GC Enhancer, 1 μL of dNTPs, 10 μM each forward and reverse primer, 60 ng of genomic DNA and enough
ddH₂O to reach 50 μL. The amplifications were performed in a ABI Applied Biosystems 9902 thermal cycler with an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of annealing and extension (each cycle consisted of 95 °C for 1 min, 50 °C for 1 min and extension at 72 °C for 1 min) and a final extension at 72 °C for 7 min. The PCR products were checked by electrophoresis on an agarose gel (1.8% agarose, 1× TBE), stained with ethidium bromide and visualized under ultraviolet light. The products from the first round of PCR were purified with VAHTSTM DNA Clean Beads. The second round of PCR was then performed in a 40 μL reaction containing 20 μL of 2 × Phusion HF MM, 8 μL of ddH₂O, 10 μM each forward and reverse primer and 10 μL of PCR product produced in the first round. The second round of PCR was run under the following conditions: initial denaturation at 98 °C for 30 s, followed by 10 cycles at 98 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s and a final extension at 72 °C for 5 min. Finally, all PCR products were quantified and pooled by Quant-iT™ dsDNA HS Reagent. High-throughput sequencing analysis of bacterial rRNA genes was performed on the purified, pooled sample by using the Illumina HiSeq 2500 platform at Biomarker Technologies Co., Ltd, Beijing, China.

Bioinformatics and statistical analysis

After sequencing, PE reads obtained with HiSeq sequencing were merged by overlapping to obtain raw tags. To obtain clean tags, the raw tags were denoised, sorted and separated by using Trimmomatic (version 0.33). The remaining sequences were filtered for redundancy, and all unique sequences in each sample were clustered into operational taxonomic units (OTUs) on the basis of 97% similarity. Low-abundance OTUs were identified and eliminated by using UCHIME v4.2. Taxonomic assignment of the OTUs was conducted with the Silva reference database. Species abundance tables were generated by QIIME, and community structures in every taxon category was plotted by R software. The relative abundances of the bacteria were determined by percentages.

Alpha diversity based on Chao1 richness and ACE richness estimators, as well as the Simpson and Shannon diversity indices, was evaluated by using the mothur v.1.11.0 program. Among these measure, Chao1 and ACE reflected species richness in the samples, the Shannon index reflected community diversity, the Simpson index reflected the dominance of species in the community, and the coverage index reflected the degree to which the sequencing results represented the actual composition of the microorganisms in the samples.
Molecular characterization and phylogenetic analyses
COI was amplified by using the forward primer LCO1490 and reverse primer HCO2198 (Nugnes et al., 2015). The 16S rRNA gene of Rickettsia was amplified by using the primers listed in Table S1. The PCR program for both genes (COI and 16S rRNA) was as follows: 3 min of initial denaturation at 94 °C, 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min and a final extension of 5 min at 72 °C. PCR products were observed by using 1.0% agarose gel electrophoresis, and the amplified fragments were directly sequenced by TsingKe Biological Technology Co., Ltd, Beijing, China. Representative sequences of other regions were downloaded from GenBank, and sequence alignment was completed by using Clustal X. The neighbour-joining method was used to construct a consensus phylogenetic tree with MEGA7 software. To evaluate the branch support of the phylogenetic tree, bootstrap analysis of 1000 replicates was performed.

Accession numbers
Data is available at NCBI SRA, accession numbers: SRR9591039, SRR9591038. The COI and 16S rRNA sequences determined in this study have been deposited in the GenBank database with Accession number MN524231 and MN524230, respectively.

Results
Sex of L. invasa specimens in this study
All female and male specimens were identified on the basis of morphology. In this study, a total of 656 females and 51 males were collected (Table S2). The materials were deposited at the Forest Conservation Laboratory, College of Forestry, Guangxi University, Nanning 530004, China.

Sequencing and classification
A total of 533266 raw tags (370680 from males and 162586 from females) were obtained for L. invasa, and 476235 clean tags (328833 from males and 147402 from females) were generated (Table S3), which were classified into different OTUs based on 97% similarity. Among the 476235 clean tags, a total of 1320 OTUs were obtained; of these 1320 OTUs, 154 were common to both sexes, and 38 and 1128 were specific to female and male adults, respectively (Fig. 1).

Analysis of Alpha diversity
Alpha diversity was estimated by five indices: Chao1, the Shannon index, the Simpson index, the ACE and coverage. The results in Table 1 show that the bacteria in L. invasa adults were diverse
in both sexes. The Chao1 (229.50 vs 1282.00) and ACE (212.84 vs 1282.28) values were lower in the females than in the males. Good agreement was also observed between the 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 being detected than of bacteria being undetected.

**The analysis of community composition and species abundance**

The bacterial community composition and species abundance in both sexes of *L. invasa* were analyzed (abundances greater than 0.1%) based on the results of the OTUs (Table 2, Fig. 2). A total of 24 phyla were detected and classified in the samples. *Proteobacteria* was the dominant bacterial phylum annotated in females and males, accounting for 95.63% and 34.99% of bacteria, respectively. At the genus level, *Rickettsia* (with an abundance of 93.67%) and *Rhizobium* (with 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).

**Molecular characterization and phylogenetic analyses**

After comparison with Genebank, the identification of *L. invasa* in this research was lineage B and the phylogenetic tree of COI also indicated that the population of this research belonged to the lineage (Fig. 3). The phylogenetic analysis of 16S rRNA genes revealed that the *Rickettsia* of *L. invasa* symbionts belonged to the *Rickettsia* transitional group (Fig. 4).

**Discussion**

**Differences in bacteria between female and male adults**

This research revealed that the bacteria harbored in *L. invasa* had high diversity, and many of the endosymbiotic bacteria were annotated in this species for the first time. Based on alpha diversity analysis, the diversity of the endosymbiotic bacteria in males was higher than that in females (Table 1). The variation in bacterial communities between males and females may be partly explained by differences in physiology structure and between the two sexes of *L. invasa*; specifically, the female wasps have ovaries, which harbor an abundance of *Rickettsia*, allowing the genus to occupy different bacterial niches than in males (*Nugnes et al., 2015*). Another possibility is that insects launch innate and systematic immune responses to cope with microbe colonization (*Leulier & Royet, 2009*) and females have stronger immune systems than males.
Comparison of the bacteria with those in other insects

Bacterial community analysis at the phylum level demonstrated that *Proteobacteria* was the dominant group in female and male wasps, and *Firmicutes, Bacteroidetes, Actinobacteria* and *Fusobacteria* were also annotated. Previous studies revealed that *Proteobacteria* were dominant in other Hymenoptera, such as *Apis cerana* and leaf-cutter ants (*Ahn et al., 2012; Zhukova et al., 2017*). In contrast, *Firmicutes and Bacteroidetes* were the major bacterial phyla detected in the guts of termites (*Miyata et al., 2007; Xiang et al., 2012*) and bees (*Mohr & Tebbe, 2006*). *Firmicutes* and *Actinobacteria* were the dominant bacteria in *A. mellifera* and bumblebees (*Ahn et al., 2012; Praet et al., 2018*).

Putative Functions of dominant endosymbiotic bacteria in *L. invasa*

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*, are 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 were 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, and resistance to pathogens and enhance the adaptability of the host (*Anand et al., 2010*). Adding *Enterobacter* to feed extended the life span of Mediterranean flies (*Behar et al., 2005, 2008*). Similarly, *Enterobacteriaceae* (*Hongoh & Ishikawa, 2000*) and *Acinetobacter* (*Broderick et al., 2004*) facilitated carbon-nitrogen metabolism and accelerated 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. 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 *Bacillus 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 sexes, and their specific functions require further study. Nevertheless, Acinetobacter was detected in L. invasa, and previous research showed that Acinetobacter produces an antiviral compound that inhibits tobacco mosaic virus (Lee et al., 2009). Moreover, members of Bacteroidetes specialize 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 for host absorption and digestion (Liu et al., 2011). In addition, Bacteroidetes also include some Azotobacter, such as Azobacteroides pseudotrichonympha, which can provide the host with amino acids for nutrition (Doda et al., 2009; Desai & Brune, 2012). Bacteroidetes involved in the 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. 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 (with an abundance of 93.67%) was the dominant bacterial genus present in female adults (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 manipulation, such as parthenogenesis inducing (PI) (Hagimori et al., 2006; Adachi-Hagimori et al., 2008; Giorgini et al., 2010) and male killing (MK) (Lawson et al., 2001; Schelenburg et al., 2001; Majerus & Maherus, 2010). During female gamete formation in Rickettsia-carrying Neochrysocharis formosa, meiotic cells underwent only one equatorial division, and meiotic recombination was absent, which demonstrated that Rickettsia could induce parthenogenesis by changing the meiosis of wasps (Adachi-Hagimori et al., 2008). Rickettsia also induced male embryo death in Adalia bipunctata and A. decempunctata (Hurst GDD et al., 1993, 1996;
Moreover, *Rickettsia* affects the fitness of the host and protects it against 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 the *Bemisia tabaci* B-biotype was faster with *Rickettsia* infection than without (Chiel et al., 2009). 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 larger proportion of daughters than did uninfected whiteflies. Males have never been recorded in Italy, Tunisia and Argentina, and rarely in Turkey (sex ratio 0-0.5%) (Nugnes et al., 2015). These results show that *L. invasa* reproduces by thelytokous parthenogenesis. In contrast, males appeared more frequently in China, India and Thailand. In this study, the sex ratio was 7.2%. In addition, Nugnes et al. (2015) found that *Rickettsia* was located in reproductive tissues in females and passed to the next generation via vertical transmission, representing a possible reason for thelytokous parthenogenesis in *L. invasa*. Female *L. invasa* play an important role in invasion and colonization (Zheng et al., 2014a). 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 require further testing. In addition, a low abundance of *Rickettsia* was present in males in this research. For Hymenoptera, the dominant reproductive mode is arrhenotoky; that is, diploid females develop from fertilized eggs, and haploid males develop from unfertilized eggs (van Wilgenburg et al., 2006). A previous investigation suggested that *Rickettsia* could be passed 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., 2001; 2010). Removing *Rickettsia* by feeding antibiotics could lead to the production of more male offspring. Giorgini et al. (2010) found that *Rickettsia*-infected *Pnigalio soemius* generated only female progeny, and after 24 h, when the *Rickettsia* was 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 *N. formosa*, a dominant parasite of leaf miners, and after removing *Rickettsia* from the adults by feeding the adults tetracycline, female offspring without *Rickettsia* were present. Therefore, future studies should clarify whether *Rickettsia* is involved in the reproductive manipulation of *L. invasa* accomplished via feeding with antibiotics. Furthermore, environmental factors could also influence the density of the bacteria, and endosymbiont
densities and functions may change with space, time and season (Bordenstein & Bordenstein, 2011; Nugnes et al., 2015). A previous study indicated that the sex ratio of the Chinese population could change with temperature, presumably because the relationship between Rickettsia strain and Chinese population is weaker than that of Western population, which could be more susceptible to temperature (Zhu et al., 2014; Nugnes et al., 2015). In addition, another plausible explanation may be the use of different host plants (the host of lineage A is E. camaldulensis but in this research, it was DH201-2), which has been demonstrated in other systems (Ferrari et al., 2007; Biere and Tack 2013). Therefore, it is also essential to compare the differences in bacteria between L. invasa that parasitize different hosts.

**Conclusions**

The results of this study obtained by high-throughput revealed the bacterial diversity and differences between sexes in L. invasa, suggesting an abundant endosymbiotic bacterial community, and some bacteria were reported in L. invasa for the first time. Moreover, the males harbored a more diverse bacterial community than did the females. The next research should focus on the bacteria found in this study to identify their specific ecological functions and the specific sex-based regulatory mechanism of Rickettsia occurrence in 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 members of the Guangxi Key Laboratory of Forest Ecology and Conservation.
References

Ahn JH, Hong IP, Bok JI, Kim BY, Song J, Weon HY. 2012. Pyrosequencing analysis of the bacterial communities in the guts of Honey Bees Apis cerana and Apis mellifera in Korea. Journal of Microbiology 50(5): 735-745 DOI 10.1007/s12275-012-2188-0.

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.

Biere A, Tack AJM. 2013. Evolutionary adaptation in three-way interactions between plants, microbes and arthropods. Function Ecology 27: 646-660. DOI 10.1111/1365-2435.12096.

Bordenstein SR, Bordenstein SR. 2011. Temperature affects the tripartite interactions between bacteriophage WO, Wolbachia, and cytoplasmic incompatibility. PLoS ONE 6: e29106 DOI 10.1371/journal.pone.0029106.

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 62(1): 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
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.

Dittrich-Schröder G, Hoareau TB, Hurley BP, Wingfield MJ, Lawson S, Nahrung HF, Slippers B. 2018. Population genetic analyses of complex global insect invasions in managed landscapes: a *Leptocybe invasa* (Hymenoptera) case study. *Biological Invasions* **20**(9): 2395-2420. DOI 10.1007/s10530-018-1709-0.

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.
Ferrari J, Scarborough CL, Godfray HCJ. 2007. Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. *Oecologia* 153: 323-329 DOI 10.2307/40210868.

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. 2001. Induction of males in thelytokous populations of *Encarsia meritoria* and *Encarsia protransvena*: a systematic tool. *BioControl* 46(4): 427-438 DOI 10.1023/A:1014181431482.

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.

Gualtieri L, Nugnes F, Nappo AG, Gebiola M, Bernardo U. 2017. Life inside a gall: closeness does not favour horizontal transmission of *Rickettsia* between a gall wasp and its parasitoid. *FEMS Microbiology Ecology* 93(7): fix087 DOI 10.1093/femsec/fix087.

Hagimori T, Abe Y, Date S, Miura K. 2006. The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. *Current Microbiology* 52(2): 97-101 DOI 10.1007/s00284-005-0092-0.

Hammer TJ, Bowers MD. 2015. Gut microbes may facilitate insect herbivory of chemically defended plants. *Oecologia* 179(1): 1-14 DOI 10.1007/s00442-015-3327-1.

Hurst GDD, Majerus MEN, Walker LE. 1993. The importance of cytoplasmic male killing elements in natural populations of the two spot ladybird, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). *Biological Journal of the Linnean Society* 49(2): 195-202.

Hurst GDD, Walker LE, Majerus MEN. 1996. Bacterial infections of hemocytes associated with the maternally inherited male-killing trait in British populations of the two spot ladybird, *Adalia bipunctata*. *Journal of Invertebrate Pathology* 68(3): 286-292. DOI 10.1006/jipa.1996.0098.

Himler AG, Adachi-Hagimori T, Bergen JE, Kozuch A, Kelly SE, Tabashnik BE, Chiel E, Duckworth VE, Dennehy TJ, Zchori-Fein E, Hunter MS. 2011. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness...
benefits and female bias. *Science* **332**(6026): 254-256 DOI 10.1126/science.1199410.

Hongoh Y, Ishikawa H. 2000. Evolutionary studies on uricases of fungal endosymbionts of aphids and planthoppers. *Journal of Molecular Evolution* **51**(3): 265-277 DOI 10.1007/s002390010088.

Huang MY, Ge XZ, Shi HL, Tong YG, Shi J. 2019. Prediction of Current and Future Potential Distributions of the Eucalyptus Pest *Leptocybe invasa* (Hymenoptera: Eulophidae) in China Using the CLIMEX Model. *Pest management science* DOI10.1002/ps.5408.

Huang XF, Chaparro JM, Reardon KF, Judd TM, Vivanco JM. 2016. Supplementing blends of sugars, amino acids, and secondary metabolites to the diet of Termites (*Reticulitermes flavipes*) drive distinct gut bacterial communities. *Microbial Ecology* **72**(3): 497-502 DOI 10.1007/s00248-016-0792-y.

Huang ZY, Li J, Lu W, Zheng XL, Yang ZD. 2018. Parasitoids of the eucalyptus gall wasp *Leptocybe* spp.: a global review. *Environmental Science and Pollution Research* **25**(30): 29983-29995 DOI 10.1007/s11356-018-3073-0.

Konig H. 2006. *Bacillus* species in the intestine of termites and other soil invertebrates. *Journal of Applied Microbiology* **101**: 620–627 DOI 10.1111/j.1365-2672.2006.02914.x.

Kumari KN, Kulkarni H, Vastrad AS, Goud KB. 2010. Biology of eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae). *Karnataka Journal of Agricultural Sciences* **23**: 211-212.

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.

Makonde HM, Boga HI, Osiemo Z, Mwirichia R, Mackenzie LM, Goker M, Klenk HP. 2013. 16S-rRNA-based analysis of bacterial diversity in the gut of fungus-cultivating termites (Microtermes and Odontotermes species). Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology 104(5): 869-883 DOI 10.1007/s10482-013-0001-7.

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.

Miyata R, Noda N, Tamaki H, Kinjyo K, Aoyagi H, Uchiyama H, Tanaka H. 2007. Influence of Feed Components on Symbiotic Bacterial Community Structure in the Gut of the Wood-Feeding Higher Termite Nasutitermes takasagoensis. Bioscience Biotechnology and Biochemistry 71(5): 1244-1251 DOI 10.1271/bbb.60672.

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 Genetics* 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.

Praet J, Parmentier A, Schmid-Hempel R, Meeus I, Smagghe G, Vandamme P. 2018. Large-scale cultivation of the bumblebee gut microbiota reveals an underestimated bacterial species diversity capable of pathogen inhibition. *Environmental Microbiology* 20(1): 214-227 DOI: 10.1111/1462-2920.13973.

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. *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.

Schulenburg JHGV, Habig M, Slogget 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.

Shi WB, Syrenne R, Sun JZ, Yuan JS. 2010. Molecular approaches to study the insect gut symbiotic microbiota at the 'omics' age. *Insect Science* 17(3): 199-219. DOI 10.1111/j.1744-7917.2010.01340.x.

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.

van Wilgenburg E, Driessen G, Beukeboom L W. 2006. Single locus complementary sex
determination in Hymenoptera: an “unintelligent” design. *Frontiers in Zoology* **3(1):** 1-15
DOI 10.1186/1742-9994-3-1.

Wang SB, Ghosh AK, Bongio N, Stebbings KA, Lampe DJ, Jacobs-Lorena M. 2012. Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America* **109(31):** 12734-12739. DOI 10.1073/pnas.1204158109.

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.

Werren JH, Hurst GDD, Zhang W, Breeuwer JA, Stouthamer R, Majerus ME. 1994. Rickettsial relative associated with male killing in the ladybird beetle (*Adalia bipunctata*). *Journal of Bacteriology*, **176(2):** 388-394.

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. 2014a. 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, Yang ZD, Li J, Xian ZH, Yang J, Liu JY, Su S, Wang XL, Lu W. 2014b. Rapid identification of both sexes of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae): a morphological perspective. *African Entomology* 22(3): 643-650 DOI 10.4001/003.022.0326.

Zheng XY, Zhang DJ, Li YJ, Yang C, Wu Y, Liang X, Liang YK, Pan XL, Hu LC, Sun Q, Wang XH, Wei YY, Zhu J, Qian W, Yan ZQ, Parker AG, Gilles JRL, Bourtzis K, Bouyer J, Tang MX, Zheng B, Yu JS, Liu JL, Zhuang JJ, Hu ZG, Zhang MC, Gong JT, Hong XY, Zhang ZB, Lin LF, Liu QY, Hu ZY, Wu ZD, Baton LA, Hoffmann AA, Xi ZY. 2019. Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature* DOI 10.1038/s41586-019-1407-9.

Zhu FL, Ren SX, Qiu BL, Wu JH. 2015. Effect of temperature on life table parameters of *Leptocybe invasa* (Hymenoptera: Eulophidae). *Austral Entomology* 54: 71-78. DOI 10.1111/aen.12094.

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 genu (E) in females and males of *Leptocybe invasa*. 
Figure 3

Phylogenetic tree of different *Leptocybe invasa* populations based on COI sequences.
Figure 4

Phylogenetic analysis of different *Rickettsia* groups based on their 16S rRNA sequences.
Table 1 (on next page)

Statistics of alpha diversity indices of the bacteria in female and male adults of *Leptocybe invasa*. 
Table 1:

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

| 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     |
Table 2 (on next page)

Basic composition of the bacterial colonies in female and male adults of *Leptocybe invasa*.
### Table 2:

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

| 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   |
Table 3 (on next page)

Relative abundance of dominate bacteria at the levels of genus in female and male adults of *Leptocybe invasa*.
Table 3:
Relative abundance of dominate bacteria at the levels of genus in female and male adults of *Leptocybe invasa*

| 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 |