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A combination of *Tropilaelaps mercedesae* and imidacloprid negatively affects survival, pollen consumption and midgut bacterial composition of honey bee

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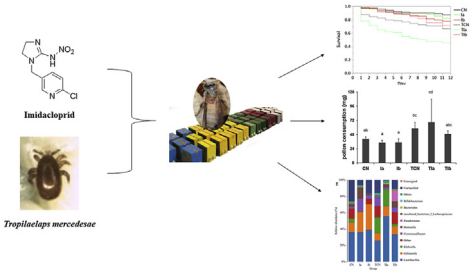
HIGHLIGHTS

- *Tropilaelaps mercedesae* impacted *Apis mellifera* survival.
- *Tropilaelaps mercedesae* and 50 μg/L imidacloprid impacted *Apis mellifera* survival and pollen consumption.
- *Tropilaelaps mercedesae* infestation affected the midgut bacterial composition of *Apis mellifera*.
- *Tropilaelaps mercedesae* and 25 μg/L imidacloprid affected the midgut bacterial composition of *Apis mellifera*.

GRAPHICAL ABSTRACT



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ABSTRACT

Tropilaelaps mercedesae is not only a major threat to honey bees in Asia but also a potential risk to global apiculture due to trade. Imidacloprid is a systemic insecticide that negatively affects individual bees. Moreover, the health of honey bees may be threatened by imidacloprid exposure and *T. mercedesae* infestation. We studied the effects of *T. mercedesae* and imidacloprid on the survival, food consumption and midgut bacterial diversity of *Apis mellifera* in the laboratory. Illumina 16S rRNA gene sequencing was used to determine the bacterial composition in the honey bee midgut. *T. mercedesae* decreased survival in parasitized honey bees compared with nonparasitized honey bees, but there was no significant difference in food consumption. The imidacloprid 50 μg/L diet significantly decreased syrup consumption of *A. mellifera* compared with the control diet. The combination of *T. mercedesae* infestation and imidacloprid 50 μg/L exposure reduced survival and increased pollen consumption of *A. mellifera*. *T. mercedesae* infestation or a combination of *T. mercedesae* infestation and exposure to 25 μg/L imidacloprid affected the midgut bacterial composition of honey bees. *T. mercedesae* infestation and imidacloprid exposure may reduce the survival and affect honey bee health.

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1. Introduction

Tropilaelaps mercedesae in the genus *Tropilaelaps* (Acari: Laelapidae) is considered a major threat to *Apis mellifera* colony health in Asia (Anderson and Morgan, 2007; Khongphinitbunjong et al., 2016). *T. mercedesae* was originally a parasite of the giant honey bee *Apis dorsata* (Anderson and Morgan, 2007) and successfully adapted to a new host, *A. mellifera* (Chantawannakul et al., 2018). *T. mercedesae* mites are similar to *Varroa,* primarily reproducing and completing their lifecycles within capped brood cells (de Guzman et al., 2017). Compared to *Varroa* mites, the smaller size, characteristically rapid locomotion, shorter duration of lay eggs, faster reproductive rate, and ability to mate outside of brood cells of *T. mercedesae* suggest that their populations grow even more rapidly than do those of *Varroa* populations (de Guzman et al., 2017; Chantawannakul et al., 2016, 2018). *T. mercedesae* feeds on the hemolymph of developing honey bees, and the parasites complete their lifecycle within the capped brood cells of their host (Khongphinitbunjong et al., 2016). Unfortunately, *A. mellifera* lacks behavioral defense mechanisms against severe *T. mercedesae* infestations (Khongphinitbunjong et al., 2012). *T. mercedesae* has been found to infest both tropical and temperate zones *of A. mellifera* in mainland Asia (de Guzman et al., 2017) and has been collected from *A. mellifera* colonies in all districts of China (Luo et al., 2011a, 2011b). In severe cases, *T. mercedesae* significantly decreased the survival of *A. mellifera* (Ma et al., 2018). *T. mercedesae* is capable of acting as a vector for diseases such as *acute bee paralysis virus* (ABPV) and may be a vector of *deformed wing virus* (DWV) (Forsgren et al., 2009; Khongphinitbunjong et al., 2016; Wu et al., 2017; Chanpanitkitchote et al., 2018). Furthermore, *T. mercedesae* infestations in brood cells can be high, and surviving *A. mellifera* adult bees often display deformed or missing legs, reduced abdominal size, deformed wings, and shorter life spans when compared with healthy, uninfested bees (Kavinseksan and Wongsiri, 2016). These malformations can lead to low honey yield or outright colony death (de Guzman et al., 2017). Of concern, the risk of global transmission of *T. mercedesae* has increased substantially as a result of trade globalization (Chantawannakul et al., 2018).

Imidacloprid is a systemic insecticide that is absorbed by plants and spread to all tissues through their vascular system (Cycoń et al., 2013; Brandt et al., 2016). Thus, imidacloprid can be present in pollen, nectar and guttation fluids (Brandt et al., 2016), and worker honey bees can become exposed to imidacloprid residues in pollen and nectar (Sánchez-Bayo and Goka, 2014). The residue levels of imidacloprid are incredibly broad in pollen and nectar. For example, a field residual amount of imidacloprid from seed-dressed cotton was reported to range from not detectable to 1.8 μg/kg in nectar and from 1.61 to 64.58 μg/kg in pollen (Jiang et al., 2018). In another study, the mean residue level of imidacloprid was 19.7 μg/kg in pollen and 6 μg/kg in nectar, with maximum residue levels of 912.0 μg/kg in the former and 72.8 μg/kg in the latter (Sánchez-Bayo and Goka, 2014). There has been an increasing amount of evidence that imidacloprid negatively affects individual bees (Brandt et al., 2016; Dai et al., 2017, 2019a). Indeed, honey bee olfactory learning and memory abilities were impaired and caused a delay in homing and foraging behavior after consumption of certain concentration of imidacloprid (Yang et al., 2012). Sublethal doses of imidacloprid affect the individual immunocompetence of honey bees by reducing hemocyte densities, encapsulation responses, and antimicrobial activity and may lead to impaired disease resistance (Brandt et al., 2016).

The microbial community in the digestive tract of insects is abundant, and both the microbes and host function synergistically to prevent the proliferation of pathogens and to preserve the symbiotic relationships between the host and microbiota, allowing the host to maintain proper physiology, nutrition, development, digestion, immunity and overall fitness (Evans and Armstrong, 2006; Crotti et al., 2013; Zhao et al., 2018). The honey bee hosts hundreds of gut bacterial species that are distributed throughout the entire digestive tract; the midgut is the primary location for the digestion of nutrients, to which gut-associated microbes contribute (Ludvigsen et al., 2015). In recent years, studies have shown a tradeoff in bee colonies between pathogen threat and the maintenance of potentially beneficial bacterial symbionts (Evans and Schwarz, 2011; Evans and Armstrong, 2006). The honey bee gut microbiota consists mainly of Alpha-, Beta-, and Gammaproteobacteria, Firmicutes, Bacteroidetes and Actinobacteria, which represent the core gut bacterial components of honey bees worldwide (Crotti et al., 2013; Dai et al., 2018). Gut bacteria facilitate the digestion of food and the breakdown of toxic dietary compounds and produce metabolites that can be exploited to actively confer resistance against honey bee pathogens and parasites or to enhance bee immune functions, thus enhancing the protection of honey bee health (Crotti et al., 2013; Ludvigsen et al., 2015; Dai et al., 2018).

In general, the gut bacterial composition and diversity may be influenced negatively by multiple factors (Zhao et al., 2018). Mites, chalkbrood, microsporidiosis, American foulbrood disease and pesticides have severe effects on the health of honey bees through impacts on the diversity and richness of the gut bacteria (Dai et al., 2018; Zhao et al., 2018; Ma et al., 2019). Previous research has shown that exposure to sublethal doses of imidacloprid and pathogens or parasites has a significantly negative effect on the survival and health of honey bees (Alaux et al., 2010; Abbo et al., 2017).

In this study, survival, pollen and syrup consumption, and midgut bacterial diversity after exposure to *T. mercedesae* and imidacloprid were analyzed in *A. mellifera*. High-throughput 16S rRNA gene sequencing was used to identify the microbial communities associated with worker adult honey bees, and we evaluated the effects of both imidacloprid and *T. mercedesae* on honey bee health.

2. Materials and methods

2.1. Honey bees

Honey bee workers were collected from *A. mellifera* colonies located in the Institute of Apicultural Research apiaries (40°00′28″N, 116°12′18″E), Chinese Academy of Agricultural Sciences, Beijing, from September to October 2018. Queens from the same breeding line were used in these colonies, and the colonies were of mixed-race, European-derived stock.

As *T. mercedesae* mite donors, five colonies with mites were left untreated for the entire year. Queens of the other five colonies were caged for one month and until all brood emerged from the experimental healthy colonies. The colony, as an auxiliary group, provided a large number of healthy mite-free larvae for the experiment. Each queen was caged on an empty comb for 24 h to lay eggs. The combs with eggs were divided into small pieces and fixed onto a small frame, constituting a special comb that was replaced into the original colony (Fig. S1). One foundress of *Tropilaelaps mercedesae* was introduced into a brood cell with a 5th larval instar (LS) after sealing (Odemer, 2020), and mites were

inoculated into newly sealed brood cells with the transfer technique (Khongphinitbunjong et al., 2014; Buawangpong et al., 2015; Lin et al., 2018). All inoculum mites were randomly obtained from highly infested *A. mellifera* colonies. Other combs with open and closed brood cells without mites were used as controls.

A total of 10 brood combs, five infested combs and five controls were placed in a dark incubator (34 ± 0.5 °C, 40 ± 10% RH) for 9 days. Emerging bees with and without *T. mercedesae* infestation were collected within 2 h, and 20 bees were randomly placed in a cage (9 × 9 × 10 cm) with mesh on two sides. Each cage was supplied with sufficient imidacloprid syrup (50% w/v sucrose solution) and pollen paste (two thirds of the corbicular pollen is mixed with one-third 50% sugar syrup). Pollen from field-grown *Brassica napus* was collected by colonies in Hubei Province in 2018. To ensure freshness, the pollen was stored at −20 °C, and the freshly collected pollen was determined not to be contaminated by *Nosema ceranae* or *Ascosphaera apis* by microscopic examination.

2.2. Experimental design

We mixed imidacloprid in syrup to identify the pesticide-parasite-induced stress responses of honey bees infested with T. mercedesae. Imidacloprid (98.5%, Lot# d1701044, Aladdin, Shanghai, China) was weighed using a precision balance (Sartorius, Reading precision: 0.0001 g, Germany). Imidacloprid was dissolved in acetone to prepare a stock solution (2.5 mg/L and 5 mg/L) which were kept covered to avoid concentration changes due to solvent evaporation. Two nominal concentrations were chosen based on available pollen residue data (Sánchez-Bayo and Goka, 2014; Jiang et al., 2018): 25 μg/L (1 × mean residue level in pollen, 1/40th × maximum residue level in pollen reported by Sánchez-Bayo and Goka, 2014; 1/3rd × maximum residue level in pollen reported by Jiang et al., 2018) and 50 μg/L (2 × mean residue level in pollen, 1/20th × maximum residue level in pollen reported by Sánchez-Bayo and Goka, 2014; 2/3rd × maximum residue level in pollen reported by Jiang et al., 2018). We dissolved 0.1 mL stock solution into 9.9 mL sugar syrup (50% (w/v)), and the solvent accounted for 1% of the volume in the final diets. Imidacloprid/syrup combinations were mixed thoroughly using a vortex mixer. Sugar syrup with 1% acetone was used as the control diet. Six treatments were as follows: (1) workers without mite infestation fed the control diet (CN), (2) workers without mites fed the 50 μg/L imidacloprid diet (Ia), (3) workers without mites fed the 25 μg/L imidacloprid diet (Ib), (4) workers infested with T. mercedesae fed the control diet (TCN), (5) workers infested with T. mercedesae fed the 50 μg/L imidacloprid diet (TIa), and (6) workers infested with T. mercedesae fed the 25 μg/L imidacloprid diet (TIb). Five replicates from five different colonies were performed for each treatment, with 20 bees per replicate. A total of 600 workers of A. mellifera were used for the control and treatments.

The number of dead bees in each cage was recorded and removed daily (Table S1). The pollen and treatment syrup were replaced daily and weighed daily in the feeder before and after it was placed in the cage. Five empty cages were placed with five feeders containing sugar syrup and five feeders for pollen paste and weighed (Reading precision: 0.001 g, Shanghai Yue Ping Scientific Instrument Co., LTD) in the same manner as the other treatments to estimate the amount of desiccation and reduction in quality of the pollen paste in the incubator. Pollen and sugar consumption was calculated by comparing the difference in weight between the treatments and the empty cages (Dai et al., 2019b). Honey bees in the treatment groups were fed imidacloprid sugar syrup for 12 d. Five individuals randomly collected from each cage were surface-sterilized and dissected according to the protocol (Disayathanoowat et al., 2012). The midguts of the honey bees were stored in 1.5-mL Eppendorf tubes at −80 °C until sequencing.

2.3. HiSeq sequencing of 16S rRNA gene amplicons

Total bacterial DNA was extracted from samples using a Power Soil DNA Isolation Kit (Lot# A-12888-100, Biolabs, America) according to the manufacturer's protocol. DNA quality and quantity were assessed by visualization at 260 nm/280 nm and 260 nm/230 nm, respectively, using a Nanodrop ND 2000. The DNA was stored at −80 °C until further processing. The V3–V4 region of the bacterial 16S rRNA gene was amplified using common primer pairs combined with adapter sequences and barcode sequences according to the protocol described by Dai et al. (2019b). All PCR products were quantified by a Quant-iT™ dsDNA HS kit and pooled together. High-throughput sequencing analysis of the bacterial rRNA genes was performed on the purified, pooled samples using the Illumina HiSeq 2500 platform (2 × 250 paired ends) at Biomarker Technologies Corporation, Beijing, China.

2.4. Sequence analyses

Raw fastq files were aligned with overlapping regions within paired-end reads with the spliced sequences based on FLASH v1.2.1 (Magoč and Salzberg, 2011). Low-quality sequences were screened for chimeric sequences by Trimmomatic v0.33 (Bolger et al., 2014) software, which identified high-quality clean tags, and the remaining sequences were removed using UCHIME v8.1 (Edgar et al., 2011) with the obtained effective tags. The normalized sequences with 97% similarity were classified using USEARCH v10.0 (Edgar, 2013) into operational taxonomic units (OTUs). Taxonomy was then assigned to the OTUs via blast searches against 16S: Silva (Quast et al., 2013) (Release128) with default parameters. The highest resolution of taxonomy was projected using RDP Classifier v2.2 (Qiong et al., 2007) based on the ribosomal database. Linear discriminant analysis effect size (LEfSe) analysis was applied for species with significant differences in abundance among groups.

2.5. Statistical analysis

One-way ANOVA with Tukey's honestly significant difference (HSD) multiple-test correction with the software package SPSS 22 was used to compare pollen and syrup consumption among the experimental groups. Kaplan-Meier analysis (JMP 13) was employed to compare survival rates associated with the different treatments. Alpha diversity was assessed by the Chao richness estimator, and the Shannon diversity index was calculated with Mothur (Version v.1.30). For the statistical tests in this study, $\alpha = 0.05$ was considered significant. Beta diversity including the Bray-Curtis distance between the experimental groups was performed with QIIME (Version 1.8.0).

3. Results

3.1. Survival

The survival of workers infested with *T. mercedesae* (TCN) was significantly lower than that of the control (CN) ($\chi^2 = 8.4892$, $P = 0.0036$, Fig. 1). There was no significant difference in the survival of worker bees fed 25 μg/L (Ib) and 50 μg/L (Ia) imidacloprid compared to control bees (CN) ($\chi^2 = 0.0015$, $P = 0.9689$; $\chi^2 = 1.0682$, $P = 0.3014$). The survival workers infested with

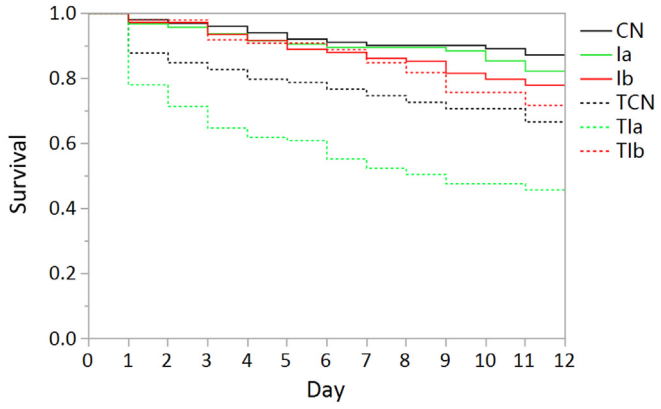

Fig. 1. The total survival of honey bees exposed to a combination of *T. mercedesae and imidacloprid*. CN – Worker without mite infestation fed the control diet; Ia – Worker without mites fed the 50 μg/L imidacloprid diet; Ib – Worker without mites fed the 25 μg/L imidacloprid diet; TCN – Worker infested with *T. mercedesae* fed the control diet; TIa – Worker infested with *T. mercedesae* fed the 50 μg/L imidacloprid diet; TIb – Worker infested with *T. mercedesae* fed the 25 μg/L imidacloprid diet (n = 5 replicates of 20 workers of A. mellifera/replicate, or 100 workers of A. mellifera, per test substance). The data analysis corresponds to Table S2.

T. mercedesae and fed 50 μg/L imidacloprid (TIa) was significantly lower than that of the control (CN), workers infested with *T. mercedesae* (TCN) and worker bees fed 50 μg/L imidacloprid (Ia) (all $P < 0.05$, Table S2).

3.2. Syrup and pollen consumption

Daily sugar consumption by honey bees infected with *T. mercedesae* fed to 50 μg/L imidacloprid (TIa) was significantly higher than that of honey bees without mite infestation fed to imidacloprid 50 μg/L (Ia) on D6, lower than that of honey bees infested with *T. mercedesae* (TCN) or without *T. mercedesae* (CN) on D8, and lower than honey bees infested with *T. mercedesae* (TCN) on D9 (Fig. S2A, Table S3). The total sugar consumption of honey bees without mite infestation fed to imidacloprid 50 μg/L (Ia) was significantly lower than that of honey bees infested with *T. mercedesae* (TCN) or without *T. mercedesae* (CN) ($F = 2.0119$, $P = 0.1132$, Fig. 2A).

Overall, the daily pollen consumption by honey bees infected with *T. mercedesae* fed to 50 μg/L imidacloprid (TIa) was significantly higher than that of honey bees without mite infestation fed to imidacloprid 50 μg/L (Ia) and the control (CN) on D6 and D11 (Fig. S2B, Table S4). The cumulative pollen consumption by honey bees infected with *T. mercedesae* fed to 50 μg/L imidacloprid (TIa) was significantly higher than those without mite infestation fed to imidacloprid (25 μg/L (Ib), 50 μg/L (Ia)) and control bees (CN) ($F = 3.3855$, $P = 0.0187$, Fig. 2B).

3.3. Bacterial 16S rRNA gene sequencing

A total of 1,581,493 raw sequences were obtained from 30 samples (Table S5), and 1,285,459 effective sequences were retained after trimming of barcodes and primers and filtering chimeras. The sample showed an average of 42,848 clean reads, with an average length of 425 bp. The average value of Q30 (%) was 94.15%, and the average value of effectiveness (%) was 81.24%. The total number of OTUs was 101 after OTU selection with 97% similarity.

3.4. Bacterial community composition and diversity

Bacteria were classified into different taxa (phylum, class, order, family, and genus levels) according to QIIME with default settings; the midgut bacterial communities at different levels are summarized in Fig. 3. On the basis of the average relative abundance, *Lactobacillus* (Firmicutes, 37.59%), *Gilliamella* (Gammaproteobacteria, 16.74%), *Klebsiella* (Gammaproteobacteria, 8%), *Orbus* (Gammaproteobacteria, 7.81%), *Commensalibacter* (Alphaproteobacteria, 6.94%), *Bartonella* (Alphaproteobacteria, 4.86%) and *Pseudomonas* (Gammaproteobacteria, 2.27%) were the most abundant genera.

For alpha diversity analysis, we compared the Chao estimator and Shannon index among the different sample groups, as illustrated in Fig. S3. No significant difference for the Chao estimator ($F = 0.3593$, $P = 0.8712$, Fig. S3A) or Shannon index ($F = 1.2453$, $P = 0.3207$, Fig. S3B) was detected among the treatments.

The beta diversity analysis compared species diversity similarity among the different samples (Fig. 4). In this case, a statistically significant decrease in the beta diversity of workers without mite infestation fed the control diet, 50 μg/L or 25 μg/L imidacloprid diet compared to workers infested with *T. mercedesae* fed the 50 μg/L or 25 μg/L imidacloprid diet was observed.

LEfSe analysis identified specific taxa in the gut microbiota that can serve as biomarkers, with significant differences among the different groups (Fig. 5). Indeed, the relative abundances of *Pseudomonas* (LDA = 5.0563 and $P = 0.0063$) and *Acinetobacter* (LDA = 4.1146 and $P = 0.0414$) in workers exposed to 25 μg/L imidacloprid and ectoparasitic *T. mercedesae* mites were significantly higher than those in the other honey bee groups.

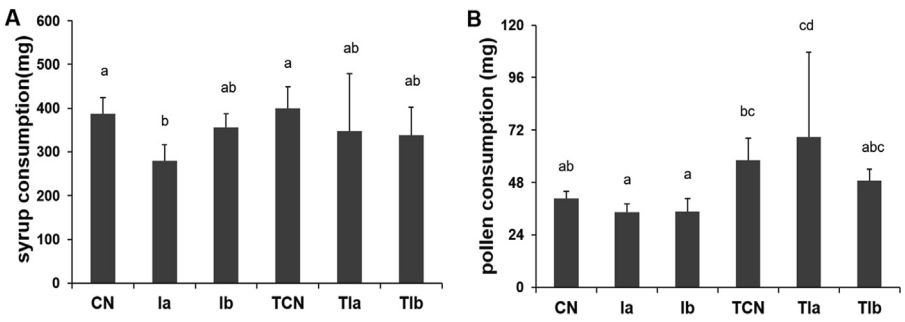

Fig. 2. Cumulative syrup (A) and pollen (B) consumption of A. mellifera exposed to *T. mercedesae* and imidacloprid. CN – Worker without mite infestation fed the control diet; Ia – Worker without mites fed the 50 μg/L imidacloprid diet; Ib – Worker without mites fed the 25 μg/L imidacloprid diet; TCN – Worker infested with *T. mercedesae* fed the control diet; TIa – Worker infested with *T. mercedesae* fed the 50 μg/L imidacloprid diet; TIb – Worker infested with *T. mercedesae* fed the 25 μg/L imidacloprid diet. The same letter in bars means no significant difference (P > 0.05).

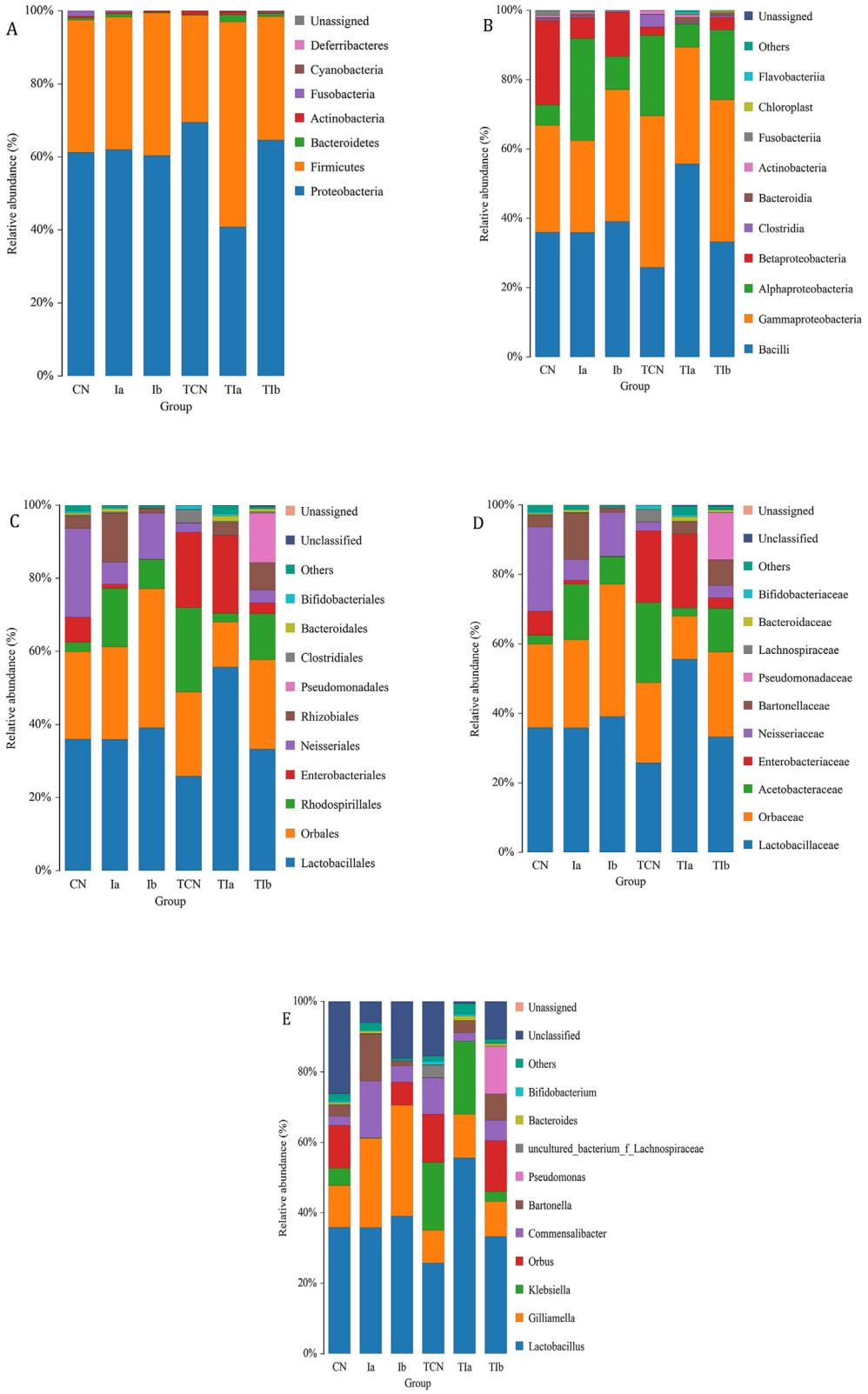

Fig. 3. Relative abundance of the dominant midgut bacterial communities in *A. mellifera* at the phylum (A), class (B), order (C), family (D), and genus (E) levels. CN – Worker without mite infestation fed the control diet; Ia – Worker without mites fed the 50 μg/L imidacloprid diet; Ib – Worker without mites fed the 25 μg/L imidacloprid diet; TCN – Worker infested with *T. mercedesae* fed the control diet; TIa – Worker infested with *T. mercedesae* fed the 50 μg/L imidacloprid diet; TIb – Worker infested with *T. mercedesae* fed the 25 μg/L imidacloprid diet.

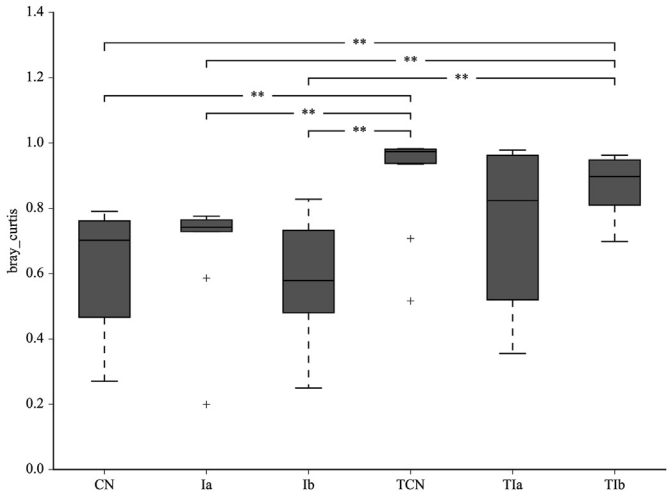

Fig. 4. Box plot of Bray-Curtis distances of the midgut microbiota among the six treatments. CN – Worker without mite infestation fed the control diet; Ia – Worker without mites fed the 50 μg/L imidacloprid diet; Ib – Worker without mites fed the 25 μg/L imidacloprid diet; TCN – Worker infested with *T. mercedesae* fed the control diet; TIa – Worker infested with *T. mercedesae* fed the 50 μg/L imidacloprid diet; TIb – Worker infested with *T. mercedesae* fed the 25 μg/L imidacloprid diet. ** indicates significance at the 0.01 level.

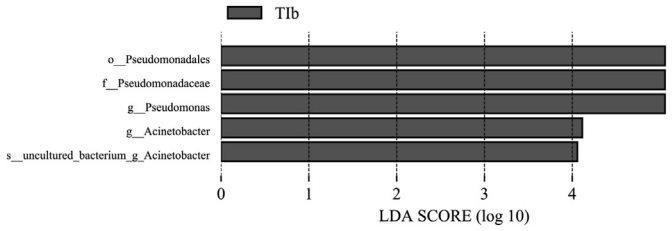

Fig. 5. LEfSe analysis illustrating the differentially abundant bacteria in honey bees exposed to 25 μg/L imidacloprid and *T. mercedesae*. The threshold of the LDA score was 4.0. TIb – honey bee exposure to 25 μg/L imidacloprid and ectoparasitic *T. mercedesae* mites.

4. Discussion

T. mercedesae is widespread in *A. mellifera* populations across much of Asia (Buawangpong et al., 2015; Khongphinitbunjong et al., 2016); these mites have a high reproductive ability in infested colonies and the potential to transfer bee viruses (Buawangpong et al., 2015; Khongphinitbunjong et al., 2015; Wu et al., 2017). *T. mercedesae* infestation significantly reduced *A. mellifera* longevity (Khongphinitbunjong et al., 2016). We provide experimental evidence showing that the survival of worker bees infested with *T. mercedesae* (TCN) was significantly lower than that of bees without mite infestation (CN). There was no significant difference in syrup and pollen consumption between CN and TCN. Our results showed that *T. mercedesae* infestation significantly reduced survival but not affected food consumption of *A. mellifera.*

Imidacloprid at 25 μg/L and 50 μg/L had no significant effect on the survival of honey bees compared with control bees, which was consistent with another study (van Dooremalen et al., 2018). A relatively high concentration of imidacloprid reportedly has a repellent effect on honey bees (Bortolotti et al., 2003). In our study, the imidacloprid 50 μg/L diet significantly decreased syrup consumption by *A. mellifera* compared with the control diet, which may be attributed to this repellent effect.

Honey bees exposed to a combination of *T. mercedesae* and 50 μg/L imidacloprid presented increased mortality and pollen consumption under laboratory-rearing conditions. Infection by the microsporidian *Nosema* and exposure to imidacloprid also increases mortality rates and feeding consumption in laboratory-reared bees (Mondet and Brunet, 2009). In addition, midgut ultrastructural analysis has revealed that exposure to a sublethal concentration of imidacloprid alters the midgut cells of bees, with changes including nuclear damage, increases in vacuoles and loss of mitochondrial matrix, double membrane and cristae (Catae et al., 2018). Honey bee exposure to *Nosema* and imidacloprid decreased the activity of glucose oxidase (Mondet and Brunet, 2009), and parasitic mites induce variations in phenoloxidase activity in honey bees (Mondet and Brunet, 2009). The phenoloxidase activation is an energy-consuming metabolic process (Park and Kim, 2014), therefore honey bees exposed to a combination of *T. mercedesae* and 50 μg/L imidacloprid increased pollen consumption.

A. mellifera workers harbor gut microbiota that may play a beneficial role in host health (Martinson et al., 2012; Engel et al., 2016). *Gilliamella*, *Lactobacillus* and *Bifidobacterium* genus clusters formed the core microbiota of the honey bee gut (Anderson et al., 2011; Kwong and Moran, 2016), with Alphaproteobacteria genera *Bartonella* and *Commensalibacter*, Gammaproteobacteria genera *Pseudomonas*, *Orbus* and *Klebsiella*, and the Bacteroidetes genus *Bacteroides* being less numerous and less prevalent (Evans and Armstrong, 2006; Anderson et al., 2011; Kwong and Moran, 2016; Steele et al., 2017).

T. mercedesae affects the midgut bacterial community composition of honey bees, whereas imidacloprid does not significantly affect the midgut bacteria. Our results revealed a statistically significant increase in beta diversity in honey bees infested with *T. mercedesae* and fed the control diet compared to those without mite infestation. Pusceddu (2016) reported that *Varroa* infestation affects the honey bee midgut bacterial community composition. Although the number of microbiota bacterial taxa in *Varroa* mites is less than that in honey bees, but *Varroa* mites and honey bee populations share bacteria (Hubert et al., 2016). *T. mercedesae* possibly affects the composition of the honey bee midgut bacterial community by sharing bacteria. Imidacloprid 25 μg/L and 50 μg/L did not impact the midgut bacterial composition of honey bee, and it was consistent with the previous work that imidacloprid did not affect the composition of the honey bee gut microbiome (Raymann et al., 2018).

There was a significant increase in the midgut microbiota community structure in honey bees fed the 25 μg/L imidacloprid diet and infested with *T. mercedesae* compared to those fed the 25 μg/L imidacloprid diet without infestation, but the survival rate of infested bees fed the imidacloprid-free diet did not decrease. *Pseudomonas* in the midgut of honey bees infested with *T. mercedesae* and fed a 25 μg/L imidacloprid diet was affected. *Pseudomonas* is present in the guts of honey bees (Mohr and Tebbe, 2006) and in honey bee food (Donkersley et al., 2018). *Pseudomonas* is able to transform imidacloprid, and thiamethoxam is transformed to nitrosoguanidine, desnitro, and urea metabolites under microaerophilic conditions (Pandey et al., 2009). These molecules can be further converted to a nontoxic urea metabolite through desnitro/guanidine intermediate metabolism (Pandey et al., 2009). *Pseudomonas* in honey bee guts can detoxify harmful neonicotinoid insecticides, but honey bees artificially infected with *Pseudomonas* exhibit a decrease in overall health (Papadopoulou-Karabela et al., 1993; Ishii et al., 2014). Walderdorff et al. (2018) showed that an additive interaction of imidacloprid and pathogens through simultaneous immune activation with LPS intensifies hemocyte activity. Imidacloprid has an impact on honey bee cellular immune activation under pathogen challenge (Walderdorff et al., 2018); it

also acts through *Pseudomonas*-mediated transformation of metabolic functions to weaken the overall damaging effects of the insecticide and parasitic mites. Detoxification is an energy-consuming metabolic process in which exposure to sublethal doses of imidacloprid may lead to increased energy usage in honey bees (Abbo et al., 2017). Exposure to 50 μg/L imidacloprid increased the pollen consumption of worker bees infested with *T. mercedesae*. The elemental building blocks (phosphorous, nitrogen, and co-factors) of pollen are necessary for microbial reproduction and persistence (Anderson et al., 2011), and the simple sugars present in nectar may also temporarily support energy usage in honey bees for detoxification and immune responses.

5. Conclusions

In conclusion, a combination of *T. mercedesae* infestation and imidacloprid 50 μg/L exposure increased mortality and pollen consumption, and a combination of *T. mercedesae* and 25 μg/L imidacloprid affected the midgut bacterial composition of honey bees under laboratory-rearing conditions. Our tests represent a likely worst-case scenario of exposure of honey bees to imidacloprid approximately at the residue levels found in pollen. It seems unlikely that the pesticide levels found in honey bee food will approach the maximum residues found in pollen under normal environmental conditions, though this does not preclude the possible existence of any sublethal impacts these compounds may have on developing bees. For honey bees, it is known that the entity of the colony has an enormous buffering capacity to counter stressors such as pesticides or pests (Straub et al., 2015; Sponsler and Johnson, 2017). Indeed, effects assessed at the individual level do not necessarily translate to the colony level (Rolke et al., 2016). Under field conditions, most of the consequences of pesticide exposure fail to appear when compared to laboratory studies. The results of laboratory experiments cannot draw conclusions from these experiments and translate them equally to the colony for example the food consumption. Furthermore, there is no absolute guarantee that there is no pesticide residue in the pollen used in experiments. Nevertheless, laboratory experiments play an important role in the study of the effects of pesticide exposure and brood mite infestation, as many factors may be different under field conditions. Field-level experiments can be biased by many uncontrolled factors, such as weather, pests and management, but the rearing of honey bees allows for more controlled experiments. Our data highlight the importance of considering risk when estimating the interaction of pesticides and pests on honey bees.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Abbo, P.M., Kawasaki, J.K., Hamilton, M., Cook, S.C., DeGrandi-Hoffman, G., Li, W.F., Liu, J., Chen, Y.P., 2017. Effects of imidacloprid and *Varroa destructor* on survival and health of European honey bees, *Apis mellifera*. Insect Sci. 24, 467–477. https://doi.org/10.1111/1744-7917.12335.

Alaux, C., Brunet, J.L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L.P., Le Conte, Y., 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honey bees (*Apis mellifera*). Environ. Microbiol. 12, 774–782. https://doi.org/10.1111/j.1462-2920.2009.02123.x.

Anderson, D.L., Morgan, M.J., 2007. Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (Acari: Laelapidae): new and re-defined species. Exp. Appl. Acarol. 43, 1–24. https://doi.org/10.1007/s10493-007-9103-0.

Anderson, K.E., Sheehan, T.H., Eckholm, B.J., Mott, B.M., DeGrandi-Hoffman, G., 2011. An emerging paradigm of colony health: microbial balance of the honey bee and hive (*Apis mellifera*). Insectes Soc. 58, 431–444. https://doi.org/10.1007/s00040-011-0194-6.

Bolger, A.M., Marc, L., Bjoern, U., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Stefano, M.S., Porrini, C., 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bull. Insectol. 56, 63–67.

Brandt, A., Gorenflo, A., Siede, R., Meixner, M., Büchler, R., 2016. The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). J. Insect Physiol. 86, 40–47. https://doi.org/10.1016/j.jinsphys.2016.01.001.

Buawangpong, N., de Guzman, L.I., Khongphinitbunjong, K., Frake, A.M., Burgett, M., Chantawannakul, P., 2015. Prevalence and reproduction of *Tropilaelaps mercedesae* and *Varroa destructor* in concurrently infested *Apis mellifera* colonies. Apidologie 46, 779–786. https://doi.org/10.1007/s13592-015-0368-8.

Catae, A.F., Roat, T.C., Pratavieira, M., Silva Menegasso, A.R.D., Palma, M.S., Malaspina, O., 2018. Exposure to a sublethal concentration of imidacloprid and the side effects on target and nontarget organs of *Apis mellifera* (Hymenoptera, Apidae). Ecotoxicology 27, 109–121. https://doi.org/10.1007/s10646-017-1874-4.

Chanpanitkitchote, P., Chen, Y., Evans, J.D., Li, W., Li, J., Hamilton, M., Chantawannakul, P., 2018. *Acute bee paralysis virus* occurs in the Asian honey bee *Apis cerana* and parasitic mite *Tropilaelaps mercedesae*. J. Invertebr. Pathol. 151, 131–136. https://doi.org/10.1016/j.jip.2017.11.009.

Chantawannakul, P., de Guzman, L.I., Li, J.L., Williams, G.R., 2016. Parasites, pathogens, and pests of honeybees in Asia. Apidologie 47, 301–324. https://doi.org/10.1007/s13592-015-0407-5.

Chantawannakul, P., Ramsey, S., VanEngelsdorp, D., Khongphinitbunjong, K., Phokasem, P., 2018. *Tropilaelaps* mite: an emerging threat to European honey bee. J. Invertebr. Pathol. 26, 69–75. https://doi.org/10.1016/j.jip.2017.11.009.

Crotti, E., Sansonno, L., Prosdocimi, E.M., Vacchini, V., Hamdi, C., Cherif, A., Gonella, E., Marzorati, M., Balloi, A., 2013. Microbial symbionts of honeybees: a promising tool to improve honeybee health. N. Biotech. 30, 716–722. https://doi.org/10.1016/j.nbt.2013.05.004.

Cycoń, M., Markowicz, A., Borymski, S., Wójcik, M., Piotrowska-Seget, Z., 2013. Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. J. Environ. Manag. 131, 55–65. https://doi.org/10.1016/j.jenvman.2013.09.041.

Dai, P.L., Jack, C.J., Mortensen, A.N., Ellis, J.D., 2017. Acute toxicity of five pesticides to *Apis mellifera* larvae reared *in vitro*. Pest Manag. Sci. 73, 2282–2286. https://doi.org/10.1002/ps.4608.

Dai, P.L., Yan, Z.X., Ma, S.L., Yang, Y., Wang, Q., Hou, C.S., Wu, Y.Y., Liu, Y.J., Diao, Q.Y., 2018. The herbicide glyphosate negatively affects midgut bacterial communities and survival of honey bee during larvae reared *in vitro*. J. Agric. Food Chem. 66, 7786–7793. https://doi.org/10.1021/acs.jafc.8b02212.

Dai, P.L., Jack, C.J., Mortensen, A.N., Bustamante, T.A., Bloomquist, J.R., Ellis, J.D., 2019a. Chronic toxicity of clothianidin, imidacloprid, chlorpyrifos, and dimethoate to *Apis mellifera* L. larvae reared *in vitro*. Pest Manag. Sci. 75, 29–36. https://doi.org/10.1002/ps.5124.

Dai, P.L., Wang, M.Y., Geng, L.L., Yan, Z.X., Yang, Y., Guo, L., Guo, L., Ma, S.L., 2019b. The effect of Bt Cry9Ee toxin on honey bee brood and adults reared *in vitro, Apis*

mellifera (Hymenoptera: apidae). Ecotoxicol. Environ. Saf. 181, 381–387. https://doi.org/10.1016/j.ecoenv.2019.06.031.
de Guzman, L.I., Williams, G.R., Khongphinitbunjong, K., Chantawannakul, P., 2017. Ecology, life history, and management of *Tropilaelaps* mites. J. Econ. Entomol. 110, 319–332. https://doi.org/10.1093/jee/tow304.
Disayathanoowat, T., Young, J.P.W., Helgason, T., Chantawannakul, P., 2012. T-RFLP analysis of bacterial communities in the midguts of *Apis mellifera* and *Apis cerana* honey bees in Thailand. FEMS Microbiol. Ecol. 79, 273–281. https://doi.org/10.1111/j.1574-6941.2011.01216.x.
Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C., Wilson, K., 2018. Bacterial communities associated with honeybee food stores are correlated with land use. Ecol. Evol. 8, 4743–4756. https://doi.org/10.1002/ece3.3999.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996. https://doi.org/10.1093/bioinformatics/btr381.
Edgar, R.C., Haas, B.J., Clemente, J.C., Christopher, Q., Rob, K., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194. https://doi.org/10.1093/bioinformatics/btr381.
Engel, P., Kwong, W.K., McFrederick, Q., Anderson, K.E., Barribeau, S.M., Chandler, J.A., Cornman, R.S., Dainat, J., de Miranda, J.R., Doublet, V., Emery, O., Evans, J.D., Farinelli, L., Flenniken, M.L., Granberg, F., Grasis, J.A., Gauthier, L., Hayer, J., Koch, H., Kocher, S., Martinson, V.G., Moran, N., Munoz-Torres, M., Newton, I., Paxton, R.J., Powell, E., Sadd, B.M., Schmid-Hempel, P., Schmid-Hempel, R., Song, S.J., Schwarz, R.S., vanEngelsdorp, D., Dainat, B., 2016. The Bee Microbiome: impact on bee health and model for evolution and ecology of host-microbe interactions. mBio 7, e02164. https://doi.org/10.1128/mBio.02164-15.
Evans, J.D., Armstrong, T., 2006. Antagonistic interactions between honey bee bacterial symbionts and implications for disease. BMC Ecol. 6, 4. https://doi.org/10.1186/1472-6785-6-4.
Evans, J.D., Schwarz, R.S., 2011. Bees brought to their knees: microbes affecting honey bee health. Trends Microbiol. 19, 614–620. https://doi.org/10.1016/j.tim.2011.09.003.
Forsgren, E., de Miranda, J.R., Isaksson, M., Wei, S., Fries, I., 2009. *Deformed wing virus* associated with *Tropilaelaps mercedesae* infesting European honey bees (*Apis mellifera*). Exp. Appl. Acarol. 47, 87–97. https://doi.org/10.1007/s10493-008-9204-4.
Hubert, J., Kamler, M., Nesvorna, M., Ledvinka, O., Kopecky, J., Erban, T., 2016. Comparison of *Varroa destructor* and worker honeybee microbiota within hives indicates shared bacteria. Microb. Ecol. 72, 448–459. https://doi.org/10.1007/s00248-016-0776-y.
Ishii, K., Hamamoto, H., Sekimizu, K., 2014. Establishment of a bacterial infection model using the European honeybee, *Apis mellifera* L. PloS One 9, e89917. https://doi.org/10.1371/journal.pone.0089917.
Jiang, J.G., Ma, D.C., Zou, N., Yu, X., Zhang, Z.Q., Liu, F., Mu, W., 2018. Concentrations of imidacloprid and thiamethoxam in pollen, nectar and leaves from seed-dressed cotton crops and their potential risk to honeybees (*Apis mellifera* L.). Chemosphere 201, 159–167. https://doi.org/10.1016/j.chemosphere.2018.02.168.
Kavinseksan, B., Wongsiri, S., 2016. Life history and control of the parasitic bee mite, *Tropilaelaps mercedesae* Anderson and Morgan (Acari: Laelapidae): a Review. Basic Research Journal of Agricultural Science and Review 5, 56–71.
Khongphinitbunjong, K., de Guzman, L.I., Burgett, M.D., Rinderer, T.E., Chantawannakul, P., 2012. Behavioral responses underpinning resistance and susceptibility of honeybees to *Tropilaelaps mercedesae*. Apidologie 43, 590–599. https://doi.org/10.1007/s13592-012-0129-x.
Khongphinitbunjong, K., de Guzman, L.I., Buawangpong, N., Rinderer, T.E., Frake, A.M., Chantawannakul, P., 2014. Observations on the removal of brood inoculated with *Tropilaelaps mercedesae* (Acari: Laelapidae) and the mite's reproductive success in *Apis mellifera* colonies. Exp. Appl. Acarol. 62, 47–55. https://doi.org/10.1007/s10493-013-9728-0.
Khongphinitbunjong, K., de Guzman, L.I., Tarver, M.R., Rinderer, T.E., Chantawannakul, P., 2015. Interactions of *Tropilaelaps mercedesae*, honey bee viruses and immune response in *Apis mellifera*. J. Apicult. Res. 54, 40. https://doi.org/10.1080/00218839.2015.1041311.
Khongphinitbunjong, K., Neumann, P., Chantawannakul, P., Williams, G.R., 2016. The ectoparasitic mite *Tropilaelaps mercedesae* reduces western honey bee, *Apis mellifera*, longevity and emergence weight, and promotes *Deformed wing virus* infections. J. Invertebr. Pathol. 137, 38–42. https://doi.org/10.1016/j.jip.2016.04.006.
Kwong, W.K., Moran, N.A., 2016. Gut microbial communities of social bees. Nat. Rev. Microbiol. 14, 374–384. https://doi.org/10.1038/nrmicro.2016.43.
Lin, Z.G., Qin, Y., Page, P., Wang, S., Li, L., Wen, Z.S., Hu, F.L., Neumann, P., Zheng, H.Q., Dietemann, V., 2018. Reproduction of parasitic mites *Varroa destructor* in original and new honeybee hosts. Ecol. Evol. 8, 2135–2145. https://doi.org/10.1002/ece3.3802.
Ludvigsen, J., Rangberg, A., Avershina, E., Sekelja, M., Kreibich, C., Amdam, G., Rudi, K., 2015. Shifts in the midgut/pyloric microbiota composition within a honey bee apiary throughout a season. Microb. Environ. 30, 235–244. https://doi.org/10.1264/jsme2.ME15019.
Luo, Q.H., Zhou, T., Dai, P.L., Song, H.L., Wu, Y.Y., Wang, Q., 2011a. Prevalence, intensity and associated factor analysis of *Tropilaelaps mercedesae* infesting *Apis mellifera* in China. Exp. Appl. Acarol. 55, 135–146. https://doi.org/10.1007/s10493-011-9459-z.
Luo, Q.H., Zhou, T., Wang, Q., Dai, P.L., Wu, Y.Y., Song, H.L., 2011b. Identification of *Tropilaelaps* mites (Acari, Laelapidae) infesting *Apis mellifera* in China. Apidologie 42, 485–498. https://doi.org/10.1007/s13592-011-0028-6.
Ma, S., Yang, Y., Diao, Q., Fu, Z., Dai, P., 2018. Effects of *Tropilaelaps mercedesae* on the survival and food consumption of honey bees (*Apis mellifera*). Chinese Journal of Applied Entomology 55, 1066–1073. https://doi.org/10.7679/j.issn.2095-1353.2018.128.
Ma, S.L., Yang, Y., Jack, C.J., Diao, Q.Y., Fu, Z.M., Dai, P.L., 2019. Effects of *Tropilaelaps mercedesae* on midgut bacterial diversity of *Apis mellifera*. Exp. Appl. Acarol. 79, 169–186. https://doi.org/10.1007/s10493-019-00424-x.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.
Martinson, V.G., Moy, J., Moran, N.A., 2012. Establishment of characteristic gut bacteria during development of the honeybee worker. Appl. Environ. Microbiol. 78, 2830–2840. https://doi.org/10.1128/AEM.07810-11.
Mohr, K.I., Tebbe, C.C., 2006. Diversity and phylotype consistency of bacteria in the guts of three bee species (Apoidea) at an oilseed rape field. Environ. Microbiol. 8, 258–272. https://doi.org/10.1111/j.1462-2920.2005.00893.x.
Mondet, F., Brunet, J., 2009. *Nosema* and imidacloprid synergy affects Immune-strength-related enzyme activity in the honey bee, *Apis mellifera* (Rapport de stage, vol. 17. Université Claude Bernard (Lyon 1), Villeurbanne, FRA).
Odemer, R., Rosenkranz, P., 2020. Chronic exposure to a neonicotinoid pesticide and a synthetic pyrethroid in full-sized honey bee colonies. J. Apicult. Res. 59, 2–11. https://doi.org/10.1080/00218839.2019.1675337.
Pandey, G., Dorrian, S.J., Russell, R.J., Oakeshott, J.G., 2009. Biotransformation of the neonicotinoid insecticides imidacloprid and thiamethoxam by *Pseudomonas* sp. 1G. Biochem. Bioph. Res. Commun. 380, 710–714. https://doi.org/10.1016/j.bbrc.2009.01.156.
Papadopoulou-Karabela, K., Iliadis, N., Liakos, V., 1993. Haemocyte changes in honeybee (*Apis mellifera* L) artificially infected by *Pseudomonas aeruginosa*. Apidologie 24, 81–86. https://doi.org/10.1051/apido:19930110.
Park, J., Kim, Y., 2014. Prostaglandin mediates down-regulation of phenoloxidase activation of Spodoptera exigua via plasmatocyte-spreading peptide-binding protein. Arch. Insect Biochem. 85, 234–247. https://doi.org/10.1002/arch.21156.
Pusceddu, M., 2016. Social Immunity in honeybee: behavioral, chemical and microbiological aspects. Italy, Università degli Studi di Sassari 61–78.
Qiong, W., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. https://doi.org/10.1093/nar/gks1219.
Raymann, K., Motta, E.V.S., Girard, C., Riddington, I.M., Dinser, J.A., Moran, N.A., 2018. Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome. Appl. Environ. Microbiol. 84, e00545.
Rolke, D., Fuchs, S., Grünewald, B., Gao, Z., Blenau, W., 2016. Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*). Ecotoxicology 25, 1648–1665.
Sánchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees – a risk assessment. PloS One 9. https://doi.org/10.1371/journal.pone.0094482 e94482.
Sponsler, D.B., Johnson, R.M., 2017. Mechanistic modeling of pesticide exposure: the missing keystone of honey bee toxicology. Environ. Toxicol. Chem. 36, 871–881. https://doi.org/10.1002/etc.3661.
Steele, M.I., Kwong, W.K., Whiteley, M., Moran, N.A., 2017. Diversification of type VI secretion system toxins reveals ancient antagonism among bee gut microbes. mBio 8, e01630. https://doi.org/10.1128/mBio.01630-17.
Straub, L., Williams, G.R., Pettis, J., Fries, I., Neumann, P., 2015. Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors. Curr. Opin. Insect Sci. 12, 109–112. https://doi.org/10.1016/j.cois.2015.10.010.
van Dooremalen, C., Cornelissen, B., Poleij, H.A.C., Blacquière, T., 2018. Single and interactive effects of *Varroa destructor, Nosema spp*., and imidacloprid on honey bee colonies (*Apis mellifera*). Ecosphere 9, e2378. https://doi.org/10.1002/ecs2.2378.
Walderdorff, L., Laval-Gilly, P., Bonnefoy, A., Falla-Angel, J., 2018. Imidacloprid intensifies its impact on honeybee and bumblebee cellular immune response when challenged with LPS (lippopolysacharide) of *Escherichia coli*. J. Insect Physiol. 108, 17–24. https://doi.org/10.1016/j.jinsphys.2018.05.002.
Wu, Y., Dong, X., Kadowaki, T., 2017. Characterization of the copy number and variants of *Deformed Wing Virus* (DWV) in the pairs of honey bee pupa and infesting *Varroa destructor* or *Tropilaelaps mercedesae*. Front. Microbiol. 8, 1558. https://doi.org/10.3389/fmicb.2017.01558.
Yang, E.C., Chang, H.C., Wu, W.Y., Chen, Y.W., 2012. Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval sage. PloS One 7, e49472. https://doi.org/10.1371/journal.pone.0049472.
Zhao, Y.Z., Chen, Y.P., Li, Z.G., Peng, W.J., 2018. Environmental factors have a strong impact on the composition and diversity of the gut bacterial community of Chinese black honeybees. J. Asia Pac. Entomol. 21, 261–267. https://doi.org/10.1016/j.aspen.2018.01.002.