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Infestation of *Apis mellifera* workers and larvae with *Varroa destructor* affects gut bacterial diversity

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Abstract

The present work aims to investigate the midgut bacteria of honey bee (*Apis mellifera* lamarckii) workers and larvae infested with *Varroa destructor*. To achieve this task, uninfested and infested honey bee larvae and workers with the parasitic mites were obtained from natural bees apiaries in Qaliubiya Governorate, Egypt. Aerobic bacteria were isolated from the midgut of uninfested and infested *A. mellifera* on nutrient agar medium and identified using a culture-dependent isolation along with 16S rRNA sequences. The results revealed that both uninfested and *V. destructor* infested workers and larvae harbored gut bacteria belonging to Gamma-proteobacteria strains. The uninfested workers contained *Enterobacter cloacae* and *Serratia nematodiphila*, while *V. destructor* infested workers harbored *Enterobacter* species. Uninfested larvae contained *E. hormaechei* and *Klebsiella variicola*. *V. destructor* infested larvae harbored *K. oxytoca* as a result of infection. We concluded that the honeybee microbiome composition is likely to be impacted through isolates variation by stage and diversity disruption of isolates by *V. destructor* infection.

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Introduction

The honeybee, *Apis mellifera* L., is a social insect that lives in a colony of comprising individuals with varying tasks (Winston, 1987). *Varroa* mites, *Varroa destructor* are the most devastating global pests of immature and adult honey bees, *A. mellifera* as mites reduce adult bee body weight, life span, and immunity to pathogens (Jong *et al.,* 1982; Martin, 1994; Büchler, 2015; Roberts *et al.,* 2017). There are multiple combined factors contributing to colony mortality and decline of the honey bee population; including *Varroa* parasitism (Chantawannakul *et al.,* 2016; Roberts *et al.,* 2017) together with extensive honey bee viruses infection (McMenamin and Genersch, 2015), pesticide poisoning (Stanley *et al.,* 2015), changes of land-use (Otto *et al.,* 2016) and low genetic diversity (Mattila & Seeley, 2007). Undetected or untreated infestation by mite populations in honey bee colonies would usually lead to their collapse within a year (Büchler, 2015). Honeybee colonies decrease significantly around the world, and this has drawn the attention to investigate factors that can affect bees' health. Gut microbiomes are considered an essential part of a honey bee system (Alatawy *et al.,* 2020). The honey bee gut microbiota has a great potential as a model for studying natural bacterial populations due to the remarkably simple and conserved composition of the community (Martinson *et al.,* 2011; Moran *et al.,* 2012). The honey bee harbors a core gut microbiota of eight abundant phylotes, which accounts for ~95% of all gut bacteria. Also, the gut microbiota is distributed throughout the entire diges-

tive tract, in which the midgut harbors approximately 1-4% and the ileum/rectum over 90% of the most dominant bacteria found in honey bees (Martinson *et al.,* 2012). Knowledge of microbiota composition, persistence, and transmission as well as the overall function of the bacterial community are important and may be linked to honey bee health (Hroncova *et al.,* 2019). Bacterial symbionts associated to honey bees might provide novel avenues for *Varroa* control (Chandler *et al.,* 2011) and additionally known to produce a variety of bioactive molecules that have been suggested to play a protective role against honey bee pathogens (Saccà & Lodesani, 2020).

An array of bacteria belonging to different genera and species were characterized based on 16S rRNA sequences. The bacterial distribution and diversity in many insects were studied based on16S rRNA sequences (Rajagopal, 2009). Most studies on bacterial gut diversity have been taxon-specific, in insects such as termites (Boucias *et al.,* 2013), ants (Poulsen & Sapountzis, 2012), fire bugs (Salem *et al.,* 2013), beetles (Arias-Cordero *et al.,* 2012) and bees (Mohr & Tebbe, 2006; Cox-Foster *et al.,* 2007; Martinson *et al.,* 2011; Engel *et al.,* 2012), leaving a need for broader and systematic characterization besides comparison across all insects. The gut bacteria of *A. cerana* and *A. andreniformis* have been profiled (Martinson *et al.,* 2011; Disayathanoowat *et al.,* 2012).

The present work aims to use a culture-dependent system along with 16S rRNA sequences to characterize the gut symbiont viability of honey bees infested with *V. destructor* for the first time and determine to what extent the *V. destructor* infection affects the change of microbiome composition as well as aiding in establishing effective microbial control strategies.

Materials and methods

Insect collection

For larvae, 25 *V. destructor* infested, and 5 un-infested larvae (last instar) were collected from each apiary. For workers; 30 *V. destructor* infested and 5 un-infested workers (about 10~12 days old) were collected from each apiary. Each larval or worker gut was isolated separately. They were obtained from nine different apiaries in Qaliubiya Governorate, Egypt. Larvae were transferred with their honey comb. While workers were transferred to a small jar and used for midgut isolation at the laboratory of the Department of Entomology, Faculty of Science, Benha University. *V. destructor* infested workers and larvae were collected based on varroosis symptoms including visible dark mites within the immature brood, punctured holes on worker brood cells, or deformed adults crawling around inside or outside of the hive as described by (Sammataro *et al.,* 2000). They were maintained under laboratory conditions (32°C and 70% RH) and fed with a sugar water mixture (1/1) at libitum. Six replicates from each larvae and workers group were performed, each contained ten individuals.

Midgut collection

Fresh honey bee samples (workers and larvae) were surface-sterilized with 7% sodium hypochlorite thrice in sterile plates (Inglis *et al.,* 1998). The larvae were starved for 12 hours and then dissected with a sterile blade. The worker was held by the head and the stinger was pulled out as described by Ludvigsen *et al.* (2015). After the appearance of intact digestive tract, the midgut was separated and immediately frozen in liquid nitrogen, and stored at –80°C.

Isolation of gut bacteria flora

The collected midgut tissues were opened with forceps and homogenized in 1 ml of phosphate-buffered saline (PBS) for 30 sec. Homogenized suspensions were then serially diluted from 10^{-3} to 10^{-6} (Anjum *et al.,* 2018). Approximately 100 µL of diluted suspension were inoculated onto a nutrient agar plate using spread plate technique and incubated at 37°C for 24-48 h (Anjum *et al.,* 2018). On the basis of different colony characteristics (*e.g.* size, edge, consistency) discrete colonies were selected for identification. Selected colonies were purified as pure culture through repeated subculturing onto a nutrient agar plate and maintained at 4°C (Anjum *et al.,* 2018). All isolated cells were harvested in a microcentrifuge tube after centrifuging for 10 min at 5000g.

DNA extraction

Genomic DNA was isolated from bacterial pellets using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA quality was tested by running the samples on 1% agarose gel, and DNA purity was quantified using a spectrophotometer and recording the ratios of absorbance at 260 and 280 nm (Sambrook, 2001).

16S rRNA gene amplification and sequencing

The 16S rRNA gene (1500 bp) was amplified using 27 primers forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 primer reverse (5′-GGTT ACCTTGTTACGACTT-3′) according to Miller *et al.* (2013). Standard PCR reactions were conducted in a final reaction volume of 20 µL, contained 0.1 µL Pfu DNA polymerase, 2 µL DNA polymerase buffer, 1.5 µL MgSO4, 0.25 µL forward and reverse primers, and 1 µL DNA template. RNase-free water (14.9 µL) was added to reach the final volume. PCR were conducted in the following conditions: initial DNA denaturation for 3 min at 95°C for 1 cycle, 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final extension cycle of 72°C for 10 min (Tajabadi *et al.,* 2013). The PCR products were eluted on the gel and partially sequenced using 27F and 1492R primers, at sequencing facility of Solgent Co. Ltd. (South Korea). Sequence results were checked and compared directly with other 16S rRNA sequences registered in GenBank using BLASTN tool (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences were aligned using ClustalX algorthim. Phylogenetic analysis was conducted by a neighbor-joining method using the program Mega 6 (Tamura *et al.,* 2013).

Results

Bacterial isolation and characterization

Totally, nine bacterial isolates on nutrient agar plates were characterized and subcultured to obtain pure cultures for Gram staining. In the case of larvae, three isolates were spread as follows, two isolated colonies from uninfested larvae and one isolated colony from *V. destructor* infested ones. On the other hand, six isolates were separated from the workers as follow two isolated colonies from uninfested workers and four randomly isolated colonies from *V. destructor* infested ones. The morphological characters of the colony (*e.g.* form, elevation, margin, and color) were recorded for all isolated bacteria. The colonies of isolated bacteria were circular, raised, and Gram negative.

16S rRNA Amplification

An intense single DNA band was resolved in 1% agarose gel

in all wells along with the DNA marker. The extracted DNA was used as a template for amplification of 16S rRNA gene and observed by the high intense band in agarose gel with molecular size of approximately 1.5 kb (Figure 1).

Sequence analysis and identification

Similarly, the PCR amplified the 16S rRNA gene from all six bacterial isolates from both uninfested and infested honey bee workers partially ranged from 862, 891, 895, 935, 1394 and 1404 bp, respectively (Figure 1A,B). The maximum identity of the sequence was 99-100%. Genera *Enterobacter* were found to be predominant in both infested and non-infested workers. The determined bacterial communities in uninfested honey bee workers were *E. cloacae* and *Serratia nematodiphila* while *Enterobacter sp.*1, *Enterobacter sp.*2, *Enterobacter sp.*3 and *Enterobacter sp.*4 were detected in *V. destructor* infested workers.

A partial sequence was obtained from the three bacterial isolates from both uninfested and infested honey bee larvae ranged from 1392 and 1400bp (Figure 1A,C). The maximum identity of the sequence was 99.78-100%. The determined bacterial communities in uninfested honey bee larvae were *K. variicola* and *E. hormaechei* whereas, *K. oxytoca* was found to be predominant in infested larvae. The nine bacterial isolates appeared distributed separately, different and variable between honey bee larvae and workers in uninfested and infection conditions as summarized in Table 1.

The results revealed that both uninfested and *V.destructor* infested workers and larvae harbored gut bacteria belonging to phylum Proteobacteria, order Gammaproteobacteria and family Enterobacteriaceae but *S. nematodiphila* belonging to Yersiniaceae family.

Phylogenetic analysis

Concerning *Enterobacter* sp., tree topology showed two major divisions. One isolate from *V. destructor* infested workers and uninfested larvae were located in one lineage. Meanwhile, the other lineage includes uninfested workers and three isolates from *V. destructor* infested ones. The latter was located in species-collective sister clades supporting the probability of their similarity at the evolutionary level (Figure 2). Regarding *Klebsiella* sp; tree's topology showed two major divisions where *K. variicola* isolate from uninfested larvae was located in the first lineage, and the second lineage included *K. oxytoca* isolated from *V. destructor* infested larvae (Figure 3). *Serratia nematodihilia* isolated from midgut

Table 1. Comparison of occurrence of different bacterial species in Apis mellifera larvae and worker (uninfested and Varoa destructor infected) according to partial 16S rRNA gene sequences.

Bacterial species	Uninfested larvae	Infected larvae	Uninfested workers	Infected workers	Sequence similarity, %
Klebsiella variicola	+	-	-	-	99.93
Enterobacter hormaechei	+	-	-	-	99.93
Enterobacter cloacae	-	-	+	-	100
Serratia nematodiphila	-	-	+	-	100
Klebsiella oxytoca	-	+	-	-	99.93
Enterobacter sp1	-	-	-	+	99.78
Enterobacter sp2	-	-	-	+	99.88
Enterobacter sp3	-	-	-	+	99.89
Enterobacter sp4	-	-	-	+	99.79

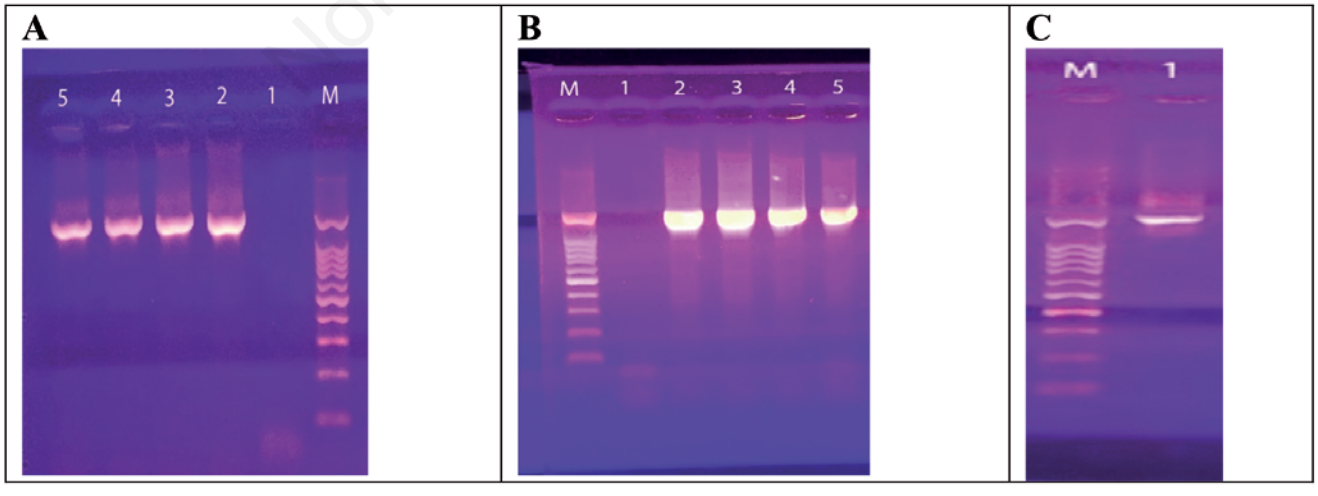

Figure 1. A): 1% Agarose gel electrophoresis of genomic DNA from four bacterial isolates from (uninfested) larvae and workers of *A. mellifera*. Lane (M): Marker (1500 bp). Lane 1 no sample, Lane 2-3 uninfested workers and Lane 4-5 uninfested larvae. B) 1% Agarose gel electrophoresis of 16S rRNA PCR amplicon. Lane (M): Marker (1500 bp). Lane 1 no sample, Lane 2-5 *V. destructor* infested workers. C) 1% Agarose gel electrophoresis of 16S rRNA PCR amplicon. Lane (M): Marker (1500 bp). Lane 1: *V. destructor* infested larvae.

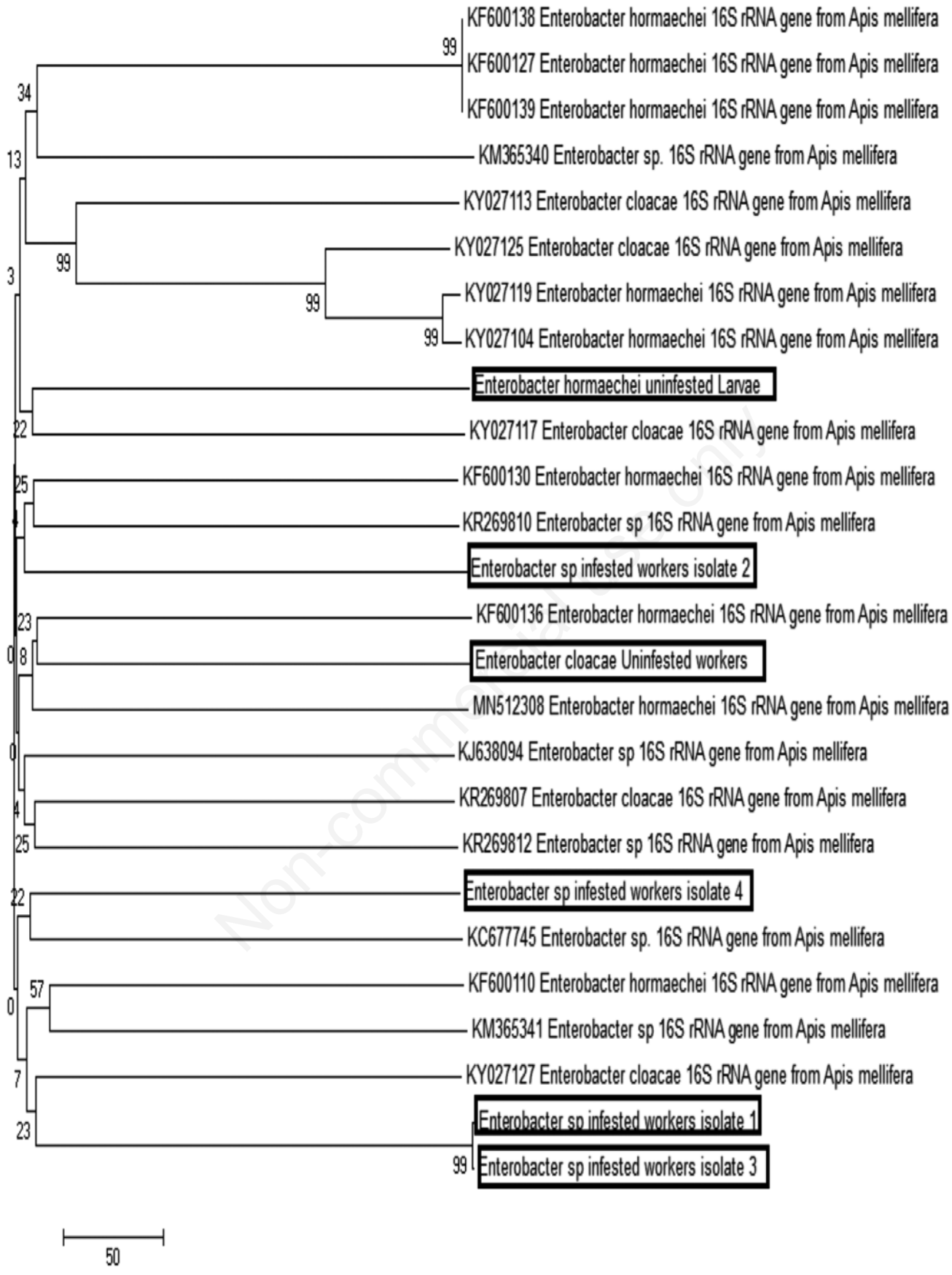

Figure 2. Neighbor joining Phylogenetic tree based on 16S rRNA gene sequences of *Enterobacter* sp. from midgut of *A. mellifera* (uninfested and *V. destructor* infested) larvae and workers.

of *A. mellifera* uninfested workers was clustered in a separate lineage in one monophyletic clade (Figure 4).

Discussion

In the present study, the identified bacterial isolates from uninfested and infested honey bee larvae and workers were found inhabiting many insect species; *Enterobacter cloacae* from *Lutzomyia longipalpis* (Gouveia *et al.,* 2008), *Spodoptera litura* (Thakur *et al.,* 2015) *Aedes albopictus* and *Aedes aegypti* (Yadav *et al.,* 2015), *K. variicola* from *Odontotermes formosanus* (Guo *et al.,* 2014), *K. oxytoca* from *Bactrocera cacuminata* (Thaochan *et al.,* 2010) and from *Aspidimorpha miliaris* (Shil *et al.,* 2014), *Enterobacter sp.* from *Bombyx mori* (Anand *et al.,* 2010) and from *Culex gelidus* (Gunathilaka *et al.,* 2020). In the current study, the midgut microbiome of uninfested and infested honey bee larvae and workers with *V. destructor* were detected using culture dependent methods. Nine bacterial guts were isolated, and most of their 16S rRNA sequences were identical or highly similar to sequences that were previously found in culture-dependent studies of honey bees (Mohr & Tebbe, 2006; Olofsson & Vasquez, 2008; Yoshiyama & Kimura, 2009; Tajabadi *et al.,* 2011; Anderson *et al.,* 2013; Corby-Harris *et al.,* 2014; Ludvigsen *et al.,* 2015). In this study, all the bacterial isolates from both *A. mellifera* larvae and workers either uninfested or infested with *V. destructor* belonged to Proteobacteria. This was in accordance with the results of Ahn *et al.* (2012), who reported the dominance of Proteobacteria in *A. cerana and A. mellifera* gut. Most of the Gamma-proteobacteria from both honey bee larvae and workers either uninfested or infested with *V. destructor* were similar to those that were detected in previous culture-dependent (Gilliam, 1997; Mohr & Tebbe, 2006; Yoshiyama & Kimura, 2009; Anderson *et al.,* 2013; Vojvodic *et al.,* 2013; Ludvigsen *et al.,* 2015) and culture-independent studies (Cox-Foster *et al.,* 2007; Martinson *et al.,* 2011; Ahn *et al.,* 2012; Disayathanoowat *et al.,* 2012; Sabree *et al.,* 2012; Corby-Harris *et al.,* 2014; Horton *et al.,* 2015).

The three bacterial isolates of *A. mellifera* larvae determined that genus *Klebsiella* was predominant. In case of the workers, the six bacterial isolates determined that genera *Enterobacter* was predominant, these results were in accordance with studies on microbial communities present in the midgut of *A. cerana indica and A. mellifera jemenitica*, respectively by Disayathanoowat *et al.* (2012) and Khan *et al.* (2017). Enterobacteria of the genera *Escherichia, Enterobacter, Proteus, Hafnia, Klebsiella*, and *Erwinia* were most commonly isolated from *A. mellifera* intestine (Gilliam *et al.,* 1988). Vassart *et al.* (1988) isolated *K. oxytoca*

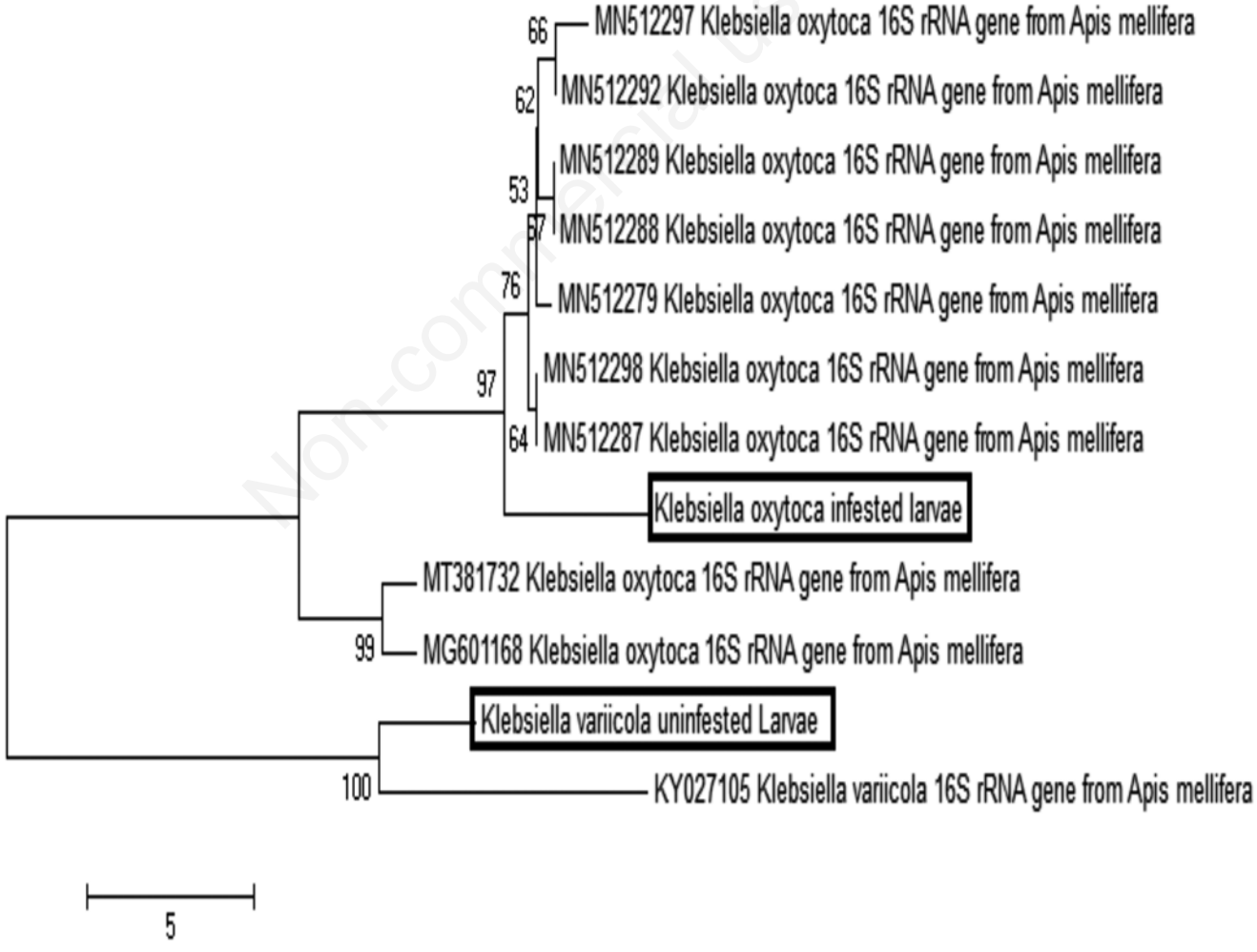

Figure 3. Neighbor joining Phylogenetic tree based on 16S rRNA gene sequences of *Klebsiella* sp. from midgut of *A. mellifera* (uninfested and *V. destructor* infested) larvae.

from a diseased bee family. *A. mellifera* has been reported to harbor numerous *Enterobacter* species in the gut. The abundant species of *Enterobacter* constitute the complex of *E. aerogenes*, *E. agglomurans* and *E. cloacae* that were found in the bee gut (Disayathanoowat *et al.,* 2012). The insect gut bacteria could promote nutrient uptake and produce some enzymes (Dillion & Dillion, 2004) and in addition, in enzymatic assays Disayathanoowat *et al.* (2012) observed that *K. pneumoniae* and some isolates of the genus *Enterobacter* produced protease and lipase enzymes. Bacteria from the family *Enterobacteriacea* were prevalent in gut microflora of *A. mellifera* (Moran *et al.,* 2012; Sabree *et al.,* 2012; Corby-Harris *et al.,* 2014). Honey bee gut bacteria could serve as resistive microorganisms, where *E. cloacae, K. oxytoca* and *S. marcescens* isolates displayed the antimicrobial resistance (Gasper *et al.,* 2017). Genus *Serratia* especially *S. marcescens* and *S. nematodiphila* species can be found and isolated from different organisms such as plants (Cho *et al.,* 2020; Elateek *et al.,* 2020), and nematode (Zhang *et al.,* 2008). *S. nematodiphila* has many beneficial uses as a microbial control agent against insect pests (Townsend *et al.,* 2003) and as biocontrol agent against plant-pathogens because carrying many genes involved in nematotoxic activity (Proença *et al.,* 2012) and producing a secondary metabolite known as 'prodigiosin' as nematicide on juvenile stages of *Radopholus similis* and *Meloidogyne javanica* (Rahul *et al.,* 2014).

The molecular methods differed among the sequencing studies in which results were similar, revealing a very characteristic bacterial species in honey bee gut (Jeyaprakash *et al.,* 2003; Mohr & Tebbe, 2006; Babendreier *et al.,* 2007; Cox-Foster *et al.,* 2007; Martinson *et al.,* 2011). These differences between the midgut microbiome of honey bee larvae and workers may be due to multiple conditions that have been reported to influence the bacterial gut community, including the environment, host age, habitat, pathogens and diet (Dillon *et al.,* 2010; Evans and Schwarz, 2011; Yun *et al.,* 2014). The commensally bacterial gut community in bees was found to be developed with age and expands to include more numerous bacteria groups until it reaches a stable composition (Palmer *et al.,* 2007; Martinson *et al.,* 2011).

Perturbation of the honey bee gut microbiota with *V. destructor* may lower immune responses and elevated susceptibility to other pathogens that invade through the midgut epithelium ((Hubert *et al.,* 2017). Many of the rarer bacterial species in honey bee guts likely represent opportunistic organisms able to invade as pathogens. Commonly sampled groups included species of Enterobacteriaceae, such as *Enterobacter* and *Klebsiella* (Raymann & Moran, 2018).

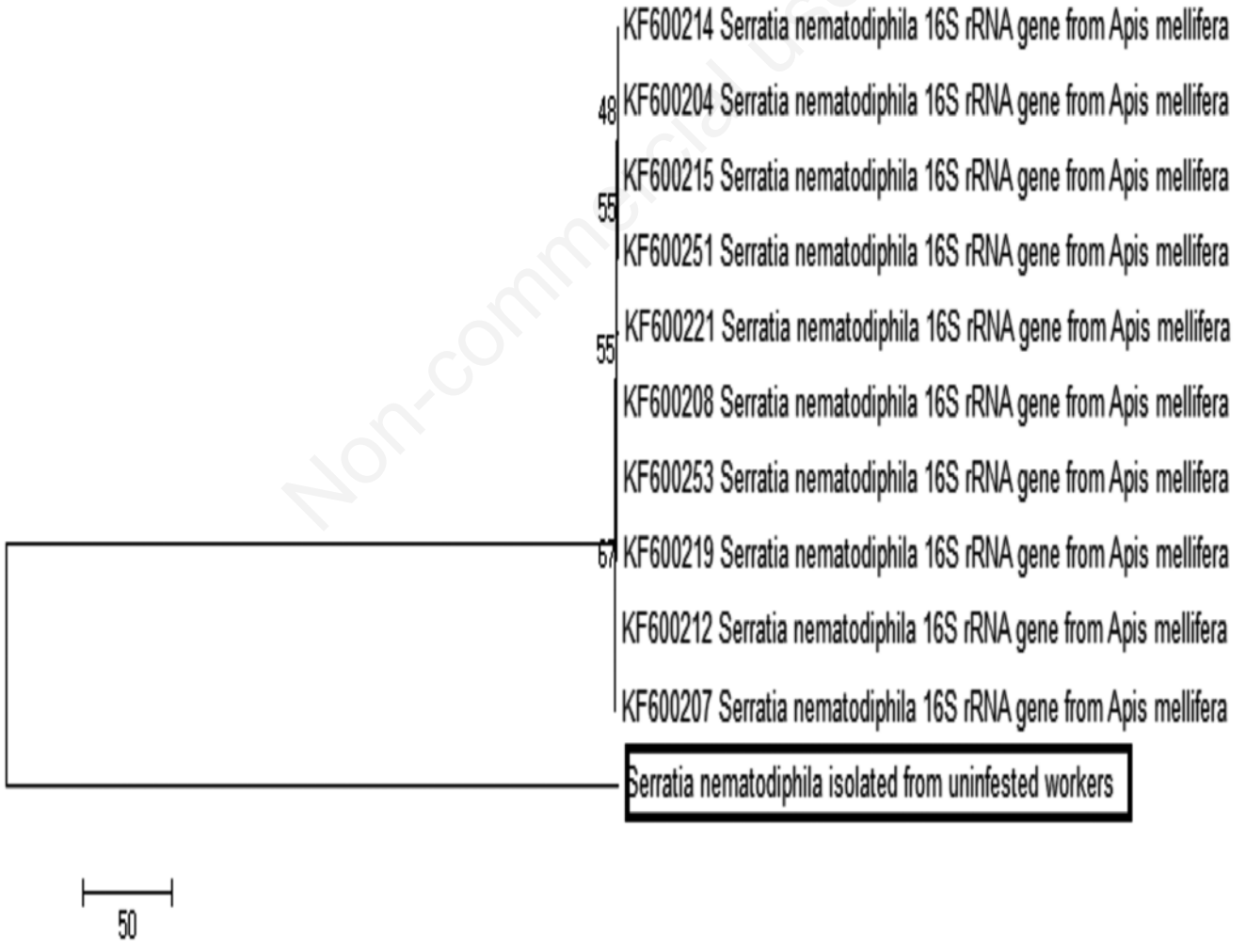

Figure 4. Neighbor joining Phylogenetic tree based on 16S rRNA gene sequences of *Serratia* sp. from midgut of *A. mellifera* uninfested workers.

The present study revealed the occurrence of several bacterial species in the midgut of *A. mellifera*. The occurrence of common gut bacterial genera *Enterobacter* and *Serratia* in many insect guts and their role in the host fitness attributes was reported by Broderick *et al.* (2004). *Enterobacter* genera isolated from midgut of *A. mellifera* workers infested with *V. destructor* (which represents an environmental stress) could perturb the gut community and lead to higher susceptibility of the hosts (Engel & Moran, 2013).

Conclusions

To our knowledge, this is the first report towards a comprehensive study of midgut microbiota of uninfested *A. mellifera* larvae and workers and those infested with *V. destructor*. *Enterobacter* genera were found to be the predominant culturable gut bacteria in both uninfested and infested ones. Changes in the isolated gut bacteria in relation to infection status and honey bee stage were observed. Such results may give insight towards a better understanding of microbiota involvement either directly or indirectly in the immune response.

References

AHN J.H., HONG I.P., BOK J.I., 2012 - Pyrosequencing analysis of the bacterial communities in the guts of honey bees *Apis cerana* and *Apis mellifera* in Korea. - J Microbiol. 50: 735–745.

ALATAWY M., AL-ATTAS S.G., ASSAGAF A.I., AL-SHEHRI A., ALGHAMDI K.M., BAHIELDIN A., 2020 - Gut microbial communities of adult honey bee workers (*Apis mellifera*). - Biosci. Biotech. Res. Asia. 17: 353-362.

ANAND A.A.P., VENNISON S.J., SANKAR S.G., PRABHU D.I.G., VASAN P.T., RAGHURAMAN T., GEOFFREY C.J., VENDAN S.E., 2010 - Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. – J. Insect Sci. 10: 107.

ANDERSON K.E., SHEEHAN T.H., MOTT B.M., MAES P., SNYDER L., SCHWAN M.R. WALTON A., JONES B.M., CORBY-HARRIS V., 2013 - Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). – PLoS One. 8: e83125.

ANJUM S.I., SHAH A.H., AURONGZEB M., KORI J., AZIM M.K., ANSARI M.J., BIN L., 2018 - Characterization of gut bacterial flora of *Apis mellifera* from north-west Pakistan. - Saudi J Biol Sci. 25: 388-392.

ARIAS-CORDERO E., PING L., REICHWALD K., DELB H., PLATZER M., BOLAND W., 2012 - Comparative evaluation of the gut microbiota associated with the below- and above ground life stages (larvae and beetles) of the forest cockchafer, *Melolontha hippocastani*. - PLoS One. 7: e51557.

BABENDREIER D., JOLLER D., ROMEIS J., BIGLER F., WIDMER F., 2007 - Bacterial community structures in honey bee intestines and their response to two insecticidal proteins. FEMS Microbiol Ecol. 59: 600-610.

BOUCIAS D.G., CAI Y., SUN Y., LIETZE V.U., SEN R., RAYCHOUDHURY R., SCHARF M.E., 2013 - The hindgut lumen prokaryotic microbiota of the termite *Reticulitermes flavipes* and its responses to dietary lignocellulose composition. Mol. Ecol. 22: 1836-1853.

BRODERICK N.A., RAFFA K.F., GOODMAN R.M., HANDELSMAN J., 2004 - Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture independent methods. - Appl Environ Microbiol. 70: 293–300.

BUCHLER R., 2015 - *Varroa* tolerance in honey bees occurrence, characters and breeding. - Bee World. 75: 54–70.

CHANDLER D., SUNDERLAND K.D., BALL B.V., DAVIDSON G., 2011 - Prospective biological control agents of *Varroa destructor* n. sp., an important pest of the European honeybee, *Apis mellifera*. - Biocontrol Sci Technol. 11: 429-448.

CHANTAWANNAKUL P., DE GUZMAN L.I., LI J., WILLIAMS G.R., 2016 - Parasites, pathogens, and pests of honeybees in Asia. - Apidologie. 47: 301–3243.

CHO G.S., STEIN M., BRINKS E., RATHJE J., LEE W., SUH S.H., FRANZ C.M.A.P., 2020 - *Serratia nevei sp*. nov. and *Serratia bockelmannii* sp. nov., isolated from fresh produce in Germany and reclassifica-tion of *Serratia marcescenssubsp. sakuens*is Ajithkumar *et al*. 2003 as a later heterotypic synonym of *Serratia marcescens subsp.* - Marcescens. Syst Appl Microbiol. 43: 126082.

CORBY-HARRIS V., MAES P., ANDERSON K.E., 2014 - The bacterial communities associated with honey bee (*Apis mellifera*) foragers. - PLoSHYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/24740297" One. 9: e95056.

COX-FOSTER D.L., CONLAN S., HOLMES E.C., PALACIOS G., EVANS J.D., MORAN N.A., QUAN P.L., BRIESE T., HORNIG M., GEISER D.M., MARTINSON V., VANENGELSDORP D., KALKSTEIN A.L., DRYSDALE A., HUI J., ZHAI J., CUIN L., HUTCHISON S.K., FREDRIK SIMONS J., EGHOLM M., PETTIS J.S., LIPKIN W.I., 2007 - A metagenomic survey of microbes in honey bee colony collapse disorder. - Science. 318: 283-287.

DILLON R.J., DILLON M., 2004 - The gut bacteria of insects: nonpathogenic interactions. - Annu. Rev. Entomol. 49: 71-92.

DILLON R.J., WEBSTER G., WEIGHTMAN A.J., CHARNLEY A.K., 2010 - Diversity of gut microbiota increases with aging and starvation in the desert locust. - Antonie Van Leeuwenhoek. 97: 69-77.

DISAYATHANOOWAT T., YOSHIYAMA M., KIMURA K., CHANTAWANNAKUL P., 2012 - Isolation and characterization of bacteria from the midgut of the Asian honey bee (*Apis cerana indica*). - J. Apic. Res. 51: 312-319.

ELATEEK S.Y., ABDEL-RAZIK A.B., IBRAHIM S.A., 2020 - Initial clustering of some Egyptian nematicides *Serratia* isolates based on their 16S rRNAs gene sequences. - Egypt. J. Genet. Cytol. 49: 59-68.

ENGEL P., MARTINSON V.G., MORAN N.A., 2012 - Functional diversity within the simple gut microbiota of the honey bee. - Proc Natl Acad Sci USA 109: 11002-11007.

ENGEL P., MORAN N.A., 2013 - The gut microbiota of insects diversity in structure and function. - FEMS Microbiol. Rev. 37: 699-735.

EVANS J.D., SCHWARZ R.S., 2011 - Bees brought to their knees: microbes affecting honey bee health. - Trends Microbiol. 19: 614-620.

GASPER J., TERENTJEVA M., KANTOR A., IVANISOVA E., KNAZOVICKA V., KLUZ M., KACANIOVA M., 2017 - *Enterobacteriaceae* in gut of honey bee (*Apis mellifera*) and the antibiotic resistance of the isolates. - J. Anim. Sci. Biotechnol. 50: 69-72.

GILLIAM M., 1997 - Identification and roles of non-pathogenic microflora associated with honey bees. - FEMS Microbiol- Lett. 155: 1-10.

GILLIAM M., LORENZ B.J., RICHARDSON G.V., 1988 -

Digestive Enzymes and microorganisms in honey bees (*Apis melifera* L.) influence of Streptomycin, Age, Season and Pollen. - Microbios. 55: 95-114.

GOUVEIA C., MARISE D., ASENSI M.D, ZAHNER V., RANGEL E.F., DE Oliveira S.M.P., 2008 - Study on the bacterial midgut microbiota associated to different Brazilian populations of *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae). – Neotrop. Entomol. 37: 597-601.

GUNATHILAKA N., RANASINGHE K., AMARASINGHE D., RODRIGO W., MALLAWARACHCHI H., CHANDRASENA N., 2020 - Molecular characterization of culturable aerobic bacteria in the midgut of field-caught *Culex tritaeniorhynchus*, *Culex gelidus*, and *Mansonia annulifera* mosquitoes in the Gampaha district of Sri Lanka. - BioMed Res. Int. 1-13.

GUO C., SUN L., KONG D., SUN M., ZHAO K., 2014 - *Klebsiella variicola*, anitrogen fixing activity endophytic bactrium isolated from the gut of *Odontotermes formosanus*. – Afr. J. Microbiol. Res. 8: 1322-1330.

HORTON M.A., OLIVER R., NEWTON I.L., 2015 - No apparent correlation between honey bee forager gut microbiota and honey production. - Peer J. 3: e1329.

HRONCOVA Z., KILLER J., HAKL J., TITERA D., HAVLIK J., 2019 - In-hive variation of the gut microbial composition of honey bee larvae and pupae from the same oviposition time. - BMC Microbiol. 19: 110.

HUBERT J., BICIANOVA M., LEDVINKA O., KAMLER M., LESTER P.J., NESVORNA M., KOPECKY J., ERBAN T., 2017 - Changes in the bacteriome of honey bees associated with the parasite *Varroa destructor*, and pathogens *Nosema* and *Lotmaria passim*. - Microb Ecol. 73: 685–698.

INGLIS G.D., YANKE L.J., GOETTEL M.S., 1998 - Anaerobic bacteria isolated from the alimentary canals of alfalfa leafcutting bee larvae. - Apidologie. 29: 327-332.

JEYAPRAKASH A., HOY M.A., ALLSOPP M.H., 2003 - Bacterial diversity in worker adults of *Apis mellifera capensis* and *Apis mellifera scutellata* (Insecta: Hymenoptera) assessed using 16S rRNA sequences. - J. Invertebr. Pathol. 84: 96-103.

JONG D.D., JONG P.H.D., GONCALVES L.S., LOSS W., 1982 - Damage to developing worker honeybees from infestation with *Varroa Jacobsoni*. - J. Apicultural Research. 21: 165-167.

KHAN K.A., ANSARI M.J., AL-HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Al-Ghamdi%20A%5BAuthor%5D&cauthor=true&cauthor_uid=28663705"GHAMDI HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Al-Ghamdi%20A%5BAuthor%5D&cauthor=true&cauthor_uid=28663705" A., NURU A., HARAKEH S., IQBAL J., 2017 - Investigation of gut microbial communities associated with indigenous honey bee (*Apis mellifera jemenitica*) from two different eco-regions of Saudi Arabia. - Saudi J. HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/28663705"Biol.HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/28663705" Sci. 24: 1061-1068.

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.

MARTIN S.J., 1994 - Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. - Exp. Appl. Acarol. 18: 87-100.

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.

MARTINSON V.G., DANFORTH B.N., MINCKLEY R.L., RUEPPELL O., TINGEK S., MORAN N.A., 2011 - A simple and distinctive microbiota associated with honey bees and bumble bees. - Mol Ecol. 20: 619-628.

MATTILA H.R., SEELEY T.D., 2007 - Genetic diversity in honey bee colonies enhances productivity and fitness. - Science 317: 362-4.

MCMENAMIN A.J., GENERSCH E., 2015 - Honey bee colony losses and associated viruses. – Curr. Opin. Insect Sci. 8: 121–129.

MILLER C.S., HANDLEY K.M., WRIGHTON K.C., FRISCHKORN K.R., THOMAS B.C., BANFIELD J.F., 2013 - Short-read assembly of full-length 16S amplicons reveals bacterial diversity in subsurface sediments. - PloS One. 8: e56018.

MOHR K.I., TEBBE C.C., 2006 - Diversity and phylotype consistency of bacteria in the guts of three bee species (Apoida) at an oilseed rape field. – Eviron. Microbiol. 8: 258-272.

MORAN N.A., HANSEN A.K., POWELL J.E., SABREE Z.L., 2012 - Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. - PloS One 7: e36393.

OLOFSSON T.C., VASQUEZ A., 2008 - Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honey bee *Apis mellifera*. - Curr Microbiol. 57: 356-363.

OTTO C.R., ROTH C.L., CARLSON B.L., SMART M.D., 2016 - Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains. - Proc Natl Acad Sci USA 113: 10430-10435.

PALMER C., BIK E.M., DIGIULIO D.B., RELMAN D.A., BROWN P.O., 2007 - Development of the human infant intestinal microbiota. - PLoS Biol. 5: e177.

POULSEN M., SAPOUNTZIS P., 2012 - Behind every great ant, there is a great gut. - Mol. Ecol. 21: 2054-2057.

PROENCA D.N.S., GRASS C.E., MORAIS G., PAULA V., 2012 - Draft genome sequence of *Serratia sp*. Strain M24T3, isolated from pinewood disease nematode *Bursaphelenchus xylophilus*. - J Bacteriol. 194: 3764.

RAHUL K.S., PATIL C.D., HEMANT B., PATIL S.V., 2014 - Nematicidal activity of microbial pigment from *Serratia marcescens*. - Nat. Prod. Res. 28: 1399-404.

RAJAGOPAL R., 2009 - Beneficial interactions between insects and gut bacteria. - Indian J Microbiol. 49: 114-119.

RAYMANN K., MORAN N.A., 2018 - The role of the gut microbiome in health and disease of adult honey bee workers. – Curr. Opin. Insect. Sci. 26: 97-104.

ROBERTS J.M.K., ANDERSON D.L., DURR P.A., 2017 - Absence of deformed wing virus and *Varroa destructor* in Australia provides unique perspectives on honeybee viral landscapes and colony losses. - Sci Rep. 7: 1-11.

SABREE Z.L., HANSEN A.K., MORAN N.A., 2012 - Independent studies using deep sequencing resolve the same set of core bacterial species dominating gut communities of honey bees. - PloS One. 7: e41250.

SACCA M.L., LODESANI M., 2020 - Isolation of bacterial microbiota associated to honey bees and evaluation of potential biocontrol agents of *Varroa destructor*. – Benef. Microbes. 11: 641-654.

SALEM H., KREUTZER E., SUDAKARAN S., KALTENPOTH M., 2013 - Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). - Environ. Microbiol. 15: 1956-1968.

SAMBROOK J., 2001 - Molecular cloning: a laboratory manual. Cold Spring Harbor, N Y: Cold Spring Harbor Laboratory Press.

SAMMATARO D., GERSON U., NEEDHAM G., 2000 - Parasitic

Mites of Honey Bees: Life History, Implications, and Impact. - Annu. Rev. Entomol. 45: 519-548.

SHIL R.K., MOJUMDER S., SADIDA F.F., UDDIN M., SIKDAR D., 2014 - Isolation and identification of cellulolytic bacteria from the gut of three phytophagus insect species. - Braz. Arch. Biol. Technol. 57: 927-932.

STANLEY J., SAH K., JAIN S.K., BHATT J.C., SUSHIL S.N., 2015 - Evaluation of pesticide toxicity at their field recommended doses to honeybees, *Apis cerana* and *A. mellifera* through laboratory, semi-field and field studies. - Chemosphere. 119: 668-674.

TAJABADI N., MARDAN M., MANAP M.Y., MUSTAFA S., 2013 - Molecular identification of *Lactobacillus spp.* isolated from the honey comb of the honey bee (*Apis dorsata*) by 16S rRNA gene sequencing. - J. Apic. Res. 52: 235-241.

TAJABADI N., MARDAN M., SHUHAIMI M., ABDUL MANAP M.Y., 2011 - Isolation and identification of *Enterococcus sp.* from honey stomach of honeybee based on biochemical and 16S rRNA sequencing analysis. - Int. J. Probiotics Prebiotics. 6: 95-100.

TAMURA K., STECHER G., PETERSON D., FILIPSKI A., KUMAR S., 2013 - MEGA6: molecular evolutionary genetics analysis version 6.0. - Mol. Biol. Evol. 30: 2725-2729.

THAKUR A., DHAMMI P., SAINI H.S., KAUR S., 2015 - Pathogenicity of bacteria isolated from gut of *Spodoptera litura* (Lepidoptera: Noctuidae) and fitness costs of insect associated with consumption of bacteria. - J. Invertebr. Pathol. 127: 38-46.

THAOCHAN N., DREW R.A.I., HUGHES J.M., VIJAYSEGARAN S., CHINAJARIYAWONG A., 2010 - Alimentary tract bacteria isolated and identified with API-20E and molecular cloning techniques from Australian tropical fruit flies, *Bactrocera cacuminata* and *B. tryoni.* - J. Insect Sci. 10: 131.

TOWNSEND R.J., CALLAGHAN M., JOHN-SON V.W., JACKSON T.A., 2003 - Compatibility of microbial control agents *Serratia entomophila* and *Beauveria bassiana* with selected fertilizers. - N. Z. Plant Protect. 56: 118-122.

VASSART M., THEVENON J., COTTO F., 1988 - Health study of the Neo-Caledonian apiary. - Bull. Tech. Apic. 15: 195-202.

VOJVODICHYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Vojvodic%20S%5BAuthor%5D&cauthor=true&cauthor_uid=23991051" S., REHAN S.M., ANDERSON K.E., 2013 - Microbial gut diversity of Africanized and European honey bee larval instars. - PLoSHYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/23991051" One. 8: e72106.

WINSTON M.L., 1987 - The Biology of the Honey Bee. Camb. Harvard Uni. Press. 281.

YADAV K.K., BORA A., DATTA S., CHANDELK., GOGOI H.K., PRASAD G.B.K.S., VEER V., 2015 - Molecular characterization of midgut microbiota of *Aedes albopictus* and *Aedes aegypti* from Arunachal Pradesh, India. - Paras. Vectors. 8: 641.

YOSHIIYAMA M., KIMURA K., 2009 - Bacteria in the gut of the Japanese honey bee, *A. cerana japonica*, and their antagonistic effect against *P. larvae*, the causal agent of American foulbrood. - J. Invertebr. Pathol. 102: 91-96.

YUN J., ROH S.W., WHON T.W., JUNG M., KIM M., PARK D., YOON C., NAM Y., KIM Y., CHOI J., KIM J., SHIN N., KIM S., LEE W., BAE J., 2014 - Insects gut bacterial diversity determined by host environmental habitat, diet, developmental stage and phylogeny. - Appl. Environ. Microbiol. 80: 5254-5264.

ZHANG C.X., LIU J.R., XU M.X., SUN J., YANG S.Y., AN X., GAO G.F., LIN M.S., LAI R., 2008 - *Heterorhabditidoides chongmingensis gen*. nov., sp. nov. (Rhabditida: Rhabditidae), a novel member of the entomopathogenic nematodes. - J. Invertebr. Pathol. 98: 153-168.