






# Comparative analysis of bacterial communities in olive psyllids, *Euphyllura straminea* and *Euphyllura pakistanica* expose proteobacteria dominance

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## Abstract

Insect–bacteria symbiont associations can be found in almost all insect orders which supply an array of physiological and ecological benefits to the host insect. Plant sap-feeding insects, including psyllids, have developed symbiotic relationships with a variety of bacteria to compensate nitrogen-poor diets. In this study, we determined the bacterial composition of two olive psyllid pest species, *Euphyllura straminea* and *Euphyllura pakistanica* (Hemiptera: Aphalaridae), using 16S rRNA gene sequence and qPCR analyses. Proteobacteria, including Alphaproteobacteria, Gammaproteobacteria and Betaproteobacteria, composed the primary bacterial structures of the psyllids. Alphaproteobacteria were the dominant bacterial group in *E. straminea*, while Gammaproteobacteria composed the main portion of the bacterial structure in *E. pakistanica*. The primary endosymbiont, *Carsonella*, was detected in both psyllids and *Arsenophonus* with high prevalence was the main secondary endosymbiont. Interestingly, we observed high titres of *Wolbachia* in *E. straminea* compared with *E. pakistanica*, which revealed a negative correlation between *Wolbachia* and *Carsonella* and *Arsenophonus* titres. Our results show that the limited core microbiota of olive psyllids is similar while the abundance of each bacterial symbiont can vary in different species. A possible role of *Wolbachia* in modulating bacterial composition in the psyllids was also suggested. There is the potential to utilize symbionts for developing pest control strategies by reducing the insect host's fitness such as targeting essential endosymbionts, transferring symbionts between species and parthenogenesis. Our results can be used as a basis for further research towards the implementation of novel strategies in symbiont-based biological control of the olive psyllids.

## KEYWORDS

*Arsenophonus*, bacterial symbionts, *Carsonella*, proteobacteria, *Wolbachia*

## 1 | INTRODUCTION

Bacterial and insect host associations in symbiotic contexts have been evolving for millions of years. The effects of this relationship on different biological, evolutionary and physiological

aspects of their hosts have been elucidated by many studies in recent decades. There are many examples of the importance of endosymbionts in host biology, including nutrition (Akman Gündüz & Douglas, 2009), immunity (Lee & Mazmanian, 2010), reproduction (Bagheri et al., 2019; Simon et al., 2011), behaviour (Bagheri et al., 2022; Dion et al., 2011), speciation (Shropshire &

Bordenstein, 2016), morphology (Kashkouli et al., 2020; Tsuchida et al., 2010), detoxification (Kikuchi et al., 2012) and defence (Hamilton & Perlman, 2013).

As one of the most successful animal groups on earth, insects have occupied diverse ecological niches and possess primary (P-) and secondary (S-) endosymbionts (Baumann, 2005) which assist them in various life stages. The P-endosymbionts are intracellular, mostly live in special cells known as bacteriocytes and are obligate organisms necessary for the survival or reproduction of their host. Because of their intracellular living, these endosymbionts have a reduced genome size with high AT content, are primarily vertically transmitted to the next generation and cannot survive out of their host body (Wernegreen, 2002). Maternally inheritable gene transfers throughout evolutionary times have been the primary causes of co-speciation between hosts and P-endosymbionts (Clark et al., 2000; Kikuchi et al., 2009). Moreover, most insects harbour another bacterial endosymbiont, which is classified as facultative for their respective hosts (Pontes & Dale, 2006). These endosymbionts are classified as S-endosymbionts and are generally not essential for their host survival or reproduction (Dale & Moran, 2006). However, the S-endosymbionts can affect host reproduction, such as in the case of *Wolbachia* (Bagheri et al., 2019; Guidolin & Cönsoli, 2013; Werren & Windsor, 2000), or can provide benefits for their host such as *Arsenophonus* (Ayoubi et al., 2020; Hansen et al., 2007).

There is a complex interaction between P- and S-endosymbionts within the host. In some cases, reduction in P-endosymbiont genome size can be sharp, because the presence of S-endosymbionts is necessary for complementation of their function in the host (Pérez-Brocal et al., 2006). The evolution of this relationship can lead to the elimination of P-endosymbionts and provide a more suitable environment for the S-endosymbionts. In these cases, the S-endosymbionts become obligate for normal growth of the host (Hall et al., 2016). Moreover, other research has indicated that the presence of *Wolbachia* can change the abundance of other endosymbionts in the host (Arbuthnott et al., 2016; Simhadri et al., 2017).

Among different orders of insects, blood and phloem-feeding species like psyllids are more dependent on their endosymbionts because of their restricted diet. The normal growth of these insects on this nutrient-poor diet is related to the P-endosymbiont synthesis of nutrients. Given their restricted diet, nearly all psyllids harbour *Carsonella rudii*, a P-endosymbiont, in special vesicles within bacteriocytes (Fukatsu & Nikoh, 1998; Spaulding & von Dohlen, 1998). This endosymbiont provides essential amino acids for their hosts and is vertically transmitted through generations (Nakabachi et al., 2006). Moreover, several S-endosymbionts have also been reported from different species of psyllid insects (Morrow et al., 2017; Thao et al., 2000). The presence of *Proftella* (Nakabachi et al., 2013), *Arsenophonus* (Hansen et al., 2007) and *Wolbachia* (Cooper et al., 2015) have also been published from psyllids in different studies. Moreover, some psyllids harbour other endosymbionts such as *Liberibacter* (Halbert & Manjunath, 2004) and *Phytoplasma*

(Malagnini et al., 2010), which are considered among the most important plant pathogens in agriculture.

Olive psyllids, *Euphyllura straminea*, and *Euphyllura pakistanica* (Hemiptera: Aphalaridae), are among the most important pests of olive trees. Adults and juveniles feed on leaves and buds, shoots, flowers and young fruits, thus causing major damage within orchards with large economic losses (Asadi et al., 2009). Feeding by psyllids on plant sap and secretion of honeydew causes sooty mould, which reduces photosynthesis in the plants (Santos et al., 2013). These insects are also associated with a number of symbiotic bacteria that play important roles in their survival. Considering the diverse and important roles of the microbiome in insects, this study attempted to define the bacterial composition of the olive psyllids collected from Iran. This is a first attempt to identify bacterial communities in these insect pests to highlight the importance of microbial symbionts with the hopes of developing a symbiont-based control strategy.

## 2 | METHODS AND MATERIALS

### 2.1 | Insect sampling and DNA extraction and 16S rRNA gene amplicon sequencing

The adults of *E. straminea* were collected from Tehran (Es-Te) and Tarom (Es-Ta), while adults of *E. pakistanica* were collected from Shiraz (Ep-Sh), Iran. Immediately after collection, the insects were transferred to individual vials containing 1 ml acetone and preserved at room temperature until DNA extraction. Before DNA extraction all psyllids have dipped into alcohol 95% for surface sterilization. To explore the composition of the different bacterial endosymbionts in the psyllids, total DNA was extracted from 100 adults of psyllids in every three populations (every population from each town). The concentration of DNA samples was measured by BioTek Epoch Microplate Spectrophotometer, and the quality of the sample was cleared by running on a (1%) agarose gel.

To determine the bacterial composition, PCR was performed on the V3-V4 region of 16S rRNA of two species from Shiraz and Tarom using universal primer pairs 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') under the following conditions: initial denaturation at 95°C for 1 min, followed by 30 cycles of denaturation at 95°C for 15 s, annealing temperature at 55°C for 15 s and extension at 72°C for 90 s, with a final elongation at 72°C for 10 min. All the qualified samples were used to construct libraries using fusion primers with dual index and adaptors for PCR. Fragments that were too short were removed by AMPure beads, and only the qualified libraries were used for sequencing with the Illumina Mi Seq platform (BGI). The raw data were filtered to eliminate the adapter pollution and low quality to obtain clean reads, then paired-end reads with 15 bp minimal overlapping length and  $\leq 1$  mismatch in overlap regions were merged to tags using FLASH (Magoč & Salzberg, 2011). The tag was subjected to UCHIME (v4.2.40) algorithm to detect and remove chimeric

sequencing (Edgar et al., 2011). The high-quality tags were clustered into OTU (Operational taxonomic unit), and the clustering was performed at 97% sequence similarity to the Greengene database (DeSantis et al., 2006). Taxonomic ranks were assigned to OTU representative sequence using Ribosomal Database Project (RDP) (Cole et al., 2014) and Naive Bayesian Classifier v.2.2 (Garrity et al., 2007). Metaxa2 was used to remove mitochondrial and chloroplast sequences (Bengtsson-Palme et al., 2015). To identify the complexity of species, alpha diversity was performed for samples through several indices, including observed species, Chao and Ace, Shannon and Simpson using mothur (v1.31.2) based on OTU and taxonomic ranks (Schloss et al., 2009). The curves were analysed by R software (v3.1.1).

## 2.2 | PCR and Real-Time PCR (qPCR)

To evaluate the relative abundance of different bacterial endosymbionts in the three populations of male and female olive psyllids and also confirm the high throughput amplicon sequencing results, we performed qPCR using species-specific primers (16S rRNA of *Carsonella ruddii* and *ftsZ* of *Wolbachia pipientis*) and class-specific primers (Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Bacteroidia and Firmicutes). The 18S rRNA gene was used as a reference gene (Karamipour et al., 2016). The qPCR condition was 95°C for 15 min, followed by 40 cycles of 95°C for 15 s and 15 s at the annealing temperature and followed by the melting curve (68–95°C). The annealing temperature and primers list are shown in Table 1. The qPCRs were performed by using SYBR green (Ampliqon) with a micPCR instrument (Bio Molecular systems). The qPCR data were analysed using the  $\Delta\Delta C_t$  method (Livak & Schmittgen, 2001). Moreover, the presence of *Arsenophonus* was confirmed by PCR. The PCR reactions were conducted under a temperature profile of 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 55°C for 1 min and 72°C for 1 min and a final extension at 72°C for 10 min. Eventually, the PCR products were subjected to agarose (1%) electrophoresis.

## 2.3 | Phylogenetic analysis

To develop phylogenetic trees of the bacterial endosymbionts, phylogenetic analysis was performed using the annotated sequences obtained from the 16S rRNA gene amplicon analysis. To do this, the amplicon sequencing-derived were aligned to the 16S rRNA genes from the Ribosomal Database Project (RDP) using Blastn. Then, the most similar sequences from the database along with our sequences were used for multiple sequence alignments using ClustalX v.2 program (Larkin et al., 2007). Molecular phylogenetic analyses based on maximum likelihood and neighbour-joining algorithms were performed by using MEGA 6.06 (Tamura et al., 2013). GTR+G+I model was selected as the best-fit substitution model for the maximum likelihood trees. The Bootstrap values were calculated with 1000

replications for neighbour-joining methods and 100 replications for maximum likelihood methods.

## 3 | RESULTS

### 3.1 | 16S rRNA gene sequencing analysis

Two species of the olive psyllids including, *E. straminea* and *E. pakistanica*, were collected from Tarom and Shiraz, respectively. They were used for bacterial community profiling using 16S rRNA gene amplicon sequencing (Figure 1a). In total, 123,860 pair-end reads (69,842 and 62,496 reads with about 293 lengths from Es-Ta and Ep-Sh populations, respectively) were obtained. After removing low-quality reads, the overlapping reads were merged to construct the tags. About 104,868 tags remained after cleaning (56,819 tags with 495 average lengths from the Es-Ta population and 48,049 tags with 522 average lengths from Ep-Sh populations). The chimer sequences were removed, and filtered tags were subjected to clustering into OTUs at 97% similarity. Rarefaction curves showed that most of the samples had reached the saturation plateau (Figure 1b), indicating that the data obtained from the sequencing was enough to cover all species within the community. To compare alpha diversity between Es-Ta and Ep-Sh populations, we used the Chao and Ace indexes, which assess the richness of OTUs. The mean Chao index of the microbiota of Es-Ta insects was higher than those of Ep-Sh, which showed a higher species richness of microbiota in Es-Ta populations compared with Ep-Sh populations (Mann–Whitney U test;  $p < 0.0001$ ) (Figure 1b). A similar result was also observed based on the Ace index (Mann–Whitney U test;  $p < 0.0001$ ) (Figure 1c). The Es-Ta populations showed higher species uniformity and diversity, as shown by the Shannon index (Mann–Whitney U test;  $p < 0.0001$ ) (Figure 1d). According to the clustering results, a number of 23 and 21 OTUs were identified from Es-Ta and Ep-Sh populations, respectively. Twelve OTUs were similar in both populations while there were nine and 11 specific OTUs in Shiraz and Tarom populations, respectively (Figure 1f).

### 3.2 | The olive psyllid bacterial microbiota analysis

The 16S rRNA analysis showed that the core bacterial structures of Es-Ta and Ep-Sh were mostly similar (Figure 2a–d). Both species harbour two bacterial phyla including Proteobacteria and Actinobacteria. Proteobacteria including Alphaproteobacteria, Gammaproteobacteria and Betaproteobacteria established the primary bacterial structure of the psyllids (Figure 2a). The abundances of different bacterial microbiota were different in the two psyllid species (Figure 2b–d). Alphaproteobacteria was the most abundant bacteria in Es-Ta while Gammaproteobacteria was the most abundant population of the bacterial endosymbionts in Ep-Sh (Figure 2a). *Arsenophonus* and *Carsonella* from Gammaproteobacteria and *Wolbachia* from Alphaproteobacteria composed the main part of the bacterial populations in both psyllids (Figure 2d). *Carsonella*

TABLE 1 Primers used in this study

Target	Primer	Primer sequence (5'-3')	Fwd./rev	Annealing temp. (°C)	Ref.
Universal bacterial	906F	AAACTCAAAGAATTGACGG	Fwd.	61.5	(Garrity et al., 2007)
	1062R	CTCACRRCACGAGCTGAC	Rev.		
α-proteobacteria	α682F	CIAGTGTAGAGGTGAAATT	Fwd.	61.5	(Garrity et al., 2007)
	908αR	CCCCGTCAATTCCTTTGAGTT	Rev.		
β-proteobacteria	Eub338	ACTCCTACGGGAGGCAGCAG	Fwd.	61.5	(Bengtsson-Palme et al., 2015)
	Bet680	TCACTGCTACACGYG	Rev.		
γ-proteobacteria	1080γF	TCGTCAGCTCGTGYGTGA	Fwd.	61.5	(Bengtsson-Palme et al., 2015)
	γ1202R	CGTAAGGGCCATGATG	Rev.		
Actinobacteria	Act920F3	TACGGCCGCAAGGCTA	Fwd.	61.5	(Garrity et al., 2007)
	Act1200R	TCRTCCCCACCTTCTCCG	Rev.		
Bacteroides	798cfbF	CRAACAGGATTAGATACCCT	Fwd.	61.5	(Garrity et al., 2007)
	Cfb967R	GGTAAGGTTCTCGCGTAT	Rev.		
Firmicutes	Lgc353	GCAGTAGGGAATCTTCCG	Fwd.	61.5	(Garrity et al., 2007)
	Eub518	ATTACCGGGCTGCTGG	Rev.		
18S rRNA	penta-18S rRNA-F	CCTGCGGCTTAATTTGACTC	Fwd.	57	(Pontes & Dale, 2006)
	penta-18S rRNA-R	AACTAAGAACGGCCATGCAC	Rev.		
FtsZ	ftsZ -F(RT)	AGCAGCCAGAGAAGCAAGAG	Fwd.	57	(Kaiwa et al., 2010)
	ftsZ -R(RT)	TACGTGCGCACACCTTCAAAA	Rev.		
16S r RNA	carso-16S rRNA_qPCR_F	TGAAGAAGGCCTTAGGGTTGT	Fwd.	57	This study
	carso-16S rRNA_qPCR_R	AGTTAGCCGGTGCTTCTTTT	Rev.		
<i>Arsenophonus</i>	16SA1	AGAGTTTGATCMTGGCTCAG	Fwd.		(Bacchetti De Gregoris et al., 2011)
	Ars16SR	TTAGTCCGGAGGCCACAGT	Rev.		

*rudii* was the only P-symbiont bacterial species that was identified in 16S rRNA gene amplicon sequencing. Interestingly, *Wolbachia* was the most abundant bacteria in Es-Ta. Moreover, in both psyllids the abundance of *Arsenophonus* and *Carsonella* was almost similar in both psyllids (Figure 2d).

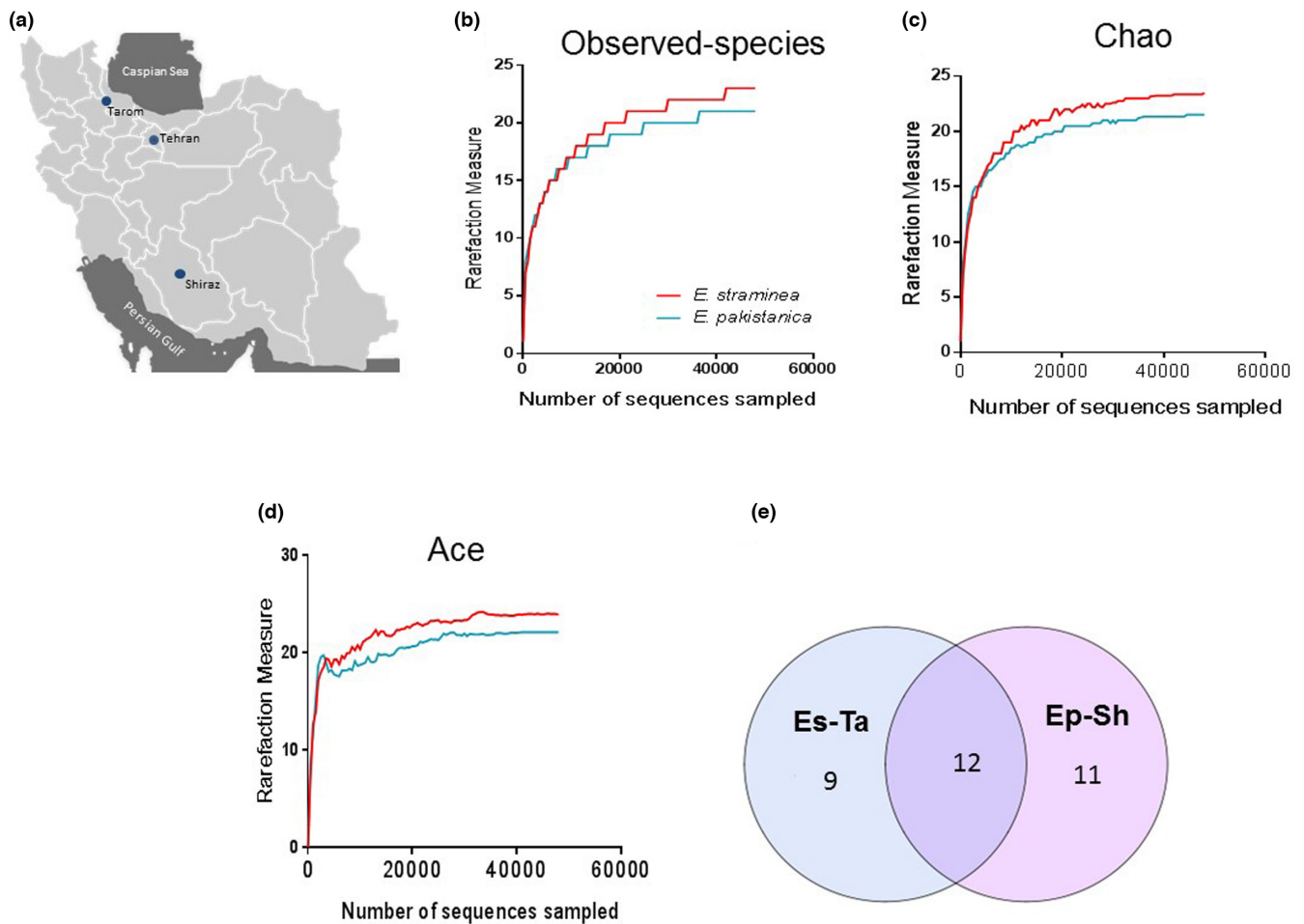
The qPCR analyses were performed to confirm the 16S rRNA gene sequencing results. The DNA samples were extracted from male and female psyllids, and the relative abundances of different classes of bacteria were quantified using the bacterial class-specific primers. *Carsonella* was detected as the P-endosymbiont of both olive psyllids. Furthermore, *Arsenophonus* and *Wolbachia* were also present in these species as facultative endosymbionts, showing similar core bacterial symbiont composition in both species. Our results showed that Gammaproteobacteria were the most abundant bacteria in Es-Ta (Figure 3a,b) and Ep-Sh populations (Figure 3c,d). Moreover, we also quantified the bacterial community of *E. straminea* from Tehran populations and found that Gammaproteobacteria were the most abundant bacterial endosymbionts in Es-Te (Figure 3e,f). The relative abundance of *Wolbachia* in Es-Ta and Es-Te were also more prevalent than Ep-Sh (Figure 4a), while, *Carsonella* and *Arsenophonus* showed higher titres in Ep-Sh (Figure 4b,c). Our results also showed that the relative abundance of the microbiota of female psyllids was higher than males (Figure 3a,c,e).

### 3.3 | Phylogenetic analysis

The phylogenetic analysis was based on the contigs obtained from the 16S rRNA gene sequencing. According to the phylogenetic analysis, *Carsonella* sequences from Ep-Sh and Es-Ta populations and *Carsonella* 16S rRNA sequences from GeneBank were placed in the same clade. Also, *Arsenophonus* sequences obtained from the 16S rRNA gene sequences were placed in a clad along with *Arsenophonus* 16S rRNA sequences derived from the database (Figure 5). *Wolbachia*, *Acinetobacter*, *Pseudomonas*, *Enterobacter* and *Micrococcus* are also shown in the figure. The results are congruent with high bootstrap values in both phylogenetic trees based on maximum likelihood and neighbour-joining algorithms.

## 4 | DISCUSSION

The microbiota of insects plays diverse roles in populations, ranging from supplying essential nutrients to defence mechanisms (Engel & Moran, 2013), to evolution and speciation (Brucker & Bordenstein, 2012; Shropshire & Bordenstein, 2016). The diverse functions of symbionts in insects can provide promising and novel strategies for the biological control of insect pests and vectors.



**FIGURE 1** Comparison of bacterial microbiota in the olive psyllids of two species, *E. straminea* and *E. pakistanica*. (a). Location of sampling sites; (b). rarefaction curves of the bacterial symbionts; (c–d). different population diversity indices for the bacterial symbionts of olive psyllids. (e). Shared OTUs between *E. straminea* and *E. pakistanica*. Overall, 12 OTUs were similar in the species from two different populations.

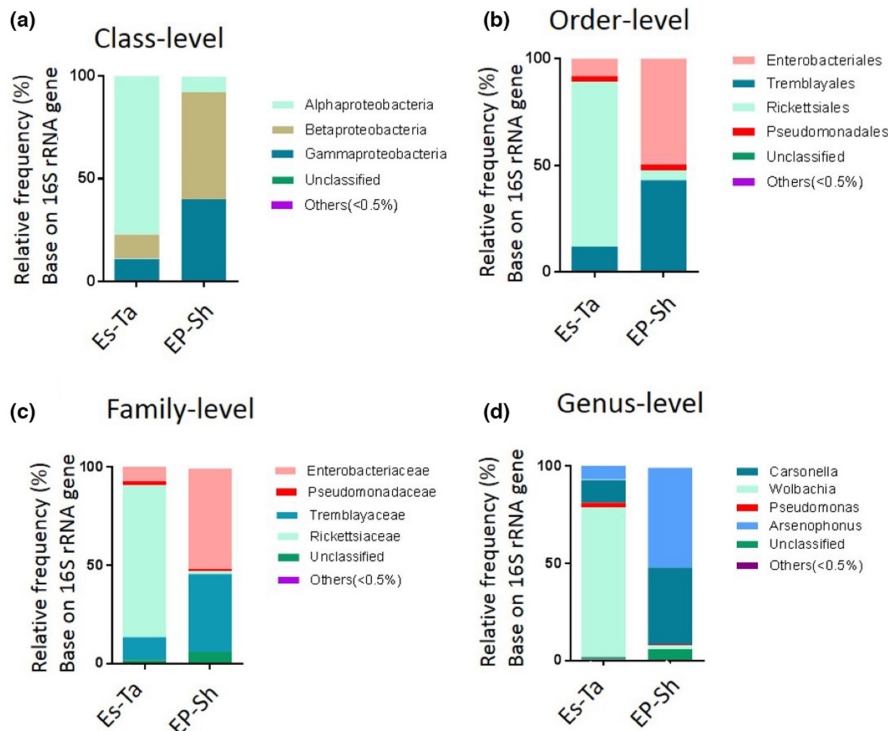
Identification and functional characterization of microbial symbionts are the key steps towards harnessing these associations for developing symbiont-based pest control.

In the present study, the whole bacterial composition of the olive psyllids, *E. straminea* and *E. pakistanica*, was investigated. Rarefaction curves indicated that the data obtained from sequencing was enough to cover all species in the community. The Es-Ta population showed higher species richness and diversity as shown by Chao, Ace and Shannon indexes. Proteobacteria (i.e. Alphaproteobacteria and Gammaproteobacteria) constructed the main part of bacterial structures in the olive psyllids. Our results revealed a small core microbiota, including *Carsonella* as a P-symbiont, and at least one Gammaproteobacteria S-symbiont (mainly *Arsenophonus*) in the olive psyllids, which is consistent with previous studies reporting overall low diversity of bacterial symbionts in psyllids (Meng et al., 2019; Morrow et al., 2017; Overholt et al., 2015).

*Carsonella* was detected in all screened samples, revealing its high rate of transmission due to its essential function through nutritional supplementation in their psyllid hosts (Hall et al., 2016; Hosseinzadeh et al., 2019; Sloan & Moran, 2012). The genome of

*Carsonella* is about 160 Kb (Nakabachi et al., 2006) and has lost genes that are responsible for amino acid synthesis (Sloan & Moran, 2012); therefore, the acquisition of other symbionts by psyllid hosts is necessary to compensate for this shortfall that may result in co-presence of the P- and S-endosymbionts (Hall et al., 2016; Morrow et al., 2017; Thao et al., 2000). In addition to the P-symbiont, we also identified *Arsenophonus*, *Wolbachia*, *Pseudomonas* and other unclassified S-endosymbionts belonging to Enterobacteriaceae. The endosymbiotic bacteria *Arsenophonus* were detected in both *E. straminea* and *E. pakistanica*, as the dominant S-symbiont, with near-complete prevalence. The 16S rRNA amplicon sequencing showed the abundances of *Carsonella* and *Arsenophonus* are almost similar, revealing the importance of *Arsenophonus* as an S-endosymbiont in the olive psyllids. The presence of *Arsenophonus* with a high prevalence in psyllids has also been reported in different species (Morrow et al., 2017), suggesting the high prevalence of this bacteria is essential for transmission to other progeny. The vertical transmission of *Arsenophonus* has also been previously reported in other species of psyllids such as *Cardiaspina* (Hall et al., 2016). A variety of functions have been reported for *Arsenophonus* in insects, such as





**FIGURE 2** Bacterial composition in *E. straminea* and *E. pakistanica* was obtained from the 16S rRNA gene analysis. (a). Comparison of different bacterial classes. (b). Comparison of different bacterial orders. (c). Family level comparison of bacteria. (d). Comparison of genus and species of bacteria.

modification of dietary scope (Wagner et al., 2015), contributing to the performance and fitness of aphids (Ayoubi et al., 2020; Tian et al., 2019; Wulff & White, 2015) and providing essential B vitamins in whiteflies (Santos-Garcia et al., 2018); however, its specific role is not yet known in psyllids. It has been suggested that *Arsenophonus* may impose a defensive role against parasitism in the lerp psyllid, *Glycaspis brimblecombei* (Hansen et al., 2007), but this effect seems to be limited to this species (Wulff et al., 2013). Considering our results and previous studies, it appears that *Arsenophonus* is probably the most common S-symbiont in psyllids, providing benefits that are yet unknown.

We also detected *Wolbachia* in all the screened samples using 16S rRNA amplicon sequencing and qPCR with specific primers. The high prevalence of *Wolbachia* has been found in other psyllids such as the gall-forming psyllid, *Trioza magnolia* (Morrow et al., 2017), and the citrus psyllid, *Diaphorina citri* (Hosseinzadeh et al., 2019). Although, *Wolbachia* is primarily an intracellular bacterium that impacts insect reproduction, in some cases, it also functions as an obligate nutritional endosymbiont (Kaiwa et al., 2010; Nikoh et al., 2014), but this relationship is limited in insects. The role of *Wolbachia* in psyllids is not well understood, but, in the potato psyllid, *Bactericera cockerelli* the cytoplasmic incompatibility effect of *Wolbachia* has been reported. (Cooper et al., 2015) In our study, the abundance of *Wolbachia* in *E. straminea* (i.e. Es-Ta and Es-Te) was higher than in *E. pakistanica* (i.e. Ep-Sh). However, the level of *Carsonella* and *Arsenophonus* in *E. pakistanica* was higher than that detected in *E. straminea*. It would appear that *Wolbachia* abundance may be negatively correlated to *Carsonella* and *Arsenophonus*. There are other reports on the effect of *Wolbachia* infection on bacterial composition in *Drosophila* species (Arbuthnott et al., 2016; Simhadri et al., 2017). Different levels

of bacterial symbionts can also be due to different environmental conditions from the original habitats of these psyllids, the host and symbiont genotypes, and the interactions between genotypes and the environment. Many symbionts confer benefits depending on the environment; therefore, the fittest symbionts may vary during time and space, leading to variation in symbiont communities in different populations.

Variation in the bacterial symbiont composition could affect different aspects of psyllid biology and ecology, including interactions with their parasites/predators (Hansen et al., 2007) and host plants. There is also evidence for S-symbiont variation and replacement in close psyllid species to complement the nutritional function of P-symbionts (Morrow et al., 2017). Therefore, variation in the bacterial symbiont levels of the olive psyllids may modulate the fitness of these pest species and could be reflected in their damage level to olive trees, a topic worthy of further investigations.

## 5 | CONCLUSION

We studied the bacterial composition of two close olive species *E. straminea* and *E. pakistanica*. The P-symbiont, *Carsonella* was detected in all screened psyllids. Moreover, *Arsenophonus* is identified as the dominant S-endosymbiont with high prevalence. *Wolbachia* was also detected with high prevalence, particularly in *E. straminea*. It seems that all three symbionts have efficient vertical transmission, which warrants their high prevalence in field populations. The nutritional function of *Carsonella* in psyllids has been suggested, while the role of *Arsenophonus* and *Wolbachia* remains to be elucidated in these insects. There is a demand for

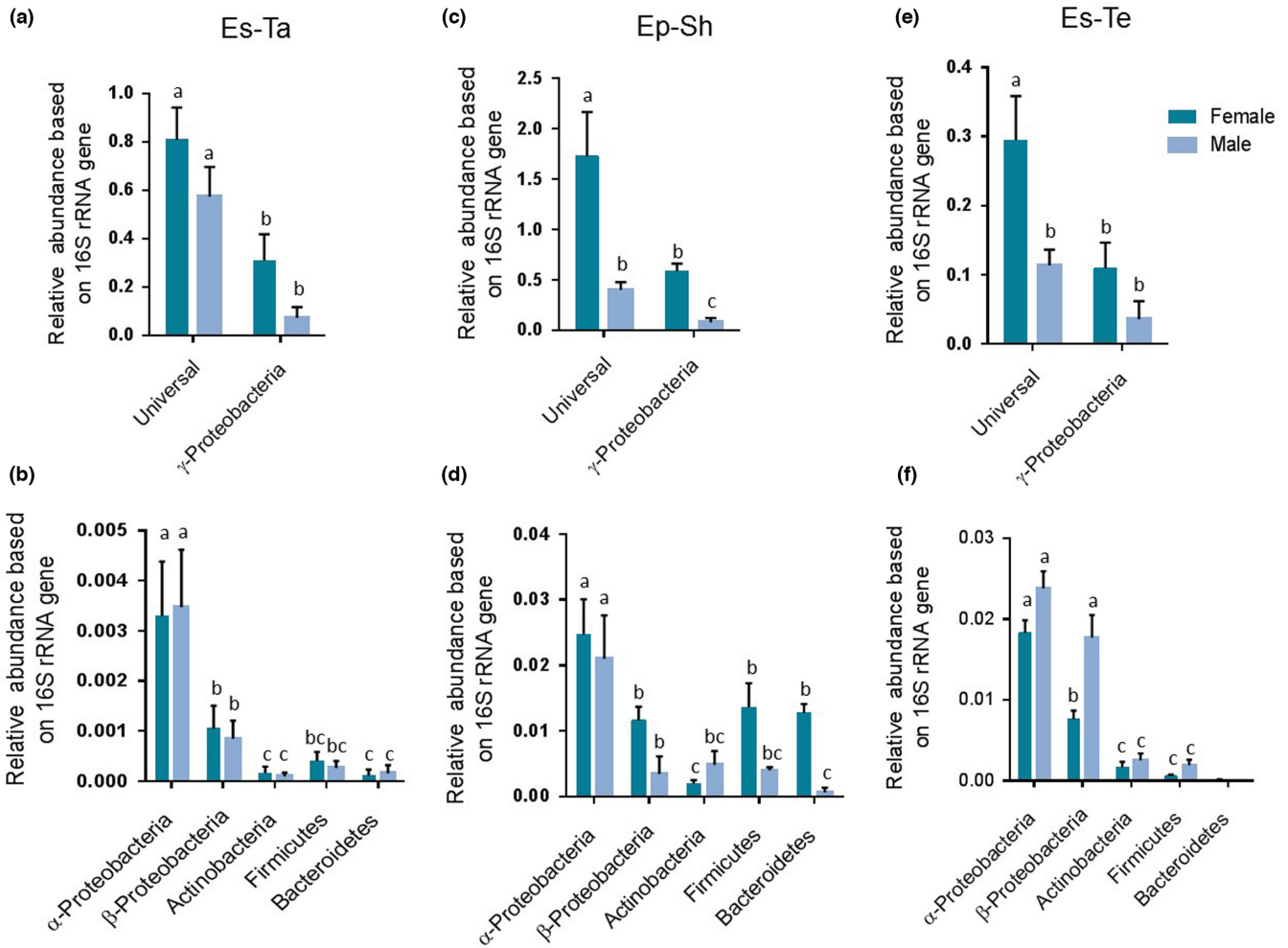


FIGURE 3 Relative abundance of the olive psyllids bacterial composition using class-specific primers. (a, b). bacterial composition in *E. straminea* from Tarom population. (c, d). bacterial composition in *E. pakistanica* from Shiraz population. (e) and (f). bacterial composition in *E. straminea* from Tehran population. Gammaproteobacteria were the dominant bacterial class in all three populations. Different letters mean significant differences,  $p < 0.05$ ; analysis of variance followed by Tukey multiple comparisons.

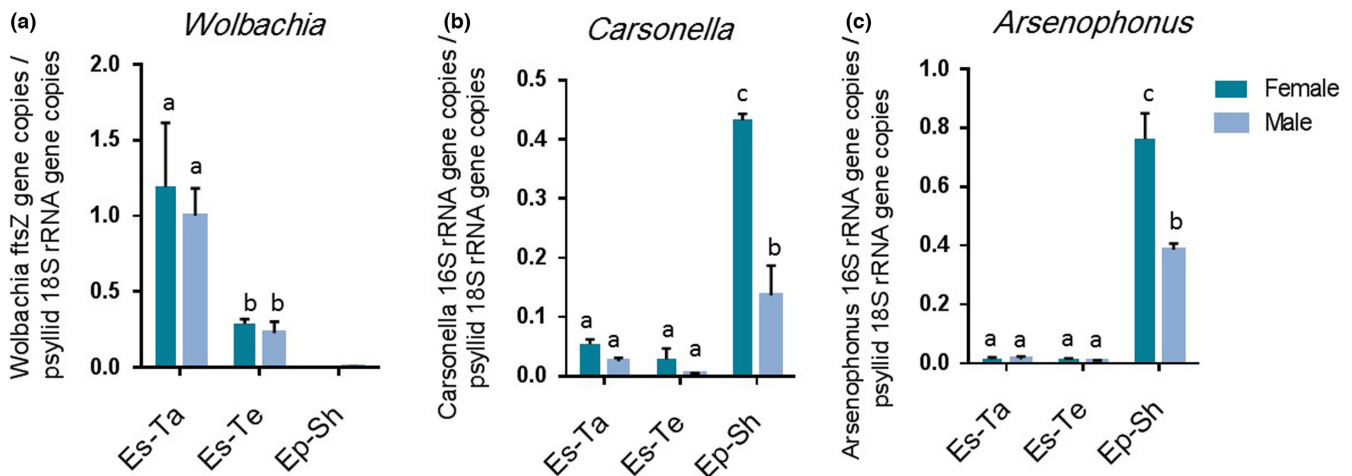
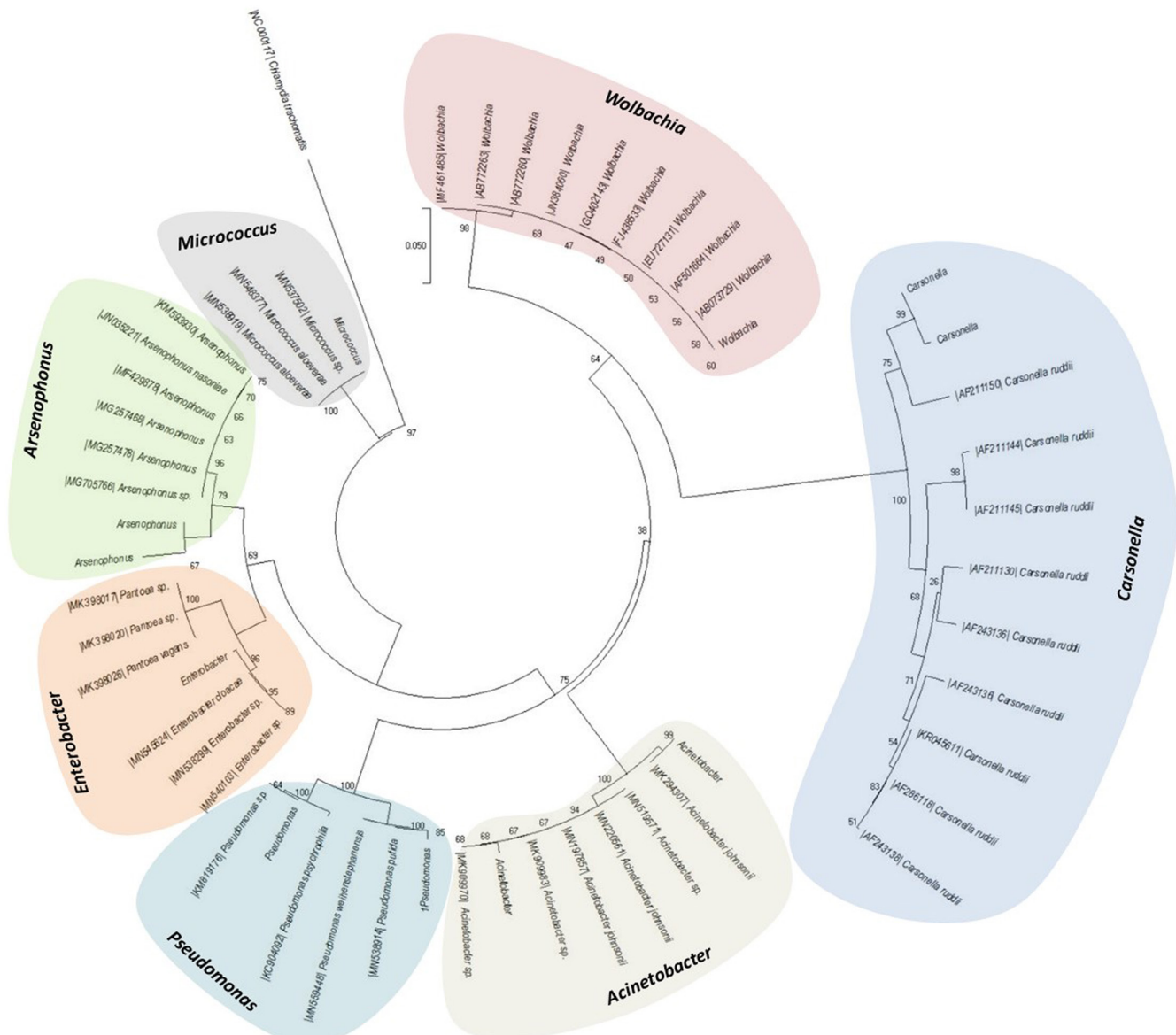


FIGURE 4 QPCR quantifications of *Carsonella*, *Wolbachia*, and *Arsenophonus* titers in the male and female olive psyllids. (a). *Wolbachia* abundance in *E. straminea* and *E. pakistanica* in three populations. (b). *Carsonella* was detected in all samples as P-endosymbiont. (c). the presence of *Arsenophonus* was confirmed using the PCR method. Different letters mean significant differences,  $p < 0.05$ ; analysis of variance followed by Tukey multiple comparisons.



**FIGURE 5** Phylogenetic tree showing the relationship of bacteria identified in the olive psyllids, *E. straminea* and *E. pakistanica* as revealed by 16S rRNA gene phylogenetic analysis. Composition of the olive psyllids, *E. straminea* collected from Tarom and *E. pakistanica* collected from shiraz as revealed by 16S rRNA gene phylogenetic analysis. Maximum likelihood method based on GTR + G + I model with 100 replications. Bootstrap probabilities are displayed on nodes and sequence accession numbers are shown in parentheses.

developing new pest management strategies and symbiotic-based pest control programmes such as targeting essential endosymbionts, transferring symbionts between species and parthenogenesis, which are promising approaches; however, these strategies require detailed information on microbial communities and their functions in the insect host. This study provides data on the bacterial microbiome in the economically important olive psyllids including primary and facultative bacterial symbionts which can be a basis for future research on developing symbiont-based control programmes for these pests.

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#### AUTHOR CONTRIBUTIONS

Experiments were designed and performed by T.F., N.K. and M.M. Laboratory space, materials/supplies and reagents were provided by A.A., A.S. and M.M. All the authors analysed the data and wrote the paper.

#### CONFLICT OF INTEREST

The authors declare no competing interests.

#### DATA AVAILABILITY STATEMENT

The data associated with this publication including workflow, bioinformatics analysis pipeline, 16S rRNA Fasta files can be accessed on zenodo (<https://doi.org/10.5281/zenodo.5910758>).



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