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Investigation of *Wolbachia* Bacteria in Different Insect Taxa

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ABSTRACT

Arthropods are the most common living species considering their population densities and distribution. However, due to the increasing world population and drought due to global warming, it is necessary to develop environmentally friendly and effective alternative strategies in terms of both health and agricultural production, especially in the fight against harmful species. In this context, *Wolbachia* is promising monitoring the effects of global warming due to its relations with its hosts. However, the symbiotic structure in arthropods varies with differences such as climate, geography and ecosystem. In this study, *Wolbachia* infection was investigated in insects that differ in their living conditions, hosts and ecological niches: *Drosohphila melanosgaster* (Diptera: Drosophilidae), *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Pulex irritans* (Siphonaptera: Pulicidae), *Eusomus ovulum* (Coleoptera: Curriculionidae) and *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *Wolbachia* was screened with a specific *Wspec* F/R primer set and identified according to the sequence data of the obtained PCR products. *Wolbachia* was observed to have a widespread incidence in the species studied. A supergroup *Wolbachia* was found in fleas, fruit fly and *E. ovulum*, and B supergroup *Wolbachia* in parasitoid bees and whiteflies. This is the first study in Turkey to report the presence of *Wolbachia* in *E. ovulum*, and it is thought that the data presented here will contribute to future studies.

Farklı Böcek Taksonlarında Wolbachia Bakterisinin İncelenmesi

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ÖZ

Eklembacaklılar, popülasyon yoğunlukları ve dağılımları göz önüne alındığında en yaygın canlı türüdür. Ancak artan dünya nüfusu ve küresel ısınmaya bağlı kuraklık nedeniyle hem sağlık hem de tarımsal üretim açısından özellikle zararlı türlerle mücadelede çevre dostu ve etkili alternatif stratejiler geliştirmeye yönelik çalışmaları zorunlu kılmaktadır. Bu bağlamda *Wolbachia*, küresel ısınmanın etkiklerinin izlenmesinde konak olarak kullandıkları canlılarla olan ilişkileri nedeniyle umut vericidir. Ancak eklembacaklılardaki simbiyotik yapı iklim, coğrafya ve ekosistem gibi farklılıklara göre değişmektedir. Bu çalışmada yaşam koşulları, konukçuları ve ekolojik nişleri bakımından farklı böcekler olan: *Drosohphila melanosgaster* (Diptera: Drosophiidae), *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Pulex irritans* (Siphonaptera: Pulicidae), *Eusomus ovulum* (Coleoptera: Curriculionidae) ve *Lariophagus distinguendus* (Hymenoptera: Pteromalidae)'da *Wolbachia* enfeksiyonu

incelenmiştir. *Wolbachia*, spesifik bir *Wspec* F/R primer seti ile taranmış ve elde edilen PCR ürünlerinin dizi verilerine göre tanımlanmıştır. *Wolbachia*'nın çalışılan türlerde yaygın bir insidansa sahip olduğu gözlendi. Pire, sirke sinekleri ve *E. ovulum*'da A süpergrubu, parasitoid arı ve beyazsineklerde ise B süpergrubu *Wolbachia* bulunduğu tespit edilmiştir. Bu çalışma, Türkiye'de *E. ovulum*'da *Wolbachia* varlığını bildiren ilk çalışma olup, burada sunulan verilerin yapılacak çalışmalara katkı sağlayacağı düşünülmektedir.

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1. Introduction

Wolbachia is a maternally inherited obligate intracellular gram-negative endosymbiotic bacterium (Werren and Windsor, 2000). *Wolbachia* is found in arthropods (40-66% of all insect species (Hilgenboecker et al., 2008; Zug and Hammerstein, 2012; Tolley et al., 2019) and fillarial nematodes (Taylor and Hoerauf, 1999). Considering the population densities and distributions of insects, *Wolbachia* is regarded as the most successful (Laidoudi et al., 2020) and the most common living species in the terrestrial ecosystem (Hilgenboecker et al., 2008; Zug and Hammerstein, 2012). *Wolbachia* can be seen in host somatic and reproductive tissues (Zouache et al., 2009) and in host phenotype (Werren, 1997), defense (Hamilton and Perlman, 2013; Zhang et al., 2020), nutrition (Bi and Wang, 2020), biology (Werren, 1997; Bi and Wang, 2020) and pathogenicity (Johnson, 2015). The most important and remarkable feature is that it affects the sex ratio of the host and thus the population structure by manipulating the reproduction of its host. It does this through cytoplasmic incompatibility, promoting parthenogenesis, induction of feminization, and malekilling (Breeuwer and Werren, 1990; Werren, 1997; Hurst at al., 1999; Weeks and Breeuwer, 2001). This can be particularly useful in the development of alternative strategies for the biological control of pests (Bourtzis, 2008; Hancock et al., 2011; Pagendam et al., 2020).

Wolbachia is transmitted mainly vertically from parents to offspring transovarially in insects (Guo et al., 2018). However, it is also transferred horizontally between phylogenetically distant taxa (Werren et al., 1995; Ahmed et al., 2013, 2015; Tolley et al., 2019), such as excretion, injury (Rigaud and Juchault, 1995) or interaction between hosts (like parasitoid-host relationship) (Vavre et al., 1999; Tijsse-Klasen et al., 2011; Le Clec'h et al., 2013; Brown and Lloyd, 2015). According to some studies, this transfer ability provides various advantages to its hosts and also plays a role in speciation (Werren, 1997; König et al., 2015; Bruzzese et al., 2021; Aikawa et al., 2022). On the other hand, *Wolbachia* is also genetically diversified (Lefoulon et al., 2020). So that there are seventeen monophyletic lineages ("supergroups" A to T) of *Wolbachia* (Lefoulon et al., 2020; Laidoudi et al., 2020). Although supergroups are controversial (Baldo and Werren, 2007; Gerth et al., 2014), A and B supergroup *Wolbachia* are generally seen in arthropods (Lo et al., 2002).

In addition to these, it has been reported that especially harmful species have moved to the north and higher region due to global warming and its consequences in recent years. This situation creates risk in

terms of human health and agricultural products. Therefore, the detection of *Wolbachia* diversity in pests of medical and agricultural importance has been quite current and is screened in different geographies (Mazur et al., 2016; Li et al., 2017; König et al., 2019; Onder et al. al., 2019; Hou et al., 2020; İpekdal and Kaya, 2020; Pagendam et al., 2020; Madhav et al., 2020). As a matter of fact, the symbiotic structure of insects can show differences in populations of the same species in different geographies with the effect of various factors such as climate and isolation. In this context, Turkey is remarkable geography with conditions suitable for the spread of insect species, and diversity of wild species and is on a migration route (for example, migratory birds). It is also a corridor for the expansion of the spread of insects on the Africa-Middle East-Eurasia-Europe route (Inci et al., 2016). From this point of view, this study aimed to examine *Wolbachia* bacterium in taxa that are phylogenetically distant from each other and have different ecological niches.

2. Material and Method

2.1. Insect samples

The insects examined in the study were sampled from Kırşehir. Sample details are given in Table 1. In this context, fleas, parasitoid bees, beetles, fruit fly and whiteflies were collected. The samples were washed directly in situ with 70% alcohol, rinsed with distilled water, and then stored in alcohol at -20 °C until working. Flea, parasitoid bee, vinegar fly and whitefly were identified by PCR method. The beetle, on the other hand, was defined according to its morphological features using a dissecting microscope (Marvaldi et al., 2018).

Table 1. Details of the location, hosts and ecological niches of the studied arthropods.

Locality	Coordinates	Date collection	Host / Field	Ecological niche
Karaboğaz Village	38°57'20" K 34°08'49" D	November, 2020	Dog	Ectoparasite
Bahçelievler District	38°10'48" K 34°18'70" D	June, 2019	Granary	Parasitoid bee
Bahçelievler District	38°10'48" K 34°18'70" D	September, 2021	Peach	Fruit pest
Bahçelievler District	38°10'48" K 34°18'70" D	September, 2021	Bean	Plant pest, vector
Çukurçayır District	39°09'18" K 34°07'32" D	August, 2019	Clover	Plant pest

2.2. DNA extraction and PCR screening

Total DNA from insects was extracted using the CTAB method (Doyle and Doyle, 1990). Primer pair LCO1490-F and HCO2198-R subunit (*COI*) of mitochondrial cytochrome *c* oxidase I was used for the identification of fleas, parasitoid bees, vinegar flies and whiteflies (Table 2). *Wspec* (F-R) was used for screening and identification of *Wolbachia* bacteria (Table 2). Mixtures with a total volume of 20 µl were used for PCR reactions. Mixtures were prepared using 1 x PCR buffer, 10 mM each of deoxynucleoside triphosphate, 1 µM of each primer, 0.1 U of Taq DNA polymerase and amplified with 1 µl of DNA. The resulting PCR products were electrophoresed on a 1% agarose gel with

negative and positive controls (Ipekdal and Kaya, 2020). *Wolbachia* positive *Sitophilus granarius* total DNA isolate was used as a positive control. Electrophoresed gels were evaluated using a UV Transilluminator (ThermoScientific). Samples that gave electrophoretic bands in the same position as the positive control were considered positive for the presence of *Wolbachia*.

Tablo 2. Primers used in studies for *Wolbachia* endosymbiont and insects and their properties (*COI:* mitochondrial Cytochrome *c* oxidase subunit I).

Primer	**Sequence (5'-3')**	**Target genus and gene region**	**PCR product (bp)**	**Annealing (^{o}C)**	**Reference**
LCO1490-F	GGTCAACAAATCATAAAGATATTGG	COI	710	52	Folmer et al., (1994)
HCO2198-R	TAAACTTCAGGGTGACCAAAAAATCA				
Wspec-F	YATACCTATTCGAAGGGATAG	*Wolbachia* 16S *r*RNA	430	53	Werren and Windsor (2000)
Wspec-R	AGCTTCGAGTGAAACCAATTC				

2.3. Sequencing and Sequence Analysis

DNA sample from at least one individual from the insect species was sequenced. Bidirectional sequencing of *Wolbachia* and insect COI PCR products was performed by Macrogen (Netherlands). Dendrograms were created from the obtained sequence data to represent taxonomic data. For this, consensus sequences were obtained using the Clustal W 2.0 algorithm (Thompson et al., 1994) in BioEdit (Hall, 1999). Consensus sequences for *Wolbachia* and insects were identified in NCBI databases using BLAST (Altschul et al., 1990). In addition, *Wolbachia* consensus sequences were compared using dendrograms created by downloading (GenBank accession numbers are in Figure 1) additional sequences from NCBI databases. Dendrograms for *Wolbachia* sequences were created using the Maximum Likelihood method. Model testing was performed for each sequence set and the Kimura 2-parameter model (Kimura, 1980) (1000 copies) was used. MEGA version X (Kumar et al., 2018) was used for evolutionary analyses.

3. Results and Discussion

In this study, five species of Siphonaptera, Hymenoptera, Coleoptera and Diptera taxa collected from Kırşehir were examined (Table 2). Consensus sequences obtained from sequence data of Siphonaptera, Hymenoptera, and Diptera samples for taxonomic identification were made according to the match in GenBank databases. According to the BLAST results, the studied arthropods *Pulex irritans* (Siphonaptera: Pulicidae), *Lariophagus distinguendus* (Hymenoptera: Pteromalidae), *Drosohphila melanogaster* (Diptera: Drosophilidae) and *Bemisia tabaci* (Hemiptera: Aleyrodidae) showed homology (Table 3). Curculionidae sample was defined as *Eusomus ovulum* (Coleoptera: Curculionidae) according to its morphological features.

Table 3. Arthropods studied, *Wolbachia* screening results and presence ratio (*pr*: *Wolbachia* positive individuals / number of individuals screened) (n: number of individuals screened).

Locality	Insect species (n)	*Wolbachia*		
		pr	GenBank	
			Similarity rate	Accession Number
Karaboğaz Village	*P. irritans* (12)	(0.83)	100	MK184277
Bahçelievler District	*L. distinguendus* (8)	(1.0)	100	KF598750
Bahçelievler District	*D. melanogaster* (20)	(1.0)	100	MK184277
Bahçelievler District	*B. tabacii* (20)	(1.0)	100	MN123078
Çukurçayır District	*E. ovulum* (15)	(1.0)	100	MK184277

PCR products of *Wolbachia* bacteria obtained with *wspec* F/R primer pair from *P. irritans*, *L. distinguendus*, *D. melanogaster*, *E. ovulum* and *B. tabacii* were sequenced for diagnostic and confirmation purposes. The sequence data showed 100% similarity to *Wolbachia* endosymbiont, according to analyzes in the GenBank database (Table 3). *Wolbachia* infection was found in 83% of *P. irritans* and in all of the screened individuals *L. distinguendus*, *D. melanogaster*, *E. ovulum* and *B. tabacii*, in other words, it had a widespread incidence (Table 3).

Dendograms were created using the Maximum Likelihood method and the Kimura 2-parameter model using *Wolbachia* consensus sequences obtained from *P. irritans*, *L. distinguendus*, *D. melanogaster*, *E. ovulum* and *B. tabacii* and DNA sequences downloaded from GenBank databases. Accordingly, the symbionts of *P. irritans*, *E. ovulum* and *D. melanogaster* were clustered in *Wolbachia* Supergroup A. On the other hand, *L. distinguendus* and *B. tabacii* showed homology with Supergroup B strains. (Figure 1).

This study shows the presence of endosymbiotic *Wolbachia* bacteria in five arthropods with different living conditions, hosts and biological cycles in Kırşehir (Turkey). Although its presence has been demonstrated in the species studied here before (Tuncbilek et al., 2015; Mazur et al., 2016; Inci et al., 2016; Gang et al., 2020), further studies are needed to understand the prevalence and role of the *Wolbachia* endosymbiont.

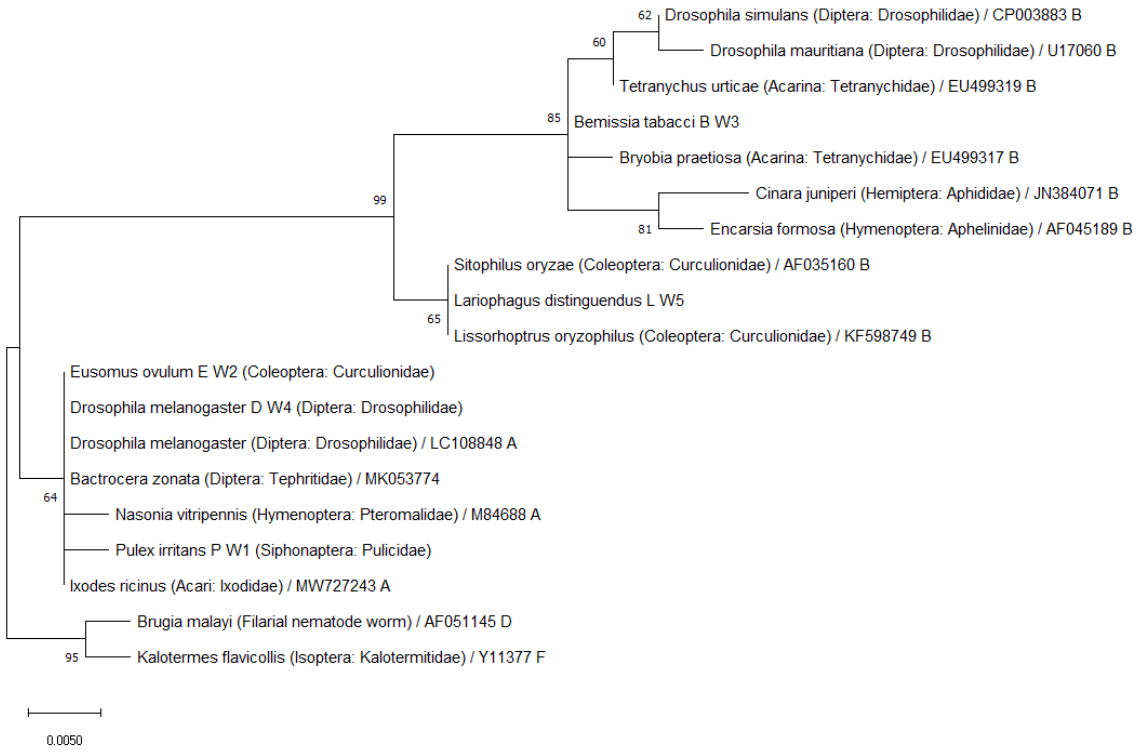

Figure 1. Maximum likelihood (ML) trees of *P. irritans*, *L. distinguendus*, *D. melanogaster*, *E. ovulum* and *B. tabacii* studied based on *Wspec*. Phylogenetic analysis was performed using consensus sequences obtained in this study and additional sequences downloaded from NCBI databases. The percentage of trees in which the relevant taxa are clustered is shown next to the branches. The last capital letter denotes the *Wolbachia* supergroup. D and F supergroups were used as outgroups.

Within the scope of the study, *Wolbachia* was detected in almost all 55 individuals from *P. irritans*, *L. distinguendus*, *D. melanogaster*, *E. ovulum* and *B. tabacii* samples, which were different in terms of phyologenetic and ecological niche. *Wolbachia* endosymbiosis in this species has been previously reported. However, the data obtained are insufficient to explain the widespread incidence of *Wolbachia*, which affects the genotype, phenotype and ecology of its host. However, the high prevalence of *Wolbachia* in the studied species may also result from examining individuals collected from a limited host (dog) or locality. However, the symbionts of insects can also vary due to nutrition, geographical conditions and/or isolation. Indeed, it has been shown that *Wolbachia* infection and its frequency within the species are geographically variable between different populations (Arthoferet al., 2009; Hughes et al., 2011; Zug and Hammerstein, 2012; Aikawa et al., 2022). The results obtained here are in agreement with previous studies of *Wolbachia* infection in five taxa and its frequency in their hosts (Oteo et al., 2014; Inci et al., 2016; Gang et al., 2020). However, *Wolbachia* infection in *E. ovulum* in Turkey was detected for the first time in this study.

On the other hand, analyzes showed supergroup A topology in hematophag *P. irritans*, parthenogenetic *E. ovulum* and fruit fly *D. melanogaster*; the parasitoid *L. distinguendus* and the pest-

vector *B. tabacii* indicate the presence of supergroup B *Wolbachia*. Although this determination was obtained with limited sequence data, it generally overlaps with the prevalence of A and B supergroup *Wolbachia* in arthropods. However, it has been observed that there are *Wolbachias* with similar homology in the taxa with which the examined species are related. For example, the symbiont of *L. distinguendus*, the parasitoid of *Sitophilus oryze* and *Lissorhoptrus oryzophilus*, have a similar topology to *Wolbachia* (Figure 1). This may not be a coincidence. Such that, this can be explained by the hosts' acquisition of *Wolbachia* through maternal inheritance as well as prey-predator, host-parasitoid, injury or contaminations between feeding meals (Gomard et al., 2021). However, more data is needed to state this definitively. On the other hand, the obtained data may contribute to the studies that will carry out the genetic differentiation in *E. ovulum* (Morozov-Leonov and Nazarenko, 2021) and the origin of *Wolbachia* in *P. irritans*.

4. Conclusion

In this study, *Wolbachia* endosymbiont was investigated in taxa that are phylogenetically distant from each other and different in niche. Considering the expansion and diversification of the distribution areas of especially harmful insect species due to global warming and drought, endosymbiotic bacteria may affect the population densities of these hosts. It is thought that the presented data will contribute to the studies to be carried out on the *Wolbachia* bacterium, which is promising especially in the fight against pests.

Conflict of Interest Statement

As the author of the article, I declare that there is no conflict of interest.

Statement of Contribution to the Study

As the author of the article, I declare that the design, sample collection, laboratory studies and analysis and writing of this study were done by me.

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