

Analysis of genetic variation in an important pest, *Tuta absoluta*, and its microbiota with a new bacterial endosymbiont

Fariba MEHRKHOU¹ , Nurper GÜZ^{2*} , Ertan Mahir KORKMAZ³ , Naciye Sena ÇAĞATAY² 

¹Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran

²Molecular Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Ankara, Ankara, Turkey

³Department of Molecular Biology and Genetics, Faculty of Science, Sivas Cumhuriyet University, Sivas, Turkey

Received: 02.06.2020 • Accepted/Published Online: 12.10.2020 • Final Version: 10.02.2021

Abstract: Tomato leaf miner is an aggressive and invasive pest that causes serious damage in tomato production, resulting in up to 100% yield losses during serious outbreaks. The management of tomato leaf miner with synthetic pesticides is difficult due to its leaf mining habit, short life span, high reproductive rates, and insecticide resistance problem. In this study, sequences of the mitochondrial *cytochrome oxidase I* (mtCOI) gene of *Tuta absoluta* specimens were analyzed and compared with the available COI sequence data members from GenBank. All the specimens of tomato leaf miner were clustered together in a single group with a high support value, suggesting the presence of a homogeneity in the *T. absoluta* specimens. Furthermore, 164 samples were analyzed in order to detect the prevalence of *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Pantoea*, *Spiroplasma*, and *Wolbachia* infection in *T. absoluta* populations. PCR analysis revealed 100% prevalence of *Wolbachia* and *Pantoea*; however, no other symbiotic bacterial infections were observed in *T. absoluta* populations. Finally, *Wolbachia* *wsp*, *Pantoea* 16S ribosomal RNA genes, and mtDNA COI haplotypes were analyzed in order to estimate the mitochondrial effects of these endosymbionts on the population structure of tomato leaf miner. The findings of high genetic homogeneity among *T. absoluta* populations with high prevalence of *Wolbachia* and *Pantoea* infections seem to imply that both bacterial infections have less impact on the mtDNA variations.

Key words: Endosymbionts, genetic variation, mitochondrial DNA, tomato leaf miner

1. Introduction

Tomato (*Lycopersicon esculentum* Miller) is one of the most widely grown vegetables in the world¹. Tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) is an aggressive pest that causes serious damage in tomato production, resulting in up to 100% yield losses during serious outbreaks (Flores et al., 2003; Saidov et al., 2018). Among the lepidopteran pests of tomato crop, *T. absoluta* is considered as a highly invasive agricultural pest, which is native to Latin America and has spread through most of Europe, Mediterranean regions, and many other regions worldwide (Hassan and Alzaidi, 2009; Mansour et al., 2018). This pest is known as an oligophagous pest attacking solanaceous crops, including eggplant, pepper, potato, sweet pepper, tobacco (Pereyra and Sánchez, 2006; Kanle Satishchandra et al., 2019), as well as solanaceous weeds (Shashank et al., 2018). Direct feeding of the pest not only damages all parts of tomato plants, but also the entrance of

pathogens through the wounds results in indirect damage (EPPO, 2005). It is difficult to manage tomato leaf miner with synthetic pesticides due to its leaf mining habit, short life span, and high reproductive rates, which results in rapid development of insecticide resistance (Guedes et al., 2019).

Symbiotic relationships between insects and microorganisms have the potential to influence the biology and ecology of their hosts. Depending on the type of symbiotic associations, they can be involved in providing nutritional benefits, host plant preference, reproductive alteration, and adaptation to environmental conditions, as well as in insecticide resistance (Kontsedalov et al., 2008). Detection of microbial fauna of insects is important to highlight the problems caused by these microorganisms to their hosts. The interactions between bacterial microorganisms and their hosts are important in terms of the host's fitness, ecology, and evolutionary biology (Gurung et al., 2019).

¹ Food and Agriculture Organization of the United Nations (FAO) (2012). FAOSTAT [online]. Website <http://www.fao.org/faostat/en/> [accessed 14 March 2020].

* Correspondence: nurperguz@agri.ankara.edu.tr

Wolbachia is widely distributed with about 70% of all insect species (Werren and O'Neill, 1997), which regulates host reproduction through cytoplasmic incompatibility (CI), feminization, male killing, and parthenogenesis (Werren, 1997; Stouthamer et al., 1999). *Wolbachia* also plays a major role in affecting the mtDNA variation of its hosts (Hurst and Jiggins, 2005; Moran et al., 2008; Charlat et al., 2009; Feldhaar, 2011; Richardson et al., 2012; Bennett and Moran, 2015; Schuler et al., 2016; Schuler et al., 2018). Mitochondrial DNA (mtDNA) has advantages compared to nuclear DNA, such as the absent or very infrequent recombination conjoint with their reduced effective population size and relatively high mutation rates. These properties make this genome suitable for characterization in the level of inter- and intraspecies (Saccone et al., 2000), as well as use in phylogeography (Hurst and Jiggins, 2005).

Pantoea is another common bacterium with more than 20 species that have adapted to diverse ecological niches, including humans, insects, plant material, soil, and water (Walterson and Stavrinides, 2015). Recent studies showed that *Pantoea* species contribute to providing essential nutrients limited in the host's diet (Kenyon et al., 2015), participating in creating a nitrogen-rich environment for developing eggs and larvae (MacCollom et al., 2009) and breakdown of plant cell-wall biopolymer (Bozorov et al., 2019). *Pantoea* isolates have been reported from different insect species, such as fruit fly (Lauzon et al. 2009) and Asian citrus psyllid (Guz et al. 2020b).

In this study, samples were collected from different geographic locations of Iran and Turkey in order to investigate genetic diversity and differentiation of *T. absoluta* populations. The nucleotide sequences of the mtCOI gene of these populations were analyzed and compared with the previously reported COI sequence data of tomato leaf miner members from GenBank. Furthermore, 164 samples were analyzed in order to detect the prevalence of *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Pantoea*, *Spiroplasma* and *Wolbachia* infection in *T. absoluta* populations. Finally, *Wolbachia* *wsp*, *Pantoea* 16S ribosomal RNA (16S rRNA) genes and COI haplotypes were analyzed to estimate the mitochondrial effects of these endosymbionts on tomato leaf miner population structures.

2. Materials and methods

Samples of *T. absoluta* were collected during 2019 and 2020 from 15 different major tomato-growing areas of West-Azerbaijan of Iran, and Turkey. The collected specimens were transferred to 70% ethanol and preserved at 4 °C until DNA extraction. The details of the collected samples are shown in Table 1.

To avoid any contamination, each larvae and adult sample was exposed to 1% sodium hypochlorite, followed by 2 times distilled water rinse prior to DNA extraction.

Table 1. Collection sites.

Location	Coordinates
Salmas	38° 11' 41" N 44° 45' 53" E
Salmas (Kochameshk)	38° 08' 59" N 44° 47' 25" E
Khoy	38° 33' 01" N 44° 57' 08" E
Urmia	37° 33' 19" N 45° 04' 21" E
Urmia (Hesar-e Torkaman)	37° 26' 34" N 45° 13' 08" E
Urmia (Qotlu)	37° 30' 24" N 45° 08' 18" E
Urmia (Tala Tappeh)	37° 43' 13" N 45° 10' 47" E
Urmia (Satlu)	37° 25' 18" N 45° 08' 54" E
Urmia (Kashtiban)	37° 33' 26" N 45° 14' 24" E
Oshnavieh	37° 02' 23" N 45° 05' 54" E
Miandoab	36° 58' 10" N 46° 06' 10" E
Naghadeh	36° 57' 19" N 45° 23' 17" E
Van, Turkey	38° 29' 39" N 43° 22' 48" E
Fethiye, Muğla, Turkey	36° 39' 33" N 29° 7' 35" E
Menemen, İzmir, Turkey	38° 36' 58" N 27° 8' 49" E

Total genomic DNA was isolated individually from 164 specimens, including 19 adults and 146 larvae, using a modified cetyltrimethylammonium bromide protocol (CTAB) with minor modifications (Doyle and Doyle, 1987; Guz et al., 2020a). Briefly, each specimen was homogenized in lysis buffer (100mM Tris, 50mM EDTA, 1.4M NaCl, 2% CTAB) and incubated at 65 °C for 12 h. After chloroform-isoamyl alcohol (24:1) extraction, the DNA samples were precipitated using isopropanol. The quality and quantity of DNA extracts were estimated using electroporation in 1% agarose gel containing Pronasafe Nucleic Acid Staining Solution (Laboratorios Conda, S.A., Madrid, Spain) and NanoDrop2000 spectrophotometer (Thermo Scientific Scientific Inc., Waltham, MA, USA). Each DNA extract was standardized to 100 ng/μL. PCR details used to amplify an mtCOI fragment and bacterial endosymbionts are given in Table 2. Fifteen purified PCR amplicons of COI gene were bidirectionally Sanger sequenced at Macrogen, Inc (Seoul, South Korea).

The forward and reverse nucleotide sequences representing 15 specimens were assembled, edited, and manually aligned, using Geneious R9 (Kearse et al., 2012). Obtained sequences were submitted to the GenBank with the accession numbers of MT328190-MT328204. A sampling dataset of the COI gene region of *T. absoluta* was generated from 594 specimens (15 from this study and 579 sequences from NCBI annotated database representing different localities) (Table 3).

The number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π) were calculated using

Table 2. Details of the PCR conditions and primers used in the study.

Target gene	Primer sequence (5'-3')	Tm (°C)	Reference
<i>Hamiltonella</i> 16S rDNA	Ham-F: TGAGTAAAGTCTGGAATCTGG	60	Chiel et al., 2007
	Ham-R: AGTTCAAGACCGCAACCTC		
<i>Arsenophonus</i> 23S rDNA	Ars23S-1: CGTTTGATGAATTCATAGTCA AA	50	Thao and Baumann, 2004
	Ars23S-2: GGTCTCCAGTTAGTGTTACC CAAC		
<i>Cardinium</i> 16S rDNA	CFB-F: GCGGTGTAATAATGAGCGTG	58	Weeks et al., 2003
	CFB-R: ACCTMTTCTTAACTCAAGCCT		
<i>Wolbachia</i> <i>wsp</i> gene	wsp81F: TGGTCCAATAAGTGATGAAGAAAC	55	Braig et al., 1998
	wsp691-R: AAAAATTAACGCTACTCCA		
<i>Pantoea</i> 16S rRNA	Panteo-F: ACGGAGGGTGCAAGCGTTAAT	56	Guz et al., 2020b
	Panteo-R: AGGTAAGGTTCTTCGCGTTGCA		
<i>Spiroplasma</i> 16S rRNA	16SA1: AGAGTTTGATCMTGGCTCAG	55	Tsuchida et al., 2002
	TKSSpR: TAGCCGTGGCTTCTGGTAA		
Universal COI	dgLCO-1490: GGTCAACAAATCATAAAGAYATYGG	45	Meyer, 2003
	dgHCO-2198: TAAACTTCAGGGTGACCAAARAAYCA		

DnaSP v5 (Librado and Rozas, 2009). The level of genetic differentiation between specimens was quantified via estimating net p-distance value, using MEGA X (Kumar et al., 2018). The principal coordinate analysis (PCoA) was performed using the GenAIEx v6.5 (Peakall and Smouse, 2006;2012) based on the matrix of pairwise p-distance values between specimens.

In order to estimate the phylogenetic relationship of the haplotypes of *T. absoluta* specimens, phylogenetic trees were constructed using neighbour-joining (NJ) and maximum likelihood (ML) approaches. *Symmetrischema lectulifera* (KY951829) was selected as an outgroup. For phylogenetic tree constructions, the best-fit model of DNA substitution and the parameter estimates were used in MEGA X program (Kumar et al., 2018). The NJ tree was constructed in MEGA X under the algorithm of Kimura 2 parameter with Gamma distribution (Gamma parameter: 0.11) with 1.000 bootstrapping. ML tree was built using PhyML v3.1 (Guindon et al., 2010) under GTR+G+I substitution model. The visualization of the trees was carried out using FigTree v1.4.2².

3. Results

Our dataset consisted of the 594 *COI* sequences representing 15 specimens from this study and 579 specimens from previously reported studies. After alignment and trimming, the remaining length of sequences was 558 bp. In total, 16 variable positions were detected including 13 singleton variable sites. Diversity indices of haplotype (Hd) and nucleotide (π) were 0.040

± 0.011 and 0.00014 ± 0.00006 , respectively. The retrieved haplotype number was 8, and 5 of them were definable by a single mutation difference and were unique to a single location. The haplotype Hap1 was the most abundant and widespread, as it was shared in 97.98% of the sequences.

The overall mean p-distance for the *COI* gene region was found as 0.0078 ± 0.0018 , and the pairwise p-distances was found as 0.0018 between these 5 haplotypes. The first 3 principal axes in the PCoA showed 57.75, 15.04, and 10.76 of the total variation, respectively, which indicated that almost all of the specimens exhibited overlapped clustering except for was clustered together except for the haplotype Hap_3 (Figure 1).

As NJ and ML analyses have resulted in the same tree topology, the tree produced under the ML approach was presented in Figure 2. All specimens of *T. absoluta* were clustered together in a single group with a high support value (100%, Figure 2) except for the haplotypes Hap_3, suggesting the presence of a homogeneity in the *T. absoluta* specimens. The tree also indicated no clustering related to geographic origin. Furthermore, no mtDNA polymorphism in entire populations infected by *Wolbachia* and *Pantoea* has been detected.

The presence and prevalence of *Wolbachia* infections were detected using the *wsp* gene in *T. absoluta* yielding an approximately 600 bp amplicon in length. The obtained *Wolbachia* sequence from *T. absoluta* has been submitted to GenBank under the accession number MT340264. BLAST analysis revealed that this sequence displayed the highest homology with 99.82% identity to *Wolbachia*

² Rambaut A (2014). FigTree v1.4.2 [online]. Website: <http://tree.bio.ed.ac.uk/software/figtree/> [accessed 13 April 2020].

Principal Coordinates (1 vs.2)

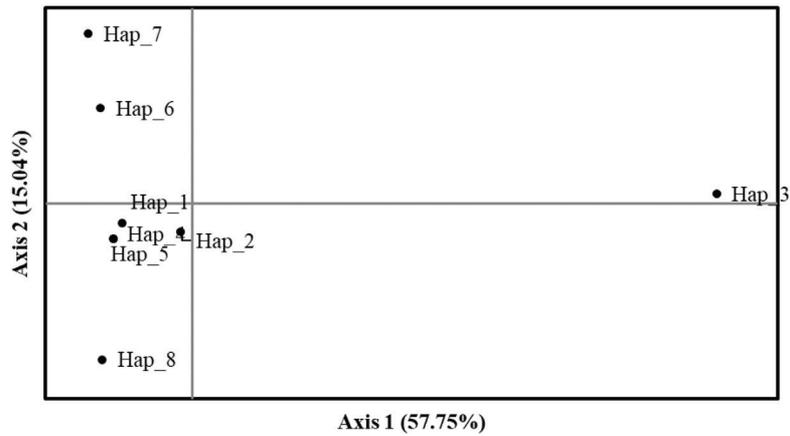


Figure 1. Principal coordinate analysis (PCoA) of the original data in 8 haplotypes of *T. absoluta*. Each dot corresponds to a haplotype of *T. absoluta* COI data. The amount of variance explained by each PC is indicated on each axis.

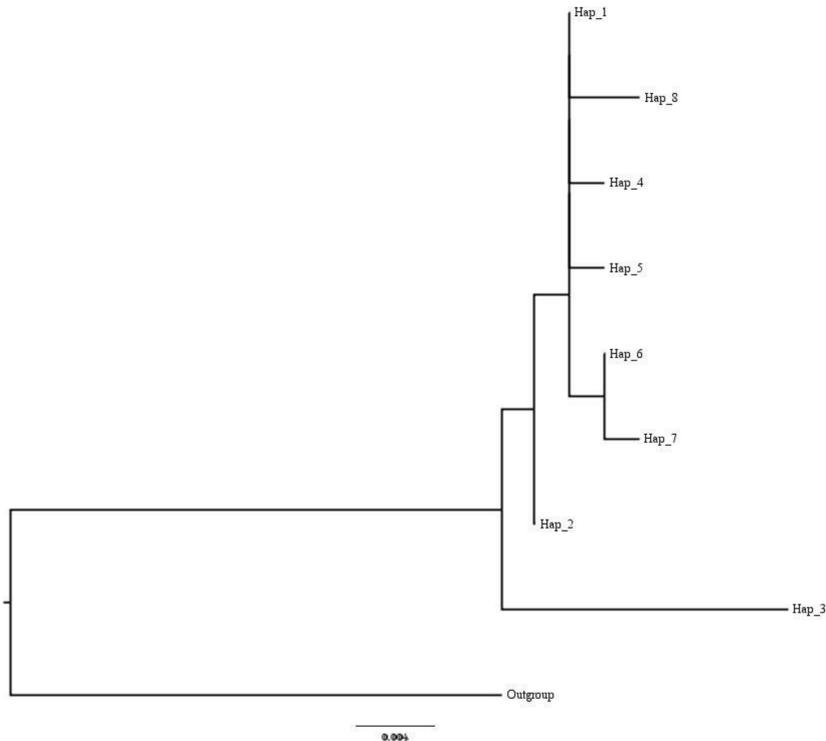


Figure 2. ML tree of haplotypes of *T. absoluta* based on the mtDNA COI data. *Symmetrischema lectulifera* was used as an outgroup.

endosymbiont of *Operophtera brumata* (KY587652.1), *Epirrita christyi* (JX310335.1), *Mamestra brassicae* (AB094375.1), *Operophtera brumata* (KY587653.1). To evaluate the phylogenetic relationship between *Wolbachia* strains isolated from various insects, we constructed a phylogenetic tree (Figure 3) of a total of 18 arthropod sequences under the NJ method, and the tree topology

of all subjected sequences revealed 2 supergroups (A and B). The first cluster, supergroup B, consisted of the orders including Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera, whereas the second cluster, supergroup A, exhibited a relationship between Formicidae, Diptera, Lepidoptera, Hymenoptera, and Coleoptera. The phylogenetic tree showed that the *Wolbachia* sequences

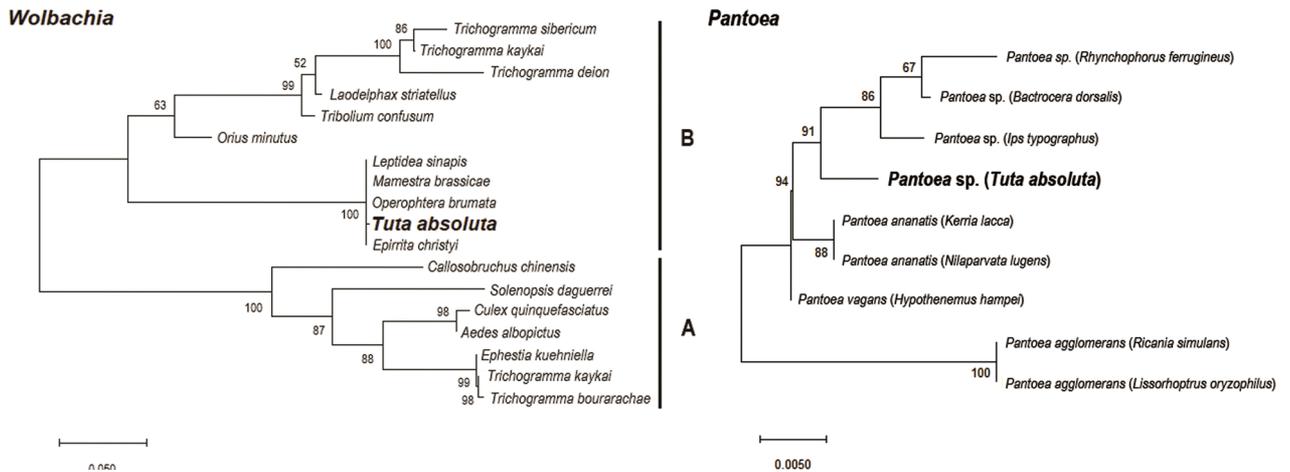


Figure 3. Phylogenetic analysis of *Wolbachia* and *Pantoea* infections in *T. absoluta* species in relation to other insect species. The species and GenBank accession numbers of the reference sequences used to construct the phylogenetic trees are as follows: Uncultured *Pantoea* sp. (*Rhynchophorus ferrugineus*) KF125498.1; Uncultured *Pantoea* sp. (*Bactrocera dorsalis*) MK749919.1; *Pantoea* sp. (*Ips typographus*) DQ309419.1; *Pantoea* sp. (*T. absoluta*) MT326636; *P. ananatis* (*Kerria lacca*) KF717513.1; *P. ananatis* (*Nilaparvata lugens*) KJ655537.1; *P. vagans* (*Hypothenemus hampei*) KF913828.1; *P. agglomerans* (*Ricania simulans*) KM102494.1; *P. agglomerans*; *Lissorhoptrus oryzophilus* (KC754748.1); *Trichogramma sibericum* (AF071923.1); *Trichogramma kaykai* (AF071924.1); *Trichogramma deion* (AF020084.1); *Laodelphax striatellus* (WSTR_RS03110); *Tribolium confusum* (AF020083.1); *Orius minutus* (AB094363.1); *Leptidea sinapis* (KC137222.1); *Mamestra brassicae* (AB094375.1); *Operophtera brumata* (KY587656.1); *T. absoluta* (MT340264); *Epirrita christyi* (JX310339.1); *Callosobruchus chinensis* (MG255145.1); *Solenopsis daguerrei* (AY878105.1); *Culex quinquefasciatus* (KX650071.1); *Aedes albopictus* (KJ140127.1); *Ephesthia kuehniella* (AF071911.1); *Trichogramma kaykai* (AF071912.1); *Trichogramma bourarachae* (AF071913.1).

from *T. absoluta* and different Lepidopteran species were clustered with the *Wolbachia* supergroup B. PCR analysis revealed 100% prevalence *Wolbachia* infection frequencies for the *Wolbachia* out of 164 (including 19 adults and 146 larvae) samples in *T. absoluta* populations.

Investigation of the presence of *Pantoea* in *T. absoluta* populations was performed using a set of primers amplifying the 16S rRNA gene fragment (421 bp) of *Pantoea*. The sequence of *Pantoea* sp. from *T. absoluta* has been submitted to GenBank under the accession number MT326636, which is the first record of infection by *Pantoea* within tomato leaf miner populations. The sequence homology showed >99% nucleotide identity with 16S rRNA encoded with the *Pantoea agglomerans* (NR_116751.1), *Pantoea brenneri* (NR_116748), *Pantoea eucalypti* (NR_116112), and *Pantoea vagans* (NR_116115) with a variety of different hosts in the database of BLAST. Figure 3 depicts the tree constructed by NJ analysis using sequences of 16S rRNA genes from *Pantoea* originating only from insects. According to the phylogenetic tree, there were 2 major branches based on *Pantoea* sequences. Among these sequences, *P. agglomerans* formed the main branch and differed from all other taxa. *Tuta Pantoea* was grouped in *Pantoea* sp. and diverged from *P. ananatis* and *P. vagans*. PCR analysis revealed 100% prevalence of *Pantoea* infection frequencies for the *Pantoea* out of

164 adult and larval samples of *T. absoluta* populations. On the contrary, no other symbiotic bacteria comprising *Arsenophonus*, *Cardinium*, *Hamiltonella*, and *Spiroplasma* infections were found in any of the tomato leaf miner populations tested.

4. Discussion

The results of this study exhibited high genetic homogeneity with a low nucleotide diversity value among different specimens of *T. absoluta* across their distribution range. The presence of high genetic homogeneity was also observed in the PCoA analysis and phylogenetic tree. This low nucleotide diversity concurs well with previous results of pinworm populations from different parts of its distribution range (Shashank et al., 2018; Guedes et al., 2019). The mtCOI has been shown to be very useful and informative for estimating inter- and intraspecific levels; however, high genetic homogeneity observed in *T. absoluta* populations might be mainly related to the occurrence of genetic drift on this invasive species (Korkmaz et al., 2011; Duman et al. 2015). As reported in many studies on invasive species (Shashank et al., 2018; Hawley et al., 2006; Lindholm et al., 2005; Tsutsui et al., 2000), in small populations, the genetic diversity can be lost due to high genetic drift settled in new parts of the invasive range (Hagenblad et al., 2015). The biggest

limitation of this study is the sole use of *COI*; however, no genetic variation has been observed in *Tuta* populations collected from several countries using mtCOI, ITS rDNA, and microsatellite markers (Cifuentes et al., 2011; Duric et al., 2014; Guillemaud et al., 2015; Yukselbaba and Gocmen, 2016; Shashank et al., 2018; Kinyanjui et al., 2018). Although great variance between nuclear-nuclear and mito-nuclear incompatibilities has been detected in insects (Telschow et al., 2019), further analyses with a wide range of samples need to be performed to enable a better estimation of the degree of population differentiation of *T. absoluta* using nuclear genes and microsatellites, which has already been developed (Bettaibi et al., 2013; Tabuloc et al., 2019).

Recently, insecticide resistant phenotypes have been discovered in *T. absoluta* in different studies (Siqueira et al., 2000; Lietti et al., 2005; Yalcin et al., 2015; Karaagac et al., 2015; Zibaee et al., 2016; Barati et al., 2018; Guedes et al., 2019). The populations examined in this study need to be confirmed in terms of insecticide susceptibility since a single genetically uniform *Tuta* population has been related to higher insecticide resistance in Mediterranean Basin and South America (Cifuentes et al., 2011).

On the other hand, mtDNA is still commonly used as a molecular marker for identification, phylogeny, and population structure of insect species (Guz et al. 2013; Hebert et al., 2003; Krishnamurthy and Francis, 2012; Yatkin and Guz 2018). This is of significant importance as the spread of *Wolbachia* might influence polymorphism in mtDNA (Turelli et al., 1992; Solignac et al., 1994; Ballard et al., 1996; Keeling et al., 2003; Charlat et al., 2009; Graham and Wilson, 2012; Richardson et al., 2012; Schuler et al., 2016). In this study, the findings of high genetic homogeneity among *T. absoluta* populations with high prevalence of *Wolbachia* and *Pantoea* infections seems to imply that both bacterial infections have less impact on the mtDNA variations. Our results are in accordance with the findings about the populations reported in Brazil (Carvalho et al., 2018). Even though these results differ from previous studies that report that *Wolbachia* spread affects the mtDNA diversity of its host through indirect selection, which means if a population gains any reproductive advantage or fitness from *Wolbachia*, the initial mtDNA type will hitchhike through the population and change the uninfected haplotypes (Hurst and Jiggins, 2005; Schuler et al., 2016). Therefore, the inference about lack of genetic diversity is more precise when the uninfected *Wolbachia* population of *T. absoluta* is analyzed. To our knowledge, this is the first report for *Pantoea* in terms of mtDNA haplotype diversity, which needs to be interpreted with the insights into the temporal and spatial spread of *Pantoea* in *T. absoluta*.

Wolbachia is the most common bacterium with an approximately 66% infection in all insects (Hilgenboecker et al., 2008). In a broad survey, *Wolbachia* infected majority of neotropical insect orders, including Hemiptera, Orthoptera, Diptera, Hymenoptera, Coleoptera, and Lepidoptera (Werren et al., 1995). Other comprehensive studies about *Wolbachia* infection in Lepidopteran families showed 58.3% in 120 species of Siberian populations (Ilinsky and Kosterin, 2017); 45% of 49 Japanese species (Tagami and Miura, 2004); 52% of 29 Indian species (Salunke et al., 2012), and 79% of 24 *Acraea* species from Uganda (Jiggins et al., 2001). In our study, all tomato leaf miner populations displayed 100% of *Wolbachia* infection, which is consistent with the populations from Brazil, Croatia, and Montenegro (Škaljac et al., 2012; Carvalho et al., 2018). Although *Wolbachia* is mostly known bacteria, *Spiroplasma* were present at low levels while *Rickettsia* was also not commonly found with only one species across Lepidopterans. However, none of the samples harbored *Arsenophonus*, *Cardinium*, or *Hamiltonella* in any host (Russel et al., 2012; Weinert et al., 2015). On the other hand, *Bacillus*, *Enterobacter*, *Enterococcus*, *Pseudomonas*, and *Staphylococcus* were detected as the most common bacterial genera >70% of the 30 different caterpillar species (Voirol et al., 2018). In particular, in *T. absoluta* *Rickettsia*, *Hamiltonella*, *Arsenophonus*, *Cardinium*, and *Fritshea* were not detected in Croatian populations (Škaljac et al., 2012). These findings are congruent with our results since all the other bacteria we have tested comprising *Arsenophonus*, *Spiroplasma*, *Hamiltonella*, and *Cardinium* were negative in *Tuta* populations.

In this study, we have detected 100% *Pantoea sp.* in *T. absoluta* populations for the first time. The *Pantoea* is a member of the *Enterobacteriaceae*, with more than 20 species, which are adapted to diverse ecological niches, including humans, insects, plant material, soil, and water (Walterson and Stavrinos, 2015). It has been reported to colonize in several Lepidopteran species (Broderick et al., 2004; Robinson et al., 2010; Pinto-Tomás et al., 2011; Tang et al., 2012; He et al., 2013; Chen et al., 2016; Sevim et al., 2016). Recent studies have shown that *Pantoea* species contribute to the provision of essential nutrients limited in the host's diet (Kenyon et al., 2015), participating in creating a nitrogen-rich environment for developing eggs and larvae (MacCollom et al., 2009) and the breakdown of plant cell-wall biopolymer (Bozorov et al., 2019). To our knowledge, no previous research has investigated the impact of *Pantoea* on *T. absoluta*, which would be of interest to further determine the ecological benefits of this symbiotic bacteria.

Currently, based on phylogenetic reconstructions, 17 *Wolbachia* supergroups (A-Q) have been recognized (Glowska et al., 2015). The multilocus sequence typing

(MLST) system became a standard protocol in determining *Wolbachia* strains using the substitutions in housekeeping loci (*ftsZ*, *gatB*, *coxA*, *hcpA*, and *fbpA*) (Baldo et al., 2006). In this study, we have detected *T. absoluta* using *wsp* gene sequencing, which has been used in phylogenetic studies and for microtaxonomic subdivision (Van Meer et al., 1999). Our result is congruent with other pinworm populations in which 2 *Wolbachia* strains were detected, including ST41 and ST354, and classified in the supergroup B (Carvalho et al., 2018). *Wolbachia* sequences of *T. absoluta* analyzed in this study are grouped with the other Lepidopterans belonging to supergroup B, which is suitable with Croatian populations, as well (Škaljac et al., 2012). However, the predominance of supergroup B in Lepidopteran species is still discussed to determine whether there is a congruence between Lepidoptera and B group or whether the groups are ancestrally associated (Choi et al., 2015, Ahmed et al., 2016). Therefore, for *T. absoluta* populations, pyrosequencing-based MLST method for large-scale screening of multiple *Wolbachia* STs is suggested to be fully investigated due to the limitation in application in infections with a single strain of *Wolbachia*.

In Lepidoptera endosymbiotic bacteria are known to alter host reproduction via feminization, male killing, and CI (Duplouy and Hornett, 2018). *Wolbachia* induced feminization occurred in *Bombyx mori*, *Eurema*, and *Ostrinia* species (Hiroki et al., 2002; Fujii et al., 2001; Kageyama et al., 2003, 2004). Male killing has been reported in Lepidoptera generated by *Wolbachia* as well as *Spiroplasma* (Duplouy and Hornett, 2018). CI has been observed at high frequency in various Lepidopteran families (Sasaki et al., 2002; Hornett et al., 2008). *Wolbachia* strains detected in Brazilian pinworms found to be associated with CI (Carvalho et al., 2018). Apart from *Wolbachia*, tomato leaf miner populations showed deuterotokous parthenogenesis in mating experiments under laboratory conditions (Caparros Megido and Haubruge, 2018). The low genetic variation has been attributed to parthenogenetic reproduction; however,

more genetic polymorphism was observed in asexual species than sexual species (Chevasco et al., 2012). The fitness of parthenogenetic ancestries are clearly needed to confirm the deuterotokous parthenogenesis in *T. absoluta*. Moreover, endosymbiont manipulation, impact upon host fitness, and additional effects of the bacterial microbiota in *T. absoluta* need to be further assessed.

Here, we detected *Wolbachia* and *Pantoea* infection in both adult and larval stages in the entire populations of *T. absoluta*, verifying the findings that some bacterial taxa can persist through the life cycles of insects (Hammer et al., 2014). However, in a large-scale study, it is outlined that host phylogeny, developmental stage, environmental habitat, and diet could influence the host's microbiota (Yun et al., 2014). As expected, the bacterial communities might substantially differ between larval and adult stages of the same species (Staudacher et al., 2016, Xia et al., 2017) since almost all lepidopteran larvae feed on plant material and the majority of adults feed on floral nectar and other liquid substances (Krenn et al., 2010). The widespread abundance of *Wolbachia* infection in *T. absoluta* might be due to the shared natural enemies and food sources as potential routes of horizontal transmission (Ahmed et al., 2016). Although whiteflies and tomato leaf miner populations share common habitat, host plant, and natural enemies, we confirmed in this study that the symbiotic bacterial composition of 2 pests differed except *Wolbachia*. It remains unclear why the composition of bacterial communities varies in distinct phytophagous species. Further experimental investigations are needed to estimate the transmission routes, which might have a critical role in evolutionary biology of *T. absoluta* populations.

As a result, characterization of the microbiota of the tomato leaf miner as well as factors influencing symbiotic composition and the impact of symbiotic bacteria to its host, such as nutrition, physiology, and behavior, are important in developing alternative control strategies for this invasive pest.

References

- Ahmed MZ, Breinholt JW, Kawahara AY (2016). Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. *BMC Evolutionary Biology* 16 (1): 118. doi: 10.1186/s12862-016-0660-x
- Baldo L, Hotopp JCD, Jolley KA, Bordenstein SR, Biber SA et al. (2006). Multilocus sequence typing system for the endosymbiont *Wolbachia pipiensis*. *Applied and Environmental Microbiology* 72 (11): 7098-7110. doi: 10.1128/AEM.00731-06
- Ballad JWO, Hatzidakis J, Karr TL, Kreitman M (1996). Reduced variation in *Drosophila simulans* mitochondrial DNA. *Genetics* 144 (4): 1519-1528.
- Barati R, Hejazi MJ, Mohammadi SA (2018). Insecticide susceptibility in *Tuta absoluta* (Lepidoptera: Gelechiidae) and metabolic characterization of resistance to diazinon. *Journal of Economic Entomology* 111 (4): 1551-1557. doi: 10.1093/jeet/toy134
- Bennett GM, Moran NA (2015). Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. *Proceedings of the National Academy of Sciences* 112 (33): 10169-10176. doi: 10.1073/pnas.1421388112
- Bettaibi A, Mezghani-Khemakhem M, Soltani Z, Makni H, Makni M (2016). Development of polymorphic microsatellite loci for the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) *Journal of Genetics* 93 (2): 110-112.

- Bozorov TA, Rasulov BA, Zhang D (2019). Characterization of the gut microbiota of invasive *Agrilus mali* Matsumura (Coleoptera: Buprestidae) using high-throughput sequencing: uncovering plant cell-wall degrading bacteria. *Scientific Reports* 9 (1): 1-12. doi:10.1038/s41598-019-41368-x
- Braig HR, Zhou W, Dobson SL, O'Neill SL (1998). Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *Journal of Bacteriology* 180: 2373-2378. doi: 10.1128/JB.180.9.2373-2378.1998
- Broderick NA, Raffa KF, Goodman RM, Handelsman J (2004). Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Applied and Environmental Microbiology* 70 (1): 293-300. doi: 10.1128/AEM.70.1.293-300.2004
- Carvalho GAS, Corrêa A, De Oliveria LO, Chediak M, Siqueira HÁ et al. (2018). *Wolbachia* strains, and lack of genetic diversity and parthenogenesis in Brazilian populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Applied Entomology* 142 (9): 905-910. doi: 10.1111/jen.12531
- Charlat S, Duploux A, Hornett EA, Dyson EA, Davies N et al. (2009). The joint evolutionary histories of *Wolbachia* and mitochondria in *Hypolimnas bolina*. *BMC Evolutionary Biology* 9 (1): 64. doi: 10.1186/1471-2148-9-64
- Chen J, Chia N, Kalari KR, Yao JZ, Novotna M et al. (2016). Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Scientific Reports* 6 (1): 1-10. doi:10.1038/srep28484
- Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N et al. (2007). Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bulletin of Entomological Research* 97: 407-413. doi: 10.1017/S0007485307005159
- Choi, JY, Bubnell JE, Aquadro CF (2015). Population genomics of infectious and integrated *Wolbachia pipientis* genomes in *Drosophila ananassae*. *Genome Biology and Evolution* 7 (8): 2362-2382. doi: 10.1093/gbe/evv158
- Cifuentes D, Chynoweth R, Bielza P (2011). Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest Management Science* 67 (9): 1155-1162. doi: 10.1002/ps.2166
- Caparros-Megido R, Haubruge E, Verheggen FJ (2012). First evidence of deuterotokous parthenogenesis in the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Pest Science* 85: 409-412. doi: 10.1007/s10340-012-0458-6
- Chevasco V, Elzinga JA, Viinikainen SM, Mappes J, Galarza JA (2012). Unexpected genetic variability in the parthenogenetic bag worm moth *Dahlia fennicella* (Lepidoptera: Psychidae) revealed by novel microsatellite markers. *Conservation Genetics Resources* 4 (1): 159-162. doi: 10.1007/s12686-011-9498-0
- Doyle JJL, Doyle JL (1987). Genomic plant DNA preparation from fresh tissue-CTAB method. *Phytochemical Bulletin* 19 (11): 11-15.
- Duman M, Guz N, Sertkaya E (2015) DNA barcoding of sunn pest adult parasitoids using cytochrome c oxidase subunit I (COI) *Biochemical Systematics and Ecology* 59: 70-77. doi: 10.1016/j.bse.2015.01.003
- Đurić Z, Delić D, Hrnčić S, Radonjić S (2014). Distribution and molecular identification of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera, Gelechiidae) populations in Bosnia and Herzegovina and Montenegro. *Polish Journal of Entomology* 83 (2): 121-129. doi: 10.2478/pjen-2014-0009
- Duploux A, Hornett EA (2018). Uncovering the hidden players in Lepidoptera biology: the heritable microbial endosymbionts. *PeerJ* 6: e4629.
- EPP0 (2005). Data sheets on quarantine pests: *Tuta absoluta*. In: EPP0 Bulletin. Paris, France: EPP0.
- Feldhaar H (2011). Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology* 36 (5): 533-543. doi: 10.1111/j.1365-2311.2011.01318.x
- Flores LV, Gilardon E, Gardenal CN (2003). Genetic structure of populations of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *Journal of Basic and Applied Genetics* 15 (2): 47-54.
- Fujii Y, Kageyama D, Hoshizaki S, Ishikawa H, Sasaki T (2001). Transfection of *Wolbachia* in Lepidoptera: the feminizer of the adzuki bean borer *Ostrinia scapularis* causes male killing in the Mediterranean flour moth *Ephestia kuehniella*. *Biological Sciences Proceedings of the Royal Society of London* 268 (1469): 855-859. doi: 10.1098/rspb.2001.1593
- Graham RI, Wilson K (2012). Male-killing *Wolbachia* and mitochondrial selective sweep in a migratory African insect. *BMC Evolutionary Biology* 12 (1): 204. doi: 10.1186/1471-2148-12-204
- Glowska E, Dragun-Damian A, Dabert M, Gerth M (2015). New *Wolbachia* supergroups detected in quill mites (Acari: Symbiontida). *Infection, Genetics and Evolution* 30: 140-146. doi: 10.1016/j.meegid.2014.12.019
- Guedes RNC, Roditakis E, Campos MR, Haddi K, Bielza P et al. (2019). Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. *Journal of Pesticide Science* 92: 1329-1342. doi: 10.1007/s10340-019-01086-9
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W et al. (2010). New algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59 (3): 307-321. doi: 10.1093/sysbio/syq010
- Gurung K, Wertheim B, Falcao Salles J (2019). The microbiome of pest insects: it is not just bacteria. *Entomologia Experimentalis et Applicata* 167 (3): 156-170. doi: 10.1111/eea.12768

- Guz, N, Kocak, E, Kilincer, N (2013). Molecular phylogeny of *Trissolcus* species (Hymenoptera: Scelionidae). *Biochemical Systematics and Ecology* 48: 85-91. doi: 10.1016/j.bse.2012.12.010
- Guz N, Arshad M, Cagatay NS, Dageri A, Ullah MI (2020a). Detection of *Wolbachia* (Rickettsiales: Anaplasmataceae) and *Candidatus Liberibacter asiaticus* (Rhizobiales: Rhizobiaceae) associated with *Diaphorina citri* (Hemiptera: Liviidae) from *Citrus reticulata* (Sapindales: Rutaceae) and alternate host, *Cordia myxa* (Boraginales: Boraginaceae). *Journal of Economic Entomology*. doi: 10.1007/s00284-020-01969-6
- Guz N, Arshad M, Cagatay NS, Dageri A (2020b). High prevalence of *Pantoea* in *Diaphorina citri* (Hemiptera: Liviidae): vector of citrus huanglongbing disease. *Current Microbiology*. doi: 10.1007/s00284-020-01969-6
- Guillemaud T, Blin A, Le Goff I, Desneux N, Reyes M et al. (2015). The tomato borer, *Tuta absoluta*, invading the Mediterranean Basin, originates from a single introduction from Central Chile. *Scientific Reports* 5: 8371. doi: 10.1038/srep08371
- Hagenblad J, Hülskötter J, Acharya KP, Brunet J, Chabrerie O et al. (2015). Low genetic diversity despite multiple introductions of the invasive plant species *Impatiens glandulifera* in Europe. *BMC Genetics* 16 (1): 103. doi: 10.1186/s12863-015-0242-8
- Hammer TJ, McMillan WO, Fierer N (2014). Metamorphosis of a butterfly-associated bacterial community. *PLoS One* 9 (1). doi: 10.1371/journal.pone.0086995
- Hassan MN, Alzaidi S (2009). *Tuta absoluta*-A serious pest advancing in the Mediterranean region. Roll of pheromones in management strategies. *International Pest Control* 51 (2): 85-87.
- Hawley DM, Hanley D, Dhondt AA, Lovette IJ (2006). Molecular evidence for a founder effect in invasive house finch (*Carpodacus mexicanus*) populations experiencing an emergent disease epidemic. *Molecular Ecology* 15: 263-275. doi: 10.1111/j.1365-294X.2005.02767.x
- He C, Nan X, Zhang Z, Li M (2013). Composition and diversity analysis of the gut bacterial community of the Oriental armyworm, *Mythimna separata*, determined by culture-independent and culture-dependent techniques. *Journal of Insect Science* 13 (1): 165. doi: 10.1673/031.013.16501
- Hebert PD, Ratnasingham S, De Waard JR (2003). Barcoding animal life: cytochrome oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B* 270: S96-S99. doi: 10.1098/rsbl.2003.0025
- Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH (2008). How many species are infected with *Wolbachia*?: a statistical analysis of current data. *FEMS microbiology letters* 281 (2): 215-220. doi: 10.1111/j.1574-6968.2008.01110.x
- Hiroki M, Kato Y, Kamito T, Miura K (2002). Feminization of genetic males by a symbiotic bacterium in a butterfly, *Eurema hecabe* (Lepidoptera: Pieridae). *Naturwissenschaften* 89 (4): 167-170. doi: 10.1007/s00114-002-0303-5
- Hornett EA, Duploux AM, Davies N, Roderick GK, Wedell N et al. (2008). You can't keep a good parasite down: evolution of a male-killer suppressor uncovers cytoplasmic incompatibility. *Evolution: International Journal of Organic Evolution* 62 (5): 1258-1263.
- Hurst GD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society B: Biological Sciences* 272 (1572): 1525-1534. doi: 10.1098/rspb.2005.3056
- Ilinsky Y, Kosterin OE (2017). Molecular diversity of *Wolbachia* in Lepidoptera: prevalent allelic content and high recombination of MLST genes. *Molecular Phylogenetics and Evolution* 109: 164-179. doi: 10.1016/j.ympev.2016.12.034
- Jiggins FM, Hurst GD, Schulenburg JHG, Majerus ME (2001). Two male-killing *Wolbachia* strains coexist within a population of the butterfly *Acraea encedon*. *Heredity* 86 (2): 161-166. doi: 10.1046/j.1365-2540.2001.00804.x
- Kageyama D, Ohno S, Hoshizaki S, Ishikawa Y (2003). Sexual mosaics induced by tetracycline treatment in the *Wolbachia*-infected adzuki bean borer, *Ostrinia scapulalis*. *Genome* 46: 983-989. doi: 10.1139/G07-041
- Kageyama D, Traut W (2004). Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*. *Proceedings of the Royal Society B: Biological Sciences* 271: 251-258. doi: 10.1098/rspb.2003.2604
- Kanle Satishchandra N, Chakravarthy AK, Özgökçe MS, Atlıhan R (2019). Population growth potential of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato, potato, and eggplant. *Journal of Applied Entomology* 143 (5): 518-526. doi: 10.1111/jen.12622
- Karaağaç SU (2015). Enzyme activities and analysis of susceptibility levels in Turkish *Tuta absoluta* populations to chlorantraniliprole and metaflumizone insecticides. *Phytoparasitica* 43 (5): 693-700. doi:10.1007/s12600-015-0476-z
- Keeling MJ, Jiggins FM, Read JM (2003). The invasion and coexistence of competing *Wolbachia* strains. *Heredity* 91 (4): 382-388.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12): 1647-1649. doi: 10.1093/bioinformatics/bts199
- Kenyon LJ, Meulia T, Sabree ZL (2015). Habitat visualization and genomic analysis of "*Candidatus Pantoea carbekii*," the primary symbiont of the brown marmorated stink bug. *Genome Biology and Evolution* 7 (2): 620-635. doi: 10.1093/gbe/evv006
- Kinyanjui G, Khamis FM, Ombura FLO, Kenya EU, Ekesi S et al. (2019). Infestation levels and molecular identification based on mitochondrial COI Barcode region of five invasive gelechiidae pest species in Kenya. *Journal of Economic Entomology* 112 (2): 872-882. doi: 10.1093/jee/toy357

- Kontsedalov S, Zchori-Fein E, Chiel E, Gottlieb Y, Inbar M et al. (2008). The presence of *Rickettsia* is associated with increased susceptibility of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides. *Pest Management Science* 64: 789-792. doi: 10.1002/ps.1595
- Korkmaz EM, Budak M, Başbüyük HH (2011). Utilization of cytochrome oxidase I in *Cephus pygmeus* (L.) (Hymenoptera: Cephidae). *Turkish Journal of Biology* 35: 713-726. doi: 10.3906/biy-1003-65
- Krenn HW (2010). Feeding mechanisms of adult Lepidoptera: structure, function, and evolution of the mouthparts. *Annual Review of Entomology* 55: 307-327. doi: 10.1146/annurev-ento-112408-085338
- Krishnamurthy PK, Francis RA (2012). A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodiversity and Conservation* 21 (8): 1901-1919. doi: 10.1007/s10531-012-0306-2
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35 (6): 1547-1549. doi: 10.1093/molbev/msy096
- Librado P, Rozas J (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452. doi: 10.1093/bioinformatics/btp187
- Lindholm AK, Breden F, Alexander HJ, Chan WK, Thakurta SG et al. (2005). Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Molecular Ecology* 14: 3671-3682. doi: 10.1111/j.1365-294X.2005.02697.x
- Lietti MM, Botto E, Alzogaray RA (2005). Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 34 (1): 113-119. doi: 10.1590/S1519-566X2005000100016
- Lauzon CR, McCombs SD, Potter SE, Peabody NC (2009). Establishment and vertical passage of *Enterobacter (Pantoea) agglomerans* and *Klebsiella pneumoniae* through all life stages of the Mediterranean fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America* 102: 85-95. doi: 10.1603/008.102.0109
- MacCollom GB, Lauzon CR, Sjogren RE, Meyer WL, Olday F (2009). Association and attraction of blueberry maggot fly Curran (Diptera: Tephritidae) to *Pantoea (Enterobacter) agglomerans*. *Environmental Entomology* 38 (1): 116-120. doi: 10.1603/022.038.0114
- Mansour R, Brévault T, Chailleux A, Cherif A, Grissa-Lebdi K et al. (2018). Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomologia Generalis* 38 (2): 83-112. doi: 10.1127/entomologia/2018/0749
- Moran NA, McCutcheon JP, Nakabachi A (2008). Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics* 42: 165-190. doi: 10.1146/annurev-genet.41.110306.130119
- Meyer CP (2003). Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society* 79 (3): 401-459. doi: 10.1046/j.1095-8312.2003.00197.x
- Peakall ROD, Smouse PE (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6 (1): 288-295. doi: 10.1111/j.1471-8286.2005.01155.x
- Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 6 (1): 288-295.
- Pereyra PC, Sánchez NE (2006). Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 35 (5): 671-676. doi: 10.1590/S1519-566X2006000500016
- Pinto-Tomás AA, Sittenfeld A, Uribe-Lorio L, Chavarría F, Mora et al. (2011). Comparison of midgut bacterial diversity in tropical caterpillars (Lepidoptera: Saturniidae) fed on different diets. *Environmental Entomology* 40 (5): 1111-1122. doi: 10.1603/EN11083
- Richardson MF, Weinert LA, Welch JJ, Linheiro RS, Magwire MM et al. (2012). Population genomics of the *Wolbachia* endosymbiont in *Drosophila melanogaster*. *PLoS Genetics* 8 (12). doi: 10.1371/journal.pgen.1003129
- Robinson CJ, Schloss P, Ramos Y, Raffa K, Handelsman J (2010). Robustness of the bacterial community in the cabbage white butterfly larval midgut. *Microbial Ecology* 59 (2): 199-211. doi: 10.1007/s00248-009-9595-8
- Russell JA, Funaro CF, Giraldo YM, Goldman-Huertas B, Suh D et al. (2012). A veritable menagerie of heritable bacteria from ants, butterflies, and beyond: broad molecular surveys and a systematic review. *PLoS One* 7 (12): e51027. doi: 10.1371/journal.pone.0051027
- Saccone C, Gissi C, Lanave C, Larizza A, Pesole G et al. (2000). Evolution of the mitochondrial genetic system: an overview. *Gene* 261 (1): 153-159. doi: 10.1016/S0378-1119(00)00484-4
- Saidov N, Srinivasan R, Mavlyanova R, Qurbonov Z (2018). First report of invasive South American tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tajikistan. *Florida Entomologist* 101 (1): 147-149. doi: 10.1653/024.101.0129
- Salunke BK, Salunke RC, Dhotre DP, Walujkar SA, Khandagale AB et al. (2012). Determination of *Wolbachia* diversity in butterflies from Western Ghats, India, by a multigene approach. *Applied and Environmental Microbiology* 78 (12): 4458-4467. doi: 10.1128/AEM.07298-11
- Sasaki T, Kubo T, Ishikawa H (2002). Interspecific transfer of *Wolbachia* between two lepidopteran insects expressing cytoplasmic incompatibility: a *Wolbachia* variant naturally infecting *Cadra cautella* causes male killing in *Ephesia kuehniella*. *Genetics* 162 (3): 1313-1319.

- Schuler H, Köppler K, Daxböck-Horvath S, Rasool B, Krumböck S et al. (2016). The hitchhiker's guide to Europe: the infection dynamics of an ongoing *Wolbachia* invasion and mitochondrial selective sweep in *Rhagoletis cerasi*. *Molecular Ecology* 25 (7): 1595-1609. doi: 10.1111/mec.13571
- Schuler H, Egan SP, Hood GR, Busbee RW, Driscoe AL et al. (2018). Diversity and distribution of *Wolbachia* in relation to geography, host plant affiliation and life cycle of a heterogonic gall wasp. *BMC Evolutionary Biology* 18 (1): 37. doi: 10.1186/s12862-018-1151-z
- Sevim A, Sevim E, Demirci M, Sandalli C (2016). The internal bacterial diversity of stored product pests. *Annals of Microbiology* 66 (2): 749-764. doi: 10.1007/s13213-015-1155-5
- Shashank PR, Twinkle S, Chandrashekar K, Meshram NM, Suroshe SS et al. (2018). Genetic homogeneity in South American tomato pinworm, *Tuta absoluta*: a new invasive pest to oriental region. *3Biotech* 8 (8): 350. doi: 10.1007/s13205-018-1374-0
- Solignac M, Vautrin D, Rousset F (1994). Widespread occurrence of the proteobacteria *Wolbachia* and partial cytoplasmic incompatibility in *Drosophila melanogaster*. *Comptes rendus de l'Académie des sciences. Série 3, Sciences de la vie* 317: 461-470.
- Siqueira HAA, Guedes RNC, Picanço MC (2000). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology* 2 (2): 147-153. doi: 10.1046/j.1461-9563.2000.00062.x
- Staudacher H, Kaltenpoth M, Breeuwer JA, Menken SB, Heckel DG et al. (2016). Variability of bacterial communities in the moth *Heliothis virescens* indicates transient association with the host. *PLoS One* 11 (5). doi: 10.1371/journal.pone.0154514
- Stouthamer R, Breeuwer JA, Hurst GD (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* 53 (1): 71-102. doi: 10.1146/annurev.micro.53.1.71
- Škaljac M, Kostanjšek R, Žanić K (2012). The presence of *Wolbachia* in *Tuta absoluta* (Lepidoptera: Gelechiidae) populations from coastal Croatia and Montenegro. *African Entomology* 20 (1): 191-194. doi: 10.4001/003.020.0125
- Tagami Y, Miura K (2004). Distribution and prevalence of *Wolbachia* in Japanese populations of Lepidoptera. *Insect Molecular Biology* 13 (4): 359-364. doi: 10.1111/j.0962-1075.2004.00492.x
- Tang X, Freitag D, Vogel H, Ping L, Shao Y et al. (2012). Complexity and variability of gut commensal microbiota in polyphagous lepidopteran larvae. *PLoS One* 7 (7): e36978. doi: 10.1371/journal.pone.0036978
- Tabuloc CA, Lewald KM, Conner WR, Lee Y et al. (2019). Sequencing of *Tuta absoluta* genome to develop SNP genotyping assays for species identification. *Journal of Pest Science* 92 (4): 1397-1407. doi: 10.1007/s10340-019-01116-6
- Telschow A, Gadau J, Werren JH, Kobayashi Y (2019). Genetic incompatibilities between mitochondria and nuclear genes: effect on gene flow and speciation. *Frontiers Genetics* 10: 62. doi: 10.3389/fgene.2019.00062
- Thao M, Baumann P (2004). Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. *Applied and Environmental Microbiology* 70: 3401-3406. doi: 10.1128/AEM.70.6.3401-3406.2004
- Tsuchida T, Koga R, Shibao H, Matsumoto T, Fukatsu T (2002). Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrtosiphon pisum*. *Molecular Ecology* 11: 2123-2135. doi: 10.1046/j.1365-294X.2002.01606.x
- Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000). Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences* 97: 5948-5953. doi: 10.1073/pnas.100110397
- Turelli M, Hoffmann AA, McKechnie SW (1992). Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics*, 132 (3): 713-723.
- Van Meer MMM, Witteveldt J, Stouthamer R (1999). Phylogeny of the arthropod endosymbiont *Wolbachia* based on the *wsp* gene. *Insect Molecular Biology* 8 (3): 399-408. doi: 10.1046/j.1365-2583.1999.83129.x
- Voirol LRP, Frago E, Kaltenpoth M, Hilker M, Fatouros NE (2018). Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. *Frontiers Microbiology* 9: 1-14. doi: 10.3389/fmicb.2018.00556
- Walterson AM, Stavrinides J (2015). *Pantoea*: insights into a highly versatile and diverse genus within the *Enterobacteriaceae*. *FEMS Microbiology Reviews* 39 (6): 968-984. doi: 10.1093/femsre/fuv027
- Weinert LA, Araujo-Jnr EV, Ahmed MZ, Welch JJ (2015). The incidence of bacterial endosymbionts in terrestrial arthropods. *Proceedings of the Royal Society B: Biological Sciences* 282 (1807). doi: 10.1098/rspb.2015.0249
- Werren JH, O'Neill SL (1997). The evolution of heritable symbionts. *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. New York, NY, USA: Oxford University Press, pp. 1-41.
- Werren JH (1997). Biology of *Wolbachia*. *Annual Review of Entomology* 42 (1): 587-609.
- Werren JH, Zhang W, Guo LR (1995). Evaluation of *Wolbachia*: reproductive parasites of arthropods. *Proceedings of the Royal Society B: Biological Sciences* 261 (1360).
- Weeks AR, Velten R, Stouthamer R (2003). Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proceedings of the Royal Society B: Biological Sciences* 270: 1857-1865. doi: 10.1098/rspb.2003.2425
- Xia X, Gurr GM, Vasseur L, Zheng D, Zhong H et al. (2017). Metagenomic sequencing of diamondback moth gut microbiome unveils key holobiont adaptations for herbivory. *Frontiers Microbiology* 8: 663. doi: 10.3389/fmicb.2017.00663
- Yalcin M, Mermer S, Kozaci LD, Turgut C (2015). Insecticide resistance in two populations of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) from Turkey. *Turkish Journal of Entomology* 39 (2): 137-145. doi: 10.16970/ted.63047

- Yatkin G, Guz N (2018). The use of DNA barcoding in entomology. *Yüzüncü Yıl University Journal of Agricultural Sciences* 28 (1): 126-134. doi: 10.29133/yyutbd.350146
- Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS et al. (2014). Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied Environmental Microbiology* 80 (17): 5254-5264. doi: 10.1128/AEM.01226-14
- Yükselbaba U, Göçmen H (2016). Determination of genetic variation of tomato leafminer [*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)] populations from west Mediterranean region of Turkey based on mitochondrial cytochrome oxidase I (mtCOI). *Mediterranean Agricultural Sciences* 29 (1): 5-7.
- Zibae I (2016). The expression profile of detoxifying enzyme of tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) to chlorpyrifos. *Arthropods* 5 (2): 77.