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Original article (Orijinal araştırma)

Phylogenetics of *Buchnera aphidicola* Munson et al., 1991 (Enterobacteriales: Enterobacteriaceae) based on 16S rRNA amplified from seven aphid species[1]

Farklı yaprak biti türlerinden izole edilen *Buchnera aphidicola* Munson et al., 1991 (Enterobacteriales: Enterobacteriaceae)'nın 16S rRNA'ya göre filogenetiği

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Abstract

The obligate symbiont, *Buchnera aphidicola* Munson et al., 1991 (Enterobacteriales: Enterobacteriaceae) is important for the physiological processes of aphids. *Buchnera aphidicola* genes detected in seven aphid species, collected in 2017 from different plants and altitudes in Adana Province, Turkey were analyzed to reveal phylogenetic interactions between *Buchnera* and aphids. The 16S rRNA gene was amplified and sequenced for this purpose and a phylogenetic tree built up by the neighbor-joining method. A significant correlation between *B. aphidicola* genes and the aphid species was revealed by this phylogenetic tree and the haplotype network. Specimens collected in Feke from *Solanum melongena* L. was distinguished from the other *B. aphidicola* genes on *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) with a high bootstrap value of 99. *Buchnera aphidicola* in *Myzus* spp. was differentiated from others, and the difference between *Myzus cerasi* (Fabricius, 1775) and *Myzus persicae* (Sulzer, 1776) was clear. Although, *B. aphidicola* is specific to its host aphid, certain nucleotide differences obtained within the species could enable specification to geographic region or host plant in the future.

Keywords: Aphid, genetic similarity, phylogenetics, symbiotic bacterium

Öz

Obligat simbiyont, *Buchnera aphidicola* Munson et al., 1991 (Enterobacteriales: Enterobacteriaceae), yaprak bitlerinin fizyolojik olaylarının sürdürülmesinde önemli bir rol oynar. Adana (Türkiye)' dan 2017 yılında farklı bitki ve yüksekliklerden toplanan yedi yaprak biti türünde saptanan *B. aphidicola* genleri ile yaprakbiti türleri arasındaki filogenetik etkileşimi ortaya çıkarmak için analiz edilmiştir. Bu amaçla, 16S rRNA'nın gen bölgeleri kullanılmış ve filogenetik ağaç, neighbor-joining ile oluşturulmuştur. *Buchrena aphidicola* genleri ve aphid türleri arasında filogenetik ağaç ve haploid networke göre anlamlı bir korelasyon tespit edilmiştir. *Solanum melongena* L. toplanan Feke örneği, diğer *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)'lerdeki *B. aphidicola* genlerinden çok yüksek bir boostrap değeri (99) ile ayrılmıştır. *Myzus* cinsindeki *B. aphidicola* genleri diğer cinslerden ayrı dallanmış ve *Myzus cerasi* (Fabricus, 1775) ve *Myzus persicae* Sulzer, 1776 arasındaki ayrım belirgindir. *Buchera aphidicola* konukçu yaprakbiti türüne özelleşmiş olsa da tür içinde elde edilen bazı nükleotid farklılıkları ilerde coğrafik bölgeye ya da bitkiye de özelleşmeye neden olabilir.

Anahtar sözcükler: Yaprak biti, genetik benzerlik, filogenetik, simbiyotik bakteri

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Introduction

Aphids (Hemiptera: Aphididae) are a group of diverse insect species highly adapted to feed on plants by sucking sap from phloem (Blackman & Eastop, 2000). Turkey has more than 500 aphid species across its different geographic and climatic regions, with more than 80 aphid species have been recorded in Adana Province (Uygun et al., 2001; Çalışkan et al., 2012; Görür et al., 2017). Worldwide there are about 5000 aphid species, and most are considered have an intracellular bacterial symbiont (Fukatsu, 2001). These symbionts can be obligating like *Buchnera aphidicola* Munson et al., 1991 (Enterobacteriales: Enterobacteriaceae) or secondary symbionts such as *Rickettsia* spp. da Rocha-Lima, 1916*, Wolbachia* spp. Hertig & Burt 1924 (Rickettsiales: Rickettsiaceae), *Hamiltonella defensa* Moran et al., 2005*, Regiella insecticola* Moran et al., 2005, and *Serratia symbiotica* Moran et al., 2005 (Enterobacteriales: Enterobacteriaceae) (Peccoud et al., 2014). Endosymbionts have active roles in nutritional support (Akman Gündüz & Douglas, 2009; Chen et al., 2009), insecticide resistance (Martine et al., 2013; Pan et al., 2013), reproductive behavior (Baldo et al., 2006; Simon et al., 2011), predator-prey and parasitoid-prey interactions (Tsuchida et al., 2010; Vorburger et al., 2010; Telesnicki et al., 2012; Martine et al., 2013), adaptation of the insects to external conditions (Russell & Moran, 2006), and the physiology of host-associated populations of polyphagous insects (Brady & White, 2013). Physiological processes, such as development and reproduction in aphids, depend on the existence of the obligate symbiont *B. aphidicola* (Baumann et al., 1995; Douglas, 2003; Peccoud et al., 2014). Aphids need *B. aphidicola* for synthesizing essential amino acids and riboflavin, nutrients that are usually not present in phloem sap (Akman Gündüz & Douglas, 2009; Chen et al., 2009; Liu et al., 2013). Without *B. aphidicola*, aphids produce dwarfed offspring, or have lower or no capacity to reproduce. The benefit for *B. aphidicola* is that aphids create a safe and stable environment for the bacterium in specialized bacteriocytes (Chen et al., 2009). Therefore, the relationship between aphids and *B. aphidicola* is mutualistic (Baumann et al., 1995).

For the evolutionary role of such mutualisms, the term symbiogenesis was first used by Mereschkowsky (1909) and then in the context of the work of Buchner (1912) on hemipteran symbionts. In 1924, Kozo-Polyansky (see Kozo-Polyansky & Fet, 2010) proposed a role for symbiont organisms in the evolutionary process of the all living organisms. Within this context, *B. aphidicola* is a highly fascinating symbiont. It is located in maternal bacteriocytes and transmits through eggs or embryos (Liu et al., 2013). Improved understanding the relationship between aphids and their symbionts, a relationship that evolved 150-200 million years ago (Jousselin et al., 2009), will undoubtedly provide deeper insights into the biology of both organisms. There have been a range of studies on this coevolution published (e.g., Fukatsu, 2001; Liu et al., 2013, 2014). In order to reveal the coevolutionary process between the aphid species and *B. aphidicola*, molecular examination of the 16S rRNA gene has been used (Munson et al., 1991; Baumann et al., 1997; Liu et al., 2014). These studies suggest that *B. aphidicola* has lost some genes and/or aphids acquired some highly transcribed genes including LD-carboxypeptidases (LdcA1, LdcA2 and yLdcA), lipoprotein As (RlpA1-5), DNA polymerase III alpha chain (yDnaE) and ATP synthase delta chain (yAtpH) from *B. aphidicola* (Nikoh et al., 2010; Liu et al., 2013; Lagos et al., 2014; Güz et al., 2015). Given their close evolutionary relationship, phylogenetics of the host and its symbiont mirror each other in deeper evolutionary divergences. Therefore, data obtained from the symbionts can be used to reconstruct the evolutionary process of hosts (Nováková et al., 2013).

Satar et al. (2013) studied *Aphis gossypii* Glover, 1877 on different host plant species in the eastern Mediterranean Region, and discovered that *A. gossypii* on cucurbits had distinctly different biology. However, it is known if *B. aphidicola* contributes to this observed biological diversity. Also, it is not known whether different aphid taxa on the same host plant possess similar or diverse *B. aphidicola* genotypes. Both are possible because host-plant relationships in polyphagous insects are important in the evolution of the insect. In addition, relationships between aphids and *B. aphidicola* have generally been studied on

a wide geographic scale, with geography potentially acting as a major driver for differentiation of *B. aphidicola*. Therefore, research focusing on a smaller geographic scale may provide valuable information by eliminating the wider geographic effects on climate, host plants and natural enemies.

Therefore, the aim of this study was to examine the genetic interaction between *B. aphidicola* and seven different aphid species collected from different plants at different altitudes in Adana Province, Turkey, which represents the first genetic investigation of this symbiotic system in Turkey. Additionally, this study aimed to increase the genetic database on *B. aphidicola*, and provide deeper insights into the coevolutionary process between this obligate bacterium and its aphid hosts.

Material and Methods

Sampling of aphids

Aphid populations were collected in 2017 from a range of plants (trees, weeds and vegetables) and altitudes in Adana Province, Turkey (Table 1, Figure 1). Aphids were removed from infested plants with a fine brush and transferred to Eppendorf tubes containing 96% alcohol. Collection date, geographic location and host plant species were recorded, and samples stored at -80ºC until DNA extraction. Aphid species were determined morphologically by Dr. Işıl Özdemir (Plant Protection Central Research Institute, Ankara, Turkey) according to Blackman & Eastop (2019). Seven aphid species were determined in three genera of the Aphidinae subfamily, and used for constructing molecular phylogeny of *B. aphidicola* (Table 1). According to morphological identification, the specimens were *Aphis craccivora* Koch, *Aphis fabae* Scopoli, 1763, *A. gossypii*, *Aphis pomi* De Geer, 1773, and *Rhopalosiphum maidis* (Fitch, 1856) from the Aphidini tribe, and *Myzus cerasi* (Fabricius, 1775) and *Myzus persicae* Sulzer, 1776 from the Macrosiphini tribe.

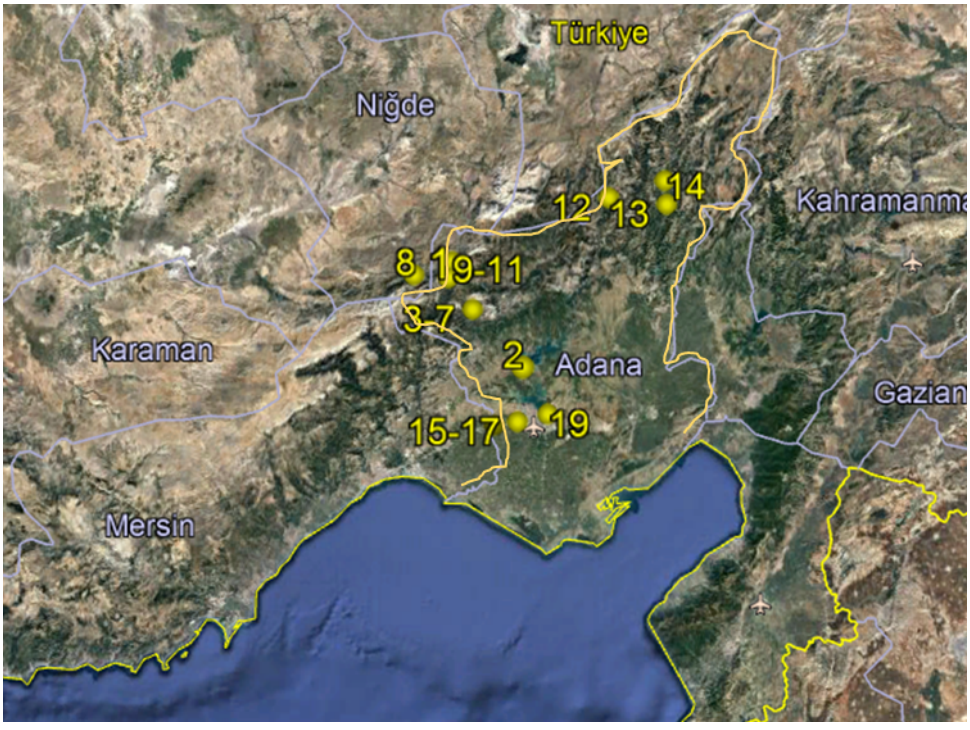

Figure 1. Collection locations of aphid samples from Adana Province, Turkey (satellite image from Anonymous, 2019a).

Table 1. Sample numbers, sampling date, location, altitude, host plants, and host aphid species for detection of genetic diversity of *Buchnera aphidicola*

Sample number	Date	Location	Altitude (m)	Host plant	Aphid species
1	27.07.2017	Fındıklı/Pozantı	1139	*Cucumis sativus* L.	*Aphis gossypii*
2	17.08.2017	Sağdıkali/Karaisalı	141	*Abelmoschus esculentus* L.	*A. gossypii*
3	17.08.2017	Kızıldağ	1666	*Prunus avium* L.	*Myzus cerasi*
4	17.08.2017	Kızıldağ	1666	*Zea mays* L.	*Rhopalosiphum maidis*
5	17.08.2017	Kızıldağ	1650	*A. esculentus*	*A. gossypii*
6	17.08.2017	Kızıldağ	1650	*Malus communis* L.	*Aphis pomi*
7	17.08.2017	Kızıldağ	1650	*Phaseolus vulgaris* L.	*Aphis fabae*
8	07.09.2017	Çiftehan	950	*P. avium*	*M. cerasi*
9	07.09.2017	Kamışlı Pozantı	1220	*Robinia pseudoacacia* L.	*Aphis craccivora*
10	07.09.2017	Kamışlı Pozantı	1220	*M. communis*	*A. pomi*
11	07.09.2017	Kamışlı Pozantı	1220	*Capsicum annuum* L.	*Myzus persicae*
12	20.09.2018	Görbeyaz-Feke	1050	*C. annuum*	*M. persicae*
13	20.09.2017	Feke-Akkaya	772	*Z. mays*	*R. maidis*
14	20.09.2017	Düşmüş-Feke	620	*Solanum melongena* L.	*A. gossypii*
15	03.11.2017	Seyhan	27	*C. annuum*	*A. gossypii* *A. craccivora*
16	01.11.2017	Seyhan	23	*Vigna unguiculata* (L.)	*A. craccivora*
17	01.11.2017	Seyhan	23	*Cucumis melo* L.	*A. gossypii*
18	24.02.2018	Zeytinli	24	*Capsella bursa-pastoris* (L.)	*A. craccivora*
19	27.02.2018	Balcalı	137	*C. bursa-pastoris*	*M. persicae*

DNA extraction and amplification

Genomic DNA for 37 specimens, representing all 19 populations (Table 1), were extracted from parasitoid free single aphids with a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions in order to analyze *B. aphidicola* 16S rRNA gene (Table 2). The primer pairs, 16sF (5'-AGAGTTTGATCATGGCTCAGATTG-3') and 16sR (5'-TACCTTGTTACGACTTCACCCCAG-3') belonging to 16S rRNA gene region were used for PCR (Liu et al., 2013). The reaction mixture was prepared to achieve a final volume of 25 µl with inclusion of Taq buffer (10X), 2.5 mM $MgCl_2$, 250 µM dNTPs, 1 µM primer, 0.5 U Taq and 10 µM DNA template. The thermocycler conditions were: 5 min at

94ºC for predenaturation, followed by 35 cycles at 94ºC for 1 min, 50ºC for 1 min, at 72ºC for 1 min, and a final period at 72ºC for 7 min. The PCR products were run on 1% agarose gel, stained with ethidium bromide and viewed with a gel imaging system before two-way sequencing by a commercial company (Molgentek, Adana, Turkey).

Table 2. Country of origin, aphid species, and sample number (this study) or GenBank accession number used for examine of 16S rRNA gene of *Buchnera aphidicola*

Country	Aphid species	n	Sample number
Turkey (this study)	*Aphis craccivora*	7	9-1, 9-2, 15-1 15-2, 15-10, 16-1, 16-2
	Aphis fabae	2	7-1 7-2
	Aphis gossypii	12	1-1, 1-2, 2-1, 2-2, 5-1, 5-2, 14-1*, 14-2*, 15-20, 17-1, 17-2, 18-1
	Aphis pomi	3	3-2, 8-1, 8-2
	Myzus cerasi	3	10-2, 122-1,122-2
	Myzus persicae	6	11-1 11-2 12-1 12-2 19-1 19-2
	Rhopalosiphum maidis	4	4-1 4-2, 13-1 13-2
India	*A. fabae*	7	KT175935.1, KT175936.1, KT175937.1, KT175938.1, KT175939.1, KT175941.1, KT175940.1
	A. gossypii	18	KT175910.1, KT175911.1, KT175912.1, KT175913.1, KT175914.1, KT175915.1, KT175916.1, KT175917.1, KT175918.1, KT175919.1, KT175920.1, KT175921.1, KT175922.1, KT175923.1, KT175924.1, KT175925.1, KT175926.1, KT175927.1
USA	*A. fabae*	1	AY518294.1
	M. persicae	1	M63249.1
Taiwan	*A. craccivora*	1	EF614236.1
China	*R. maidis*	1	JX998123.1

* Different haplotype of *B. aphidicola* in *A gossypii* from Adana, Turkey.

Data analysis

The multiple alignments were made using ClustalW, and MEGA6 software (Tamura et al., 2013) was used to make necessary comparisons. Two-way sequences were controlled for each different base, edited on the Finch TV (FinchTV, 2019) and combined with MEGA6 software (Tamura et al., 2013). The reference sequences for the 16S rRNA gene of *B. aphidicola* from different countries obtained from GenBank were compared with these new results. DnaSP 5 software was used to detect haplotypes (Librado & Rozas, 2009.). Along with the reference sequences, 9, 4, 2, 1, 1, 2, and 2 haplotypes were used for construction of a phylogenetic tree for *A. gossypii*, *A. fabae, A. craccivora*, *A. pomi*, *M. cerasi*, *M. persicae*, and *R. maidis,* respectively (Table 2). The seven haplotypes were added to GenBank with accession number MK676083-90.

The neighbor-joining method based on Kimura 2-parameter (K2P) and Gamma distributed (5 categories, +G, parameter = 0.4153), and the best-fit model for the sequences in MEGA6, was used to reconstruct phylogenetic tree (Tamura et al., 2013). The bootstrap consensus tree inferred from 500 replicates (Nei & Kumar, 2000) was taken to represent the evolutionary history of the taxa analyzed. *Escherichia coli* T. Escherich, 1885 (Enterobacteriales: Enterobacteriaceae) (Migula, 1895) was selected

as an out-group. Nucleotide distance matrix based on the K2P calculated according to Kimura (1980) and Tamura et al. (2013) in MEGA6 software. All genes from this study and corresponding reference genes were used to establishment the 16S rRNA gene-specific haplotype network using PopArt (Anonymous, 2019b) software by the median-joining method (Bandelt et al., 1999). Nucleotide distance matrix based on the K2P was calculated according to Kimura (1980) and Tamura et al. (2013) in MEGA6 software.

Results and Discussion

Amplification of the 16S rRNA gene of *B. aphidicola* from the seven aphid species yielded 1500-bp products for 37 specimens on the agarose gel. After cleaning and editing of the specimen sequences, 66 sequences (1357-bp) were included from this study and reference sequences were analyzed to construct the phylogenetic tree (Table 2, Figure 2). Specimens belonging to the same haplotypes are shown in the brackets at the same tip on the phylogenetic tree.

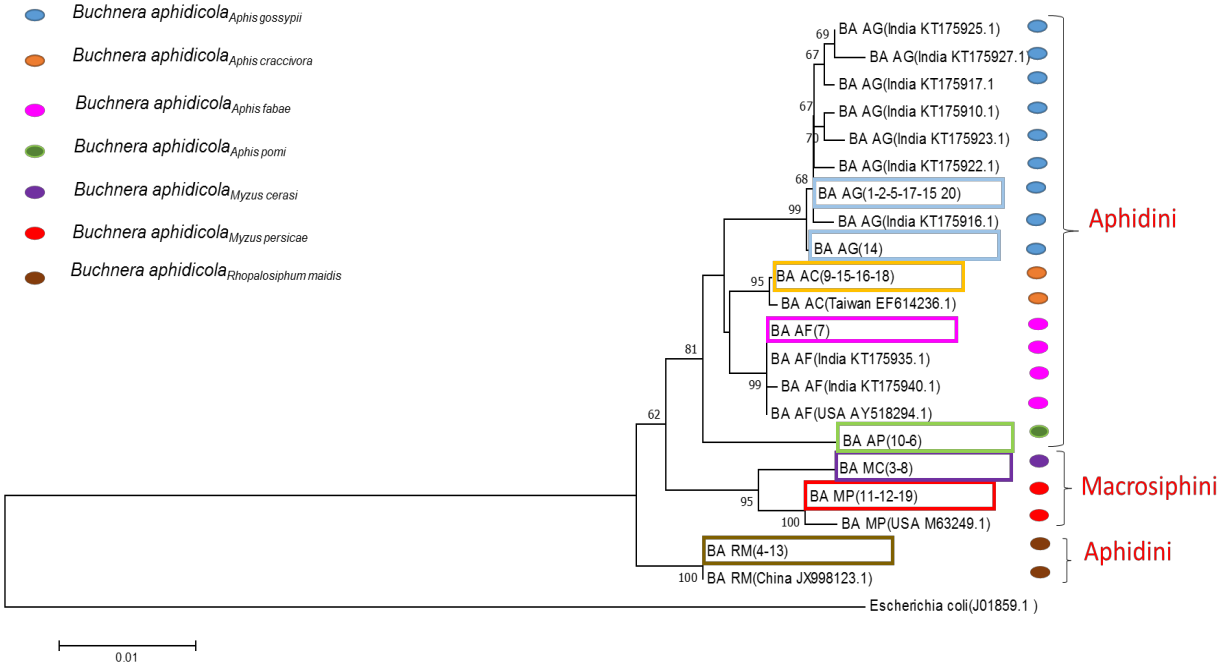

Figure 2. Phylogenetic tree of *Buchnera aphidicola* (BA) from seven aphid species based on neighbor-joining method (bootstrap 500). The genes used in this study, highlighted with different colors, came from to *B. aphidicola* from seven aphid species: AC, *Aphis craccivora;* AF, *Aphis fabae;* AG, *Aphis gossypii;* AP, *Aphis pomi*; MC, *Myzus cerasi*; MP, *Myzus persicae*; and RM, *Rhopalosiphum maidis*.

The *E. coli* sequence, used as the out-group, was well separated from the *B. aphidicola* 16S rRNA sequences. *Buchnera aphidicola* is a close relative of *E. coli*, but its genome size is only one seventh of *E. coli* (Shigenobu et al., 2000). Differentiation of Aphidini and Macrosiphini tribes is evident in the tree (Figure 2). However, *R. maidis* belonging to Aphidini tribe was separated from the *Aphis* spp. in the same tribe. This might relate to the feeding of these two genera on different host plants. *Rhopalosiphum maidis* feeds on monocotyledonous plants, whereas, *Aphis* spp. feed exclusively on dicotyledonous plants (Holman, 2009; Betsiashvili et al., 2014). *Buchnera aphidicola* provides essential amino acids to aphids, and this might create genetic distance between monocotyledonous and dicotyledonous plants because of their different composition of metabolites (Schobert et al., 1998; Qi et al., 2018). The range of the amino acids provided and the metabolic versatility of *B. aphidicola* may vary between higher aphid taxa or between aphid species that have narrow and broad host plant ranges (Douglas, 1998).

Buchnera aphidicola genes are distinguished according to aphid genus in the phylogenetic tree (Figure 2). *Myzus* spp. are differentiated from *Aphis* spp. with bootstrap value of 62. Two *Myzus* spp. are also distinguished from each other with a high bootstrap value. Species within *Aphis* was distinguished with low bootstrap value except for *A. pomi*. When *A. gossypii* was evaluated intraspecifically, *A. gossypii* samples collected from Adana Province were clustered in a group with homologous sequences from specimens from India. However, the *B. aphidicola* sample collected from *Solanum melongena* L. (14) in Feke District was distinguished from others with a bootstrap value of 99. Although one nucleotide difference was determined among the specimens from Turkey, higher numbers of differences were found in Indian samples (Figures 2 and 3). Aphids are holocyclic in regions with warmer climates including the coast areas of the East Mediterranean Region of Turkey. However, they are heterocyclic in colder areas like plateaus of the same region. Holocyclic aphid populations consist of females only, and the offspring are genetically identical to their parent. However, heterocyclic aphids that overwinter under harsher climatic conditions as eggs have both females and males. Therefore, genetic differentiation can occur more readily in colder regions. Samples collected in Feke District, a transition zone between upland and coastal areas, have higher variation than the *B. aphidicola* in *A. gossypii* specimens, which is probably a resulted of to the mating individuals from the two different areas. Chong & Moran (2016) showed that the same *B. aphidicola* haplotype in different aphid genotypes differently affected the fitness cost of aphid clones. The intraspecific genotypic variation of aphids could be important for the potential long-term evolution of *B. aphidicola* and aphids. In this respect, molecular studies alone are insufficient for explaining this interaction.

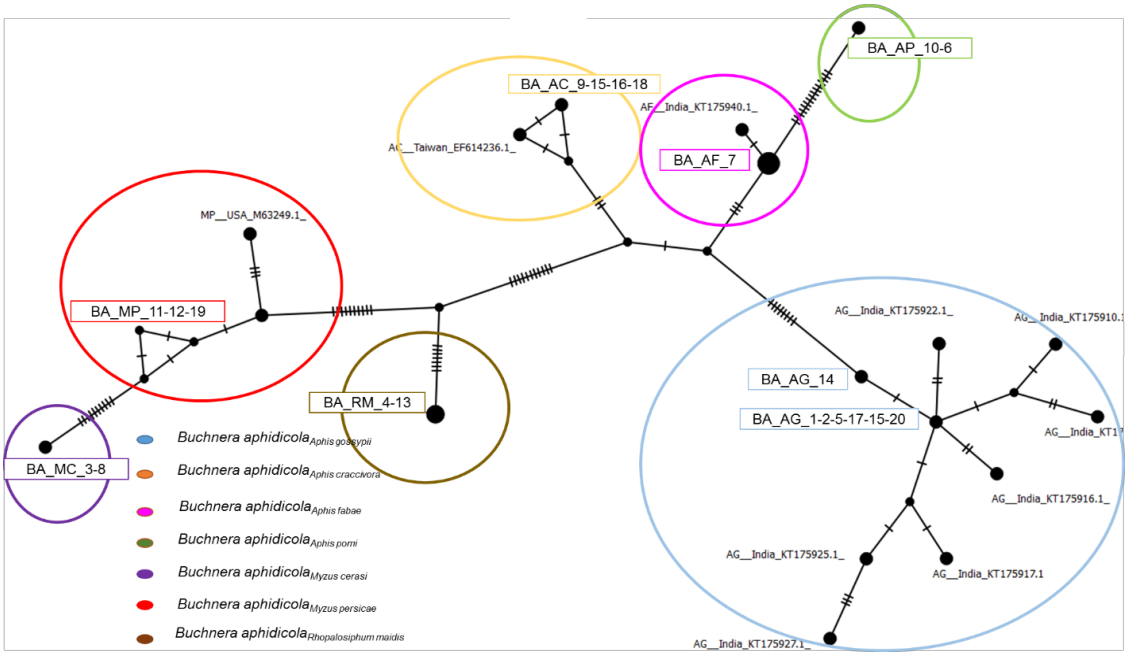

Figure 3. Haplotype network of *Buchnera aphidicola* (BA) 16S rRNA gene from seven host aphid species: AC, *Aphis craccivora*; AF, *Aphis fabae*; AG, *Aphis gossypii*; AP, *Aphis pomi*; MC, *Myzus cerasi*; MP, *Myzus persicae*; and RM, *Rhopalosiphum maidis*.

Buchnera aphidicola 16S rRNA genes from seven aphid species were grouped separately on the haplotype network in parallel with the phylogenetic tree. While *B. aphidicola* in *A. gossypii* and *M. cerasi* are the furthest species with more than 28 single nucleotide shifts, *A. fabae* was closest to *A. craccivora* with only eight to nine single nucleotide shifts (Figure 3). The pairwise K2P nucleotide distance analyses showed that interspecific and intraspecific mean distance values were 1-2% and 0%, respectively. General pairwise distance is ranged up to 3% (Table 3). In general, low genetic distance was observed between *B. aphidicola* in the seven-aphid species.

Although *A. gossypii* and *A. craccivora* species were collected from same *Capsicum annuum* L. plant (15), their *B. aphidicola* appears to be specific to the aphid species (Table 1). Also, no specific differences were observed in relation to altitude or plant species. For example, the samples on *C. annuum* collected from the coastline (15) and upland area (11 and 12) were found to be identical in terms of genetic structure. Satar et al. (2013) studied *A. gossypii* on different host plant species and found that *A. gossypii* has distinctly different biology on cucurbits compared to other host plants. However, it was found that *B. aphidicola* from *A. gossypii* on cucurbits (1 and 17) did not have any nucleotide differences from other *A. gossypii* specimens in the region, except for the *S. melongena* specimens. Thus, it appears that host plant species is not a driver of *B. aphidicola* diversity.

As Turkey is at the intersection between Europe and Asia, it is rich in terms of aphid biodiversity compared to the neighboring countries (Kök et al., 2016). It has diverse geographical features and climate types that have led to many endemic aphid species and genetic variation within these species. Over 540 aphid species have been recorded in Turkey, as significant proportion of the 5000 aphidofauna and 13 subspecies belong to 141 genera recorded worldwide (Görür et al., 2017). The present study was by intent restricted to a narrow geographic area and limited number of aphid species. However, it is the first study to demonstrate the relationship between aphid species and *B. aphidicola* in Turkey. Future studies of a wider range of aphids from different ecosystems could potentially provide reasons for the genetic variation such as seen in the Feke specimen.

Table 3. Nucleotide distance matrix based on Kimura 2-parameter model of *Buchnera aphidicola* (BA) 16S rRNA gene on seven host aphid species: AC, *Aphis craccivora*; AF, *Aphis fabae*; AG, *Aphis gossypii*; AP, *Aphis pomi*; MC, *Myzus cerasi*; MP, *Myzus persicae*; and RM, *Rhopalosiphum maidis*

No	Specimen Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	BA_AG (1, 2, 5, 15_20, 17)																					
2	BA_AG (14)	0.00																				
3	BA_AG (India_KT175910.1)	0.00	0.00																			
4	BA_AG (India_KT175916.1)	0.00	0.00	0.00																		
5	BA_AG (India_KT175917.1)	0.00	0.00	0.00	0.00																	
6	BA_AG (India_KT175922.1)	0.00	0.00	0.00	0.00	0.00																
7	BA_AG (India_KT175923.1)	0.00	0.00	0.00	0.00	0.00	0.00															
8	BA_AG (India_KT175925.1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00														
9	BA_AG (India_KT175927.1)	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00													
10	BA_AC (9, 15, 16, 18)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01												
11	BA_AC (Taiwan_EF614236.1)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00											
12	BA_MC (3, 8)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02										
13	BA_AP (6, 10)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02									
14	BA_RM (4, 13)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02								
15	BA_RM (China_JX998123.1)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00							
16	BA_MP (11, 12, 19)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.01	0.02	0.01	0.01						
17	BA_MP (USA_M63249.1)	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.01	0.02	0.02	0.02	0.00					
18	BA_AF (7)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02				
19	BA_AF (India_KT175935.1)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.00			
20	BA_AF (India_KT175940.1)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.00	0.00		
21	BA_AF (USA_AY518294.1)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.00	0.00	0.00	
22	*Escherichia coli* (J01859.1)	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	0.12	0.11	0.13	0.12	0.11	0.11	0.12	0.13	0.12	0.12	0.12	0.12

Theories on the coevolution and coexistence of aphid species and *B. aphidicola* have been offered by a number of researchers (Munson et al., 1991; Funk et al., 2000; Liu et al., 2013), although there is no universal agreement on these ideas (van Ham et al., 1997). Aphids and *B. aphidicola*, which coevolved 150-200 million years ago (Jousselin et al., 2009), may have been affected by many factors, such as host plant, environment and geographic region. However, detailed investigations of aphid fitness cost, effects of environmental factors, geographic differences are needed to fully understand this symbiotic relationship. Although, *B. aphidicola* is specific to host aphid, some intraspecific nucleotide differences may be found to be related to geographic region or host plant in future studies. In conclusion, sampling from different geographic regions and different plants at different intervals would be helpful to further examine the coevolutionary processes.

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