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POTENTIAL PSYLLID VECTORS OF *CANDIDATUS* PHYTOPLASMA MALI AND *CANDIDATUS* PHYTOPLASMA PYRI IN TURKEY

Kamuran Kaya[1,*], Çigdem Ulubas Serce[2], Mona Gazel[1], Kadriye Caglayan[1] and Nicolas Sauvion[3]

[1]Mustafa Kemal University, Faculty of Agriculture, Plant Protection Department, Antakya/Hatay, Turkey;
[2]Agricultural Science and Technology Faculty, Crop Production and Technologies Department, Nigde, Turkey;
[3]INRA, Unite Mixte de Recherche Biologie et Genetique des Interactions Plante-Parasite, Montpellier, France.
[*]Corresponding author's e-mail: kayakamuran@gmail.com

Psyllids are vectors of phytoplasma, which cause important diseases of pome fruit trees. Psyllid species reported as phytoplasma vectors were captured during 2010–2011 in several pome fruit growing regions in Turkey. Potential psyllid vectors of '*Candidatus* Phytoplasma mali' were collected from *Malus* spp. (apple), *Cydonia oblonga* (quince), *Crataegus* spp. (hawthorn) and also from the overwintering hosts, whereas those of '*Candidatus* Phytoplasma pyri' were collected from wild and cultured forms of *Pyrus* spp. (pear) trees. The psyllids were identified morphologically as *Cacopsylla picta*, *C. melanoneura-C. affinis* complex, *C. crataegi*, *C. pyrisuga*, *C. pyri*, *C. pyricola* and other *Cacopsylla* species. The highest natural phytoplasma infection rate was found in *C. picta* followed by *C. pyri*, *C. melanoneura-C. affinis* complex and *C. crataegi* with rates of 4.36, 3.84, 2.77 and 1.67%, respectively. No phytoplasma were detected in *C. pyrisuga*, *C. pyricola*, or the other *Cacopsylla* spp. '*Ca*. P. mali' was detected in *C. picta*, *C. melanoneura-C. affinis* complex and *C. pyri*; '*Ca*. P. pyri' was detected in *C. picta*, *C. crataegi*, *C. melanoneura-C. affinis* complex and *C. pyri* individuals. To our knowledge, this is the first report on the possible psyllid vectors of '*Ca*. P. mali' in Turkey.
Keywords: Fruit tree diseases, apple proliferation, pear decline, epidemiology, *Cacopsylla* spp.

INTRODUCTION

Fruit tree diseases caused by phytoplasma have great economic effects on fruit production, especially in Europe. A major phytoplasma is '*Candidatus* Phytoplasma mali', which causes apple proliferation (AP) disease mainly in cultured and wild forms of apple trees (Seemuller *et al*., 2011a); however, there has been a report of different hosts, including *Prunus avium* (L.) L., *P. armeniaca* L., and *P.domestica* L. (Mehle *et al*., 2007). '*Ca.* Phytoplasma pyri' causes pear decline (PD) disease that is found mainly in cultured and wild forms of pear (*Pyrus* spp.) and quince (*Cydonia oblonga* Mill.) (Seemüller *et al*., 2011b). These phytoplasma are taxonomically classified into the 16SrX group (or AP-group) of phytoplasmas and constitute closely related subgroups (Seemüller and Schneider, 2004).

Phytoplasmas are mainly spread by vegetative propagation or the grafting of infected plant material and phloem feeding insects, primarily leafhoppers, planthoppers and psyllids (Weintraub and Beanland, 2006). Only one genus of this last one, *Cacopsylla* spp., transmit AP-group phytoplasmas to pome and stone fruit trees. In apple orchards, *'Ca*. P. mali' can be transmitted by two psyllid species. *Cacopsylla (Thamnopsylla) picta* (Foerster, 1848) (syn. *C. costalis*) has been reported main vector in Germany (Jarausch *et al.*, 2003, 2011) and northern Italy (Frisinghelli *et al*., 2000; Carraro *et al*., 2008), while *Cacopsylla (Thamnopsylla) melanoneura* (Foerster, 1848) was identified as main vector in Aosta Valley (Tedeschi *et al*., 2002). *C. picta* is monophagous on *Malus* spp. and until now this species have been described only in Europe (Burckhardt, 1994; Ossiannilsson, 1992; Ouvrard, 2014) and Turkey (Klimaszewski and Lodos, 1977, 1979; Drohojowska and Burckhardt, 2014). *C. melanoneura* has a Palaearctic distribution and is oligophagous on *Rosaceae*, its principal host plant being a common shrub, hawthorn (*Crataegus monogyna* L.) (Ouvrard, 2014). In most of studied cases, both psyllid species are present in apple orchards (Jarausch *et al*., 2003; Delic *et al*., 2005; Carraro *et al*., 2008; Mattedi *et al*., 2008). Two others species living on hawthorn, *Cacopsylla peregrina* (Foerster, 1848) and *Cacopsylla (Thamnopsylla) affinis* (Low, 1880), were found able to harbor the phytoplasmas of the AP-group, in particular *'Ca*. P. mali' (Tedeschi *et al*., 2009). Their transmission ability was not proven but this result highlights the potential role as vector of these psyllid species. In pear orchards, until recently, two psyllid species were known as vector of '*Ca*. P. pyri'. *Cacopsylla (Hepatopsylla) pyricola* (Foerster, 1848) has been reported for Great Britain (Davies *et al*., 1992) and North America (Jensen *et al*., 1964), while *Cacopsylla (Hepatopsylla) pyri* (Linne, 1758) was described as the vector in France (Lemoine 1984), Italy (Carraro *et al*., 1998a) and Spain (Garcia-Chapa *et al*., 2005). *C. pyri* is widespread in Europe, in the Caucasus, Georgia, the Middle Asia, including Turkey (Klimaszewski and Lodos, 1979; Burckhardt and

Hodkinson, 1986; Ossiannilsson, 1992; Burckhardt and Onuçar, 1993; Güçlü and Burckhardt, 1996; Ulubaş Serçe *et al*., 2006; Drohojowska and Burckhardt, 2014), while *C. pyricola* naturally occurs in the whole Palaearctic, from Europe to eastern Siberia, south Korea and Japan (Burckhardt and Hodkinson, 1986; Ossiannilsson, 1992; Inoue, 2010; Ouvrard, 2014) and was introduced into the eastern United States in the early 1800s. The morphological identification of the *Pyrus*-feeding psyllids must be considered with a particular attention. In west Palaeartic, they form a complex of related but distinct seven species with overlapping geographical distributions (Ouvrard, 2014) and entomologists have usually applied names *pyri* or *pyricola* to all members of the complex and have failed to recognize morphological and biological differences between the species (Burckhardt and Hodkinson, 1986). Typically, Burckhardt and Onuçar (1993) have shown that the records of *C. pyricola* from Turkey (Klimaszewski and Lodos, 1977, 1979) were misidentifications and concerned *Cacopsylla (Hepatopsylla) notata* (Flor, 1861). Another uncertainty could be *Cacopsylla chinensis* (Yang and Li, 1981), a psyllid responsible of severe damages in pear orchards in Taiwan and China (Yang *et al.*, 2004; Lee et al., 2008) and recently introduced in Japan (Katoh *et al*., 2013); comparisons of COX1 sequences prove that this species cannot be confused with the west Palaeartic *C. pyri* (Sauvion com. pers.). Others *Pyrus*-feeding psyllids have been still neglected as potential vectors of '*Ca*. P. pyri', in particular, *Cacopsylla (Thamnopsylla) pyrisuga* (Foerster, 1848) and *Cacopsylla (Hepatopsylla) bidens* (Šulc, 1907), two species reported from Turkey (Burckhardt and Hodkinson, 1986; Burckhardt and Onuçar, 1993; Ozgen *et al*., 2012; Drohojowska and Burckhardt, 2014).

The presence of fruit tree diseases caused by phytoplasma in Turkey was first reported in 1999 (Çağlayan and Gazel, 1999). Phytoplasma diseases have been reported in most of the germplasm sources and commercial orchards in the Mediterranean, Marmara and Central Anatolia regions (Sertkaya *et al*., 2005; Ulubaş Serçe *et al*., 2006; Gazel *et al*., 2007; Canik and Ertunç, 2007). Despite of the economic impact of these phytoplasma diseases, there is very few information on the vectors responsible of their spread. Only *C. pyri* individuals from Bursa collected on a local pear cultivar (cv. Deveci) were detected with AP-phytoplasma, and there are no data available on '*Ca*. P. mali' vectors in Turkey (Ulubaş Serçe *et al*., 2006). By a large scale sampling, this work highlights the different psyllid species present on apple, pear and wild host plants in Turkey and shows the potential spread risk of '*Ca*. P. pyri' and '*Ca*. P. mali' by several of these species.

MATERIALS AND METHODS

***Sampling and identification of psyllids*:** Surveys were performed in the Eastern Mediterranean (Adana, Mersin, Hatay and Kahramanmaraş), Marmara (Bursa and Yalova), Central Anatolia (Ankara and Niğde) and Eastern Anatolia (Malatya and Erzurum) regions where phytoplasma infections were previously reported and/or pome fruit growing is very important (Fig. 1). Psyllids were collected during 2010-2011 from cultivated, wild pome and stone fruit species (*Malus* spp., *Pyrus* spp., *Crataegus* spp. and *Prunus* spp.) in early spring and in overwintering sites on conifers (pine, fir, cedar) from late summer to autumn. They were captured using mouth exhausters and from 0.5 m^2 white trays placed under tree branches that were then struck repeatedly. The psyllids were placed in different falcon tubes and labeled according to the host and province. Then, the insects were conserved in 95% alcohol at 4°C for morphological and molecular analysis. For a correct identification of the specimens, different keys have been used: the handbooks published by Hodkinson and White (1979), and Ossiannilsson (1992); an electronic key focused on the Central European *Cacopsylla* spp. developing on Rosaceae (Burckhardt, 2007); and a short illustrated diagnoses of the adults of the three species associated with apple (Burckhardt and Lauterer, 2009). The differentiation of the both species *C. melanoneura* and *C. affinis* was problematic: females cannot be distinguished on morphology and the males could be discriminated only by observation of the apical part of the aedeagus and the shape of the parameres, yet these characters show inter-individual variability that could be source of errors. So, we have used the primers designed by Tedeschi and Nardi (2010) to tentatively discriminate the psyllids previously identified *C. affinis* or *C. melanoneura* by morphological characters, but unsuccessfully. Finally we gave up differentiating the two species and denoted them as the *C. melanoneura-C. affinis* complex.

***PCR and RFLP analysis of phytoplasma from individual psyllids*:** Total DNAs were extracted from whole psyllids individually using the High-Salt method based on TNES (10 mM Tris, pH 7.5, 400 mM NaCl, 100 mM EDTA, 0.6 % SDS) buffer in semi-deep well plates (Peccoud *et al*., 2013). DNA was diluted in 60 µL of sterile water and stocked at -20°C before to be used for phytoplasma detection by PCR analysis. Phytoplasma-specific primers P1 5′-AGAGTTTGATCCTGGCTCAGGA-3′ (Deng and Hiruki, 1991) and P7 5′-CGTCCTTCATCGGCTCTT-3′ (Smart *et al*., 1996) amplifying an approximately 1,800 bp fragment were used for general identification of phytoplasma by conventional PCR. Nested PCR assays were employed using the universal primer pair for the AP group, F01 5′-CGGAAACTTTTAGTTTCAGT-3′ and R01 5′-AAGTGCCCAACTAAATGAT-3′, designed to amplify a 1,050 bp portion of the16S rRNA gene (Lorenz *et al*., 1995). Nucleic acid samples were diluted in sterile deionized water to obtain a final concentration of 20 $ng.\mu L^{-1}$. Conventional PCR and nested PCR reactions were performed in mixtures with 50 µL final volumes containing 5 µL 10×PCR buffer

(100 mM Tris-HCl pH 8.3, 500 mM KCl, 0.01% gelatin) 1.5 mM $MgCl_2$, 250 µM each dNTP, 20 pmol.µL^{-1} each primer, 2 U *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania) and 1 µL DNA. Conventional PCR products were diluted to 1:50 and 1 µL DNA was used for nested PCR. The following amplification conditions were used: first cycle 94°C for 2 min; 35 cycles 94°C for 1 min (30 s for nested PCR) denaturation, 55°C for 2 min (30 s for nested PCR) annealing, 72°C for 3 min (1 min for nested PCR) extension, and final cycle 72°C for 5 min. Nested PCR products, including reference isolates of the phytoplasma 16S rDNA sequence, were subjected to RFLP analysis. PCR products (10 µL) were separately digested overnight at 37°C with restriction endonucleases *Rsa*I and *Ssp*I (Fermentas). The digested products were analyzed by electrophoresis using 2% agarose gels, which were then stained with ethidium bromide. The products were visualized with an ultraviolet transilluminator and then photographed.

Phytoplasma-infected positive control plant materials (ESFY, AP and PD isolates) were kindly provided from Dr. B. Schneider (Germany).

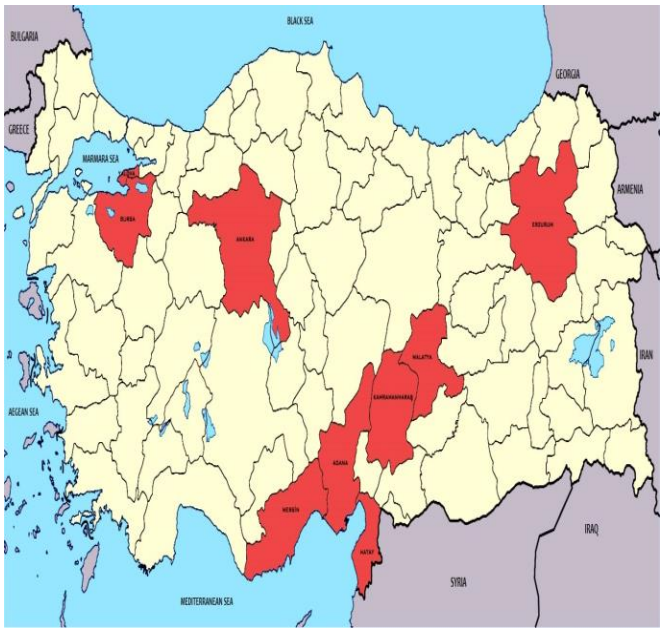

Figure 1. Surveyed provinces where potential psyllid vectors of apple proliferation and pear decline diseases were collected.

RESULTS

Psyllids were collected from *Malus* spp., *Pyrus* spp., *Cydonia oblonga*, as well as surrounding wild vegetation, mainly *Crataegus* spp., wild *Pyrus* spp. and some conifer species. The first *Cacopsylla* spp. individuals were found on hawthorn at the altitude of 950 m and on apple trees at the altitude of 1,390 m. in the second half of April in Mersin province. In the fallow months, different *Cacopsylla* spp. was captured from cultured and wild forms of pome fruit trees in different provinces. Collected psyllids were identified morphologically as *Cacopsylla crataegi* (Schrank, 1801), *C. picta*, *C. melanoneura-C. affinis* complex, *C. pyrisuga*, *C. pyri*, *C. pyricola* and *Cacopsylla* spp. (Table 1).

C. picta individuals were collected from apple, pear and hawthorn plants in all sampling provinces and this was the most abundant species of psyllid. They were found only on apple trees in overwintering (OW) and new generation (NG) forms. *C. melanoneura-C. affinis* complex and *C. crataegi* were found only in the OW form on different wild and cultivated plants during the survey period.

C. pyri individuals were collected from pear, quince, apple and plum trees in the OW and NG forms. It was the most abundant *Cacopsylla* spp. among the possible vectors of PD disease. *C. pyricola* and *C. pyrisuga* were not as widespread as *C. pyri* in the sampling provinces.

A PCR analysis of these psyllids showed that the highest phytoplasma infection rate was in *C. picta* followed by *C. pyri*, *C. melanoneura-C. affinis* complex and *C. crataegi* with rates of 4.36, 3.84, 2.77 and 1.67%, respectively. No phytoplasma infection was detected in *C. pyrisuga*, *C. pyricola* or other uncharacterized *Cacopsylla* spp. (Table 1, Figure 2). Phytoplasma were detected in the OW and NG forms of different psyllid species but no phytoplasma were detected in any *Cacopsylla* spp. collected from conifers.

Phytoplasma-infected *C. picta* were only found in Mersin, which had a high psyllid incidence among the sampled provinces (Table 2). A similar correlation between the high psyllid population and the phytoplasma infection rate was also observed for *C. melanoneura-C. affinis* complex and *C. crataegi*, which were collected from different provinces.

RFLP profiles of PCR products from *C. picta* individuals and amplified by the F01-R01 primer pair exhibited '*Ca*. P. mali' patterns. Interestingly, single '*Ca*. P. prunorum' and '*Ca*. P. pyri' RFLP profiles were obtained from the *C. picta* collected from an apple tree in Mersin province. One PCR amplicon obtained from a *C. crataegi* collected in Erzurum province also showed the '*Ca*. P. pyri' RFLP pattern (Figure 3). '*Ca*. P. pyri' was detected in *C. pyri* samples collected from pear and in five *C. melanoneura-C. affinis* complex collected from hawthorn and blackthorn trees. Interestingly, two *C. pyri* individuals exhibited '*Ca.* P. prunorum' profiles and one individual had a '*Ca.* P. mali' RFLP profile (Fig. 4,5; Table 3).

Cacopsylla pyri samples collected from plum, quince and apple trees were not infected by any phytoplasma. Among the phytoplasmas-infected psyllids, both genders had similar infection rates although more female psyllids were captured.

Table 1. The *Cacopsylla* species collected from different provinces of Turkey and the phytoplasma infection rate in psyllids

Collected *Cacopsylla* species	Province	Plant species	Stage	Infected/total tested				Infection rate (%)
				Female	Male	Nymph	Total	
C. picta	Mersin	Apple	OW	5/151	5/112	0	10/263	3.80
	Mersin	Apple	NG	2/33	1/26	1/3	4/62	6.45
	Mersin	Hawthorn	OW	0/4	1/4	0	1/8	12.50
	Bursa	Pear	OW	0/3	0/5	0	0/8	0
	Niğde	Apple	OW	0/2	0/1	0	0/3	0
Total							15/344	4.36
C. melanoneura- C. affinis complex	Bursa	Wild plum	OW	0/1	0	0	0/1	0
	Bursa	Hawthorn	OW	0/17	0/6	0	0/23	0
	Bursa	Blackthorn	OW	1/13	1/10	0	2/23	8.69
	Bursa	Fir	OW	0/20	0/11	0	0/31	0
	Erzurum	Hawthorn	OW	1/43	1/35	0	2/78	2.56
	Hatay	Wild plum	OW	0/2	0	0	0/2	0
	Hatay	Pine	OW	0/2	0	0	0/2	0
	Hatay	Hawthorn	OW	0/1	0	0	0/1	0
	Adana	Hawthorn	OW	3/48	0/43	0	3/91	3.29
Total							7/252	2.77
C. crataegi	Hatay	Hawthorn	OW	0/8	0/10	0	0/18	0
	Hatay	Wild plum	OW	0/1	0	0	0/1	0
	Hatay	Pine	OW	0/9	0/6	0	0/15	0
	Hatay	Cedar	OW	0/1	0/1	0	0/2	0
	Bursa	Blackthorn	OW	0	0/1	0	0/1	0
	Erzurum	Hawthorn	OW	1/9	0/10	0	1/19	5.26
	Kahramanmaraş	Hawthorn	OW	0/2	0	0	0/2	0
	Malatya	Hawthorn	OW	0/1	0/1	0	0/2	0
Total							1/60	1.67
C. pyrisuga	Bursa	Hawthorn	OW	0/1	0	0	0/1	0
	Bursa	Fir	OW	0/1	0	0	0/1	0
	Erzurum	Pear	OW	0/8	0/4	0	0/12	0
Total							0/14	0
C. pyri	Adana	Quince	OW	0/3	0/1	0	0/4	0
	Ankara	Pear	OW	1/37	0/28	0	1/65	1.54
	Malatya	Pear	OW	0/1	0	0	0/1	0
	Yalova	Pear	OW	8/55	2/26	1/22	11/103	10.68
	Mersin	Plum	NG	0/3	0	0	0/3	0
	Niğde	Apple	NG	0	0/2	0	0/2	0
	Niğde	Pear	NG	2/117	1/78	0	3/195	1.54
	Bursa	Pear	OW	2/45	2/76	0	4/121	3.30
Total							19/494	3.84
C. pyricola	Hatay	Pear	NG	0/6	0/5	0	0/11	0
	Hatay	Pine	OW	0	0/1	0	0/1	0
	Adana	Quince	OW	0/1	0/1	0	0/2	0
Total							0/14	0
Cacopsylla spp.	Malatya	Hawthorn	OW	0/12	0/4	0	0/16	0
	Malatya	Unknown	OW	0/29	0/11	0	0/40	0
	Malatya	Wild plum	OW	0/3	0	0	0/3	0
Total							0/59	0
OVERALL TOTAL				26/693	14/519	2/25	42/1237	

OW: Overwintered, NG: New generation

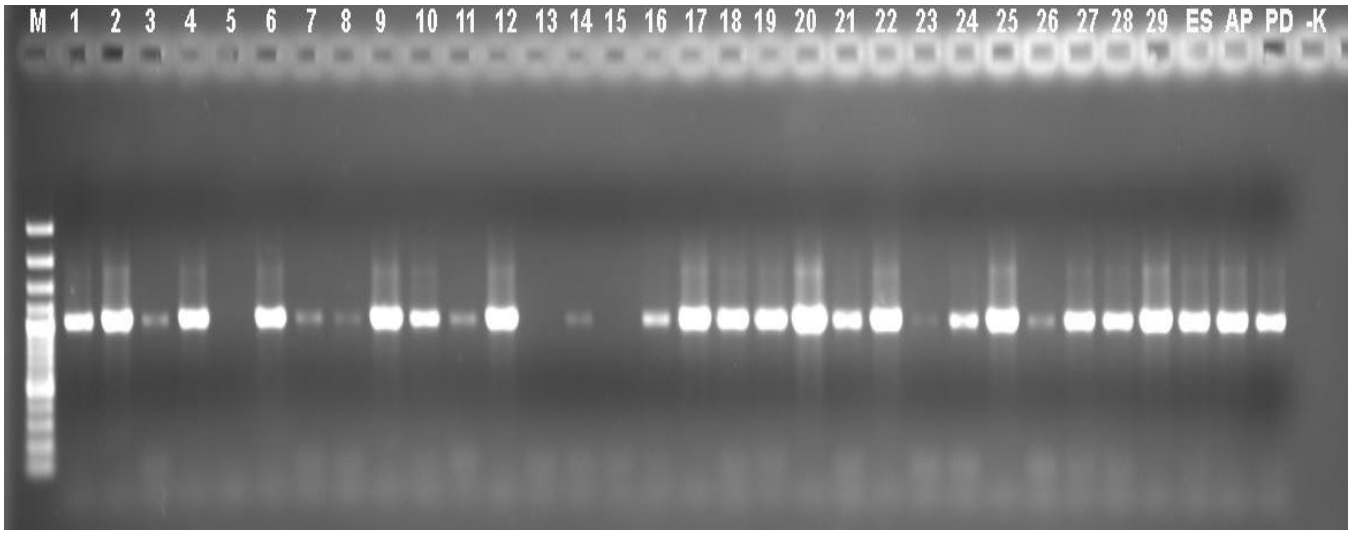

Figure 2. PCR analysis using the F01-R01 primer pair to the 16SrX rRNA apple proliferation group of phytoplasma in *Cacopsylla picta* (lines 1–13), *Cacopsylla crataegi* (lines 14–15), and *Cacopsylla pyri* (lines 16–29) DNAs. M, Gene Ruler DNA ladder mix (MBI Fermentas); ES, '*Candidatus* Phytoplasma prunorum'; AP, '*Candidatus* Phytoplasma mali' and PD, '*Candidatus* Phytoplasma pyri' positive controls; and -K, water control.

Table 2. Prevalence of *Cacopsylla* species collected from different provinces of Turkey and their infection rates by apple proliferation phytoplasma group.

Provinces	Collected psyllid species					
	C. picta		*C. melanoneura - C.affinis* complex		*C. crataegi*	
	Prevalance (%)	Infec.rate (%)	Prevalance (%)	Infec.rate (%	Prevalance (%)	Infec.rate (%)
Mersin	96.80	4.50	-	-	-	-
Bursa	2.33	0.00	30.95	2.56	1.67	0.00
Niğde	0.87	0.00	-	-	-	-
Hatay	-	-	1.98	0.00	60.00	0.00
Adana	-	-	36.11	3.29	-	-
Erzurum	-	-	30.95	2.56	31.67	5.26
K.Maraş	-	-	-	-	3.33	0.00
Malatya	-	-	-	-	3.33	0.00
Total	100	4.36	100	2.77	100	1.67

-: no insect found

Table 3. The number of different *Cacopsylla* species carrying the phytoplasmas belonging to 16SrX group.

	Ca. P. prunorum	*Ca.* P. mali	*Ca.* P. pyri	Total numbers of phytoplasma infected psyllids
C. picta	1	13	1	15
C. melanoneura-C. affinis complex	0	2	5	7
C. crataegi	0	0	1	1
C. pyri	2	1	16	19
C. pyrisuga	0	0	0	0
C. pyricola	0	0	0	0

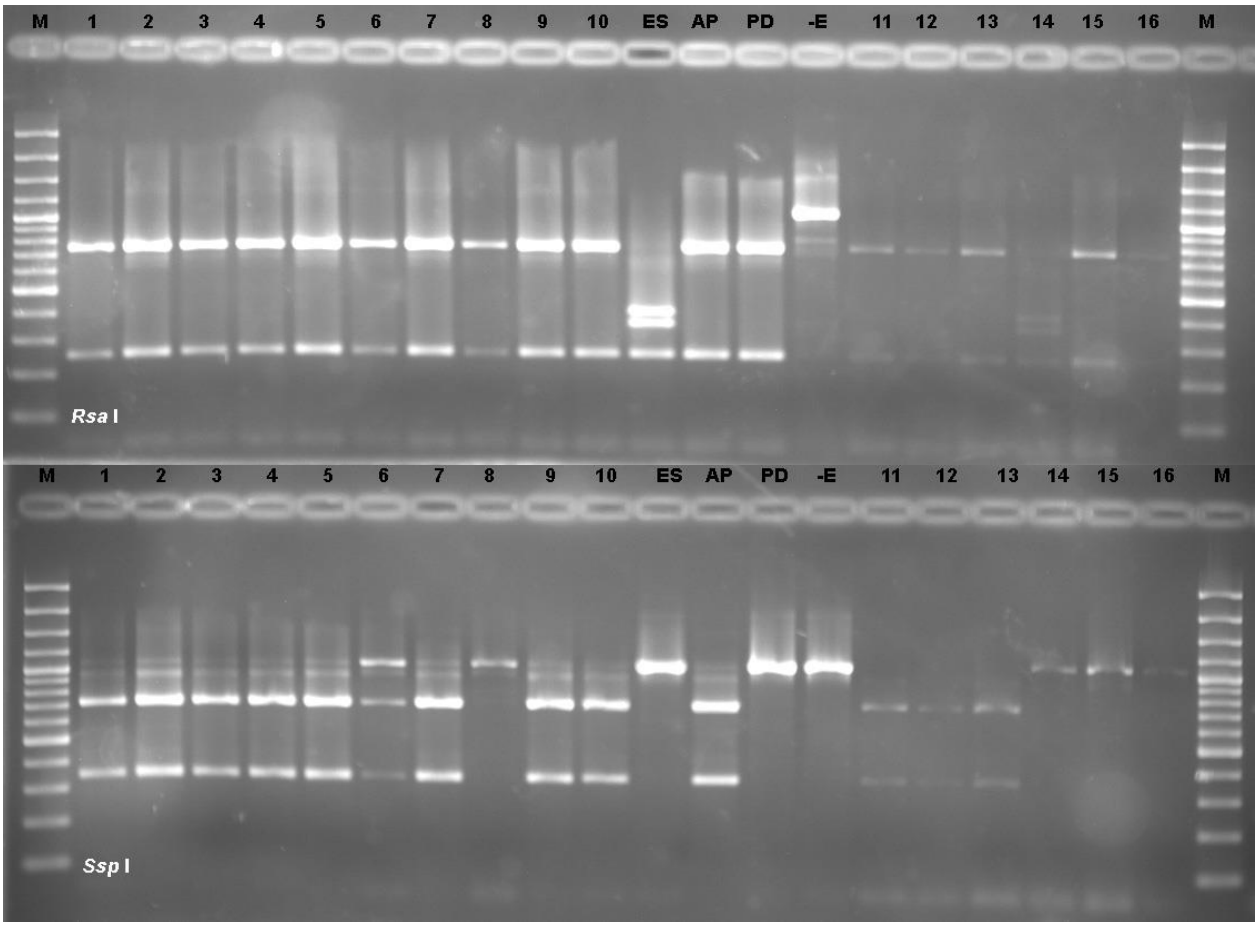

Figure 3. Restriction analysis, using *Rsa*I and *Ssp*I restriction enzymes, of PCR amplicons generated by the F01-R01 primer pair to the 16SrX rRNA apple proliferation group of phytoplasma. The phytoplasma were isolated from *Cacopsylla picta* (lines 1–14, 16) and *Cacopsylla crataegi* (line 15). M, Gene Ruler DNA ladder mix (MBI Fermentas); ES, '*Candidatus* Phytoplasma prunorum'; AP, '*Candidatus* Phytoplasma mali' and PD, '*Candidatus* Phytoplasma pyri' positive controls; and -E, control without enzyme.

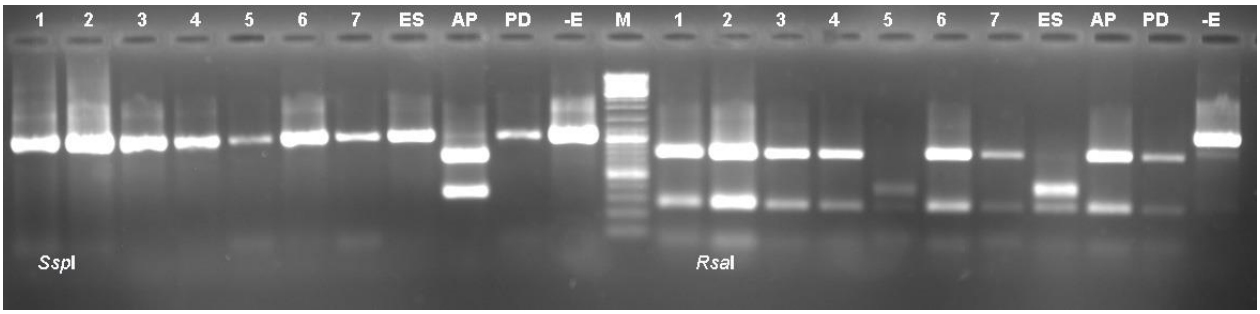

Figure 4. Restriction analysis, using *Rsa*I and *Ssp*I restriction enzymes, of PCR amplicons generated by the F01-R01 primer pair to the 16SrX rRNA apple proliferation group of phytoplasma. The phytoplasma were isolated from *Cacopsylla melanoneura-C.affinis* complex collected from hawthorn (lines 1, 2,6) and blackthorn (lines 3, 4) and *Cacopsylla pyri* collected from pear (lines 5, 7). M, Gene Ruler DNA ladder mix (MBI Fermentas); ES, '*Candidatus* Phytoplasma prunorum'; AP, '*Candidatus* Phytoplasma mali' and PD, '*Candidatus* Phytoplasma pyri' positive controls; and -E, control without enzyme.

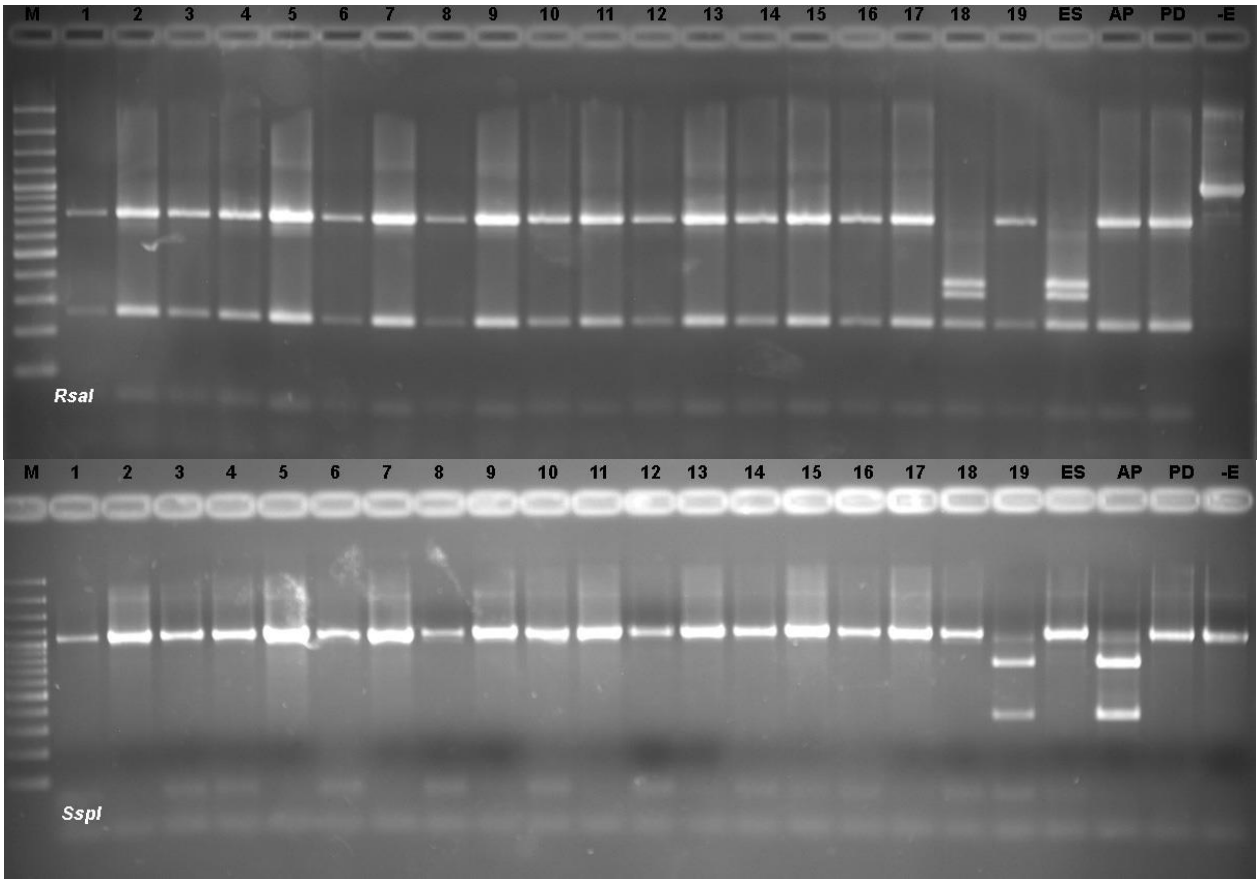

Figure 5. Restriction analysis, using *Rsa*I and *Ssp*I restriction enzymes, of PCR amplicons from phytoplasma isolated from *Cacopsylla pyri* and generated by the F01-R01 primer pair to the 16SrX rRNA apple proliferation group of phytoplasma. The M, Gene Ruler DNA ladder mix (MBI Fermentas); ES, '*Candidatus* Phytoplasma prunorum'; AP, '*Candidatus* Phytoplasma mali' and PD, '*Candidatus* Phytoplasma pyri' positive controls; and -E, control without enzyme.

DISCUSSION

In total five psyllid species, *C. picta*, *C. crataegi*, *C. pyrisuga*, *C. pyri* and *C. pyricola* and a psyllid complex of *C. melanoneura*-*C. affinis*, known as potential vectors of phytoplasmas that cause AP and PD diseases were collected from cultivated and wild varieties of stone and pome fruit trees as well as conifers in Turkey. There were already some reports on the presence and distribution of *C. picta* on *Malus* spp. (Klimaszewski and Lodos, 1977, 1979); *C. pyri* and *C. pyricola* on *Pyrus* spp., *Malus* spp. and *Armeniaca vulgaris* (Klimaszewski and Lodos, 1979; Burckhardt and Onuçar, 1993); *C. affinis* on *Mespilus* spp., *Pyrus* spp. and *Crateagus* spp. (Burckhardt and Onuçar 1993) and *C. pyrisuga* on *Solanum melongena* (Burckhardt and Hodkinson, 1986; Burckhardt and Onuçar, 1993). To our knowledge, until now there were no report on *C. crataegi* and *C. melanoneura* -*C. affinis* complex in Turkey.

Despite already being reported in Turkey, there was no detailed study on the *Cacopsylla* species as vectors. Most psyllid studies focused on *C. pyri,* which is a very important pest in the pear orchards of Turkey. These studies were mainly concerned with its life cycle, its tolerance to different pear cultivars and the biological management of this insect (Gençer, 1999; Yanik and Uğur, 2004; Erler, 2004a; Erler, 2004b). Studies related to *C. pyri* as a vector of PD disease were also recently reported (Gazel *et al*., 2007; Çağlayan *et al*., 2010). However, there have been no reports on the possible vectors of AP disease. In this study, the prevalence of different *Cacopsylla* species as potential vectors of AP in different hosts and provinces was reported for the first time.

The phytoplasma infection rate in *C. pyri* individuals in this study was fairly low at 3.84%, whereas in a previous study in Turkey it was reported as 17.5% (Çağlayan *et al*., 2010). Additionally, the '*Ca*. P. pyri' infection rate was reported as 55% in *C. pyri* individuals in Italy (Carraro *et al*., 1998b; 2001). This difference may be because of different phytoplasma strains or pear cultivars in the two countries.

Despite the widespread distribution of *C. pyricola* in Turkey (Lodos, 1986), the presence of *C. pyricola* and *C. pyrisuga* was very rare in the regions investigated in this study and no phytoplasma were detected in these insect species. Previously, naturally infected *C. pyrisuga* individuals were found by Kucerova *et al*. (2007), but their ability to transmit phytoplasma has not been proven until now (Jarausch and Jarausch, 2010). *C. pyricola* was described as the vector of PD in North America and England (Jensen *et al*., 1964; Hibino *et al*., 1971; Davies *et al*., 1992), while *C. pyri* is considered as the main vector in the rest of Europe (Carraro *et al*., 2001; Garcia-Chapa *et al*., 2005). Our present results seem to confirm these previous observations.

The phytoplasma infection rate in *C. picta* individuals varied according to their locations (provinces) and hosts. In our study, *C. picta* infection rate was 4.36% and this low rate could be correlated to the low incidence of AP disease in Turkey (Çağlayan *et al*., 2014). In Germany, the infection rate has been reported as 10% in OW psyllids collected from apple orchards (Mayer *et al*., 2009) and a high infection rate of '*Ca*. P. mali' (40%) in apple trees was detected (Bliefernicht and Krczal, 1995). The highest prevalence and infection rate for '*Ca.* P. mali' was found in *C. picta* individuals among the *Cacopsylla* spp. collected in the surveyed provinces. Although most of the *C. picta* individuals were infected by '*Ca*. P. mali', unexpectedly, single '*Ca*. P. prunorum' and '*Ca*. P. pyri' RFLP profiles were obtained. Similarly, the RFLP analysis of amplified fragments from *C. melanoneura-C. affinis* complex individuals exhibited '*Ca*. P. mali' and '*Ca*. P. pyri' profiles. *C. melanoneura-C. affinis* complex and *C. crataegi* showed only '*Ca*. P. pyri' profiles. Studies in different countries on *C. melanoneura* psyllids collected from hawthorn plants found they were infected by '*Ca*. P. pyri' as well as '*Ca*. P. mali' (Tedeschi *et al*., 2009; Tedeschi and Nardi, 2010). Out of 21 *C. affinis* psyllids collected from hawthorn in Italy, one has been reported as infected by '*Ca*. P. pyri' phytoplasma. In another study, '*Ca*. P. prunorum' was identified in *C. affinis* (Tedeschi *et al*., 2009; Tedeschi and Nardi, 2010). Even though some of the *Cacopsylla* spp. are specific to hosts, they might visit different hosts and acquire different phytoplasma species during feeding activity. According to our results, *C. picta* is a good candidate as a potential vector of '*Ca*. P. mali' in Turkey. However, other psyllids collected from apple and hawthorn were always found to be phytoplasmas free. This result was supported by a study from Germany that found the infection rate of *C. melanoneura* by '*Ca*. P. mali' was very low at 0.5–0.6% and the psyllid failed in both acquisition and transmission of this phytoplasma (Mayer *et al*., 2009).

***Conclusion*:** In this study, it has been confirmed that *C. pyri* is the main vector of '*Ca.* P. pyri' and that *C. picta* is the main potential vector of '*Ca*. P. mali', which has been reported in Turkey for the first time. To give more data on the epidemiological aspect of diseases caused by phytoplasma, these results should be supported by experimental transmission trials.

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