

DNA Barcoding Data of Aphids (Hemiptera: Aphidomorpha) in Safflower (*Carthamus tinctorius* L.) with New Host Plant Records in Turkey

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is an important oilseed plant that is grown as vegetable oil source in the world and in Turkey due to its use as a quality edible oil and biodiesel raw material obtained from its seeds. In Ankara and Eskişehir Provinces, in areas where are safflower dense plantations, aphids were sampled from the leaves, stems and roots of each plant observed once or twice a week between May-June and September-October in 2015-2017.

As a result of the study, it was determined that there are infested with harmful aphids in safflower plantations areas. Eight aphid species were identified morphologically; *Aphis craccivora*, *A. fabae*, *Brachycaudus (Prunaphis) cardui*, *B. helichrysi*, *Myzus persicae*, *Uroleucon aeneum*, *U. carthami* and *U. jaceae*. *Brachycaudus (P.) cardui* and *Uroleucon aeneum* were recorded on safflower for the first time. In addition, DNA barcoding of safflower associated aphids were performed for confirming the identification of aphids and COI sequences for the pest aphids were uploaded to the BOLD database. Identification key to *Carthamus tinctorium*-feeding aphids in Turkey based on apterous viviparous females is provided.

Key words: Safflower, Aphididae, new host plant record, Central Anatolia, COI, mtDNA.

INTRODUCTION

The aphids (Hemiptera: Aphidomorpha), which represents an important group with its vector ability among plant pests, are polymorphic species and cause serious losses in terms of both quality and quantity by stinging and absorbing them in many cultured plants of economic importance. Aphids are among the important plant pests in our country as well as all over the world. Many of them feed on cereals, fruits and vineyards, forest trees, park and ornamental plants, causing significant damage. Especially the damage they give to agricultural products due to their primer and seconder damage is also economically important as product loss. Oilseed plants are plants having strategic importance in human and animal nutrition due to the vegetable oils, proteins, carbohydrates, minerals and vitamins they contain. The remaining pulps of oilseed plants after obtaining fat are an absolute source of protein for meat, milk and egg production (Anonymous, 2021). Due to these crucial properties, oilseed plants constitute the main source of both vegetable oil and mixed feed sector. In recent years, biodiesel sectors have been included in these two main sectors. The most widely known oilseed is sunflower in Turkish conditions. However, the land and climatic conditions of Turkey are extremely suitable for oilseed production in different alternatives. Soy, rapeseed and safflower stand out as alternative sources of oilseeds for Turkey (Anonymous, 2021).

The Safflower (*Carthamus tinctorius* L.) is annual oil plant with a pile root system, with between 30-50% fat in its seeds, whose oil can be used to make biodiesel, whose pulp is considered as animal feed (Öğüt & Oğuz, 2005). Turkey's agricultural potential is increasing day by day due to its characteristics such as being a potential source of raw materials for vegetable oil and mixed feed sectors, being able to grow in alternative areas, entering into debate, evaluating barren areas (İşler & Karaosmanoğlu, 2010).

Global climate change leads to negative consequences in nature, while it produces positive changes in some groups of insects, such as aphids. In order to monitor the effects of global warming and climatic changes, it is necessary to know the current status of the species, their spread, the complexes they have created in different ecologies and even their past situations (Görür, 2008). Aphids are among the important plant pests in Turkey as well as all over the world.

There are 5668 species of 733 genera in the world, while 591 aphid species are known from Turkey (Favret, 2021; Blackman & Eastop, 2021; Kök & Özdemir, 2021). The following 20 aphid species are reported on *Carthamus tinctorius* in the world: *Acyrtosiphon ilka*, *Amphorophora* sp., *Aphis craccivora*, *A. fabae*, *A. gossypii*, *Aulacorthum solani*, *Brachycaudus helichrysi*, *Capitophorus elaeagni*, *Macrosiphum euphorbiae*, *Myzus persicae*, *Protaphis anuraphoides*, *P. carthami*, *P. pseudocardui*, *Protrama flavescens*, *Sitobion akebiae*, *Uroleucon carthami*, *U. compositae*, *U. gobonis*, *U. jaceae* and *U. sonchi* (Holman, 2009; Blackman & Eastop, 2021).

The aphids are named afit, püseron, zenk, cute, kezbi, kevzi in Turkey. They are important due pest insect to its negative effects on the vegetative development and productivity of plants. Especially the effect due to the primary and secondary

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damages in agricultural plants causes harvest loss and this is economically important. Aphids secrete saliva during feeding in the plant tissue, which causes molting or over proliferation of cells. This secretion, called honey substance, offers a suitable fattening environment for the development of saprophyte fungi in temperate regions, forming blackbal disease or fumajin. Aphids are the most important vector groups carrying plant virus diseases (Toros, 1973; Blackman & Eastop, 2021).

In agricultural management and plant quarantine, a rapid and accurate aphid identification to the species level is a critical task. However, high taxonomical expertise is required and morphological identification methods are time-consuming (Hebert, Cywinska, Ball, deWaard, 2003). Polymorphism, morphological plasticity, cryptic species and damaged specimens and immature stages are the other challenges. Molecular methods with easy DNA-based identification tool called DNA barcoding have been performed to resolve this problem (Hebert et al, 2003). The DNA barcoding identifies target species using short DNA sequences as barcodes (Hebert et al, 2003), a 658-bp fragment of the mitochondrial cytochrome c oxidase (CO1) gene. Since DNA Barcoding is an emerging tool, databases should be constructed on the basis of specimens identified by specialists to make identification comprehensive and reliable (Jalali, Rakshit, & Venkatesan, 2015).

MATERIAL AND METHODS

Material

In 2015-2017, aphids were sampled from the areas where safflower plant cultivation is carried out in Ankara and Eskişehir province. The specimens were studied using a LEICA DM LB2 compound light microscope and morphological characters were measured using LAS 4.1 version software. Measurements of morphological characters were done according to Blackman & Eastop (2006, 2021).

Methods

Field studies

Sampling was carried out in Ankara and Eskişehir Provinces and districts, where safflower plant were sowed densely. Collected specimens localities were given. Each locality shows safflower field areas which were given with their size in the result part. The samples were collected from the leaves, stems and root parts of each plant observed infested with pests. The samplings were performed once a week or every two weeks between May-June and September-October, and in other months when necessary according to suitable the climatic situation. The samplings were performed between 2015 and 2017.

The aphid and infested host plant organs were cut with pruning shears, first wrapped in a piece of paper to remove moisture. Then the samples were put in polyethylene bags and wrote the date, place and host plant number on them, and these bags were placed in the ice box and brought to the laboratory. The adults in the samples were

placed into 96% alcohol and the nymphs were maintained in the cages with the plant parts (16:8) under controlled conditions (16: 8 D/L, 25±2°C and a 70±10% H) , until they became adults. After the alate and aptera individuals reached to the adult period, they were placed into 96% ethyl alcohol for identification.

Laboratory studies

Slide mounting of aphids were done using the method applied by Hille Ris Lambers (1950).

Molecular studies

DNA of the species was extracted by dissecting abdomen and three pairs legs and using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) with slight modifications as described by Magoga et al (2016). Extracted DNA was used as template for a 658-bp fragment of the mitochondrial CO1 gene amplified by PCR using universal primers LCO1490 (5'-GGT CAA CAAATC ATAAAG ATA TTG G) and HCO2198 (5'-TAAACT TCA GGG TGA CCAAAAAT CA) (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). PCR reactions were performed in a 25µl final volume reaction mix and PCR thermal profile as in Montagna et al (2017). Successful amplifications were determined by gel electrophoresis and sequenced bidirectionally by ABI Technology (Applied Biosystems, Foster City, CA, USA). The electropherograms obtained were manually edited, checked for double peaks and frameshifts by using Geneious Pro 5.5 (Biomatters Ltd., Auckland, New Zealand), and primers were removed. Each sequence was translated to protein in the EMBOSS transeq tool (www.ebi.ac.uk/Tools/emboss/transeq) to be sure that they complied with an open reading frame. All the COI sequences obtained in this study were clustered using the Barcode Index Number (BIN) in BOLD (Ratnasingham & Hebert, 2013). Furthermore, a dataset composed of our sequences and sequences of close species to our three species mined from BOLD database was built. A neighbor joining cluster analysis (NJ; Saitou & Nei, 1987) was performed for a representing the genetic differences between sequences and clusters of sequences in the dataset by using MEGA 6 (Molecular Evolutionary Genetics Analysis Version 6.0). *Myzus persicae* (Sulzer, 1776) was included as the outgroup. Tamura & Nei (1993) was used as the model of nucleotide substitution, and 1000 bootstrap replicates were performed. Finally, the sequences were deposited in the BOLD system (sequence page from CENK001-21 to CENK005-21). based on K2P distances using MEGA 6 (Tamura, Stecher, Peterson, Filipowski, Kumar, 2013). Sequences were aligned using MUSCLE (Edgar, 2004), implemented in MEGA 6. Nonparametric bootstrap support values were obtained by resampling and analyzing 1000 replicates (Felsenstein, 1985).

RESULTS

List of investigated aphids and new aphid-plant interactions is as follows.

***Aphis craccivora* Koch, 1854 (Aphidinae: Aphidini)**

Detected for the first time in safflower in Turkey (Yücel, Özdemir, Ertürk, Şahin, 2014).

Material examined: Oltan, Ayaş, Ankara, 27.05.2015, Area 1, 39°57'13 N 32°09'55 E 827 m, 35 da; Area 2, 39°59'16 N 32°12'30 E 798 m, 30 da; Ortabereket, 27.05.2015, Area 1, 40°06'54 N 32°24'53 E 1028 m, 28 da; Sinanlı, 27.05.2015, Area 1 39°59'23 N 32°18'37 E 823 m, 25 da; Oltan, 25.05.2016, Area 1, 39°57'17 N 32°08'56 E 860 m, 15 da; Area 2, 39°57'18 N 32°07'59 E 893 m, 15 da; Sinanlı, 25.05.2016, Area 1, 40°00'25 N 32°15'46 E 932 m, 17 da; Kaçarlı, Şereflikoçhisar, 28.05.2015, Area 1, 39°05'10 N 33°31'16 E 980 m, 70 da; Area 2, 39°05'53 N 33°31'55 E 1010 m, 30 da; Gülhüyük, 28.05.2015, Area 3, 39°06'54 N 33°34'39 E 970 m, 60 da; Area 4, 39°07'26 N 33°34'48 E 914 m, 20 da.

Comments on coloration and morphology: *Aphis craccivora* is one of the bright black Aphids and is quite complex in relation with its hosts. Young individuals are seen as slightly waxy secreted. In wingless viviparous females, the head and body are black in color and have dark and typical patterned sclerotization, starting from methanotum and covering the abdomen dorsum. In winged viviparous females, the head and body are black in color. Their antennae are half the length of the body. Abdomen is bright brownish black.

Host plants of this species detected in Turkey: *Acacia* sp., *Alhagi pseudalhagi*, *Amaranthus retroflexus*, *Anthemis* sp., *Begonville* sp., *Brassica oleracea*, *Bromus japonicus*, *Capsicum annuum*, *Capsella bursa pastoris*, *Cardaria draba*, *Catalpa* sp., *Centaurea* sp., *Chenopodium album*, *Cichorium intybus*, *Cicer arietinum*, *Convolvulus arvensis*, *Crepis foetida*, *Cucumis melo*, *Cucurbita pepo*, *Cydonia vulgaris*, *Dipsacus laciniatus*, *Galium aparine*, *Gossypium herbaceum*, *Heracleum* sp., *Hibiscus esculentus*, *Lens esculentum*, *Lycopersicum esculentum*, *Malus domestica*, *Medicago sativa*, *Portulaca oleraceae*, *Prunus* sp., *Pyrus communis*, *Ribes rubrum*, *Rosa* sp., *Rumex* sp., *Sanguisorba minor*, *Senecio vulgaris*, *Solanum lycopersicum*, *S. melongena*, *Taraxacum officinale*, *Urtica urens*, *Vicia faba* and *Vitex agnus castus* (Düzgüneş & Toros, 1978; Tuatay, 1993; Ölmez & Ulusoy, 2002; Toros, Yaşar, Özgökçe, Kasap, 2002; Aslan, 2002; Özdemir, 2004; Ayyıldız & Atlıhan, 2006; Kocadal, 2006; Kaygın, Görür, & Çota, 2008).

Virus transmission: This species is known to be a vector of approximately 30 plant viruses (Blakman & Eastop, 1984, 2000). Examples of nonpersistent viruses include bean yellow mosaic virus, beet mosaic virus, cucumber mosaic virus and pea mosaic virus. Peanut badge virus and peanut mottle virus as examples of viruses transmitted in a persistent way (Kennedy, Day, Eastop, 1962; Blakman & Eastop, 2021).

***Aphis fabae* Scopoli, 1763 (Aphidinae: Aphidini)**

It was recorded for the first time on *Carthamus* in Ankara and Eskişehir Provinces.

Material examined: Aşağıhacıbekir, Bala, Ankara, 28.05.2015, Area 1, 39°23'44 N 33°17'38 E 844 m, 60 da; Area 2, 39°24'16 N 33°17'59 E 826 m, 75 da; Bala, Tarım, 28.05.2015, Area 3, 39°30'46 N 33°16'14 E 1007 m, 20 da; Area 4, 39°29'43 N, 33°17'01 E 940 m, 400 da; Kesikköprü, Area 1, 39°22'33 N 33°24'03 E 762 m, 30 da; Area 2, 39°25'54 N 33°21'00 E 910 m, 20 da; Aşağıhacıbekir, 28.05.2016, Area 1, 39°28'08 N 33°21'07 E 876 m, 30 da; Mahmudiye, Işıklar, Eskişehir, 20.05.2015, Area 1, 39°27'54 N 30°58'21 E 933 m, 30 da; Area 2, 39°26'43 N 30°57'50 E 904 m, 50 da; Mesudiye, 20.05.2015, Area 1, 39°31'50 N 30°55'10 E 891 m, 30 da

Comments on coloration and morphology: In the winged viviparous female, the body is of colors ranging from brown to black. There are with areas dark green blackish irregular pattern on the abdomen. In the wingless viviparous female, the body is brown, greenish. There are irregular dark pigmented areas on the abdomen. *Aphis fabae* is matte black or very dark coffee color in living individuals. White pleural candle secretions are present in dots and almost always present in wingless individuals and rarely seen in young individuals. Winged individuals have sclerit in the abdomen 4th and 5th tergite, and the cauda is dark in color.

Host plants of this species detected in Turkey: *Pimpinella anisum*, *Vitis vinifera*, *Viburnum* sp. (Bodenheimer & Swirski, 1957), *Solanum dulcamara* (Tuatay & Remaudiere, 1964), *Arbitus verachne* (Canakçioğlu, 1967), *Solanum lycopersicum*, *Cucurbita pepo*, *Ranunculus* sp., *Zea mais* (Canakçioğlu, 1966; 1975), *Papaver* sp., *Nicotiana tabacum*, *Vicia fabae*, *Philadelphus coronarius*, *Matricaria* sp., *Amaranthus* sp., *Lactuca sativa* (Giray, 1974), *Foeniculum vulgare* and *Ferula* sp. (Tuatay, Kalkandere, Aysev, 1972; Düzgüneş, Toros, Kılınçer, Kovancı, 1982); *Papaver* sp., *Phaseolus* sp., *Pimpinella anisum*, *Vicia faba*, *Beta vulgaris*, *Phaseolus vulgaris*, *Solanum lycopersicum*, *Solanum nigrum*, *Urtica urens*, *Heracleum sphondylium*, *Beta vulgaris*, *Portulaca oleracea*, *Anthemis arvensis*, *Cucurbita pepo*, *Zea mays*, *Papever somniferum*, *Nicotiana tabacum*, *Foeniculum vulgare*, *Matricaria* sp., *Amaranthus* sp., *Lactuca sativa*, *Chrysanthemum* sp., *Dianthus* sp., *Impatiens balsamina*, *Kniphofia hybrida*, *Portulaca grandiflora* ve *Zinnia elegans* were registered as hosts of this species (Tuatay & Remaudiere, 1964; Çanakçioğlu, 1967; Giray, 1974; Çanakçioğlu, 1975; van Harten, 1975; Düzgüneş et al, 1982; Zeren, 1989; Akkaya & Uygun, 1996; Toros, Yaşar, Özgökçe, & Kasap, 1996; Özdemir & Toros 1997; Ölmez, 2000; Özdemir, Toros, Kılınçer, & Gürkan, 2006; Toros et al, 2002).

In European conditions, it chooses other woody shrubs as primary hosts like *Euonymus europaeus* and *Viburnum opulus* and *Philadelphus coronarius* and switch to numerous herbaceous secondary hosts (Stroyan, 1984). Regularly visited by ants (Blackman & Eastop, 1984, 2021).

Virus transmission: Examples of viruses that transplanting nonpersistent way are yellow mosaic, sugar beet mosaic, Dahlia mosaic, cucumber mosaic viruses. Beet yellow mosaic viruses, and potato leaf curly viruses as examples of viruses transplanted in a persistent way (Kennedy et al, 1962).

***Brachycaudus (Prunaphis) cardui* (Linnaeus, 1758) (Aphidinae: Aphidini)**

This aphid is recorded for the first time on Safflower in the world (Blackman & Eastop, 2021; Holman, 2009).

Material examined: Gölbaşı, Günalan, 21.05.2018, Area 1 39°36'55 N 32°52'52 E 930 m, 15 da; Area 2 39°37'13 N 32°54'05 E 901 m, 10 da; İkizce, 21.05.2018, Area 1 39°36'33 N 32°40'22 E 862 m, 50 da; Area 2 39°35'52 N 32°41'59 E 844 m, 15 da; Dikilitaş, 21.05.2018, Area 1 39°33'56 N 32°43'54 E 870 m, 20 da; Mahmutiye, Işıklar, Eskişehir, 20.05.2015, Area 1, 39°27'54 N 30°58'21 E 933 m, 30 da; Area 2, 39°26'43 N 30°57'50 E 904 m, 50 da; Mesudiye, 20.05.2015, Area 1, 39°31'50 N 30°55'10 E 891 m, 30 da

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Comments on coloration and morphology: The first record in Turkey was made in Ankara in 1939 through *Prunus domestica* and *Carduus* sp. (Bodenheimer & Swirski, 1957). There are with areas dark brownish and dark green blackish irregular pattern on the abdomen. In the wingless viviparous female, the body is brown, greenish

Host plants of this species detected in Turkey: Düzgüneş & Tuatay (1956) were found on *Carduus* sp. It chooses many plants from the Malvaceae family as hosts. In Turkey, it is reported on *Heliotropium*, *Achillea* (Tuatay & Remaudiere, 1964), *Eryngium* sp. (Canakçioğlu, 1966), *Cynara scolymus* (Giray, 1974), *Prunus domestica*, *Carduus crispus*, *Prunus mahaleb*, thorns and weeds (Düzgüneş et al, 1982), *Anchusa* sp., *Artemisia* sp., *Carlina* sp., *C. vulgaris*, *Cirsium cephalotes*, *C. arvense inconium* and *Carduus acanthoides* (Tuatay, 1988). It is found on *Salix* sp., *Centaurea* sp., *Cirsium* sp., *Onopardium illyricum*, *Prunus spinosa*, *Silybum marianum*, *Cistus crveicus*, *Circus benedicus*, *Cirsium spinasissinum* and *Cirsium* in the Eastern Mediterranean region (Toros et al, 2002).

B. (P.) cardui, which is heavily visited by ants, feeds on plum leaves in spring, causing longitudinal severe curls along the middle vein. In summer it forms dense colonies in the pots and flower heads of secondary hosts.

Virus transmission: It also transferring beans yellow mosaic, cabbage black-ringed stain, cucumber mosaic, onion yellow dwarfism (Kennedy et al, 1962) and Sharka viruses in a non-persistent way (Blackman & Eastop, 1984, 2000, 2021).

***Brachycaudus helichrysi* (Kaltenbach, 1843) (Aphidinae: Aphidini)**

It was recorded for the first time on *Carthamus* in Ankara and Eskişehir Provinces (Yücel et al, 2014).

Material examined: Mahmudiye, Işıklar, Eskişehir, 20.05.2015, Area 1, 39°27'54 N 30°58'21 E 933 m, 30 da; Area 2, 39°26'43 N 30°57'50 E 904 m, 50 da; Mesudiye, 20.05.2015, Area 1, 39°31'50 N 30°55'10 E 891 m, 30 da.

Comments on coloration and morphology: *B. helichrysi*, which is about 2 mm in size, has a slightly waxy bright appearance on its primary host, *Prunus domestica*, as in wingless form of the spring population. These wingless individuals are green, coffee or brownish yellow in color. The population in secondary hosts are very small in size and are green, pale yellow or whitish (Blackman & Eastop, 1984). The cornicle is light, short and flat and it does not have any pattern on it.

Host plants of this species detected in Turkey: In this study of *B. helichrysi*, first mentioned by Tuatay & Remaudiere (1964) in Turkey.

Some hosts in Turkey are *Taraxacum officinalis*, *Carthamus tinctorius*, *Carthamus dentatus*, *Chrysanthemum leucanthemum*, *Prunus* sp., *Senecio vernalis*, *Centaurea* sp., *Chrysanthemum* sp., *Helianthus annuus*, *Matricaria* sp., *Prunus* sp., *Prunus persicae*, *Senecio* sp. and *Helianthus* sp. (Giray, 1974; Tuatay, 1988), *Aster alpinus*, *Dahlia hybrida*, *Zinnia elegans* (Özdemir & Toros, 1997). Ölmez (2000) reported on *Prunus domestica* and *Calendula officinalis* as the first record for Diyarbakır province. It was determined on *Matricaria chamomilla*, *Prunus spinosa*, *Prunus domestica* in the eastern Mediterranean region (Toros et al, 2002).

B. helichrysi causes significant damage to plums due to toxic saliva. It is also known as an important pest of *Chrysanthemum* in the greenhouse. It is not visited by ants.

Virus transporting: It is known as a vector of Cineraria mosaic, cucumber mosaics, Dahlia mosaics and Sharka viruses (Kennedy, Day, & Eastop, 1962). Sometimes it can also transmit nonpersistent viruses to plants that do not have hosts due to heavy flights (Blackman & Eastop, 1984; 2000).

***Myzus (Nectarosiphon) persicae* (Sulzer, 1776) (Aphidinae: Aphidini)**

Yücel, Özdemir, Ertürk, & Şahin (2014) determined this aphid species on Safflower for the first time in Turkey.

Material examined: Sivrihisar, Mülkköy, Eskişehir, 21.05.2015, Area 1 39°29'57 N 31°47'59 E 897 m, 20 da; Hamamkarahisar, 21.05.2015, Area 1 39°29'25 N 31°47'16 E 835 m, 15 da; Area 2 39°29'06 N 31°46'41 E 821 m, 15 da; Çifteler, Eminekin, Eskişehir, 21.05.2015, Area 1, 39°21'55 N 31°08'54 E 852 m, 10 da; Area 2, 39°20'53 N 31°07'17 E 921 m, 20 da; Şereflikoçhisar, Yazısöğüt, 26.05.2015, Area 1 39°05'26 N 33°35'26 E 1169 m, 25 da; Kaçarlı, 13.06.2017, Area 1 39°07'03 N 33°31'09 E 843 m, 15 da

Comments on coloration and morphology: In wingless viviparous females, body color varies from whitish yellowish green to grayish-green, pale yellow green or pinkish and reddish green. Their antennae are slightly shorter than the body, and the antennae tubercles are distinct. The antennae and cornical ends are dark shaded and cornical is cylindrical or slightly bulging. Cauda is sharp and shorter than corniculus. Winged viviparous females also have a rather bright abdomen, head and thorax is in blackish color. Antennae are dark brown or black and about the average body length. The cornicle color is dark brownish and cylindrical in shape. Cauda is slightly muffled and has three pairs of hairs on the sides (Düzgüneş & Tuatay, 1956; Blackman & Eastop, 1984).

Host plants of this species detected in Turkey: Some hosts of this species in Turkey are *Allium sativum*, *Althea rosa*, *Antirrhinum* sp., *Asparagus* sp., *Atropa belladonna*, *Beta vulgaris*, *Brassica oleraceae*, *Capsella bursa-pastoris*, *Capsicum annuum*, *Cardaria draba*, *Carduus pycnocephalus*, *Carthamus tinctorius*, *Cirsium arvense*, *Crataegus* sp., *Cucurbita pepo*, *Cucumis melo*, *Daucus carota*, *Foeniculum vulgare*, *Helianthus annuus*, *Hordeum vulgare*, *Lactuca sativa*, *Lycopersicum esculentum*, *Malus communis*, *Malva neglecta*, *Nicotiana tabacum*, *Petroselinum hortense*, *Phaseolus vulgaris*, *Portulago oleraceae*, *Prunus avium*, *Pyrus communis*, *Rhaphanus raphanistrum*, *Sesamum indicum*, *Solanum melongena*, *Spinacia oleracea*, *Triticum* sp., *Tulipa* sp., *Viola tricolor* and *Zea mays* (Düzgüneş & Tuatay, 1956; Bodenheimer & Swirski, 1957; Tuatay & Remaudiere, 1964; Giray, 1974; Çanakçıoğlu, 1975; Düzgüneş & Toros 1978; Düzgüneş et al, 1982; Karaat & Göven, 1986; Zeren, 1989; Tuatay, 1991; Önuçar & Ulu, 1993; Kiran, 1994; Akkaya & Uygün, 1996; Toros et al, 1996; Özdemir & Toros, 1997; Ölmez, 2000; Çobanoğlu, 2000; Toros et al, 2002; Özdemir, 2004; Ayyıldız & Atlıhan, 2006; Kocadal, 2006).

Virus transmission: It is stated that this species can transmit more than 100 plant virus diseases through persistent and nonpersistent ways. Of these, it is especially important by transporting potato and tobacco viruses that cause economic damage. *M.*

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persicae, a polyfag species, has been reported that it transfers tobacco leaf bending virüs and tomato yellow web virüs as persistent viruses. In addition, potato acuba mosaic virus, potato A virus, potato Y virus and tobacco wilt virüs are transferred in a nonpersistent way (Kennedy et al, 1962).

Uroleucon (Uromelan) aeneum (Hille Ris Lambers, 1939) (Aphidinae: Aphidini)

This aphid is recorded for the first time on Safflower in the world (Blackman & Eastop, 2021; Holman, 2009). It was recorded for the first time on *Carthamus* in Ankara and Eskişehir Provinces (Yücel et al, 2014).

Material examined: Güzelyurt, Kalecik, Ankara, 29.05.2015, 40°08'26 N 33°23'59 E 721 m; 40°08'54 N 33°25'13 E 773 m; Güzelyurt, Hacıköy, Ankara, 26.06.2015, 40°11'39 N 33°26'47 E 846 m; 40°11'39 N 33°26'47 E 846 m; 40°10'53 N 33°26'01 E 861 m.

Comments on coloration and morphology: Wingless and winged are shiny black in color, have a metallic appearance. Adults are large and striking. It is dark brown in color in the pre-adult period.

Host plants of this species detected in Turkey: Its hosts are *Carduus crispus*, *Carduus* sp., *Cirsium arvense* and *Cirsium* sp. (Canakçioğlu, 1975). In addition, it was reported in Van/Tatvan on *Cirsium* sp. (Tuatay, 1967).

Virus transporting: *U. (U.) aeneum* was transmit Moroccan watermelon mosaic virus (MWMV; Potyvirus, Potyviridae) (Chatzivassiliou, Papapanagiotou, Mpenardis, Perdikis, Menexes, 2016).

Uroleucon (Uromelan) jaceae (Linnaeus, 1758) (Aphidinae: Aphidini)

Yücel et al (2014) recorded this aphid species on Safflower for the first time in Turkey.

Material examined: Eskikarsak, Polatlı, Ankara, 27.05.2015, Area 1 39°53'30 N 32°04'43 E 692 m, 40 da; Sarıoba 27.05.2015; 25.06.2015), Area 1 39°51'40 N 32°05'39 E 706 m, 30 da; Eskipolatlı 25.05.2016, Area 1 39°33'36 N 32°11'43 E 892 m, 18 da; Area 2 39°32'14 N 32°10'15 E 816 m, 10 da; Şeyhali 25.05.2016, Area 1 39°30'58 N 32°17'02 E 875 m, 10 da; Area 2 39°29'17 N 32°18'24 E 937 m, 15 da; Şabanözü 25.05.2016, Area 1 39°43'01.5" N 32°03'50.5"E 762 m, 20 da

Comments on coloration and morphology: Its color is ranging from dark reddish brown to black. The antennae, legs, cornicle and cauda are black. Wingless individuals are 2.5- 3.5 mm long (Blackman & Eastop, 1984; 2000).

Host plants of this species detected in Turkey: *Acroptilon pieris*, *A. repens*, *Carduus nutans*, *Carduus* sp., *Centaurea calcitrapa*, *C. coroniformis*, *C. solstitialis*, *Centaurea* spp., *Carlina vulgaris*, *Cirsium arvense*, *Onopordium anatolicum* and Compositae (Boschma, 1939; Bodenheimer & Swirski, 1957; Tuatay, Gül, Demirtola, Kalkandelen, & Çağatay, 1967; Giray, 1974; Tuatay, 1991; Aslan, 2002; Özdemir, 2004; Yücel et al, 2014).

Virus transmission: This species transmits the cucumber mosaic virus in a nonpersistent way (Kennedy et al, 1962).

***Uroleucon (Uromelan) carthami* (Hille Ris Lambers, 1948) (Aphidinae: Aphidini)**

It was recorded for the first time on *Carthamus* in Eskişehir provinces (Yücel et al, 2014).

Material examined: Güzelyurt, Kalecik, Ankara, 26.06.2015, Area 2, 40°08'54 N 33°25'13 E 773 m, 25 da; Hacıköy 26.06.2015, Area 3 40°11'39 N 33°26'47 E 846 m, 70 da; Area 4 40°10'53 N 33°26'01 E 861 m, 35 da; Oltan, Ayaş, Ankara, 27.05.2015, Area 1 39°57'13 N 32°09'55 E 827 m, 35 da; Area 2 39°59'16 N 32°12'30 E 798 m, 30 da; Ortabereket, 27.05.2015, Area 1 40°06'54 N 32°24'53 E 1028 m, 28 da; Sinanlı, 27.05.2015, Area 1 39°59'23 N 32°18'37 E 823 m, 25 da; Oltan, 25.05.2016, Area 1 39°57'17 N 32°08'56 E 860 m, 15 da; Büyükboyalık, Area 1, 39°33'06 N 33°13'47 E 897 m, 25 da; Üçem, Area 1, 39°32'17 N 33°12'54 E 928 m, 15 da; Ergin, Area 1, 39°37'50 N 33°09'32 E 914 m, 15 da; Area 2, 39°39'14.6 N 33°07'35 E 865 m, 18 da; Kerişli, 14.06.2017, Area 1, 39°41'51 N 33°07'19 E 935 m, 20 da; Karaali, 14.06.2017, Area 1, 39°39'40 N 32°57'50 E 945 m, 20 da; Area 2, 39°39'59 N 32°58'28 E 912 m, 15 da.

Comments on coloration and morphology: The color of the apterapy varies from dark brown to blackish-brown according to Papapanagiotou et al (2012), the body length is 2.1-3.3 mm. *Carthamus* spp. in southern and central Europe. Algeria (Laamari, Coeur d'acier, & Jousselin, 2013), Israel, Lebanon, Turkey and Pakistan and India (Kashmir) in the east. Most of the records from *C. tinctorius* in India are probably referred to as *U. compositae*. There is also confusion in European literature with the very closely related *U. jaceae* and *U. aeneum*, which are normally on the other Cynareae but can sometimes also be fed in *Carthamus* (Nieto Nafria, Remaudiere, & Mier Durante, 1986). Sexual morphs and life cycle are unknown. In Turkey, It was determined on 29.6.1963 in Ankara, Beypazarı-Nallıhan road; on 5.6.1963 in Mersin, Narlıkaya and on *C. dentatus* in Muğla, Marmaris on 11.6.1963 (Tuatay & Remaudiere, 1964).

Host plants of this species detected in Turkey: It was determined on 29.06.1963 in Ankara, Beypazarı-Nallıhan road; on 5.6.1963 in Mersin, Narlıkaya and on *C. dentatus* in Muğla, Marmaris on 11.6.1963 (Tuatay & Remaudiere, 1964).

Virus transmission: There is no record of this aphid being a virus vector.

Molecular analysis

In molecular studies, four CO1 sequences (ranged from 619 to 661 bp) were successfully obtained after performing quality control analysis. As it is known for arthropods, our sequence data also revealed a high AT-content for the DNA barcode region with a base composition of A = 35%; C = 13.7%; G = 10.1%; T = 41.1%. This study provided CO1 sequences of *Brachycaudus helichrysi* (1 sequence), *Uroleucon aeneum* (1 sequence) and two *Uroleucon jaceae* (2 sequences) species. In BIN analysis, unique BINs were revealed for our sequences with less than 1% nucleotide distances. In addition, certain morphological identifications of our specimens performed by the second author, which supports the accuracy of the sequences. Dataset includes for neighbour joining tree 56 close sequences to our species and one outgroup as *M. persicae*. The neighbour joining tree confirmed the results previously obtained on the basis of the BIN analyses, and all the species possessing at least one conspecific were monophyletic and supported with high bootstrap values (Fig. 1).

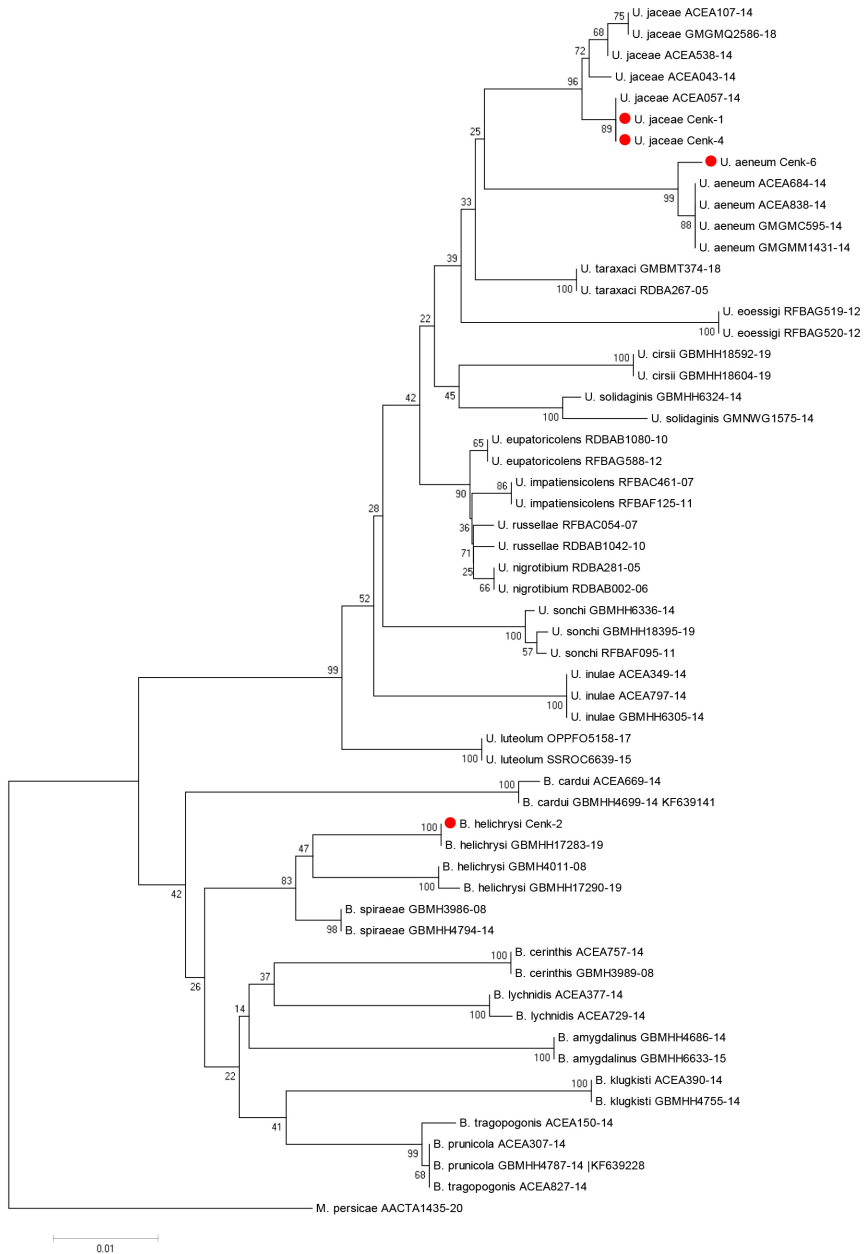
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Fig. 1. Neighbour-joining tree inferred on dataset representative sequences of close species available in BOLD. Bootstrap values are reported above the main lineages; sequences with red dot are our sequences in this study.

DISCUSSION

In this study, for providing confirmation of aphid species identification, DNA barcoding was performed. As a result, confirmation of our DNA sequences and neighbour-joining tree results with the morphological identifications gives promising results for future studies. Aphids are a group of plant sap sucking insects, including many pests in agriculture. Therefore, an accurate and rapid taxonomic identification of individuals must to be regarded as crucial for management strategies for crop pests in Turkey. Due to morphological plasticity aphid identification is considered to be difficult. Traditional morphological identification requires high quality slides and specimens; however, even for taxonomists, their small body size and difficulty in slide making procedure cause to a time consuming process of species identification. Moreover, morphological characteristics of aphids can be influenced by biotic factors such as life cycle, natural enemy, feeding site, and host plant, as well as abiotic factors (Tamura et al, 2013; Li et al, 2020). In addition, high phenotypical similarity and intraspecific variation among species could interrupt the identification of aphid species. For these reasons, supporting and checking the identification of aphid species with the DNA barcoding is vital with new prospective informations, valuable for future studies and applications.

Fourteen aphid species are given in the world aphid host plant catalogue (Holman, 2009). Eight species were identified in this study and eight species were detected morphologically; *Aphis craccivora* Koch, *Aphis fabae* Scopoli, *Brachycaudus (Prunaphis) cardui* (Linnaeus), *Brachycaudus helichrysi* (Kaltenbach), *Myzus (Nectarosiphon) persicae* (Sulzer), *Uroleucon (Uromelan) jaceae* (Linnaeus), *Uroleucon (Uromelan) aeneum* (Hille Ris Lambers) and *Uroleucon (Uromelan) carthami* (Hille Ris Lambers) are reported from Safflower (*Carthamus tinctorius* L.). All of these species is associated with Asteraceae plants worldwide (Holman, 2009; Blackman & Eastop, 2021).

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