

Updated status of whiteflies (Hemiptera: Aleyrodidae) in Jordan with emphasis on the *Bemisia tabaci* species complex

Actualización del estatus de las moscas blancas (Hemiptera: Aleyrodidae) en Jordania con énfasis en el complejo *Bemisia tabaci*

IHAB GHABEISH¹; MAIS SWEISS²; GHANDI ANFOKA³

¹ Ph. D. Entomology Al-Balqa Applied University, As-Shoubak University College, Department of Agricultural Sciences, Al-Shoubk 711910, Jordan, ghabeish@bau.edu.jo, <https://orcid.org/0000-0002-3985-827X>. ² Ph. D. Algae and Plant Biotechnology, Al-Balqa Applied University, Faculty of Agricultural Technology, Department of Biotechnology, Al-Salt 19117, Jordan, m.swies@bau.edu.jo, <https://orcid.org/0000-0002-1512-9224>. ³ Ph. D. Plant Virology, Al-Balqa Applied University, Faculty of Agricultural Technology, Department of Biotechnology, Al-Salt 19117, Jordan, anfoka@bau.edu.jo, <https://orcid.org/0000-0002-7826-356X>.

Abstract: Whiteflies are economically important plant pests that cause damage to crops worldwide. This study aimed to update the status of whiteflies in Jordan by combining the classical morphological identification and the DNA markers using the *mitochondrial cytochrome oxidase I (mtCOI)* gene. Over the course of three consecutive years, 111 whiteflies were collected from different geographical regions and different plant hosts in Jordan. The results showed that, in addition to *Bemisia tabaci*, another nine different whitefly species were identified, including two species that were recorded for the first time in Jordan: *Africaleurodes coffeacola*, and *Tetraleurodes neemani*. A special focus has been given to economically important plant pests like the *B. tabaci* species complex. Three different diagnostic techniques were used to identify *B. tabaci* putative species based on *mtCOI* gene. All the collected samples of *B. tabaci* species complex were identified as Middle East-Asia Minor 1 (MEAM1) putative species.

Keywords: Molecular identification, whiteflies, pests, MEAM1, *mtCOI*, *Bemisia tabaci*.

Resumen: Las moscas blancas son plagas de plantas de importancia económica, que causan daños a cultivos en todo el mundo. Este estudio tuvo como objetivo actualizar el estado de conocimiento sobre las moscas blancas en Jordania, combinando la identificación morfológica clásica y la técnica del gen de *citocromo oxidasa I mitocondrial (mtCOI)* como un marcador de ADN. En el transcurso de tres años consecutivos se recolectaron 111 moscas blancas de diferentes regiones geográficas, y de diferentes plantas hospederas. Los resultados mostraron que, además de *Bemisia tabaci*, existen nueve especies diferentes de mosca blanca; incluso se registraron por primera vez en Jordania dos especies: *Africaleurodes coffeacola* y *Tetraleurodes neemani*. Se hizo especial énfasis en el complejo de especies de *B. tabaci* por su importancia económica. Se utilizaron tres técnicas de diagnóstico diferentes para identificar especies cercanas a *B. tabaci* basadas en el gen *mtCOI*. Sin embargo, todas las muestras recolectadas del complejo de especies de *B. tabaci* se identificaron como especies del complejo de Oriente Medio-Asia Menor 1 (MEAM1).

Palabras clave: Identificación molecular, moscas blancas, plagas, MEAM1, *mtCOI*, *Bemisia tabaci*.

Corresponding author

Mais Sweiss. Ph. D. Algae and Plant Biotechnology, Al-Balqa Applied University, Faculty of Agricultural Technology, Department of Biotechnology, Al-Salt 19117, Jordan, m.swies@bau.edu.jo, <https://orcid.org/0000-0002-1512-9224>.

Suggested citation

GHABEISH, I.; SWEISS, M.; ANFOKA, G. 2021. The updated status of whiteflies (Hemiptera: Aleyrodidae) in Jordan with emphasis on the *Bemisia tabaci* species complex. Revista Colombiana de Entomología 47 (1): e8944. <https://doi.org/10.25100/socolen.v47i1.8944>

Received: 21-Feb-2020

Accepted: 01-Sep-2020

Published: 21-May-2021

Revista Colombiana de Entomología

ISSN (Print): 0120-0488

ISSN (On Line): 2665-4385

<https://revistacolombianaentomologia.univalle.edu.co>

Open access



BY-NC-SA 4.0
creativecommons.org/licenses/by-nc-sa/4.0/deed.es

Publishers: Sociedad Colombiana de Entomología
SOCOLEN (Bogotá, D. C., Colombia)

<https://www.socolen.org.co>

Universidad del Valle (Cali, Colombia)

<https://www.univalle.edu.co>

© 2021 Sociedad Colombiana de Entomología -
SOCOLEN y Universidad del Valle - Univalle

Introduction

Whiteflies (Hemiptera: Aleyrodidae) are insects that feed on plants by sucking large quantities of sap. Sucking plant sap can cause early wilting, stunted growth, premature defoliation, and eventually yield loss (Shukla *et al.* 2016). In addition, whiteflies secrete honeydew that causes sooty mould growth on plants leaves and fruits and reduces their market values. Whiteflies have also been reported to act as vectors for plant viruses (Byrne and Bellows 1991; Jones 2003; Shukla *et al.* 2016). There are around 1556 species of whiteflies in 161 genera (Martin 2004), however, only a few acts as a vector for plant viruses. *Bemisia tabaci* (Gennadius, 1889) transmits around 212 viruses from five genera *Begomovirus*, *Crinivirus*, *Ipomovirus*, *Carlavirus*, and *Torradovirus* (Navas-Castillo *et al.* 2011; Polston *et al.* 2014). *Trialeurodes vaporariorum* (Westwood, 1856), the greenhouse whitefly, transmits viruses from two genera *Crinivirus* and *Torradovirus* (Navas-Castillo *et*

al. 2011). *Trialeurodes abutiloneus* (Haldeman, 1850, the banded-winged whitefly) transmits viruses of the genera *Crinivirus* and *Torradovirus* (Mlynarek and Labbé 2018). *Bemisia afer* (*sensu lato*) transmits the sweet potato chlorotic stunt virus of *Crinivirus* (Gamarra *et al.* 2010; Navas-Castillo *et al.* 2011). *Trialeurodes ricini* (Misra, 1924, the castor bean whitefly) could be a vector for the tomato yellow leaf curl virus (TYLCV) in Egypt (Idriss *et al.* 1997).

It is important to study the diversity of the different whitefly populations to be able to manage and design effective control methods for these pests. During the period between 1985-1994, a study was conducted to identify the whitefly species in Jordan and report some of their natural enemies. Eleven species were morphologically identified based on the characteristics of their pupa. These species were *Acaudaleyrodes citri* (Priesner and Hosny, 1934) *Aleurocanthus zizyphi* (Priesner and Hosny, 1934), *Aleurolobus niloticus* (Priesner and Hosny, 1934), *Aleurolobus olivinus* (Silvestri, 1911), *Aleyrodes prolella* (Linnaeus, 1758), *Aleyrodes singularis* (Danzig, 1964), *B. tabaci*, *Siphoninus phillyreae* (Haliday, 1835), *Trialeurodes lauri* (Signoret, 1882), *T. ricini* (Misra), and *T. vaporariorum* (Allawi 1994). In addition to the aforementioned whiteflies, *Acaudaleyrodes rachipora* (Singh, 1931, Babul whitefly) and *Aleurolobus marlatti* (Quaintance, 1903) have been also reported in Jordan (Ghahari *et al.* 2009). Nonetheless, the classical taxonomy of whiteflies based on the morphology of the puparium (fourth instar) is complicated since the intraspecific variability in the morphology such as the size, shape, number of setae and papillae, the perianal structure, and the body size, can be affected by the variations in the environment (Ko *et al.* 2005).

The “superbug” *Bemisia tabaci* is one of the most damaging insects known in the agricultural world (Barinaga 1993). It is broadly polyphagous, feeding on an estimated 600 plant species (European and Mediterranean Plant Protection Organization 2004). In Jordan, around 339 plant species in 64 families were reported as hosts for *B. tabaci* (Sharaf and Allawi 1980). *B. tabaci* was described as a species complex having many putative species that are morphologically indistinguishable (Dinsdale *et al.* 2010). Reliable morphological markers which can distinguish between the different genetic groups of the *B. tabaci* species complex are not known (Rosell *et al.* 1997). As a result, the molecular markers can be an important tool to study the variation in populations of the species complex of *B. tabaci* (Cervera *et al.* 2000).

Phylogenetic studies comparing the sequence of a region of the DNA such as the 16S ribosomal subunit (Frohlich *et al.* 1999), ribosomal internal transcribed spacer-1 ITS1 sequence (De Barro *et al.* 2000; Wu *et al.* 2003), and mitochondrial cytochrome oxidase I (*mtCOI*) gene (Frohlich *et al.* 1999; Kirk *et al.* 2000; Luo *et al.* 2002) have been carried out to determine the genetic relationships among *B. tabaci* species complex. Dinsdale *et al.* (2010) performed a study based on the analysis of sequence pairwise divergence and the Bayesian phylogenetic analysis of *mtCOI* gene to study the *B. tabaci* species complex. Based on the genetic species concept to distinguish between the putative species, besides the mating experiment, they concluded that *B. tabaci* is a species complex consisting of 11 groups containing 24 species (Dinsdale *et al.* 2010). Boykin (2014) concluded that *B. tabaci* species complex seems to be made up of more than one species. This was based on data obtained from mating compatibility (Xu *et al.* 2010; Liu *et al.* 2012; Sun *et al.* 2011), genomes (Wang *et al.*

al. 2011; Wang *et al.* 2013), and *mtCOI* phylogenetic analysis (Boykin *et al.* 2007; Dinsdale *et al.* 2010; Boykin *et al.* 2012; Tay *et al.* 2012).

This study is concerned with the characterization of the most invasive putative species of *B. tabaci*, which are the Middle East–Asia Minor 1 (MEAM1; previously described as biotype B), and the Mediterranean putative species (MED; previously known as biotype Q). Both of them are important from a biosecurity perspective, as they are resistant to a wide range of insecticides (Prabhaker *et al.* 1988), globally invasive, and cause huge economic losses (Oliveira *et al.* 2001; Boykin *et al.* 2012). MEAM1 invaded at least 54 countries around the world (Broadbent *et al.* 1989; Cheek and MacDonald 1994, De Barro *et al.* 2011). Whilst MED putative species invaded at least ten countries worldwide such as United States, China, Japan, and New Zealand (De Barro *et al.* 2011).

Since the putative species MEAM1 and MED of *B. tabaci* are morphologically indistinguishable (Liu *et al.* 2016) many studies were performed to understand their dispersal behaviour, insecticide resistance, plant-host preference, endosymbiont composition, fecundity, and efficiency in plant viruses transmission (Brown *et al.* 1995a; Horowitz *et al.* 2005; Bing *et al.* 2012; Liu *et al.* 2016; Shi *et al.* 2018; Watanabe *et al.* 2019; Yang *et al.* 2020). MED putative species is characterized by its resistance to a wide variety of insecticides (Nauen *et al.* 2002; Horowitz *et al.* 2005; Nauen and Denholm 2005; Ghanim and Kontsedalov 2007; Yang *et al.* 2013).

In Jordan, the presence of MEAM1 was documented by (Brown *et al.* 1995b). Subsequently, a study carried out using RAPD-PCR to identify the *B. tabaci* species complex in Jordan found MEAM1 and the New World putative species that is formerly known as biotype A (Sharaf and Hasan 2003). The highly invasive putative species MED was not reported in Jordan before, although it was reported in neighbour countries such as Syria, Palestine/Israel, and Egypt (Horowitz *et al.* 2003; Khasdan *et al.* 2005; De Barro *et al.* 2011). This work aims to combine the classical morphological identification method with the DNA barcoding marker (*mtCOI*) for the first time to identify and document the presence of the whitefly species in Jordan.

Materials and methods

Sample collection and morphological identification. To learn more about the status of whiteflies in Jordan, around 111 different whiteflies samples were collected during the years 2009-2012 (Table 1). Of the 111 samples, around 85 were of *B. tabaci*. The samples of *B. tabaci* were collected from more than 23 hosts of cultivated plants (e.g. tomato, cucumber, cauliflower, okra, watermelon, eggplant, and squash), non-cultivated (e.g. basil), and non-food crops (e.g. cotton, poinsettia, and *Lantana camara* L.). The host plants were grown in both greenhouses and open fields in different geographical regions in Jordan including the Jordan Valley area (32°19'1.20"N 35°34'7.19"E), and another six different provinces: Amman (31°35'1.28"N 36°20'0.06"E), AL-Balqa` (32°00'0.00"N 35°40'0.01"E), Madaba (31°34'59.99"N 35°40'0.01"E), Jerash (32°15'0.00"N 35°55'0.01"E), Ma'an (30°19'59.99"N 36°34'59.99"E), and Al-Mafraq (32°19'59.99"N 37°55'0.01"E). Pupal stages were collected and reared until adults emerged. The collected adults were preserved in 70 % ethanol to be subjected to DNA isolation, whilst the empty pupal cases were sent for morphological

identification by Professor Dan Gerling (Tel Aviv University). Two references for the putative species MEAM1 and MED were kindly provided by Dr Rami Horowitz (the Institute of Plant Protection, Gilat Research Centre).

DNA extraction and amplification of the *mtCOI* gene.

DNA was extracted from a single whitefly adult or pupa for DNA barcoding of the collected samples according to (Cenis *et al.* 1993) with modifications recommended by (Khasdan *et al.* 2005). After the DNA extraction, a PCR reaction was performed to amplify 816 bp fragment of the *mtCOI* gene (Khasdan *et al.* 2005) with some modifications. The PCR reaction contained around 20 ng total DNA in 1X buffer, one unit of the *Taq* DNA polymerase and 0.2 μ M dNTPs (Promega Corporation, USA), 2.5 mM MgCl₂, 0.4 μ M of the forward primer C1-J-2195 5' TTGATTTTTGGT-CATCCAGAAGT3' and 0.4 μ M reverse primer L2-N-3014 5' TCCAATGCACTAATCTGCCATATTA3' (Frohlich *et al.* 1999). PCR was performed in a PTC200 thermocycler (MJ Research Inc., USA). The PCR program was composed of an initial denaturation at 94 °C for 3 min, 40 cycles of one min at 94 °C followed by one min at 52 °C and one min at 72 °C, and a final extension step at 72 °C for seven min. The PCR products were analysed on 1 % agarose gel stained with 0.5 μ g/ml of ethidium bromide. A part of the amplified *mtCOI* gene was sent for sequencing and another part was analysed in the following step.

Cleaved Amplified Polymorphic Sequences (CAPS) for (*mtCOI*) sequences.

To reduce the number of the samples that will be sent for sequencing, two diagnostic techniques were used to distinguish between *B. tabaci* species complex. The first technique was CAPS which distinguishes between MEAM1 and MED putative species that are likely to present in Jordan. To perform CAPS, a part of *mtCOI* gene, which was amplified in the previous step, was subjected to digestion by restriction endonuclease *VspI* according to (Khasdan *et al.* 2005). Only a short fragment (41 bp) was cut out of MEAM1, while PCR products of MED yielded three fragments of about 436 bp, 292 bp, and 41 bp.

Bidirectional PCR amplification of *mtCOI* fragments.

The second diagnostic technique was used to distinguish between *B. tabaci* species complex was the bidirectional PCR (Tsagkarakou *et al.* 2007). Four primers were used in each PCR reaction. The two outer primers, the forward primer C1-J-2195 (5' TTGATTTTTGGT-CATCCAGAAGT 3'; Frohlich *et al.* 1999) and the reverse primer tRNA-1576 (5' TATAAATCTTAAATTTACTGCA 3'; Tsagkarakou *et al.* 2007) they yielded around 879 bp control fragment for all the *B. tabaci* species complex. The two inner primers, LQ 5' AAGGGCCTGAATTTATTG 3' and RB5' CTACTTTGG-GTGAATAAAGTCT 3' were designed and tested to distinguish between MEAM1 and MED (Tsagkarakou *et al.* 2007). In the case of MEAM1 putative species, RB/tRNA1576 primers amplify the 609 bp fragment. Whilst, in the case of MED LQ/C1-J-2195 primers will amplify the 310 bp fragment. If only the control band is obtained, it may indicate that it belongs to another putative species of the *B. tabaci* such as the New World or other putative species which were known previously as C, E, and G biotypes and the exact biotype will be confirmed by the sequencing of the *mtCOI* gene. The PCR reaction was performed in mostly the same way as above,

with the exception that in this reaction, four primers were used and the PCR program was, initial denaturation at 94 °C for 3 min, 40 cycles of 45 sec at 94 °C, one min at 50 °C and one min at 72 °C, and a final extension step at 72 °C for 10 min.

Sequencing and phylogenetic analysis of *mtCOI* gene.

Representative samples of *B. tabaci* species complex and the other whitefly species were sent for sequencing at Macrogen, (Seoul, South Korea). Analysis of the sequences was performed using MEGA X (Kumar *et al.* 2018) and the Nucleotide Basic Local Alignment Search Tool (Nucleotide BLAST) service provided by the National Center for Biotechnology Information (NCBI) (Zhang *et al.* 2000; Morgulis *et al.* 2008). The obtained sequences were submitted to GenBank. To illustrate the relationship between the sequences of the whiteflies obtained from Jordan and other whiteflies sequences from all over the world, a phylogenetic tree was constructed. The sequences of whiteflies from Jordan were aligned and trimmed to the same length beside other sequences of whiteflies from throughout the world obtained from the GenBank. Then the phylogenetic tree was built using Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993).

Results

Ten species of whiteflies were morphologically identified in Jordan.

As the aim of this study is to combine the morphological and molecular identification tools to identify the whitefly species in Jordan; the morphological identification was the main tool for identifying the whitefly species other than *B. tabaci*. According to the morphological classification of the whitefly species, nine different whitefly species were identified in addition to *B. tabaci* (Table 1). The identified species were *Trialeurodes lauri* (Signoret), *Trialeurodes ricini* (Misra), *Aleyrodes singularis* (Danzig), *Aleurolobus niloticus* (Priesner and Hosny), *Aleurolobus olivinus* (Silvestri), *Acaudaleyrodes rachipora* (Singh), *Africaleurodes coffeacola* (Dozier, 1934), *Siphoninus phillyreae* (Haliday) and *Tetraleurodes neemani* (Bink-Moenen, 1992). They belong to seven different genera. The two whitefly species *Africaleurodes coffeacola* and *T. neemani* had not been recorded before in Jordan.

Molecular and phylogenetic analysis.

For the purpose of barcoding of the collected whitefly samples, *mtCOI* gene was amplified and analysed. Around 816 bp fragment of the *mtCOI* gene was amplified for all the collected samples of whitefly species and *B. tabaci* species complex (Fig. 1A). Representative samples of the different whitefly species and *B. tabaci* were sent for sequencing. Additionally, this PCR product was subjected to CAPS for further analysis of *B. tabaci* samples.

CAPS and the bidirectional PCR for *mtCOI* sequence revealed the presence of only MEAM1. In spite of the different hosts, geographical regions, and the time of collection; all the collected *B. tabaci* samples during the period 2009-2012 showed MEAM1 pattern in CAPS (Fig. 1B). The same results were confirmed by the bidirectional PCR, the second DNA marker used as it is presented (Fig. 1C).

After the morphological identification of the whitefly species that were collected from Jordan, the sequences of *mtCOI* for some of these whiteflies -except *B. tabaci*- were submitted to GenBank under the accession numbers:

KP418768-KP418780. Whereas representative samples of *B. tabaci mtCOI* sequences were submitted to GenBank under the accession numbers: KC789925-KC789962. All of the *B. tabaci* samples showed high identity with MEAM1 putative species throughout the world.

To illustrate the relationship within the collected whiteflies samples from Jordan and the other related whiteflies from the world, two phylogenetic trees were constructed. The first tree illustrated the relationship among the different whitefly species from Jordan including *B. tabaci* species complex and the other related whitefly species from the world that are available in the GenBank (Fig. 2). The tree confirmed the results obtained from the morphological and molecular identifications. The second phylogenetic tree was constructed for the *B. tabaci* species complex only (Fig. 3). It showed that all the samples from Jordan are very closely

related to the MEAM1 putative species from around the world such as MEAM1 from Japan, Morocco, and Cuba. This as well confirms that all the collected *B. tabaci* samples from Jordan are MEAM1.

Discussion

This study aimed to combine the classical morphological identification method of the different whitefly species with the molecular DNA barcoding method for faster and easier identification. Ten different whitefly species were identified mainly based on the morphology. This was due to the poor database of the *mtCOI* that is available for the whitefly species other than *B. tabaci* in GenBank. The obtained sequences were submitted to GenBank, so that, in the future, they would be helpful for the purpose of whitefly identification.

Table 1. Updated whitefly species in Jordan, host plants, location and method(s) used in species identification.

| Whitefly species | Host | Location | Accession No. (GenBank) | Identification method |
|----------------------------------|--|--|----------------------------------|--------------------------|
| <i>Acaudaleyrodes rachipora</i> | <i>Citrus limon</i> (L.) Osbeck <i>Olea europaea</i> L. | Amman | KP418775 KP418776 KP418780 | Morphology and molecular |
| <i>Africaleurodes coffeacola</i> | <i>Ziziphus spina-christi</i> (L.) Desf | Al-Balqa` | | Morphology |
| <i>Aleurolobus niloticus</i> | <i>Punica granatum</i> L. | Al-Balqa` | KP418772 KP418773 | Morphology and molecular |
| <i>Aleurolobus olivinus</i> | <i>Olea europaea</i> L. | Amman, Al-Balqa` | KP418774 KP418778 KP418779 | Morphology and molecular |
| <i>Aleyrodes singularis</i> | <i>Lactuca serriola</i> L. | Amman, AL-Balqa` | KP418769 KP418770 KP418771 | Morphology and molecular |
| <i>Bemisia tabaci</i> (MEAM1) | <i>Abelmoschus esculentus</i> (L.) Moench <i>Althaea rosea</i> L. <i>Brassica oleracea</i> var. <i>botrytis</i> L. <i>Brassica oleracea</i> var. <i>capitata</i> L. <i>Brugmansia</i> sp. <i>Capsicum</i> sp. <i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai <i>Cucumis melo</i> var. <i>flexuosus</i> (L.) Naudin <i>Cucumis sativus</i> L. <i>Cucurbita pepo</i> var. <i>melopepo</i> L. Harz. <i>Cucurbita pepo</i> var. <i>pepo</i> L. <i>Euphorbia pulcherrima</i> Willd. ex Klotzsch <i>Gossypium</i> sp. <i>Helianthus annuus</i> L. <i>Lagenaria siceraria</i> (Molina) Standl. <i>Lantana camara</i> L. <i>Lycopersicon esculentum</i> Mill. <i>Ocimum basilicum</i> L. <i>Ricinus communis</i> L. <i>Solanum melongena</i> L. <i>Solanum tuberosum</i> L. | Amman, AL-Balqa`, Al-Mafraq, Jerash, Jordan Valley, Ma`an, Madaba | KC789925- KC789962 | Morphology and molecular |
| <i>Siphoninus phillyreae</i> | <i>Punica granatum</i> L. | Al-Balqa` | | Morphology |
| <i>Tetraeurodes neemani</i> | <i>Punica granatum</i> L. | Amman, Al-Balqa` | | Morphology |
| <i>Trialeurodes lauri</i> | <i>Arbutus andrachne</i> L. | Jerash | KP418768 | Morphology and molecular |
| <i>Trialeurodes ricini</i> | <i>Ricinus communis</i> L. | Al-Balqa` | KP418777 | Morphology and molecular |

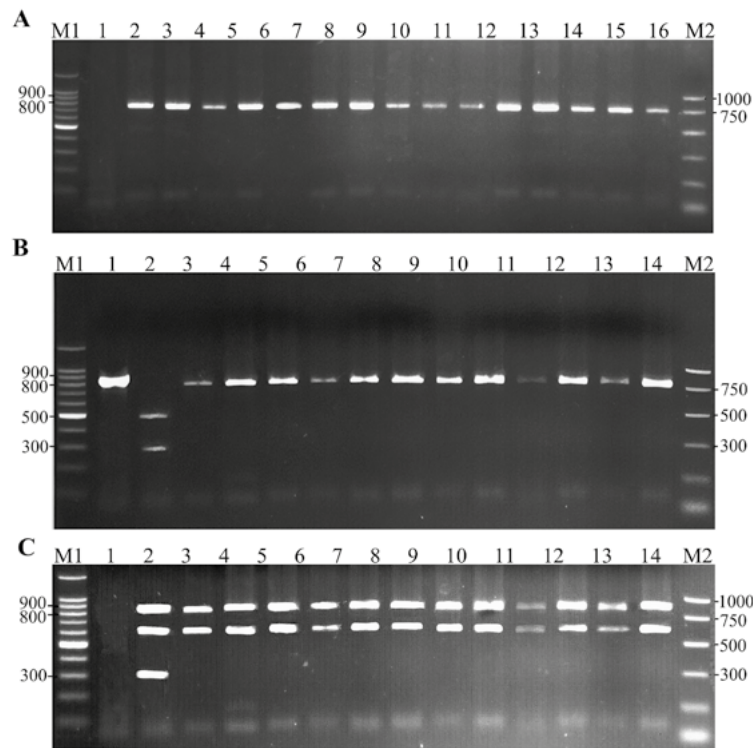


Figure 1. Amplification and analysis of *mtCOI*. **A.** Amplification of 816 bp of *mtCOI* gene for the whitefly species including *Bemisia tabaci* species complex. Sample 1, negative control; 2, positive control; 3-16, amplified part of *mtCOI* gene. **B.** CAPS applied on the amplified *mtCOI* gene (816 bp) for *B. tabaci* species complex. Sample 1, undigested PCR product; 2, CAPS pattern of MED reference; 3, CAPS pattern of MEAM1 reference; 4-14, samples of *B. tabaci* from Jordan. **C.** The bidirectional PCR analysis. Sample 1, negative control; 2, MED reference; 3, MEAM1 reference; 4-14, samples of *B. tabaci* from Jordan. Samples were analysed on 1 % agarose gel stained with 0.5 µg/ml of ethidium bromide M1, 100 bp DNA ladder; M2, PCR markers.

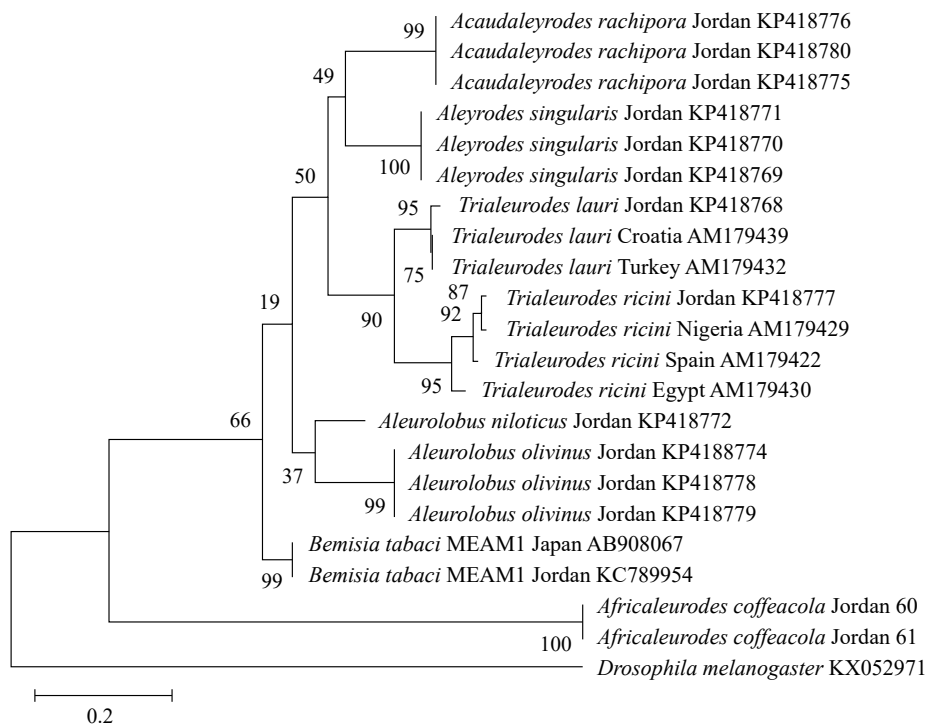


Figure 2. Phylogenetic tree analysis by Maximum Likelihood method based on the Tamura-Nei model for a part of *mtCOI* gene for different whitefly species collected from Jordan and other parts around the world, *Drosophila melanogaster* (Meigen, 1830) was used as an outgroup taxon. The number at the nodes represents the bootstrapping value and the scale represents the genetic distance. The analyses were conducted using MEGA-X (Kumar *et al.* 2018).

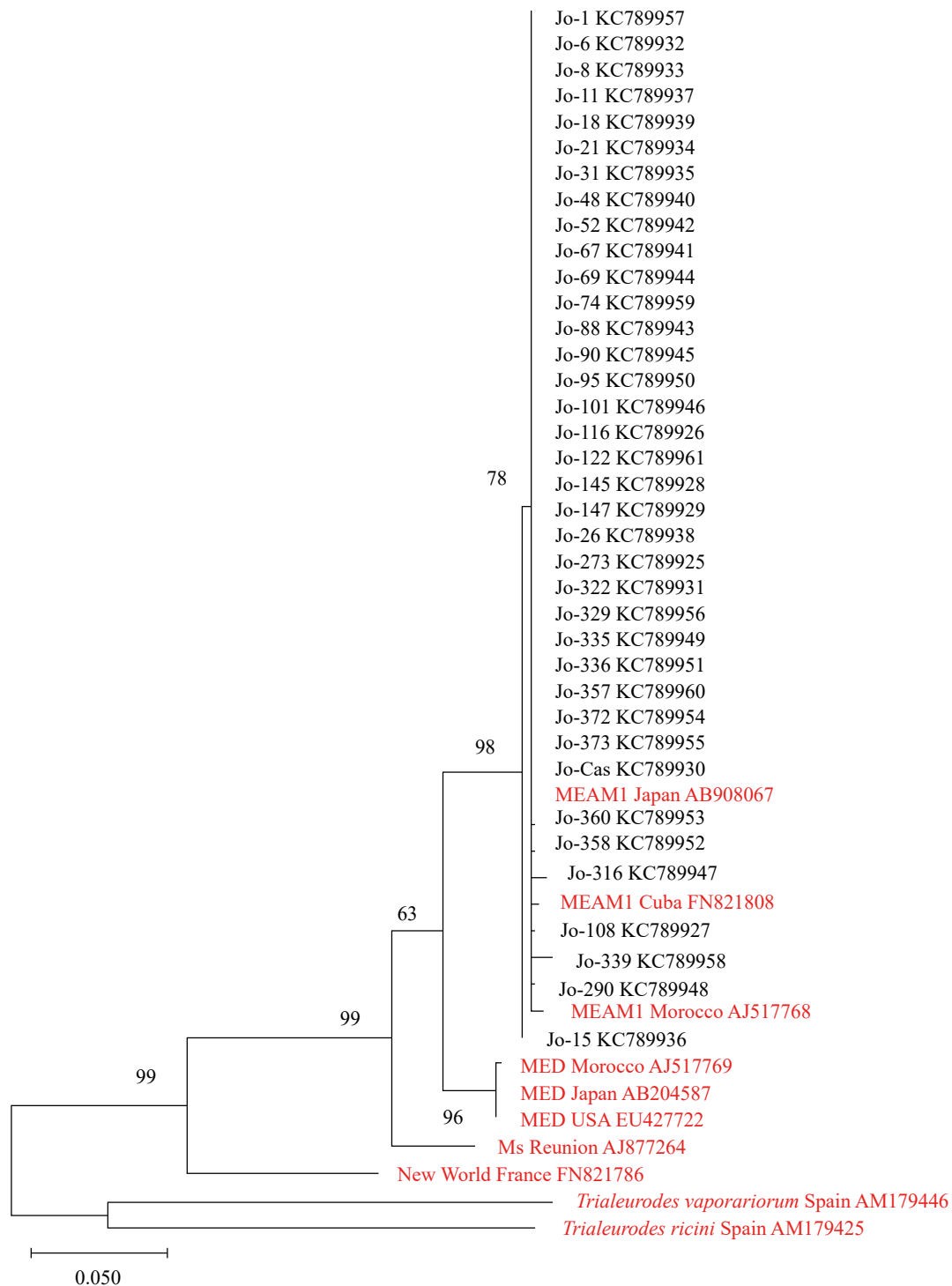


Figure 3. Phylogenetic analysis by Maximum Likelihood method based on the Tamura-Nei model, for a part of *mtCOI* gene for *Bemisia tabaci* samples collected from Jordan indicated with (Jo-), the other *B. tabaci* from the world are colored in red. *Trialeurodes vaporariorum* and *Trialeurodes ricini* were used as outgroup taxa. The number at the nodes represents the bootstrapping value and the scale represents the genetic distance. The analyses were conducted using MEGA-X (Kumar *et al.* 2018).

Of the ten species, two species were recorded in Jordan for the first time; these species were *T. neemani* and *Africaleurodes coffeacola*. *T. neemani* was also reported in Palestine/Israel (Martin and Mound 2007), China and Iran (Wang *et al.* 2016). Whilst, *Africaleurodes coffeacola* has been reported in Nigeria (Oyelade and Ayansola 2015) and Congo (Martin and Mound 2007).

This study especially focused on studying the genetic diversity of the invasive whitefly *B. tabaci* species complex using three techniques based on *mtCOI* gene sequence (bi-directional PCR, CAPS, and sequencing). In the literatures, MEAM1 and New World putative species have been identified in Jordan using only RAPD technique (Sharaf and Hasan 2003). However, in this study the samples collected during

three consecutive years on more than 23 different hosts and from different geographical regions in Jordan confirmed the presence of only MEAM1. It is worth mentioning that the samples were collected from the same places where the New World had been reported earlier as well as the fact that MEAM1 putative species is not new to Jordan as it is known to originate from this area (Broadbent *et al.* 1989; Cheek and Macdonald 1994; De Barro *et al.* 2011). MEAM1 putative species are polyphagous, and the females are known for their high fecundity and lower immature mortality (Brown 2007; Costa and Brown 1991; Horowitz *et al.* 2005; Zhang *et al.* 2005); this may help this putative species to displace any other species present. In addition to the previous points, the agricultural practices in Jordan could favour the dominance of MEAM1 and the displacement of New World putative species.

Conclusions

Nine species of whiteflies in addition to *B. tabaci* were identified in Jordan. Identification was primarily based on the morphology and supported by the sequences of the *mtCOI* gene. Two species were reported in Jordan for the first time. Depending on the results, it is important to evaluate the damage caused especially by the newly reported species in Jordan, their hosts and if they could transmit any plant viruses. Also, this study was concerned with updating the status of *B. tabaci* species complex in Jordan to help in designing effective control methods. The results confirmed the presence of only MEAM1 putative species. As a result, it is important to consider the control methods of this pest, since MEAM1 is an invasive pest, that resists many insecticides and is a vector for many important plant viruses.

Acknowledgement

The authors acknowledge Prof. Dan Gerling for the morphological identification of the whiteflies, Dr Rami Horowitz for providing us with reference samples for MEAM1 and MED, and Dr. Fatima AlHaj-Ahmad and Dr. Wafa'a Odeh for their help in measuring the DNA concentration and sample collection.

Literature cited

- ALLAWI, T. R. 1994. Whitefly species in Jordan. Arab Journal Plant Protection 12 (1): 30-32.
- BARINAGA, M. 1993. Is devastating whitefly invader really a new species? Science 259 (5091): 30-31. <https://doi.org/10.1126/science.8418492>
- BING, X. L.; RUAN, Y. M.; RAO, Q.; WANG, X. W.; LIU, S. S. 2012. Diversity of secondary endosymbionts among different putative species of the whitefly *Bemisia tabaci*. Insect Science 20 (2): 194-206. <https://doi.org/10.1111/j.1744-7917.2012.01522.x>
- BOYKIN, L. M. 2014. *Bemisia tabaci* nomenclature: lessons learned. Pest Management Science 70 (10): 1454-1459. <https://doi.org/10.1002/ps.3709>
- BOYKIN, L. M.; SHATTERS JR, R. G.; ROSELL, R. C.; MCKENZIE, C. L.; BAGNALL, R. A.; DE BARRO, P.; FROHLICH, D. R. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. Molecular Phylogenetic and Evolution 44 (3): 1306-1319. <https://doi.org/10.1016/j.ympev.2007.04.020>
- BOYKIN, L. M.; ARMSTRONG, K. F.; KUBATKO, L.; DE BARRO, P. 2012. Species delimitation and global biosecurity. Evolutionary Bioinformatics 8: 1-37. <https://doi.org/10.4137/EBO.S8532>
- BROADBENT, A. B.; FOOTIT, R. G.; MURPHY, G. D. 1989. Sweet potato whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), a potential insect pest in Canada. Canadian Entomology 121 (11): 1027-1028. <https://doi.org/10.4039/Ent1211027-11>
- BROWN, J. K. 2007. The *Bemisia tabaci* complex: genetic and phenotypic variation and relevance to TYLCV-vector interactions. pp. 25-56. In: Czosnek, H. (Ed.). Tomato yellow leaf curl virus disease. Springer. Jerusalem, Israel. 447 p. https://doi.org/10.1007/978-1-4020-4769-5_3
- BROWN, J. K.; FROHLICH, D. R.; ROSELL, R. C. 1995a. The sweet potato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annual Review of Entomology 40 (1): 511-534. <https://doi.org/10.1146/annurev.en.40.010195.002455>
- BROWN, J. K.; COATS, S. A.; BEDFORD, I. D.; MARKHAM, P. G.; BIRD, J.; FROHLICH, D. R. 1995b. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). Biochemical Genetics 33: 205-214. <https://doi.org/10.1007/BF02401851>
- BYRNE, D. N.; BELLOWS JR, T. S. 1991. Whitefly biology. Annual Review Entomology 36 (1): 431-457. <https://doi.org/10.1146/annurev.en.36.010191.002243>
- CENIS, J. L.; PEREZ, P.; FERERES, A. 1993. Identification of aphid (Homoptera: Aphididae) species and clones by random amplified polymorphic DNA. Annals of the Entomological Society of America 86 (5): 545-550. <https://doi.org/10.1093/aesa/86.5.545>
- CERVERA, M. T.; CABEZAS, J. A.; SIMON, B.; MARTINEZ-ZAPATER, J. M.; BEITIA, F.; CENIS, J. L. 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. Bulletin of Entomological Research 90 (5): 391-396. <https://doi.org/10.1017/S0007485300000523>
- CHEEK, S.; MACDONALD, O. 1994. Statutory controls to prevent the establishment of *Bemisia tabaci* in the United Kingdom. Pest Science 42 (2): 135-142.
- COSTA, H. S.; BROWN, J. K. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. Entomologia Experimentalis et Applicata 61 (3): 211-219. <https://doi.org/10.1111/j.1570-7458.1991.tb01553.x>
- DE BARRO, P. J.; DRIVER, F.; TRUEMAN, J. W. H.; CURRAN, J. 2000. Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. Molecular Phylogenetic and Evolution 16 (1): 29-36. <https://doi.org/10.1006/mpev.1999.0768>
- DE BARRO, P. J.; LIU, S. S.; BOYKIN, L. M.; DINSDALE, A. B. 2011. *Bemisia tabaci*: a statement of species status. Annual Review of Entomology 56: 1-19. <https://doi.org/10.1146/annurev-ento-112408-085504>
- DINSDALE, A.; COOK, L.; RIGINOS, C.; BUCKLEY, Y. M.; DE BARRO, P. 2010. Refined global analysis of *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) mitochondrial COI to identify species level genetic boundaries. Annals of the Entomological Society of America 103 (2): 196-208. <https://doi.org/10.1603/AN09061>
- EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION. 2004. *Bemisia tabaci*. Bulletin OEPP/EPP Bulletin 34: 281-288. <https://doi.org/10.1111/j.1365-2338.2004.00729.x>
- FROHLICH, D. R.; TORRES-JEREZ, I.; BEDFORD, I. D.; MARKHAM, P. G.; BROWN, J. K. 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Molecular Ecology 8 (10): 1683-1691. <https://doi.org/10.1046/j.1365-294x.1999.00754.x>
- GAMARRA, H. A.; FUENTES, S.; MORALES, F. J.; GLOVER, R.; MALUMPHY, C.; BARKER, I. 2010. *Bemisia afersensu lato*, a

- vector of Sweet potato chlorotic stunt virus. *Plant Diseases* 94 (5): 510-514. <https://doi.org/10.1094/PDIS-94-5-0510>
- GHAHARI, H.; ABD-RABOU, S.; ZAHRADNIK, J.; OSTOVAN, H. 2009. Annotated catalogue of whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) from Arasbaran, Northwestern Iran. *Journal of Entomology and Nematology* 1 (1): 007-018. <https://doi.org/10.5897/JEN.9000004>
- GHANIM, M.; KONTSEDALOV, S. 2007. Gene expression in pyriproxyfen-resistant *Bemisia tabaci* Q biotype. *Pest Management Science: formerly Pesticide Science*. 63 (8): 776-783. <https://doi.org/10.1002/ps.1410>
- HOROWITZ, A. R.; DENHOLM, I.; GORMAN, K.; CENIS, J. L.; KONTSEDALOV, S.; ISHAAYA, I. 2003. Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica* 31: 94-98. <https://doi.org/10.1007/BF02979772>
- HOROWITZ, A. R.; KONTSEDALOV, S.; KHASDAN, V.; ISHAAYA, I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Archives of Insect Biochemistry and Physiology* 58 (4): 216-225. <https://doi.org/10.1002/arch.20044>
- IDRISS, M.; ABDALLAH, N.; AREF, N.; HARIDY, G.; MADKOUR, M. 1997. Biotypes of the castor bean whitefly *Trialeurodes ricini* (Misra) (Hom., Aleyrodidae) in Egypt: biochemical characterization and efficiency of geminivirus transmission. *Journal of Applied Entomology* 121 (1-5): 501-509. <https://doi.org/10.1111/j.1439-0418.1997.tb01440.x>
- JONES, D. R. 2003. Plant viruses transmitted by whiteflies. *European Journal of Plant Pathology* 109 (3): 195-219. <https://doi.org/10.1023/A:1022846630513>
- KHASDAN, V.; LEVIN, I.; ROSNER, A.; MORIN, S.; KONTSEDALOV, S.; MASLENIN, L.; HOROWITZ, A. R. 2005. DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population dynamics. *Bulletin of Entomological Research* 95 (6): 605-613. <https://doi.org/10.1079/BER2005390>
- KIRK, A. A.; LACEY, L. A.; BROWN, J. K.; CIOMPERLIK, M. A.; GOOLSBY, J. A.; VACEK, D. C.; WENDEL, L. E.; NAPOMPETH, B. 2000. Variation in the *Bemisia tabaci* species complex (Hemiptera: Aleyrodidae) and its natural enemies leading to successful biological control of *Bemisia* biotype B in the USA. *Bulletin of Entomological Research* 90 (4): 317-327. <https://doi.org/10.1017/S0007485300000444>
- KO, C.; CHANG, S.; HU, C. 2005. Survey of whiteflies and their transmission of plant viruses in Taiwan. Taipei: ASPAC Food and Fertilizer Technology Centre. https://www.fttc.org.tw/htmlarea_file/library/20110712181840/eb571.pdf
- 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. <https://doi.org/10.1093/molbev/msy096>
- LIU, S.; COLYIN, J.; DE BARRO, P. J. 2012. Species concepts as applied to the whitefly *Bemisia tabaci* systematics: how many species are there? *Journal of Integrative Agriculture* 11 (2): 176-186. [https://doi.org/10.1016/S2095-3119\(12\)60002-1](https://doi.org/10.1016/S2095-3119(12)60002-1)
- LIU, G.; MA, H.; XIE, H.; XUAN, N.; GUO, X.; FAN, Z.; RAJASHEKAR, B.; ARNAUD, P.; OFFMANN, B.; PICIMBON, J-F. 2016. Biotype characterization, developmental profiling, insecticide response and binding property of *Bemisia tabaci* chemosensory proteins: role of CSP in insect defense. *PLoS ONE* 11 (5): e0154706. <https://doi.org/10.1371/journal.pone.0154706>
- LUO, C.; YAO, Y.; WANG, R.; YAN, F.; HU, D.; ZHANG, Z. 2002. The use of mitochondrial cytochrome oxidase I (mt CO I) gene sequences for the identification of biotypes of *Bemisia tabaci* (Gennadius) in China. *Acta Entomologica Sinica* 45 (6): 757-763. <https://europepmc.org/article/cba/381891>
- MARTIN, J. H. 2004. Whiteflies of Belize (Hemiptera: Aleyrodidae). Part 1-introduction and account of the subfamily Aleyrodinae Quaintance & Baker. *Zootaxa* 681 (1): 1-119. <https://doi.org/10.11646/zootaxa.681.1.1>
- MARTIN, J. H.; MOUND, L. A. 2007. An annotated check list of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). *Zootaxa* 1492 (1): 1-84. <https://doi.org/10.11646/zootaxa.1492.1.1>
- MLYNAREK, J. J., LABBÉ, R. M. 2018. *Trialeurodes abutiloneus* (Haldeman) (Hemiptera: Aleyrodidae), a species long present but never officially recorded in Canada. *The Canadian Entomologist* 150 (4): 532-538. <https://doi.org/10.4039/tce.2018.26>
- MORGULIS, A.; COULOURIS, G.; RAYTSELIS, Y.; MADDEN, T. L.; AGARWALA, R.; SCHAFFER, A. A. 2008. Database indexing for production MegaBLAST searches. *Bioinformatics* 24 (16): 1757-1764. <https://doi.org/10.1093/bioinformatics/btn554>
- NAUEN, R.; DENHOLM, I. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Archive of Insect Biochemistry and Physiology* 58 (4): 200-215. <https://doi.org/10.1002/arch.20043>
- NAUEN, R.; STUMPF, N.; ELBERT, A. 2002. Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science* 58 (9): 868-875. <https://doi.org/10.1002/ps.557>
- NAVAS-CASTILLO, J.; FIALLO-OLIVE, E.; SANCHEZ-CAMPOS, S. 2011. Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology* 49: 219-248. <https://doi.org/10.1146/annurev-phyto-072910-095235>
- OLIVEIRA, M. R. V.; HENNEBERRY, T. J.; ANDERSON, P. 2001. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection* 20 (9): 709-723. [https://doi.org/10.1016/S0261-2194\(01\)00108-9](https://doi.org/10.1016/S0261-2194(01)00108-9)
- OYELADE, O. J.; AYANSOLA, A. A. 2015. Diversity and distribution of whiteflies in south-western Nigeria. *African Crop Science Journal* 23 (2): 135-149. <https://www.ajol.info/index.php/acsj/article/view/117735>
- POLSTON, J. E.; DE BARRO, P.; BOYKIN, L. M. 2014. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science* 70 (10): 1547-1552. <https://doi.org/10.1002/ps.3738>
- PRABHAKER, N.; COUDRIET, D. L.; TOSCANO, N. C. 1988. Effect of synergists on organophosphate and permethrin resistance in sweet potato whitefly (Homoptera: Aleyrodidae). *Journal of Economic Entomology* 81 (1): 34-39. <https://doi.org/10.1093/jee/81.1.34>
- ROSELL, R. C.; BEDFORD, I. D.; FROHLICH, D. R.; GILL, R. J.; BROWN, J. K.; MARKHAM, P. G. 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Annals of Entomological Society of America* 90 (5): 575-589. <https://doi.org/10.1093/aesa/90.5.575>
- SHARAF, N.; ALLAWI, T. F. 1980. Studies on whiteflies on tomato in Jordan Valley I. Host range of the tobacco whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae). *Dirasat* 7 (1): 53-63.
- SHARAF, N.; HASAN, H. 2003. The identification of two biotypes of *Bemisia tabaci* in Jordan. *Dirasat* 30 (1): 101-108. <https://www.cabdirect.org/cabdirect/abstract/20033042084>
- SHI, X.; TANG, X.; ZHANG, X.; ZHANG, D.; LI, F.; YAN, F.; ZHANG, Y.; ZHOU, X.; LIU, Y. 2018. Transmission efficiency, preference and behavior of *Bemisia tabaci* MEAMI and MED under the influence of Tomato chlorosis virus. *Frontiers in Plant Science* 8: 1-9. <https://doi.org/10.3389/fpls.2017.02271>
- SHUKLA, A. K.; UPADHYAY, S. K.; MISHRA, M.; SAURABH, S.; SINGH, R.; SINGH, H.; THAKUR, N.; RAI, P.; PANDEY, P.; HANS, A. L.; SRIVASTAVA, S. 2016. Expression of an insecticidal fern protein in cotton protects against whitefly. *Nature Biotechnology* 34 (10): 1046-1051. <https://doi.org/10.1038/nbt.3665>
- SUN, D. B.; XU, J.; LUAN, J. B.; LIU, S. S. 2011. Reproductive incompatibility between the B and Q biotypes of the whitefly *Bemisia tabaci* in China: genetic and behavioural evidence. *Bulletin of Entomological Research* 101 (2): 211-220. <https://doi.org/10.1017/S0007485310000416>

- TAMURA, K.; NEI, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10 (3): 512-526. <https://doi.org/10.1093/oxfordjournals.molbev.a040023>
- TAY, W. T.; EVANS, G. A.; BOYKIN, L. M.; DE BARRO, P. J. 2012. Will the real *Bemisia tabaci* please stand up? *PLoS One* 7 (11): e50550. <https://doi.org/10.1371/journal.pone.0050550>
- TSAGKARAKOU, A.; TSIGENOPOULOS, C. S.; GORMAN, K.; LAGNEL, J.; BEDFORD, I. D. 2007. Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Greece: mitochondrial DNA and microsatellites. *Bulletin of Entomological Research* 97 (1): 29-40. <https://doi.org/10.1017/S000748530700466X>
- WANG, X-W.; LUAN, J-B.; LI, J-M.; SU, Y-L.; XIA, J.; LIU, S-S. 2011. Transcriptome analysis and comparison reveal divergence between two invasive whitefly cryptic species. *BMC Genomics* 12 (1): 1-12. <https://doi.org/10.1186/1471-2164-12-458>
- WANG, H-L.; YANG, J.; BOYKIN, L. M.; ZHAO, Q-Y.; LI, Q.; WANG, X-W.; LIU, S-S. 2013. The characteristics and expression profiles of the mitochondrial genome for the Mediterranean species of the *Bemisia tabaci* complex. *BMC Genomics*. 14 (1): 1-15. <https://doi.org/10.1186/1471-2164-14-401>
- WANG, J.; DU, Y.; XU, Z. 2016. Six newly recorded species of whitefly (Hemiptera: Aleyrodidae) from China. *Zoological Systematics* 41 (4): 427-438. <https://doi.org/10.1186/zs.201647>
- WATANABE, L. F. M.; BELLO, V. H.; DE MARCHI, B. R.; SILVA, F. B.; FUSCO, L. M.; SARTORI, M. M. P.; PAVAN, M. A.; KRAUSE-SAKATE, R. 2019. Performance and competitive displacement of *Bemisia tabaci* MEAM1 and MED cryptic species on different host plants. *Crop Protection* 124: 104860. <https://doi.org/10.1016/j.cropro.2019.104860>
- WU, X.; LI, Z.; HU, D.; SHEN, Z. 2003. Identification of Chinese populations of *Bemisia tabaci* (Gennadius) by analyzing ribosomal ITS1 sequence. *Progress in Natural Science* 13 (4): 276-281. <https://doi.org/10.1080/10020070312331343530>
- XU, J.; DE BARRO, P. J.; LIU, S. S. 2010. Reproductive incompatibility among genetic groups of *Bemisia tabaci* supports the proposition that the whitefly is a cryptic species complex. *Bulletin of Entomological Research* 100 (3): 359-366. <https://doi.org/10.1017/S0007485310000015>
- YANG, N.; XIE, W.; YANG, X.; WANG, S.; WU, Q.; LI, R.; PAN, H.; LIU, B.; SHI, X.; FANG, Y.; XU, B.; ZHOU, X.; ZHANG, Y. 2013. Transcriptomic and proteomic responses of sweet potato whitefly, *Bemisia tabaci*, to thiamethoxam. *PLoS One* 8 (5): e61820. <https://doi.org/10.1371/journal.pone.0061820>
- YANG, J.; XIE, W.; LIU, B.; WANG, S.; WU, Q.; HE, Y.; ZHANG, Y.; JIAO, X. 2020. Phenolics, rather than glucosinolates, mediate host choice of *Bemisia tabaci* MEAM1 and MED on five cabbage genotypes. *Journal of Applied Entomology* 144 (4): 287-296. <https://doi.org/10.1111/jen.12737>
- ZHANG, Z.; SCHWARTZ, S.; WAGNER, L.; MILLER, W. 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7 (1-2): 203-214. <https://doi.org/10.1089/10665270050081478>
- ZHANG, L. P.; ZHANG, Y. J.; ZHANG, W. J.; WU, Q. J.; XU, B. Y.; CHU, D. 2005. Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. *Journal of Applied Entomology* 129 (3): 121-128. <https://doi.org/10.1111/j.1439-0418.2005.00950.x>

Origin and funding

The present research was self-funded project.

Author contribution

Ihab Ghabeish: samples collection, morphological identification and writing.

Mais Sweiss: molecular identification, results analysis and writing.

Ghandi Anfoka: samples collection and sequencing of the samples.