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Diversity and molecular identification of endosymbionts of the white-flies *Bemisia tabaci* and *Trialeurodes vaporariorum*

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Abstract

The infection of insects with symbiotic bacteria has significant implications for the evolution and ecology of the hosts. Maternally inherited symbionts associated with *B. tabaci* and *T. vaporariorum* whiteflies play a vital role in their fitness and survival. Whitefly symbionts have been identified in many countries, but no study has been undertaken in Iraq and the UK. For the first time in both countries, the molecular identification and diversity of the symbionts of both whiteflies have been investigated in the present study. Fourteen populations of *B. tabaci* from Iraq and twenty populations of *T. vaporariorum* from the UK were used to detect and identify seven common endosymbiont bacteria associated with whitefly using the 16S rRNA and 23S rRNA nuclear markers. All females and males of B. tabaci harboured one primary symbiont, Portiera aleyrodidarum, and almost all of both sexes of all B. tabaci species have the two secondary symbionts Hamiltonella sp. and Rickettsia sp. The primary symbiont P. aleyrodidarum was also detected in both sexes of T. vaporariorum, whereas only one secondary symbiont, *Arsenophonus* sp., was detected in almost all females but not in the males. Additionally, an investigation into genetic diversity using three genes of the Arsenophonus sp. populations showed no variation among different populations. The results supported the notion that Arsenophonus sp. might play an important role in the survival of T. vaporariorum females and maybe a killer of male whiteflies. Also, secondary symbionts Hamiltonella sp. and Rickettsia sp. with B. tabaci could support their host's fitness and survival. These findings reveal the endosymbionts associated with *B. tabaci* and *T. vaporariorum* in Iraq and the UK, respectively. Further investigation is needed to understand the roles of these symbionts in both countries .

Keywords: *Bemisia tabaci*, *Trialeurodes vaporariorum*, endosymbiont bacteria, 16S rRNA and 23S rRNA

Introduction

Endosymbiosis is vital in insect-plant interactions, affecting numerous aspects of herbivorous ecology. [1] described hundreds of different bacterial endosymbionts of herbivores through their anatomy. Symbiotic bacteria have traditionally been classified as primary or secondary endosymbionts. Relations among hosts and primary symbionts are often ancient, with an expected history of 30–250 million years [2]. Primary symbionts are inherited entirely vertically through the germline to offspring. They are normally considered mutualistic symbioses and are commonly required for host fitness, survival, and reproduction. The endosymbionts are adapted to the hosts' diet by supplying vital nutrients, which are obligated to both partners [3]. Obligate symbionts are located in particular host cells that might constitute a larger organ-like structure called the bacteriome. It has been reported that 15% of insect species harbour a primary symbiont [1].

Secondary symbionts are considered facultative endosymbionts from the host's perspective and have a shorter coevolutionary history with the host species [4]. Some secondary symbionts are uncommon, whereas others are fixed in their hosts [5, 6]. Facultative symbionts are usually located in specific host tissues, such as fat bodies, muscle, nervous tissue, and the gut, but they might also be found in the haemocoel of their host, and they occur at lower titres than primary endosymbionts [7, 8]. Secondary symbiotic bacteria are commonly transmitted vertically, but in some cases, horizontal transmission between hosts might occur [9, 4, 10].

Whiteflies are known to host the obligatory symbiont *Portiera aleyrodidarum*, which has a long coevolutionary history with all species of the Aleyrodinae subfamily [11]. In addition to the primary endosymbiont, whiteflies contain a range of secondary symbionts, including species of *Hamiltonella* sp., *Cardinium* sp. (Bacteroidetes), *Fritschea* sp., *Wolbachia* sp., *Arsenophonus* sp., and *Rickettsia* sp. (Rickettsiales) [12,13]. Both the endosymbiotic bacteria and mtDNA are vertically transmitted and are linked with the evolutionary history of their hosts, and consequently might be used to shed light on evolutionary processes relating to both sides of symbiosis [14, 15].

Endosymbiotic bacteria have been reported to have effects on various aspects of host biology, including genetic diversity, nutrition, survival, reproduction, insecticide resistance, and the ability to cope with environmental factors [16, 17]. The primary symbiont *Portiera* s Supplements the hosts' diet with essential nutrients like amino acids and carotenoids that provide significant anti-oxidant action [18]. Additionally, secondary symbionts contribute to pest hosts and may play negative or even decisive roles in the survival of their hosts. For instance, secondary symbionts such as *Wolbachia* sp. can provide nutrients [19], initially increase host resistance to parasitic wasps and pathogens [20], and may also increase tolerance to heat stress [21]. However, at the same time, some secondary endosymbionts, such as *Wolbachia* sp., *Arsenophonus* sp., *Cardinium* sp. and *Rickettsia* sp., have been reported to be parasitic rather than useful to their hosts [22]. Endosymbionts influence the reproductive sys-

tems of insects by imposing asexuality, being male-killers, and feminising genetic males. Also, the endosymbionts encourage cytoplasmic incompatibility (CI) together with parthenogenesis; all these aspects help the symbionts to spread their infections in host populations [23, 15,24].

In the case of whitefly, secondary endosymbionts have been found to affect several aspects of the performance of their hosts, for instance, in increased resistance to parasitoids [25], tolerance to high temperatures [26], the capacity to transmit viruses [27], and susceptibility to pesticides [28,29]. [30] revealed that the MEAM1 genetic group of *B. tabaci* infected with *Rickettsia* in the US exhibited significantly increased fitness. Also, there was an increase in female bias in their host populations. The symbionts could perform two functions, being mutualistic and reproductive manipulators for their host insect, which could positively affect the host population size, and spread the symbiont in the field. Additionally, the secondary endosymbionts *Cardinium* and⁄ or *Arsenophonus* in *B. tabaci* might influence interbreeding among whitefly biotypes [31].

The secondary symbionts *Rickettsia* sp. and *Hamiltonella* sp. are known to be harboured by specific *B. tabaci* biotypes and play important roles in their fitness. For instance, *Rickettsia* sp. linked with *B. tabaci* MEAM1 genetic group has been reported as unable to synthesise some nutritional substances such as amino acids. Therefore, *Rickettsia* sp. in biotype B needs to obtain nutrition from its host [32]. In addition, the secondary symbiont *Hamiltonella* sp. also increases its host's resistance to parasitoid wasps [33]. Also, *Hamiltonella* sp. linked with *B. tabaci* MEAM1 might play an important role in assisting the invasion of MEAM1 throughout the world [34] and is suggested to increase the transmission capacity of plant viruses, especially TYLCV [27, 35].

Bacterial diversity in whitefly has been studied in several regions of their distribution, but there is as yet no data concerning *T. vaporariorum* and *B. tabaci* symbionts in the UK and Iraq. Thus, this study aims to investigate the endosymbionts associated with *T. vaporariorum* and *B. tabaci* populations from the UK and Iraq, respectively. The results report the presence of primary and secondary symbionts of whitefly in both countries. The results might improve our understanding of the role of symbiotic bacteria in whitefly and may support the development of better whitefly management.

Materials and Methods

Field sampling

The locations and host plants of samples of whiteflies collected from Iraq and the UK are described and detailed in Table (1).

Table .خطأ! لا يوجد نص من النمط المعين في المستند **(1):** Collection sites, population codes, dates of collection, host plants, and coordinates for the glasshouse whitefly *T. vaporariorum* and *B. tabaci* sampled from the UK and Iraq examined in this study.

Glasshouse whitefly *T. vaporariorum* populations from the UK						
Locality	**Code**	**Year**	**Host**	**Plant family**	**Latitude (°N)**	**Longitude (°E)**
Billingham East/Teesside	BE14	2014	Tomato	Solanaceae	54.604285	-1.257358
Dundee	DU1_14	2014	Eupatorium	Asteraceae	56.456253	-3.025183
Dundee	DU15	2015	Eupatorium	Asteraceae	56.456253	-3.025183
East Riding of York-shire	ERYS15	2015	Cucumber	Cucurbitaceae	53.741930	-0.731197
East Riding of York-shire	ERYS14	2014	Cucumber	Cucurbitaceae	53.750523	-0.732015
East York	EYO14	2014	Cucumber	Cucurbitaceae	53.771412	-0.748213
Essex	ES15	2015	Tomato	Solanaceae	51.933305	1.022727
Essex	ES14	2014	Tomato	Solanaceae	51.933305	1.022727
Herefordshire	HE2_14	2014	Cape gooseberry	Solanaceae	52.162737	-2.996278
Herefordshire	HE3_14	2014	Basil	Lamiaceae	52.162737	-2.996278
Herefordshire	HE4_14	2014	Chili peppers	Solanaceae	52.162737	-2.996278
Herefordshire	HE15	2015	Squash	Cucurbitaceae	52.162737	-2.996278
Isle of Wight	IW14	2014	Unknown	-	50.657994	-1.227233
Kent County	KE15	2015	Tomato	Solanaceae	51.283319	1.295062
Lab colony	LC15	2015	Eggplant	Solanaceae	54.980320	-1.615713
Norfolk	NO15	2015	Tomato	Solanaceae	52.560526	0.442994
Norfolk	NO3_14	2014	Tomato	Solanaceae	52.560526	0.442994
Orkney	Or14	2014	Pelargonium	Geraniaceae	59.052969	-3.293660
Orkney	Or15	2015	Pelargonium	Geraniaceae	59.052969	-3.293660
West Sussex	WS15	2015	Tomato	Solanaceae	50.832853	-0.027808
Sweet potato whitefly *B. tabaci* populations from Iraq						
Locality	**Code**	**Year**	**Host**	**Plant family**	**Latitude (°N)**	**Longitude (°E)**
Basra	BAS-Tom-15	2015	Tomato	Solanaceae	29.975	48.474
Hillah	HI-Tom-15	2015	Tomato	Solanaceae	32.406	44.405
Karbala 1	KA1-Tom-15	2015	Tomato	Solanaceae	32.676	44.164
Karbala 2	KA2-Pep-15	2015	Pepper	Solanaceae	32.676	44.164
Karbala 3	KA3-Tom-16	2016	Tomato	Solanaceae	32.512	44.052
Kufa	KU-Tom-16	2016	Tomato	Solanaceae	32.108	44.392
Mosayib	MO-Tom-16	2016	Tomato	Solanaceae	32.778	44.290
Muthanna1	MU1-Tom-16	2016	Tomato	Solanaceae	31.533	45.200

Muthanna2	MU2-Tom-15	2015	Cucumber	Cucurbitaceae	31.533	45.200
Muthanna3	MU3-Aln-Tom-16	2016	Tomato	Solanaceae	31.666	45.183
Muthanna4	MU4-SA-Cum-16	2016	Cucumber	Cucurbitaceae	31.483	45.166
Najaf 1	NA1-Tom-15	2015	Tomato	Solanaceae	32.019	44.338
Najaf 2	NA2-Pep-15	2015	Pepper	Solanaceae	32.019	44.338
Najaf4	NA4-Eggp-16	2016	Eggplant	Solanaceae	32.019	44.338

Confirming the identity of whitefly molecularly and morphologically

The morphological and molecular techniques used to identify the *B. tabaci* and *T. vaporariorum* are described [36, 37, 38].

Molecular identification and sequencing of endosymbionts

The total gDNA of 10 males and ten females from each population of B. tabaci and T. vaporariorum was used to detect the presence of obligate and facultative bacterial symbionts. The PCR was performed using species-specific markers for the 16S rRNA genes in Portiera sp., Wolbachia sp., Rickettsia sp., Hamiltonella sp., and Cardinium sp. and the 23S rRNA genes in Arsenophonus sp. and Fritschea sp. (Table 2). The protocol of PCR amplification, as in [36, 37], was used. Additionally, to check the quality of DNA extraction, samples that tested negative for all symbiotic bacteria were cross-checked for the primary endosymbiont P. aleyrodidarum using primers 518f and 799r of the 16S rRNA gene to check the DNA quality. Also, adults of both B. tabaci and T. vaporariorum positive for secondary symbionts were included to test for the reliability of the PCR testing. The following conditions for PCR reactions were used: initial denaturation at 93 °C for 2 min, followed by 35 cycles of 93 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The PCR products were visualised on 2% agarose gels containing ethidium bromide and were purified using ExoSap as described in [39].

Characterisation of Arsenophonus sp. diversity

One to two *Arsenophonus* sp. positive individuals were randomly chosen from each *T. vaporariorum* sample (representing both UK mtCOI haplotypes and all geographic locations) for use in multi-locus sequence typing (MLST). The PCR and sequencing of three housekeeping genes of *Arsenophonus* sp. (ftsK, yaeT, and fbaA) were carried out using the primers described in Table 2 [40,41]. The same PCR reac-

tion was used as described in [39] (Kareem 2018), and the appropriate annealing temperature was used for each reaction, as indicated in Table 2.

Sequence alignment and phylogenetic analysis

All of the symbiont DNA sequencings were performed and visualised on a 3130XL Genetic Analyzer as described in the mtCOI sequencing procedure. The sequences obtained were checked using Geneious, version 6.1.4 [42]. All sequences were compared with those in the GenBank database using the NCBI BLAST algorithm. Single sequences of primary and secondary endosymbionts of B. tabaci were deposited in NCBI GenBank under accession numbers KY465885, KX679579, and KX679580, respectively. Also, sequences of a primary and a secondary endosymbiont of *T. vaporariorum* were deposited in GenBank under accession numbers KY457224 and KY243936. Additionally, the *Arsenophonus* sp. gene sequences were deposited in NCBI GenBank under the accession numbers KY626170-KY626172 for fbaA, ftsK and yaeT genes, respectively. The phylogenies were estimated using maximum likelihood (ML) using MEGA 6, as described in [39].

Table (2): Primers are used to screen the primary and secondary symbionts in whitefly species [41]. Ann.: annealing temperature. Amp. Size: amplification product size.

Targeted taxon	Targeted gene	Primers	Sequences (5' - 3')	An. (C)	Amp. size (bp)
Portiera	16S rRNA	28F	TGCAAGTCGAGCGGC	55	1000-1100
		1098R	AAAGTTCCCGCCTTATGCGT		
Arsenophonus	23S rRNA	Ars23S.1	CGTTTGATGAATTCATAGTCAAA	55	600
		Ars23S.2	GGTCCTCCAGTTAGTGTTACCCAAC		
Wolbachia	16S rRNA	Wol16S-f	CGGGGGAAAAATTTATTGCT	55	600
		Wol16S-r	AGCTGTAATACAGAAAGTAAA		
Hamiltonella	16S rRNA	Ham_F	TGAGTAAAGTCTGGAATCTGG	55	700
		Ham_R	AGTTCAAGACCGCAACCTC		
Rickettsia	16S rRNA	RB_F	GCTCAGAACGAACGCTATC	55	900
		RB_R	GAAGGAAAGCATCTCTGC		
Cardinium	16S rRNA	CFB_F	GCGGTGTAAAATGAGCGTG	55	400
		CFB_R	ACCTMTTCTTAACTCAAGCCT		
Fritschea	23S rRNA	U23F	GATGCCTTGGCATTGATAGGCGATGAAGGA	55	600
		23SIGR	TGGCTCATCATGCAAAAGGCA		
Portiera	16S rRNA	518f	CCAGCAGCCGCGGTAAT	55	1000-1100
		799r	CMGGGTATCTAATCCKGTT		
Arsenophonus	*fbaA*	fbaAf	GCYGCYAAAGTTCRTTCTCC	58	~800
		fbaAr	CCWGAACCDCCRTGGAAAACAAAA		
Arsenophonus	*yaeT*	YaeTF496	GGCGATGAAAAAGTTGCTCATAGC	55	500
		YaeTR496	TTTTAAGTCAGCACGATTACGCGG		
Arsenophonus	*ftsK*	ftsKFor1	GCCGATCTCATGATGACCG	59	400
		ftsKRev1	CCATTACCACTCTCACCCTC		

Results and Discussion

Confirmation of the identity of specimens molecularly and morphologically

Both whitefly species have been confirmed morphologically and molecularly as *T. vaporariorum* and B. tabaci in the UK and Iraq, respectively.

Symbionts

The results for the symbionts of *T. vaporariorum* showed that the primary symbiont *P. aleyrodidarum* was identified in almost all samples of both sexes, indicating that the DNA extracts were good quality. The infection status of *T. vaporariorum* was 96.6% for one secondary symbiont, *Arsenophonus* sp., in the females, and it was not present in any of the males. At the same time, no PCR products were found for the other symbionts (Table 3). The PCR products were sequenced to confirm the genus and species of symbiotic bacteria using the corresponding NCBI GenBank databases, the sequence length of Arsenophonus sp. 23S rRNA was 447 bp, whereas the sequence for the primary endosymbiont P. aleyrodidarum 16S rRNA was 784 bp in length.

The analyses of the *P. aleyrodidarum* and *Arsenophonus* sp. sequences from 20 *T. vaporariorum* populations showed no polymorphisms within species. All the *P. aleyrodidarum* and *Arsenophonus* sp. sequences obtained from 20 populations of *T. vaporariorum* are identical to those sequences deposited in GenBank under accession numbers KY457224 and KY243936, respectively. The *P. aleyrodidarum* sequence matched 100% with the GenBank sequences with accession numbers CP004358 and Z11928 [43], and secondary symbionts *Arsenophonus* sp. matched 99% with the *Arsenophonus* sp. isolated from India with the accession number KJ541957.

Table (3): Numbers of male and female individuals of *T. vaporariorum* infected by each of the seven endosymbiotic bacteria tested using specific primers for whitefly symbiotic bacteria. Ten females and ten males were tested for each endosymbiont.

Locality	Codes	N* ♀+♂	*Portiera* ♀+ ♂		*Wolbachia* ♀ + ♂		*Hamiltonella* ♀+ ♂		*Arsenophonus* ♀+ ♂		*Rickettsia* ♀+ ♂		*Cardinium* ♀+ ♂		*Fritschea* ♀+ ♂	
Herefordshire	HE2_14	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-
Herefordshire	HE3_14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Herefordshire	HE4_14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Herefordshire	HE15	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Orkney	Or14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Orkney	Or15	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Dundee	DU14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Dundee	DU15	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
East York's	EYO14	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-
East Riding of Yorkshire	ERYS15	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-
East Riding of Yorkshire	ERYS14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Essex	ES15	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-
Essex	ES14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
West Sussex	WS15	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-
Norfolk	NO15	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Norfolk	NO3_14	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-
Isle of Wight	IW14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Billingham East /Teesside	BE14	20	10	10	-	-	-	-	10	-	-	-	-	-	-	-
Kent County	KE15	20	10	9	-	-	-	-	10	-	-	-	-	-	-	-
Lab Colony	LC15	20	10	9	-	-	-	-	9	-	-	-	-	-	-	-

For Iraqi *B. tabaci*, the primary symbiont *P. aleyrodidarum* was identified in all samples in both sexes, again indicating that the DNA extracts were of good quality. The infection status of *B. tabaci* was 96.4% for the secondary symbionts *Hamiltonella* sp. and *Rickettsia* sp. in both sexes, while no PCR products were found for the other symbionts considered (Table 3). The PCR products on the gels were sequenced to confirm the secondary species of symbiotic bacteria. Sequences for *P. aleyrodidarum, Hamiltonella* sp. and *Rickettsia* sp. matched 100% to the corresponding sequences of each of the symbiont species available in NCBI GenBank [43,34]. The analyses of the *P. aleyrodidarum, Hamiltonella* sp. and *Rickettsia* sp. from 14 *B. tabaci* populations showed no polymorphisms within species. All the 16S rRNA sequences of the *P. aleyrodidarum, Hamiltonella* sp. and *Rickettsia* sp. were identical to those sequences deposited in GenBank under accession numbers KY465885, KX679580 and KX679579 with total lengths 623, 676, and 768 bp, respectively.

Genetic characterisation of *Arsenophonus* sp.

The sequences of three housekeeping genes of the secondary endosymbiont *Arsenophonus* sp. of glasshouse whitefly *T. vaporariorum* showed a 100% match to the NCBI GenBank database sequences. In the three bacterial genes investigated for MLST analysis, with total lengths of 587, 382, and 335 bp for *fbaA*, *yaeT*, and *ftsK*, respectively, no polymorphism was detected in 20 populations of whitefly collected from the UK. For the first time, this study presents the identification of endosymbionts of *T. vaporariorum* in the UK and *B. tabaci* populations in Iraq, respectively. *T. vaporariorum* populations from the UK harboured just one secondary symbiont, *Arsenophonus* sp., in females but not males. This finding is identical to that of another study that showed that males of *T. vaporariorum* from Japan did not harbour *Arsenophonus* sp., even though females from this population in various countries were all infected [41] (Kapantaidaki *et al.*, 2015). In contrast, populations of this species in Croatia, Bosnia, and Herzegovina harboured both *Arsenophonus* sp. and *Hamiltonella* bacterial symbionts found in females [44,45]. A more diverse community of bacterial symbionts was recorded in *T. vaporariorum* populations from Montenegro, where the populations harboured *Rickettsia, Hamiltonella, Arsenophonus, Wolbachia, and Cardinium* [46].

Table (4): Numbers of both sexes of *B. tabaci* infected by each of the seven endosymbiotic bacteria were tested using specific primers for whitefly symbiotic bacteria. Ten females and ten males were tested for each endosymbiont.

Locality	Codes	N ♀+♂	*Portiera* ♀+ ♂		*Wolbachia* ♀ + ♂		*Hamiltonella* ♀+ ♂		*Arsenophonus* ♀+ ♂		*Rickettsia* ♀+ ♂		*Cardinium* ♀+ ♂		*Fritschea* ♀+♂	
Basra	BAS_15	20	10	10	-	-	10	9	-	-	9	10	-	-	-	-
Hillah	HI_15	20	10	10	-	-	10	9	-	-	10	9	-	-	-	-
Karbala1	KA1_15	20	10	10	-	-	9	10	-	-	9	10	-	-	-	-
Karbala2	KA2_15	20	10	10	-	-	10	10	-	-	10	9	-	-	-	-
Karbala3	KA3_16	20	10	10	-	-	10	9	-	-	10	9	-	-	-	-
Kufa	KU_16	20	10	10	-	-	9	10	-	-	10	10	-	-	-	-
Mosayib	MO_16	20	10	10	-	-	10	10	-	-	9	10	-	-	-	-
Muthanna1	MU1_16	20	10	10	-	-	9	10	-	-	10	9	-	-	-	-
Muthanna2	MU2_16	20	10	10	-	-	10	9	-	-	9	10	-	-	-	-
Muthanna3	MU3-Aln_16	20	10	10	-	-	9	10	-	-	10	10	-	-	-	-
Muthanna4	MU4-SA_16	20	10	10	-	-	9	10	-	-	10	9	-	-	-	-
Najaf_1	NA1_15	20	10	10	-	-	9	10	-	-	9	10	-	-	-	-
Najaf_2	NA2_15	20	10	10	-	-	10	10	-	-	10	10	-	-	-	-
Najaf_4	NA4_16	20	10	10	-	-	10	10	-	-	10	10	-	-	-	-

However, *B. tabaci* populations from Iraq harboured the same obligatory primary symbiont *P. aleyrodidarum* and the two secondary symbiotic bacteria *Hamiltonella* sp. and *Rickettsia* sp. This finding was similar to other studies [47,32]. Other mtCOI biotypes of *B. tabaci* harboured different species of secondary symbionts. For example, the symbiotic bacteria of the Mediterranean (MED) species (including the common biotype (Q) vary among regions. French and Uruguayan populations of MED Q1 were infected with *Cardinium* sp. and *Hamiltonella* sp. at high frequencies [6]. However, in Greek and West African populations and a laboratory population representing MED Q1 in China, approximately 100% infection was found with *Hamiltonella* sp. but not with *Cardinium* sp. [48,49,50]. There are doubts about the role of *Hamiltonella* sp. with *B. tabaci* biotypes. For instance,[35] demonstrated a link between the capacity of *B. tabaci* biotypes to harbour *Hamiltonella* sp. and to transmit TYLCV. In contrast, the MED population in the same study without the secondary symbiont *Hamiltonella* sp. was ineffective in transmitting the virus. Therefore, it would be interesting to know the role of secondary symbionts since new strains of TYLCV have recently been recorded in Iraq and might be transmitted by new biotypes harbouring *Hamiltonella* sp. As a result, the primary role of *Hamiltonella* sp. in virus transmission in the various *B. tabaci* biotypes needs to be further investigated.

It has been reported that *B. tabaci* biotypes that harbour *Rickettsia* sp. might be linked to insecticide resistance, increased host resistance against parasitoid wasps, and increased whitefly fitness and female bias [28, 25, 30]. *Rickettsia* sp. of *B. tabaci* MEAM1 isolated was confirmed to be linked with a reduced capacity of whitefly to resist pesticides and immunoreactions against parasitic wasps [28, 25]. Therefore, *Rickettsia* sp. linked with Iraqi *B. tabaci* could play the same role as above to make its host more fit and able to survive.

The results of a further investigation of the genetic diversity in secondary symbionts of the UK whitefly showed no genetic diversity within *Arsenophonus* sp. infecting *T. vaporariorum*, despite its prevalence in this species. The sequences obtained from the *fbaA*, *ftsK*, and *YaeT* housekeeping genes were identical for all our positive samples of *Arsenophonus* sp. in *T. vaporariorum*. On the other hand, the sequence analysis of *fbaA*, *ftsK*, and *YaeT* revealed genetic diversity within *Arsenophonus* infecting *B. tabaci*, but this diversity was highly correlated with the different *B. tabaci* biotypes [40] (Mouton *et al.*, 2012). In the same study, almost no polymorphism was found in the *Arsenophonus* gene sequences from African *T. vaporariorum* samples, which was identical to the present finding.

The low polymorphism of the secondary symbiont *Arsenophonus* sp. within *T. vaporariorum* populations, alongside its high occurrence in *T. vaporariorum*, is consistent with an established and vertically transmitted endosymbiont. The information concerning the symbionts and mtCOI diversity of *T. vaporariorum* confirmed and supported the idea that there are no biotypes in *T. vaporariorum*. However, more sec-

ondary symbionts and high mtCOI diversity reported from Iraqi whitefly are consistent with the complex species of *B. tabaci*[40,51,52, 53].

These findings provide an initial database for further investigating symbiotic bacteria associated with whiteflies in the UK and Iraq. Further study of the role of these symbionts and their diversity is needed to update the status of *T. vaporariorum* and *B. tabaci*. The outcomes may potentially influence the management of Whitefly.

The presence of *Portiera* sp., an obligate endosymbiont in *T. vaporariorum* haplotypes H1 and H3 in the UK, was found in both sexes, whereas the facultative symbiont *Arsenophonus* sp. was detected in females but not males. However, analysis of the four *B. tabaci* biotypes in Iraq showed the presence of *Portiera* sp., an obligate endosymbiont. In contrast, the facultative symbionts *Hamiltonella* sp. and *Rickettsia* sp. were also detected in most individuals.

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References

1) Buchner, P. (1953) Endosymbiose der Tiere mit pflanzlichen Mikroorganismen. Basel: Birkhäuser Verlag.

2) Baumann, P. (2005) 'Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects', Annual Review of Microbiology, 59, 155-89.

3) Baumann, P., Moran, N.A. and Baumann, L. (2006) 'Bacteriocyte-associated endosymbionts of insects', in Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H. and Stackebrandt, E. (eds.) The prokaryotes, volume 1: symbiotic associations, biotechnology, applied microbiology. 3rd edn. New York: Springer, 403-438.

4) Dale, C. and Moran, N.A. (2006) 'Molecular interactions between bacterial symbionts and their hosts', Cell, 126(3), 453-465.

5) Simon, J.-C., Carre, S., Boutin, M., Prunier–Leterme, N., Sabater–Muñoz, B., Latorre, A. and Bournoville, R. (2003) 'Host–based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts', Proceedings of the Royal Society of London B: Biological Sciences, 270(1525), 1703-1712.

6) Gueguen, G., Vavre, F., Gnankine, O., Peterschmitt, M., Charif, D., Chiel, E., Gottlieb, Y., Ghanim, M., Zchori-Fein, E. and Fleury, F. (2010) 'Endosymbiont metacommunities, mtDNA diversity and the evolution of the Bemisia tabaci (Hemiptera: Aleyrodidae) species complex', Molecular Ecology 19(19), 4365-4376.

7) Dobson, S.L., Bourtzis, K., Braig, H.R., Jones, B.F., Zhou, W., Rousset, F. and O'Neill, S.L. (1999) 'Wolbachia infections are distributed throughout insect somatic and germ line tissues', Insect Biochemistry and Molecular Biology, 29(2), 153-160.

8) Moran, N.A., McCutcheon, J.P. and Nakabachi, A. (2008) 'Genomics and evolution of heritable bacterial symbionts', Annual Review of Genetics, 42, 165-190.

9) Russell, J.A., Latorre, A., Sabater-Muñoz, B., Moya, A. and Moran, N.A. (2003) 'Side‐stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea', Molecular Ecology, 12(4), 1061-1075.

10) Oliver, K.M., Degnan, P.H., Burke, G.R. and Moran, N.A. (2010) 'Facultative symbionts in aphids and the horizontal transfer of ecologically important traits', Annual Review of Entomology, 55, 247-266.

11) Thao, M.L. and Baumann, P. (2004) 'Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts', Applied and Environmental Microbiology, 70(6), 3401-3406.

12) Zchori-Fein, E. and Brown, J.K. (2002) 'Diversity of prokaryotes associated with Bemisia tabaci (Gennadius)(Hemiptera: Aleyrodidae)', Annals of the Entomological Society of America, 95(6), 711-718.

13) Nirgianaki, A., Banks, G.K., Frohlich, D.R., Veneti, Z., Braig, H.R., Miller, T.A., Bedford, I.D., Markham, P.G., Savakis, C. and Bourtzis, K. (2003) 'Wolbachia infections of the whitefly Bemisia tabaci', Current Microbiology 47(2), 93-101.

14) Hurst, G.D. and Jiggins, F.M. (2005) 'Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts', Proceedings of the Royal Society of London B: Biological Sciences, 272(1572), 1525-1534.

15) Werren, J.H., Baldo, L. and Clark, M.E. (2008) 'Wolbachia: master manipulators of invertebrate biology', Nature Reviews Microbiology, 6(10), 741-751.

16) Saridaki, A. and Bourtzis, K. (2010) 'Wolbachia: more than just a bug in insects genitals', Current Opinion in Microbiology, 13(1), 67-72.

17) Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. and Fukatsu, T. (2012) 'Symbiont-mediated insecticide resistance', Proceedings of the National Academy of Sciences of the United States of America, 109(22), 8618-8622.

18) Santos-Garcia, D., Farnier, P.A., Beitia, F., Zchori-Fein, E., Vavre, F., Mouton, L., Moya, A., Latorre, A. and Silva, F.J. (2012) 'Complete genome sequence of "Candidatus Portiera aleyrodidarum" BT-QVLC, an obligate symbiont that

supplies amino acids and carotenoids to Bemisia tabaci', Journal of Bacteriology, 194(23), 6654-6655.

19) Brownlie, J.C., Cass, B.N., Riegler, M., Witsenburg, J.J., Iturbe-Ormaetxe, I., McGraw, E.A. and O'Neill, S.L. (2009) 'Evidence for metabolic provisioning by a common invertebrate endosymbiont, Wolbachia pipientis, during periods of nutritional stress', PLoS Pathogens, 5(4), p. e1000368.

20) Vorburger, C., Gehrer, L. and Rodriguez, P. (2010) 'A strain of the bacterial symbiont Regiella insecticola protects aphids against parasitoids', Biology Letters, 6(1), 109-111.

21) Montllor, C.B., Maxmen, A. and Purcell, A.H. (2002) 'Facultative bacterial endosymbionts benefit pea aphids Acyrthosiphon pisum under heat stress', Ecological Entomology, 27(2), 189-195.

22) Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstädter, J. and Hurst, G.D. (2008) 'The diversity of reproductive parasites among arthropods: Wolbachia do not walk alone', BMC Biology, 6(1), p. 27.

23) Werren, J.H. (1997) 'Biology of Wolbachia', Annual Review of Entomology, 42, 587-609.

24) Engelstädter, J. and Hurst, G.D.D. (2009) 'The ecology and evolution of microbes that manipulate host reproduction', Annual Review of Ecology, Evolution, and Systematics, 40, 127-149.

25) Mahadav, A., Gerling, D., Gottlieb, Y., Czosnek, H. and Ghanim, M. (2008) 'Parasitization by the wasp Eretmocerus mundus induces transcription of genes related to immune response and symbiotic bacteria proliferation in the whitefly Bemisia tabaci', BMC Genomics, 9(1), p. 342.

26) Brumin, M., Kontsedalov, S. and Ghanim, M. (2011) 'Rickettsia influences thermotolerance in the whitefly Bemisia tabaci B biotype', Insect Science, 18(1), 57-66.

27) Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Kontsedalov, S., Skaljac, M., Brumin, M., Sobol, I., Czosnek, H., Vavre, F., Fleury, F. and Ghanim, M. (2010) 'The transmission efficiency of Tomato Yellow Leaf Curl Virus by the whitefly Bemisia tabaci is correlated with the presence of a specific symbiotic bacterium species', Journal of Virology, 84(18), 9310-9317.

28) Kontsedalov, S., Zchori-Fein, E., Chiel, E., Gottlieb, Y., Inbar, M. and Ghanim, M. (2008) 'The presence of Rickettsia is associated with increased susceptibility of Bemisia tabaci (Homoptera : Aleyrodidae) to insecticides', Pest Management Science, 64(8), 789-792.

29) Ghanim, M. and Kontsedalov, S. (2009) 'Susceptibility to insecticides in the Q biotype of Bemisia tabaci is correlated with bacterial symbiont densities', Pest Management Science, 65(9), 939-942.

30) Himler, A.G., Adachi-Hagimori, T., Bergen, J.E., Kozuch, A., Kelly, S.E., Tabashnik, B.E., Chiel, E., Duckworth, V.E., Dennehy, T.J., Zchori-Fein, E. and Hunter, M.S. (2011) 'Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias', Science, 332(6026), 254-256.

31) Thierry, M., Becker, N., Hajri, A., Reynaud, B., Lett, J.M. and Delatte, H. (2011) 'Symbiont diversity and non-random hybridization among indigenous (Ms) and invasive (B) biotypes of Bemisia tabaci', Molecular Ecology, 20(10), 2172-2187.

32) Zhu, D.T., Xia, W.Q., Rao, Q., Liu, S.S., Ghanim, M. and Wang, X.W. (2016) 'Sequencing and comparison of the Rickettsia genomes from the whitefly Bemisia tabaci Middle East Asia Minor I', Insect Science, 23(4), 531-42

33) Oliver, K.M., Russell, J.A., Moran, N.A. and Hunter, M.S. (2003) 'Facultative bacterial symbionts in aphids confer resistance to parasitic wasps', Proceedings of the National Academy of Sciences, 100(4), 1803-1807.

34) Fujiwara, A., Maekawa, K. and Tsuchida, T. (2015) 'Genetic groups and endosymbiotic microbiota of the Bemisia tabaci species complex in Japanese agricultural sites', Journal of Applied Entomology, 139(1-2), 55-66.

35) Su, Q., Pan, H., Liu, B., Chu, D., Xie, W., Wu, Q., Wang, S., Xu, B. and Zhang, Y. (2013) 'Insect symbiont facilitates vector acquisition, retention, and transmission of plant virus', Scientific Reports, 3, p. 1367.

36) Kareem, A.A., Port, G. and Wolff, K., 2019. Population structure of the glasshouse whitefly Trialeurodes vaporariorum (Hemiptera: Aleyrodidae) shows multiple introductions to the UK. Agricultural and Forest Entomology, 21(2), 139-148.

37) Kareem, A.A., Logan, S.A., Port, G. and Wolff, K., 2020. Bemisia tabaci in Iraq: Population structure, endosymbiont diversity and putative species. Journal of Applied Entomology, 144(4), 297-307.

38) ABD, D.A., KAREEM, A.A. and LAHUF, A.A., 2021. Molecular identification of sweet potato whitefly Bemisia tabaci putative species in Karbala province, Iraq and possibility control it using the nanoparticles of MgO and ZnO. Plant Cell Biotechnology and Molecular Biology, 175-184.

39) Kareem, A.A., 2018. Population genetic structure and symbionts of whitefly Trialeurodes vaporariorum and Bemisia tabaci (Hemiptera: Aleyrodidae), in the UK and Iraq (Doctoral dissertation, Newcastle University).

40) Mouton, L., Thierry, M., Henri, H., Baudin, R., Gnankine, O., Reynaud, B., Zchori-Fein, E., Becker, N., Fleury, F. and Delatte, H. (2012) 'Evidence of

diversity and recombination in Arsenophonus symbionts of the Bemisia tabaci species complex', BMC Microbiology, 12(Suppl. 1), p. S10.

41) Kapantaidaki, D.E., Ovcarenko, I., Fytrou, N., Knott, K.E., Bourtzis, K. and Tsagkarakou, A. (2015) 'Low levels of mitochondrial DNA and symbiont diversity in the worldwide agricultural pest, the greenhouse whitefly Trialeurodes vaporariorum (Hemiptera: Aleyrodidae)', Journal of Heredity, 106(1), 80-92.

42) Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A. (2012) 'Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data', Bioinformatics, 28(12), 1647-1649.

43) Clark, M.A., Baumann, L., Munson, M.A., Baumann, P., Campbell, B.C., Duffus, J.E., Osborne, L.S. and Moran, N.A. (1992) 'The eubacterial endosymbionts of whiteflies (Homoptera: Aleyrodoidea) constitute a lineage distinct from the endosymbionts of aphids and mealybugs', Current Microbiology, 25(2), 119-123.

44) Skaljac, M., Zanic, K., Ban, S.G., Kontsedalov, S. and Ghanim, M. (2010) 'Co-infection and localization of secondary symbionts in two whitefly species', BMC Microbiology, 10(1), p. 142.

45) Skaljac, M., Zanic, K., Hrncic, S., Radonjic, S., Perovic, T. and Ghanim, M. (2013) 'Diversity and localization of bacterial symbionts in three whitefly species (Hemiptera: Aleyrodidae) from the east coast of the Adriatic Sea', Bulletin of Entomological Research 103(1), 48-59.

46) Prijovic, M., Skaljac, M., Drobnjakovic, T., Zanic, K., Peric, P., Marcic, D. and Puizina, J. (2014) 'Genetic variation of the greenhouse whitefly, Trialeurodes vaporariorum (Hemiptera: Aleyrodidae), among populations from Serbia and neighbouring countries, as inferred from COI sequence variability', Bulletin of Entomological Research, 104(3), 357-366.

47) Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M. and Ghanim, M. (2007) 'Biotype-dependent secondary symbiont communities in sympatric populations of Bemisia tabaci', Bulletin of Entomological Research, 97(4), 407-13.

48) Tsagkarakou, A., Mouton, L., Kristoffersen, J.B., Dokianakis, E., Grispou, M. and Bourtzis, K. (2012) 'Population genetic structure and secondary endosymbionts of Q Bemisia tabaci (Hemiptera: Aleyrodidae) from Greece', Bulletin of Entomological Research 102(3), 353-365.

49) Bing, X.L., Ruan, Y.M., Rao, Q., Wang, X.W. and Liu, S.S. (2013) 'Diversity of secondary endosymbionts among different putative species of the whitefly Bemisia tabaci', Insect Science, 20(2), 194-206.

50) Gnankine, O., Mouton, L., Henri, H., Terraz, G., Houndete, T., Martin, T., Vavre, F. and Fleury, F. (2013) 'Distribution of Bemisia tabaci (Homoptera: Aleyrodidae) biotypes and their associated symbiotic bacteria on host plants in West Africa', Insect Conservation and Diversity, 6(3), 411-421.

51) Lee, W., Park, J., Lee, G.S., Lee, S. and Akimoto, S.I., 2013. Taxonomic status of the Bemisia tabaci complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. PloS one, 8(5), p.e63817.

52) Salman, M. D. and Lahuf, A. A. (2022). A new procedure to identify plant RNA viruses associated with the whitefly (*Bemisia tabaci*) using next-generation sequencing. Arab Journal of Plant Protection, 40(2):169-174.

53) Abd, D.A., Kareem, A.A. and Lahuf, A.A. (2021). Molecular identification of sweet potato whitefly *Bemisia tabaci* putative species in Karbala province, Iraq and possibility control it using the nanoparticles of MgO and ZnO. Plant Cell Biotechnology and Molecular Biology, 22(3 & 4):175-184.