Population genetic structure and symbionts of whitefly *Trialeurodes vaporariorum* and *Bemisia tabaci* (Hemiptera: Aleyrodidae), in the UK and Iraq

Thesis submitted for the degree of Doctor of Philosophy,
School of Natural and Environmental Sciences

By

ALI ABDULHUSIEN KAREEM

September 2018

بسم الله الرحمن الـرحيم

وَيَسْـَٔلُونَكَ عَنِ ٱلرُّوحِ ۖ قُلِ ٱلرُّوحُ مِنْ أَمْرِ رَبِّى وَمَآ أُوتِيتُم مِّنَ ٱلْعِلْمِ إِلَّا قَلِيلًا

(85) And they ask you, [O Muhammad], about the soul. Say, "The soul is of the affair of my Lord. And mankind have not been given of knowledge except a little."

Holy Quran القرآن الكريم

Al-Israa (85) سورة الاسراء

Dedication

I would like to dedicate this modest effort to the martyrs of Iraq who irrigated the motherland with their innocent blood and became a symbol of defiance and steadfastness. As well as for my loving children Zaid, Ruqaya, Ridha and Kumail who brighten my life. A special feeling of gratitude to my parents, wife and brothers who have supported me throughout the entire doctorate programme.

ALI A. KAREEM

September 2018

DECLARATION

This thesis is submitted to Newcastle University for the degree requirements of Doctor of Philosophy in Biology (Insect Molecular Taxonomy and Genetic Diversity of Insects). The research detailed within was performed during the period of 2014-2018 and was conducted in Newcastle University laboratories under the supervision of Dr Gordon Port and Dr Kirsten Wolff.

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere for any award. Where other sources of information have been used, they have been acknowledged.

ALI A. KAREEM

September 2018

CERTIFICATE OF APPROVAL

I confirm that, to the best of my knowledge, this thesis is from the student's own work and effort, and all other sources of information used have been acknowledged. This thesis has been submitted with my approval for the PhD degree.

Dr Gordon Port and Dr Kirsten Wolff

September 2018

Acknowledgements

In the first place, all praise to Allah for this accomplishment. I am indebted to the Ministry of Higher Education and Scientific Research and the Agriculture College / University of Kerbala in Iraq for providing me with the scholarship and support to study for a PhD degree at Newcastle University.

I am extremely grateful to my supervisors, Dr Gordon Port and Dr Kirsten Wolff, for their valuable supervision, directional guidance, suggestions and time throughout the study period, including their advice for experimental designs, techniques and laboratory use, statistical and genetic software analysis and the final write-up. They also gave me the opportunity to attend workshops, meeting and conferences, which helped me to broaden my perspective and provided me with great experience and skills. This study would never have been accomplished without my supervisors' support. A special thanks to Professor John Colvin and Dr Ethan Hack for having been my examiners and their valuable feedback. Also, thanks to Professor John Bythell and Professor Steven Rushton for having been my panel throughout my study.

I would like to thank Dr Samuel Logan and Dr Nicholas Levsen for useful discussions and assistance in my project. Especial thanks go to Dr Irina Ovcarenko from Finland for her advice. Also big thanks to the providers of whitefly samples across the UK and Iraq, without whom this study would not have been possible. Also, I am grateful to all of the staff and friends in the School of Natural and Environmental Sciences (Biology) for their kindness, support and friendship. Special thanks go to Roselyn Brown, Mark Bendall, Matthew Peake, and Helen Martin for their help with lab techniques and their advice. I must express my warmest gratitude to all my friends and colleagues, Dr Imen, Dr Nadeer, Dr Taha, Ibrahim, Saddam, Rana, and Dr Baida for their support throughout the study period.

Finally, my most special thanks I save until last, these go to my wife Sienaa Al-Zurfi. She was always at my side throughout my long journey in higher education. Without her love, patience and encouragement this study could not have been completed.

I will never forget you!

Publications:

Kareem, A., Port, G., and Wolff, K. 2017. Population structure of glasshouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), shows multiple introductions to the UK and effects of glasshouse management. Accepted by *Agricultural and Forest Entomology* journal.

Kareem, A., Port, G., and Wolff, K. 2017. *Bemisia tabaci* in Iraq: population structure, endosymbiont diversity, and biotypes. [*Manuscript]*

Poster presentation:

Kareem A., Port, G., and Wolff, K. (2015). Population genetic structure of glasshouse whitefly *T. vaporariorum* in the UK. *ENTO 2015 Conference*, Dublin, Ireland.

Kareem, A., Port, G., and Wolff, K. (2016). Population structure of glasshouse whitefly *T. vaporariorum* and its symbionts in the UK. *ICE 2016 Conference*, Orlando, US. (Awarded first place in relevant session).

Abstract

Whiteflies are major pests of many crops worldwide. The population structure and symbionts of whitefly species have been studied in different regions, but there is little knowledge about *Trialeurodes vaporariorum* in the UK and *Bemisia tabaci* in Iraq.

MtCOI sequencing and microsatellite genotyping were used to investigate the population structure and endosymbionts of *T. vaporariorum* from the UK and *B. tabaci* from Iraq. The study aimed to answer questions about the occurrence of haplotypes/biotypes, genetic differentiation among populations, identity of symbionts, and if agricultural management might affect their genetic diversity.

MtCOI sequencing showed that *T. vaporariorum* had a low level of variation, with only two mitochondrial haplotypes (mtH) with one nucleotide difference. The results revealed a new record of haplotype mtH3 from Essex and Norfolk in the UK. However, the mtCOI sequencing of *B. tabaci* suggested a high level of polymorphism, with 31 variable nucleotide sites. Four haplotypes of *B. tabaci* (B, B2, Unknown, and MEAM2) were identified in Iraq.

The genotyping results suggested clustering of *T. vaporariorum* in the UK which was linked to location, but not to host plant. The population structure suggests that glasshouse agroecosystems restrict gene flow between populations. However, the genotyping of *B. tabaci* showed low genetic clustering, which was linked to location and time of collection, but not host plant.

The results for symbionts showed that all female and male *B. tabaci* harboured one primary symbiont, *Portiera aleyrodidarum*, and most (96%) had two secondary symbionts: *Hamiltonella* sp. and *Rickettsia* sp. *P. aleyrodidarum* was detected in both sexes of *T. vaporariorum*, whereas one secondary symbiont, *Arsenophonus* sp., was detected in almost all females, but not males.

The new haplotypes of *B. tabaci* might be linked with new strains of plant virus in Iraq. The new mtH3 of *T. vaporariorum* might be important for growers in the UK. These findings both present challenges and may be opportunities for the improved management of these pests in both countries.

Keywords: *Trialeurodes vaporariorum*, *Bemisia tabaci*, genetic diversity, microsatellite, mitochondrial haplotype, biotype, mtCOI, population structure, endosymbionts.

List of Abbreviations

A_e	Effective number of alleles
ABI	Applied Biosystems Genetic Analysis Systems
AMOVA	Analysis of molecular variance
A_r	Allelic richness
BAPS	Bayesian Analysis of Population Structure (software)
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of life data system
bp	Base pair(s)
BPYV	Beet pseudo yellows virus
CABI	Centre for Agriculture and Bioscience International
CI	Cytoplasmic incompatibility
CLCuV	Cotton leaf curl virus
COI	Cytochrome oxidase subunit I mitochondrial gene
Defra	The Department for Environment, Food and Rural Affairs
dNTP	Deoxynucleoside triphosphates
FAO	Food and Agriculture Organization of the United Nations
F_{st}	Measure of population differentiation
Genn.	Gennadius
H	Haplotype
H_e	Expected heterozygosity
H_o	Observed heterozygosity
IBD	Isolation by distance
IPM	Integrated pest management
IRM	Insecticide-resistance management
MCMC	Markov chain Monte Carlo (statistical method)
MEAM	Middle East Asia Minor
MED	Mediterranean
MEGA	Molecular Evolutionary Genetics Analysis (software)
ML	Maximum likelihood

MLG	Multilocus genotype
MLST	Multilocus sequence typing
mtCOI	Mitochondrial cytochrome oxidase subunit I
mtDNA	Mitochondrial DNA
mtH	Mitochondrial haplotype
NCBI	National Center for Biotechnology Information
N_a	Number of alleles
nt	Nucleotide(s)
LSD	Least significant difference
PCOa	Principal coordinates analysis
PCR	Polymerase chain reaction
PI	Parthenogenesis induction
PI	Probability of identity
PYV	Potato yellowing virus
PYVV	Potato yellow vein virus
Q-BANK	Quarantine bank
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
SAP	Shrimp alkaline phosphatase
SNPs	Single nucleotide polymorphism(s)
sp.	Species (singular)
spp.	Species (plural)
SS	Secondary endosymbiont
SSR	Simple sequence repeat(s) or Microsatellites
STR	Short tandem repeats
Taq	Thermus aquaticus
TBE	Tris boric acid EDTA buffer
TICV	Tomato infectious chlorosis virus
ToCV	Tomato chlorosis virus
TYLCV	Tomato yellow leaf curl virus
U	Unit
UK	United Kingdom

West. Westwood

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Chapter 1. Introduction and literature review

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1.1 Genetic diversity

Genetic diversity is the foundation of biodiversity. The term genetic diversity is commonly used to describe the amount of heritable variation within and among populations of organisms (Brown, 1983; Lowe *et al.*, 2004). There are two processes, mutation and recombination, which lead to genetic diversity in organisms. Selection, genetic drift and gene flow of alleles amongst different populations are the main reasons for variation within populations (Rao and Hodgkin, 2002). For any species, genetic diversity is vital for survival and adaptation to the environment. Furthermore, genetic diversity is vital for species and populations to colonise new ecological and environmental niches (Dlugosch and Parker, 2008; Crawford and Whitney, 2010).

The study of population genetics involves investigations of the frequency of alleles and differentiation within and between populations of organisms. The diversity of species is quantified at the molecular level following basic principles and assuming the occurrence of processes such as mutation, gene flow, Mendelian inheritance and natural selection. Studies of population genetic structure and diversity can subsequently identify the levels of genetic diversity in specific species of any type of organism. Genetic analyses provide evidence about current gene flow amongst lineages and can also highlight historical demographic developments in terms of both the ancestral population spreading out and divergence over time from a common ancestor (Hadjistylli *et al.*, 2016).

1.2 Factors influencing genetic diversity

It is accepted that genetic diversity and population structure change over time (Loveless and Hamrick, 1984). The variation and distribution of genetic diversity in plants and animals depend on many factors, such as ecological and geographical aspects, the breeding system, bottlenecks and human activities (Rao and Hodgkin, 2002). Changes in geographical location are associated with differences in ecological landscapes, such as latitude, altitude, temperature and moisture. These characteristics are essential in determining the distribution of genetic diversity and population genetic structure. Factors such as climatic fluctuations during post-glacial periods, host plants, and agricultural activities are believed to have had a significant influence on the genetic diversity, distributional range, and variations in many species living on the Earth

(Hewitt, 2000). In natural environments, the habitat might define the characteristics of populations in which characters have been selected for survival. Even with small habitat changes, the adaptive genetic difference often reacts with high sensitivity (Rao and Hodgkin, 2002). The breeding behaviours and reproduction systems of some insect species is another factor that significantly affects the range and distribution of genetic diversity (Loveless and Hamrick, 1984).

Bottleneck situations reduce genetic variation. A population bottleneck happens when the population size is reduced, which could be the result of various events such as environmental conditions or the effects of human activities including management of the organism. A bottleneck within a population means that some of the alleles that were originally present in the population are lost. Thus, the remaining population has lower genetic diversity. The smaller the population size and the longer it remains small, the more genetic variation will be lost (Hoffmann and Willi, 2008). The remaining population is faced with a high level of genetic drift, which can be described as random changes in allele frequencies in a population. Infrequently occurring alleles have a higher chance of being lost in a small population. The loss of genetic diversity in a new population can result in a population that is genetically distinct from the original population, and this may accelerate the evolution of new species (William and Catton, 2009).

Different types of reproduction in insects include sexual, non-sexual and parthenogenetic reproduction. For example, in aphids, the clonal (asexual) reproduction system tends to decrease the genetic diversity within natural populations of these insects, and in addition can cause an increase in the populations of adapted clonal genotypes, which then take over more of the resources available (Vrijenhoek and Parker, 2009). Another kind of reproduction system occurs in whiteflies. They are parthenogenetic (haplodiploid) species in which non-fertilized eggs grow into males and fertilised eggs into females (Hoy, 2003). Haplodiploidy means that the male whitefly has half the number of chromosomes than the female, which has 22 (Mittler, 1946). The haplodiploidy of whiteflies might lead to decreased genetic diversity due to many factors. Firstly, selection against slightly deleterious alleles may increase, as all alleles are shown in the hemizygous haploid males (Crozier, 1970). Secondly, balanced polymorphism can be problematic and may increase in the sex-linked genetic system (Menken, 1991). Thirdly, reduced effective population size increases the effects of genetic drift and therefore decreases genetic diversity (Lester and Selander, 1979; Owen, 1985). The

capacity of introduced populations to adapt to different environments could be a result of a preadaptation for phenotypic plasticity rather than natural selection acting on genetic diversity (Facon *et al.*, 2006; Ward *et al.*, 2008). This capacity might be reduced by high rates of reproduction and large population size (Loxdale, 2008). Therefore, clonal reproduction and haplodiploidy in some insects might increase or decrease their ability to show rapid and widespread adaptation to different environments.

1.3 Phenotypic plasticity in invasive species

Successful invasion by non-native species could be facilitated by differences in the gene pool in the source population, wide-ranging tolerance and phenotypic plasticity (Lee, 2002). The ability of a single genotype to produce various phenotypes in different environments is called phenotypic plasticity (Bradshaw, 1965; Zhou *et al.*, 2012). There is a high chance that invasive species of high phenotypic and genetic diversity will become established in new regions due to their ability to adapt to environments and a lower susceptibility to predation and disease (Forsman, 2014). Invasive species with genetic and phenotypic variation have greater chances of becoming established in new environments due to this lower susceptibility and higher adaptation potential in varying abiotic conditions (Forsman, 2014). Conversely, many non-native species have low genetic variation in the new area compared to their native environment (Grapputo *et al.*, 2005). This can be seen as an apparent contradiction since genetic diversity is often clearly correlated with fitness and survival (Reed and Frankham, 2003; Frankham, 2005).

However, genetic variation might be less important for the survival of non-sexually reproducing insects populating glasshouses. Parthenogenetic reproduction systems in some insects may allow survival under strong selection pressures in different environments by enabling the fixation of genes linked to resistance to pesticides and this can lead to the prevalence of homogeneous resistant populations (Dunley and Croft, 1992; Denholm *et al.*, 2008; Karunker *et al.*, 2008). Low levels of genetic diversity in homogeneous resistant populations might be outweighed by advantageous resistance characteristics contributing to introduction and invasion success. Also, phenotypic plasticity is not always reduced in line with low genetic diversity (Ovcarenko *et al.*, 2014). For instance, *Adelges cooleyi* (Gillette) (Hemiptera: Adelgidae) in the US has considerably reduced genetic and phenotypic variation, but despite that, it is capable of retaining phenotypes similar to those found in the possible donor region (Ahern *et al.*,

2009). Also, morphological phenotypic plasticity, behaviour and life history characteristics that contribute to successful invasion could continue in hereditarily homogeneous clonal populations of successful asexually reproducing invaders (Gray, 1986; Görür, 2000). Moreover, phenotypic plasticity may be preferred over genetic variation in conditions of short-term, seasonal exposure to stochastically varying environments (Görür, 2000; Moczek, 2010). Varying environmental conditions are usually encountered by invasive populations dispersing from glasshouses to field habitats in boreal areas. The removal of mating barriers amongst these indoor and outdoor populations, such as through active dispersal at the end of the crop season, might lead to the development of large pest colonies (Cox, 2004). Additionally, it has been found that species introduced to novel environments are particularly likely to evolve rapidly (Cox, 2004). Thus, agroecosystems might serve as fertile grounds for evolutionary change (Via, 1990).

1.4 Molecular systematics and its application

Studies in systematics involve the investigation of phylogeny and classification characteristics. Taxonomy is used to separate descriptive classifications and allows the identification of taxa. Advances in DNA techniques allow the most direct examination of the genetic material and are appropriate for systematics projects. Advanced molecular techniques are widely used, such as in the past restriction analyses of DNA, and now DNA sequencing. Each technique has advantages and limitations in terms of the information provided and cost, and time constraints.

1.4.1 DNA sequencing

Sequences of DNA and RNA have been studied since their discovery in the last century. The first attempts to derive sequence information were achieved with proteins in the early 1950s (Sanger and Tuppy, 1951). RNA sequences were first reported in the mid-1960s, whereas DNA sequences were identified from the mid-1970s onwards (Sanger and Coulson, 1975). Discovery of the polymerase chain reaction (PCR) has improved DNA sequencing by making it less costly and time-consuming. DNA sequencing can be used in phylogenetic studies in order to assess the evolution of specific DNA sequences or gene families, to estimate evolutionary variations within species, and to construct phylogenies among species (Hoy, 2003). Single copy genes obtained from mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA) can be used for DNA sequencing. DNA sequencing can be used to study most areas in systematics from

intraspecific variability to the phylogeny of animals. DNA sequencing is an appropriate method for the analysis of intraspecific differences, complex species, geographical variation, reproductive behaviour, and heterozygosity assessments (Hoy, 2003).

The mitochondrial (mt) gene coding for cytochrome c oxidase subunit I (mtCOI) is the most frequently sequenced region in many different groups of insects in identification and evolutionary studies (Caterino *et al.*, 2000). The 5' region of the mtCOI gene has been recommended for use in DNA barcoding for the identification of many organisms, including insects (Hebert *et al.*, 2003). Currently, it is easy to compare the mtCOI region with those available in databases such as the NCBI GenBank, the Barcode of Life Data (BOLD) system, and Quarantine-bank (Q-bank). There is also the DNA barcoding project generated by BOLDSYSTEMS, which uses known mtCOI sequences to help researchers in identifying unknown sequences as shown in Figure 1.1.

Ribosomes are a main structure in living cells and are involved in translating messenger RNA (mRNA) into proteins. They consist of ribosomal RNA (rRNA) and proteins. Ribosomal RNA is commonly used to assess evolutionary relationships between species. RNAs contain conserved and variable regions that can serve for both slow and fast molecular clocks. Nuclear ribosomal genes in eukaryotes, including the 18S (small subunit) and 28S (large subunit) rRNAs, are clustered as tandem repeats in the nucleolus-organising regions of the nuclear chromosomes, but two ribosomal genes are also found in the mitochondria (Hoy, 2003). Meanwhile, rDNA is a region in the nuclear genome, and thus it is inherited by both sexes. There are numerous other beneficial properties of this nuclear region in its use for phylogenetic inferences. Using universal primers can help in the identification and phylogenetic study of many species.

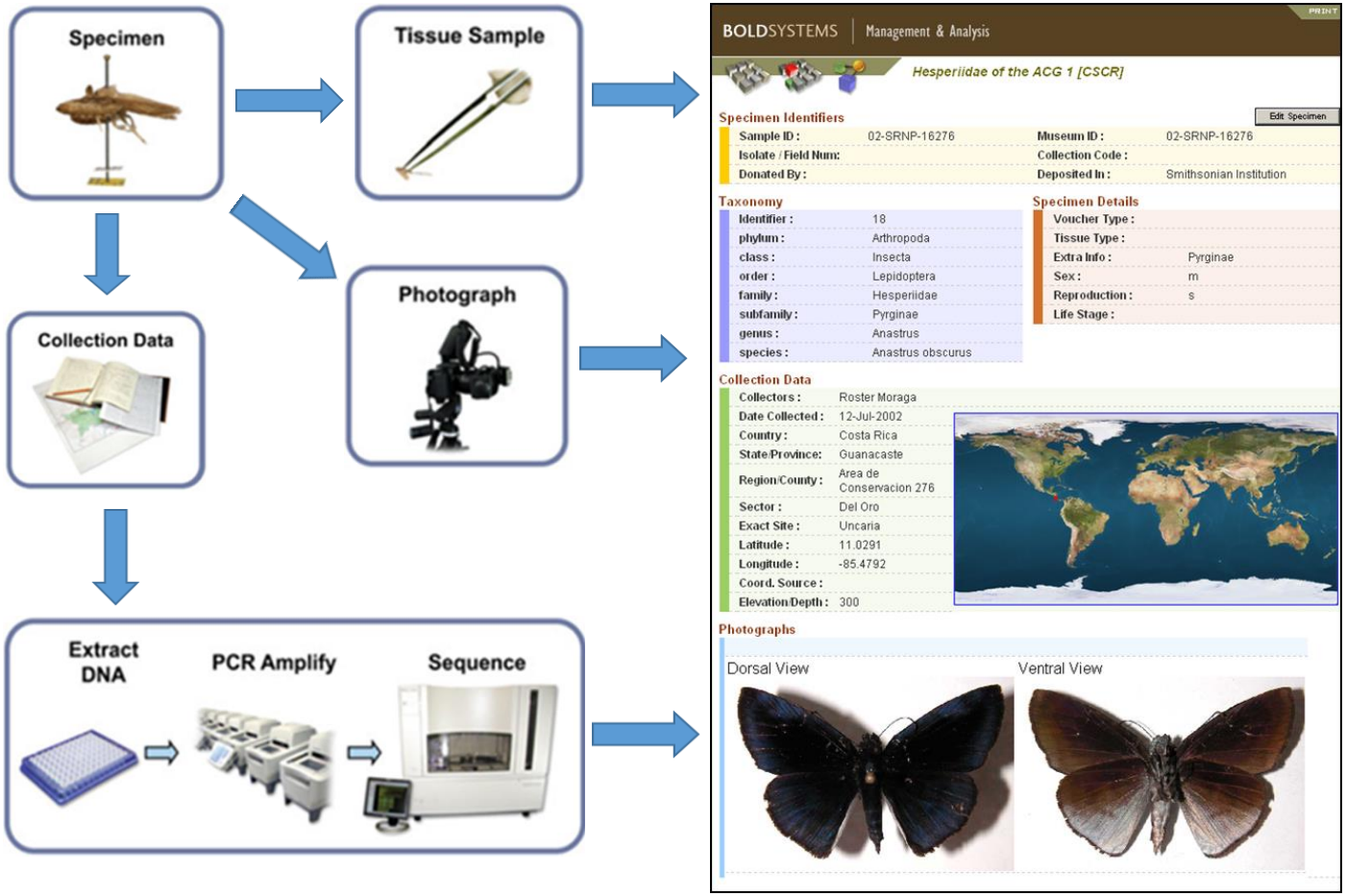


Figure 1.1. Workflow of DNA barcoding using the mtCOI gene. The leg of an insect could be taken from a specimen to generate a DNA sequence. A photograph of the original specimen is taken to be kept as a voucher. All information collected including the image is kept with the DNA barcode sequence in the BOLD database. Source Floyd *et al.* (2010).

1.4.2 Satellite DNA

Satellite DNA can be a large proportion of the DNA in an insect. Short repeated regions of the genome (microsatellites) are found in many organisms. They have been used to assess genetic differentiation between populations of the same and closely related species due to their high polymorphism and representation across the nuclear genome (Schlötterer, 2001). Other names for these nuclear DNA markers are short tandem repeats (STRs) or simple sequence repeats (SSRs), where the former are sets of non-coding repetitive DNA sequences found in large quantities in the genome of most taxa. Microsatellite markers have numerous advantages over other kinds of molecular indicators, such as that a small amount of DNA is required leading to the great simplicity of the procedure, low costs of analysis, and an ability to identify genetic variations even between closely related individuals (Cooke *et al.*, 2003). Microsatellites are composed of motifs of 1-6 nucleotide tandem repeats (Tautz and Renz, 1984; Selkoe and Toonen, 2006). They typically range between 5 and 40 sequence repeats (Selkoe and Toonen, 2006). These repeat numbers in microsatellites occur mainly as a result of slippage and errors within DNA replication. Slippage in replication occurs more commonly than point mutations. Therefore microsatellites tend to be highly variable. In general, the long repeats of microsatellites are more polymorphic than those with shorter repeats (Ellegren, 2004). In microsatellites, rates of mutation vary amongst different species of organisms, ranging from 10^{-6} in *Drosophila* (Schug *et al.*, 1998) to 10^{-3} in humans (Brinkmann *et al.*, 1998) with an average of 5×10^{-4} per locus per generation (Schlötterer, 2000). The mutation rates of a microsatellite vary considerably amongst repeat types. Microsatellite DNA markers have been used increasingly since their discovery for many applications, such as studies of population structure, molecular ecology, the construction of genetic maps, DNA fingerprinting, hybrid detection and parentage investigations (Ellegren, 2004; Jones *et al.*, 2010; Guichoux *et al.*, 2011).

1.5 History and nomenclature of whitefly

Whitefly are tiny sap-sucking insects belonging to the family Aleyrodoidae. The whitefly family belongs to the Order Hemiptera which includes a single superfamily, Aleyrodoidea, within the suborder Sternorrhyncha. The well-known term whiteflies refers to the powdery wax secretions that are produced in the adults and the puparia of the Aleyrodidae family. The unique structure of this family existing in all phases apart from the eggs includes the presence of the vasiform opening which contains the lingula and operculum. The whitefly adults bear a remarkable superficial resemblance to tiny

moths. For example, the European cabbage whitefly, *Aleyrodes proletella*, was first described as a moth by Linnaeus (1758). They were considered as Hemiptera by Latreille (1795) (Mound and Halsey, 1978). The family Aleyrodidae has the fewest species among the four groups of sternorrhynchous Hemiptera by a wide margin, with about 1450 species. However, sampling of tropical whiteflies in south-east Asia and Central America shows that only a very small proportion of species have been described (Martin, 1999). They are highly polyphagous. There are some important damaging species such as the cotton whitefly (commonly known as the sweet potato whitefly), *Bemisia tabaci* (Gennadius), and the greenhouse whitefly (commonly known as the glasshouse whitefly), *Trialeurodes vaporariorum* (Westwood) (Fig. 1.2). Both whiteflies are about 1-3 mm long. They normally have short life cycles depending on environmental conditions and have numerous generations annually (Martin *et al.*, 2000).

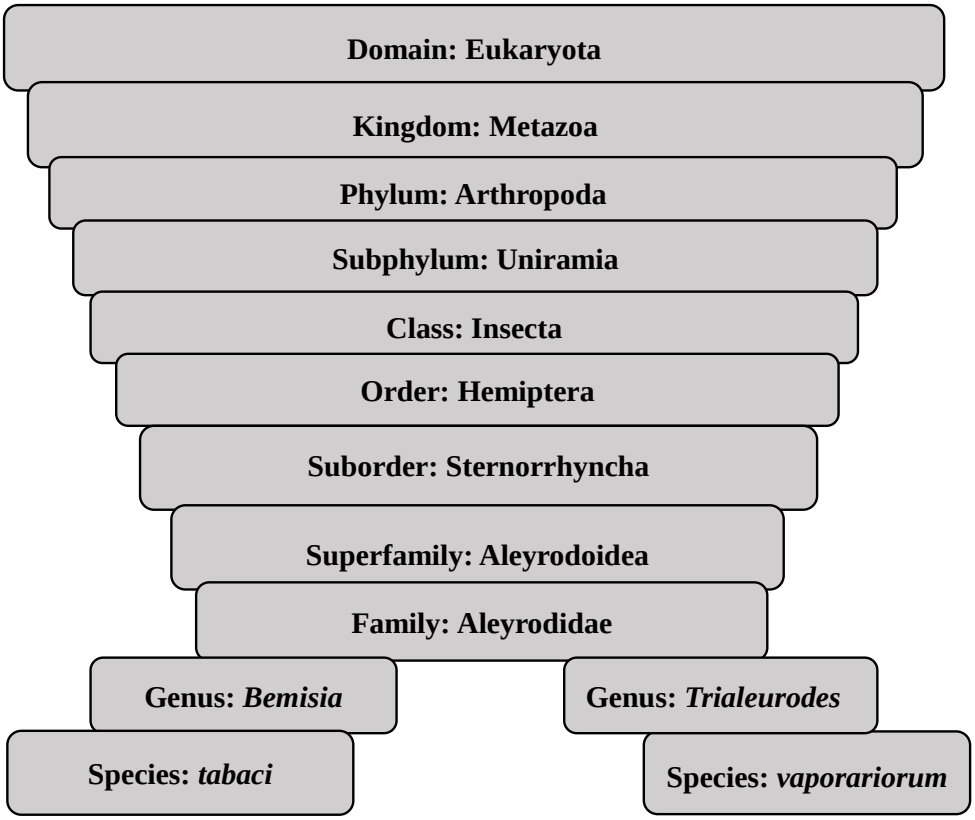


Figure 1.2. Classification of glasshouse whitefly *Trialeurodes vaporariorum* and sweet potato whitefly *Bemisia tabaci.*

1.6 Life cycle of whitefly

The whitefly species *T. vaporariorum* and *B. tabaci* have similar life cycles which start with the laying of eggs on the lower surfaces of new plant leaves. In some cases the eggs of *T. vaporariorum* are laid in partial circles whereas those of *B. tabaci* are laid in complete circles (Martin *et al.*, 2000). The fresh eggs take about four to 12 days to incubate depending on temperature, and then they hatch into the crawler stage (Curry and Pimentel, 1971). The crawlers begin feeding immediately on plant sap using their piercing-sucking mouthparts and depending on environmental conditions, within six weeks the nymphs grow and moult until they reach the adult stage, as shown in Figures 1.3 and 1.4 (Gill and Brown, 2009). The adults live for approximately one to two months (Karatolos, 2011). There are several generations of *B. tabaci* annually, the number depending on environmental conditions such as temperature, humidity, and host plants. For instance, in the Middle East including Iraq, *B. tabaci* produces 10 to 15 generations annually.

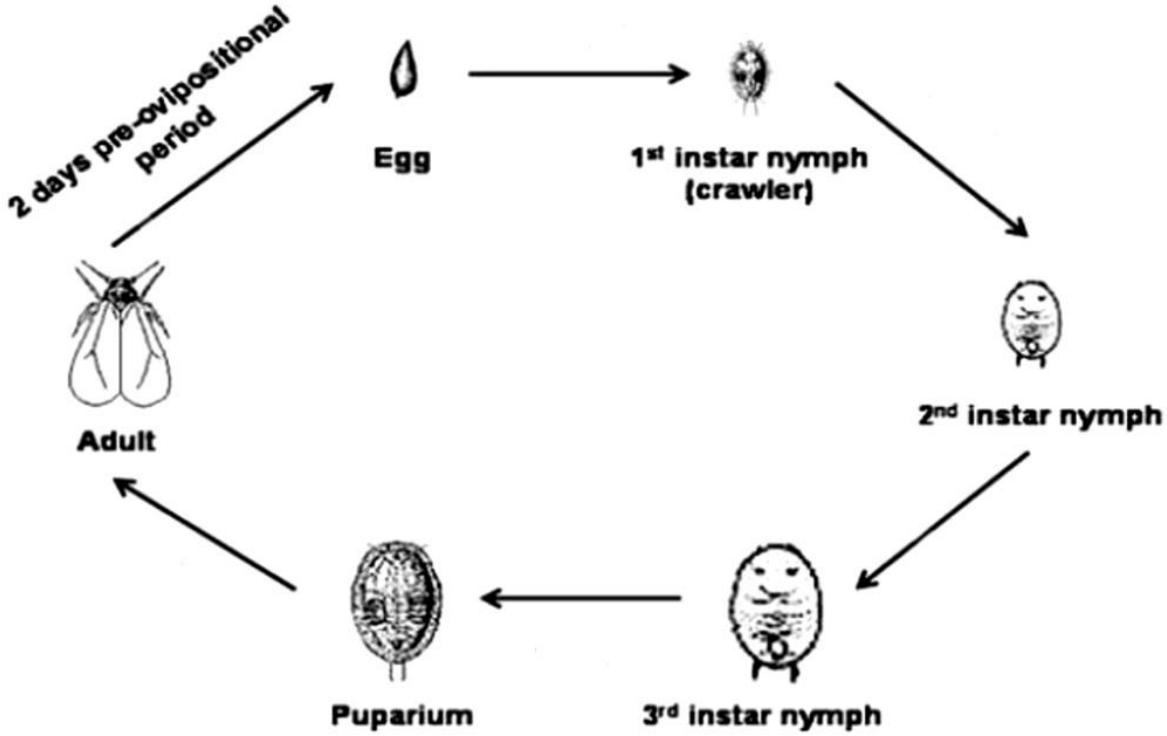


Figure 1.3. The life cycle of *T. vaporariorum*. Source Karatolos (2011).

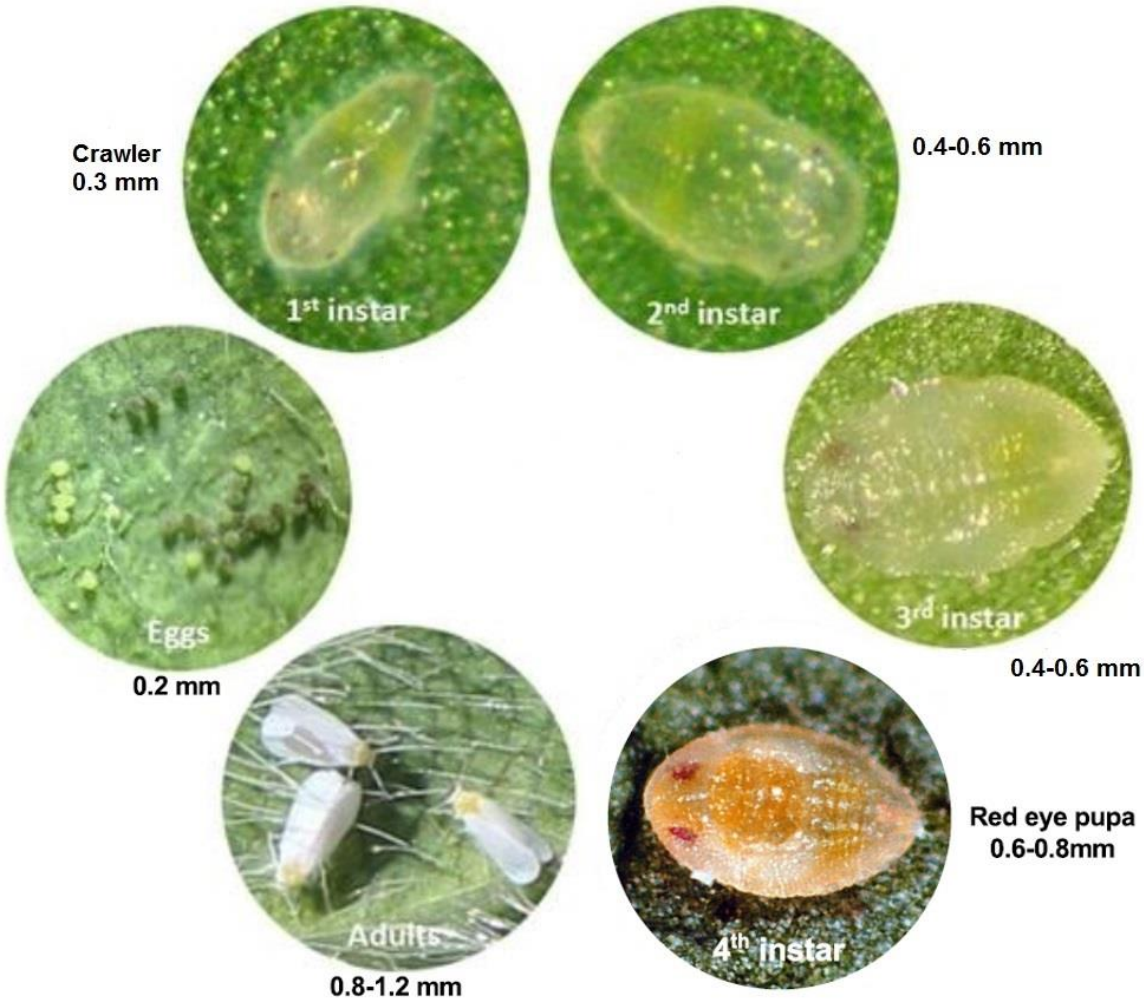


Figure 1.4. The life cycle of *B. tabaci*. Adapted from García (2014).

1.7 Biotypes, haplotypes and species complexes

The concept of biotypes within species has been shown to be relevant in many organisms. The term "biotype" is used to refer to a distinguishable population within a species, without indicating precisely how distinctive it is (Drès and Mallet, 2002). Biotypes have been recognised widely in different organisms' taxonomy; particularly herbivorous pests might exhibit adaptations to different host species, in addition to differences in resistance to both biotic and abiotic stressor factors. Biotypes in insects have been reported from several orders, such as Hemiptera, Lepidoptera, Coleoptera, Diptera, and Hymenoptera (Drès and Mallet, 2002). The differences between biotypes cannot necessarily be determined morphologically. Therefore, molecular tools including DNA markers have been used to distinguish between them. In some cases, it has been

concluded that biotypes of a single named species actually represent separate species. It has been found that in insects, there are many morphologically identical species, which have been reported as species complexes, such as in aphids and whiteflies (Ferrari *et al.*, 2012).

1.7.1 Bemisia tabaci

Bemisia tabaci is considered a key pest of many vegetable and ornamental crops. It has been reported as an invasive species in many countries around the world and causes damage to plants directly by feeding via phloem-sucking or indirectly by transmitting deleterious plant viruses, mainly from the *Begomovirus*, *Crinivirus* and *Closterovirus* genera (Czosnek *et al.*, 1990; Jones, 2003; De Barro *et al.*, 2011; CABI, 2018a). Over the past decades, *B. tabaci* has been reported to cause considerable damage to crops planted in fields and polythene tunnels in Middle East countries including Iraq (Ahmed *et al.*, 2011; Al-ani *et al.*, 2011). The Centre for Agriculture and Bioscience International CABI (2018a) reported that *B. tabaci* is present in Iraq, but no further details have been given (see Fig. 1.7).

Bemisia tabaci is considered to be a cryptic species complex (Brown *et al.*, 1992; Boykin *et al.*, 2012), which means that it is a group of closely associated species referred to as putative species (Mound, 1963; Rosell *et al.*, 1997; Calvert *et al.*, 2001; Gill and Brown, 2009). While morphologically indistinguishable, sister species of this complex differ at the molecular level and exhibit full or partial reproductive isolation (Oliveira *et al.*, 2001; Maruthi *et al.*, 2004; De Barro *et al.*, 2011). The *B. tabaci* species complex has been found to vary in terms of host range (Chu *et al.*, 2006; Sun *et al.*, 2013), insecticide resistance (Costa *et al.*, 1993; Horowitz *et al.*, 2005; Luo *et al.*, 2010), and behaviour (Liu *et al.*, 2007; Crowder *et al.*, 2010; Wang *et al.*, 2010). Additionally, the species complex has shown a variable capacity for virus transmission (Bedford *et al.*, 1994; Li *et al.*, 2005), and in interactions with viruses and host plants (Colvin *et al.*, 2006; Liu *et al.*, 2009; De Barro and Bourne, 2010).

The criteria for classifying *B. tabaci* have been debated for a long time, with different approaches to its taxonomy proposed (Russell, 1957; Mound and Halsey, 1978; De Barro *et al.*, 2011). Additional confusion has arisen due to the abundance of sibling and/or biotype descriptions, many of which are based on molecular tools rather than on biological data that discriminate among biotype or sibling boundaries. This has led to a

misuse of the term "biotype" and the designation of many biotypes that do not represent biologically significant variation (Boykin *et al.*, 2007; De Barro *et al.*, 2011). The precise ecological reasons that are involved in the high levels of variation in *B. tabaci* biotypes are not yet fully understood, but it has been suggested that some lineages of *B. tabaci* diverged millions of years ago following separation worldwide (Gill and Brown, 2009).

Currently, *B. tabaci* is considered to be a complex of eleven major genetic groups and at least 39 putative species (De Barro *et al.*, 2011; Liu *et al.*, 2012; Boykin and De Barro, 2014; Alemandri *et al.*, 2015). It has been proposed that putative species should be separated by a minimum of 3.5% or 4% in mtCOI nucleotide divergence (Fig. 1.6) (Dinsdale *et al.*, 2010; Lee *et al.*, 2013). Pupal (nymph) stages of *B. tabaci* genetic groups exhibit some phenotypic variation, such as diverse shapes, sizes, and colours of setae and pores, at least some of which may be a response to leaf surface morphology of the plant (Neal and Bentz, 1999; Li *et al.*, 2013). The significant differences in *B. tabaci* across the complex and the variation in ability to develop resistance to insecticides make awareness of individuals' identity critical for the development of effective control measures (Ahmed *et al.*, 2012). The most common and important putative species in the *B. tabaci* complex are Middle East-Asia Minor 1 (MEAM1) (which includes the common biotype B) and Mediterranean (MED) (which includes the common biotype Q), which are globally invasive (Perring, 2001). It has been suggested that *B. tabaci* biotype B is originally from the Old World (Brown *et al.*, 1995; Frohlich *et al.*, 1999), and most likely from the Middle East and eastern and northern Africa (Brown, 2007; Mugerwa *et al.*, 2018). Hadjistylli (2010) concluded that historical divergence of the most invasive *B. tabaci* biotypes B and Q coincided with eras of wide human movement and trade of agricultural goods in Africa, the Middle East, and the Mediterranean during the Bronze and Iron Ages and the Roman period (Fig. 1.5).

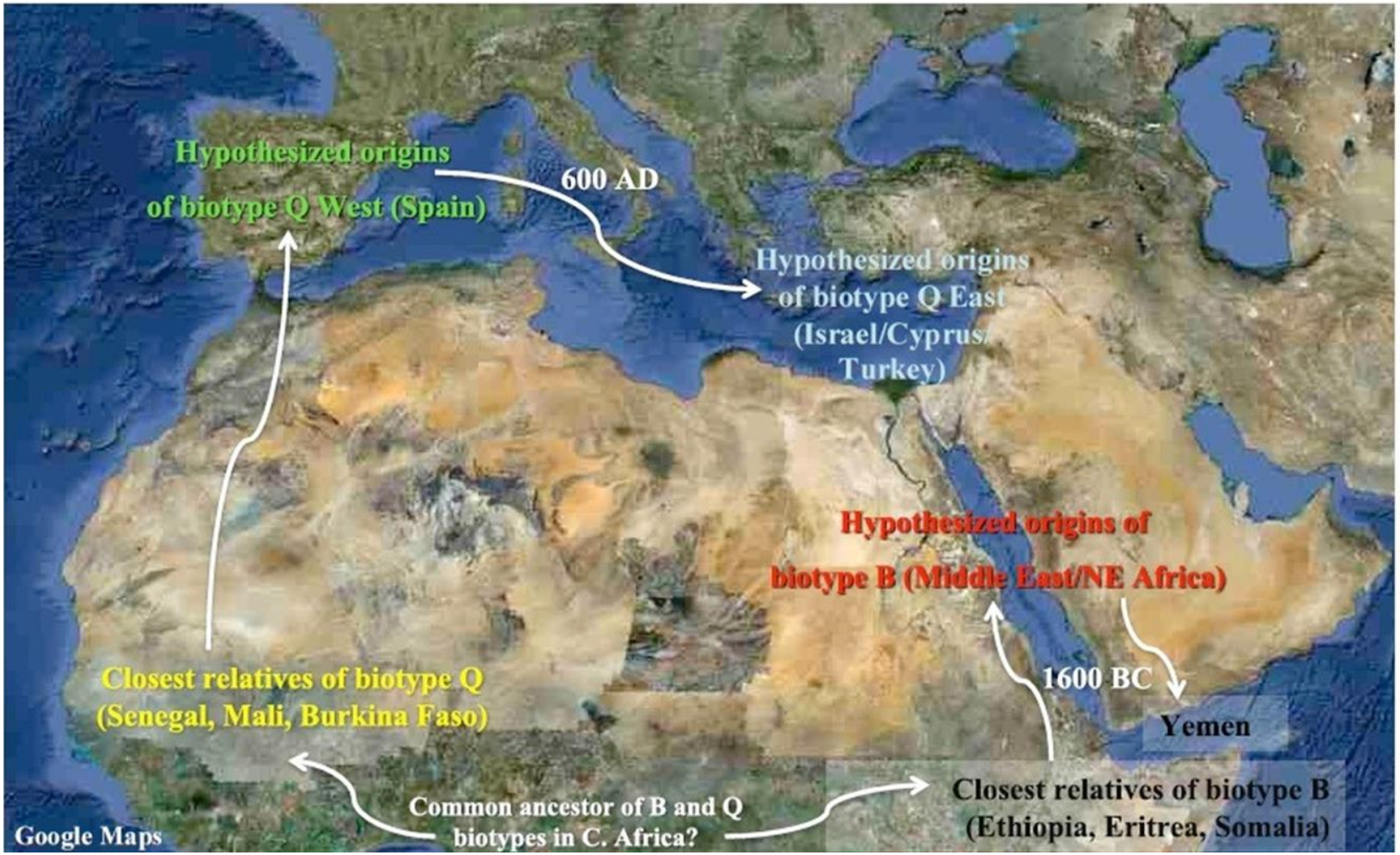


Figure 1.5. The map suggests the origin of *B. tabaci* biotypes B and Q and their closest relatives in the Mediterranean. Arrows display the hypothesised patterns and routes of divergence of both biotypes and their closest relatives from their ancestors. Source Hadjistylli (2010).

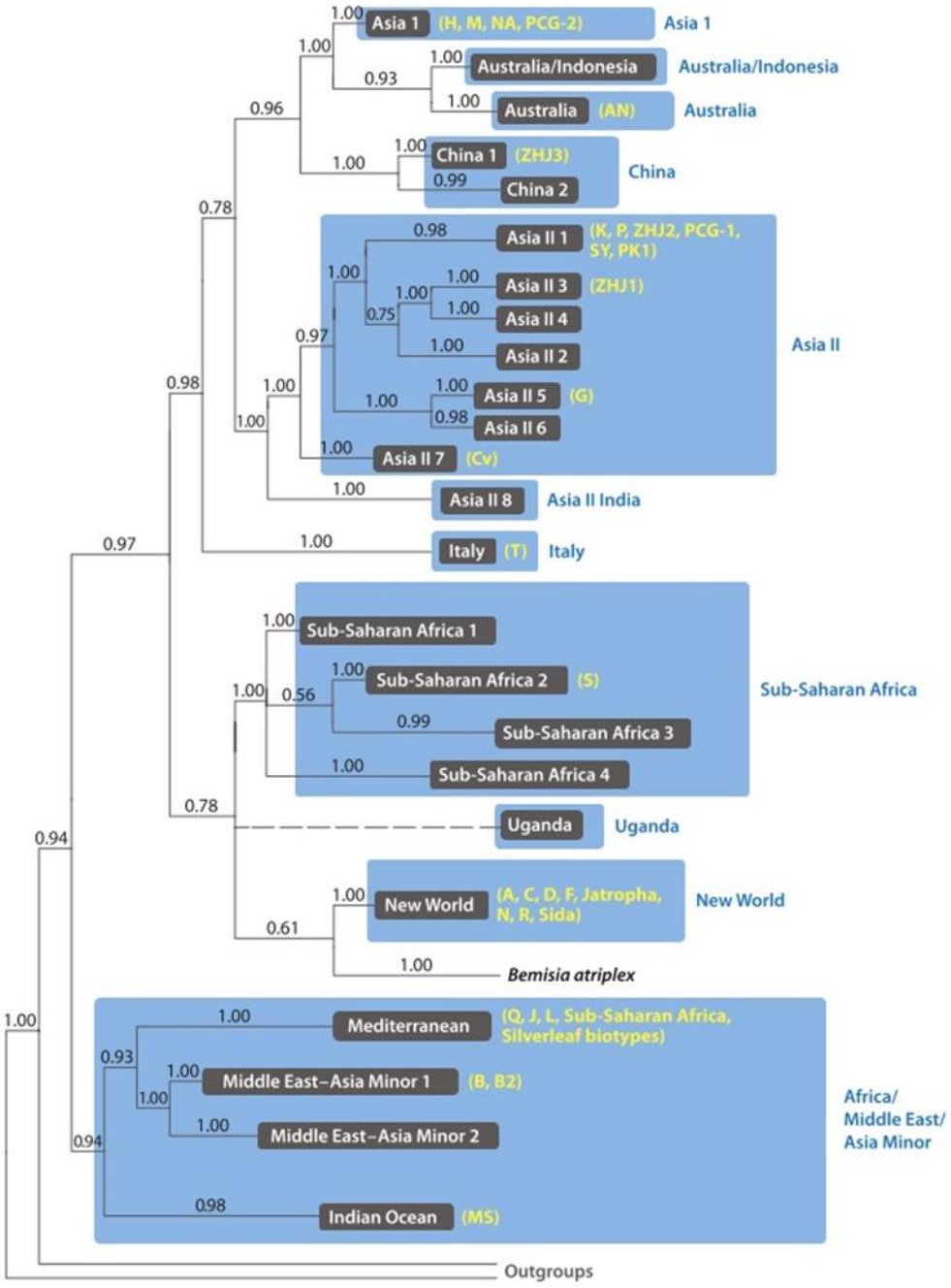


Figure 1.6. Phylogenetic analyses of *B. tabaci* based on mtCOI using Bayesian analysis. Posterior probabilities are shown on the branches. *B. tabaci* can be grouped into 11 high-level (blue boxes) and 24 low-level (black boxes) groups. The low-level groups are considered to be species. The names of *B. tabaci* biotypes are displayed in yellow within parentheses. The position of *Bemisia atriplex* within the *B. tabaci* group may be an artefact based on the limited number of outgroups, which will influence topology but not grouping. Source De Barro *et al.* (2011).

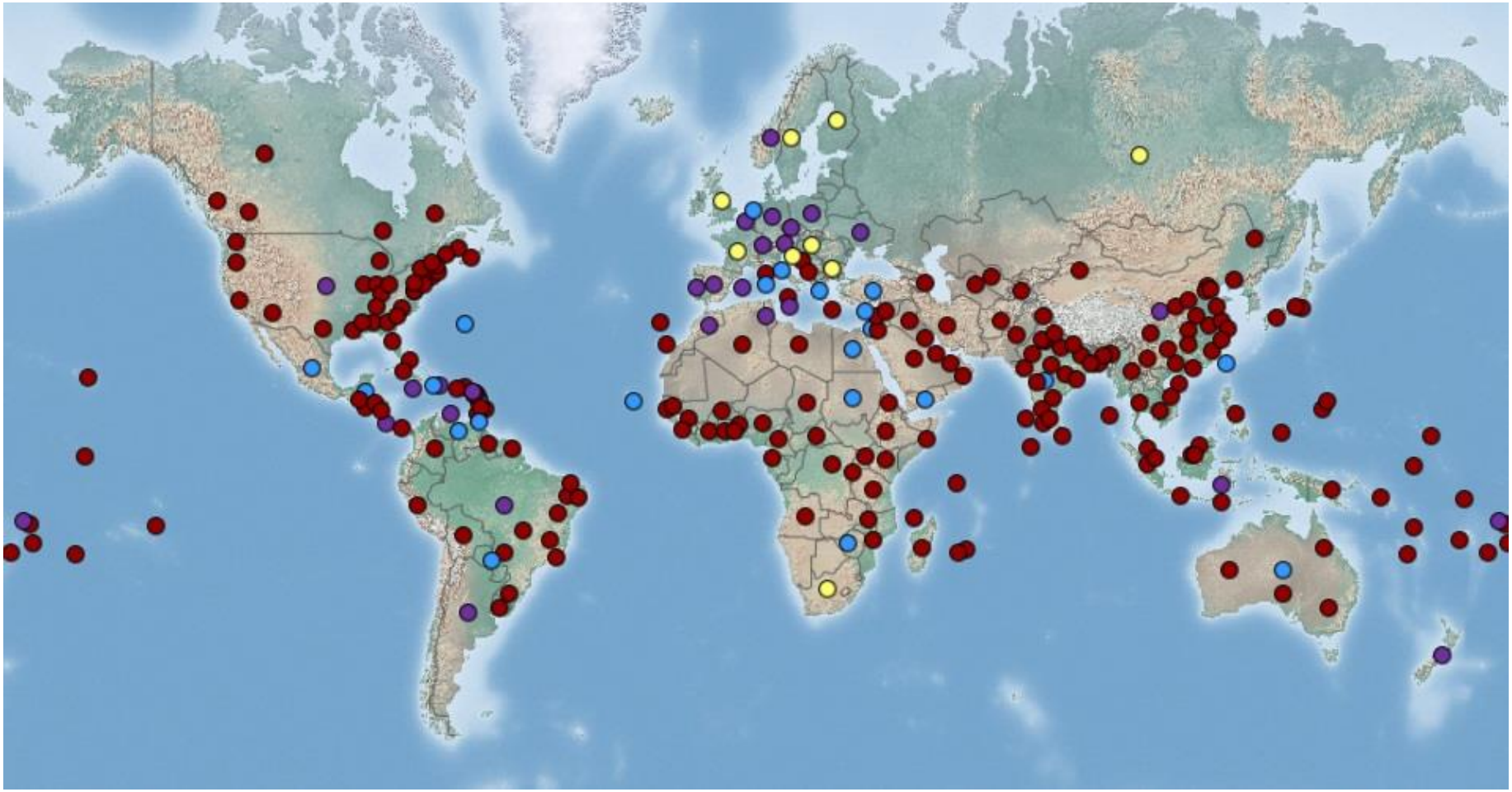


Figure 1.7. Distribution map of *B. tabaci* across the world. Blue dots (widespread locally), red dots (present, no further details), purple dots (localised), yellow dots (occasional or few reports). Source CABI (2018a).

1.7.2 Trialeurodes vaporariorum

The glasshouse whitefly, *T. vaporariorum*, is a well-known pest occurring in glasshouses and field crops worldwide. The glasshouse whitefly is more stable and dispersed in northern latitude countries than in southern ones. Cold winters prevent the naturalisation of *T. vaporariorum* outdoors at high latitudes since it neither has a diapause resting stage nor is freeze tolerant. *T. vaporariorum* was first identified in Europe (UK) in 1856 (Mound and Halsey, 1978). It is commonly distributed throughout European countries. In the UK and northern countries, it is usually found in crops grown in glasshouses (Martin *et al.*, 2000). The distribution of the glasshouse whitefly in winter is limited to glasshouses with environmental control systems. Additionally, dispersal between populations may occur from spring to late autumn, when diverse outdoor habitats may provide host plants for the species. Although *T. vaporariorum* is known for its polyphagous diet and morphological and phenotypic plasticity in response to host plants (Neal and Bentz, 1999; Li *et al.*, 2013), a prolonged period of host experience sometimes leads to specialisation on the host and the formation of host races (Roditakis, 1990; Lei *et al.*, 1998; Bezerra *et al.*, 2004). Thus, the ability of this species to utilise host plants other than greenhouse crops in the boreal climate zone is uncertain. The status of invasion of this herbivore at northern latitudes also remains unclear.

It has been reported that *T. vaporariorum* is able to transmit several virus diseases to plants, such as beet pseudo yellow virus (BPYV) (Closterovirus) (Duffus, 1965; Tzanetakis *et al.*, 2003), potato yellow vein virus (PYVV) (Alba, 1950), tomato infectious chlorosis virus (TICV), and tomato chlorosis virus (ToCV) (Duffus *et al.*, 1996; Wisler *et al.*, 1998). Furthermore, the genetic diversity of *T. vaporariorum* populations and the degree of connectivity regionally or on a global scale have only rarely been analysed. CABI (2018b) reported that *T. vaporariorum* in the UK is present but gave no further details (see Fig. 1.8).

In contrast with *B. tabaci* the glasshouse whitefly *T. vaporariorum* whitefly has no biotypes reported. However, 16 to 19 global mitochondrial haplotypes of *T. vaporariorum* have been described (Prijovic *et al.*, 2014; Wainaina *et al.*, 2017). There has been a lack of information on mtCOI sequences and haplotypes of *T. vaporariorum* in the UK.

Figure 1.8. Distribution map of *T. vaporariorum* across the world. Blue dots (widespread locally), red dots (present, no further details). Source CABI (2018b).

1.8 Endosymbionts

Many animals, including arthropods, live in symbiotic associations with one or more bacteria, and a wide diversity of bacterial associations are found ranging from mutualism to parasitism (Moran, 2007; Moya *et al.*, 2008). Symbionts are categorised depending on their location, with ectosymbionts externally located on the host body and endosymbionts located inside the host body. Furthermore, endosymbionts can be located extracellularly in internal cavities or intracellularly freely in the cytoplasm (Fig. 1.9) (García, 2014). Plant phloem-feeding insects belong to the suborder Sternorrhyncha, which includes aphids, whiteflies, psyllids, scales and mealybugs, and they may harbour symbiotic bacteria in both obligate (primary) and facultative (secondary) associations (Moran, 2001). Symbiotic bacteria are usually located in their host vesicles within specialised cells called bacteriocytes that form a combined area inside the body cavity called a bacteriome (Baumann, 2005; Baumann *et al.*, 2006). Both types of symbiotic bacteria are transmitted maternally to the insect's offspring. Primary symbionts produce important non-dietary metabolites and are vertically transmitted to the next generation (Buchner, 1965). For example, an obligate prokaryotic symbiont associated with whitefly is *Portiera aleyrodidarum.*

A range of facultative symbionts are also associated with phloem-feeding insects, and especially whitefly. Seven secondary symbionts have been reported in *B. tabaci*, which are *Rickettsia* sp., *Wolbachia* sp., *Hamiltonella defensa*, *Cardinium* sp., *Arsenophonus* sp., *Fritschea bemisiae* and, recently, *Hemipteriphilus asiaticus* (Zchori-Fein and Brown, 2002; Baumann, 2005; Gottlieb *et al.*, 2006; Bing *et al.*, 2013b; Kapantaidaki *et al.*, 2015). These symbionts are not necessarily localised in the bacteriocytes; they might be found throughout their insect host (Moran and Telang, 1998; Skaljac *et al.*, 2013; Marubayashi *et al.*, 2014). The main method of transmission of secondary endosymbionts is vertical. However, some of them, such as *Wolbachia* sp., might be transmitted horizontally when contact is made with other infected insects. (Buchner, 1965; Baumann *et al.*, 2006; Clark *et al.*, 2010).

In addition to the essential support from endosymbionts for the insect's diet, there are other important roles they can play in the ecology and evolution of host insects, such as a capacity to provide resistance to insecticides, plant virus transmission and influencing the reproductive system and coevolution (Moreira *et al.*, 2009; Gottlieb *et al.*, 2010). Furthermore, reproductive manipulation has been documented for *Wolbachia* sp.,

Cardinium sp. and *Arsenophonus* sp. (Werren and Windsor, 2000; Engelstädter and Hurst, 2009).

Symbiotic bacteria may play a vital role in the evolution of their host organism. Endosymbionts in particular are thought to act as reproductive manipulators that are the source of reproductive isolation and speciation. Furthermore, the putative species of *B. tabaci* identified using mtCOI can also be specifically linked with their symbionts (Chiel *et al.*, 2007; Gueguen *et al.*, 2010).

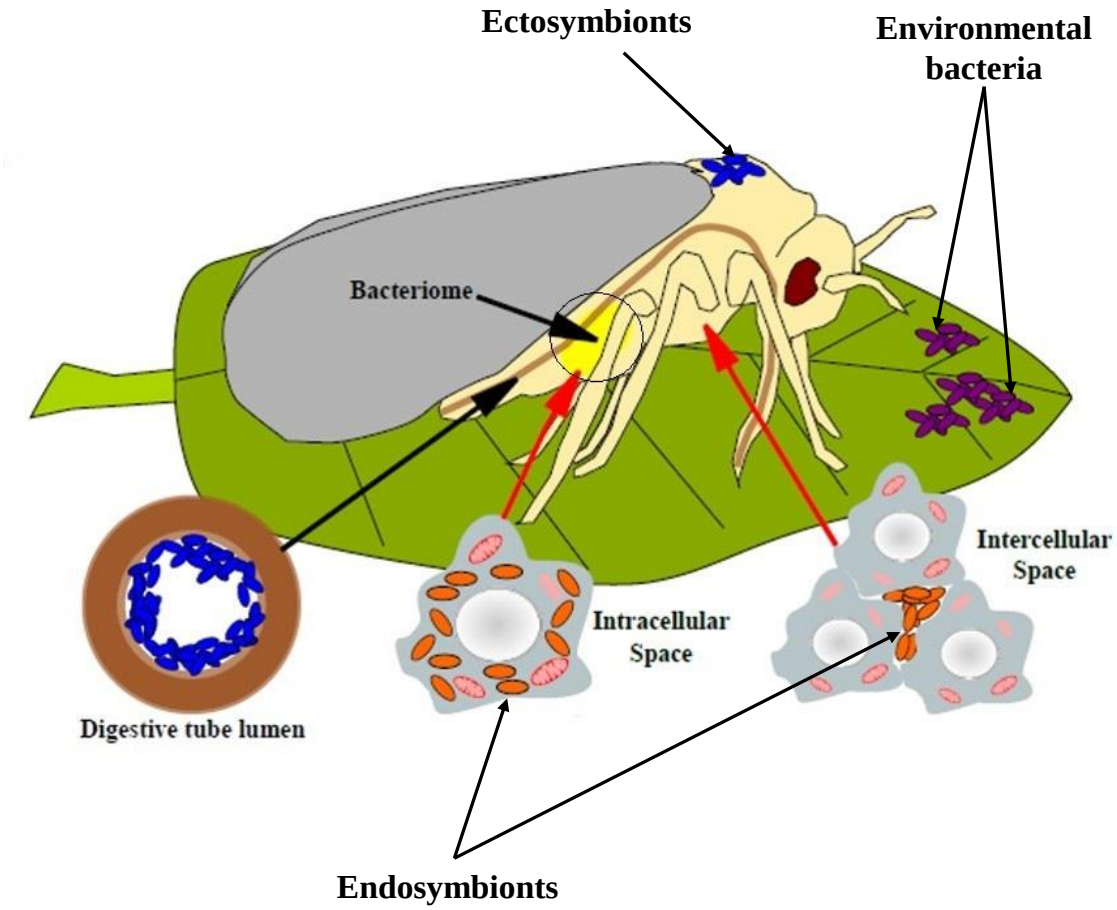


Figure 1.9. Different kinds of symbiotic bacteria hosted by whitefly. Adapted from García (2014).

1.8.1 Portiera aleyrodidarum

The primary symbiont *P. aleyrodidarum* was described by Costa *et al.* (1993) as a pleomorphic bacterium associated with *T. vaporariorum* and *B. tabaci*. Both whiteflies are phytophagous, phloem-feeding pests. The phloem contains sugars and non-essential amino acids (Sandström and Pettersson, 1994; Douglas, 2006). Also, whitefly feed on the plant's xylem, which includes minerals that have small amounts of non-essential amino acids and inorganic compounds (Andersen *et al.*, 1989). All primary endosymbionts from insects in the suborder Sternorrhyncha, including whitefly, supply the lacking nutrients, such as vitamins and amino acids for the whitefly's diet (Shigenobu *et al.*, 2000). Therefore, whitefly cannot survive without their primary symbionts.

1.8.2 Rickettsia

The secondary symbiont *Rickettsia* sp. was first identified in *B. tabaci* by Gottlieb *et al.* (2006). It can be found in both B and Q biotypes and, unlike other endosymbionts, can be found in most of the whitefly pest's tissues and organs as opposed to being confined to the bacteriome. Himler *et al.* (2011) reported that the secondary symbiont *Rickettsia* sp. could be associated with enhanced whitefly fitness through increased fecundity, more rapid growth, better survival into adulthood and the presence of a higher proportion of females.

Rickettsia sp. has additionally been revealed to confer heat tolerance (Brumin *et al.*, 2011), but *B. tabaci* infected with *Rickettsia* sp. have greater susceptibility to some insecticides (Kontsedalov *et al.*, 2008). Furthermore, the highest susceptibility to some insecticides such as acetamiprid, thiamethoxam, sporimesifen and pyripoxen was observed in strains doubly infected with *Rickettsia-Arsenophonus* or *Rickettsia-Wolbachia* (Ghanim and Kontsedalov, 2009). This increased susceptibility has been demonstrated in both B (Kontsedalov *et al.*, 2008) and Q biotypes (Ghanim and Kontsedalov, 2009).

1.8.3 Wolbachia

Many insects contain secondary endosymbionts in the genus *Wolbachia* (α-Proteobacteria). *Wolbachia pipientis*, which is found in mosquitoes, is vertically transmitted through the cytoplasm of the egg cells. Also, it has been reported to be transmitted horizontally in some cases between insects. *Wolbachia* species are located

in their hosts' reproductive tissues (Laven, 1967; Poinsot *et al.*, 1998), and are well-known as reproductive manipulators. Four major reproductive irregularities have been recognised by Stouthamer *et al.* (1999).

1. Cytoplasmic incompatibility (CI): Here the outcome of mating between symbiont-infected males and uninfected females is a failure to produce offspring, increasing the relative fitness of infected females and leading to a greater share of the symbiont in the host population (Penz et al., 2012).
2. Parthenogenetic induction (PI): infected virgin females produce daughters in many arthropod species (Stouthamer *et al.*, 1993; Schilthuizen and Stouthamer, 1997).

3. Feminization: infected genetic males reproduce as females. Also, infected insects have high fecundity and fertility (Poinsot *et al.*, 1998).

4. Male-killing: the infected female will produce just females and male embryos do not survive (Werren, 1997).

1.8.4 Hamiltonella

Hamiltonella defensa is a mutualistic symbiont with a host-dependent metabolism, relying on the host's obligate endosymbionts for its nutritional requirements and increasing host resistance to parasitoid wasps (Degnan *et al.*, 2009). Also, it has been found that *H. defensa* increases the ability of *B. tabaci* to transmit the tomato yellow leaf curl virus (TYLCV), through the production of the GroEL protein which cooperates with the TYLCV coat proteins in the *B. tabaci* B biotype (Gottlieb *et al.*, 2010). Also, Degnan *et al.* (2009) found that *H. defensa* confers protection against parasites, encoding toxins, effector proteins and virulence factors. It has been confirmed in the *B. tabaci* B biotype, but not the Q biotype (Chiel *et al.*, 2007).

1.8.5 Arsenophonus

Arsenophonus sp. (γ-Proteobacteria) is known in numerous sap-feeding arthropods including whiteflies, louse flies, psyllids and aphids (Baumann, 2005). It plays an important role in virus transmission in *B. tabaci* as revealed by Rana *et al.* (2012), who confirmed that the GroEL protein of *Arsenophonus* sp. interacts with the coat protein of cotton leaf curl virus (CLCuV). *Arsenophonus* sp. has been confirmed in *T. vaporariorum* and *B. tabaci* Q biotype, but not the B biotype (Chiel *et al.*, 2007).

1.8.6 Cardinium

Cardinium hertigii is a secondary intracellular symbiont bacterium which has been found in some sap-feeding insects including whitefly, producing cytoplasmic incompatibility (CI) as in *Wolbachia* sp. (Weeks *et al.*, 2003). However, although they both share some proteins with the possibility to restrict eukaryotic cell cycle regulation, there does not appear to be a shared evolutionary origin for CI in the two bacteria, as they have different molecular mechanisms for CI (Penz *et al.*, 2012).

1.8.7 Fritschea

The secondary symbiont *Fritschea bemisiae* (Chlamydiales) is located in the gut of *B. tabaci*. These symbiotic bacteria live within bacteriocyte cells and are transmitted directly to offspring via oocytes. The first identification of *Fritschea* sp. was in 2003 (Thao and Baumann, 2004). It has been detected in many *B. tabaci* biotypes but not yet in the MEAM1 genetic group (Everett *et al.*, 2005).

1.8.8 Hemipteriphilus

Hemipteriphilus asiaticus was identified for the first time as an endosymbiont in whitefly by Bing *et al.* (2013b) in the China 1 *B. tabaci* biotype. There are no reports yet of the role of this symbiont species in whitefly or from other countries.

1.9 Aims of the study

The biotype/haplotype-specific distribution of symbionts is important because of their consequences for the phenotype and fitness of the whiteflies *T. vaporariorum* and *B. tabaci*. The development of resistance in insects to pesticides, which in some countries have widely been used as the main strategy against the whitefly, may be affected by endosymbionts associated with whiteflies, which influence their adaptation and evolution. For example, *Rickettsia* sp. is linked with low resistance to insecticides in the *B. tabaci* Q biotype (Ghanim and Kontsedalov, 2009). Therefore, it is important to investigate the symbiont bacteria associated with *T. vaporariorum* and *B. tabaci*. PCR and sequencing methods are used in this study to identify the endosymbionts associated with both whiteflies in Iraq and the UK.

Studies have been carried out in whiteflies to assess their genetic diversity and population structure, but no such study has been conducted on *B. tabaci* in Iraq and *T. vaporariorum* in the UK. So far it has been suggested that about 19 mitochondrial haplotypes of mtCOI have been identified for *T. vaporariorum*, whereas more than 39

biotypes of *B. tabaci* are globally known. In this study, the population structure and genetic diversity for both *B. tabaci* and *T. vaporariorum* whitefly in Iraq and the UK have been investigated to bring up to date knowledge of the status of these species.

For this study, two approaches have been used. DNA barcoding using mitochondrial cytochrome oxidase subunit I gene (mtCOI) is discussed in Chapter 2, and microsatellite markers, which are sets of non-coding repetitive DNA sequences, have been used to assess genetic differentiation and are discussed in Chapter 3 for the UK whitefly *T. vaporariorum,* and Chapter 4 for the Iraqi whitefly *B. tabaci*. Also, DNA markers are used to identify the symbiotic bacteria associated with both whiteflies, and this work is discussed in Chapter 5.

The general details and aims of each chapter are as follows:

In Chapter 2, DNA barcoding and phylogenetic tree of both species of whitefly, *T. vaporariorum* and *B. tabaci* (Hemiptera: Aleyrodidae).

Chapter 3 considers the population structure of the sweet potato whitefly, *T. vaporariorum*, in the UK, while Chapter 4 looks at the population structure of sweet potato whitefly, *B. tabaci* in Iraq.
In Chapter 5, the diversity and molecular identification of endosymbionts in the two species of whitefly *B. tabaci* and *T. vaporariorum* are examined, and in Chapter 6, the findings of this study are discussed in a wider context.

2 Chapter 2. Barcoding DNA and phylogenetic trees of two species of whitefly, *Trialeurodes vaporariorum* and *Bemisia tabaci* (Hemiptera: Aleyrodidae), in Iraq and the UK, respectively

2.1 Abstract

Whiteflies are major pests of many crops worldwide. The DNA barcoding and phylogenetic structure of whitefly species have been studied in different regions in the world, but there are no intensive studies on *T. vaporariorum* in the UK and *B. tabaci* in Iraq.

The objectives of this study were to perform mtCOI sequencing of the most common whiteflies collected from the UK and Iraq and to try to answer questions about the haplotypes and/or biotypes of whiteflies that occur and how habitat and agricultural management affect their genetic diversity.

MtCOI sequencing showed that *T. vaporariorum* has a low level of variation, and only two haplotypes with one variable nucleotide were found. The results revealed a new record of mitochondrial haplotype mtH3 from the counties of Essex and Norfolk in the UK. However, the mtCOI sequencing of the *B. tabaci* species complex suggested a high level of genetic polymorphism with 31 variable sites, and four biotypes of *B. tabaci* (B, B2, Unknown, and MEAM2) were identified in Karbala and other cities in Iraq. The most common was biotype B2 of the Middle East-Asia Minor1 (MEAM1) genetic group according to the global dataset of this species complex.

The low level of mtCOI diversity in *T. vaporariorum* and diverse population substructure (see Chapter 3) suggest that multiple but limited numbers of introductions of *T. vaporariorum* have occurred, mainly from countries nearest to the UK. However, the results for *B. tabaci* mtCOI sequencing might also indicate that multiple introductions of *B. tabaci* biotypes into Iraq have occurred, mainly from neighbouring countries.

The new biotypes of *B. tabaci* may be linked with new strains of the tomato yellow leaf curl virus plant disease (TYLCV), which may have been transmitted by *B. tabaci* in Iraq. The new mitochondrial haplotype of *T. vaporariorum* should alert growers in the UK about new haplotypes which might be introduced. These findings may highlight challenges for the management of these pests in both countries. Furthermore, imposing adequate quarantine restrictions to avoid the introduction and spread of genetically diverse and potentially more invasive and adaptable strains become much more important. More studies are needed to update the status of these pests in the UK and Iraq.

2.2 Introduction

Whiteflies (Hemiptera: Aleyrodidae) are considered major pests of many crops worldwide. More than 1450 species have been described in two subfamilies, and the most economically significant pest species are members of the Aleyrodidae (Bink-Moenen, 1990; Martin *et al.*, 2000). The most critical and severe whitefly species are the sweet potato whitefly, *B. tabaci* (Gennadius) and the glasshouse whitefly, *T. vaporariorum* (Westwood). They are considered as primary insect pests of many vegetable and ornamental crops and cause damage to plants either directly by feeding or indirectly by transmitting viruses (CABI, 2018b). Whiteflies typically have short life cycles, which are dependent on climatic conditions, and especially temperature. In normal conditions, they produce several generations every year (Martin *et al.*, 2000). The rapid population growth of whiteflies is due in part to an arrhenotokous parthenogenesis system in which non-fertilized eggs grow into males and fertilised eggs into females. Therefore, males are homozygous, as they have only half of the alleles of their mother, whereas the female's offspring are heterozygous and have a full set of alleles (Horowitz *et al.*, 2003).

A pest's resistance to different insecticides is an evolutionary phenomenon. It is an inherited characteristic selected by insecticide management that allows an insect to survive chemical control. Insecticide resistance allows pests to survive doses that would usually kill susceptible individuals of the same species (Onstad, 2008). Haplodiploid breeding systems could support the selection of resistance genes for pesticides. Insect resistance genes arising by mutation have been shown to be selected from the outset in hemizygous males, irrespective of the inherent dominance or recessiveness of genes (Denholm *et al.*, 1998; Horowitz *et al.*, 2003). It has been suggested that the males of *B. tabaci* and *T. vaporariorum* whiteflies show less tolerance than females to pesticides (Horowitz *et al.*, 1988; Sanderson and Roush, 1992; Carrière, 2003).

2.2.1 Trialeurodes vaporariorum

The glasshouse whitefly *T. vaporariorum* is a highly polyphagous pest of glasshouse vegetable and ornamental crops in most temperate regions in the world. It is commonly dispersed throughout Europe and is found and survives on crops growing in glasshouses or polytunnel plastic greenhouses in northern European countries including the UK

(Martin *et al.*, 2000). *T. vaporariorum* causes indirect damage by transmitting numerous plant viruses, which cause major economic losses for growers (Chapter 1).

The genetic diversity of *T. vaporariorum* has been studied in a few geographical regions worldwide. The first study on *T. vaporariorum* populations, which originated from India used the mitochondrial cytochrome c oxidase subunit I gene (mtCOI) and Internal Transcribed Spacer 1 (ITS-1) markers to examine genetic diversity (Roopa *et al.*, 2012). Later, Prijovic *et al.* (2014) indicated six mitochondrial DNA haplotypes of *T. vaporariorum* using mtCOI sequencing in Serbia and surrounding countries, but 19 mtCOI haplotypes based on the GenBank dataset have been reported from different countries around the world (Wainaina *et al.*, 2017). Kapantaidaki *et al.* (2015) then reported a low level of variation of *T. vaporariorum* using the mtCOI sequence collected from 18 countries. Finally, a study using available mtCOI sequences reported just 16 mitochondrial haplotypes in global populations (Wainaina *et al.*, 2017). There are as yet, not enough data and mtCOI sequences reported from the UK. Also little is known about the current mitochondrial haplotypes which occur in the UK since the glasshouse whitefly was established in about 1856 (Mound and Halsey, 1978).

2.2.2 Bemisia tabaci

Bemisia tabaci is considered a major pest of many crops worldwide. It is found and survives outdoors in tropical and sub-tropical countries, whereas it survives only in glasshouses or plastic tunnels in temperate regions (Martin *et al.*, 2000). More than 900 host plants have been reported for *B. tabaci,* and the members of the species complex collectively transmit more than 100 plant viruses (Polston and Capobianco, 2013; CABI, 2018a). It is suggested that *B. tabaci* has spread across the world through international trade in plant products infested by whiteflies. *B. tabaci* has been reported to cause considerable damage to field and plastic tunnel crops in the Middle East region, including Iraq (Ahmed *et al.*, 2011; Al-ani *et al.*, 2011).

The concept of biotypes of *B. tabaci* became prominent after it invaded the southern United States, where differences in behaviour compared to the native population were observed (De Barro *et al.*, 2011). The invading *B. tabaci* had a different esterase enzyme and host plant range from the local population (Bird, 1957), and was referred to as the B biotype, which belongs to the Middle East-Asia Minor (MEAM) genetic group. The native US population was called the A biotype, which belongs to the New World

genetic group (Costa and Brown, 1991; Brown *et al.*, 1992; Bedford *et al.*, 1994). A more comprehensive study compared a range of biological characteristics in eleven biotypes of *B. tabaci* (A, B, B2, D, E, G, H, K, J, L, M), including the capacity to transmit viruses, host plant, and the ability to produce female offspring following inter-biotype mating trials (Bedford *et al.*, 1994). The complex of 39 *B. tabaci* putative species can be grouped into eleven major groups. The putative species are defined by separation by a minimum of 3.5% mitochondrial cytochrome c oxidase I gene (mtCOI) nucleotide divergence (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011; Boykin and De Barro, 2014; Alemandri *et al.*, 2015). The most common putative species of whitefly is MEAM1 (which includes biotype B), which is listed as the most invasive putative species in the world by the International Union for the Conservation of Nature and Natural Resources (IUCN) and the Invasive Species Specialist Group (ISSG, 2017). Therefore, it is important to understand which species are present in order to develop effective control measures (De Barro *et al.*, 2011; Ahmed *et al.*, 2012).

The use of molecular identification for *B. tabaci* is a valuable approach to highlight genetic variation among morphologically similar individuals (Firdaus *et al.*, 2013; Wang *et al.*, 2014; Hadjistylli *et al.*, 2015). As a consequence of the status of *B. tabaci*, the regular monitoring of its species is needed to understand new invasive populations. In Iraq, for example, no such study has assessed the genetic diversity of *B. tabaci* populations or reported the species which occur and their level of genetic diversity.

2.2.3 The application of DNA systematics

Advances in molecular tools and protocols play a vital role in improving the taxonomy and identification of organisms. Using DNA has become an easier and more accurate approach for identification. There are many targets of DNA analysis, such as nuclear, mitochondrial, and ribosomal DNA, which have been utilised in systematics studies (Caterino *et al.*, 2000). The application of DNA taxonomy is supported by many authors (Godfray, 2002; Pilgrim *et al.*, 2002; Tautz *et al.*, 2002; Hebert *et al.*, 2003; Tautz *et al.*, 2003). It is quite clear that DNA taxonomy has particular benefits in species with only slight morphological distinctions and high economic importance, such as whitefly. To date, many techniques have been applied to distinguish between the species and siblings of whiteflies using the electrophoresis of allozymes, analysis of randomly amplified polymorphic DNAs (RAPDs) and nucleic acid sequence comparisons of nuclear or mitochondrial DNA markers (Calvert *et al.*, 2005). Many researchers have used these

approaches to distinguish between closely related species. For example, in China, one study used molecular methods to identify the B biotype of *B. tabaci* (Li *et al.*, 2005). Similarly, other research has analysed data based on sequences of mtCOI DNA and rDNA ITSl recorded in the US National Center for Biotechnology Information (NCBI) GenBank to identify the genetic distinctions between five different geographical populations of *B. tabaci* in the world (Chu *et al.*, 2007; De Barro, 2012). The RAPD technique has also been used to distinguish the B biotype from other biotypes of *B. tabaci* (De Barro and Driver, 1997). However, in the case of *T. vaporariorum* there have been few studies on the genetic variation in populations of this species (mentioned in Section 2.2.1).

2.2.4 DNA barcoding for identification

The use of well-known DNA fragments to identify and classify species has been used for many applications across all forms of life. The term DNA barcode has been used for more than a decade (Kress and Erickson, 2012). The most used approach is the 'barcoding' of mtCOI, which is a mitochondrial coding gene that has been recommended as a global identification standard for organisms (Hebert *et al.*, 2003), and the partial mtCOI gene sequence has been commonly utilized in the identification of various species of animal. The method uses a short known gene sequence of about 700 base pairs (bp) to identify species based on available references to DNA sequences in datasets. In insects, DNA barcoding involves sequencing a short fragment of the mtCOI gene to identify unknown specimens and for comparisons with a reference library of barcodes of known species (Floyd *et al.*, 2010). There are many user-friendly online barcode libraries, such as the NCBI GenBank, Barcode of Life Data System (BOLD), and Quarantine-bank (Q-bank), which can be used as a reference to help identify unknown sequences. The known sequences of mtCOI in barcoding libraries are informed by place of collection, sequence data, Linnaean names, and images. The DNA barcoding approach represents a remarkable advance in datasets of species, which increases the ability to identify unknown specimens.

2.2.5 Mitochondrial haplotypes and biotypes

The use of molecular tools for identification is advancing in taxonomy and classification. For example, recent molecular research on *B. tabaci* whitefly led to the conclusion that it is a complex of more than thirty-nine putative species (De Barro *et al.*, 2011; Liu *et al.*, 2012; Boykin and De Barro, 2014; Alemandri *et al.*, 2015). They have

been described worldwide on the basis of insecticide resistance, morphology, behaviour and/or mtCOI (Boykin *et al.*, 2012; Boykin *et al.*, 2013). The complex of species in *B. tabaci* is important for several reasons, including the development of insecticide resistance in response to selection pressure, host differences, and geography, all of which might impact on the vector potential for various viruses (Bird, 1957; Mound, 1963; Costa and Russell, 1975; Bird and Maramorosch, 1978). However, in the *T. vaporariorum* whitefly, 16 to 19 global mitochondrial haplotypes have been reported. There has been a lack of information, data and mtCOI sequences of both whiteflies, *B. tabaci* and *T. vaporariorum*, reported from Iraq and the UK.

This study aimed to increase the understanding of how the genetic diversity of both whitefly pests depends on different factors such as geographical location, environmental conditions, and crop management. The following questions were posed: Which putative species/biotypes/haplotypes of whitefly occur? Does the geographical distribution of these whiteflies affect their genetic diversity? I hypothesised that there would be many putative species of *B. tabaci* in Iraq and haplotypes of *T. vaporariorum* in the UK because of the invasive ability of whitefly. This chapter presents the first extensive data on and phylogenetic analysis of mtCOI sequences of *B. tabaci* in Iraqi and *T. vaporariorum* in UK populations. The findings may improve our understanding of genetic diversity and the factors which might affect both species, thus supporting the development of improved management strategies in Iraq and the UK.

2.3 Materials and Methods

2.3.1 Field sampling

Adults of glasshouse whitefly, *T. vaporariorum*, were collected from tomato, cucumber, and ornamental crops from commercial glasshouses in 12 locations throughout the UK during the summer and autumn, and some locations were sampled in both 2014 and 2015 (Table 2-1 and Fig. 2.1). In addition, a laboratory colony of *T. vaporariorum* was used; this was taken from a mixed-age colony maintained at Newcastle University (UK) on aubergine (*Solanum melongena*). This colony was obtained from Rothamsted Research and was initially collected in 1960 in Kent from French bean plants (Brogan, pers. comm.). At least 20 adult whitefly specimens were collected from whitefly-colonised plants at each location. Additionally, more than 30 adults of *B. tabaci* were collected from 14 populations with different host plants, such as tomato, cucumber, pepper, and eggplant, grown in plastic tunnel greenhouses at eight locations from the

middle and south of Iraq during summer 2015 and autumn 2016 (Table 2-1 and Fig. 2.1). Male and female adults of both whitefly species were collected from whitefly-colonised plants at each location and stored in 95% ethanol at −20 °C until DNA was extracted.

Table 2-1**.** 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 Yorkshire	ERYS15	2015	Cucumber	Cucurbitaceae	53.741930	-0.731197
East Riding of Yorkshire	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

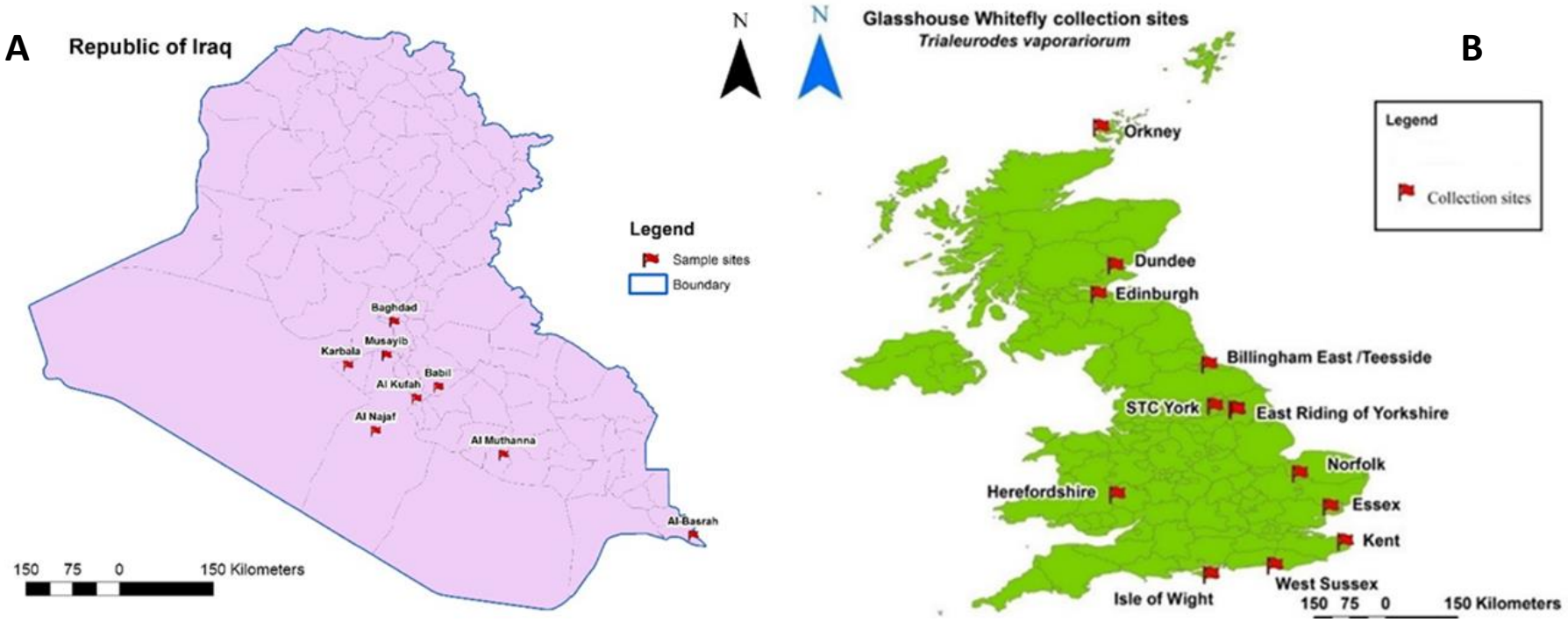


Figure 2.1. Collection sites of *B. tabaci* from Iraq (A) and *T. vaporariorum* from the UK (B).

2.3.2 Confirming the identity of specimens morphologically

Several specimens were collected from each whitefly population in order to confirm the species. Specimens were slide-mounted with Canada balsam using the procedure described by Brown (1997). The taxonomic characteristics used for identification of both whitefly were based on Hill (1969).

For *T. vaporariorum*: "Forewings with anterior margin curved anteriorly and rounded distally. Compound eyes divided with no lenses between the two groups. No pit sensorium on 6th antennal segment. No stout sensory setae on segment 3. Stout spines on mesothoracic legs arranged in two lateral 'tufts'".

For *B. tabaci*: "Anterior margin of forewings straight. Rounded distally. Eyes divided: with single lens forming a 'bridge' between the two groups. Antennae with a pit sensorium on segment 6 and a stout sensory seta on segment 3. Spines on mesothoracic legs arranged randomly; not in lateral tufts" (Hill, 1969).

2.3.3 MtCOI amplification and sequencing

The total genomic DNA (gDNA) of adult whiteflies was extracted as described in Tsagkarakou *et al.* (2007). The whiteflies were placed in a 1.5 ml microcentrifuge tube and ground with a pestle in 50 ml of ice-cold lysis buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0) containing 0.4 mg ml^{-1} of proteinase K. The extracts were incubated at 55 °C for 1 h and at 85 °C for 5 min prior to a 5 min centrifugation (13,000*g*) to pellet debris. The supernatant was used as the DNA source for the polymerase chain reaction (PCR).

Three individuals of *B. tabaci* for each population, giving a total of 42 samples, were sequenced. The PCR of mtCOI was performed using the primers C1-J-2195 5'-TTGATTTTTTGGTCATCCAGAAGT-3' (Frohlich *et al.*, 1999) and tRNA-1576 5'-TATAAATCTTAAATTTACTGCA-3' (Tsagkarakou *et al.*, 2007). For *T. vaporariorum*, at least four individuals for each location and both years from each site, giving in total 96 samples, were sequenced. The PCR of mtCOI was performed using specific primers for this region, COI-F: 5'-GCCTGGTTTTGGCATTA-3', and COI-R: 5'-GCTTATTTAGCACCCACTCTA-3'), which produced a ~752 bp product (Gao *et al.*, 2014). PCR reactions for both whiteflies were carried out in a 10 μl volume containing 1 μl of DNA template, 0.5 μl of each primer (0.2-0.4 μM), 2 μl 5× PCR MyTaq reaction buffer, and 0.5 units of MyTaq DNA polymerase (Bioline). The PCR

products were visualised on 2% agarose gels containing 1X TBE buffer and 0.2 µg ml^{-1} ethidium bromide and purified using ExoSAP kits (New England BioLabs) according to the manufacturer's instructions. The reactions were incubated in a thermocycler at 37 °C for 40 min and 80 °C for 15 min. The purified PCR products were sequenced using ABI Prism BigDye® Terminator Version 3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, California, USA). Sequencing reactions were performed in 10 µl containing 1.5 µl of 5X sequencing buffer, 0.5 pmol of mtCOI forward or reverse primer, 1 µl of BigDye terminator sequencing mix and 1 µl of purified PCR product. The reactions were carried out by 35 cycles at 96 °C for 10 s, 52 °C for 5 s and 60 °C for 4 min. Sequencing products were purified via ethanol precipitation. The sequences were visualised on a 3130XL Genetic Analyzer (Applied Biosystems).

2.3.4 Sequence alignment and phylogenetic analysis

The sequences from each whitefly in both directions were separately checked manually and aligned using Geneious, version 6.1.4 (Kearse *et al.*, 2012), and compared with those available in GenBank using the BLAST algorithm of the National Center for Biotechnology Information (NCBI). MtCOI sequences were aligned using ClustalW (Higgins *et al.*, 1996).

Trees were constructed with the sequences obtained in this study and other representative sequences from GenBank. The phylogenies were estimated using maximum likelihood (ML) using MEGA 6 with 1000 bootstrap replications performed to assess the robustness of branches and the Kimura 2-parameter model (Tamura *et al.*, 2013). To root phylogenetic trees, whitefly of another genus and species, such as *Trialeurodes lauri, T. abutilonea* and *Tetraleurodes acaciae,* were used as outgroup. Variable sites and number of mitochondrial haplotypes of both whiteflies were generated separately using DnaSP Version 5.10 (Librado and Rozas, 2009). Evolutionary analyses were conducted in MEGA 6 (Tamura *et al.*, 2013).

2.4 Results

2.4.1 Species identification

Morphological examination confirmed that the species were *T. vaporariorum* from the UK and *B. tabaci* from Iraq (Fig. 2.2).

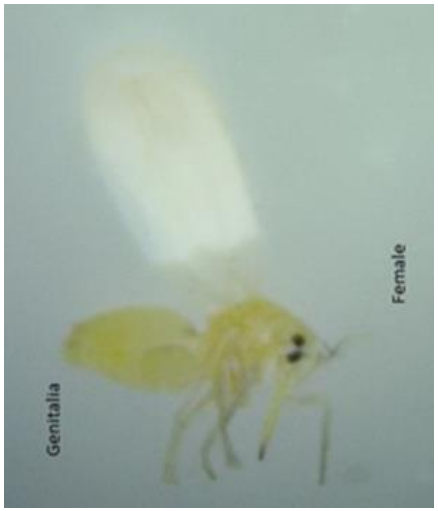
2.4.2 MtCOI sequences

Ninety-one out of the 96 UK *T. vaporariorum* samples were mitochondrial haplotype mtH1, with sequence lengths ranging from 626 to 657 base pairs (bp). Interestingly, mitochondrial haplotype mtH3 was found, which is new for UK records, with sequencing lengths of 530 and 525 bp from Essex and Norfolk, respectively (Fig. 2.3). Only one variable nucleotide was found, with haplotype diversity Hd 0.262, and corresponding nucleotide diversity $\boldsymbol{\pi}$, 0.00057 amongst the *T. vaporariorum* populations (Table 2-2). The mtH1 and mtH3 haplotypes are described by Prijovic *et al.* (2014) in the NCBI dataset, and differ by a single nucleotide C-T substitution at position 154. Two mitochondrial haplotype mtH3 sequences and one mtH1 sequence were deposited in GenBank with accession numbers KX679578 (ES14), KY048293 (NO15), and KX679581 (DU1_14), respectively (Appendices 1-3). It has been reported that there are 16 to 19 mitochondrial haplotypes of *T. vaporariorum* worldwide (Prijovic *et al.*, 2014; Wainaina *et al.*, 2017).

The phylogenetic results indicated 17 haplotypes, which has confirmed that the UK whitefly haplotypes are H1 and H3. The phylogenetic tree result using maximum likelihood indicated that there are two genetic clades of the 17 *T. vaporariorum* haplotypes. The first clade includes H3, H7, H8, H9, H10, H11, H12, H13, H14, and H16, whereas the second clade includes H1, H2, H4, H5, H6, H15, and H 17 (Fig. 2.4). Regarding UK *T. vaporariorum* haplotypes H1 and H3, the results showed that H1 (KX679581) was matched to the mtCOI haplotype (AM179444) previously recorded from the UK. Also, the results confirmed that H1 was most common, whereas H3 (KY048293) was recorded in the UK for the first time and is the same as the haplotype from Costa Rica (JF682884) (Fig. 2.4).

In Iraq, out of 42 mtCOI *B. tabaci* sequences, four haplotypes were identified with 31 variable sites. The value of haplotype diversity Hd is 0.143, and corresponding nucleotide diversity $\boldsymbol{\pi}$, 0.00308 amongst the *B. tabaci* populations (Table 2-3). Sequences were 761-801 bp in length, and 39 out of the 42 corresponded to species

MEAM1 (biotype B2), which was present in all populations collected. Three other haplotypes, corresponding to MEAM1 biotype B, MEAM2 and a previously unknown haplotype, were found in populations collected in Karbala city (Fig. 2.5). The four haplotype sequences were deposited at NCBI GenBank with accession numbers and sequence details shown (Appendices 4-7). The detection of biotypes of *B. tabaci* species based on global mtCOI sequences available in GenBank is shown in the phylogenetic tree (Fig. 2.6). The common Iraqi *B. tabaci* biotype was found to be identical to the mtCOI MEAM1 B2 biotypes from Al-Hasakeh (WF30) in Syria and Pakistan with accession numbers AB473559 and GU977267, respectively (Ahmed *et al.*, 2009; Fujiie *et al.*, 2009) (Fig. 2.6). However, the other three mtCOI haplotypes were not phylogenetically close to the mtCOI biotype as indicated above (Fig. 2.6). The mtCOI biotype isolate KA2-3 from Karbala city (KX679575) was found to be close to isolates of mtCOI MEAM1 (biotype B) from Iran (EU547770), the United Arab Emirates (DQ133382) (Shoorcheh, 2008; Muniz *et al.*, 2011), and previous Iraqi specimens isolated in 2010 (HM070413). Interestingly, a unique mtCOI isolate, KA1-5, was recorded in Karbala city (KX679574), which was not close to other haplotypes and was named as an Unknown. The Unknown Iraqi biotype was different in having high bootstrap values compared to MEAM1 and MEAM2 at 99 and 85, respectively (Fig. 2.6). Surprisingly, a single mtCOI sequence, from isolate KA2-5 (KX679576) of *B. tabaci*, had a 99% match to a MEAM2 sequence from Reunion (AJ550177) (Delatte *et al.*, 2005) (Figs. 2.6 and 2.7). The variable nucleotide positions and neighbour joining tree of mtCOI and related sequences are shown in Figs. 2.7 and 2.8. Notably, in the results for both whiteflies no connection was found between the biotypes/mtCOI haplotype and the host plants that the whiteflies were sampled from. The two mtCOI haplotypes of *T. vaporariorum* and four biotypes of *B. tabaci* infested different vegetable crops.



A

B

C

Figure 2.2. *T. vaporariorum* (A) females, (B) males and *B. tabaci* (C) Credit: Stephen Ausmus.

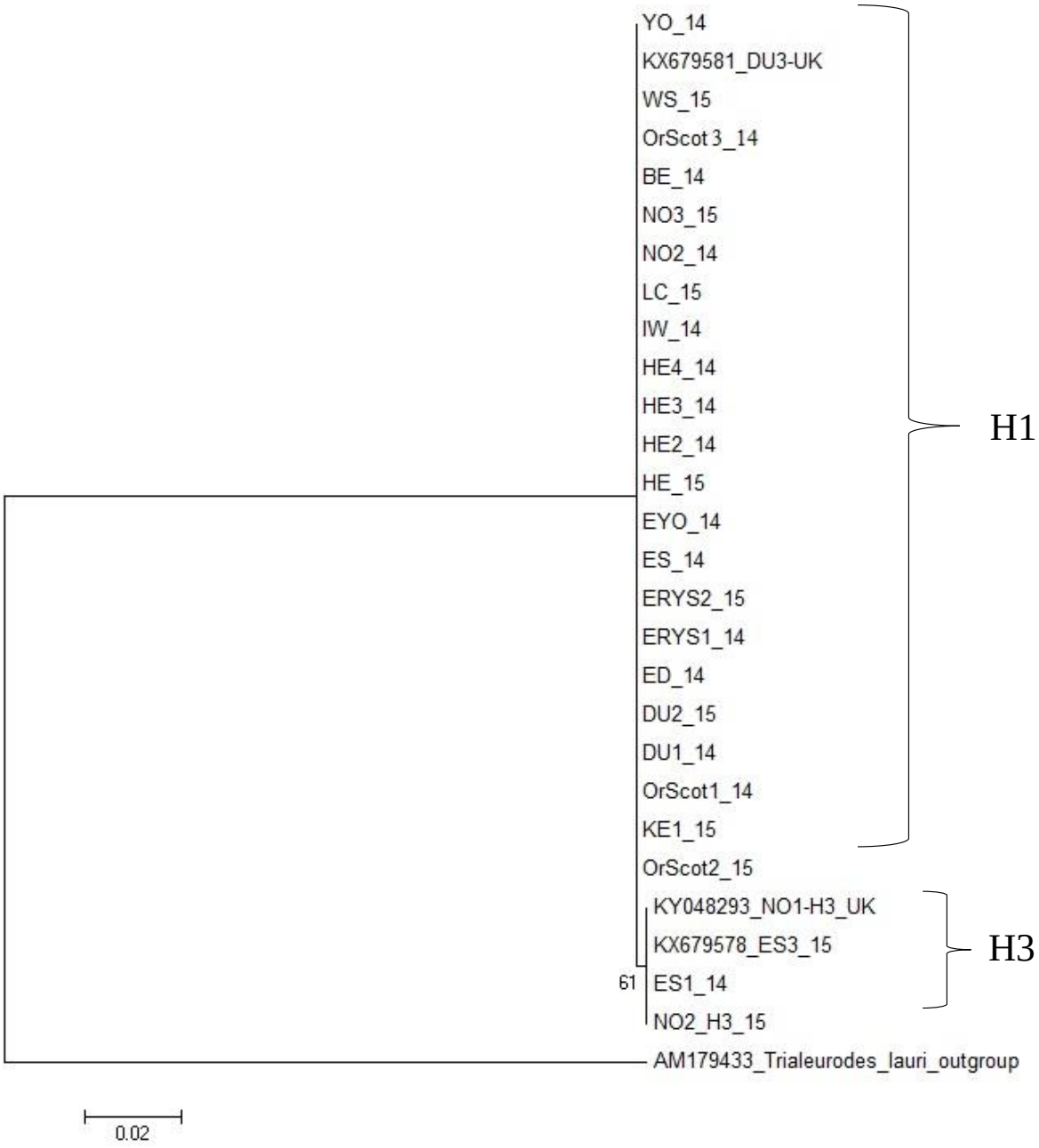


Figure 2.3. Rooted ML tree showing the phylogenetic relationships of the UK *T. vaporarioum* mtCOI sequences. *Trialeurodes lauri* was used as an outgroup. The analysis was based on 456 sites, and likelihood-ratio tests indicated by the Kimura 2-parameter model (Kimura, 1980). Phylogenetic analyses were carried out with MEGA6 (Tamura *et al.*, 2013). Haplotype (H) is indicated.

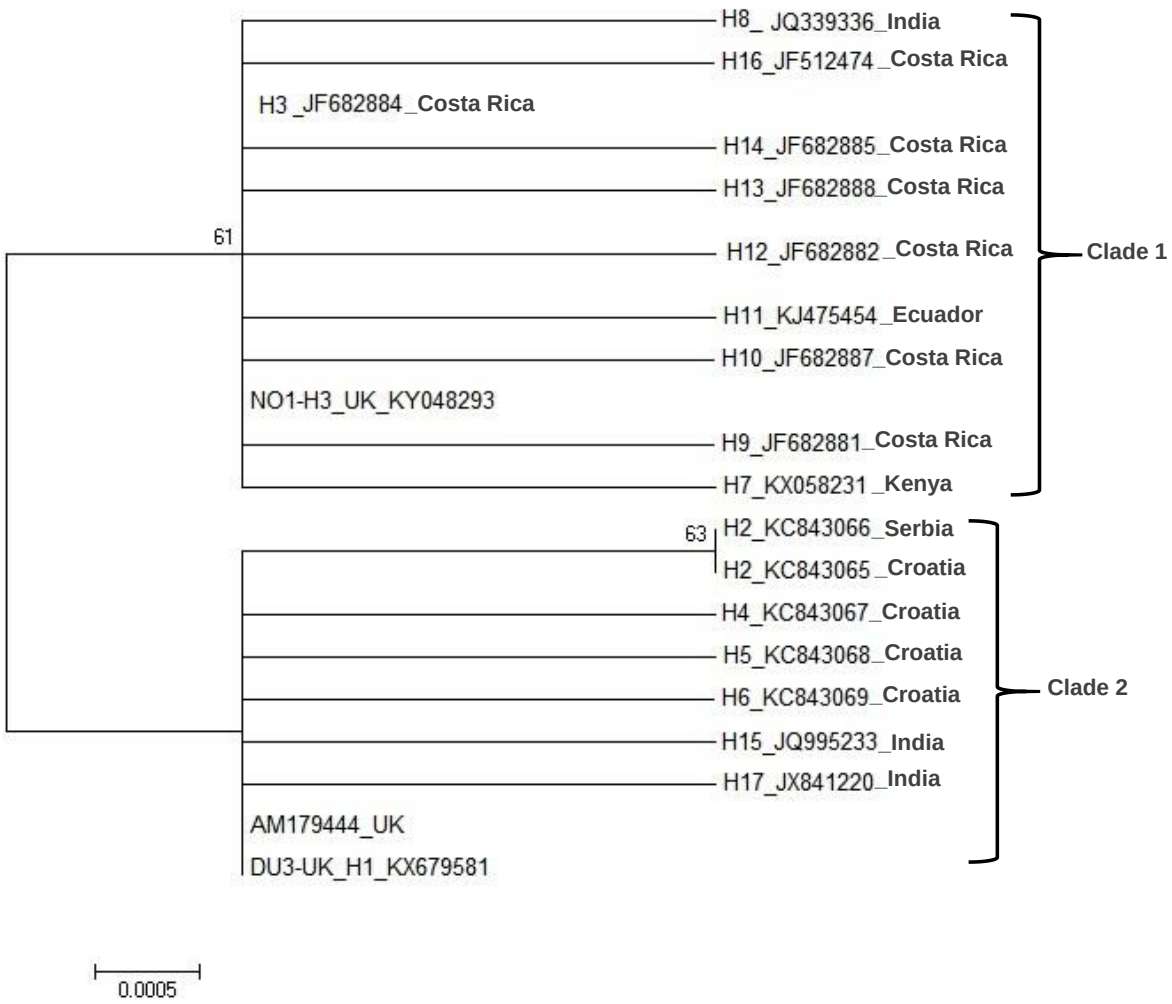


Figure 2.4. Unrooted ML tree showing the phylogenetic relationships of the 17 global haplotype mtCOI sequences of *T. vaporarioum*. The analysis was based on 443 sites, and likelihood-ratio tests indicated by the Kimura 2-parameter model (Kimura, 1980). Phylogenetic analyses were carried out with MEGA6 (Tamura *et al.*, 2013). Haplotype (H) is indicated.

Table 2-2. Variation in the COI sequences of *T. vaporariorum* specimens from the UK.

Number of sequences	27
Sequence length (bp)	456
Variable sites	1
Singleton variable sites	0
Parsimony informative sites	1
No. of haplotypes	2
Haplotype diversity (Hd) ± SD	0.262 ± 0.097
Nucleotide diversity $\boldsymbol{\pi}$ (Pi)	0.00057
Neutrality tests	
Tajima's D	0.01659 NS
Fu's Fs statistic	0.479 NS

Statistical significance: NS – not significant

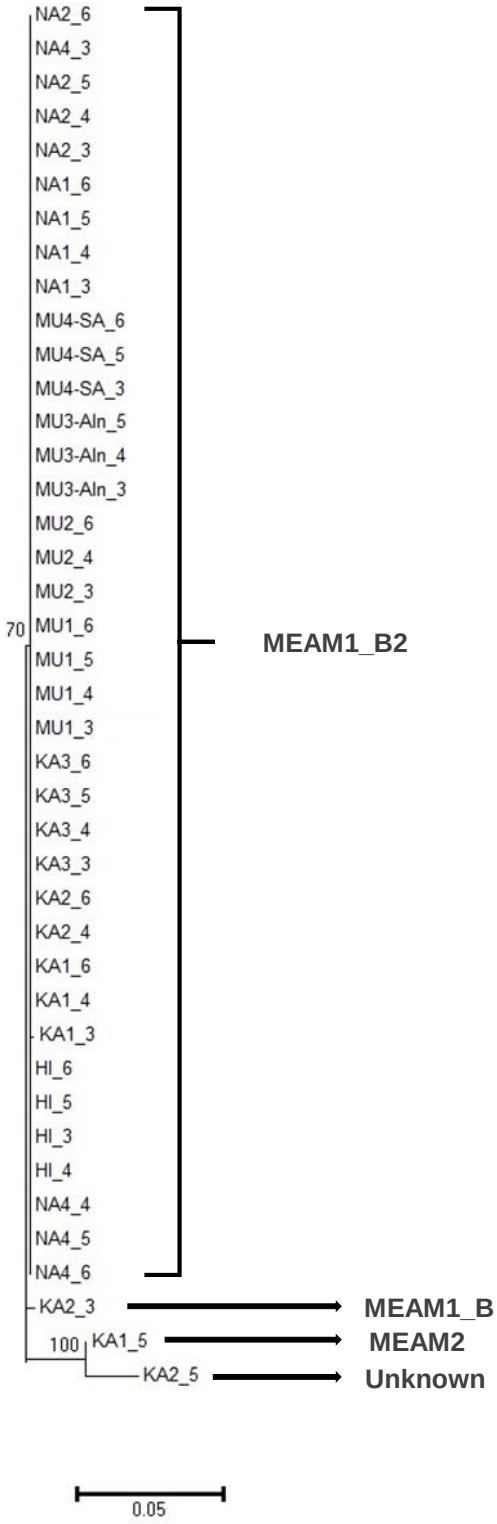


Figure 2.5. Unrooted ML tree showing the phylogenetic relationships of 41 *B. tabaci* COI haplotype sequences generated in this study. The analysis was based on ~ 846 sites, and likelihood-ratio tests indicated by the Kimura 2-parameter model (Kimura, 1980). Phylogenetic analyses were carried out with MEGA6 (Tamura *et al.*, 2013).

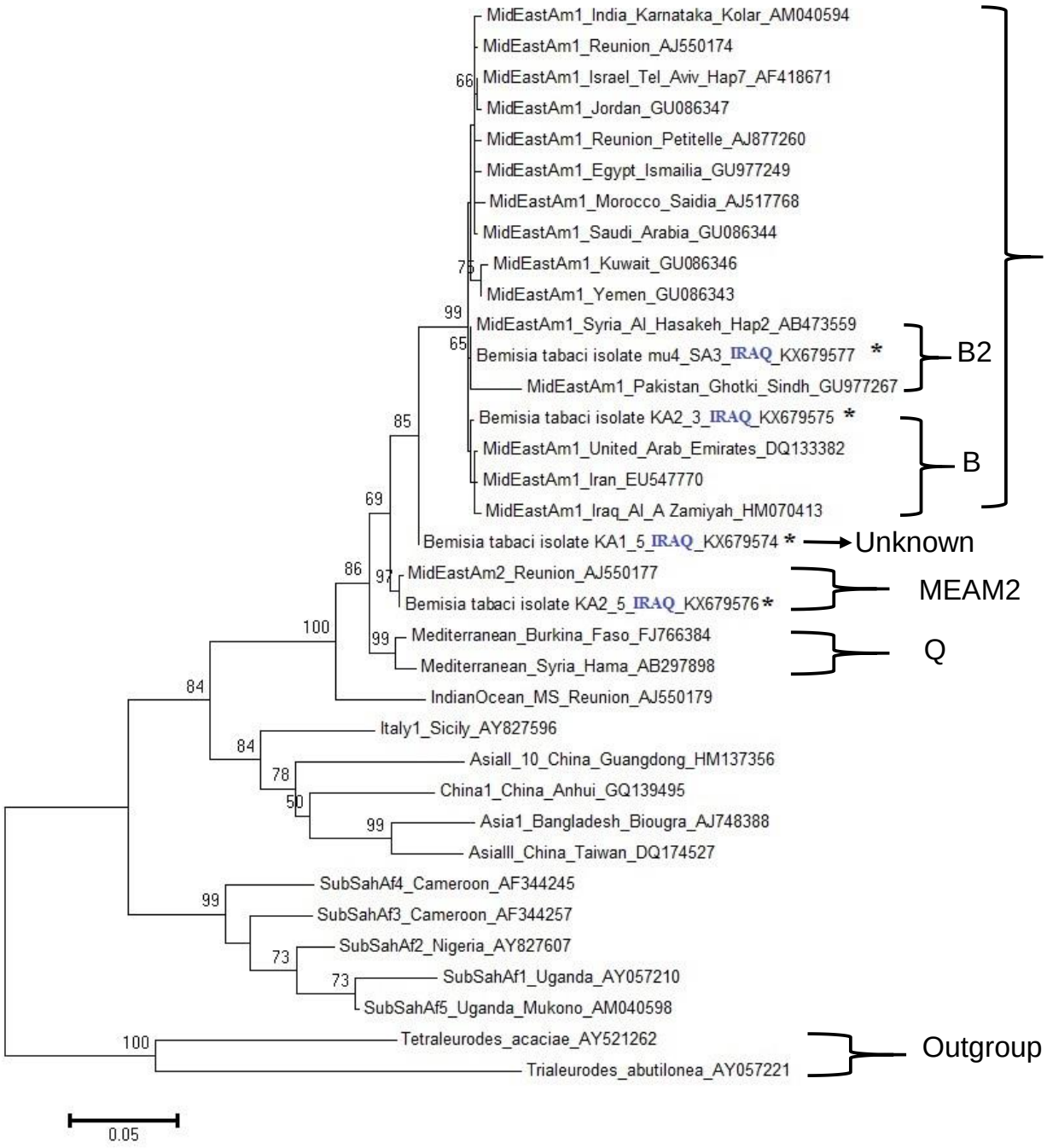
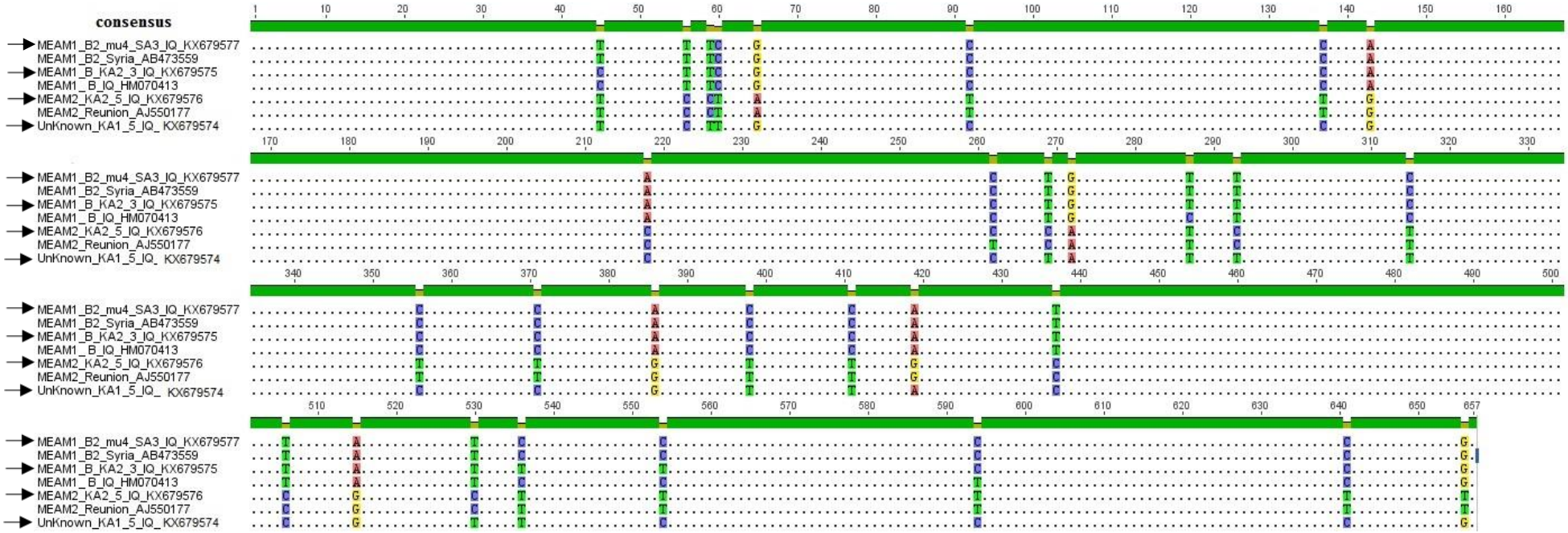


Figure 2.6. Maximum likelihood phylogenetic tree based on 52 COI sequences showing the relationships of the four Iraqi *B. tabaci* COI biotypes and other biotype sequences obtained from CSIRO (De Barro and Boykin, 2013); sequences generated in this study are indicated by "IRAQ" in blue and by asterisks. *Trialeurodes abutilonea* and *Tetraleurodes acaciae* were used as an outgroup. The analysis was based on ~801 sites and likelihood-ratio tests indicated by the Kimura 2-parameter model (Kimura, 1980). Bootstrap values below 50 are not shown. Biotypes have been indicated by codes or by the bracket in the sequences.

Table 2-3. Variations in the mtCOI sequences of *B. tabaci* specimens from Iraq.

Number of sequences	42
Sequence length (bp)	761
Variable sites	31
Singleton variable sites	14
Parsimony informative sites	17
No. of haplotypes	4
Haplotype diversity (Hd) ± SD	0.143 ± 0.073
Nucleotide diversity $\boldsymbol{\pi}$ (Pi)	0.00308
Neutrality tests	
Tajima's D	-2.32565 **
Fu's Fs statistic	3.643 NS

Statistical significance: **, 0.01 < P < 0.02; NS – not significant.



2.7. Variable nucleotide sites among four *B. tabaci* mtCOI haplotypes from Iraq (KX679574-KX679577) with most similar sequences from GenBank. Four Iraqi biotypes indicated by arrows.

Table 2-4. Evolutionary divergence between four *B. tabaci* mtCOI biotypes from Iraq (KX679574-KX679577) and the most similar sequences from GenBank.

	Biotype and accession number	1	2	3	4	5	6
1	MEAM1_B2_mu4_SA3_IQ_KX679577						
2	MEAM1_B2_Syria_AB473559	0.000					
3	MEAM1_B_KA2_3_IQ_KX679575	0.005	0.005				
4	MEAM1__B_IQ_HM070413	0.005	0.005	0.006			
5	MEAM2_KA2_5_IQ_KX679576	0.044	0.044	0.042	0.046		
6	MEAM2_Reunion_AJ550177	0.046	0.046	0.044	0.047	0.002	
7	UnKnown_KA1_5_IQ_ KX679574	0.020	0.020	0.022	0.025	0.022	0.024

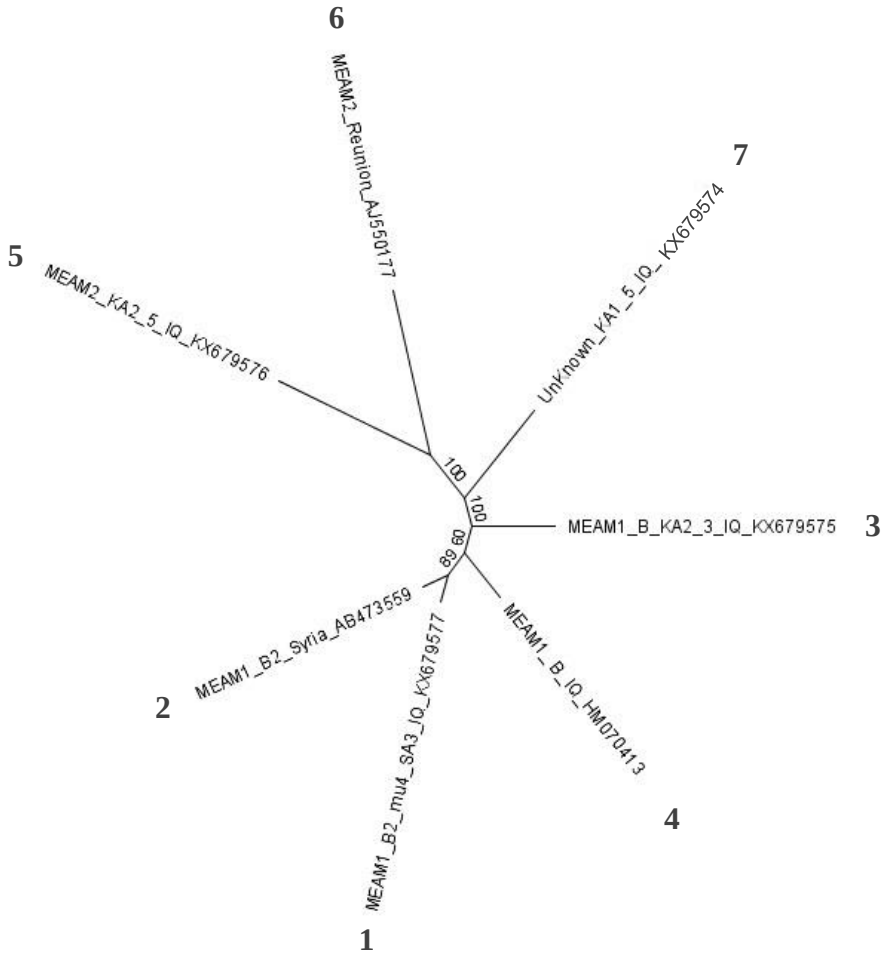


Figure 2.8. Neighbour-joining consensus tree of four *B. tabaci* mtCOI biotypes from Iraq (KX679574-KX679577) and the most similar sequences from GenBank. Numbers refer to biotypes in Table 2-4.

2.5 Discussion

This chapter presents, for the first time, extensive data on the genetic diversity of *T. vaporariorum* and *B. tabaci* populations collected from the UK and middle and south of Iraq, respectively. The results show diversity using mtCOI markers in *B. tabaci*, but very low diversity at the mitochondrial level in *T. vaporariorum*.

Most of the mtDNA COI sequences (91) of *T. vaporariorum* individuals present in the UK belonged to mitochondrial haplotype mtH1, which is also common in the Netherlands and France (Malumphy *et al.*, 2007; Kapantaidaki *et al.*, 2015). Haplotype mtH3 was recorded in the UK for the first time, namely in Essex and Norfolk in the southeast of England. The mtH3 is most common in the USA and was also previously recorded in Spain on the Canary Islands, Costa Rica and Serbia (Malumphy *et al.*, 2007; Prijovic *et al.*, 2014). The likely recent introduction of mtH3 into the UK might be a result of human activities, such as plant import from Europe or the USA. In Serbia and its neighbouring European countries, six mitochondrial haplotypes of glasshouse whitefly, including mtH1 and mtH3, have been recorded, while nineteen mtCOI haplotypes have been detected in the global populations of this species (Prijovic *et al.*, 2014). However, the phylogenetic results for *T. vaporariorum* mtCOI from GenBank showed 17 unique haplotypes, which is fewer than the haplotypes recorded in Prijovic *et al.* (2014). The different numbers of *T. vaporariorum* haplotypes reported could be due to the selection criteria used in different studies. Many of the mtCOI haplotypes identified overlap across geographical regions and this might indicate a single source of *T. vaporariorum* lineage that is easily adapted to altered niche habitats (Kapantaidaki *et al.*, 2015).

The low level of variation found in mtCOI sequences of *T. vaporariorum* from the UK populations was similar to the results of Kapantaidaki *et al.* (2015), who found that most mtCOI sequences collected from the USA and some European countries belonged to haplotype 1 (H1), and a single individual belonged to H3. Also, a low level of genetic variation has been found for this species in Serbia and its neighbouring countries (Prijovic *et al.*, 2014) and in India (Roopa *et al.*, 2012).The likely explanation for the absence of significant mtCOI sequence variation in our data could be the recent introduction of this species in the UK in about 1856 (Mound and Halsey, 1978). There might not have been enough time for the generation of variation within UK whiteflies,

and the variation in *T. vaporariorum* is most likely, therefore, to have arisen through imports.

For *B. tabaci* in Iraq, the results of mtCOI sequencing showed that the MEAM1 *B. tabaci* biotype B2 was predominant in the study area and one B biotype was found. Two mtCOI haplotypes (Unknown and MEAM2) were recorded for the first time in Iraq. All the individuals in Iraq belong to the Africa/Middle East Asia Minor genetic group. Previous studies have reported MEAM2 from Japan, Peru, Turkey, and Egypt since the first time it was recorded in La Réunion (Delatte *et al.*, 2005; Ueda *et al.*, 2009; Karut *et al.*, 2015). However, Tay *et al.* (2017) reported on the basis of high-throughput sequencing that the mtCOI sequence defining MEAM2 as a putative species could be a PCR artefact and that the true mtCOI sequence of the "MEAM2" whiteflies they studied was the same as the MEAM1 sequence. The MEAM2 sequence length from this study is just 799 bp and Tay *et al.* (2017) found a stop codon at the 905 position of some of their MEAM2-like sequences. Therefore, more sampling and whole mitogenome sequencing are needed to determine whether MEAM2 exists in Iraq. The "Unknown" sequence differs by less than 3.5% from the MEAM1 sequences (Table 2-4) and so may represent a new haplotype of MEAM1 or, like MEAM2, may be a PCR artefact.

The MEAM1 B2 and B biotypes recorded in the present study were slightly different from the previous B biotype (HM070413) recorded in Iraq in 2010 (Fig. 2.6). This was based on polymorphic sites in the mtCOI sequences of Iraqi *B. tabaci* in comparison with the relevant mtCOI sequences, as shown in Table 2-4 and Fig. 2.7. The presence of multiple haplotypes is likely a consequence of DNA mutations and plants imported from countries surrounding Iraq. This could be facilitated by human activities in moving *B. tabaci*-infested crops from multiple source populations around the world. Most Iraqi whiteflies were grouped with other MEAM1 sequences from the countries neighbouring Iraq such as Syria, Iran, and the United Arab Emirates. MEAM1 has been found in tropical and subtropical countries around the world (Boykin *et al.*, 2007; Chu *et al.*, 2010; De Barro *et al.*, 2011; De Barro, 2012). The introduction of new biotypes from different sources could increase genetic diversity by the introduction of genes for resistance to pesticides, which arose by mutation (Zidana *et al.*, 2009; Verhoeven *et al.*, 2011). The global adaptive diversity of the *B. tabaci* B biotype might be due to a large effective population size (Hadjistylli *et al.*, 2016). The results for both whiteflies also showed no connection between biotypes/mtCOI haplotype and host plant species. The

two mtCOI haplotypes of *T. vaporariorum* and four biotypes of *B. tabaci* were found in different crops. This finding was similar to that of Prijovic *et al.* (2014), who found no link between mtCOI haplotypes of *T. vaporariorum* and host plant.

Invasion patterns involving multiple introductions have been demonstrated in many invasive species, including mosquitofish *Gambusia spp.* and the caprellid *Caprella scaura* (Sanz *et al.*, 2013; Cabezas *et al.*, 2014). The introduction of non-native pests is often associated with successful invasions by some species (Roman and Darling, 2007; Suarez and Tsutsui, 2008). Numerous introductions of *B. tabaci* putative species and *T. vaporariorum* mitochondrial haplotypes have probably occurred in Iraq and the UK via international trade, which might have helped their successful invasion.

The low level of genetic variation in *T. vaporariorum* mtCOI could be a consequence of extensive insect control measures that include the removal of infestation sources, biological control, and the use of insecticides to reduce and/or eradicate this insect. Also there could be a possible influence of *T. vaporariorum* endosymbionts and possibly indirect selection on mtDNA since the UK *T. vaporariorum* populations harbour just one secondary symbiont, *Arsenophonus* sp. (Chapter 5). It is more likely that the introductions of *T. vaporariorum* populations could be from the same region, leading to low mtDNA and symbiont diversity. Extensive sampling of populations, particularly from the west and north of the UK, is needed to confirm the low level of mtDNA diversity, which might help extend our understanding of the biology, ecology, and spread of this damaging and invasive insect pest in the UK. Also, more studies are needed to investigate the role of *B. tabaci* biotypes in transmitting different strains of TYLV virus as recently recorded in Iraq. A sampling of *B. tabaci* is needed to monitor new biotypes which might be introduced. This might help improve our understanding of the biology, ecology, and spread of this damaging and invasive whitefly insect in both countries.

2.6 Conclusion

Populations of *T. vaporariorum* in the UK showed low genetic diversity of partial mtCOI sequences and all *T. vaporariorum* individuals in the north and midlands of the UK belonged to mtH1, while mtH3 has been recorded in the UK for the first time in the south-east of England. This indicates that there have been at least two introductions of *T. vaporariorum* in the UK. This is less diversity than I had predicted. Taken together

with the results from Chapters 3 and 5, it seems that the glasshouse agroecosystem and imports from limited regions have contributed to variation at the nuclear but not at the cytoplasmic level. The glasshouse agroecosystem has likely contributed to the population genetic structure through restricting gene flow between locations.

The mtCOI results confirmed that the *B. tabaci* biotype MEAM1 B2 predominates in Iraq and a new haplotype of this species has been recorded for the first time. Again, this is less diversity than I had predicted. More information on genetic diversity of *B. tabaci* in Iraq gained from a combination of genetic characterisation and biological and ecological research might help in developing sustainable management policies for *B. tabaci*.

3 Chapter 3. Population structure of glasshouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), in the UK

3.1 Abstract

This study determines the genetic differentiation and structure of twenty *T. vaporariorum* populations, samples of which were collected from commercial glasshouses across the UK. Nine pairs of microsatellite markers were used for genetic analysis.

The objectives of this chapter are to investigate the population genetic characteristics of *T. vaporariorum* and to answer questions about the number of introductions of *T. vaporariorum* in the UK and how habitat and agricultural management affect the pest populations.

Genotyping results indicated two, six or ten genetic clusters which are, to a limited extent, linked to location but not to host plant. The population structure suggests that glasshouse agroecosystems restrict gene flow between whitefly populations and that the movement of the glasshouse whitefly is linked to human-assisted spread. The high nuclear diversity indicates multiple introductions, combined with little gene flow between populations, which is probably due to glasshouse confinement and management. Taken together with the results for nuclear and cytochrome diversity presented in Chapter 2, multiple but limited numbers of introductions of *T. vaporariorum* are suggested. This conclusion indicates challenges for the management of this pest in the UK. It is important to understand their invasion history and how they can be controlled, such as via glasshouse management. These findings may highlight opportunities for better management of this pest in the UK.

3.2 Introduction

3.2.1 Glasshouse whitefly

Glasshouse or greenhouse whitefly *T. vaporariorum* (West.) (Hemiptera: Aleyrodidae) is a polyphagous species of temperate areas. It has been reported widely throughout Europe, even though in northern Europe including the UK, the climate limits *T. vaporariorum* to vegetable crops planted in a glasshouse (Martin *et al.*, 2000). *T. vaporariorum* was first reported in the UK in 1856 (Mound and Halsey, 1978). The glasshouse whitefly causes economic losses to many plant families, such as Asteraceae, Fabaceae, Euphorbiaceae, Brassicaceae, Rosaceae, Cucurbitaceae, and Solanaceae (CABI, 2018b). Also, there are many weed species used by *T. vaporariorum* as hosts (Mound and Halsey, 1978; Byrne *et al.*, 1990; Albajes *et al.*, 2000). The attention comes to glasshouse whitefly as a severe pest due its wide host range, tiny size, high reproductive rate, short life cycle and high fecundity (CABI, 2018b). The life cycle of glasshouse whitefly is shown in Chapter 1. Females of whitefly typically lay their eggs on the undersides of leaves in a circle (Martin *et al.*, 2000). Within 24 hours after emergence, females are able to mate and can lay about 30 eggs every day.

Adult and immature stages of *T. vaporariorum*, except for the eggs, cause damage to their host plants by feeding on plant sap and excreting honeydew. *T. vaporariorum* is also an important vector of many plant viruses which belong to the *Closteroviridae* family (Byrne *et al.*, 1990; Wisler *et al.*, 1998; Jones, 2003).

3.2.2 Genetic diversity of whitefly

Within insect pest species a complex of biotypes and haplotypes can often be described by insecticide resistance, morphology, behaviour and/or the DNA sequence of the mitochondrial cytochrome oxidase I (mtCOI) gene. For example, from recent research on *B. tabaci* it has been concluded that the species is a complex with more than thirty-nine relevant biotypes (Boykin *et al.*, 2012; Boykin *et al.*, 2013; Alemandri *et al.*, 2015), while in *T. vaporariorum* nineteen mtCOI haplotypes have been detected in the global population of this species (Prijovic *et al.*, 2014). The complexity of whitefly species is important for several reasons, including the development of insecticide resistance in response to selection pressure, host differences, and geography, all of

which might impact on its vector potential for various viruses (Bird, 1957; Mound, 1963; Costa and Russell, 1975; Bird and Maramorosch, 1978). In comparison to *B. tabaci*, fewer studies on population structure and phylogeny have been carried out with *T. vaporariorum*. One study used mtCOI sequencing and nuclear markers to assess the genetic diversity and phylogenetic structure of glasshouse whitefly in India (Roopa *et al.*, 2012), indicating low mtCOI sequence and nuclear marker differentiation between populations. These findings have been confirmed by Kapantaidaki *et al.* (2015) and Prijovic *et al.* (2014), who assessed glasshouse whitefly populations in Europe and the USA. Furthermore, two studies have described the population structure of non-native *T. vaporariorum* from different regions and habitat in Finland, Greece, and China (Gao *et al.*, 2014; Ovcarenko *et al.*, 2014).

3.2.3 Invasive species of whitefly

Biological invasions and introductions of non-native species are separate by-products of human-mediated travel and trade activities. This issue is important for biodiversity and crops (Pimentel *et al.*, 2001). Although many records of alien species in the literature are based on the observation of collections, introduction and distribution pathways often go unnoticed in sample collection, especially when introduction might have occurred following failures in quarantine at borders (Estoup and Guillemaud, 2010). Research on the population structure and genetic diversity of species introduced into non-native areas can assist in explaining their origin, and routes and times of introduction (Lombaert *et al.*, 2014) and provide details essential for management and the avoidance of future non-native introductions (Signorile *et al.*, 2014). Various introductions, secondary expansions of introduced populations and management efforts can lead to structured populations (Berthouly-Salazar *et al.*, 2013; Cao *et al.*, 2016), while gene flow among introduced populations ultimately decreases levels of population differentiation (Tsuchida *et al.*, 2014), except at loci with selection. No study has yet assessed the genetic structure and introduction of glasshouse whitefly in the UK since the species became established here.

This study aims to assess the genetic differentiation and population structure of *T. vaporariorum* from ten commercial and two private glasshouses across the UK using nine nuclear microsatellite markers. The following questions are asked. Have the current populations of this species arisen from a few or multiple introductions? Do

habitat and agricultural practices structure pest populations? What is the main cause of the movement of the insects across the UK? I hypothesised that multiple introductions and glasshouse habitat do affect population structure of *T. vaporariorum* in the UK. The answers to these questions might improve our understanding of gene flow and patterns of population genetic structure and the factors that affect them. The findings, in turn, may help in improving pest management strategies.

3.3 Materials and Methods

3.3.1 Field sampling

Adults of glasshouse whitefly were collected from tomato, cucumber, and ornamental crops from commercial glasshouses in twelve locations throughout the UK during summer and autumn, and some locations were sampled in both 2014 and 2015 (Table 3-1). In addition, a laboratory colony was used, which was taken from a mixed-age colony maintained at Newcastle University (UK) on aubergine (*Solanum melongena*). This colony was obtained from Rothamsted Research and was originally collected in 1960 in Kent from French bean plants (B. Brogan pers. comm.). At least 20 adult whitefly specimens were collected from whitefly-colonised plants at each location. A total of 400 individuals from 20 populations (20 individuals per population) were genotyped (Table 3-1). The whitefly specimens were stored in 95% ethanol at −20 °C until DNA was extracted.

3.3.2 Confirming the identity of specimens morphologically and genetically

The techniques used to identify the *T. vaporariorum* morphologically and molecularly are described in section 2.3.2 **(Chapter 2)**.

3.3.3 Microsatellite genotyping

Nine microsatellite primer pairs (Tvap-3-3, Tvap-1-4, Tvap-1-5, Tvap-1-1C, Tvap-1-2, Tvap-3-1, Tvap-2-2C, Tvap-3-2, and Tvap-4-2) (Table 3-2), as described in Ovcarenko *et al.* (2013), were used to amplify microsatellite loci using *T. vaporariorum* DNA as the template. Four hundred females from 20 populations were assessed with three sets of multiplex amplification reactions: set 1 (Tvap-3-3, Tvap-1-4, and Tvap-1-5); set 2 (Tvap-1-1C, Tvap-1-2, and Tvap-3-1); and set 3 (Tvap-2-2C, Tvap-3-2, and Tvap-4-2). The PCR amplification was performed in 10 µl containing 5 ng DNA, 2 µl 5× PCR reaction buffer, 2 mM $MgCl_2$, 0.2 µM dNTPs, 0.2–0.4 µM of each primer, and 0.5 units

MyTaq DNA polymerase (Bioline). PCR reactions were run in conditions of initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The PCR products were separated on a 3130XL Genetic Analyzer and the allele sizes were determined using GeneScan™ ROX500 size standard, using GeneMapper software version 3.2 (Applied Biosystems), and were confirmed manually.

Table 3-1. Collection sites, population codes, dates of collection, host plants, and genetic diversity indices for the glasshouse whitefly *T. vaporariorum* populations from the UK examined in this study. The following genetic diversity indices are indicated: an average number of alleles per locus (N_a), the effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A_r), fixation index (F; $F = [H_e - H_o] / H_e = 1 - [H_o / H_e]$).

Locality	Code	Year	Host	Plant family	Latitude	Longitude	N_a	A_e	H_o	H_e	A_r	F
Billingham East /Teesside	BE14	2014	Tomato	Solanaceae	54.604285	-1.257358	2.444	1.934	0.539	0.476	2.44	-0.106
Dundee	DU1_14	2014	Eupatorium	Asteraceae	56.456253	-3.025183	3.556	2.438	0.506	0.558	3.56	0.120
Dundee	DU15	2015	Eupatorium	Asteraceae	56.456253	-3.025183	2.667	2.197	0.594	0.495	2.67	-0.176
East Riding of Yorkshire	ERYS15	2015	Cucumber	Cucurbitaceae	53.741930	-0.731197	2.444	1.972	0.678	0.455	2.44	-0.469
East Riding of Yorkshire	ERYS14	2014	Cucumber	Cucurbitaceae	53.750523	-0.732015	3.222	1.945	0.517	0.444	3.22	-0.138
East York	EYO14	2014	Cucumber	Cucurbitaceae	53.771412	-0.748213	3.111	2.243	0.583	0.507	3.11	-0.125
Essex	ES15	2015	Tomato	Solanaceae	51.933305	1.022727	2.667	2.063	0.672	0.487	2.67	-0.360
Essex	ES14	2014	Tomato	Solanaceae	51.933305	1.022727	3.444	2.312	0.456	0.537	3.44	0.176
Herefordshire	HE2_14	2014	Cape gooseberry	Solanaceae	52.162737	-2.996278	3.111	2.197	0.650	0.482	3.11	-0.325
Herefordshire	HE3_14	2014	Basil	Lamiaceae	52.162737	-2.996278	3.000	1.969	0.478	0.417	3.00	-0.120
Herefordshire	HE4_14	2014	Chili peppers	Solanaceae	52.162737	-2.996278	3.000	1.982	0.556	0.430	3.00	-0.270
Herefordshire	HE15	2015	Squash	Cucurbitaceae	52.162737	-2.996278	2.222	1.951	0.589	0.443	2.22	-0.306
Isle of Wight	IW14	2014	Unknown	-	50.657994	-1.227233	3.333	2.362	0.544	0.464	3.33	-0.147
Kent County	KE15	2015	Tomato	Solanaceae	51.283319	1.295062	2.667	1.930	0.539	0.438	2.67	-0.205
Lab colony	LC15	2015	Eggplant	Solanaceae	54.980320	-1.6157134	2.000	1.718	0.450	0.366	1.78	-0.204
Norfolk	NO15	2015	Tomato	Solanaceae	52.560526	0.442994	2.667	2.130	0.678	0.496	2.67	-0.343
Norfolk	NO3_14	2014	Tomato	Solanaceae	52.560526	0.442994	3.444	2.458	0.672	0.541	3.44	-0.219
Orkney	Or14	2014	Pelargonium	Geraniaceae	59.052969	-3.293660	3.222	2.564	0.683	0.576	3.22	-0.162
Orkney	Or15	2015	Pelargonium	Geraniaceae	59.052969	-3.293660	2.889	2.037	0.533	0.476	2.89	-0.096
West Sussex	WS15	2015	Tomato	Solanaceae	50.832853	-0.027808	2.333	1.967	0.600	0.459	2.33	-0.285
Mean							2.872	2.118	0.575	0.477	2.86	-0.188

Table 3-2. Nine pairs of microsatellite primers used in this study as described by Ovcarenko *et al.* (2013). The number of alleles, annealing temperature used in PCR, the expected size for each marker, and the dyes (FAM, HEX) used to distinguish the loci in multiplex PCR.

Locus (GenBank Accession No.)	Primer (5'–3') (F: [dye]-forward; R: reverse)	No. of alleles	Annealing Temperature (°C)	Size range (bp)
Tvap-1-2 (GF112025)	F: [FAM] - CTGTGAATCCCTCAGAAATC R: TGACCTCTCTCAGGCTTTTA	2	55	232-236
Tvap-2-2C (GF112021)	F: [FAM] - CTGAAAGTCTTATTAGAGCC R: CTAACTGATTCCATAGTCG	4	55	211-217
Tvap-3-3 (GF112019)	F: [HEX] - CGCAAATCATACTTCCTTTC R: AAATACAGGCGACTCATGTC	3	55	233-235
Tvap-3-2 (GF112017)	F: [FAM] - GGAGGTCATTACTCATTTCG R: CATAAATTTTCGGCTCACTC	3	55	181-185
Tvap-1-1C (GF112015)	F: [HEX] - GAGACTCCACGATGTCTGTC R: TTCCCCTATCGTATGTTCAC	2	55	195-214
Tvap-1-5 (GF112028)	F: [HEX] - CAGTTGTGGTAGTGTGGTG R: CTCATCGGCTCATACATTC	10	55	123-139
Tvap-1-4 (GF112020)	F: [FAM] - GATTTAGCCCAGTTCATTTG R: CTTCAGTTGAGCTGCTGATG	3	55	246-264
Tvap-3-1 (GF112016)	F: [HEX] - GAGATGGACAAACTACAACG R: GATTGGATGTCGTGGTTG	3	55	226-228
Tvap-4-2 (GF112027)	F: [HEX] - GGTGGTATTGTGGCGTC R: CTGCCTCTTATGACTCTTCC	6	55	294-312

3.3.4 Data analysis of population genetic structure and genetic diversity

For each of the 20 populations of *T. vaporariorum*, the average number of alleles per locus (N_a), effective number of alleles (A_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using GenAlEx v. 6.5 (Peakall and Smouse, 2012). The differences in H_e between *T. vaporariorum* populations were statistically analysed using the Fisher LSD Test in Minitab® v17 software (2013 Minitab Inc.). Weir and Cockerham's estimator of the fixation index F_{st} (Weir and Cockerham, 1984) was calculated and the differences from zero tested statistically using GENEPOP v.3.4 (Raymond and Rousset, 1995). The program FSTAT 2.9.3.2 was used to calculate allelic richness (Ar) (Franks *et al.*, 2011). The population differentiation approach was based on F_{st} values. The distribution of genetic variation was investigated by performing an analysis of molecular variance (AMOVA) and a Principal Coordinates Analysis (PCO) was carried out using GenAlEx v. 6.5.

The genetic clustering of samples was examined using STRUCTURE v.2.3.2 (Pritchard *et al.*, 2000) with a burn-in of 150,000 iterations and 500,000 Markov chain Monte Carlo (MCMC) repetitions under the no admixture ancestry model and using prior allele frequency information. Twenty independent runs were performed for each K value, ranging from K = 1 to 21, and ΔK was used to calculate the optimal number of genetic clusters (K) using Structure Harvester (Earl and Vonholdt, 2012). The results were combined and visualised using online POPHELPER software (Francis, 2017).

3.4 Results

3.4.1 Identity of specimens morphologically and genetically

Morphological and mitochondrial DNA examination showed that the species was *T. vaporariorum*. The most common mitochondrial haplotype was mtH1, and the presence of mitochondrial haplotype mtH3 in Essex and Norfolk is new for UK records (Chapter 2).

3.4.2 Genetic diversity

All populations showed high genetic diversity (Table 3-1). The average number of alleles per locus (N_a) ranged from 2 (Lab colony, LC15) to 3.556 (Dundee, DU14), and

the effective number of alleles (A_e) ranged from 1.718 (Lab colony, LC15) to 2.456 (Orkney, Or14). The expected heterozygosity (H_e) ranged from 0.366 (Lab colony, LC15) to 0.576 (Orkney, Or14), while the observed heterozygosity (H_o) ranged from 0.450 (Lab colony, LC15) to 0.683 (Orkney, Or14). Values of H_o in all populations were higher than H_e, so that fixation indices were negative. The population from Orkney (Or14) had the highest value of H_e, at 0.576, while the Lab colony (LC15) had the lowest value at 0.366. The populations from Scotland (Orkney/Or14; Dundee, DU14), East York (EYO14), Essex (ES14), and Norfolk (NO3_14) exhibited H_e values higher than the mean value of 0.477. There was significant ($P < 0.05$) genetic diversity in expected heterozygosity (H_e) between *T. vaporariorum* populations Or14, DU14, NO14, and ES14, which were significantly different from the lab colony (LC15).

In terms of differences in genetic structure between populations, 97.5% of pairwise F_{st} comparisons (195 out of 200) were significantly different from zero (Table 3-3). Only geographically close populations or those from the same location did, in some cases, not exhibit significant differentiation; for example, among the Herefordshire (HE2_14-HE4_14) samples. Estimates of pairwise F_{st} / (1-F_{st}) values ranged from 0.02 (HE2_14/ HE4_14) to 0.33 (LC15/ERYS14). Evidence of isolation by distance was weak based on the Mantel test for correlation between pairwise F_{st} and geographic distance ($R^2 = 0.04$, $P > 0.05$) (Fig 3.1). The AMOVA revealed genetic differentiation among populations, explaining 17% of the total genetic variance, while the remainder of the variation (83%) was within populations (Table 3-4).

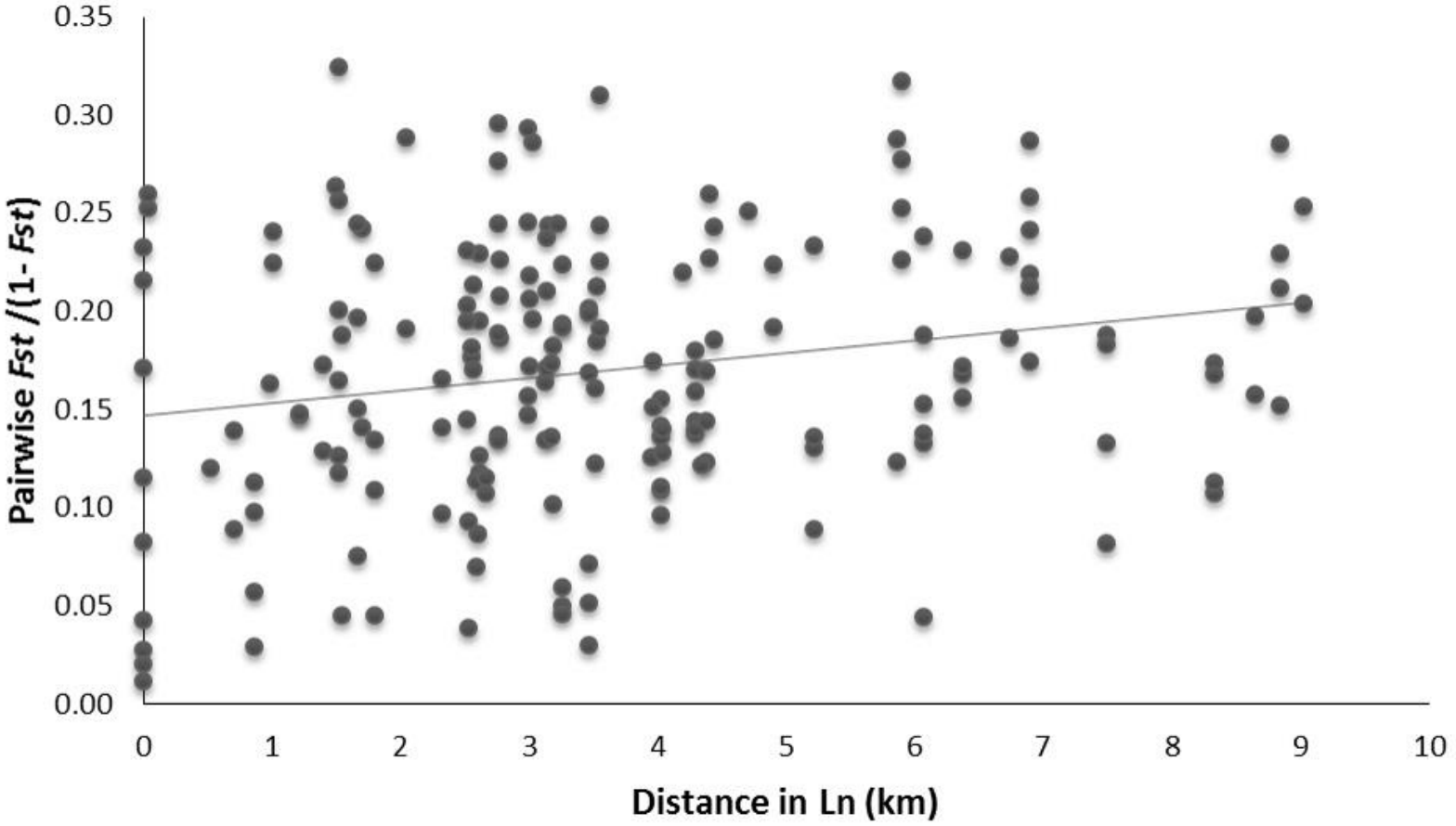


Figure 3.1. Correlation between genetic distance (based on pairwise F_{st} / (1 – F_{st})) and log (ln) geographic distance (based on pairwise distance in km) of *T. vaporariorum*. The line represents the regression line, R^2 =0.04.

Table 3-3. Pairwise estimates of genetic distance F_{st} / (1 – F_{st}) values between 20 *T. vaporariorum* populations over the nine microsatellite loci. Significant values (P < 0.05) are in **bold**.

POP	HE2_14	HE3_14	HE4_14	HE15	Or14	Or15	DU14	DU15	EYO14	ERYS15	ERYS14	ES15	ES14	WS15	NO15	NO14	IW14	BE14	KE15
HE3_14	0.03																		
HE4_14	0.02	0.02																	
HE15	**0.05**	**0.05**	**0.06**																
Or14	**0.17**	**0.22**	**0.21**	**0.21**															
Or15	**0.24**	**0.29**	**0.26**	**0.29**	**0.17**														
DU14	**0.14**	**0.15**	**0.14**	**0.16**	**0.12**	**0.13**													
DU15	**0.17**	**0.19**	**0.16**	**0.17**	**0.21**	**0.23**	**0.09**												
EYO14	**0.19**	**0.21**	**0.23**	**0.21**	**0.13**	**0.29**	**0.19**	**0.22**											
ERYS15	**0.14**	**0.19**	**0.14**	**0.15**	**0.23**	**0.28**	**0.21**	**0.23**	**0.26**										
ERYS14	**0.25**	**0.31**	**0.31**	**0.25**	**0.26**	**0.32**	**0.25**	**0.31**	**0.26**	**0.24**									
ES15	**0.11**	**0.16**	**0.14**	**0.13**	**0.18**	**0.17**	**0.16**	**0.19**	**0.18**	**0.21**	**0.24**								
ES14	**0.11**	**0.14**	**0.11**	**0.18**	**0.11**	**0.12**	0.05	**0.14**	**0.19**	**0.15**	**0.21**	**0.05**							
WS15	**0.21**	**0.22**	**0.21**	**0.22**	**0.23**	**0.16**	**0.18**	**0.23**	**0.29**	**0.16**	**0.31**	**0.21**	**0.17**						
NO15	**0.17**	**0.21**	**0.21**	**0.22**	**0.19**	**0.11**	**0.13**	**0.24**	**0.25**	**0.21**	**0.25**	**0.12**	**0.11**	**0.11**					
NO3_14	**0.03**	**0.07**	**0.06**	**0.05**	**0.14**	**0.21**	**0.09**	**0.14**	**0.14**	**0.08**	**0.16**	**0.06**	**0.03**	**0.14**	**0.12**				
IW14	**0.11**	**0.17**	**0.14**	**0.15**	**0.21**	**0.16**	**0.14**	**0.24**	**0.25**	**0.14**	**0.17**	**0.12**	**0.07**	**0.15**	**0.11**	0.04			
BE14	**0.18**	**0.21**	**0.22**	**0.18**	**0.23**	**0.21**	**0.17**	**0.22**	**0.17**	**0.23**	**0.25**	**0.13**	**0.17**	**0.16**	**0.11**	**0.12**	**0.13**		
KE15	**0.12**	**0.17**	**0.15**	**0.13**	**0.21**	**0.26**	**0.19**	**0.23**	**0.24**	**0.11**	**0.19**	**0.14**	**0.09**	**0.18**	**0.19**	**0.05**	**0.09**	**0.22**	
LC15	**0.17**	**0.24**	**0.21**	**0.19**	**0.26**	**0.23**	**0.21**	**0.29**	**0.27**	**0.26**	**0.33**	**0.13**	**0.14**	**0.25**	**0.18**	**0.14**	**0.13**	**0.12**	**0.25**

Table 3-4. AMOVA analysis using nine microsatellite loci of all 20 *T. vaporariorum* populations.

Source of variation	d. f.	Sum of squares	Variance components	Est. Var.	Percentage of variation
Among populations	19	385.533	20.291	0.452	17%
Within populations	780	1718.7	2.203	2.203	83%
Total	799	2104.233	22.494	2.656	

3.4.3 Genotyping analysis

The PCO approach showed some grouping of individuals by population (Fig. 3.2), with 32.03% of total variation explained by the first two axes (11.90% and 22.91%, respectively). For example, HE2_14-H4_14 and HE15 populations are on the right-hand side, while the Orkney (Or14 and Or15) populations are on the left in the graph (Fig. 3.2).

The clustering method implemented in STRUCTURE determined three optimal groupings of *T. vaporariorum* individuals (Fig 3.3) with two, six and ten genetic clusters as indicated by high values of Delta K (ΔK) against K (Fig. 3.4). Thus, the groupings at these K values were examined. K = 2 revealed seemingly random clusters of populations from the UK (Fig. 3.3). K = 6 and 10 gave some information based on geographical patterns, as visualised by their proportional Q values (Fig. 3.5). In some cases samples from the same location but different years grouped together, whereas in other cases they did not. For example, samples from Herefordshire (HE2_14, HE3_14, HE4_14, and HE15) grouped. Samples from Dundee (DU15 and DU14) partially share STRUCTURE groupings. On the other hand, samples from some places did not group together, for Orkney (Or14, Or15), Norfolk (NO14, NO15), and East Riding Yorkshire (ERYS15, ERYS14), despite coming from the same host plant. There is no overall effect of crop plant on the genetic clustering of this pest (Fig. 3.3).

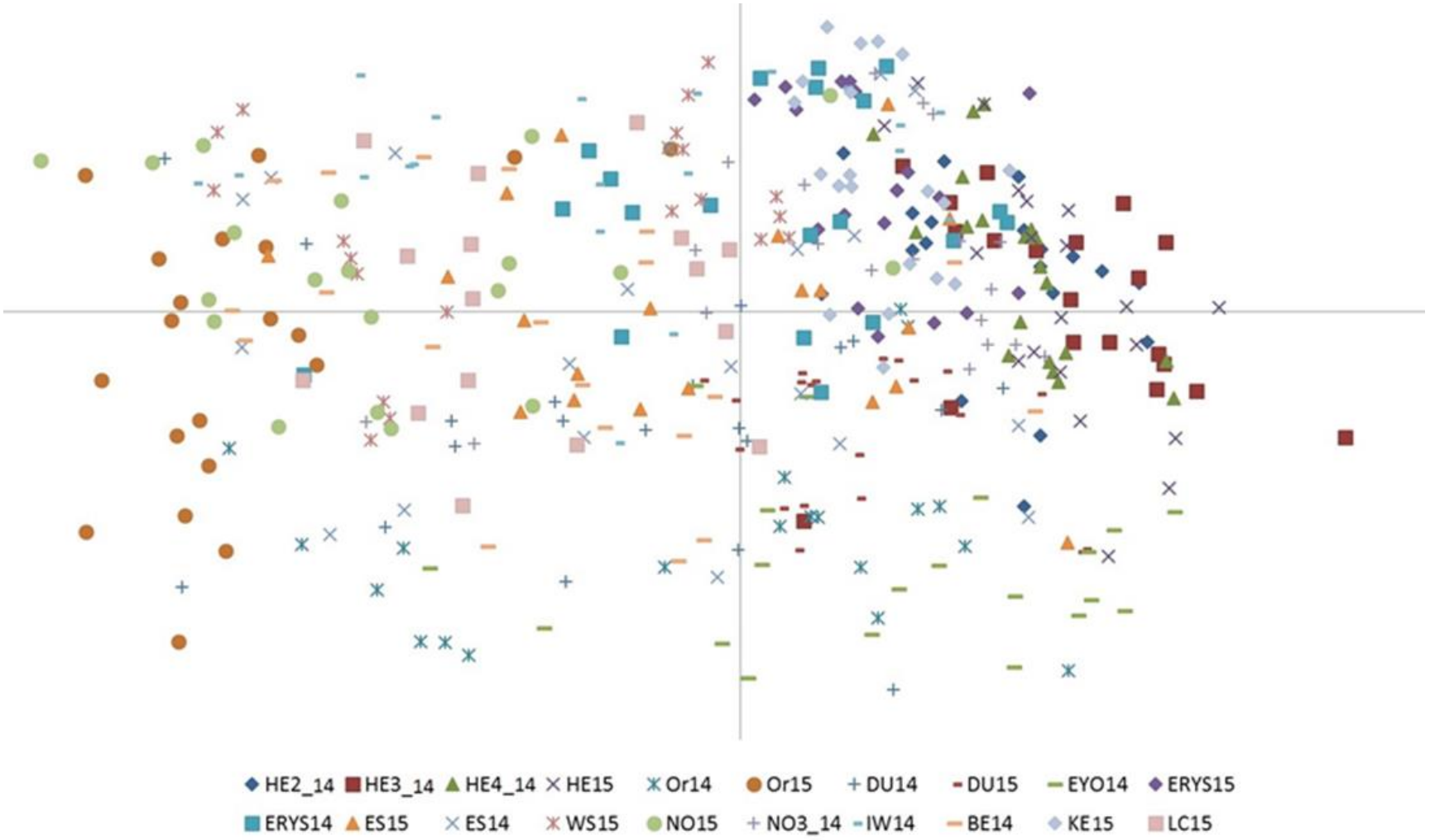


Figure 3.2. Principal coordinate analysis (PCO) of individuals from 20 UK populations of glasshouse whitefly using nine microsatellite markers. See Table 3-1 for locality codes.

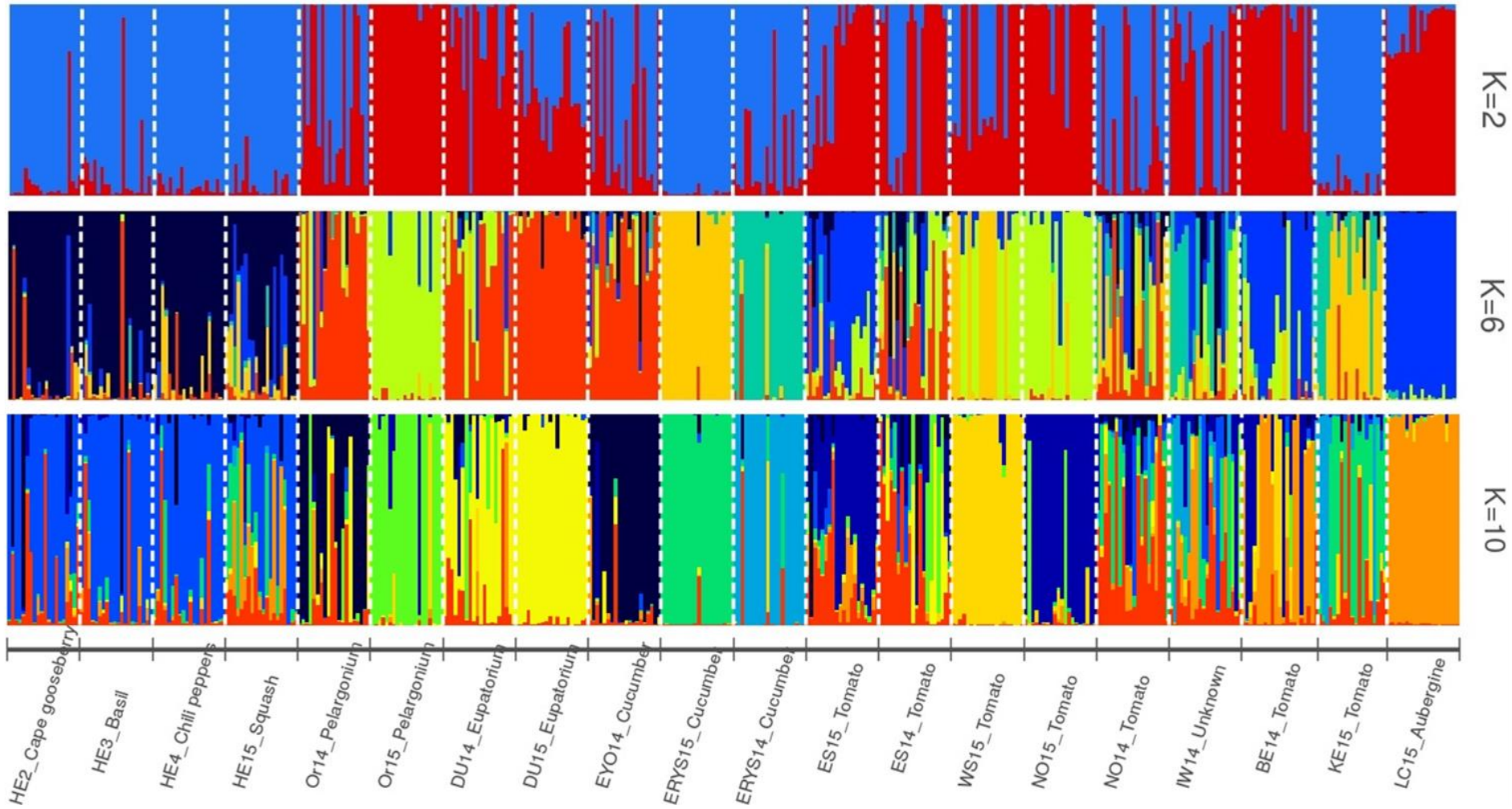


Figure 3.3. Genetic structure of 400 *T. vaporariorum* individuals (20 populations) based on nine microsatellite markers using the program STRUCTURE at K=2, 6 and 10. Each vertical bar represents the assignment of an individual. Colours indicate cluster assignment. Codes indicate location, year and host plant collections (see Table 3-1).

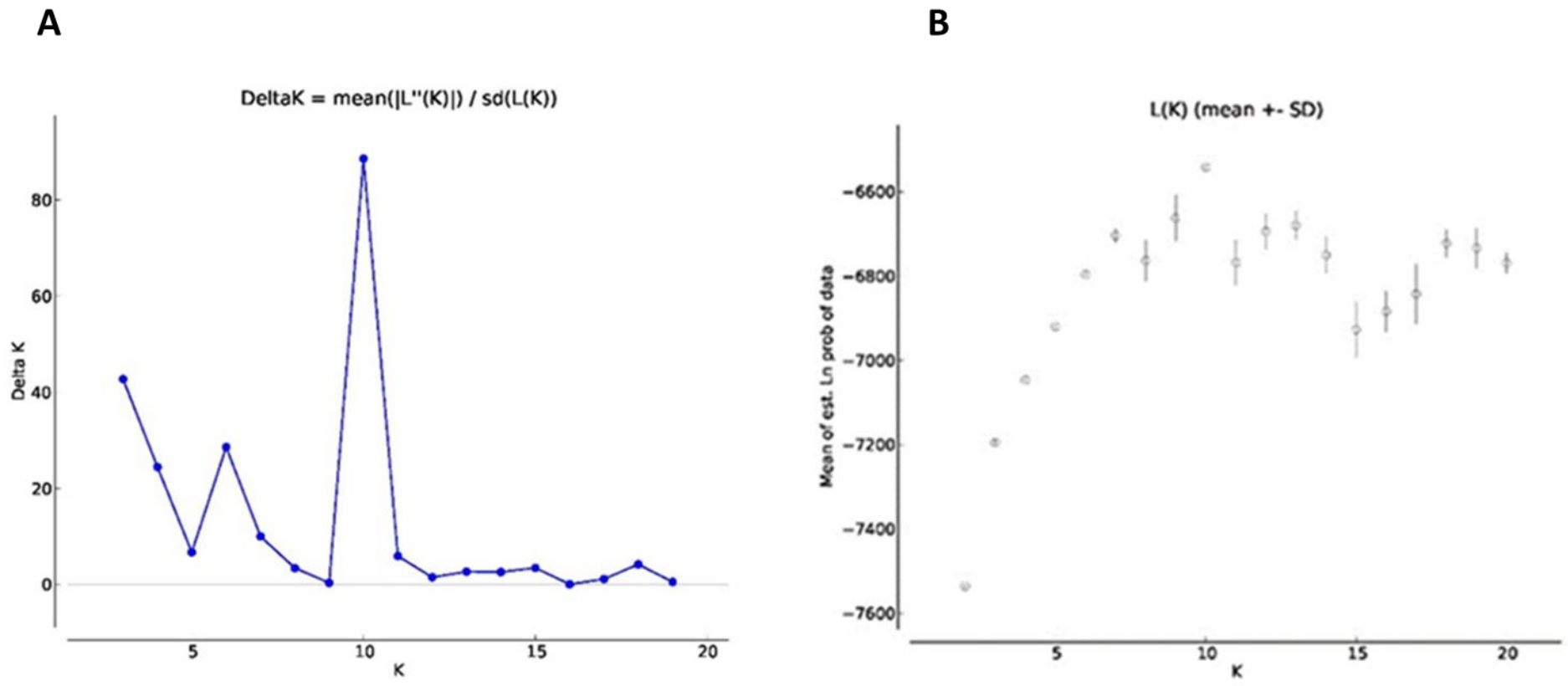


Figure 3.4. (A) Mean likelihood ΔK plotted against K to detect the number of K groups that best fit the dataset from 400 *T. vaporariorum* individuals, genotyped for nine microsatellite loci; (B) means of the estimated natural logarithm probability of the data against K.

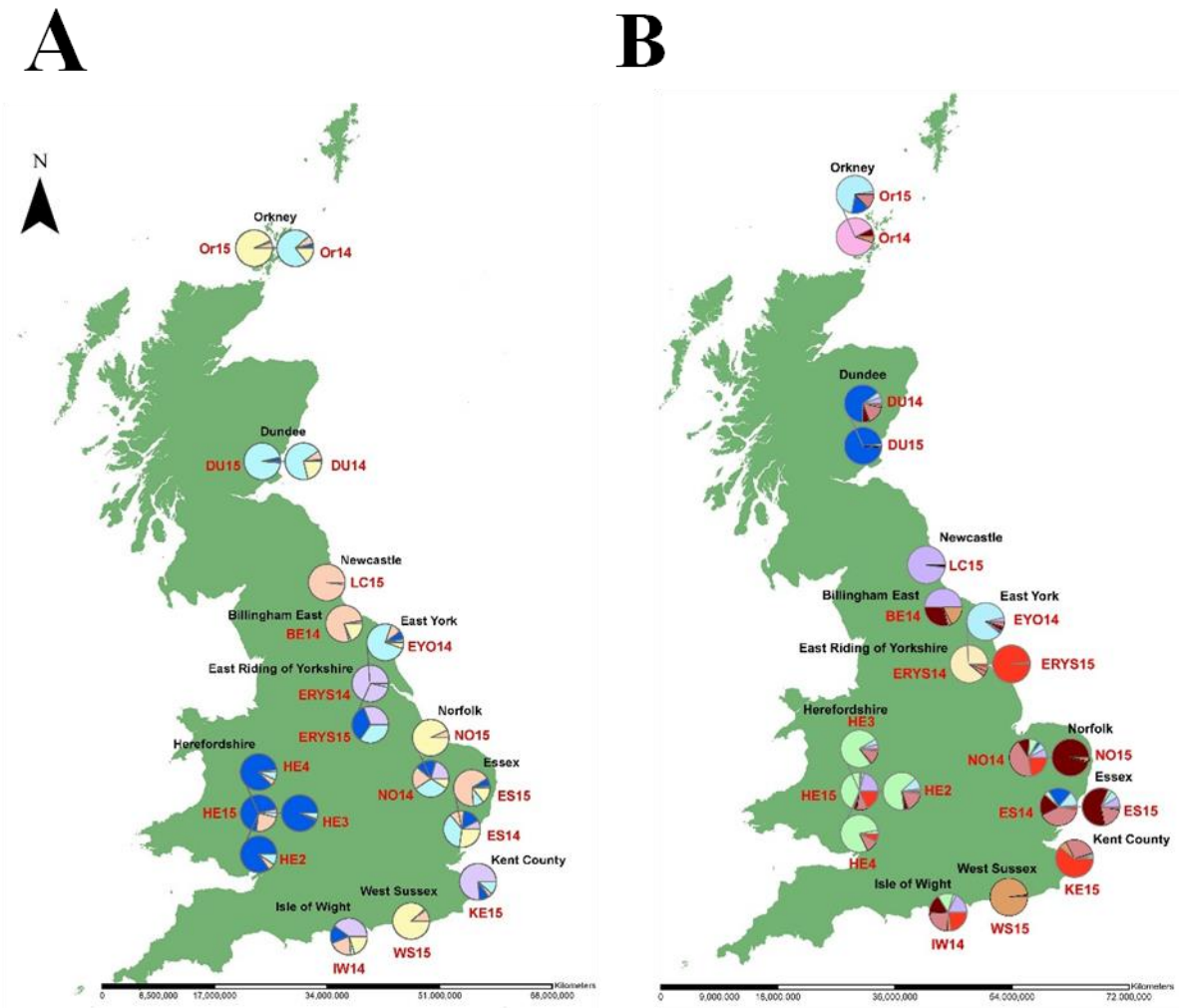


Figure 3.5. Geographical distribution and genetic structure of *T. vaporariorum* populations revealed by STRUCTURE analysis with K: 6 (A), 10 (B) (Figure 3.3). The pie chart represents proportional Q (STRUCTURE analysis) values and the codes of populations are listed in Table 3-1.

3.5 Discussion

This study presents, for the first time, extensive data on the population structure and genetic differentiation of *T. vaporariorum* in the UK, collected from 12 locations and different host plants over two years. The results show high diversity using a panel of nine microsatellite markers.

UK populations of whitefly collected from glasshouses exhibit significant genetic diversity. The mean value of H_e in UK *T. vaporariorum* populations (0.477) was similar to those in China, Greece, and Finland (0.368, 0.459 and 0.443, respectively) (Gao *et al.*, 2014; Ovcarenko *et al.*, 2014). A lower genetic diversity was indicated in the laboratory colony, which might be explained by selection and/or a prolonged bottleneck. Most of our samples were from commercial glasshouses, which are often characterised by intense management compared to field crops (Ovcarenko *et al.*, 2014). Enclosure in glasshouses affects the genetic diversity of insects due to restrictions on gene flow (Hoffmann and Willi, 2008). Crop management practices and regular population management can cause population bottlenecks, leading to potentially strong effects of random genetic drift and decreases in heterozygosity as well as increasing population genetic differentiation (Tsagkarakou *et al.*, 1998). A high level of variation was recorded among Korean *T. vaporariorum* populations using biochemical and allozyme analysis (Shin *et al.*, 2013). Therefore, the genetic clustering of *T. vaporariorum* based on microsatellites showed some structure of populations in the UK in some cases related to geography, but not related to host plant. The absence of evidence for host-plant linkage to population structure suggested that polyphagous habit of *T. vaporariorum* is very common amongst populations. Similarly, structural results based on geographical patterns were also indicated in glasshouse whitefly populations in China, Finland, and Greece (Gao *et al.*, 2014; Ovcarenko *et al.*, 2014). The results of STRUCTURE are supported by significant F_{st} values, indicating genetic differentiation between populations. In addition, in some cases STRUCTURE showed different clusters for the two years of collection from the same location. This means that some growers were able to eradicate whitefly from previous seasons, presumably through sanitising the glasshouse and plants, and new infestations were brought in with new crops. However, for other growers, whitefly populations persisted from one season to another. Thus, a combination of cleaning and checking new crops might contribute to *T. vaporariorum* management.

The genetic diversity and structure in invasive species have been studied in various insect taxa. For example, genetic diversity patterns involving multiple introductions have been demonstrated in sweet potato whitefly *B. tabaci* and thrips *Frankliniella occidentalis* (Delatte *et al.*, 2006; Cao *et al.*, 2017). For invasive species, multiple introductions are regarded as the main source of genetic variation (Reem *et al.*, 2013), which is often associated with more successful invasions (Roman and Darling, 2007; Suarez and Tsutsui, 2008). Therefore, it can be speculated that multiple introductions of glasshouse whitefly have occurred in the UK, which might have been helpful for its successful establishment and survival. This led to high diversity and significant population structure at the nuclear level. The low level of genetic variation of mtCOI (Chapter 2) could be a consequence of extensive insect control measures that include the removal of infestation sources, biological control, and the use of insecticides to reduce and/or eradicate *T. vaporariorum*. The high nuclear diversity indicates multiple introductions, combined with little gene flow between populations, which was probably due to glasshouse confinement and management. Thus, the glasshouse agroecosystem has likely contributed to the population genetic structure by restricting gene flow between locations.

3.6 Conclusion

The results support my hypothesis that multiple introductions and glasshouse habitat affect population structure of *T. vaporariorum* in the UK. Populations of *T. vaporariorum* in the UK exhibit genetic differentiation, demonstrating the possibility that multiple introductions of *T. vaporariorum* into the UK have occurred. The results showed some structure of populations, with clustering by geographical location and not by crops. The glasshouse agroecosystem and repeated imports have evidently contributed to variation at the nuclear level. The glasshouse agroecosystem has likely contributed to the population genetic structure by restricting gene flow between locations.

4 Chapter 4. Population structure of sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), in Iraq

4.1 Abstract

Whiteflies (Hemiptera: Aleyrodidae) are major pests of many crops worldwide. *Bemisia tabaci* is a cryptic species complex composed of at least 39 putative species. In Iraq, for the first time, the genetic diversity and population structure of *B. tabaci* are investigated using nuclear microsatellite markers.

Fourteen populations of *B. tabaci* were collected from glasshouse and field environments in the middle and south of Iraq. Genetic diversity was analysed using eight microsatellite markers and mtCOI sequencing (Chapter 2) to determine the genetic differentiation and structure of the Iraqi whitefly.

From analyses using STRUCTURE, the *B. tabaci* population could be grouped into two or three distinct genetic groups, whereas 7 clusters were identified using BAPS (data not shown). The genotyping results indicated low genetic clustering, which was in some case linked to location, but not host plant. Surprisingly, some clustering was linked to the time of collection. Also, the overall results suggested high levels of gene flow among populations may result in low values of F_{st}. The results provide evidence of the contemporary genetic characteristics of and differences among *B. tabaci* populations which might be useful for better understanding the population genetic and spread of this important pest. Therefore, monitoring and further sampling to cover all parts of the country are needed to confirm the population genetic structure in field and glasshouse.

4.2 Introduction

Bemisia tabaci is considered a major pest of many crops worldwide. It is suggested that this insect has spread across the world through the international trade in plants or via infected products (De Barro *et al.*, 2011). The species has been reported on all continents excluding Antarctica. *B. tabaci* is a polyphagous pest which transmits more than 100 plant virus species (Polston and Capobianco, 2013; CABI, 2018a). It causes severe damage and economic losses directly through feeding and indirectly through the transmission and spread of plant viruses such as the *Begomovirus* genus in the *Geminiviridae* family (Czosnek *et al.*, 1990; De Barro *et al.*, 2011). For example, *B. tabaci* has caused considerable damage to field and greenhouse crops in the Middle East region, including in Iraq (Ahmed *et al.*, 2011; Al-ani *et al.*, 2011).

Plant viruses belonging to the *Begomovirus* genus are transmitted by *B. tabaci* from infected to healthy plants. However, the viruses are not passed on from one generation of insects to the next. For example, the tomato yellow leaf curl virus (TYLCV) (genus *Begomovirus*) severely reduces yields in the production of tomatoes as well as other vegetables in both the greenhouse and outdoor environments in Iraq (Al-Fadhal, 2012). TYLCV can cause economic losses of between 50-90%, especially when plants are infected in the early stages of growth (Al-ani *et al.*, 2011). Recently, new strains of TYLCV have been recorded in Iraq carried by *B. tabaci* (Al-Abedy *et al.*, 2018), and these have been deposited in GenBank at the US National Center for Biotechnology Information (NCBI).

4.2.1 Genetic diversity of Bemisia tabaci

Currently, *B. tabaci* is considered to be a complex of eleven major genetic groups which together comprise at least 39 putative species. These genetic groups have been defined as being separated by a minimum of 3.5% or 4% mitochondrial cytochrome c oxidase I gene (mtCOI) nucleotide divergence (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011; Lee *et al.*, 2013; Boykin and De Barro, 2014; Alemandri *et al.*, 2015). The genetic differences among *B. tabaci* species might include the ability of some of them to develop insecticide resistance, the capacity to transmit various plant viruses and host plant range. Therefore, it is essential to understand which species are present to develop effective control measures (De Barro *et al.*, 2011; Ahmed *et al.*, 2012). The most

common species of whitefly is MEAM1, which is listed as the most invasive species in the world by the International Union for the Conservation of Nature and Natural Resources (IUCN) and the Invasive Species Specialist Group (ISSG, 2017). The MEAM1 genetic group was first reported from Middle East countries such as Iran, Israel, Jordan and Yemen in the 1990s (CABI, 2018a). The use of molecular markers for *B. tabaci* is a valuable approach to highlight genetic variation within morphologically similar biotypes/haplotypes (Firdaus *et al.*, 2013; Wang *et al.*, 2014; Hadjistylli *et al.*, 2015). As a consequence of the status of *B. tabaci*, the regular monitoring of its species is needed to understand new invasive populations.

4.2.2 Population genetic structure of B. tabaci

Microsatellite markers have been isolated from the species complex *B. tabaci* by De Barro *et al.* (2003), Tsagkarakou and Roditakis (2003) and Dalmon *et al.* (2008). These markers have been used to study population genetic variations in *B. tabaci* biotypes (De Barro, 2005; Do Valle *et al.*, 2011; Hsieh *et al.*, 2011; Tsagkarakou *et al.*, 2012; Dickey *et al.*, 2013; Hadjistylli *et al.*, 2015). Furthermore, the molecular markers can be used to recognise hybridisation among sympatric invasive and indigenous biotypes in a region (Delatte *et al.*, 2006). Additionally, the capacity of *B. tabaci* biotypes to resist different insecticides (Gauthier *et al.*, 2014), their endosymbiont composition (Gnankine *et al.*, 2013; Gauthier *et al.*, 2014), and the sources and routes of the spreading of non-native biotypes in new areas (Hadjistylli *et al.*, 2015) can be identified using microsatellite markers.

Whiteflies are arrhenotokous parthenogenetic (haplodiploid) species in which non-fertilised eggs grow into males and fertilised eggs into females (Hoy, 2003). The haplodiploid state means that female whitefly has the complete number of chromosomes (22), while the male has only half that number (Mittler, 1946). The haplodiploidy could potentially decrease the genetic diversity. The reasons could include: increased selection against slightly deleterious alleles, as all alleles are showing in the hemizygous haploid males (Crozier, 1970); balanced polymorphism can be difficult to gain in sex-linked genetic systems (Menken, 1991); reduced effective population sizes which increase the effects of genetic drift and therefore decrease genetic diversity (Lester and Selander, 1979; Owen, 1985).

The present study is the first to investigate the population structure of *B. tabaci* in Iraq. The objective is to establish if habitat and other agricultural factors structure the populations found. I hypothesised that geographical factors, host plants, and possibly the growing season of crops may play a role in the genetic variation of *B. tabaci* populations in Iraq. The outcome will contribute to our understanding of gene flow and patterns of population genetic structure in this species.

4.3 Materials and Methods

4.3.1 Field sampling

More than 30 adults of *B. tabaci* were collected from each of 14 populations with different host plants, such as tomato, cucumber, pepper, and eggplant, grown in plastic tunnel greenhouses at eight locations from the middle and south of Iraq during summer 2015 and autumn 2016 (Table 4-1). Male and female adult whitefly specimens were collected from whitefly-colonised plants at each location and stored in 95% ethanol at −20 °C until DNA extraction.

4.3.2 Confirming the identity of specimens morphologically

The techniques used to identify *B. tabaci* morphologically and molecularly are described in section 2.3.2 **(Chapter 2)**.

4.3.3 Microsatellite genotyping

Total genomic DNA (gDNA) of 20 adult females was extracted as described in Tsagkarakou *et al.* (2007). A total of 280 individuals from 14 populations of the 2015 and 2016 collections (20 individuals per population) were genotyped. Thirteen microsatellite primer pairs were tested. Eight of them, BT-4(FAM), BT-83(HEX), BT-t19(FAM), BEM11(FAM), BT-b34(FAM), BEM25(HEX), BT-b159(HEX), and BT-b69(FAM) (FAM and HEX are the fluorescent dyes coupled to the primers) were used as described in De Barro *et al.* (2003), Tsagkarakou and Roditakis (2003) and Tsagkarakou *et al.* (2007) (Table 4-2). The PCR amplification was performed in 10 µl containing 5 ng DNA, 2 µl of 5× PCR reaction buffer, 0.2–0.4 µM of each primer, and 0.5 units of MyTaq DNA polymerase (Bioline). PCR reactions were run in conditions of initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The PCR products were separated on a 3130XL Genetic Analyzer, and the allele size was determined using the GeneScan™ ROX500 size

standard, using GeneMapper software version 3.2 (Applied Biosystems), and checked manually.

Table 4-1**.** Collection sites, population codes, dates of collection, host plants, and genetic diversity indices for the sweet potato whitefly *B. tabaci* populations from Iraq examined in this study. The following genetic diversity indices are indicated: average number of alleles per locus (N_a), the effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A_r), fixation index (F; $F = [H_e - H_o] / H_e = 1 - [H_o / H_e]$), and the probability of identity (*PI*).

Locality	Code	Date	Host	Latitude (°N)	Longitude (°E)	N_a	A_e	H_o	H_e	A_r	F	PI
Basra	BAS-Tom-15	2015	Tomato	29.975	48.474	2.000	1.483	0.288	0.266	2.000	-0.032	1.0E-02
Hillah	HI-Tom-15	2015	Tomato	32.406	44.405	2.375	1.672	0.350	0.325	2.375	-0.049	2.5E-03
Karbala 1	KA1-Tom-15	2015	Tomato	32.676	44.164	2.125	1.451	0.219	0.271	2.125	0.143	1.0E-02
Karbala 2	KA2-Pep-15	2015	pepper	32.676	44.164	2.375	1.480	0.275	0.278	2.375	0.131	6.9E-03
Karbala 3	KA3-Tom-16	2016	Tomato	32.512	44.052	2.000	1.424	0.194	0.246	2.000	0.187	1.4E-02
Kufa	KU-Tom-16	2016	Tomato	32.108	44.392	2.500	1.697	0.244	0.338	2.500	0.194	2.1E-03
Musayib	MO-Tom-16	2016	Tomato	32.778	44.290	2.250	1.578	0.300	0.319	2.250	0.023	3.5E-03
Muthanna1	MU1-Tom-16	2016	Tomato	31.533	45.200	2.250	1.655	0.231	0.302	2.250	0.181	5.1E-03
Muthanna2	MU2-Tom-15	2015	Cucumber	31.533	45.200	2.125	1.637	0.331	0.275	2.125	-0.126	6.3E-03
Muthanna3	MU3-Aln-Tom-16	2016	Tomato	31.666	45.183	2.125	1.459	0.156	0.257	2.125	0.293	1.1E-02
Muthanna4	MU4-SA-Cum-16	2016	Cucumber	31.483	45.166	2.750	1.780	0.375	0.353	2.750	-0.104	1.4E-03
Najaf 1	NA1-Tom-15	2015	Tomato	32.019	44.338	2.625	1.583	0.313	0.307	2.625	-0.019	4.3E-03
Najaf 2	NA2-Pep-15	2015	pepper	32.019	44.338	2.125	1.557	0.181	0.249	2.125	0.107	1.0E-02
Najaf4	NA4-Eggp-16	2016	Eggplant	32.019	44.338	2.125	1.285	0.194	0.196	2.125	-0.008	3.2E-02
Mean						2.268	1.553	0.261	0.284	2.268	0.066	

Table 4-2. Characteristics of 8 forward and reverse microsatellite markers in *B. tabaci*. Codes of loci, dye, number of alleles, annealing temperature (Ann), and expected size range are shown.

Locus (Genbank Accession No.)	Primer (5'–3') (F: [dye]-forward; R: reverse)	No. of alleles	Ann (°C)	Size range (bp)	References
BT-4 (AY183673)	F: [FAM]-GAGATCATATCCCCATTGTTTC R: ATCACGGGTCATAGATCACG	3	55	280-297	(Tsagkarakou and Roditakis, 2003)
BT-83 (AY183674)	F: [HEX]-GATGCCACAGGTTGTCTGG R: GCTTGCCAGGCACTTTCTAG	3	55	132-145	(Tsagkarakou and Roditakis, 2003)
BT-t19 (DQ365854)	F: [FAM]-AGG TAT TGC TGC AAG GAA AG R: AAATACAGGCGACTCATGTC	2	55	171-173	(Tsagkarakou *et al.*, 2007)
BEM11 (AY145453)	F: [FAM]-TTCAATGATGCTTTCCTGAC R: CAAATAAATACACCATTTACA	2	55	195-202	(De Barro *et al.*, 2003)
BT-b34 (AY183675)	F: [FAM]-AAATTAACTGCCGCTCAACG R: ATATCGATACAATCTTACCCG	2	55	286-288	(Tsagkarakou and Roditakis, 2003)
BEM25 (AY145462)	F: [HEX]-AAGTATCAACAAATTAATCGTG R: TGAAGAATAAGAATAAAGAAGG	1	55	98	(De Barro *et al.*, 2003)
BT-b159 (AY183681)	F: [HEX]-ACTCCATTTGGCTTATGTGC R: ATTATCGTCTGAAAACTGGTGG	2	55	268-285	(Tsagkarakou and Roditakis, 2003)
BT-b69 (AY183678)	F: [FAM]-ATTCGGTTCGTCTTAGGGAC R: ACGATGTTTCCAAACTGAGC	2	55	160-166	(Tsagkarakou and Roditakis, 2003)

4.3.4 Data analysis of population genetic structure and genetic diversity

For each of the 14 populations of *B. tabaci*, the average number of alleles per locus (N_a), effective number of alleles (A_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). The differences in H_e between *B. tabaci* populations were analysed using the Fisher LSD Test in Minitab® v17 software (2013 Minitab Inc.).The program FSTAT 2.9.3.2 was used to calculate allelic richness, A_r (Franks *et al.*, 2011). The population differentiation approach was based on F_{st} values. Weir and Cockerham's (1984) estimator of the fixation index F_{st} was calculated using GENEPOP v.3.4 (Raymond and Rousset, 1995). The correlation between genetic differentiation and geographic distance (km) was examined via a Mantel test using GenAlEx v. 6.5. Isolation by distance (IBD) was defined by pairwise linear F_{st} / (1 - F_{st}) values, and geographic distance was defined as pairwise distances generated from geographical coordinates. The distribution of genetic variation was investigated by performing an analysis of molecular variance (AMOVA), and principal coordinates analysis (PCO) was carried out using GenAlEx v. 6.5.

The genetic clustering of samples was examined using STRUCTURE v.2.3.2 (Pritchard et al., 2000) with a burn-in of 150,000 iterations and 500,000 Markov chain Monte Carlo (MCMC) repetitions under the no-admixture ancestry model and using prior allele frequency information. Eighteen independent runs were performed for each K value, ranging from K = 1 to 20, and ΔK was used to calculate the optimal number of genetic clusters (K) (Evanno *et al.*, 2005) using Structure Harvester (Earl and Vonholdt, 2012). The results were combined and visualised using online POPHELPER software (Francis, 2017). Further structural analysis was conducted based on the initial results. Firstly, three populations from Karbala and Najaf (KA1-Tom_15, KA2-Pepp_15, and NA1-Tom_15) were analysed together. Secondly, 11 populations were analysed together using the same STRUCTURE parameters as above. In addition, an alternative analysis was done using Bayesian analysis of population structure (BAPS) software v.6 (Corander *et al.*, 2003; Corander *et al.*, 2008). The initial values of allele frequencies for population analyses, 100 iterations to estimate the admixture coefficients for the individuals tested, 50 iterations to estimate the admixture coefficients for the reference individuals and 200 reference individuals from each population were used (see BAPS Manual at http://web.abo.fi/fak/mnf/mate/jc/software/BAPS5manual.pdf).

Potential clonal reproduction was determined for all populations, and individuals with matching multilocus genotypes (MLG) within populations, using the 'Find Clones' function in GenAlEx v. 6.5. The probability of identity (*PI*), that is, the average probability of two distinct individuals within a randomly mating population sharing the same MLG by chance, was estimated. The values were used to assess the power of microsatellite markers, where lower values are more supportive of using the markers (Waits *et al.*, 2001; Peakall and Smouse, 2012).

4.4 Results

4.4.1 Genetic diversity

All populations showed low genetic diversity (Table 4-1). The average number of alleles per locus (N_a) ranged from 2 (Karbala, KA3-Tom_16 and Basra, BAS-Tom_15) to 2.75 (Muthanna, MU4-SA- Cucum_16), and the effective number of alleles (A_e) ranged from 1.28 (Najaf, NA4-Eggp_16) to 1.78 (Muthanna, MU4-SA- Cucum_16). Observed heterozygosity (H_o) ranged from 0.156 (Muthanna, MU3-Aln-Tom_16) to 0.37 (Muthanna, MU4-SA- Cucum_16), while expected heterozygosity (H_e) ranged from 0.196 (Najaf, NA4_Eggp-16) to 0.353 (Muthanna, MU4-SA-Cucum_16). The population from Muthanna (MU4-SA- Cucum_16) had the highest values of H_e and H_o at 0.37 and 0.35, while the populations from Najaf city (NA4-Eggp_16) and Muthanna (MU3-Aln-Tom_16) had the lowest values at 0.196 and 0.156 respectively. There was no significant ($P < 0.05$) difference in expected heterozygosity (H_e) indicated between all populations of *B. tabaci*.

Regarding genetic distance between populations, 80.3% of pairwise F_{st} comparisons (73 out of 91) showed significant population differentiation amongst samples (Table 4-3). Only geographically close populations or those from the same location and year did, in some cases, not exhibit significant differentiation; for example, samples from Karbala (KA1-Tom_15/KA2-Pep_15), and Muthanna (MU2- Cucum_15/MU1-Tom_15, and MU4_SA- Cucum_16). Estimates of pairwise $F_{st} / (1 - F_{st})$ values ranged from 0.007 (MU4_SA- Cucum_16/ MU3_Aln-Tom_16) to 0.28 (KA3-Tom_16/ NA1-Tom_15). There was no evidence of isolation by distance (IBD), based on the Mantel test for correlation between pairwise F_{st} and geographic distance ($R^2 = 0.002$, P<0.4) (Fig. 4.1). The AMOVA revealed that genetic differentiation between populations explained 13% of the variation, while the remainder (87%) was within populations (Table 4-4).

4.4.2 Microsatellite genotyping

The values of probability of identity (*PI*) for individuals within populations were low (*PI* <0.001. Table 4-1), except for individuals from Najaf (NA4-Eggp_16) (*PI* >0.001). The result means that using eight microsatellite markers is sufficient to identify identical individuals across populations. Forty-two matching multilocus genotypes (clonal) and 177 unique ones were observed across all populations. The PCO approach showed no clear grouping of individuals by geographical location of population, with 51.01% of total variation explained by the first two axes (21.25% and 36.92 % respectively) (data not shown).

The results show low genetic differentiation between the *B. tabaci* populations (Table 4-3). The clustering method implemented in STRUCTURE suggested weak groupings of *B. tabaci* individuals, with low genetic clusters indicated by the value of Delta K (ΔK) against K (Fig. 4.2), when the structural analyses were examined. K = 2 revealed a cluster of populations KA1-Tom_15, KA2-Pep_15, and NA1-Tom_15 from Karbala and Najaf, which were collected in the same year, whereas the rest of the populations could be partially grouped (Fig. 4.3). K = 3 might be giving more geographic patterns (Fig. 4.3). In some cases, samples from the same location were grouped together, while in other cases they were not. For example, samples from Karbala and Najaf (KA1-Tom_15, KA2-Pep_15, and NA1-Tom_15) were grouped together. Also, samples collected from the south of Muthanna (MU1-Tom_15, MU2- Cucum_15), Hilla (HI-Tom_15) and Basra (BAS-Tom_15) from the same year were partially grouped together. On the other hand, samples from the same place but in different years were not grouped together in Najaf (NA1-Tom_15, NA2-Pep_15, and NA4-Eggp_16) or Karbala (KA1-Tom_15, KA2-Pep_15, and KA3-Tom_16). There is no overall effect of crop plant on the genetic clustering of this pest for both potential genetic clusters (Fig. 4.2).

Further STRUCTURE analysis was conducted separately to detect substructures within the two K2 clusters. No genetic differentiation was found in the three populations (KA1_Tom, KA2_Pep, and NA1-Tom_15) which were collected in 2015 from the two cities of Karbala and Najaf. On the other hand, the analysis of 11 *B. tabaci* populations from both years of the study partially grouped separately (Fig. 4.4 A, B). Samples of NA2-Pap_15, MU1-Tom_15, MU2-Cucum_15, HI-Tom_15, and BAS-Tom_15, which were collected in 2015 from Najaf, Muthanna, Hillah, and Basra, were partially grouped, whereas samples of NA4-Eggp_16, KA3-Tom_16, MU3_Aln-Tom_16, MU4_SA-Cucum_16, MO-Tom_16, and KU-Tom_16, which were collected in 2016

from Najaf, Karbala, Muthanna, Mosayib, and Kufa, were also partially clustered together. Again there was no effect of host plant on the genetic grouping of the populations.

However, the results using the BAPS programme told a different story. Seven genetic clusters have been indicated by BAPS, in some cases linked with the geographical location of the population (data not shown because the STRUCTURE programme is more recommended and reliable for analysis of population structure).

Table 4-3. Pairwise estimates of genetic distance F_{st} / (1 – F_{st}) values between 14 *B. tabaci* populations using the eight microsatellite loci. Codes of populations shown and significant values at P<0.05 are in **bold**.

POP	KA1	KA2	NA1	NA2	MU1	MU2	HI	BAS	NA4	KA3	MU3_Aln	MU4_SA	MO
KA2	0.023												
NA1	**0.008**	**0.009**											
NA2	**0.005**	**0.037**	**0.036**										
MU1	**0.158**	**0.148**	**0.211**	**0.123**									
MU2	**0.079**	**0.127**	**0.15**	0.064	0.029								
HI	**0.133**	**0.167**	**0.19**	**0.154**	**0.1**	**0.069**							
BAS	**0.19**	**0.196**	**0.24**	**0.168**	**0.055**	**0.075**	**0.017**						
NA4	**0.109**	**0.088**	**0.148**	**0.148**	**0.075**	**0.087**	**0.065**	**0.099**					
KA3	**0.268**	**0.191**	**0.28**	**0.25**	**0.186**	**0.241**	**0.147**	**0.106**	**0.156**				
MU3_Aln	**0.082**	**0.124**	**0.131**	**0.175**	**0.195**	**0.106**	**0.074**	**0.173**	0.042	**0.298**			
MU4_SA	**0.016**	**0.029**	**0.043**	0.064	**0.127**	0.071	**0.069**	**0.134**	**0.031**	**0.204**	0.007		
MO	**0.016**	**0.008**	**0.015**	0.015	**0.139**	0.087	**0.152**	**0.194**	**0.087**	**0.26**	0.01	0.02	
KU	**0.06**	**0.1**	**0.19**	0.073	0.031	0.012	0.037	**0.066**	**0.027**	**0.213**	0.026	0.017	0.057

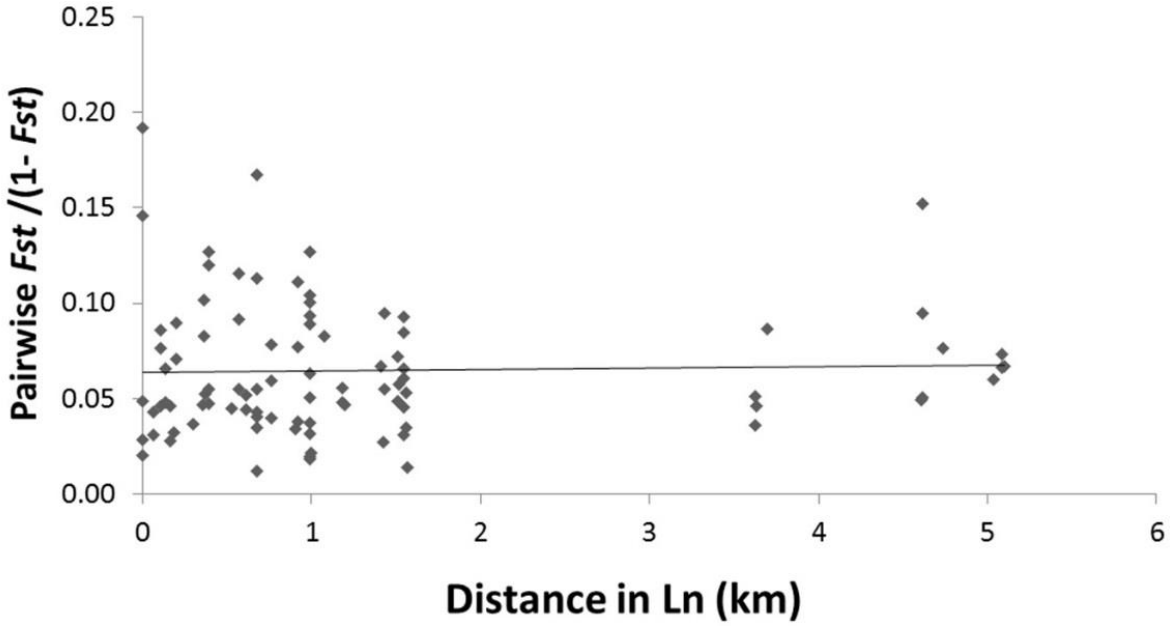


Figure 4.1**.** Correlation between genetic distance (based on F_{st} / (1 – F_{st}) and log (ln) geographic distance (based on pairwise distance in km) of *B. tabaci*. R^2 = 0.002, not significant.

Table 4-4**.** AMOVA analysis using eight microsatellite loci of 14 *B. tabaci* populations. A value of F_{st} / (1 – F_{st}) of 0.126 was calculated between populations (P<0.001).

Source of variation	d. f.	Sum of squares	Variance components	Est. Var.	Percentage of variation
Among populations	13	102.538	7.888	0.168	13%
Within populations	546	637.125	1.167	1.167	87%
Total	559	739.663	9.055	1.335	

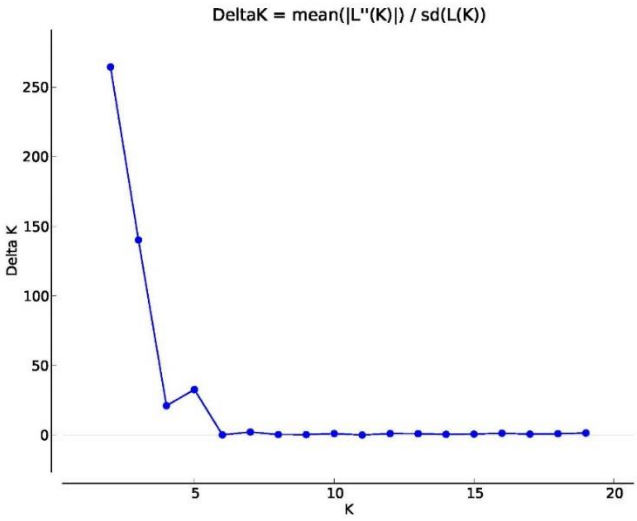


Figure 4.2. Mean likelihood ΔK plotted against K to detect the number of K groups that best fit the dataset from 280 *B. tabaci* individuals (14 populations) genotyped for eight microsatellite loci.

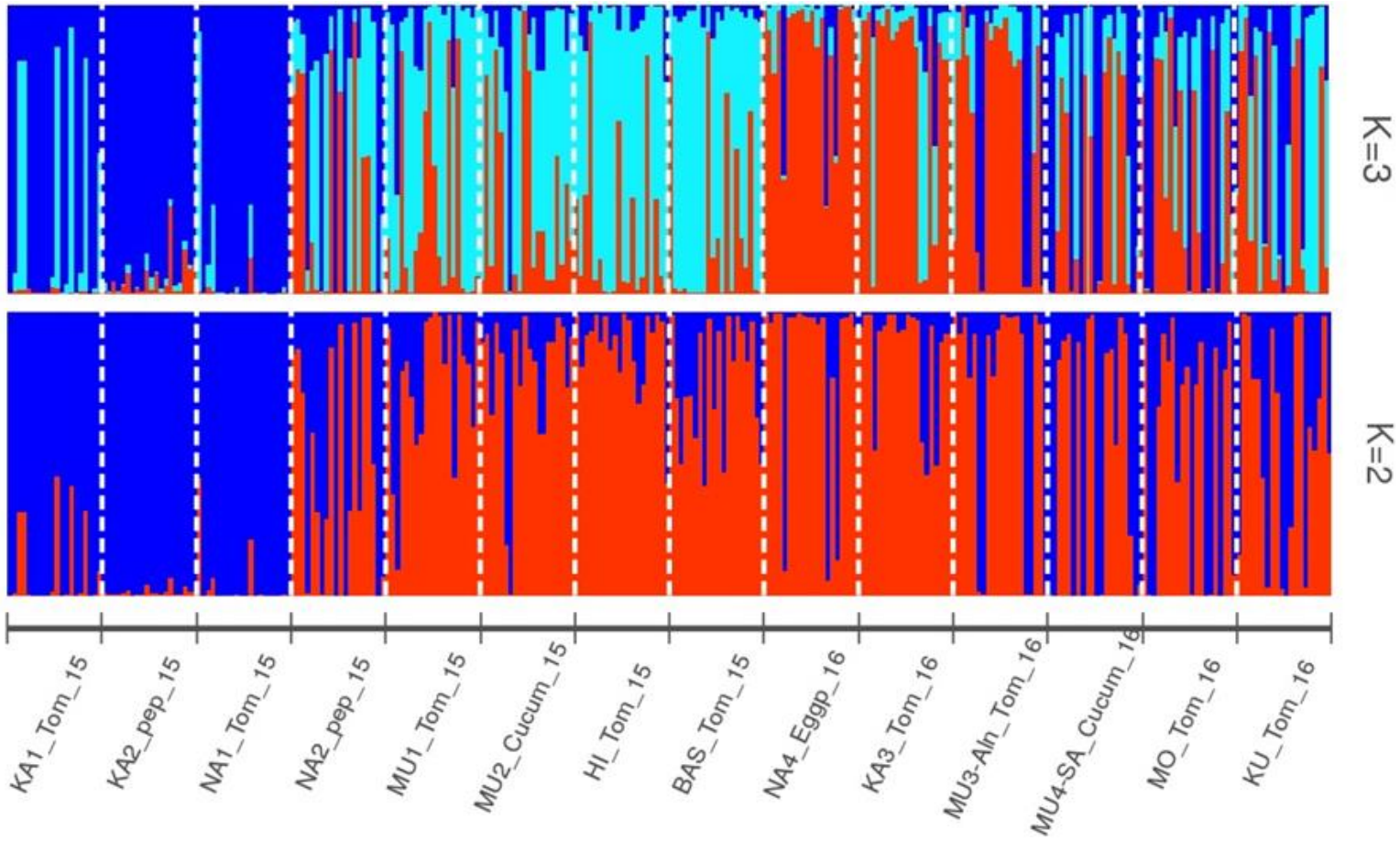


Figure 4.3**.** Genetic structure of 280 *B. tabaci* individuals (14 populations) based on eight microsatellite markers using the program STRUCTURE at K=2, and 3. Each vertical bar represents the assignment of an individual. Colours indicate cluster assignment. Codes indicate location, year and host plant collections (see Table 4-1).

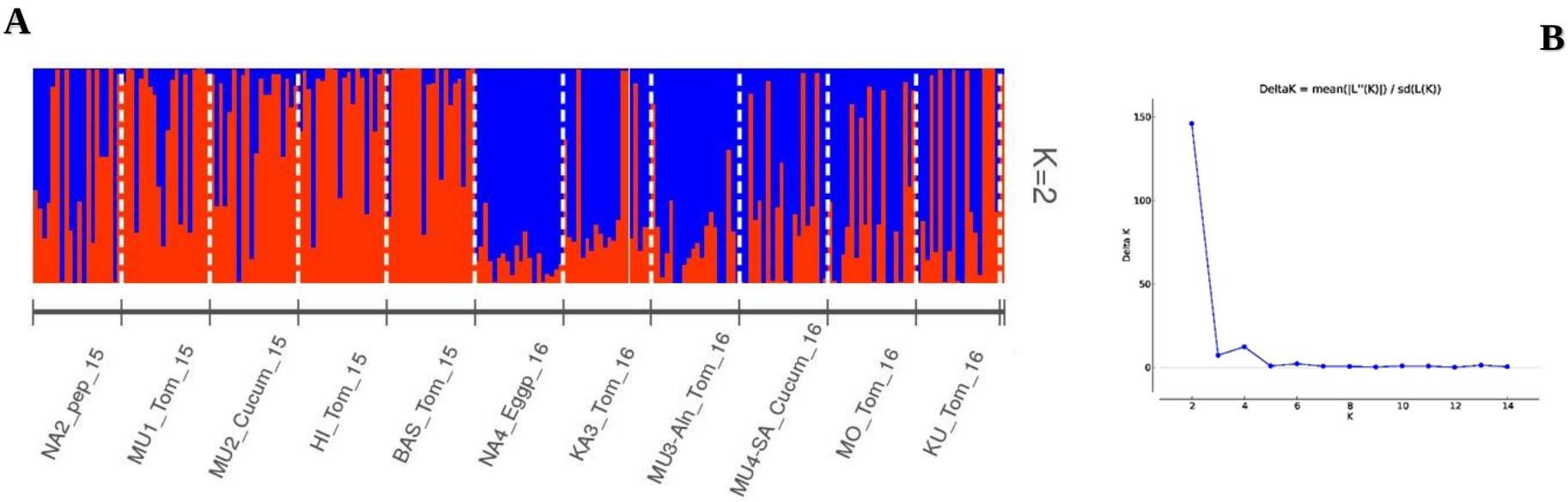


Figure 4.4**.** (A) Genetic structure of 220 *B. tabaci* individuals (11 populations) based on eight microsatellite markers using the program STRUCTURE at K=2. Each vertical bar represents the assignment of an individual. Colours indicate cluster assignment. Codes indicate location, year and host plant collections (see Table 4-1). (B) Mean likelihood ΔK plotted against K to detect the number of K groups that best fit the dataset.

4.5 Discussion

This study has reported, for the first time, extensive data on the genetic diversity and population structure of *B. tabaci* collected from the middle and south of Iraq. Altogether 14 populations were collected from eight locations and different host plants, and *B. tabaci* biotype identity, genetic diversity, and population structure were assessed.

Low genetic differentiation between the *B. tabaci* populations was observed using the microsatellite loci. The mean value of H_e in Iraqi *B. tabaci* populations (0.284) was close to those values in the US collected from field and glasshouse populations of 0.30 and 0.33, respectively (Dickey *et al.*, 2013). Also, low levels of variation and values of H_e in relevant biotype B have been reported in some populations collected from Cyprus and Egypt (Hadjistylli *et al.*, 2016). However, higher values of H_e in *B. tabaci* biotype B have been recorded, at 0.462 and 0.624, in Israel and Yemen, respectively (Hadjistylli *et al.*, 2016). The low level of differentiation among Iraqi *B. tabaci* populations could be a result of extensive control and management, including the removal of infestation sources and the use of extra insecticides to decrease population levels of this insect and might be consequently more selective on certain genotypes. Also, haplodiploidy could affect the population size which consequently increases the effects of genetic drift and then decreases the genetic differentiation (Owen, 1985). Other explanations for the low genetic variation found among *B. tabaci* populations could be insufficiently powerful microsatellite markers, mutation, and dispersal of insects from distribution centre to different growing locations. The present results are consistent with those of Dickey *et al.* (2013), who found the *B. tabaci* MEAM1 biotype showed lower population differentiation (lower H_e values) than the Mediterranean biotype across the USA.

The results of the *B. tabaci* population analysis, based on eight microsatellite loci, indicated a low level of variation. *B. tabaci* populations showed some possible genetic clusters based on the STRUCTURE analysis linked in some cases to a location, not to the host plant. Surprisingly, the result of further structure analysis exhibited some population substructure based on year of collection. However, seven possible genetic clusters were shown by BAPS analysis, which was linked to location but not host plant (data not shown). The difference in the clustering results between analysis using STRUCTURE and BAPS might be due to the underlying model of admixture applied by each software suite. Both population genetic clustering analyses showed that Iraqi *B. tabaci* populations exhibit genetic differentiation, which might suggest that there have been multiple exchanges of *B. tabaci* biotypes between Iraq and its neighbouring

countries since the *B. tabaci* biotypes were reported in the last century in some Middle East regions (CABI, 2018a). However, it is possible that *B. tabaci* has a longer history in Iraq; it has been estimated that divergence of the most invasive *B. tabaci* B (MEAM1) and Q (MED) biotypes in Africa, the Middle East, and the Mediterranean took place during the Bronze and Iron Ages and the Roman period (Hadjistylli, 2010). Similar results using STRUCTURE for the population structure of the invasive *B. tabaci* (MEAM1) biotype in the United States were found by Dickey *et al.* (2013), who reported low genetic differentiation and substructure among the MEAM1 biotype B, except in two populations from Florida. However, twenty-eight genetic groups and population substructure of *B. tabaci* have been identified using BAPS software (Hadjistylli *et al.*, 2016). Additionally, our results were similar to those of Hadjistylli (2010), who found that *B. tabaci* biotype B had lower population differentiation than biotype Q in samples from five countries, including the USA, where it was suggested that sampling and choice of microsatellite markers might have influenced the results. It has been suggested that biotype B, which has been introduced into most regions worldwide since the 1980s, is much more genetically homogeneous than biotype Q, which is likely a result of its global invasion. High levels of identical genotypes (clones) were observed, which is probably one reason for low genetic differentiation (F_{st}) among populations. A second reason could be the movement on plants of the eggs, nymphs or adults of the insect between sites via distribution centres in Iraq. A third reason could be an increase in resistant genotypes of Iraqi whitefly as a result of intensive pesticide applications.

Invasion patterns involving multiple introductions have been demonstrated in many invasive species, including mosquitofish *Gambusia* spp. and the caprellid *Caprella scaura* (Sanz *et al.*, 2013; Cabezas *et al.*, 2014). Multiple introductions are regarded as the primary source of genetic variation (Reem *et al.*, 2013), which is often associated with successful invasions (Roman and Darling, 2007; Suarez and Tsutsui, 2008). Multiple introductions of *B. tabaci* B biotype from the same source have probably occurred in some Middle East countries, including Iraq, via international trade, which might have helped its successful invasion (CABI, 2018a). The low level of variation across populations could be a result of the high levels of clonal individuals observed between and within the population, which might occur by chance or via the movement of whiteflies. Growers in most areas of the study use continuous planting and do not

have a break time during the year. This means that a tunnel plastic greenhouse could be used to grow different crops with different suitability for whitefly. Taken together the present and DNA barcoding (Chapter 2) results might improve the management of *B. tabaci* populations as genetically different populations might have inherently diverse characteristics linked to invasiveness, adaptation, and insecticide resistance compared to genetically homogeneous populations. The finding might help improve our understanding of the biology, ecology, and spread of damaging *B. tabaci* biotypes in Iraq. Further populations of *B. tabaci* should be sampled to confirm the population structure and to monitor new biotypes. The findings, in turn, could help improve management of this insect in Iraq by showing the benefits of removing infected plants, reducing use of insecticides, and also making a break time between growing seasons to prevent infection by whitefly.

4.6 Conclusion

The genotyping of *B. tabaci* populations showed limited genetic structure of populations in the cities of Karbala and Najaf, but otherwise no geographical patterns. There was no host plant effect and there were no patterns associated with growing season, but there were differences between years of study. These results do not confirm my hypothesis. More information on the populations of *B. tabaci*, gained from a combination of genetic characterisation and biological and ecological research, might help to develop management of *B. tabaci*. The result suggested that keeping plastic greenhouses clean from infected plants and also making gap time seasonally are important to prevent new infections by whitefly. Further study is needed to confirm the population structure and keep monitoring new biotypes and their capacity to transmit plant viruses.

5 Chapter 5. Diversity and molecular identification of endosymbionts of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*

5.1 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 different countries, but no study has yet 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 may be a killer of male whiteflies. Also, the presence of 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.

5.2 Introduction

Endosymbiosis plays a vital role in insect-plant interactions, affecting numerous aspects of herbivorous ecology. Buchner (1953) 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 (Baumann, 2005). Primary symbionts are inherited entirely vertically through the germline to offspring. They are normally considered to be mutualistic symbioses and are commonly required for host fitness, survival, and reproduction. The endosymbionts are adapted to the hosts' diet by supplying vital nutrients, so they are obligate for both partners (Baumann *et al.*, 2006). 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 insects species harbour a primary symbiont (Buchner, 1953).

Secondary symbionts are considered to be facultative endosymbionts from the host's perspective and have a shorter coevolutionary history with the host species (Dale and Moran, 2006). Some secondary symbionts are uncommon whereas others are fixed in their hosts (Simon *et al.*, 2003; Gueguen *et al.*, 2010). 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 (Dobson *et al.*, 1999; Moran *et al.*, 2008). Secondary symbiotic bacteria are commonly transmitted vertically, but in some cases, horizontal transmission between hosts might occur (Russell *et al.*, 2003; Dale and Moran, 2006; Oliver *et al.*, 2010).

5.2.1 Symbiotic bacteria in whitefly

Whiteflies are known to host the obligatory symbiont *Portiera aleyrodidarum*, which has a long coevolutionary history with all species of the Aleyrodinae subfamily (Thao and Baumann, 2004). 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) (Zchori-Fein and Brown, 2002; Nirgianaki *et al.*, 2003). 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 a symbiosis (Hurst and Jiggins, 2005; Werren *et al.*, 2008).

5.2.2 The role of symbiotic bacteria in whitefly

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 (Saridaki and Bourtzis, 2010; Kikuchi *et al.*, 2012). The primary symbiont *Portiera* spp. is known to supplement the hosts' diet with essential nutrients like amino acids as well as carotenoids that provide significant anti-oxidant action (Santos-Garcia *et al.*, 2012). Additionally, secondary symbionts make contributions 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 (Brownlie *et al.*, 2009), initially increase host resistance to parasitic wasps and pathogens (Vorburger *et al.*, 2010), and may also increase tolerance to heat stress (Montllor *et al.*, 2002). 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 (Duron *et al.*, 2008). Endosymbionts influence the reproductive systems 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 are helping the symbionts to spread their infections in host populations (Werren, 1997; Werren *et al.*, 2008; Engelstädter and Hurst, 2009).

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 (Mahadav *et al.*, 2008), tolerance to high temperatures (Brumin *et al.*, 2011), the capacity to transmit viruses (Gottlieb *et al.*, 2010), and susceptibility to pesticides (Kontsedalov *et al.*, 2008; Ghanim and Kontsedalov, 2009). Himler *et al.* (2011) revealed that the MEAM1 genetic group of *B. tabaci* infected with *Rickettsia* in the US exhibited significantly increased fitness and also there was an increase in the 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 host population size, and spread the symbiont in the field. Additionally, the secondary endosymbionts *Cardinium* and/or *Arsenophonus* in *B. tabaci* might even influence interbreeding among whitefly biotypes (Thierry *et al.*, 2011).

The secondary symbionts *Rickettsia* sp. and *Hamiltonella* sp. are known to be harboured by specific *B. tabaci* biotypes and play important roles for their fitness. For instance, *Rickettsia* sp. linked with *B. tabaci* MEAM1 genetic group has been reported to be unable to synthesise some nutritional substances such as amino acids. Therefore, *Rickettsia* sp. in biotype B needs to obtain nutrition from its host (Zhu *et al.*, 2016). In addition, the secondary symbiont *Hamiltonella* sp. is known to increase its host's resistance to parasitoid wasps (Oliver *et al.*, 2003). Also, *Hamiltonella* sp. linked with *B. tabaci* MEAM1 might play an important role to assist the invasion of MEAM1 throughout the world (Fujiwara *et al.*, 2015), and is suggested to increase the transmission capacity of plant viruses, especially TYLCV (Gottlieb *et al.*, 2010; Su *et al.*, 2013).

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 chapter 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 of whitefly and may support the development of better whitefly management.

5.3 Materials and Methods

5.3.1 Field sampling

The locations and host plants of samples of both whiteflies collected from Iraq and the UK are described and detailed in Chapter 2 and Table 2-1.

5.3.2 Confirming the identity of whitefly molecularly and morphologically

The techniques used to identify the *B. tabaci* and *T. vaporariorum* morphologically and molecularly are described in section 2.3.2 **(Chapter 2)**.

5.3.3 Molecular identification and sequencing of endosymbionts

To detect the presence of obligate and facultative bacterial symbionts, the total gDNA of 10 males and ten females from each population of *B. tabaci* and *T. vaporariorum* was used. 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 5-1), as described by Kapantaidaki *et al.* (2015). The same protocol of PCR amplification as for mtCOI gene sequencing (Chapter 2) 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 (Chelius and Triplett, 2001). 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 Chapter 2.

5.3.4 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 multilocus 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 5-1 (Mouton *et al.*, 2012; Kapantaidaki *et al.*, 2015). The same PCR reaction was used as described in Chapter 2, but an appropriate annealing temperature was used for each reaction as indicated in Table 5-1.

5.3.5 Sequence alignment and phylogenetic analysis

All of the symbiont DNA sequencing was performed and visualised on a 3130XL Genetic Analyzer as described in the mtCOI sequencing procedure in Chapter 2. The sequences obtained were checked using Geneious, version 6.1.4 (Kearse *et al.*, 2012). 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 (Appendices 8-10). Also, single sequences of a primary and a secondary endosymbiont of *T. vaporariorum* were deposited in GenBank under accession numbers KY457224 and KY243936 (Appendices 11-12). Additionally, the *Arsenophonus* sp. gene sequences were deposited in NCBI GenBank under the accession numbers KY626170-KY626172 for *fbaA*, *ftsK* and *yaeT* genes, respectively (Appendices 13-15). The phylogenies were estimated using maximum likelihood (ML) using MEGA 6 as described in Chapter 2.

Table 5-1. Primers used to screen the primary and secondary symbionts in whitefly species (Kapantaidaki *et al.*, 2015). Ann.: annealing temperature. Amp. Size: amplification product size.

Targeted taxon	Targeted gene	Primers	Sequences (5' - 3')	Ann. (^{o}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		

5.4 Results

5.4.1 Confirmation of the identity of specimens molecularly and morphologically

Both whitefly species have been confirmed morphologically and molecularly to be *T. vaporariorum* and *B. tabaci* in the UK and Iraq, respectively (Chapter 2).

5.4.2 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, which also indicates that the DNA extracts were of 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, while no PCR products were found for the other symbionts (Table 5-2). 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* 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 (Appendices 16 and 17). The *P. aleyrodidarum* sequnce matched 100% with the GenBank sequences with accession numbers CP004358 and Z11928 (Clark *et al.*, 1992; Sloan and Moran, 2013), and secondary symbionts *Arsenophonus* sp. matched 99% with the *Arsenophonus* sp. isolated from India with the accession number KJ541957.

Table 5-2. 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, which again indicated 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 5-3). The PCR products that appeared 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 (Clark *et al.*, 1992; Fujiwara *et al.*, 2015).

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 (Appendices 18-20).

Table 5-3. Numbers of both sexes of *B. tabaci* 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* ♀+♂	
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	-	-	-	-

5.4.3 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 sequences available in the NCBI GenBank database. 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.

5.5 Discussion

This study presents, for the first time, 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., despite the fact that females from this population in various countries were all infected (Kapantaidaki *et al.*, 2015). In contrast, populations of this species in Croatia, Bosnia, and Herzegovina harboured both *Arsenophonus* sp. and *Hamiltonella* bacterial symbionts, which were found in females (Skaljac *et al.*, 2010; Skaljac *et al.*, 2013). 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* (Prijovic *et al.*, 2014).

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 those of other studies (Chiel *et al.*, 2007; Zhu *et al.*, 2016). 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. as well as *Hamiltonella* sp. at high frequencies (Gueguen *et al.*, 2010). 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. (Tsagkarakou *et al.*, 2012; Bing *et al.*, 2013a; Gnankine *et al.*, 2013). There are doubts about the role of *Hamiltonella* sp. with *B. tabaci* biotypes. For instance, Gottlieb *et al.*

(2010) demonstrated a link between the capacity of *B. tabaci* biotypes to harbour *Hamiltonella* sp. and to transmit TYLCV. Their study showed the efficiency of the MEAM1 species in transmitting TYLCV in Israel, whereas 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 (Kontsedalov *et al.*, 2008; Mahadav *et al.*, 2008; Himler *et al.*, 2011). *Rickettsia* sp. of *B. tabaci* MEAM1 isolated in Israel was confirmed to be linked with a reduced capacity of whitefly to resist pesticides and with immunoreactions against parasitic wasps (Kontsedalov *et al.*, 2008; Mahadav *et al.*, 2008). Additionally, *Rickettsia* sp. isolated from *B. tabaci* MEAM1 in the USA appears similar to the secondary symbiont of Israeli MEAM1, but has been shown to improve the fitness of its whitefly host substantially (Himler *et al.*, 2011). 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 that there was 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 (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. Taken together, 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, the occurrence of more secondary symbionts and high mtCOI diversity reported from Iraqi whitefly is consistent with the complex species of *B. tabaci* (Mouton *et al.*, 2012; Lee *et al.*, 2013).

These findings provide an initial database for the further investigation of symbiotic bacteria associated with whiteflies in both 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.

5.6 Conclusion

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, whereas the facultative symbionts *Hamiltonella* sp. and *Rickettsia* sp. were also detected in the majority of individuals.

6 Chapter 6. General discussion and future work

6.1 General discussion

The study of divergent genetic and ecological lineages within a species provides an excellent opportunity to investigate evolutionary and demographic developments in the first stages of speciation of an organism (Losos and Glor, 2003). Ecological speciation may occur in sympatry (where species inhabit the same location) or allopatry (inhabiting separate locations), and could involve various agents of natural selection and result from a combination of adaptive processes (Schluter, 2001). Evolutionary elements in these early stages of differentiation might represent "intermediate" phases in polymorphic populations and different species, which are often defined as sibling species or biotypes (Drès and Mallet, 2002). A good example of genetic diversity and speciation could be the sweet potato whitefly species complex *B. tabaci* (Hemiptera: Aleyrodidae). It has been reported to include more than 39 cryptic species (commonly known as biotypes), which are distributed globally (Boykin *et al.*, 2007; De Barro *et al.*, 2011; Alemandri *et al.*, 2015). Pests of agriculture are known to be invasive species in non-native regions. By inhabiting new regions and adopting new crop plants, non-native species can become mobile elements in a worldwide network of agroecosystems linked through the international plant trade. Another example of a non-native species is the glasshouse whitefly *T. vaporariorum*, which is one of the most widespread invasive insects. Since 1856 the species has succeeded in surviving in the UK and may not have an inactive overwintering phase in the environment of the glasshouse habitat.

It has been suggested that some secondary symbionts associated with whitefly may increase or decrease the capacity of their hosts to resist insecticides and thus play an important role in the survival or fitness of the hosts. Unfortunately, studies of the association between whiteflies and their symbionts have been restricted due to difficulties in culturing symbiont bacteria in the laboratory (Houk and Griffiths, 1980; Douglas, 1989; Wilkinson, 1998). Studies of the functions of endosymbionts have been

restricted to *in vivo* investigations using antibiotic treatments, which can decrease or even increase levels of infection of bacteria in their host (Wilkinson, 1998). Whiteflies are interesting to study due to the serious economic damage they cause to crops. A very important reason for this damage is the capacity of whiteflies to transmit various plant viruses, especially where vegetable crops, and especially tomatoes, are widely planted.

The purpose of this study was to provide information about the genetic diversity, population structure, presence of biotypes/haplotypes, and the endosymbionts of *B. tabaci* and *T. vaporariorum* in the relatively unstudied areas of Iraq and the UK. This thesis has addressed the following questions: Have many introductions of whitefly occurred in these countries? Do habitats in both countries and other agricultural applications affect the structure of whitefly populations? Are haplotypes/biotypes/putative species present? What is the status of the presence and distribution of endosymbionts in whitefly species?

6.2 Summary of the main findings of this study

6.2.1 Chapter 2

- Four haplotypes of *B. tabaci* have been found in Iraq, two of which are in the MEAM1 putative species, one in the MEAM2 putative species, and one unknown but similar to MEAM1.
- The predominant form of *B. tabaci* is the B2 biotype of the MEAM1 putative species, with no evidence of the Q biotype from the MED putative species.
- A relatively high level of mitochondrial DNA variation was found in *B. tabaci* in Iraq, whereas there was little variation in *T. vaporariorum* in the UK.
- Two mitochondrial haplotypes of *T. vaporariorum* were indicated in the UK: mtH1was most common across the area of study, whereas mtH3 was reported for the first time in the UK.

6.2.2 Chapter 3

- Populations of *T. vaporariorum* in the UK exhibit genetic differentiation.
- It is possible that multiple introductions of *T. vaporariorum* into the UK have occurred.
- The results showed some structuring of populations, with clustering by geographical location and not by crop or year of collection.
- The glasshouse agroecosystem and repeated imports have contributed to variation at the nuclear level.
- The glasshouse agroecosystem has likely contributed to the population genetic structure by restricting gene flow between locations.

6.2.3 Chapter 4

- The *B. tabaci* populations showed limited genetic structure in Iraq.
- Low-level differentiation was found among Iraqi *B. tabaci* populations.
- There are, in some cases, links between population structure and time of collection, but not with geographical location or host plant.

6.2.4 Chapter 5

- The endosymbionts present in both sexes of *B. tabaci* were *Portiera aleyrodidarum* as a primary symbiont, whereas the secondary symbionts *Hamiltonella* sp. and *Rickettsia* sp. were also identified in the majority of individuals in Iraq.
- The presence of *Portiera aleyrodidarum*, an obligate symbiont in *T. vaporariorum* in the UK, was found in both sexes, whereas the facultative symbiont *Arsenophonus* sp. was detected in females but not males.
- Analyses of genetic variation in the secondary symbionts *Arsenophonus* sp. isolated from *T. vaporariorum* showed no polymorphism in the UK.

6.3 General conclusion

The genetic diversity and population structure in invasive species have been studied in various insect taxa. For instance, genetic diversity patterns involving multiple introductions have been demonstrated in sweet potato whitefly *B. tabaci*, *Gambusia* spp., the caprellid *Caprella scaura* and thrips *Frankliniella occidentalis* (Delatte *et al.*, 2006; Sanz *et al.*, 2013; Cabezas *et al.*, 2014; Cao *et al.*, 2017). Multiple introductions of invasive species are regarded as the main source of genetic variation (Reem *et al.*, 2013), which is often associated with successful invasions (Roman and Darling, 2007; Suarez and Tsutsui, 2008). Therefore, it can be speculated that multiple introductions of *T. vaporariorum* and *B. tabaci* putative species have occurred in the UK and Iraq, respectively, via international trade, and this might have been helpful in their successful establishment and survival. This could lead to diversity at the nuclear level, especially in *T. vaporariorum* populations in the UK. However, the low level of genetic variation in mtCOI could be a consequence of extensive whitefly control measures, and there might not have been enough time for *T. vaporariorum* to evolve. A possible explanation of the low genetic variation of mtCOI among *T. vaporariorum* populations in the UK might be that introductions from the same region have occurred, leading to low symbiont diversity as well.

Phylogenetic analyses of mtCOI DNA (coding DNA in the more conservative region) indicated new putative species of *B. tabaci* in Iraq and a new haplotype of *T. vaporariorum* in the UK (Chapter 2). These findings might help in monitoring the status and updating the distribution of the putative species of *B. tabaci* and haplotypes of *T. vaporariorum* in both countries. It is important to keep up-to-date concerning the status of *B. tabaci* as some damaging plant viruses linked with specific putative species of *B. tabaci* have been reported. So far, more than 39 putative species of *B. tabaci* and 19

haplotypes for *T. vaporariorum* have been reported worldwide which cannot be distinguished morphologically (De Barro *et al.*, 2011; Prijovic *et al.*, 2014; Alemandri *et al.*, 2015). Mitochondrial DNA (mtCOI) has been successfully used to detect the putative species and haplotypes in many insects, including whitefly. The ability of some complex species to reform as biotypes/haplotypes may increase their fitness and distribution. Taken together, the mtCOI results of *T. vaporariorum* from the UK, in line with results from other studies (Roopa *et al.*, 2012; Prijovic *et al.*, 2014; Kapantaidaki *et al.*, 2015), confirm that there are no cryptic species or biotypes in *T. vaporariorum*, but just haplotypes belonging to this species in colonised agricultural ecosystems across the UK.

The genetic diversity of the *B. tabaci* whitefly has attracted the attention of researchers because of its nature as a highly invasive and complex species. The existence of new biotypes, haplotypes and putative species might require more strategic management of whitefly insects. It has been reported that differences in *B. tabaci* putative species include differences in ability to develop insecticide resistance, in ability to transmit various plant viruses, and in host plant range (De Barro *et al.*, 2011). The results of mtCOI sequencing showed that the MEAM1 *B. tabaci* biotype B2 is predominant in Iraq, whereas one B biotype was found. Two mtCOI haplotypes (Unknown and MEAM2) were recorded for the first time in Iraq, although further investigation is required to check their authenticity. Therefore, the putative species of *B. tabaci* in Iraq might be a result of multiple introductions, especially from the countries neighbouring Iraq. Recently, new strains of the TYLCV virus have been recorded in Iraq, which are carried by *B. tabaci* (Al-Abedy *et al.*, 2018). This could be one reason for the difficulties in the control of *B. tabaci* by Iraqi growers. Further investigation is needed

to confirm the link between the dominant species of *B. tabaci* and the capacity to transmit TYLCV.

The use of microsatellite markers for non-coding DNA with high rates of mutation is very useful in understanding the population dynamics of an organism. The results of the investigation of *B. tabaci* and *T. vaporariorum* populations in Iraq and the UK, respectively, indicate that there is some genetic structure of *T. vaporariorum* in the UK based on geographical location, whereas there is low population differentiation in *B. tabaci* in Iraq. This information might be important in the monitoring of whitefly and researchers could study the link between genotype and phenotypic plasticity in this important pest.

In the UK, the genetic diversity of *T. vaporariorum* might be associated with local adaptation to the agroecosystem by the cultivation of the same crops for a long time at the same location, as has been reported with *T. vaporariorum* populations in Finland (Ovcarenko *et al.*, 2014). Therefore, the greenhouse agroecosystem may contribute to the population genetic structure of *T. vaporariorum* by restricting gene flow between locations in the UK. However, low levels of population differentiation in *B. tabaci* populations in Iraq may be a result of the intensive use of pesticides, which leads to selection pressure for resistant genotypes. Also, microsatellite markers used in this species may not be powerful enough to show genetic variation within and between Iraqi whitefly populations. Using more microsatellite markers or designing new markers for *B. tabaci* is recommended. Taken together with results of the mitochondrial DNA analysis, the idea is supported that biotype B2 is more common in Iraq than biotype B and the secondary symbiont *Rickettsia* sp. could affect resistance of *B. tabaci*, which might result in biotype B2 being more resistant than biotype B to pesticides. The

presence of the *Rickettsia* sp. symbiont together with *Hamiltonella* sp. may support the argument that biotype B2 is becoming the most common biotype in Iraq and is more difficult to manage. These factors could lead to increased adaptation in whiteflies in Iraq, making them harder for growers to manage.

The results concerning Iraqi whitefly symbionts could indicate the ability of biotype B2 to develop resistance to pesticides and parasites and to increase its capacity to transmit plant viruses. The well-represented secondary symbiont *Hamiltonella* sp., which was found in all *B. tabaci* examined, has been found to increase the host's efficiency and effectiveness in transmitting TYCLV (Gottlieb *et al.*, 2010). More information on the biotypes of *B. tabaci*, gained from a combination of genetic characterisation and biological and ecological research, might help in improving the management of *B. tabaci.*

In Iraq, pesticides are mainly used to control *B. tabaci* as it is has a very low economic threshold level, which means that growers take control action when few adult whitefly are present. The intensive use of pesticides may, as a consequence, increase resistance prevalence in *B. tabaci* MEAM1, which was the most common putative species in the area of study. However, the reduction in the *B. tabaci* biotype B may be a result of its vulnerability to pesticides due to the presence of the secondary symbiont *Rickettsia* sp., which has been linked with increased pesticide susceptibility in biotype B, but not in biotype B2 (Kontsedalov *et al.*, 2008; Chiel *et al.*, 2009). *Rickettsia* sp. also reduces the pesticide resistance of *B. tabaci* biotype Q (Ghanim and Kontsedalov, 2009). Chiel *et al.* (2007) reported that *B. tabaci* biotype B in Israel was linked exclusively with the secondary symbiont *Hamiltonella* sp. while the secondary symbiont *Wolbachia* sp. was

exclusively linked with Q. The same study also found that *Rickettsia* sp. was consistently found in both B and Q.

It appears that the current management of *B. tabaci* in Iraq, which depends on insecticides as the main method, is insufficient and needs to be improved. However, to improve the situation, pesticides could be used together with other management methods as a long-term strategy to control *B. tabaci*, especially in cases where new putative species are present, for example, *B. tabaci* MED.

The economic threshold level, which means that numbers of whitefly present require control action to be taken, and population size may play important roles in genetic differentiation among and within whitefly populations. For example, the threshold level of glasshouse whitefly in the UK is larger than that of the Iraqi *B. tabaci*, which means that the population size of *T. vaporariorum* is larger than that of *B. tabaci*. This possibility is supported by the results for population differentiation in both whiteflies, where more differentiation was found in *T. vaporariorum* due to the size of the population inhabiting glasshouses, whereas low levels of differentiation were found within and among *B. tabaci* populations in Iraq, which may be due to the small size of the population inhabiting the area of study.

The glasshouse whitefly is associated with less risk regarding the transmission of viruses, and so biological control is the main method used to control this pest in the UK. That has led to an increase in the population size of this insect and may, as a result, increase the variation within and among whitefly populations. These findings might help in improving our understanding of the biology, ecology, and spread of this damaging and invasive whitefly pest in both countries. The results, in turn, could help improve management strategies for this species in Iraq.

To reduce the potential for insect distribution to other glasshouse agroecosystems, monitoring the pest and exterminations are recommended. More effort should be taken to prevent new species or haplotypes of *B. tabaci* and *T. vaporariorum* from invading Iraq and the UK by increasing the level of quarantine at the borders in both countries.

6.4 UK glasshouse whitefly

Glasshouse construction expanded rapidly and it has been reported that there are at least two million hectares of glasshouse production worldwide (Pardossi *et al.*, 2004). In the UK about 131 thousand hectares are available to grow protected vegetables and the tomato is a major crop as recognised by Defra (2013) (the Department for Environment, Food and Rural Affairs). Glasshouse environments provide suitable conditions to produce many crops, such as tomato, cucumber, aubergine and pepper, in northern European countries, including the UK. Also, the glasshouse agroecosystem could provide some protection from insect pests, including whitefly, by limiting their access to the crop and making a physical barrier to restrict whitefly movement (Bell and Baker, 2000). However, such glasshouse conditions could also provide an optimum environment for vegetable pests to grow, including whitefly (Berlinger *et al.*, 1999). The UK glasshouse whitefly *T. vaporariorum* as an inhabitant of glasshouses should be considered as allopatric populations since its movement is restricted by the agroecosystem environment. IPM strategies including the use of biological control agents, non-chemical applications, environmentally friendly pesticides, and biopesticides are used to control *T. vaporariorum* in the UK.

This thesis presents extensive data on the population genetic structure and genetic diversity of *T. vaporariorum* across the UK. The results of analysis using microsatellite markers show high genetic diversity but limited diversity at the mitochondrial level and only one secondary symbiont species associated with *T. vaporariorum*. Most of the *T.*

vaporariorum individuals present in the UK belonged to mtH1, which is most common in countries near to the UK, namely France and the Netherlands (Malumphy *et al.*, 2007; Kapantaidaki *et al.*, 2015). The mitochondrial haplotype mtH3 was recorded in the UK for the first time in the southeast of England. The recent introduction of mtH3 into the UK might be a result of the trade in plants imported from international regions. The low level of variation found in the mtCOI sequences of *T. vaporariorum* populations was similar to the results of Kapantaidaki *et al.* (2015), who found that most mtCOI sequences collected from the US and some European countries belonged to mtH1 and only a single individual to mtH3. A similar level of genetic variation has been found for this species in Serbia and neighbouring countries (Prijovic *et al.*, 2014). The likely explanation for the absence of significant COI sequence variation in the data in the present study could be the relatively recent introduction of this species into the UK in about 1856 (Mound and Halsey, 1978), which means that there has probably not been enough time for the evolution of haplotypes within the UK, and any variation in *T. vaporariorum* is likely to have arisen through imports.

Regarding endosymbionts, the facultative symbiont *Arsenophonus* sp. has been cited as a reproductive manipulator in another pest insect (Werren *et al.*, 1986; Balas *et al.*, 1996). *T. vaporariorum* populations from the UK harboured just one secondary symbiont. *Arsenophonus* sp. was prevalent in most females, indicating near-fixation in UK *T. vaporariorum* populations, but was absent in all males. Several studies have reported that symbionts, including *Arsenophonus* sp., play a role in killing males (Gherna *et al.*, 1991; Ferree *et al.*, 2008; Duron *et al.*, 2010). Therefore, *Arsenophonus* sp. in the UK *T. vaporariorum* populations could be killing males, but further study is needed to confirm this. It would be possible to conduct laboratory experiments for *T. vaporariorum* with females having or not having the symbionts *Arsenophonus* sp. and

then to observe the sex ratios of their offspring to confirm the male-killing function of this secondary symbiont.

In general, the fact that infection with primary and secondary symbionts has reached fixation, or near fixation, suggests that the symbionts play an important role, or have a mutualistic relationship, with their insect host. The lack of diversity of secondary symbionts found in the results again possibly indicates the limited numbers of introductions of *T. vaporariorum* and/or introduction from regions only having *Arsenophonus* sp. as secondary symbionts. That was confirmed by low level of diversity in the mitochondria genes. Further investigations of the genetic variation of the secondary symbiont *Arsenophonus* sp. showed no polymorphism.

UK populations of *T. vaporariorum* exhibit significant genetic diversity, except in the laboratory colony which indicated lower genetic diversity. The low genetic variation in the laboratory colony of *T. vaporariorum* may be due to selection and/or a prolonged bottleneck (Hadjistylli *et al.*, 2016). Most of the UK whitefly samples were from commercial glasshouses, which are often more professionally managed by staff than those of private growers (Ovcarenko *et al.*, 2014). *T. vaporariorum* in the UK inhabits glasshouses, and this might affect the genetic diversity of this pest due to the restriction of gene flow (Hoffmann and Willi, 2008). Crop management applications and regular population management can cause population bottlenecks, leading to potentially strong effects of random genetic drift and decreases in heterozygosity, which may lead to increased population genetic differentiation (Tsagkarakou *et al.*, 1998). As mentioned before the management strategy for *T. vaporariorum* in the UK uses an integrated pest management (IPM) programme including sticky yellow traps to monitor and *Encarsia formosa* as biological control to reduce the population of this pest.

The genetic clustering of *T. vaporariorum* based on microsatellite data showed some structure of populations in the UK, which in some cases was related to geographical location, but not to host plant. The results of STRUCTURE are supported by significant F_{st} values indicating genetic differentiation between populations, which may indicate restricted gene flow between *T. vaporariorum* populations. Also, in some cases STRUCTURE showed different clusters for the two years of sampling from the same locations. These results may be a result of the management applied to *T. vaporariorum* in the UK. The different clusters of STRUCTURE for the two years of study imply that some growers were able to successfully eradicate *T. vaporariorum* in previous seasons and new infestations of whitefly were brought with new crops. Nevertheless, for other growers *T. vaporariorum* was maintained from one season to another.

Populations of *T. vaporariorum* in the UK exhibit genetic differentiation, demonstrating the possibility that multiple introductions of *T. vaporariorum* into the UK have occurred. The results showed some structure of populations, with clustering by geographical location and not by crops. All *T. vaporariorum* individuals in the north and midlands of the UK belong to mtH1, and mtH3 has been recorded in the UK for the first time in the south-east of England, indicating that there have been at least two introductions in the UK. The introduced haplotypes are likely to be from European countries or the US via plant material imports. The low level of mitochondrial haplotype variation in *T. vaporariorum* could be a result of insufficient time for evolution to occur in the UK. Tests revealed the presence of *Portiera* sp., an obligate endosymbiont, in both sexes, whereas the facultative symbiont *Arsenophonus* sp. was detected in females only. The glasshouse agroecosystem and repeated imports from a limited number of regions may have contributed to variation at the nuclear, but not at

cytoplasmic level. This has likely contributed to the population genetic structure by restricting gene flow between geographical locations. Therefore, an appropriate cleaning of crops seasonally, checking that new plants are free of infestation, and checking the weeds around the glasshouse can be recommended to reduce populations and limit infestation with *T. vaporariorum*.

6.5 Iraqi whitefly

Vegetable crops in Iraq are considered to be most important for food security. Tomato plants are the most commonly grown vegetable. Vegetables including tomatoes are planted in greenhouses and the field all year round in Iraq. According to the FAOSTAT (2016) database, the average area (and yield) of tomato, cucumber, and eggplant grown in Iraq reached 200712 ha (286596 tonnes), 71069 /ha (91487 tonnes), and 122609 ha (102452 tonnes), respectively. Sweet potato whitefly *B. tabaci* is considered as a major pest of vegetable crops. It can transmit many plant viruses including the highly damaging TYLCV, which can cause the total loss of a tomato plant. Growers in Iraq grow vegetable crops in simple plastic protected structures, which are less enclosed from the nearby environment. Therefore, the Iraqi *B. tabaci* inhabit both protected and outdoor environments and these should be considered as sympatric populations as there is movement between different habitats. Due to the high level of economic damage caused by *B. tabaci* at present, the intensive application of pesticides is highly recommended to control whitefly in Iraq even if only a few adults are present.

This thesis has reported data on the genetic diversity and population structure of *B. tabaci* collected from the middle and south of Iraq. The results of mtCOI sequencing showed that the *B. tabaci* species MEAM1, biotype B2, was predominant, whereas one B biotype was found. Two mtCOI haplotypes of *B. tabaci* (unknown species, but likely to be MEAM1, and MEAM2) were recorded for the first time in Iraq. It will be

necessary to sequence the whole mitogenome to confirm the identity of these isolates. The *B. tabaci* in Iraq belong to the Africa/Middle East/Asia Minor genetic group. The MEAM1 B2 and B haplotypes recorded in this study were slightly different from the previous B haplotype (HM070413) recorded in Iraq in 2010 (see Chapter 1), on the basis of the polymorphic sites that were detected in their mtCOI sequences. The presence of multiple haplotypes is likely to be a consequence of imported plants produced from the countries surrounding Iraq. Most of the Iraqi whiteflies tested were grouped with other MEAM1 sequences from the countries neighbouring Iraq such as Syria, Iran, and the United Arab Emirates. The MEAM1 putative species, including the B and B2 biotypes, has been found in tropical and subtropical countries around the world and they are believed to be dangerous non-native biotypes in many areas (Boykin *et al.*, 2007; Chu *et al.*, 2010; De Barro *et al.*, 2011; De Barro, 2012). The introduction of new biotypes from different sources could increase genetic diversity by the introduction of genes for resistance to pesticide which arise by mutation (Zidana *et al.*, 2009; Verhoeven *et al.*, 2011).

B. tabaci populations from Iraq harboured the primary symbiont *P. aleyrodidarum* and two secondary symbionts, *Hamiltonella* sp. and *Rickettsia* sp. There are doubts about the role of *Hamiltonella* sp. with putative species of *B. tabaci*. For instance, Gottlieb *et al.* (2010) demonstrated an association between the capacities of *B. tabaci* putative species to harbour *Hamiltonella* sp. and to transmit TYLCV. It has been reported that *Rickettsia* sp. might be linked to reduced insecticide resistance (Kontsedalov *et al.*, 2008), immunoreactions against wasp parasitoids (Mahadav *et al.*, 2008), and increased whitefly fitness and female bias (Himler *et al.*, 2011).

Low genetic variation between the *B. tabaci* populations was observed using microsatellite loci. The low level of variation among Iraqi *B. tabaci* populations could be a result of extensive control and management, including the removal of sources of

infestation and the overuse of insecticides to decrease population levels of this insect. Also, multilocus (clonal) genotypes have been observed in some populations, which might be the main reason for the low levels of variation among populations (Nibouche *et al.*, 2014). Another explanation for the low genetic variation found among *B. tabaci* populations could be insufficiently powerful microsatellite markers.

The results of the *B. tabaci* population analysis based on microsatellite data showed a low level of differentiation. High levels of identical genotypes between and within populations (clones) were observed, which is probably one reason for the low genetic differentiation (F_{st}) among populations. A second reason could be a movement of eggs, nymphs or adults of the insect between cities via distribution centres. That might be the reason for finding clones, which could occur by chance or might come from nearby populations of whitefly. A third reason could be the resistance genotype of *B. tabaci* as a result of intensive use of pesticides. Finally, microsatellite markers could be insufficiently polymorphic for identifying variation within and between Iraq *B. tabaci* populations.

Overall, the study of *B. tabaci* population in Iraq indicates clear patterns of evolution of Iraqi biotypes within the cytoplasm, including mitochondrial genes and endosymbionts, while low levels of variation and evolution have occurred at the nuclear level.

6.6 Future work

This following recommendations for further studies may help in improving our understanding of the biology, ecology, and spread of these damaging and invasive whitefly pests in the UK and Iraq.

- No study has yet investigated the resistance levels to pesticides of putative species and/or haplotypes of both whiteflies in Iraq and the UK, and so it is well worth conducting such a study.
- The role of endosymbionts in both whiteflies needs to be investigated further.
- The capacity of different putative species and/or haplotypes of both whiteflies to transmit plant viruses in both countries could be researched.
- The effect of plant cultivation on adaptation in glasshouse whitefly populations should be looked into.
- Further investigations of the role of different agroecosystems on the diversity and genetic structure of glasshouse whitefly are needed.
- A possible future study could investigate the phenotypic effects of the bacterial symbionts related to whiteflies in Iraq and the UK.
- Extensive sampling of glasshouse whitefly populations, particularly from the west and north of the UK, are needed to confirm the low level of mtDNA diversity, which might help in extending our understanding of the biology, ecology, and spread of this damaging and invasive insect pest in the UK.
- Further investigation is needed to understand the role of *B. tabaci* putative species in transmitting plant viruses, and particularly TYLCV, in Iraq.
- Regular sampling of *B. tabaci* is needed to monitor new putative species which might be introduced.
- The role of secondary symbionts in supporting *B. tabaci* in transmitting TYLCV in Iraq could be studied.
- The newly recorded MEAM2 putative species of *B. tabaci* in Iraq could be more studied.

- Further study should be conducted to investigate new putative species of *B. tabaci* by doing more biological work with live insects to characterise their behaviour, preferences for plant hosts and mating.
- Further study should be conducted to investigate the genetic diversity of both *T. vaporariorum* and *B. tabaci* using different mtDNA genes, such as ND5 and CYTB.
- Sequencing the whole mitogenome of *B. tabaci* is required to identify its putative species more definitively.

7 Appendices

Appendix 1.The mtCOI H1 sequence details of *T. vaporariorum* deposited in NCBI GenBank.

Trialeurodes vaporariorum isolate DU3-UK cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KX679581.1

FASTA Graphics

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            Aleyrodoidea; Aleyrodidae; Aleyrodinae; Trialeurodes.
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  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 657)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
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Appendix 2. The mtCOI H3 sequence details of *T. vaporariorum* deposited in NCBI GenBank.

Trialeurodes vaporariorum isolate ES3_UK cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KX679578.1

FASTA Graphics

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  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
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  TITLE     Direct Submission
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Appendix 3. The mtCOI H3 sequence details of *T. vaporariorum* deposited in NCBI GenBank.

Trialeurodes vaporariorum isolate NO1-H3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KY048293.1

FASTA Graphics

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  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 525)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
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Appendix 4. The mtCOI biotype sequence details of *B. tabaci* deposited in NCBI GenBank.

Bemisia tabaci isolate KA1_5 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KX679574.1

FASTA Graphics

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  TITLE     Mitochondrial, endosymbionts diversity and population structure of
            sweet potato Bemisia tabaci whitefly reveal novel invasion in Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 796)
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Appendix 5. The mtCOI biotype sequence details of *B. tabaci* deposited in NCBI GenBank.

Bemisia tabaci isolate KA2_3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KX679575.1

FASTA Graphics

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  TITLE     Mitochondrial, endosymbionts diversity and population structure of
            sweet potato Bemisia tabaci whitefly reveal novel invasion in Iraq
  JOURNAL   Unpublished
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  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
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      601 gcgggaagga ttctgagtat tatttctgtt atttatttt tatttattgt tttagaatcc
      661 tttcttcttc tgcggttagt aagatttaag cttggtgtaa gcaggcatct agaatgaaaa
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```

Appendix 6. The mtCOI biotype sequence details of *B. tabaci* deposited in NCBI GenBank.

Bemisia tabaci isolate KA2_5 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KX679576.1

FASTA Graphics

Go to:

```
LOCUS       KX679576                799 bp    DNA     linear   INV 18-OCT-2016
DEFINITION  Bemisia tabaci isolate KA2_5 cytochrome oxidase subunit I (COI)
            gene, partial cds; mitochondrial.
ACCESSION   KX679576
VERSION     KX679576.1
KEYWORDS    .
SOURCE      mitochondrion Bemisia tabaci
  ORGANISM  Bemisia tabaci
            Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta;
            Pterygota; Neoptera; Paraneoptera; Hemiptera; Sternorrhyncha;
            Aleyrodoidea; Aleyrodidae; Aleyrodinae; Bemisia.
REFERENCE   1  (bases 1 to 799)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Mitochondrial, endosymbionts diversity and population structure of
            sweet potato Bemisia tabaci whitefly reveal novel invasion in Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 799)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-AUG-2016) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /organelle="mitochondrion"
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                     /isolate="KA2_5"
                     /host="Solanum lycopersicum (tomato)"
                     /db_xref="taxon:7038"
                     /sex="female"
                     /country="Iraq: Kerbala"
                     /collection_date="2015"
                     /collected_by="Aqeel Al-Abedy"
                     /note="biotype: Middle East-Asia Minor2;
                     PCR_primers=fwd_name: C1-J-2195, rev_name: tRNA-1576"
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     CDS             <1..>799
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                     /transl_table=5
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                     DVDTRAYFTSATMIIAVPTGIKIFSWLATLGGMKSNKFSPLGLWFTGFLFLFTMGGLT
                     GIILGNSSVDVCLHDTYFVVAHFHYVLSMGIIFAIVGGVIYWFPLILGLTLNNYSLVS
                     QFYIMFIGVNLTFFPQHFLGLGGMPRRYSDYADCYLVWNKISSAGSILSIISVIYFLF
                     IVLESFLLLRLVSFKLGVSSHLEWKINKPALNHSFKELCLTFFSNVAE"
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      361 gttgttgcac attttcatta tgttttatca ataggaatta tttttgctat tgtgggagga
      421 gttatttatt gatttccatt aatcttgggt ttaaccttaa ataactatag attggtgtct
      481 caattttata tcatgtttat tggagtaaat ttaacttttt ttcctcagca tttccttggt
      541 ttgggggaa tgcctcgccg atattcagat tatgctgatt gttatctagt atgaaataaa
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      661 ttagaatctt ttcttcttct gcgtttagta agatttaagc ttggtgtaag cagacatcta
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      781 ttttctaatg tggcagaaa
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Appendix 7. The mtCOI biotype sequence details of *B. tabaci* deposited in NCBI GenBank.

Bemisia tabaci isolate mu4_SA3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: KX679577.1

FASTA Graphics

Go to:

```
LOCUS       KX679577               787 bp    DNA     linear   INV 18-OCT-2016
DEFINITION  Bemisia tabaci isolate mu4_SA3 cytochrome oxidase subunit I (COI)
            gene, partial cds; mitochondrial.
ACCESSION   KX679577
VERSION     KX679577.1
KEYWORDS    .
SOURCE      mitochondrion Bemisia tabaci
  ORGANISM  Bemisia tabaci
            Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta;
            Pterygota; Neoptera; Paraneoptera; Hemiptera; Sternorrhyncha;
            Aleyrodoidea; Aleyrodidae; Aleyrodinae; Bemisia.
REFERENCE   1  (bases 1 to 787)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Mitochondrial, endosymbionts diversity and population structure of
            sweet potato Bemisia tabaci whitefly reveal novel invasion in Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 787)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-AUG-2016) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /organelle="mitochondrion"
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                     /clone="Ali Kareem"
                     /sex="female"
                     /country="Iraq: Al-Muthanna"
                     /collection_date="2015"
                     /collected_by="Ali Ajil"
                     /note="biotype: B; PCR_primers=fwd_name: C1-J-2195,
                     rev_name: tRNA-1576"
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     CDS             <1..>787
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                     /transl_table=5
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                     /protein_id="AOW43525.1"
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                     GIILGNSSVDVCLHDTYFVVAHFHYVLSMGIIFAIVGGVIYWFPLILGLTLNNYSLVS
                     QFYIMFIGVNLTFFPQHFLGLGGMPRRYSDYADCYLVWNKISSAGSILSIISVIYFLF
                     IVLESFLLLRLVSFKLGVSSHLEWKINKPALNHSFKELCLTFFF"
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      121 cacagttgga atagatgtag atactcgagc ttatttcact tcagccacta taattattgc
      181 tgttcccaca ggaattaaaa tttttagttg gcttgctact ttgggtggaa taaagtctaa
      241 taaattaagg cctcttggcc tttgatttac aggatttta ttttattta ctataggtgg
      301 gttaactgga attattcttg gtaattcttc tgtagatgtg tgtctgcatg acacttattt
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      421 agttatctat tgatttccac taatcttagg tttaacctta aataattata gattggtgtc
      481 tcaattttat atcatgttta ttggagtaaa tttaactttt tttcctcagc attttcttgg
      541 tttaggggga atgcctcgtc gatactcaga ttatgctgat tgctatctag tatgaaataa
      601 aatttcttct gcgggaagga ttctgagtat tatttctgtt atttatttt tatttattgt
      661 tttagaatcc tttcttcttc tgcggttagt aagatttaag cttggtgtaa gcaggcatct
      721 agaatgaaaa attaataaac cagctcttaa tcacagtttt aaagagttgt gtttaacttt
      781 ttttttc
//
```

Appendix 8. The 16S rRNA sequence details of primary symbiont *Portiera* sp. isolated from *B. tabaci* and deposited in NCBI GenBank.

GenBank

Candidatus Portiera aleyrodidarum isolate NA2_IRAQ 16S ribosomal RNA gene, partial sequence

GenBank: KY465885.1

FASTA Graphics

Go to:

```
LOCUS       KY465885                623 bp    DNA     linear   ENV 23-JAN-2017
DEFINITION  Candidatus Portiera aleyrodidarum isolate NA2_IRAQ 16S ribosomal
            RNA gene, partial sequence.
ACCESSION   KY465885
VERSION     KY465885.1
KEYWORDS    ENV.
SOURCE      Candidatus Portiera aleyrodidarum
  ORGANISM  Candidatus Portiera aleyrodidarum
            Bacteria; Proteobacteria; Gammaproteobacteria; Oceanospirillales;
            Halomonadaceae; Zymobacter group; whitefly endosymbionts;
            Candidatus Portiera.
REFERENCE   1  (bases 1 to 623)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Mitochondrial and endosymbionts diversity of sweetpotato whitefly
            Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), reveal novel
            invasion in Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 623)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JAN-2017) School of Biology, Newcastle University,
            Barras Bridge, Newcastle upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /isolation_source="Greenhouse"
                     /host="Bemisia tabaci"
                     /db_xref="taxon:91844"
                     /environmental_sample
                     /country="Iraq: Alnajaf"
                     /collection_date="2015"
                     /collected_by="Mohammed Kareem"
                     /PCR_primers="fwd_name: 28F, fwd_seq: tgcaagtcgagcggc,
                     rev_name: 1098R, rev_seq: aaagttcccgccttatgcgt"
                     /note="amplified with species-specific primers"
     rRNA            <1..>623
                     /product="16S ribosomal RNA"
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      121 gaagaaggcc tttgggttgt aaagcacttt cagcgaagaa gaaaagttag aaaataaaaa
      181 gttataacta tgacggtact cgcagaagaa gcaccggcta actccgtgcc agcagccgcg
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      481 cgtggggagc aaacaggatt agataccctg gtagtccacg ctgtaaacga tatcaactag
      541 ccgttggatt cttaaagaat tttgtggcgt agctaacgcg ataagttgat cgcctgggga
      601 gtacggtcgc aaggctaaaa ctc
//
```

Appendix 9. The 16S rRNA sequence details of secondary symbiont *Rickettsia* sp. isolated from *B. tabaci* and deposited in NCBI GenBank.

GenBank

Rickettsia secondary endosymbiont of Bemisia tabaci isolate NA1_4 16S ribosomal RNA gene, partial sequence

GenBank: KX679579.1

FASTA Graphics

Go to:

```
LOCUS       KX679579                768 bp    DNA     linear   ENV 05-DEC-2016
DEFINITION  Rickettsia secondary endosymbiont of Bemisia tabaci isolate NA1_4
            16S ribosomal RNA gene, partial sequence.
ACCESSION   KX679579
VERSION     KX679579.1
KEYWORDS    ENV.
SOURCE      Rickettsia secondary endosymbiont of Bemisia tabaci
  ORGANISM  Rickettsia secondary endosymbiont of Bemisia tabaci
            Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales;
            Rickettsiaceae; Rickettsieae; Rickettsia.
REFERENCE   1  (bases 1 to 768)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Mitochondrial, endosymbionts diversity and population structure of
            sweet potato Bemisia tabaci whitefly reveal novel invasion in Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 768)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-AUG-2016) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     tabaci"
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                     /isolate="NA1_4"
                     /host="Bemisia tabaci"
                     /db_xref="taxon:1888295"
                     /environmental_sample
                     /country="Iraq: Al-Najaf"
                     /collection_date="2015"
                     /collected_by="M. Kareem"
                     /note="amplified with species-specific primers;
                     biotype: B"
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                     /product="16S ribosomal RNA"
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      121 ttggtgaggt aatggcttac caagcctacg atctgtagct ggtctgagag gatgatcagc
      181 cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtggg gaatattgga
      241 caatgggcga aagcctgatc cagcaatacc gagtgagtga tgaaggccct agggttgtaa
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      661 cgatgagtgc tagatattgg gatattttct ctcggtttcg cagctaacgc attaagcact
      721 ccgcctgggg agtacggtcg caagattaaa actcaaagga attgacgg
//
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Appendix 10. The 16S rRNA sequence details of secondary symbiont *Hamiltonella* sp. isolated from *B. tabaci* and deposited in NCBI GenBank.

GenBank

Hamiltonella secondary endosymbiont of Bemisia tabaci isolate KA2_3 16S ribosomal RNA gene, partial sequence

GenBank: KX679580.1

FASTA Graphics

Go to:

```
LOCUS       KX679580                676 bp    DNA     linear   ENV 05-DEC-2016
DEFINITION  Hamiltonella secondary endosymbiont of Bemisia tabaci isolate KA2_3
            16S ribosomal RNA gene, partial sequence.
ACCESSION   KX679580
VERSION     KX679580.1
KEYWORDS    ENV.
SOURCE      Hamiltonella secondary endosymbiont of Bemisia tabaci
  ORGANISM  Hamiltonella secondary endosymbiont of Bemisia tabaci
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Enterobacteriaceae; aphid secondary symbionts; Candidatus
            Hamiltonella.
REFERENCE   1  (bases 1 to 676)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Mitochondrial, endosymbionts diversity and population structure of
            sweet potato Bemisia tabaci whitefly reveal novel invasion in Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 676)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-AUG-2016) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /isolate="KA2_3"
                     /host="Cucumis sativus (cucumber)"
                     /db_xref="taxon:654423"
                     /environmental_sample
                     /country="Iraq: Kerbala desert"
                     /collection_date="2015"
                     /collected_by="M. Kareem"
                     /PCR_primers="fwd_name: ham_f, fwd_seq:
                     tgagtaaagtctggaatctgg, rev_name: ham_r, rev_seq:
                     agttcaagaccgcaacctc"
                     /note="amplified with species-specific primers;
                     biotype: Non-B"
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      121 cgacgatctc tagcgggtct gagaggatag cccgccacac tggaactgag acacggtcca
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      241 atgccacgtg tgtgaagaag gccttcgggt tgtaaagcac tttcagcgag gaggaagcga
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      361 gccagcagcc gcggtaatac ggagggtgcg agcgttaatc ggaataactg ggcgtaaagg
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      541 tgcgtagata tctggaggaa taccggtggc gaaggcggcc ccctggagaa agactgacgc
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      661 cgatgtcgat ttggag
//
```

Appendix 11. The 16S rRNA sequence details of primary symbiont *Portiera aleyrodidarum* isolated from *T. vaporariorum* and deposited in NCBI GenBank.

Candidatus Portiera aleyrodidarum isolate WS1_AK_UK 16S ribosomal RNA gene, partial sequence

GenBank: KY457224.1

FASTA Graphics

Go to:

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LOCUS       KY457224                784 bp    DNA     linear   ENV 18-JAN-2017
DEFINITION  Candidatus Portiera aleyrodidarum isolate WS1_AK_UK 16S ribosomal
            RNA gene, partial sequence.
ACCESSION   KY457224
VERSION     KY457224.1
KEYWORDS    ENV.
SOURCE      Candidatus Portiera aleyrodidarum
  ORGANISM  Candidatus Portiera aleyrodidarum
            Bacteria; Proteobacteria; Gammaproteobacteria; Oceanospirillales;
            Halomonadaceae; Zymobacter group; whitefly endosymbionts;
            Candidatus Portiera.
REFERENCE   1  (bases 1 to 784)
  AUTHORS   Kraeem,A.A., Port,G. and Wolff,K.
  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 784)
  AUTHORS   Kraeem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2017) School of Biology, Newcastle University,
            Barras Bridge, Newcastle upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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                     /isolation_source="Glasshouse"
                     /host="Trialeurodes vaporariorum"
                     /db_xref="taxon:91844"
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                     /collection_date="Sep-2015"
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      721 aattcgatgc aacgcgaaga accttaccta ctcttgacat ccaaagtact ttccagagat
      781 ggaa
//
```

Appendix 12. The 23S rRNA sequence details of secondary symbiont *Arsenophonus* sp. isolated from *T. vaporariorum* and deposited in NCBI GenBank.

GenBank

Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate Ars_UK 23S ribosomal RNA gene, partial sequence

GenBank: KY243936.1

FASTA Graphics

Go to:

```
LOCUS       KY243936                447 bp    DNA     linear   BCT 25-APR-2017
DEFINITION  Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate
            Ars_UK 23S ribosomal RNA gene, partial sequence.
ACCESSION   KY243936
VERSION     KY243936.1
KEYWORDS    .
SOURCE      Arsenophonus endosymbiont of Trialeurodes vaporariorum
  ORGANISM  Arsenophonus endosymbiont of Trialeurodes vaporariorum
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Arsenophonus.
REFERENCE   1  (bases 1 to 447)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 447)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-NOV-2016) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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      121 gtgttgtgaa ctcgaagagt agggcgggac acgtgttatc ctgtctgaat atgggggtac
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      241 gcgaaaagaa ccccggcgag gggagtgaaa tagaacctga aaccgtgtac gtacaagcag
      301 tggaagcacc cgaaaggggtg tgactgcgta ccttttgtat aaggggtcag cgacttatat
      361 tctgtagcaa ggttaaccgc ataggggaga cgtagggaaa ccgagtctta actgggcgtt
      421 aagttgtagg gtatagaccc gaaaccc
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Appendix 13. The sequence details of the housekeeping gene (*ftsK*) of *Arsenophonus* sp. symbiont isolated from *T. vaporariorum* and deposited in NCBI GenBank.

GenBank

Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate ES1_4_Ars DNA translocase (flsK) gene, partial cds

GenBank: KY626171.1

FASTA Graphics

Go to:

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LOCUS       KY626171                335 bp    DNA     linear   ENV 06-FEB-2018
DEFINITION  Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate
            ES1_4_Ars DNA translocase (flsK) gene, partial cds.
ACCESSION   KY626171
VERSION     KY626171.1
KEYWORDS    ENV.
SOURCE      Arsenophonus endosymbiont of Trialeurodes vaporariorum
  ORGANISM  Arsenophonus endosymbiont of Trialeurodes vaporariorum
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Arsenophonus.
REFERENCE   1  (bases 1 to 335)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 335)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-FEB-2017) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
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      181 ccttgatcaa ggtggcgctg aatctttatt aggcatgggc gatatgcttt acttaccgcc
      241 taactcctct attccaattc gtgtccatgg cgcgtttgtt cgcgatcagg aagtgcatga
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Appendix 14. The sequence details of the housekeeping gene (*yaeT*) of *Arsenophonus* sp. symbiont isolated from *T. vaporariorum* and deposited in NCBI GenBank.

GenBank

Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate ES1_4_Ars outer membrane protein assembly factor (yaeT) gene, partial cds

GenBank: KY626172.1

FASTA Graphics

Go to:

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DEFINITION  Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate
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            partial cds.
ACCESSION   KY626172
VERSION     KY626172.1
KEYWORDS    ENV.
SOURCE      Arsenophonus endosymbiont of Trialeurodes vaporariorum
  ORGANISM  Arsenophonus endosymbiont of Trialeurodes vaporariorum
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Arsenophonus.
REFERENCE   1  (bases 1 to 382)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Population structure of glasshouse whitefly, Trialeurodes
            vaporariorum (Hemiptera: Aleyrodidae) and molecular identification
            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 382)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-FEB-2017) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
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                     EASNIRVGEALDRTKLANIEKGLED"
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Appendix 15. The sequence details of the housekeeping gene (*fbaA*) of *Arsenophonus* sp. symbiont isolated from *T. vaporariorum* and deposited in NCBI GenBank.

GenBank

Arsenophonus endosymbiont of Trialeurodes vaporariorum isolate ES1_4_Ars fructose-bisphosphate aldolase class II (fbaA) gene, partial cds

GenBank: KY626170.1

FASTA Graphics

Go to:

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ACCESSION   KY626170
VERSION     KY626170.1
KEYWORDS    ENV.
SOURCE      Arsenophonus endosymbiont of Trialeurodes vaporariorum
  ORGANISM  Arsenophonus endosymbiont of Trialeurodes vaporariorum
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Arsenophonus.
REFERENCE   1  (bases 1 to 587)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Population structure of glasshouse whitefly, Trialeurodes
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            of their symbionts in the UK
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 587)
  AUTHORS   Kareem,A.A., Port,G. and Wolff,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-FEB-2017) School of Biology, Newcastle University,
            Percy Street, Newcastle Upon Tyne NE1 7RU, United Kingdom
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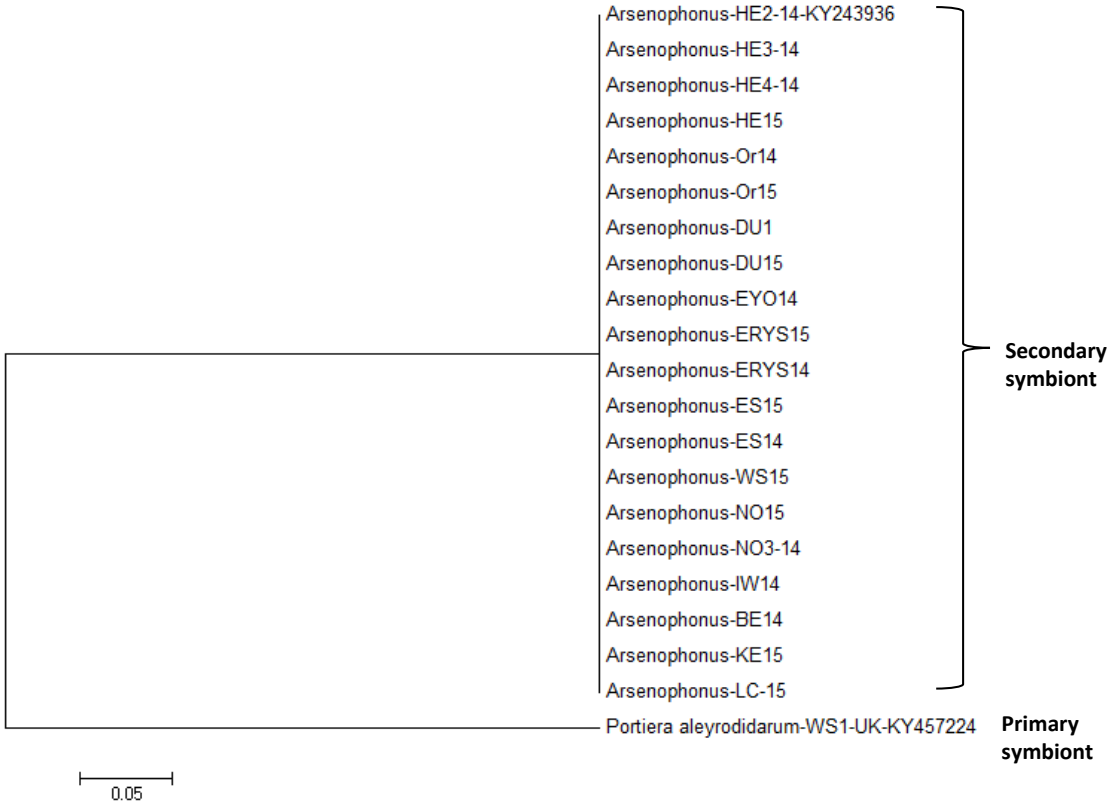
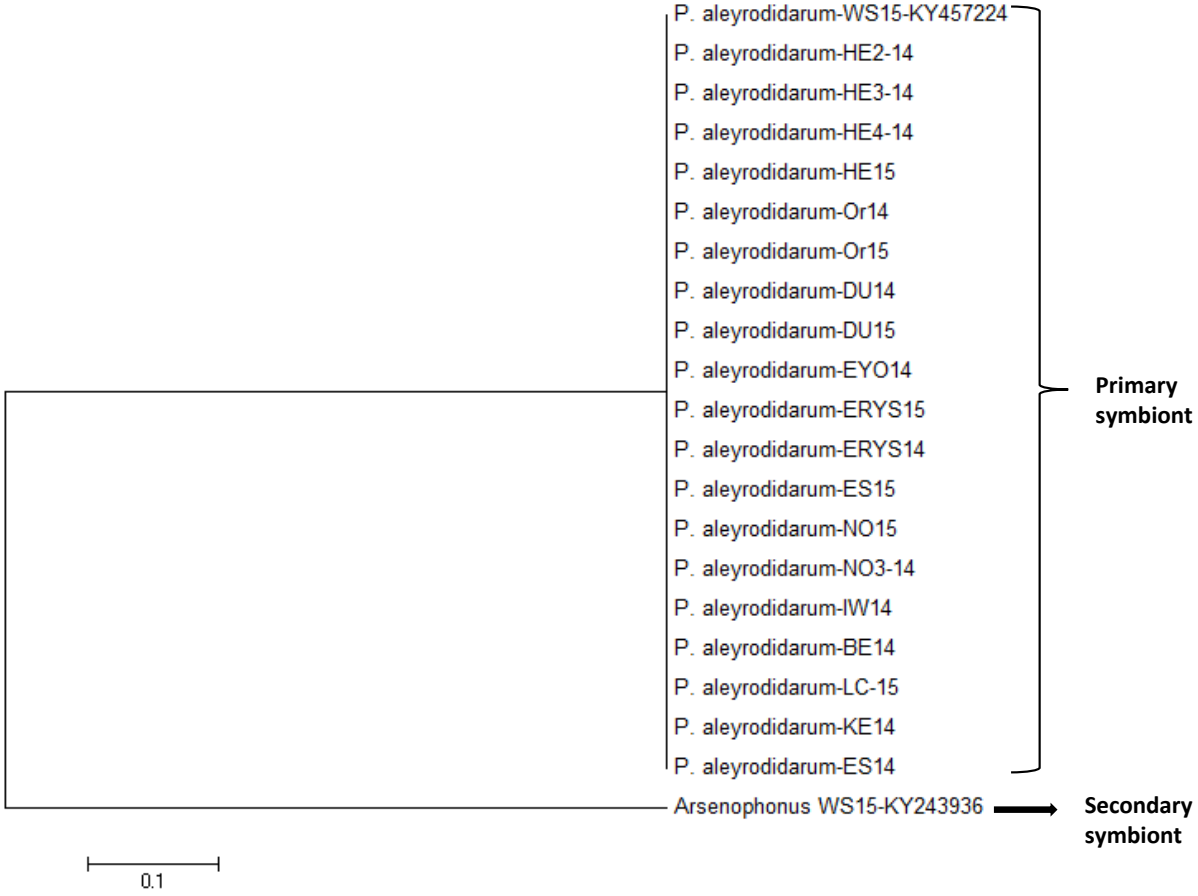
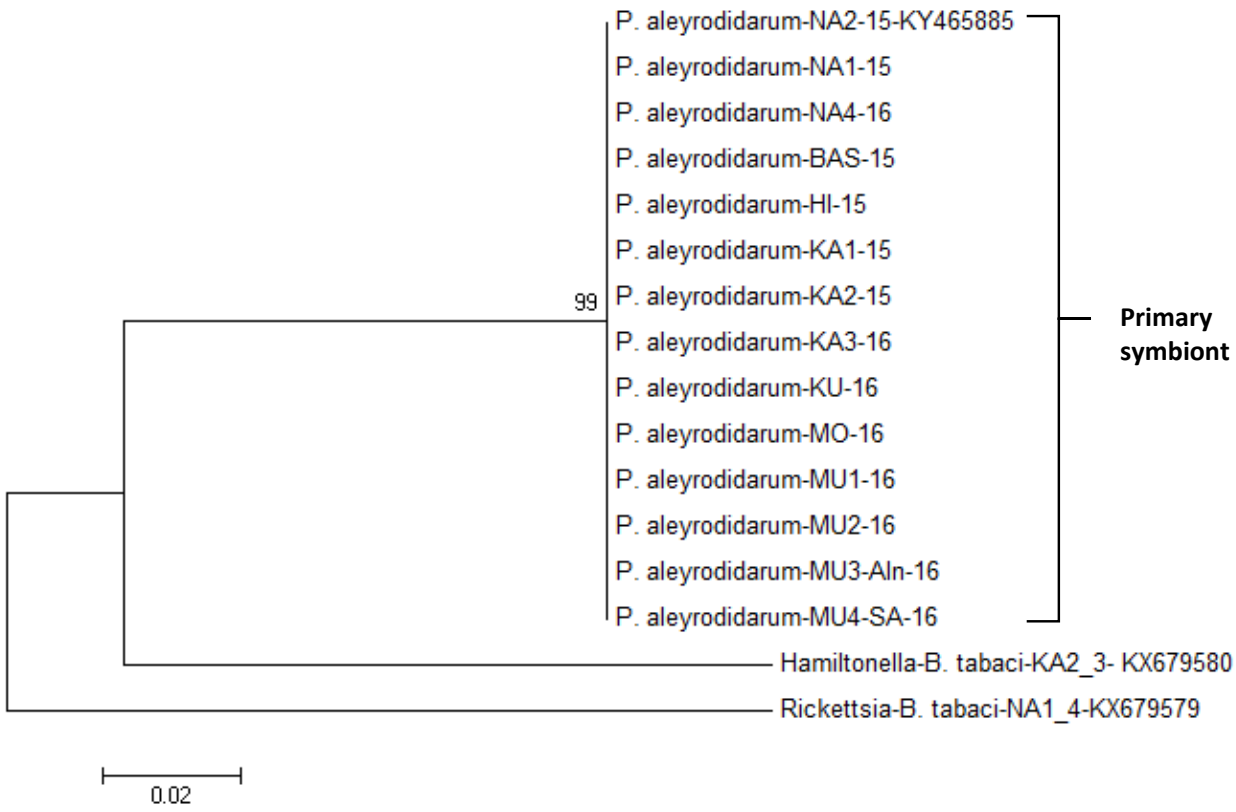
Appendix 16. Rooted molecular phylogenetic placements of secondary endosymbiont *Arsenophonus* sp. isolated from 20 *T. vaporariorum* populations based on bacterial 23S gene sequences. The analysis was based on 437 sites, and likelihood-ratio tests indicated the Kimura 2-parameter model (Kimura, 1980). *P. aleyrodidarum* was used as an outgroup**.** Phylogenetic analyses of 21 sequences with MEGA6 (Tamura *et al.*, 2013).



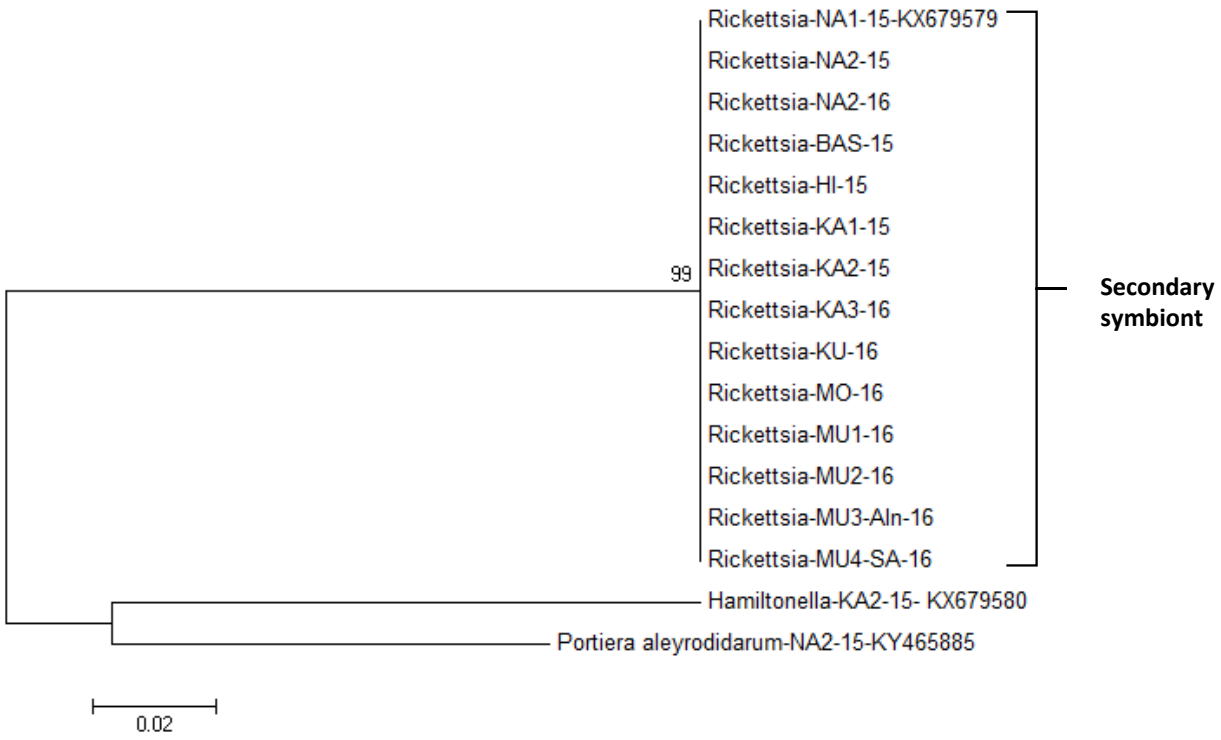
Appendix 17. Rooted molecular phylogenetic placements of primary endosymbiont *P. aleyrodidarum* isolated from 20 *T. vaporariorum* populations based on bacterial 16S gene sequences. The analysis was based on 437 sites, and likelihood-ratio tests indicated the Kimura 2-parameter model (Kimura, 1980). *Arsenophonus* sp was used as an outgroup**.** Phylogenetic analyses were for 21 sequences with MEGA6 (Tamura *et al*., 2013).



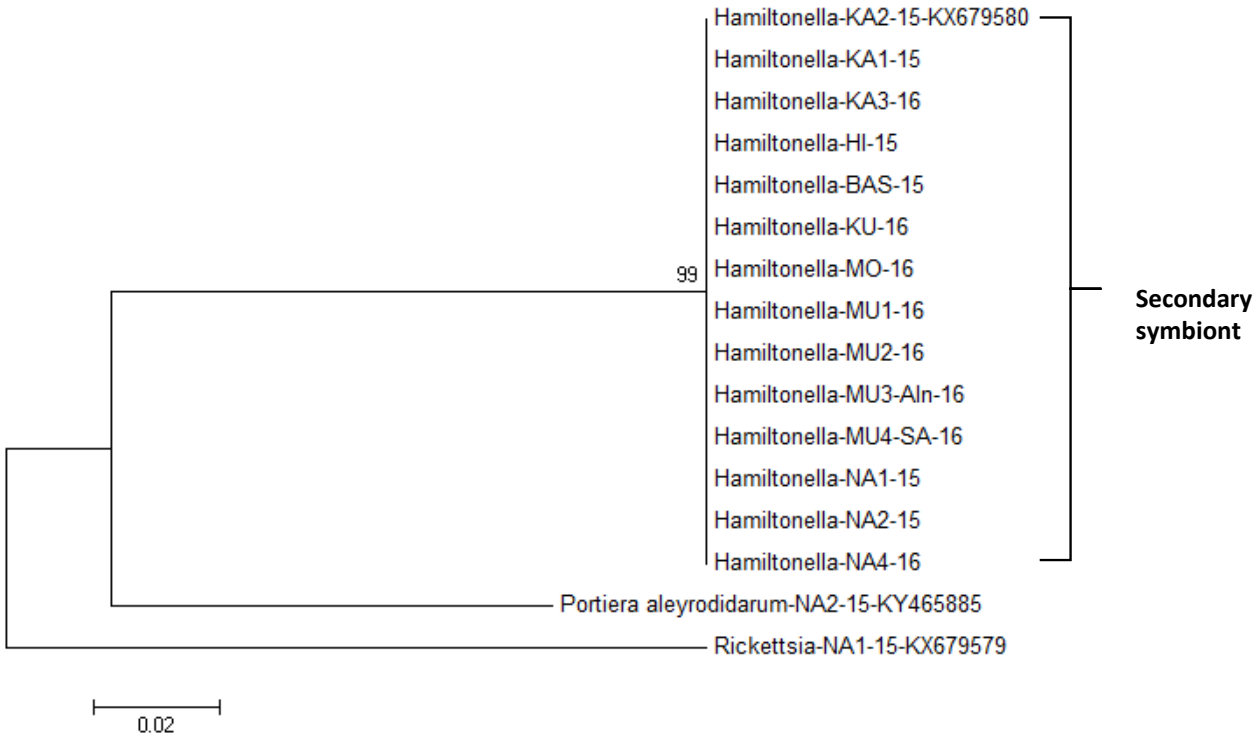
Appendix 18. Rooted molecular phylogenetic placements of primary endosymbiont *P. aleyrodidarum* isolated from 14 *B. tabaci* populations based on bacterial 16S gene sequences. The analysis was based on 518 sites, and likelihood-ratio tests indicated the Kimura 2-parameter model (Kimura, 1980). Secondary of *B. tabaci* were used as an outgroup. Phylogenetic analyses were for 16 sequences with MEGA6 (Tamura *et al*., 2013).



Appendix 19. Rooted molecular phylogenetic placements of primary endosymbiont *Rickettsia* sp. isolated from 14 *B. tabaci* populations based on bacterial 16S gene sequences. The analysis was based on 518 sites, and likelihood-ratio tests indicated the Kimura 2-parameter model (Kimura, 1980). *P. aleyrodidarum* and *Hamiltonella* sp. were used as an outgroup. Phylogenetic analyses were for 16 sequences with MEGA6 (Tamura *et al.*, 2013).



Appendix 20. Rooted molecular phylogenetic placements of primary endosymbiont *Hamiltonella* sp. isolated from 14 *B. tabaci* populations based on bacterial 16S gene sequences. The analysis was based on 518 sites, and likelihood-ratio tests indicated the Kimura 2-parameter model (Kimura, 1980). *P. aleyrodidarum* and *Rickettsia* sp. were used as an outgroup. Phylogenetic analyses were for 16 sequences with MEGA6 (Tamura *et al.*, 2013).



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