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ORIGINAL ARTICLE

Geographic distribution of *Bemisia tabaci* species in Sicily and patterns in facultative endosymbiont community composition

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Abstract

Bemisia tabaci is a group of cryptic and morphologically indistinguishable species able to cause severe damage to many agricultural and ornamental plants. Among the various species of this complex, the Mediterranean (MED) and Middle East Asia Minor 1 (MEAM1) species stand out as they are highly polyphagous, invasive, easily in acquiring resistance to many insecticides and reproduce quickly. Considering how (1) the geographic distribution of *B. tabaci* is changing with the ongoing global climate change and (2) no knowledge is presently available on phylogenetics of whitefly endosymbionts in Sicily, a population survey was conducted in the most important horticultural production areas of the island to assess the species composition within the *B. tabaci* complex, including their endosymbiont community and their geographic distribution. Our results show that the MEAM1 species and two mitochondrial variants of *B. tabaci* MED are present in pure or mixed populations, the MED Q1 presents the highest level of genetic variability within the MED populations in Sicily, having been found almost across the island and, MED Q2 was nearly exclusively detected in the Ragusa province. MEAM1 individuals were rare and exclusively detected in two localities in the Trapani province. The survey on endosymbionts community revealed the existence of a species specific composition, showing the lowest endosymbiont diversity in MED Q2 populations, typically characterized by only *Rickettsia*. Moreover, except for Portiera in MEAM1, no sequence variation was found within any endosymbiont sequence. Co-infection patterns of different endosymbiont species are discussed in the context of their needs for host cell metabolites. The present study defines an updated distribution map of cryptic species and phylogenetic groups of the *B. tabaci* complex and the first status on the endosymbiont community in Sicily; but it also represents the first report of whitefly endosymbionts sequences not only from Sicily but from Italy as a whole.

KEYWORDS
Aleyrodidae, Italy, population, survey, taxonomy, whitefly

1 | INTRODUCTION

Bemisia tabaci is a global sap-sucking insect pest causing severe damage to many agricultural crops and ornamental plant species (De Barro et al., 2011; Farina et al., 2022). The taxonomy of *B. tabaci* is an ongoing area of research, with the current consensus being that it represents a cryptic species complex consisting of over 40 morphologically indistinguishable species (Dinsdale et al., 2010; Tay et al., 2012). These species differ in life table parameters, geographic distribution, fecundity, and host range and preference, but also in insecticide resistance and virus transmission efficiency (Bedford et al., 1994; Brown et al., 1995, Milenovic et al., 2019). Originally identified as biotypes, today as cryptic species (De Barro et al., 2011), these whiteflies are in the ongoing process of speciation, as they over time evolve various adaptive traits as a response to their environment (Chu et al., 2012; Watanabe et al., 2019). Most commonly, *B. tabaci* species are discriminated on the basis of mitochondrial cytochrome oxidase subunit 1 (COI) sequence variability (Boykin & De Barro, 2014; De Barro et al., 2011; Dinsdale et al., 2010). Of many *B. tabaci* cryptic species, the Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) species stand out as fast reproducing, very invasive, highly polyphagous, globally distributed and resistant to many groups of once very effective insecticides (EFSA Panel on Plant Health (PLH), 2013). Additionally, the MED species shows a remarkable intraspecific variability and several mitochondrial variants have been identified, out of which the most common were designated as Q1, Q2, Q3, and ASL (Chu et al., 2008; Gueguen et al., 2010; Tsagkarakou et al., 2007).

The geographic distribution of different *B. tabaci* species is changing with the ongoing global climate change, aided by fast movement of plant and consequently insect material around the globe. This results in invasions of some species to new environments and displacements of others (Aregbesola et al., 2019). Although MED and MEAM1 can coexist in the same environment, during the last decade a continuous increase of MED infestations, resulting in the displacement of the MEAM1 species, has occurred in greenhouses, in open field crops, and in weed species (Bosco et al., 2006; Parrella et al., 2012; Simón et al., 2007).

In Italy, following the introduction of new begomoviruses, such as *Tomato Yellow Leaf Curl Virus* (TYLCV), *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV), and *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) (Bertin et al., 2018; Davino et al., 2006; Rapisarda & Tropea Garzia, 2002), *B. tabaci* species complex and its associated virus problems have gained greater importance. To better understand dynamics of *B. tabaci* species, various monitoring activities have been carried out in Italy during recent years, in areas where both the vector and virus species were noted and, in those ones, where the presence of *B. tabaci* was only occasional and no virus epidemics had ever occurred (Bertin et al., 2018, 2021). In Central Italy (Lazio region), zucchini plants infected with ToLCNDV and infested with both single and mixed populations of MED and MEAM1 were found (Bertin et al., 2018). In plants exclusively infested by the MED species in Lazio, the prevalent haplotype was MED Q2 (Bertin et al., 2018), as reported also by Parrella et al. (2014) in Campania and by Bertin et al. (2021) in two areas of Sicily, confirming how the spread of this haplotype is favoured by high temperatures typical of Southern Italy.

Species of *B. tabaci* complex are also characterized by a high diversity of bacterial endosymbionts (Brumin et al., 2020). Whiteflies live in obligatory symbiosis with *Candidatus* Portiera aleyrodidarum, a bacterium that synthetizes essential amino acids and carotenoids which are scarce in the phloem sap of the host plant (Santos-Garcia et al., 2012, 2015). Additionally, whitefly can, but not necessarily do, harbour additional, facultative endosymbiotic bacteria from the genera *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Hemipteriphilus*, *Rickettsia*, and *Wolbachia* (Kanakala & Ghanim, 2019). Their roles are considerably less explored compared to the obligatory endosymbiont and range from vitamin biosynthesis to potentially parasitic relationship. It has been hypothesized that they provide fitness benefits in a context dependent manner and that their diversity is at least partially responsible for the great adaptability and global success of *B. tabaci* whiteflies (Milenovic, Ghanim, et al., 2022). As such, whitefly endosymbionts are important modifiers of whitefly biology and together form one ecological unit, whitefly holobiont. Phylogenetic analysis of whitefly endosymbionts revealed that their taxonomy is sometimes non congruent with the taxonomy of their host, indicating a horizontal transfer of endosymbiont species between different whitefly species, and possibly other insects (Kanakala & Ghanim, 2019). The diversity of endosymbiont community has been extensively explored in some regions of the world (e.g., Israel), others are severely under sampled. In Italy, there are no published phylogenetic studies of whitefly endosymbionts to date.

Considering how geographic distribution of the *B. tabaci* species is changing, it is mandatory to conduct continuous monitoring. Considering the fact that there is no knowledge on phylogenetics of whitefly endosymbionts in Sicily (nor for the rest of Italy), a dedicated population survey has been realized in the most important Sicilian vegetable production areas. The aim was to assess the species composition within the *B. tabaci* complex, including their endosymbiont community and their geographical distribution.

2 | MATERIALS AND METHODS

2.1 | Sample collection

The study was conducted from April 2021 to November 2022. Samples of *B. tabaci* were collected by a mouth aspirator (John W. Hock Company) at 25 sites in Sicily on vegetable crops grown in both greenhouse and open field conditions, as well as on weeds (Table 1). The whitefly individuals were then stored in 99% ethanol at 4°C until the laboratory analysis. Each sample consisted of 3–70 specimens collected on diverse host plants and at distinct localities.

TABLE 1 Sampling Sicilian localities [town (province)], geographic coordinates (latitude, longitude) and host plants from which the samples were collected.

Locality	Geographic coordinates	Host plant
Vittoria (RG)	36.97134, 14.424505	*Solanum melongena* (L.)
Vittoria (RG)	36.905165, 14.439656	*Solanum lycopersicum* (L.)
Vittoria (RG)	36.905593, 14.482508	*Solanum melongena* (L.)
Vittoria (RG)	36.957562, 14.407787	*Solanum melongena* (L.)
Vittoria (RG)	36.983418, 14.431998	*Capsicum annuum* (L.)
Ragusa (RG)	36.831278, 14.495398	*Capsicum annuum* (L.)
Ragusa (RG)	36.828487, 14.479026	*Solanum melongena* (L.)
Santa Croce Camerina (RG)	36.811414, 14.47895	*Solanum melongena* (L.)
Marina di Ragusa (RG)	36.788314, 14.533365	*Capsicum annuum* (L.)
Ragusa (RG)	36.798317, 14.588599	*Solanum melongena* (L.)
Scicli (RG)	36.760296, 14.669167	*Cucumis sativus* (L.)
Santa Maria del Focallo (RG)	36.697643, 14.970866	*Conyza* spp. (Less)
Ispica (RG)	36.771684, 14.985896	*Capsicum annuum* (L.)
Ispica (RG)	36.771554, 14.979323	*Cucumis melo* (L.)
Gela (CL)	37.127696, 14.143832	*Solanum lycopersicum* (L.)
Gela (CL)	37.127665, 14.143848	*Solanum nigrum*
Gela (CL)	37.126001, 14.142878	*Citrullus lanatus* (Thunb.) Matsum. & Nakai
Sortino (SR)	37.122843, 14.968177	*Capparis spinosa* (L.)
Licata (AG)	37.121502, 13.877564	*Cucumis melo* (L.)
Campobello di Mazara (TP)	37.639937, 12.727814	*Sonchus asper* (L.) and *Solanum melongena* (L.)
Marsala (TP)	37.78784, 12.457519	*Phaseolus vulgaris* (L.)
Paceco (TP)	37.989567, 12.546775	*Cucumis melo* (L.)
Vittoria (RG)	36.967932, 14.479791	*Solanum melongena* (L.)
Caltanissetta (CL)	37.435092, 14.050988	*Solanum melongena* (L.)
Licata (AG)	37.1155656, 13.8640772	*Solanum lycopersicum* (L.), *Solanum melongena* (L.) and *Cucumis sativus* (L.)

2.2 | Molecular identification of *Bemisia tabaci* species and its endosymbionts

Whiteflies were individually removed from ethanol, briefly dried and DNA was extracted according to the protocol described in Milenovic, Gouttepifre, et al. (2022). Ten randomly selected, unsexed individuals were selected from each location (or less if <10 individuals were available), and DNA was extracted. Individual whiteflies were placed in 1.5 mL centrifuge tube containing 25 μL of lysis buffer (10 mM Tris–HCl pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween-20, 60 μg/mL Proteinase K) and homogenized using plastic pestle and vortex mixer. Crude lysates were spun down for 30 s at 10,000 *g* and transferred to a 96-well plate. Plates were incubated in a thermocycler for 50 min at 54°C for the optimal proteinase K activity, followed by 20 min at 80°C to heat inactivate proteinase K enzyme. Heated lid was set to 90°C. After cooling on ice, samples were diluted by adding 180 μL of PCR-grade H_2O to each well. Separate master mixes were prepared using Q5® Hot Start High-Fidelity 2× Master Mix (New England Biolabs) and a primer pair for whitefly *COX1* gene, and each of the endosymbionts as described in Milenovic, Gouttepifre, et al. (2022). To avoid human error in setting up over 1000 PCR reactions, Eppendorf epMotion 5073C (Eppendorf) automated pipetting system was programmed to distribute master mix and add DNA sample to each well. PCR conditions were as previously described. Following the PCR reaction, 5 μL of the reaction were used for gel electrophoresis using E-Gel™ 96 Agarose Gels, 2% (Thermo Fisher Scientific) with 12 min runtime. Images of the gels were taken using Syngene InGenius LHR2 Gel Imaging System (Syngene) with 400 ms exposure, aperture set to 2, and zoom adjusted to fill the frame. Images were processed using E-Editor 2.0 Software (Thermo Fisher Scientific).

Positive and inconclusive samples were identified visually based on the expected PCR product size and sent to Macrogen Europe BV for sanger sequencing in both forward and reverse direction. Raw sequencing reads were manually trimmed, and error corrected using Sequencher 5.4.6 software (Gene Codes). Unique sequences were identified, and BLAST search was performed at NCBI website using default settings.

Bemisia tabaci identity was determined by alignment to the mtCOI reference dataset (Boykin et al., 2017) followed by phylogenetic tree

construction (Figure S1) as previously described in Milenovic, Gouttepifre, et al. (2022).

Geographic distribution of *B. tabaci* species and their endosymbionts were visualized using QGIS 3.28.1 software. Base map is based on 'ESRI Terrain' map provided by the QuickMapServices 0.19.32 QGIS plugin. Chi-square test was performed using R (v4.3.1) on a contingency table of counts with sampled locations as rows and *B. tabaci* species (MEAM1, MED Q1, and MED Q2) as columns to test for random geographic distribution of *B. tabaci* species.

3 | RESULTS

The DNA was extracted from 210 whitefly individuals, out of which 204 produced a sequence of sufficient quality to determine the species. Results of the whitefly population survey show presence of two *B. tabaci* cryptic species, MED and MEAM1, with the MED representing over 92% of the analysed individuals. Within MED species, two major phylogenetic groups were detected, previously described as Q1 and Q2. All Q2 sequences in this study were identical to each other and identical to the NCBI GenBank accession MH205753.1. Similarly, MEAM1 sequences showed uniformity and identity with the previously described accession KR559508.1. Q1 sequences on the other hand were represented by eight unique sequences with various levels of similarity. These Q1 subgroups were arbitrarily numbered in the present study. Phylogenetic analysis reveals Q1_4 group as the most distant from other Q1 subgroups, while the others group into two branches with one branch consisting of closely related Q1_8, Q1_5, Q1_1, and Q1_7 and the other of closely related Q1_9, Q1_6, Q1_10. A total of 3 samples failed at various stages of the analysis, hence their identity could not be determined, and 3 samples had sequence long enough to be determined to belong to MED Q1 group, but too short to differentiate between eight different Q1 subgroups detected in the present study. A breakdown of number of samples detected for each group, as well as the best GenBank BLAST hit is presented in the Table 2.

Geographically, the detected species and phylogenetic groups show a non-random distribution (Figure 1). The visual observation was confirmed by chi-square test which rejected the null hypothesis of random distribution ($\chi^2 = 278.85$, $p = 5e{-}06$). MEAM1 samples were exclusively detected at the two localities in the Trapani province, while MED Q2 is almost exclusively detected in the Ragusa province, with only a few Q2 samples detected in the neighbouring areas. Q1 groups were detected across the island, with Q1_4 detections limited to the Ragusa province.

Whitefly endosymbiont composition was successfully determined for 200 individuals, or 98% of those with identified whitefly group. Individuals for which whitefly species was undetermined, or if any of the detected endosymbionts (by PCR product gel electrophoresis) failed to produce a sequence, were excluded from the analysis. A failure in sequencing might mean poor DNA quality or contamination, both of which could result in an overall biased result of endosymbiont composition. All detected endosymbionts showed identical sequences, with the exception of Portiera in MEAM1 whiteflies which showed a single nucleotide difference from Portiera sequences from MED whiteflies. Best NCBI GenBank BLAST hits for endosymbiont sequences are presented in the Table 3. Figure 2 showing endosymbiont composition across whitefly phylogenetic groups reveals a striking uniformity in endosymbiont composition and a clear link between endosymbiont composition and whitefly identity. As Portiera is expectedly present in all individuals, our study focuses on the facultative endosymbionts. MEAM1 whiteflies are characterized by harbouring *Rickettsia* and *Hamiltonella* (16/16 samples), while MED Q2 primarily harbours only *Rickettsia* (82/87 samples). Additionally, three specimens of MED Q2, harboured additional *Hamiltonella* or *Cardinium*, or lacked *Rickettsia*. Two MED Q2 individuals harboured *Wolbachia* in addition to *Rickettsia*. All nine MED Q1_4 individuals harboured *Wolbachia* and *Hamiltonella*, while 4/9 also harboured *Rickettsia*. This was the only Q1 population to harbour *Wolbachia*. Most Q1 samples belong to the group here named Q1_1, out of which 77/81 harboured *Rickettsia*, *Hamiltonella* and *Cardinium*. This subgroup was in fact the only one to harbour *Cardinium*. Single whitefly individuals missing either *Rickettsia*, *Hamiltonella*, or *Cardinium* were detected, as well an additional individual harbouring four endosymbionts (*Rickettsia*, *Hamiltonella*, *Cardinium* and *Wolbachia*). The other Q1 subgroups had only one or two individuals, making any conclusions, or even their existence, hard to confidently claim. Endosymbiont composition of these individuals is nevertheless presented in the Figure 2. Endosymbionts *Fritschea* and *Hemipteriphilus* were not detected in the present study.

Arsenophonus proved difficult to amplify in the present study using previously described and apparently commonly used primers. Efforts to amplify *Arsenophonus* resulted in secondary bands on the electrophoresis gel, and attempts to sequence the main product resulted in either extremely unreliable base calls or produced Portiera sequence. The issue was not resolved by varying primer annealing temperature. As a part of the troubleshooting process, we attempted to use strain-specific primers, designed for novel *Arsenophonus* strain described recently by Milenovic, Gouttepifre,

TABLE 2 Top BLAST hit sequences for 10 detected whitefly phylogenetic groups from this study, and their identity.

Group	GenBank ID	Identity
Q1_1	LN614545.1	Identical
Q2	MH205753.1	Identical
MEAM1	KR559508.1	Identical
Q1_4	HE863759.1	Identical
Q1_5	MH205752.1	Identical
Q1_6	MW604192.1	Identical
Q1_7	KX954191.1	Identical
Q1_8	MH205752.1	1 nt difference
Q1_9	MW604168.1	Identical
Q1_10	KX954191.1	1 nt difference

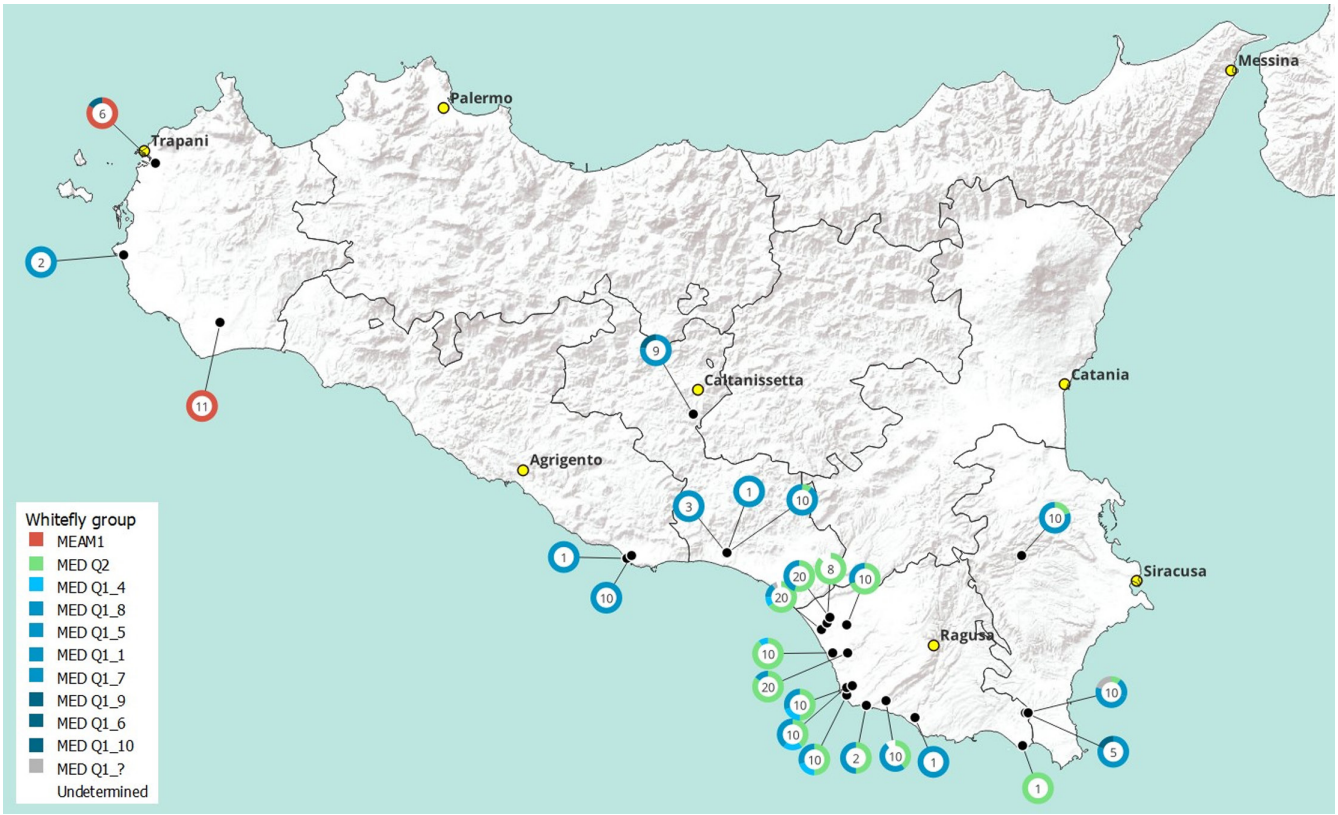

FIGURE 1 Geographic distribution of whitefly phylogenetic groups across sampling locations visualized using QGIS 3.28.1 software. Each black point represents one sampling location. The number in the callout shows the number of analysed samples, and the colours surrounding the number represent the proportion of different phylogenetic groups at each location. Groups that differ only by a single nucleotide are shown with the same colour.

TABLE 3 Top BLAST hit sequences for detected whitefly endosymbionts from this study, and their identity.

Endosymbiont	GenBank ID	Identity
Portiera MEAM1	CP007563.1	Identical
Portiera MED	CP016304.1	Identical
Hamiltonella	CP016303.1	Identical
Rickettsia	CP016305.1	Identical
Wolbachia	CP016430.1	3 nt difference
Cardinium	MH908678.1	Identical

et al. (2022), on a subset of 30 samples of the localities 3, 4, and 5 from the Table 1, which included nine Q1_1 individuals, one Q1_4, and 20 Q2 individuals. The results of this test reveal *Arsenophonus* presence only in Q2 samples, with the presence confirmed in 19/20 samples.

4 | DISCUSSION

The main horticultural production areas in Sicily are in the south and southeast where the greenhouse cultivation of tomato, eggplant, and pepper crops dominates, while the provinces of Caltanissetta, Agrigento, and Trapani are important production areas of melon, watermelon, and certain Solanaceae crops. In these areas, viral diseases are often responsible for serious reductions in crop yields, and among the viruses present, those transmitted by whiteflies on cucurbits and Solanaceae are the most common (Davino et al., 2006; Rapisarda & Tropea Garzia, 2002).

Over the years, it has been observed that the viral outbreaks in Sicily are closely correlated with the distribution of *B. tabaci* (Bosco & Caciagli, 1998). Furthermore, incidence of high densities of this pest on crops have been linked to gradual shift in its populations especially where *B. tabaci* MEAM1 was displaced by *B. tabaci* MED (Bertin et al., 2021). Previous studies have shown that in Italy the MEAM1 species of this whitefly is present at lower levels, while the MED species is gradually adapting to outdoor conditions and frequently spreads from protected cropping systems to crop fields, confirming that the geographical and genetic status of *B. tabaci* populations continues to change rapidly (Bertin et al., 2018; Bosco & Caciagli, 1998).

Our results show how two *B. tabaci* MED mitochondrial variants (Q1 and Q2) as well as the MEAM1 species are present in single or mixed populations among whitefly samples collected in some of the main cultivated areas in Sicily. Moreover, presence of MED Q2 primarily in the intense greenhouse production area in the Ragusa province support the findings of previous studies conducted in southern Italy and other Mediterranean countries, according to

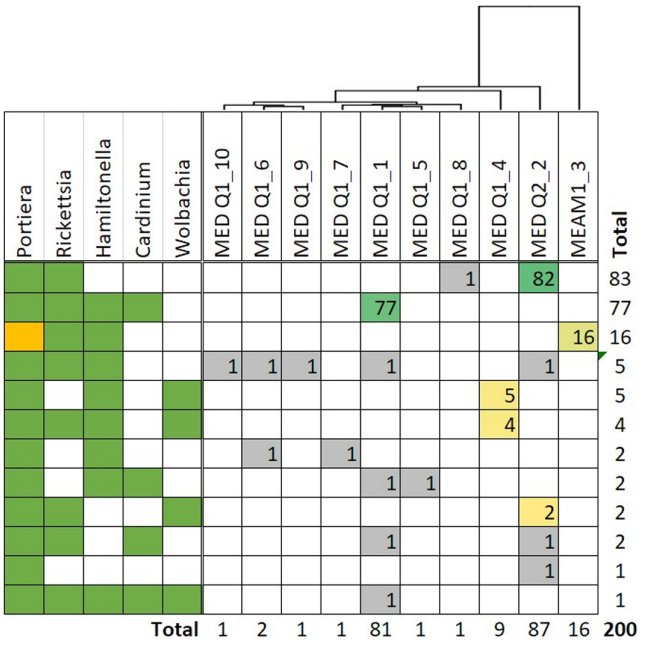

FIGURE 2 Endosymbiont composition of each of the detected whitefly phylogenetic groups. Green boxes on the left side signify the presence of the corresponding endosymbiont, while the yellow box signifies presence, but the sequence is distinct from the rest in the same column. Boxes with numbers show the number of detected individual whiteflies with the corresponding whitefly-endosymbiont combination, coloured from high to low using a green-yellow-grey gradient. Right and below the matrix are the sums per endosymbiont combination and whitefly group, respectively. Above the matrix is a maximum likelihood phylogenetic tree of the whitefly phylogenetic groups.

which MED Q2 variant is better able to adapt to the high summer temperatures that characterize greenhouses (Bertin et al., 2021; Parrella et al., 2014; Tsagkarakou et al., 2007).

The present survey also confirms that MED Q1 presents the highest level of genetic variability within the MED populations of Sicily, as MED Q2 specimens belonged to only one phylogenetic group, as indicated in several studies (Barboza et al., 2019; Chu et al., 2006, 2012; De Barro & Ahmed, 2011; Gauthier et al., 2014). This level of genetic differentiation, which differs between the two mitochondrial groups, may depend on the fact that MED Q1 was originally present in the western Mediterranean countries, whereas MED Q2 has infested this area more recently (Bertin et al., 2018; Gauthier et al., 2014). Alternatively, it is possible that multiple Q1 variants were introduced to the region. The low number (one or two individuals, Figure 2) of detected individuals for most MED Q1 groups might represent a PCR or sequencing error, therefore, more samples of these populations are needed to definitively claim their presence. In the case of here labelled MED Q1_1 and MED Q1_4 however, the confidence is high due to much larger number of detected individuals, greater sequence differences as also reflected in distances visible from the phylogenetic tree, and distinct endosymbiont composition between the two groups. The present study did not establish any clear link between whitefly biotype and host plant species.

Study of the endosymbiont composition revealed how each whitefly group harbours a distinct set of endosymbionts, with only a few exceptions. Lowest endosymbiont diversity was observed in the Q2 population which was characterized typically by only harbouring *Rickettsia* of the facultative endosymbionts. In fact, *Rickettsia* was the most detected endosymbiont presents in over 90% of all analysed whitefly individuals, which might mean that the bacteria *Rickettsia* provides fitness benefits in the environmental conditions, regardless of the whitefly genetic background. Previous studies have shown how *Rickettsia* can affect the adaptive capacity of *B. tabaci* (Brumin et al., 2011) and manipulate the defence patterns of its host plants (Shi et al., 2021). The effects of *Rickettsia* endosymbiont on the fitness of *B. tabaci* are far less clear, with different studies reporting both strong benefits and no measurable effects (Bockoven et al., 2020; Cass et al., 2015). At the same time, *Rickettsia* is known for its dynamic changes in frequency in whitefly populations over time (Bockoven et al., 2020). The group Q1_4 was the only one where absence of *Rickettsia* was detected with high confidence. Curiously, this population was the only one with apparently fixed presence of previously unreported strain of *Wolbachia*. This supports the previous observations of rare *Rickettsia-Wolbachia* coinfection, for which the working hypothesis is the fact that both bacteria are dependent on and would compete for the NAD^+ from the host cell (Opatovsky et al., 2018; Zchori-Fein et al., 2014). Except for Portiera in MEAM1, no sequence variation was found within any endosymbiont sequences. This uniformity not necessarily expected as several strains of facultative endosymbionts are known to exist. At the first glance, the uniformity might have resulted from horizontal transfer of endosymbionts between whitefly phylogenetic groups. This is supported by the previously shown evidence for the possibility of horizontal transfer (Milenovic, Ghanim, et al., 2022), as well as by the presence of mixed whitefly populations at the same locality. However, this is not possible to claim with certainty and strong natural selection for the most beneficial or the most infective strains is equally likely based solely on the present data. Presence of *Cardinium* only in Q1_1 population, in coinfection with *Hamiltonella* goes against the previously posed hypothesis that these two endosymbionts are in competition, with *Cardinium* displacing *Hamiltonella* (Zhao et al., 2018). The distinct endosymbiont communities coincide with differences in whitefly phylogeny, which makes it impossible to hypothesize if observed differences in infestation patterns are resulting from whitefly genetic background or its endosymbionts, which further corroborates the whitefly holobiont paradigm, which calls for treating whiteflies and their endosymbionts as one ecological unit (Milenovic, Ghanim, et al., 2022).

The present study defines a new map of the distribution of cryptic species and phylogenetic groups of the *B. tabaci* complex and the first state on the endosymbiont community in Sicily, where the strong presence of MED species causes several concerns due to its high insecticide resistance and efficient virus transmission (Bertin et al., 2021; Pan et al., 2012; Sánchez-Campos et al., 1999). Much is still known about the nature of the symbiosis between whiteflies and their facultative endosymbiont community, but much remains to be investigated. This research adds to the body of knowledge about their geographic distribution, coinfection patterns, and association status with different

whitefly species/phylogenetic groups for up to now unsampled region. The present study is the first to obtain sequences of whitefly endosymbionts not only from Sicily, but from Italy as a whole.

AUTHOR CONTRIBUTIONS

Milan Milenovic: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Giuseppe Eros Massimino Cocuzza:** Conceptualization; resources; writing – review and editing. **Pompeo Suma:** Conceptualization; investigation; resources; writing – review and editing. **Alessia Farina:** Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Novel sequences generated for *Bemisia tabaci* mitochondrial cytochrome oxidase subunit I (mtCOI) in this study have been deposited in the National Center for Biotechnology Information (NCBI) Nucleotide database under the accession numbers OR418428 (https://www.ncbi.nlm.nih.gov/nuccore/OR418428) and OR418429 (https://www.ncbi.nlm.nih.gov/nuccore/OR418429) for sequences MED Q1_8 and MED Q1_10, respectively. Additionally, the novel *Wolbachia* sequences obtained in this research have been made available under the accession number OR418427 (https://www.ncbi.nlm.nih.gov/nuccore/OR418427). Other sequences generated in the present study are identical to existing sequences in NCBI Nucleotide database, as described in Tables 2 and 3.

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SUPPORTING INFORMATION

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