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Studies on Whiteflies Living on Herbaceous Plants and Their Parasitoids in Tropical and Sub-Tropical Environment through Combined Morphological, Molecular and Geostatistical Approaches

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CONTENTS

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Studies on whiteflies living on herbaceous plants and their parasitoids in tropical and sub-tropical environment through combined morphological, molecular and geostatistical approaches

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Abstract

Background: In tropical and sub-tropical regions, whiteflies are common pests causing severe losses on food-crops and ornamental plants. Many of these species are tropical in origin, nowadays adapted also to sub-tropical and temperate regions. Their control involves great effort and attention, as they have also been a threat to food-security in several tropical countries. Whiteflies have been well documented in the sub-tropics; however, a critical issue at present is the lack of information regarding their spatial distribution or abundance. Moreover, scarce or outdated information is available about the current status of their parasitoid fauna. On the contrary, in several tropical regions no or incomplete information is available regarding the diversity and distribution of whiteflies and their parasitoids. The value of a geographical perspective to harmful pests and their control has long been recognized. However, it requires several steps such as identifying correctly the species occurring in a given area, studying their spatial diversity and distribution, producing maps and keeping these data updated.

Methods: This study combined morphological, molecular and geostatistical methods to assess the current diversity and distribution of the whitefly and parasitoid fauna occurring on herbaceous plants in a subtropical environment (Sicily) and in a tropical one (north-western part of Tanzania).

Generally, morphological techniques were adopted to identify specimens. Moreover, molecular markers where used to confirm doubtful or cryptic species. The phylogenetic relationships were then reconstructed through Bayesian analysis. A noteworthy aspect of this thesis is that in both tropical and sub-tropical environments, various bio-ecological aspects have been taken into account in the aim to understand which factors influence the spread of common whitefly pest species, such as the species of *Bemisia tabaci* group. For this reason, diversity and distributional information of both whitefly and parasitoid species was first accumulated to build a database. The database was then analyzed by Geographical Information System for (i) mapping richness and abundance, (ii) modeling the potential distribution of a pest species and (iii) revealing zones for parasitoid reserve selection. Finally, we showed how elevation gradient can be an additional instrument to study the cryptic species, as in the case of *B. tabaci* species complex.

Principal findings: In Sicily, a measure of the current status of whitefly and parasitoid fauna was given and the species richness and evenness were mapped. Nine whitefly species were identified on herbaceous plants and these include *Aleurolobus marlatti*, *Aleurothrixus floccosus*, *Aleyrodes elevatus*, *Aleyrodes proletella*, *Bemisia afer*, *Bemisia tabaci*, *Dialeurodes citri*, *Trialeurodes lauri* and *Trialeurodes vaporariorum*. Moreover, three cryptic species were identified in the *B. tabaci* species complex: *B. tabaci* Mediterranean, *B. tabaci* Middle East-Asia 1 and *B. tabaci* Italy .The study revealed aggregated spatial distribution for the two major pests in the region, *B. tabaci* (*s.l.*) and *Trialeurodes vaporariorum,* in cultivated lands along the southern and eastern coasts. On the contrary, no aggregated spatial distribution was observed for these species in natural or semi-natural areas or nearby surroundings. In the latter areas their population size decreased sometimes reaching almost nil. Modelling the potential distribution of these major whitefly pests at regional scale revealed the main suitable areas where they can occur. Contrary to previous studies, a striking finding in this study was the replacement of the exotic invader *B. tabac*i Middle East Minor 1 by the *B. tabaci* Mediterranean species, which is at present the predominant species. Another important finding of this research was the fact that the elevational gradient functions as a filter, selecting the species of *B. tabaci* group. In mild altitude, the three *B. tabaci* species were found to occur contemporarily whereas *B. tabaci* species Italy seemed to be the only one adapted to high

gradient. As for the parasitoid fauna, nine species were identified to parasitize the various whitefly species collected. *Encarsia pergandiella, En. lutea* and *Erermocerus mundus* were widespread. On the contrary, other species such as *En. formosa, En. inaron* and *En. tricolor* exhibited a narrow distribution range. A reserve selection model permitted to identify two main areas that seem to function as reservoir for whitefly parasitoids.

In Tanzania the diversity of whitefly species infesting cassava and other herbaceous plants was investigated. Ten whitefly species were identified in the cassava growing regions which include *Aleurodicus dispersus*; *Aleurothrixus floccosus*; *Bemisia tabaci* species group (*B. tabaci* Sub-Saharan Africa 1, *B. tabaci* Mediterranean and *B. tabaci* Indian Ocean); *Bemisia afer*, *Bemisia* sp. (formerly *Asterobemisia* sp.), *Dialeurodes citri*, *Paraleyrodes bondari* and *Trialeurodes vaporariorum*. As the study area is affected by super-abundant *B. tabaci* populations, the potential distribution of this species was modelled. The model revealed the presence of suitable areas not only in the study area but also extending further to neighbouring countries such as Zambia. Moreover, the overlay of this model with environmental data showed clearly the existence of a broad and unsuitable area serving as an environmental barrier and avoiding the spread of super-abundant population to the south-eastern part of the country. Six *Encarsia* species, four *Eretmocerus* species and one *Cales* species were identified in the surveyed areas, adding more species to the previous list of parasitoids recorded in East-Africa and leading to the discovery of a probable new species of *Eretmocerus*. For the first time in Tanzania, this study recorded *En. mineoi* and *En.* sp. pr. *circumsculpturata*. The occurrence of whitefly parasitoids in the study areas in north-western Tanzania collided with that of whiteflies. However, parasitoids have been detected at very low numbers, showing the occurrence of a relatively scarce natural enemies complex.

Conclusions: The research presented here documents the whitefly and parasitoid fauna colonizing herbaceous plants in two sites chosen as case studies for respectively tropical and sub-tropical regions, focusing on the common species that occur in both environments and that represent a threat for agriculture. The combination of morphological, molecular and bio-ecological traits was useful in understanding the diversity and distribution of these insects.

Keywords: Aleyrodidae, Africa, Aphelinidae, *Bemisia tabaci*, Bio-ecological, *Cales*, COI, Cryptic, *Encarsia*, *Eretmocerus*, GIS, Mediterranean, Sicily, Spatial analysis, Survey, Tanzania.

LIST OF FIGURES

LIST OF TABLES

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Chapter 1: INTRODUCTION

1.1 WHITEFLIES

1.1.1 Generalities

Whiteflies are small sup-sucking insects living on herbaceous and woody plants. They belong to the systematic order Hemiptera, suborder Sternorhynca, which contains also aphids, psyllids and scale insects; all of which suck sap from leaves. Within this insect group, whiteflies are ascribed to the family Aleyrodidae. Martin & Mound (2007) published a checklist of the World that includes 1,556 species of whiteflies, distributed into 161 genera. The family Aleyrodidae is subdivided into three subfamilies: Aleurodicinae, Aleyrodinae and the controversial Udamoselinae (Mound & Halsey, 1978; Martin & Mound, 2007). Among them, Aleyrodinae is the largest subfamily containing around 140 genera. Many of the economically important pest species belong to this group (Martin & Mound, 2007). The Aleurodicinae contains 20 genera and 130 described species. Between the two subfamilies, Aleurodicinae is reputed to be more primitive. Whitefly genera and species are described and distinguished by their pupal case (Martin, 1987). Unfortunately, there are not enough valid morphological characters to distinguish whitefly species through the adults, whereas, pupal case offer many characters to aid in the identification of whitefly species (Russell, 1948; Martin, 1987). In the literature, many morphological keys have been published with the aim to aid their identification. Nowadays, many of them are related to whiteflies considered as pests of particular crops. Others have to be searched in papers describing the local whitefly fauna of specific countries. However, previous studies demonstrated how the host plants might affect the morphology of the pupal case in some whitefly species (Mound, 1963). So, appropriate key were re-designed and added to the previous ones. In reference to that, when using the morphological keys, it is important to consider not only the puparia but also the host plant and leaf morphology. Similar to other insect groups, during last decades, a new concept started to be dominant also in whitefly taxonomy: "biotypes". This term is almost strictly related to *Bemisia tabaci* (Gennadius) at the moment. Initially, it was assigned when scientists noted that, within this species, the behaviour of an "invasive" population coming from other countries was quite different from the one shown by the indigenous. No morphological differences were present between the indigenous and the invasive population; but striking differences occurred in host range, their utilization and fitness (Bird, 1957; Brown *et al.*, 1992; Bedford *et al.*, 1993; Costa *et al.*, 1993). Then, even in a mixed population, the probability of mating interaction was very low. In recent studies Liu *et al.* (2012) clarified the pattern of reproductive interaction between different "population groups" of *B. tabaci* (whatever they can be considered - biotypes, genotypes or putative species) and how they are reproductively incompatible. Reproduction is usually sexual in whiteflies, with fertilized eggs producing females and unfertilized eggs producing males (arrhenotokous parthenogenesis); unmated females lay only haploid eggs. In few species [or sometimes only in peculiar geographic populations of normally bisexual species, as in the case of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood)], unfertilized eggs produce only females (thelitokous parthenogenesis). Whiteflies have six life stages in their life-cycle: the egg; the first instar or crawler; the second and third instars nymphs, which are sessile; the fourth instar nymphs, being the last young stage, where the adult insect develops inside and which is therefore referred to as pupa or puparium. After the emergence of the adult, the moulted cuticle of the fourth instar nymph is called a "pupal case." Whitefly eggs are usually attached to the bottom part of leaves by a pedicel, that adsorb water from the plant. Eggs are often laid in circles or arcs and egg batches are frequently conspicuous because of a dusting of white wax. First-instar nymphs usually crawl few centimetres in search of feeding area before they settle near the eggs from which they hatch. In most species, development occurs on the underside of leaves. After the first moult, the nymphs become immobile and cannot move. During the fourth instar, feeding ceases and the insect transforms into the winged adult. Male and female whiteflies look similar, except for the genitalia, but males are often smaller than females. Often, body and wings of the whiteflies are dusted with a powdery wax, which is produced by paired wax plates on the bottom side of the abdomen and thus adults most commonly appear whitish or greyish even if the body colour is yellow, brown, red or darkly sclerotized. In both nymphs (of all instars) and adults, the anal pore opens in a special dorsal pit, near the posterior end of the abdomen, called vasiform orifice, which comprises a depression, a dorsal flap called operculum and a tongue-shaped "lingula" that is used to flick away each droplet of anal excreta, the honeydew. Puparia are mostly oval or elongate-oval, 0.5–2mm long, their colour varying from transparent to white, brown, or black. Waxes contribute to the puparia protection and may be invisible or highly ornate, white or greyish, but physical colour (iridescence) may be surprisingly blued, turquoise, green, or pearly. Visible wax may form dorsally and/or as a marginal fringe. Whiteflies have complex host-finding and host-orientation behaviours, at least involving attraction to particular colours, especially yellow or yellow-green. They feed on plant vascular tissues and all feeding stages produce honeydew, which in few whitefly species attracts ants.

1.1.2 Economic importance

Whiteflies damage the host plants by their feeding activity and some species are known to transmit viruses, which can lead to devastating losses. The most serious Aleyrodidae pests affect orchards, protected and outdoors crops, and some ornamentals. In agricultural crops, the impact of whiteflies may cause reduction of harvestable products (Byrne *et al.*, 1992). The most famous whitefly is *Bemisia tabaci*, which occurs mainly in tropical and subtropical regions extending up to the temperate warm regions. *B. tabaci* is vector of 111 plant viruses assigned to the genera *Geminiviridae*, *Closteroviridae and Potyviridae* (Jones, 2003). Around the world, several dramatic events caused by this whitefly are reported. In USA in 1991, it was estimated to have caused combined losses of 500 million US$ to the winter vegetable crops (Perring *et al.*, 1993) through feeding damage and virus transmission. During 1990s, million ha of cotton have been destroyed in Pakistan by the cotton leaf curl geminivirus (CoLCV) (Mansoor *et al.*, 1993). In Africa, *B. tabaci* transmits viruses causing two devastating diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Both diseases caused losses exceeding 1 billion US$ annually (Thresh *et al.*, 1997; Legg *et al.*, 2006). In Africa, losses over 50% were reported from affected countries (Legg *et al.*, 2006). Oliviera *et al.* (2001) described the status of *B. tabaci* around the world. In Latin America more than twenty begomoviruses have been reported (Polston & Anderson, 1997) and many crops have been seriously damaged by a *B. tabaci*-transmitted virus (Morale & Jones, 2004). Recently, new begomoviruses have emerged in Brazil (Ribeiro *et al.*, 2003) spreading to the nearby countries. In the same genus *Bemisia, B. tuberculata* Bondar transmits viruses causing diseases such as cassava frog skin disease (CFSD) and common cassava mosaic disease. In Mediterranean countries such as Italy, Spain, France, Greece and many others, severe *B. tabaci* infestations have been reported on protected crops and open field cultivations (Patti & Rapisarda, 1981; Dantsing & Shenderovska, 1988; Traboulsi, 1994). Serious threats by *B. tabaci* are also recorded from China (Rumei, 1996). In many parts of the world, invasive *B. tabaci* populations predominate displacing the local ones (Jiao *et al.*, 2013). However, the magnitude of threats caused by *B. tabaci* transmitted-viruses can vary with crops, season and localities. Apart from *B. tabaci*, many other whitefly species occur in different agricultural systems and are considered important pests around the world. Martin (1987) provided an identification guide to 46 common whitefly pest species of the world, infesting economic plants. Among them, the genus *Trialeurodes* Cockerell includes three species know to transmit viruses. These are *T. vaporariorum* (Westwood), *T. abutiloneus* (Haldeman) and *T. ricini* (Misra). *T. vaporariorum*, called also the greenhouse whitefly, has been considered an economically important insect pest for many years. This whitefly has a cosmopolitan distribution. In temperate countries, it is considered as a key pest for many crops: egg plant, cucumber, beans, sweet peppers, tomatoes and a large number of ornamentals, including chrysanthemum, poinsettia, primula and species of *Fuchsia*, *Gerbera*, *Pelargonium* and *Solanum* (Anon., 2001a). Important viruses transmitted by *T. vaporariorum* in the European–Mediterranean region are beet pseudo-yellows virus (BPYV), which affects cucumber, melon, lettuce and sugar beet; tomato infectious chlorosis virus and tomato chlorosis virus (TICV and ToCV), both affecting tomato. The last two viruses of tomato are in the EPPO Alert List (Anon., 2001b). T. *abutiloneus* has been shown to transmit sweet potato chlorotic stunt virus (SPCSV) and ToCV. Both viruses are present in the European–Mediterranean region where T. *abutiloneus* does not occur; they are probably transmitted by other whitefly vectors, which ensure their spread. *T. ricini* has been documented as a vector of TYLCV in experiments in Egypt (Idriss *et al.*, 1997). Other *Trialeurodes* species are considered as noxious due their direct damage. This is the case of *Trialeurodes packardi* (Morrill), a key pest of cultivated strawberry in the United States. The spiralling whitefly *Aleurodicus dispersus* Russell is another dreaded whitefly, whose common name derives from its way of laying eggs in characteristic spiral patterns. It is highly polyphagous, with a wide host range covering more than 480 plants belonging to 295 genera of vegetables, fruits and ornamentals trees (Srinivasa, 2000). Important crops attacked by this pest are citrus, avocado, guava, plantain, banana, coconut, soybeans, cassava and stone fruit (John *et al.,* 2007). It is also reported as major threat to banana, other tropical fruit trees, vegetables and ornamental plants in northern Queensland (Mware *et al.*, 2010). Losses of 80% in fruit yield have been recorded in guava in Taiwan (Wen *et al.*, 1995). Heavy infestation by this whitefly also caused over 50% yield losses in cassava (Geetha, 2000). Several economically important whiteflies infest *Citrus* in many countries of the world: *Dialeurodes citri* (Ashmead), *Aleurothrixus floccosus* (Maskell), *Parabemisia myricae* (Kuwana), *Aleurocanthus spiniferus* (Quintance) and *Aleurocanthus woglumi* (Ashby). The appearance of *A. floccosus* in Mediterranean region dates back to the mid-20th century (Grandi, 1951; Della Beffa, 1961) and it has been for many years one of the most important citrus whiteflies in this area (Rapisarda & Patti, 1981; Uygun *et al.*, 1990; Rapisarda *et al.*, 1996). Its presence has also been recorded on other host plants outside the family of Rutaceae (Arzone & Vidano, 1990). *A. floccosus*, also called woolly whitefly, sucks phloem sap, causing leaves to wilt and drop when population becomes abundant. Large infestations, causing production of conspicuous amounts of honeydew, can result in the blackening of entire trees. This can reduce the photosynthesis, decreasing the fruit size. Honeydew and sooty mould can also affect the fruits. If the infestation and dust is high, then the harvest becomes slow and owners are hesitant to harvest heavily contaminated fruits. The bayberry whitefly, *P. myricae*, produces sticky honeydew on leaves and stems, developing black sooty mould fungi, and giving the foliage (even the whole plant) a sooty appearance when occurring in dense population. The honeydew may attract ants and heavy infestation and associated sooty mould development may cause defoliation. *P. myricae* is considered to be one of the six most injurious whitefly pests (Onillon, 1990). Rose & Rosen (1991) described it as serious pest causing damage to citrus. It was a noxious pest of citrus in Turkey (Sengonca *et al.*, 1993) and in Israel, until it was controlled (Swirsky *et al.*, 1985). In Florida, *P. myricae* has been recorded damaging citrus seedlings when the natural balance was disturbed by the use of chemicals (Hamon *et al.*, 1990).

However, only a few out of the many known whitefly species have been reported as economically important on various crops (Onillon, 1990). Nevertheless, unexpected whitefly species may become new emerging threat sometimes. This was the case of *Aleurocybotus occiduus* Maria and its outbreak on rice in Chitwan valley, Nepal. Though it was formerly not reported as an insect pest of rice, this whitefly infested thousands of hectares, causing vast areas of reddish-yellow rice field (Pokhrel & Thapa, 2011). Cassava, rice and many fresh vegetables or fruits are not only important economic world crops but some of them are important for food security of entire population. For example, cassava plays a key role in food security thanks to its adaptability to marginal conditions in subsistence farming. Rice is one of the oldest cultivated crops on earth and more than 90% of the world rice is grown and consumed in Asia (Narayanswamy, 2002). This means that if its production is compromised it is not only a problem of income but also a life-and-death struggle.

1.1.3 Evolution of whiteflies: An Overview

The evolution of insects dates back to more than 300 million years ago. Insects are the most diverse and dominant animals of life on Earth (Grimaldi, 2005) and data on their ages of lineages, radiations and extinctions can be provided by their fossils, where they are well preserved and compressed not only in rocks but also in carbon, phosphates, other minerals and especially in amber, being the most abundant fossils (Grimaldi, 1996). The family Aleyrodidae feed almost on angiosperms, so it is possible to assume that Angiosperm plants and Aleyrodidae have co-radiated together. The earliest whitefly fossils were described from the Early Cretaceous (135 MY) amber from Lebanon (Schlee, 1970). These were *Heidea cretacica* Schlee and *Bernaea neocomica* Schlee, in addition to other undescribed genera and species. Other fossils that have been collected from the tertiary puparia occurring in Burmese amber and other ones from the Cretaceous are still unidentified. A puparium of *Aleurochiton petri* Rietschel is reported from the Pliocene deposits of Germany (Rietschel, 1983). Another fossil species, '*Aleurodicus*' *burmiticus* Cockerell from Lower Cretaceous (Cockerell, 1919) seems to be a member of Aleurodicinae, as suggested by Shcherbakov (2000), but Schlee (1970) placed it in Aleyrodinae. Undescribed Udamoselinae are reported from Lebanese amber (Shcherbakov, 2000). Other Aleyrodidae are recorded and illustrated from Lower Cretaceous Jordanian amber (Kaddumi, 2005; Drohojowska & Szwedo, 2011), the Upper Cretaceous amber of Ethiopia (Schmidt *et al.*, 2010), Lowermost Eocene Oise amber, Eocene Baltic amber (Shcherbakov, 2000), Miocene Dominican and Mexican ambers (35 MY) (Poinar, 1992) and the Miocene amber of Peru (Antoine *et al.*, 2006). The taxonomic position of *'Aleyrodes' aculeatus* Menge, from Eocene Baltic amber (Menge, 1856), remains unclear. All these data allow us to think that the speciation of presently existing whiteflies started 65 MY ago, after the separation of Gondwanaland followed by the putative isolation of a common ancestor of Aleurodinae in the Paleotropics. There are records indicating that aleyrodines existed 55 MY ago and diverged during Oligocene 38 MY ago. Tectonic movement followed by a rapid temperature modification and reduction of the rain forest area during the Late Eocene can explain the whitefly radiation. The distribution of the recorded fossils and the molecular clock estimated support the probability that the divergence of the two known families, Aleyrodinae and Aleurodicinae, happened in concomitance with separation of Gondwanaland into Africa and Latin America (Campbell *et al.*, 1996).

1.1.4 Zoogeography

Most of the species of the family Aleyrodidae are of tropical origin and even if many of their taxa have been recorded in Mediterranean or temperate regions, they look like to have originated and then spread from the tropics. However, during the past, whiteflies have been studied mainly by researchers working in developed countries (especially Europe and USA). More recently, knowledge on tropical whiteflies has been improved, thanks to the increasing number of local scientists working in the tropics and a closer collaboration between researchers and research institutions belonging to different countries; thus many species have been described or recorded in the past years from Asia, Africa or Latin America. Here, an overview on the distribution of whiteflies is given following the data contained in the recent Catalog of Whiteflies (Hemiptera: Aleyrodidae) of the World, realized by Evans (2008).

1.1.5 Subfamily Aleurodicinae

Aleurodicinae contains 122 species in 18 genera. The species in this subfamily occur in all regions of the world, with a far greater number and diversity of species (102 species in 14 genera) in the Neotropical region, than in the other regions (Evans, 2008). In the Nearctic region (NA) 12 species are present, contained in 5 genera. Among the genera of economic importance are *Aleurodicus* Douglas and *Paraleyrodes* Quaintance. The Neotropical (NT) region includes 14 genera containing 106 species. It is possible to note that tropical areas have a great number of Aleurodicinae species compared to other eco-climatic regions, containing also genera that represent a threat for agriculture, such as: i) *Aleurodicus* Douglas*,* well represented with 26 species, among which many are crop pests; ii) *Paraleyrodes* Quaintance, containing 17 species and iii) *Metaleurodicus* Quaintance & Baker*,* with 11 species. In the Afrotropical region (AF) only 3 genera are present, including 5 species. However, though numerically rather scarce, Aleurodicinae are represented in this region by species causing huge damage, such as those ones belonging to the genera *Aleurodicus* Douglas (1 species) and *Paraleyrodes* Quaintance (2 species). In the Palaearctic region (PA), that includes also part of the Mediterranean basin, 8 whitefly genera have been recorded and only one in green houses. As in the previous regions, the genera *Aleurodicus* Douglas (2 species) and *Paraleyrodes* Quaintance (3 species) have been recorded. Peculiar and interesting genera found in this region are *Lecanoideus* Quaintance & Baker (1 species) and *Ceraleurodicus* Hempel (1 species). In the Oriental region (O), three genera containing 11 species have been recorded: *Aleurodicus* Douglas (8 species), *Nipaleyrodes* Takahashi (1 species) and *Paraleyrodes* Quaintance (2 species). The Australian region (AU) contains 4 genera and 10 species, among which the most important are: *Aleurodicus* Douglas (5 species), *Nipaleyrodes* Takahashi (1 species) and *Pseudosynaleurodicus* Gillespie (2 species). The last genus was described and recorded only in the AU region. In the Pacific Islands only 3 genera have been recorded, including 5 species: *Aleurodicus*

Douglas (3 species), *Paraleyrodes* Quaintance (1 species) and one species from the genus *Stenaleyrodes* Takahashi. Looking at the previous data and their synthesis reported in Fig. 1, it is easy to see how Aleyrodicinae is associated with tropical regions of the world, especially the Neotropical region (NT), where 106 species have been described or recorded, whereas in PA or NA regions there are only a few and in Africa only 5. The reasons of this unequal distribution can be explained as follows: (1) the subfamily Aleurodicinae and Aleyrodinae diverged from a common ancestor with separation of Gondwanaland into Africa and Latin America (Campbell *et al.*, 1996); (2) there is a gap of knowledge about the number and distribution of whiteflies especially in Tropical regions. The Afrotropical region has been much less investigated compared to the Neotropical region, where intense efforts were carried out during the last decades.

1.1.6 Subfamily Aleyrodinae

This is the largest subfamily, with 150 genera and 1438 whitefly species (fig.2) described worldwide. Some of the most harmful whitefly pests, including those ones transmitting viruses, such as *Bemisia tabaci*, *B. tuberculata*, *T. vaporariorum* and other important whiteflies, are known to cause direct damage to plants. However, this subfamily contains several genera with a conspicuous number of species. This is the case of the genus *Aleurocanthus* Quaintance & Baker, that includes 80 species; *Aleuroclava* Singh with 118 species; *Aleurolobus* Quaintance & Baker 83; *Aleuroplatus* Quaintance & Baker 75; *Aleurotrachelus* Quaintance & Baker 83; *Bemisia* Quaintance & Baker 46; *Dialeurodes* Cockrell 102 and

Dialeurolonga Dozier 53. Obviously, even if these genera include a huge number of species, this does not mean that they have a wide geographical distribution. In the NA region 29 genera and 124 species of Aleyrodinae are recorded: 31 species belong to the genera *Trialeurodes* and 26 to the genera *Aleuroplatus* Quaintance & Baker and *Tetraleurodes* Cockerell. In NT region 42 genera and 212 species are recorded. The genera *Aleuroplatus* Quaintance & Baker, *Aleurothrixus* Quaintance & Baker, *Aleurotrachelus* Quaintance & Baker, *Dialeurodes* Cockerell and *Tetraleurodes* Cockerell contain almost 40% of the total number of species known for this area. In the WPA 41 genera and 137 species are recorded. Here, no genus is particularly important and almost all genera occurring in this area are represented by only a few species, with the exclusion of the genus *Bemisia* Quaintance & Baker, which is represented in this area by more than 10 species. In the EPA 51 genera and 256 species are known to occur. The genera *Aleurotrachelus* Quaintance & Baker (18 species), *Aleyrodes* Latreille (10), *Bemisia* Quaintance & Baker (12) and *Dialeurodes* Cockerell (16) are the richest ones as for the number of species. In the AF region 54 genera and 284 species have been described or recorded. Representative genera of this region are: *Africaleurodes* Dozier (14 species), *Aleurocanthus* Quaintance & Baker (14), *Aleurolobus* Quaintance & Baker (18), *Aleuroplatus* Quaintance & Baker (29), *Aleurotrachelus* Quaintance & Baker (21), *Bemisia* Quaintance & Baker (14), *Dialeurolonga* Dozier (37) and *Tetraleurodes* Cockerell (19). The Oriental region is the zoogeographical area with the highest species richness (and also genera) in the world. In this area 543 species in 73 genera are recorded. The richest genera are: *Aleurocanthus* Quaintance & Baker (40 species), *Aleuroclava* Singh (86), *Aleurolobus* Quaintance & Baker (54), *Bemisia*

Quaintance & Baker (16) and *Dialeurodes* Cockerell (59). In the AU region 43 genera and 132 species are recorded.

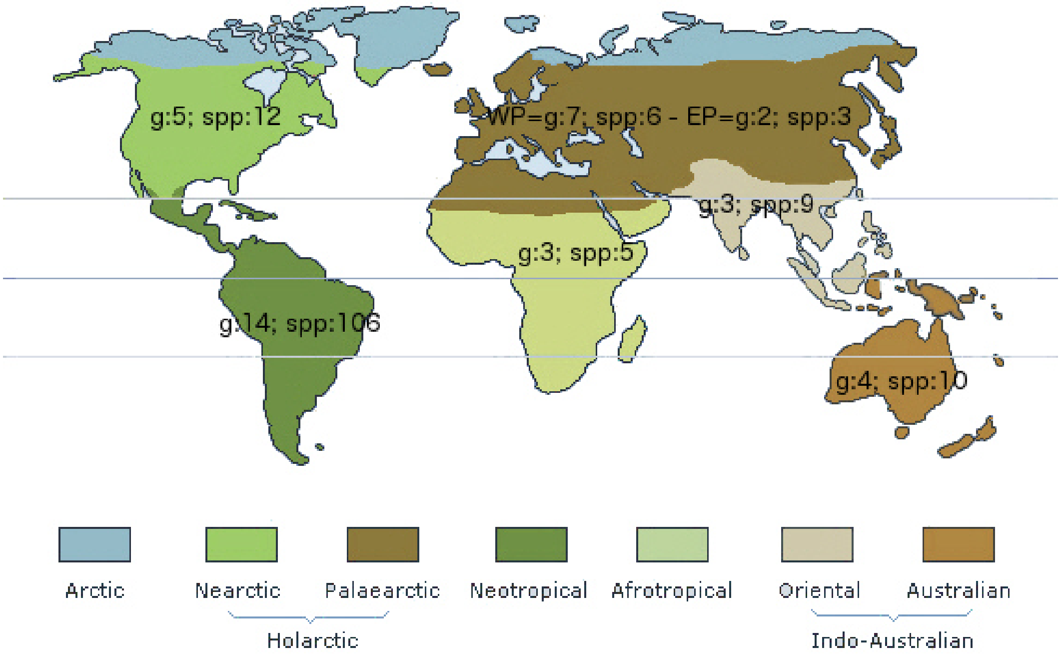

Fig. 1 Distribution of the genera and species of Aleurodicinae by Zoogeographic Region.

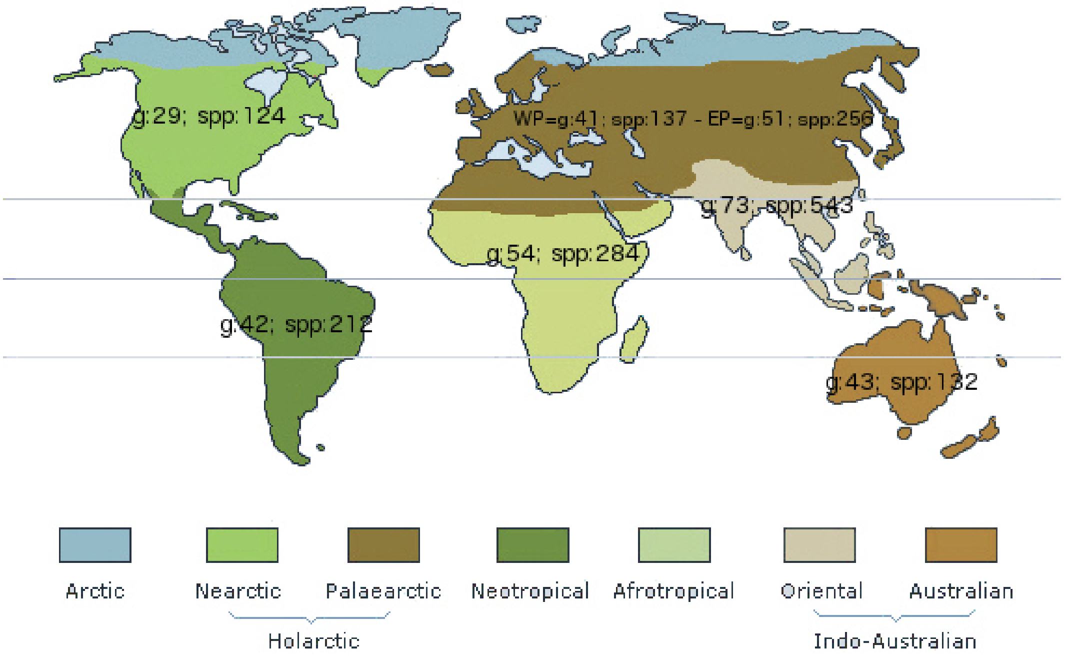

Fig. 2 Distribution of the genera and species of Aleyrodinae by Zoogeographic Region

1.1.7 The spread of whiteflies around the world

As seen in the previous section, whiteflies are distributed throughout the major zoogeographical regions of the globe, with their highest richness in tropical and subtropical regions. However, the literature about alien and invasive species of whiteflies around the world is scarce, most of the studies dealing with alien pests of economic importance such as *B. tabaci* and *T. vaporarium* (Bedford *et al.*, 1994; Martin *et al.*, 2000). Apart from this, only a few European countries and the USA are keeping updated online databases based on the latest alien insect records, including whiteflies. Many alien species of whiteflies have been introduced by plant material, spreading quickly from one country to another. However, recent introductions show that trade is the main factor of introduction and that at any time new problematic species can occur (Mifsud, 2010). On the other hand, there is not sufficient information on the movements of whiteflies within countries. Several observations indicate that in the last decades Mediterranean species expanded their distribution to the North; but it is not clear if they can establish wild populations or not. Usually these species profit from anthropogenic habitats (e.g. agricultural areas and parks) and can cause serious problem. Therefore, given the lack of knowledge, there is a need to monitor species migration carefully.

1.2 PARASITOIDS

1.2.1 General notes

Hymenoptera is one of the four great orders of insects that include more than 120,000 described species. Parasitic Hymenoptera are among the most important agents used for the biological control of pests. Despite their importance, their biology and taxonomy is poorly known. Whitefly parasitoids are classified into six families belonging to two super-families: Chalcidoidea and Platigastroidea. The six families include Aphelinidae, Encyrtidae, Eulophidae, Pteromalidae and Signiphoridae, belonging to Chalcidoidea, and Platigastridae. belonging to Platigastroidea. The superfamily Chalcidoidea is one of the largest groups of Hymenoptera, including more than 20,000 species; but recently taxonomists estimated that there could be more than 300,000 species (Goulet & Huber, 1993; Noyes, 2003). Their adults generally measure a few millimetres (2–5 mm) but they range from less than one to more than about twenty millimetres in length, showing a notable morphological differentiation (Mockford, 1997; Heraty & Schauff 1998; Gibson *et al.*, 1999). The information regarding the evolution of the two super-families is scarce. From the few data available to date and based on the fossils from Canadian amber, the diversification of Chalcidoidea occurred by the late Cretaceous (Yoshimoto, 1975). Within the families ascribed to the superfamily of Chalcidoidea, the family Aphelinidae play a very important role for the biological control of whiteflies. The World fauna consists of 1156 described species in 32 genera (Noyes, 2011). However, the phylogeny of the families or subfamilies has not been assessed and many questions about their systematics are still unanswered (Hayat, 1998; Heraty & Schauff, 1998; Gibson *et al*., 1999). In addition, molecular data have not supported any higher-level groupings in the family (Campbell *et al*., 2000; Munro *et al*., 2011). Despite their morphological issue and difficulties in defining the monophyly of Aphelinidae, understanding their species and relationships is important for their practical use in biological control (Noyes & Hayat, 1994; Rosen & De Back, 1994). Aphelinids of primary importance are ascribed in the genera *Encarsia* Foerster, *Eretmocerus* Haldeman and *Cales* Howard. Other significant genera but not common in biocontrol programs are *Ablerus* Howard, *Dirphys* Howard and *Myiocnema* Ashmead.

The genus *Encarsia* currently contains 344 described species most of which are parasitoids either of Aleyrodidae (whiteflies) or Diaspididae (armoured scale insects), but also of aphids (Aphididae) (Evans *et al.*, 1995) and eggs of Lepidoptera (Polaszek, 1991). Several *Encarsia* species have been fundamental as biological control agents (Huang & Polaszek, 1998). This genus is taxonomically intricate, including many cryptic species. In the aim to facilitate the morphological identification of the *Encarsia* species, the genus has been divided into 29 species-groups (Babcock *et al.*, 2000; Manzari *et al.*, 2002). However, not all of these species-groups are defined unambiguously on the basis of morphological characters only (Manzari *et al.*, 2002). Many of the molecular studies carried out on *Encarsia* are based on the D2 expansion region of the 28S ribosomal RNA; though still covering a limited part of all known species of *Encarsia*, results from these studies are promising because they support most of the traditionally defined groupings within the genus. *Encarsia* species are endoparasitoids (on whiteflies) and in a few cases ecto-parasitoids (on scale insects) (Hunter & Woolley, 2001).

However, many species can also be auto-parasitoids. This happen when female eggs are deposited on a primary host and male eggs deposited as parasitoids of the same or other species of *Encarsia* (Polaszek, 1991; Williams & Polaszek, 1996; Hunter & Woolley, 2001). *Encarsia* species can be uniparental or biparental. Mated females can lay both fertilized diploid eggs that become females and unfertilized haploid eggs that become males. Biparental *Encarsia* species lay the male eggs outside of the body of the larval female primary parasitoid. *Encarsia* species develop through three larval instars. The first and second instars have no functional spiracles. The third instar has open, functional spiracles and voids its meconium before moving on to the pre-pupal and pupal phases. The position of the meconium in the host body can differ with both the parasitoid and the host. More than hundreds of *Encarsia* species have been recorded on whiteflies.

The genus *Eretmocerus* has been utilized in biocontrol programs against *Bemisia tabaci* for many decades. This genus is known only to attack whiteflies, and several species have been reared from *B. tabaci.* Currently more than seventy species have been described. Despite many studies conducted on this genus, the identification at the species level often remains problematic and uncertain, due to the absence of any good distinguishing morphological characters. Comparing the literature between *Eretmocerus* and *Encarsia,* it is easy to understand how the data on *Eretmocerus* are scarce. To date, morphological and molecular techniques are not enough to distinguish and certainly identify *Eretmocerus* species (DeBarro *et al*., 2000; Guastella *et al.*, 2013 *in press*). It is interesting to note how, in a recent phylogenetic analysis of Aphelinidae, realised by means of both molecular and morphological approaches, *Eretmocerus* has been placed outside of Aphelinidae

(Kim & Heraty, 2012). In the aim to divide this genus into smaller and manageable subunits, an attempt has been done by Evans (2008), who divided the genus *Eretmocerus* into 8 species-groups, based on the number of pairs of setae on the midlobe of the mesoscutum (MS) and the length/width ratio of the antennal club (L/W). These groups have no taxonomic status till now. However, they are useful as an identification tool allowing to separate at least species having some common expression of these two characters; of course, when two species belong to the same group this does not necessarily mean that these are also phylogenetically more related to each other than to members of another group. Generally, species of the genus *Eretmocerus* are solitary endoparasitoids of whiteflies, that oviposit external to developing whitefly nymphs, complete larval development in the whitefly nymph, and pupate in the mummified fourth instar nymph. Adults of *Eretmocerus* emerge from the mummified whitefly nymphs through an exit hole cut in the dorsum of the whitefly host.

Cales is one of the smaller groups of Chalcidoidea that are parasitoids of whiteflies. Its taxonomic position has not been well defined, however, until now *Cales* is ascribed to the family Aphelinidae (Mottern *et al.*, 2011). In fact, molecular studies exclusively based on *C. noacki* (Howard) have shown *Cales* to be isolated as a unique lineage, distinct from Aphelinidae, Eulophidae or Trichogrammatidae (Campbell *et al.*, 2000). *Cales* species show a Gondwanan biogeographic pattern, with centre of diversity in the Australian region and another distinct lineage in the new tropics. Most information about the genus biology is based upon study of *C. noacki*. The species of the genus *Cales* are primarily endoparasitoids of the larval instars of various whiteflies (Dozier, 1933; DeBach & Rose, 1976; Rose & Woolley, 1984). Appertaining to another family, the genus *Amitus* (Hymenoptera: Platigastridae) is composed of small-wasps (about 1 mm), which are parasitoids of whiteflies. Platigastridae are also endoparasitoids of insect eggs, mostly of Coleoptera, as well as the later young stages of sessile hosts, such as plant hoppers, whiteflies, aphids and mealy bugs (Austin *et al*., 2005). Although the relationships of the Platigastroidea to other parasitic Hymenoptera remain unclear (Ronquist *et al*., 1999; Dowton & Austin, 2001; Austin *et al*., 2005), the monophyly of the superfamily is still undisputed. Among the few species described, *Amitus hesperidum* Silvestri, native to Asia, is one of the most effective parasitoids of the citrus blackfly, *Aleurocanthus woglumi* Ashby. Usually, this species of parasitoid is non-polyembryonic and biparental in reproduction with a sex ratio of 1:1.

1.2.2 Role of parasitoids in biological control of whiteflies

Generally, three approaches are known to the biological control by natural enemies, these are: i) the introduction and establishment of new natural enemies; ii) augmentation of existing natural enemies by releasing predators or parasitoids; iii) conservation of natural enemies by changing aspects of the environment that threaten their survival or effectiveness. The first one, mainly consisting of the importation of natural enemies, is primarily based on the understanding of the most effective natural enemies that occur in the region where the pest is native from. Efficient parasitoids are those ones that evolved with the pests and are well adapted to the pests' life cycle or behaviour. The second method consists of increasing natural enemy numbers through human intervention. For example, since parasitoids coming from outside into greenhouses will never be abundant enough to maintain the pest population below certain levels, releasing natural enemies from commercial rearing bio-farm can increase their population and strengthen the biological control. The last approach involves the conservation of natural enemies that occur naturally or that have been introduced in the frame of introduction or augmentation programs. This needs to satisfy the specific requirements for life, growth, and reproduction of these beneficial insects. Also, it is fundamental to avoid negative environmental conditions or pesticide contacts. The biological control of whiteflies through natural enemies has been attempted from long time. Some example is given by the woolly whitefly that was discovered on California citrus in the late 1960s. In the aim to control the pest, *C. noacki* was introduced from Chile and Peru (DeBach & Rose, 1976). However, the whitefly continued to spread towards the citrus-growing regions around the Mediterranean (Rodríguez, 1977; Hernandez-Suarez *et al*., 2003). For this reason, it was decided to introduce *Cales* in these areas. Example of effective control has been recorded in Italy (Sicily), where *C. noacki* was released against *A. floccosus* causing a huge reduction of the pest population (Longo *et al.*, 1985; Barbagallo *et al*., 1994). In the same region, *Encarsia lahorensis* (Howard) was released against the citrus whitefly, *Dialeurodes citri* (Ashmead), which was infesting citrus orchards of the northern countryside since long time (Liotta, 1978; Patti & Rapisarda, 1980; Barbagallo *et al.*, 1981; Barbagallo *et al.*, 1992; 1993; 1994). A few years later in Africa, *Cales noacki* was also introduced into Uganda and Kenya, where it has successfully established and has provided substantial control of woolly whitefly populations (Legg *et al*., 2003). In North America, after the introduction of *C.*

noacki, the parasitoid was able to parasitize several hosts, such as the mulberry whitefly *Tetraleurodes mori* (Quaintance); the acacia whitefly *Tetraleurodes acaciae* (Quaintance) and the red banded whitefly *Tetraleurodes perseae* Nakahara, a minor pest of avocado in California and Mexico (Rose & Woolley, 1984; Hoddle, 2006).

Another remarkable example is given by *Encarsia formosa* Gahan, a small wasp native to the semitropical areas of the New World, which was one of the first recorded parasitoids in greenhouses. It has been used since the 1920s to control the greenhouse whitefly, *Trialeurodes vaporariorum,* in warm greenhouses, for which it is widely available commercially also at present. The wasp lays its eggs in immature *T. vaporariorum*. Developing wasp larvae consume and kill their hosts before the whiteflies reach the adult stage. Like many other *Encarsia* species, the adults of *E. formosa* cause additional nymphal mortality of the whitefly by a conspicuous host-feeding activity. During the 1990s, given the huge outbreaks of *B. tabaci* in the southern part of the US, a special effort was undertaken to import, establish and augment natural enemies of *B. tabaci*. More than 50 different populations of *Encarsia* and *Eretmocerus* were received and identified (Goolsby *et al.*, 2000). These species were shown to increase the rate of parasitism from 1.5% to 86.5% in the same year (Goolsby *et al.*, 2000). During the same decades, the wasp *E. formosa* was extensively used in biological control of greenhouse whitefly in several countries around the world (van Lanteren *et al.*, 1996). In Europe, *E. formosa* was successfully released against *T. vaporariorum* on tomato and cucumber, especially in North Europe, where strong programs in biological control were carried out (van Lanteren & Woets, 1988).

The ash whitefly, *Siphoninus phillyreae* (Haliday) was first identified in California during 1988 (Stocks & Hodges, 2010). Immediately after, the infestation rapidly spread around the nearby regions. During the early 1990s, an initial effort for establishing a biological control program was carried out. Between 1989 and 1990, a team of experts introduced nine populations of natural enemies. By October 1990, the density of *S. phillyreae* was reduced several fold, becoming difficult to detect. Among the parasitoids released, *Encarisia partenopea* Masi was one of the most effective agents. It continued to constitute between the 70 - 85% of the total emergence from the parasitized *S. phillyreae* nymphs.

The Spiralling whitefly, *Aleurodicus dispersus* Russell, a native of Caribbean region and Central America was introduced into India in 1995 (Singh, 2004). This pest has spread to all the southern states causing serious damage to several crops. It has over 250 host plants belonging to more than 170 genera and 60 families. Among cultivated plants, it can be a threat to avocado, banana, cassava, guava, papaya and mango, besides several ornamental and avenue trees (Dubey & Sundararaj, 2004; Singh, 2004). *Encarsia guadeloupae* Viggiani and *E.* sp. nr. *meritoria* Gahan were collected from Central America and introduced in the affected areas of India, causing a consistent reduction of the pest population. The parasitism levels recorded from 29-70% and exceed 90% during some parts of the year (Singh, 2004). However, *E. guadeloupae* was performing better than *E*. sp. nr. *meritoria* and has displaced it almost completely in Bangalore.

The bayberry whitefly, *Parabemisia myricae* (Kuwana), is an Asian species that invaded California becoming a severe threat of citrus (Rose & Debach, 1991; 1992). Parasitoids were imported into California from Japan, mass-reared, colonized, established, and field evaluations were undertaken. *Eretmocerus debachi* Rose and Rosen, was the most effective enemy of *P. myricae* and achieved fortuitous biological control in southern California. Purposeful introduction of *E. debachi* into Mediterranean region also resulted in successful biological control. In Italy, abundant population of *P. myricae* has successfully been controlled by *E. debachi* and *Encarsia meritoria* Gahan (Longo *et al.,* 1990, 1994; Rapisarda *et al.*, 1991,1994).

More recently, the coconut whitefly, *Aleurotrachelus atratus* Hempel, has been collected on several host palms from the islands of the southwestern Indian Ocean (Borowiec *et al.*, 2010). Generally, *A. atratus* has been found abundant only on its primary host, which is the coconuts palm, observing only low level of infestation on other palm trees (Borowiec *et al.*, 2010). Obviously, a wide range of hosts has facilitated the rapid geographical dissemination of this whitefly. Several parasitoid species were found associated with the populations of this pest, but *Eretmocerus cocois* Delvare was the most dominant. *A. atratus* caused significant economic damage in the Comores during recent years; but thanks to the establishment of a biological control program (2005-2007), which also lead to the discovery of the new parasitoid species, the phytosanitary status in these islands has improved significantly (Borowiec *et al.*, 2010).

1.3 MOLECULAR TAXONOMY

Identifying an insect species only through morphological characters can be often relatively easy and fast but requires high level skills in classical taxonomy. Moreover, this kind of identification can be sometimes arduous, as in the case of intricate complexes of cryptic species. When doing identifications, taxonomists may encounter an array of cases and a wide range of needs, that can be reduced to four basic ones: i) the determination of known species, that have been collected in different regions around the globe; ii) the discovery and description of new taxa (genera or species); iii) the delimitation of species boundaries; iv) the phylogeny. Apart from the need to discriminate species belonging to cryptic complexes, molecular methods can help taxonomists to resolve taxonomic problems in one or more of these cases. Nowadays, the field of molecular insect taxonomy is extremely wide to be explained in a few pages. To this purpose, Caterino *et al.* (2000) presented an overview on the state of insect molecular systematics, referring to DNA sequences from only a small number of genes across all of insect systematics, to prevent what is called a 'Tower of Babel' where different studies are not effectively related to each other. According to the authors, the mitochondrial protein-coding genes can provide the most suitable markers at the species level; but this vision is still controversial. The present leading method involves sequencing of known regions of DNA that are conserved to allow the use of 'universal' primers and which, at the same time, have a sufficiently rapid mutation rate allowing species to be distinguished. Recent focus on mtDNA-based identification, such as DNA barcoding, has raised hopes that molecular methods will enhance the milestone of the biodiversity identification at the species level (Hebert *et al.*, 2003; Waugh, 2007). Several authors have demonstrated that not all species can easily be characterized using mtDNA sequences (Funk & Omland, 2003; Elias *et al.*, 2007; Weimers & Fiedler 2007). These problems seem to be more acute in the identification of those species for which molecular methods are most needed, such as closely related species that have limited morphological distinctions (Sperling, 2003). Obviously, information from other DNA regions are needed to definitely identify an insect species (Rubinoff & Sperling, 2004; Fallon, 2007). However, the recent increasing number of molecular markers provides hopes for the future of taxonomy. Several gene regions have been used in insect molecular studies (Simon *et al.*, 1994; Caterino *et al.*, 2000; Greenstone, 2006; Stouthamer, 2006). A few of them are most often targeted for being sequenced in insect systematics. Here below, the most important molecular methods applied in whitefly taxonomy, and therefore used also in this work, are briefly reported and explanations are given on the reasons why they are chosen.

1.3.1 Ribosomial RNA

Ribosomial RNA (rRNA) is a component of the ribosome. The DNA coding for rRNA is the rDNA. It occurs in a part of the nuclear genome, on a single or several chromosomes. Each copy of an eukaryotic nuclear rDNA is organized in three genes, these are called 18s, 5.8s and 28s. Among them, the internal transcribed spacer regions, ITS1 and ITS2 respectively, can be found dividing the region. The 5.8S rDNA is the smallest rDNA, embedded in the two internal transcribed spacers

(ITS1 and ITS2). Its length is about 150 bp, too short to contain enough information for phylogenetic study. So, it can be suggested only as additional information in insect diagnostics. 18s is one of the highly conserved rDNA regions; and for this reason it is advisable to be used in phylogenetic reconstruction of high grade taxa, such as *phyla*, classes or orders (Whiting, 1998; Caterino *et al.,* 2001). The region 28s is much bigger than 18s, with a great level of evolutionary rate. It can be used for taxonomic studies at lower level, such as orders, families and genera (Friedrich & Tautz, 1997; Hwang & Kim, 1999; Babcock *et al*., 2000). The two internal transcribed spacers (ITS1 and ITS2), since they are highly variable, are commonly used in phylogenetic studies at genera or species level (De Barro *et al*., 2000; Morris & Mound, 2004). In addition, ITS1 and ITS2 can play an important role to discriminate species or populations that are morphologically similar (Yara, 2006). However, sometime these molecular tools do not give the expected results. They seem to be applicable to some genera or species but not to others. For example, in some cases variation in ITS was correlated with geographical distribution of populations and it was not able to identify species (Vogler & De Salle, 1994; De Barro *et al*., 2000; Guastella *et al.* 2013 *in press*). Sequences of the D2 expansion of 28S rDNA region have been used to evaluate the phylogenetic results of the molecular data separately and in combination with morphological data, providing a valid data to support clades within *Encarsia* species-group or species (Babcock *et al.*, 2000; Guastella *et al.,* 2013 *in press*).

1.3.2 Mitochondrial DNA

The mtDNA consists of a molecule of 15-20 kb arranged as closed circular molecule (Warrior & Gall, 1985; Bridge *et al.*, 1992; Wolstenholme, 1992). The gene shows little variation content, since all the cases investigated show that the larger size is due to either the variation in the length of a noncoding region (Harrisson, 1989) or the iteration of some portion of the mtDNA (Moritz & Brown, 1987; Staton *et al*., 1994). Mitochondrial DNAs normally include 36 to 37 genes; 2 for the ribosomal RNAs (16S rRNA; 12S rRNA), 22 appertaining to the transfer RNAs (tRNAs) and 12-13 for subunits of multimeric proteins of the inner mitochondrial membrane (cytochrome oxidase I-III; ATP syntethasis 6 and 8; NADH dehydrogenase 1-6 and 4L and the cytochrome b apoenzyme). Since the mtDNA evolves faster than the nuclear genome, many mitochondrial protein coding genes have been used to explore phylogenetic relationships at lower taxa level such as families, genera, species or population studies (Navajas *et al.*, 1996; Hwang & Kim, 1999). More recent studies, however, demonstrated some limits of the use of mtDNA. For instance, Nazari *et al*. (2007) showed how the quality of phylogenetic information in mtDNA can usually decline rapidly beyond the generic level. Therefore, it is important to remain aware that this method can provide only preliminary evidence that needs be complemented and assayed with additional information for each group.

1.3.3 DNA Barcoding

DNA barcoding is a method that uses a standardized genomic segment (650 bp) as a marker for species identification. It requires the sequencing of a particular fragment of DNA, which involves the COI gene as a way of identifying an organism. This means that a gene, or part of it, can be used to identify a species in the same way as a retail barcode is a unique key that identifies a given product in a shop. However, the DNA barcode doesn't work in this simple way, as DNA is involved in several natural evolutionary complexities and can exhibit marked variation within species (Mallet & Willmott, 2003). Nonetheless, this method could facilitate the identification of specimens, avoiding the difficulties of other methods, speeding up also the efforts in morphological identification (Tautz *et al.*, 2002, Blaxter, 2004; Hebert & Gregory, 2005). However, if used as a single character in the identification of a specimen, the DNA barcoding may have limited phylogenetic application; unless it is combined with morphological and ecological data, especially in such cases as identification of cryptic species complexes (Moritz & Cicero, 2004; Dasmahapatra & Mallet, 2006). Using these few nucleotides, thousands of specimens have been quickly processed and unambiguously analysed by computer programs. Then, the sequencing results are used to search a DNA database. A close match quickly identifies a species that is already represented in the database. However, some barcodes will be entirely new, and identification may rely on placing the unknown species in a phylogenetic tree with near relatives. Novel DNA barcodes can be submitted to the database at the Barcode of Life Data System (BOLD) (www.boldsystems.org) at the University of Guelph (Canada)

1.4 BIOINFORMATICS

1.4.1 Data management

In the aim to store data from projects, experiments or surveys, these are kept in workbooks or on a computer, where we usually store information in paper sheets or extended and confusing spreadsheet files. In this way, only one user (i.e. the one who collected and stored them) can manage these data. If the data coming from a research project should be manipulated by an entire team, each of them can have their own way of managing and manipulating different data files. In this way, it is probable to produce huge amount of errors or inconsistency, such as names, values or positions. Moreover, keeping organizational data following this approach has a number of disadvantages, such as:

- *Redundancy*: different files (or the same one) can be created repeatedly by several users over time leading to problems in data inconsistency and resulting in various copies of the same data (Nguyen *et al.*, 2009);
- *Data isolation*: implies the difficulty to retrieve the appropriate data, when these are stored in various files;
- *Security:* problems regarding accessing file privileges;
- *Concurrency and access anomalies*: data may be accessed by many users/applications that have not been coordinated previously so it is not easy to provide a strategy to support multiple users to update data simultaneously.

A well-defined Data Base (DB) allows preserving data safely, while providing an easy and fast access to the data, also from previous works. But, what is a DB and how can it help in research activities? A DB is a shared collection of related information, which is defined once and then is accessed by various users. Several users (a professor, statistician, lab manager) working on the same project, might visualize only the data of interest, allowing also a multiple access to the database at the same time and having updated data constantly (data integrity). This is simply traduced in easy data storing and management by the users and hence with less data errors. Also, it is possible to create relational DBs, which means that data are stored in several separate tables by subject, but the data are related and can be brought together in ways that the user specifies. DBs consist of objects, such as Tables - to store data by subjects; Queries - by which it is possible to retrieve and elaborate data; Form - to control data entry and its visualization; Reports - for summarizing and printing; and Data export - to interpolate the needed data and export it as a table in worksheet.

1.4.2 Available “Open/Free” Tools

The Open Source community is often composed of motivated developers, mainly unpaid, but disciplined people that believe in a common scope. Sometimes, the quality of the products by the Open Source community can exceed the ones produced by commercial organizations (Peeling *et al.*, 2001). Here some OS open source are listed through it is possible to implement a full-functional station for bio-informatics analyses. The first one is Ubuntu, the most popular OS Linux among researcher. Thanks to the wide community that nourishes this project, it is easy to find any help and solution to start and add all the software that the user needs. In addition, a Molecular Dynamics package, as AMBER and Desmond, are available. They run generally without posing problems. Other available tools are (adapted from the Ubuntu community website):

- DNA Linux: is a Virtual Machine with bioinformatics software preinstalled.
- Bioknoppix: is a customized distribution of Knoppix Linux. It comes with applications targeted for molecular biologist. Besides using some RAM, Bioknoppix doesn't touch the host computer (because it's a Live-CD), and is ideal for demonstrations, molecular biology students, workshops, etc.
- Vigyaan: This is an electronic workbench for bioinformatics, computational biology and computational chemistry. It has been designed to meet the needs of both beginners and experts. Vigyaan CD is a live Linux CD containing all the required software to boot the computer with ready to use modelling software. Vigyaan CD is based on KNOPPIX v3.7.
- VLinux: it is a Linux distribution and appliance for students and researchers in Bioinformatics. It is based on OpenSUSE and is built using Novell's Suse Studio.
- BioSLAX: is a new live CD/DVD suite of bioinformatics tools that has been released by the resource team of the BioInformatics Center (BIC), National University of Singapore (NUS). Bootable from any PC, this CD/DVD runs the compressed SLACKWARE flavour of the LINUX operating system also known as SLAX.

- Bio-Linux 6.0: is a fully featured, powerful, configurable and easy to maintain bioinformatics workstation. Bio-Linux provides more than **500 bioinformatics programs** on an Ubuntu Linux 10.04 base. There is a graphical menu for bioinformatics programs, as well as easy access to the Bio-Linux bioinformatics documentation system and sample data useful for testing programs. One can also install Bio-Linux packages to handle new generation sequence data types.

These information listed above can be useful if we want to change our habit or the need to have a system completely free and where we can install adequate software for our research without constriction, having the complete control of our machine. However, if we want to keep other OS such as Windows or Mac, but we have not enough economic funds to buy commercial software, several free software are available on Internet. A list of the tools for phylogenetic analysis is available on *http://en.wikipedia.org/wiki/List_of_phylogenetics_software*. Among them, the most famous softwares are R-Project, Armadillo, BEAST, Mr Bayes, PALM, PAUP, PHYLIP and EPOS. Tools as R-project, BEAST, MR Bayes are very effective and stable softwares, but tiresome for many of the “mouse-click” people. However, there exist also tools that are Open Source including a pleasant graphic interface (GUI). These are EPOS and Armadillo. EPOS, by Griebel *et al.* (2008), is a software for phylogenetic analysis that supports data management (e.g. data store, sequences visualization, modification and alignment), computational methods and tree visualizations. EPOS fills the gap of the powerful CLS tools by combining a powerful graphical user interface (GUI) with plugins that allow simple integration of new algorithms, visualizations and data structures. It offers an easy method to include new modules into the framework. In fact, the system itself is made by a set of modules, which allows extensions in all directions. The easy way to install plugin allows users or developers to easily integrate their own tools or algorithm, but in the same way, to benefit from method provided by others. The connection with a new algorithm to data and data to visualization is completely implemented by the system. Also, users can use several or even new computational methods without adapting to a new software environment (Griebel *et al.,* 2008). Another interesting software - "Armadillo" is a platform dedicated to phylogenetic as well as general bioinformatics analysis by Lord *et al.* (2012). Armadillo platform is an open-source tool that allows users to develop their own modules or integrate existing computational methods. Several biological tools have been included in this software, these include: i) the multiple sequence alignment (MSA); ii) the evolutionary model inference; and iii) inference of phylogenetic trees using Distance, Maximum Likelihood, Maximum Parsimony and Bayesian methods. In addition, in these tools many other operations are included, from data storage and management to on-line sequences comparison. Other important free tools used not only for sequences alignment are ClustalX (Thompson *et al.*, 1997), BioEdit (by Tom Hall, North Carolina State University), Chromas (Goodstadt & Ponting, 2001) and SeaView (Gouy *et al.*, 2010). Among them, SeaView reserves a little bit more attention. In fact, this tool is a graphical multiple sequence alignment editor. It is able to read various alignment formats (MSF, CLUSTAL, FASTA, PHYLIP, MASE) and allows manual editing of the alignment and also to run DOT-PLOT or CLUSTAL programs to locally improve the alignment.

1.4.3 Models and methods in phylogenetic study

Molecular phylogeny and its applications are obviously popular and useful tools for making comparative investigations in insect genetics. However, for those people who have not a background in computer science or in bioinformatics, the field of molecular evolution and phylogenetics can be a zoo, and maybe without a full understanding of which tools are available or better for their studies or cases, they just choose the easiest way or the latest trend. Maybe, someone will apply the same model to different cases, such as using different molecular markers or in analysing DNA sequences with high variability. Constructing phylogenetic trees is not always straightforward. In the following pages, brief information is given on which models are available for entomological phylogenies and when to apply each of them. For phylogenetic reconstruction, several methods exist that are classified based on two criteria: 1) the type of data they use to reconstruct the tree(s) (such as distance matrices or discrete characters) and 2) the reconstruction strategy (algorithmic or optimality criterium). Distance matrix methods compute a matrix of pairwise "distances" between sequences that approximate evolutionary distance. Distance-based methods can be fast in practice (Durbin *et al.*, 1998; Dowell, 2008), using clustering techniques to calculate the evolutionary distances for all pairs of taxa. The phylogenetic trees built by this method utilize algorithms based on distance values (Dowell, 2008). Several distance methods exist, such as the *Unweighted Pair-Group Method with Arithmetic Mean (UPGMA),* which involves a clustering algorithm; the *Transformed Distance Method,* which is taking in reference an out-group, then applies the UPGMA methods; the *Neighbor-Relations*

Method, which applies 4-point condition to adjust the distance matrix, then applies UPGMA; and the *Neighbor-Joining Method,* which arranges operational taxonomic units (OTUs) in a star, the finds neighbours sequentially to minimize total length of tree. OTUs represents the operational taxonomic units, and practically correspond to the molecular sequences or taxa (species) from which the tree was inferred Durbin *et al.*, 1998).

Some of the more commonly used methods include neighbour joining (NJ) (Saitou & Nei, 1987), maximum parsimony (MP) (Fitch, 1970) and maximum likelihood (ML) (Felsenstein, 1981). Moreover, new methods that employ a Bayesian statistical approach (Larget & Simon, 1999; Ronquist & Huelsenbeck, 2003) have generated much interest. Neighbor Joining, ML and Bayesian methods are based on explicit statistical models of evolution to reconstruct evolutionary trees. The Neighbor Joining algorithm is different from ML and Bayesian methods, because it uses the model to calculate pairwise genetic distances between sequences, and reconstructs a topology based on those distances. Maximum likelihood and Bayesian methods use the sequence data directly to reconstruct a tree, thereby utilizing information in specific nucleotide differences instead of summarizing changes with a genetic distance. Due to these differences, ML offers noteworthy statistical properties in comparison with genetic distance-based methods, but is much more computationally intensive (Kuhner & Felsenstein, 1994; Yang, 1994; Huelsenbeck, 1995). While NJ and ML methods are well understood and their uses are common in the literature, Bayesian methods are relatively new. The Bayesian method is related to ML method because they both utilize the likelihood function. In Bayesian analysis the preferred result is the one that maximizes the posterior probability, which is determined by the prior distribution and the likelihood of that tree (Bos & Posada, 2005). Whereas other methods produce a unique best estimation of evolutionary relationships and ignore uncertainty outcomes, Bayesian methods produce a set of trees of where the one with the highest posterior probability is accepted as the preferred tree. Bayesian methods can be faster than ML methods, and also offer the advantage of automatically incorporating an estimate of phylogenetic uncertainty (Larget & Simon, 1999). However, several aspects of Bayesian phylogenetic estimation have not been completely explored; these methods offer the same benefits from employing statistical models as ML and NJ (Ronquist & Huelsenbeck, 2003). These benefits include the flexibility to incorporate a wide range of models, easy hypothesis testing, and improvements on estimates of numbers of substitutions, efficiency and robustness (Huelsenbeck, 1995). Regarding the Maximum Parsimony (MP), it is an optimization criterion that adheres to the principle that the best explanation of the data is the simplest, which the one requiring the fewest assumptions. In practical terms, the MP tree is the shortest, the one with less changes (Brinkman & Leipe, 2001). However, the MP method performs poorly when there is substantial among-site rate heterogeneity (Huelsenbeck, 1995). It is possible to fix this by several approaches: i) include only sites that exhibit little or no heterogeneity as determined by likelihood estimation; ii) recursively reweight positions according to their propensity to change as observed in preliminary trees. The reason why distance and ML tree methods tend to arrive at a single best tree is that their calculations involve division and decimals, whereas MP merely counts discrete steps. Unfortunately, whereas MP users conventionally present strict consensus (and sometimes consensus of trees one or two steps worse), ML users typically do not. Simulation studies have shown that MP performs no better than others and worse than ML when the amount of sequence lineages divergence is much greater than the amount of divergence that occurred between lineage splits (Huelsenbeck, 1995; Swofford *et al.*, 1996). In this way it is possible to produce ''long branch attraction'', but the long branches are artificially connected since the non-homologous similarities of the sequences exceed the homologous similarities retained (Swofford *et al.*, 1996).

Another important point in phylogenetic study involves the choice of nucleotides substitution models. Each model varies according to the number of parameters involved in the analysis. Simple model can summarize the nucleotide substitution using few parameters, (one or two), while others can include more than about fifty parameters. These parameters reflect the differences in nucleotide frequencies, substitution rate and rate variation among sites. Combining different parameters has resulted in a large number of models, but many of them share several parameters. The Jukes & Cantor (1969) model (known as JC69 model) is the simplest nucleotide substitution model; it assumes that independent nucleotide substitutions occur at all sites with equal probability. A base is independent of its identity, and when it changes there is an equal probability of ending up with each of the other three bases. Jukes and Cantor's distance performs better in phylogenetic reconstruction than distances based on more complicated models when the nucleotide sequences are short (Zharkikh, 1994). Felsenstein (1981) suggested a model in which probabilities of nucleotide changes were determined by the equilibrium nucleotide frequencies. Kimura (1980) proposed a model that utilizes a relatively simple substitution matrix that allows for two different rates: one for transitions and the other for transversions. Kimura's (1980) distance is based on his two-parameter model, which was proposed to take into account the recognized fact that transitions and transversions occur at rather different rates, with the former occurring significantly more frequently than the latter. Kimura (1981) has formulated models that incorporate more than two rates in the substitution matrix, thus enabling models to account for different rates of change between all of the possible nucleotide pairs. In an effort to make models more representative of empirical observations, Hasegawa *et al.* (1985), Felsenstein (1995), Tamura & Nei (1993) and Rodriguez *et al.* (1990) each created models that incorporate multiple aspects of sequence evolution. These models combine parameters for differences in substitution rates and differences in nucleotide frequency. Several models are available, that can be used for particular data to obtain accurate phylogenetic estimations (Bos & Posada, 2005). The simplest way to statistically represent the site rate variation is to divide sites into two classes: those that vary and those that are invariable. To better understand wide rate differences among sites, several models have been used (Tamura & Nei, 1993; Sullivan *et al.*, 1995). Among the different models, the use of "gamma distribution" is one of the most used (Yang, 1994; Gu & Zhang, 1997). The gamma distribution can be approximated with as little as four categories, and the statistical representation of rate variation is independent of substitution models like those described above and can simply be added to any pre-existing model (for example, we can specify a HKY+I or JC69+T model). The performance of a model-based phylogenetic method may depend on the fit of the model to the data (Brower *et al.*, 1996). For example, the efficiency of distance-based methods is dependant on the accuracy of model- based estimates of genetic distance (Nei, 1996). For data-sets of sequences with low levels of polymorphism, the model may have little effect on the outcome of analysis. Instead, if working with more divergent sequences, the use of one model or another can alter the results of analysis. Adopting a particular model versus another often changes the outcome of analysis, and sometime the choice of models can be more important than the method of phylogenetic reconstruction. Given that the model plays a great role in the results of analysis, it seems that the choice of one model over another should be justified in some way. So, the question is, *which model is appropriate for a particular data set and how can that model be justified?* Methods for selecting the most appropriate model for a particular data set have been proposed, by rigorous statistical framework in which to select and justify the best-fit model (Rzhetsky & Nei, 1995). These tests are independent of evolutionary time and do not require a priori phylogeny on which to base inference. Some method uses the likelihood ratio test (LRT) to compare models. The LRT can be performed on any model, but it requires a priori input phylogeny to estimate the likelihood of the models (Sethuraman & Sanjayan, 2013). It is also possible to test several models against each other in a series of LRTs. Another way of selecting the most appropriate model for a data set is to use the Akaike information criterion (AIC), which select the more appropriate model selection calculating the likelihood between models, and scoring a penalty based on the number parameters implemented in the model (Sethuraman & Sanjayan, 2013). Alternatively to LTR, the Bayesian information criterion (BIC) can be used. This method enables comparisons of multiple models. The posterior probabilities of

Bayesian statistics are used to discriminate between phylogenetic trees and these measures can also be used to choose among multiple models. Bayesian method allows estimation of model uncertainty and allows estimation of a phylogeny using a set of candidate models. In Bayesian statistics the calculation of likelihoods proceeds differently, so the results obtained are different from those used in AIC or LRT. The impact of models on phylogenetic analysis is very significant, affecting branch lengths and the tree topology as well. For this reason the use of a particular model should be justified or reported in the literature.

1.4.3.1 Summary of the activity on how to select the correct method

Sequences selected for phylogenetic analysis should be aligned with each other along their entire lengths, or by a common set of patterns that shows an evolutionary relatedness (Mount, 2008) (fig.3). A Phylogenetic analysis should be performed when the multiple sequence alignment shows a conserved region(s). Obviously, it is normal to observe variation in the multiple alignments; however, if it is too high, the multiple alignments can be ambiguous. This means that the multiple alignments should not include so many gaps. Anyway, if in the multiple alignments a conserved region is present, and then the analysis can be focused only on this. Part of the columns in the multiple alignments may have the same residue in all sequences; other columns may include variation. If the level of variation is small but definitely present, these sequences are then suitable for MP analysis. For MP analysis, the trees that best fit the observed variations in the columns of the alignment are found. The best results are obtained if no extremely different sequences are present.

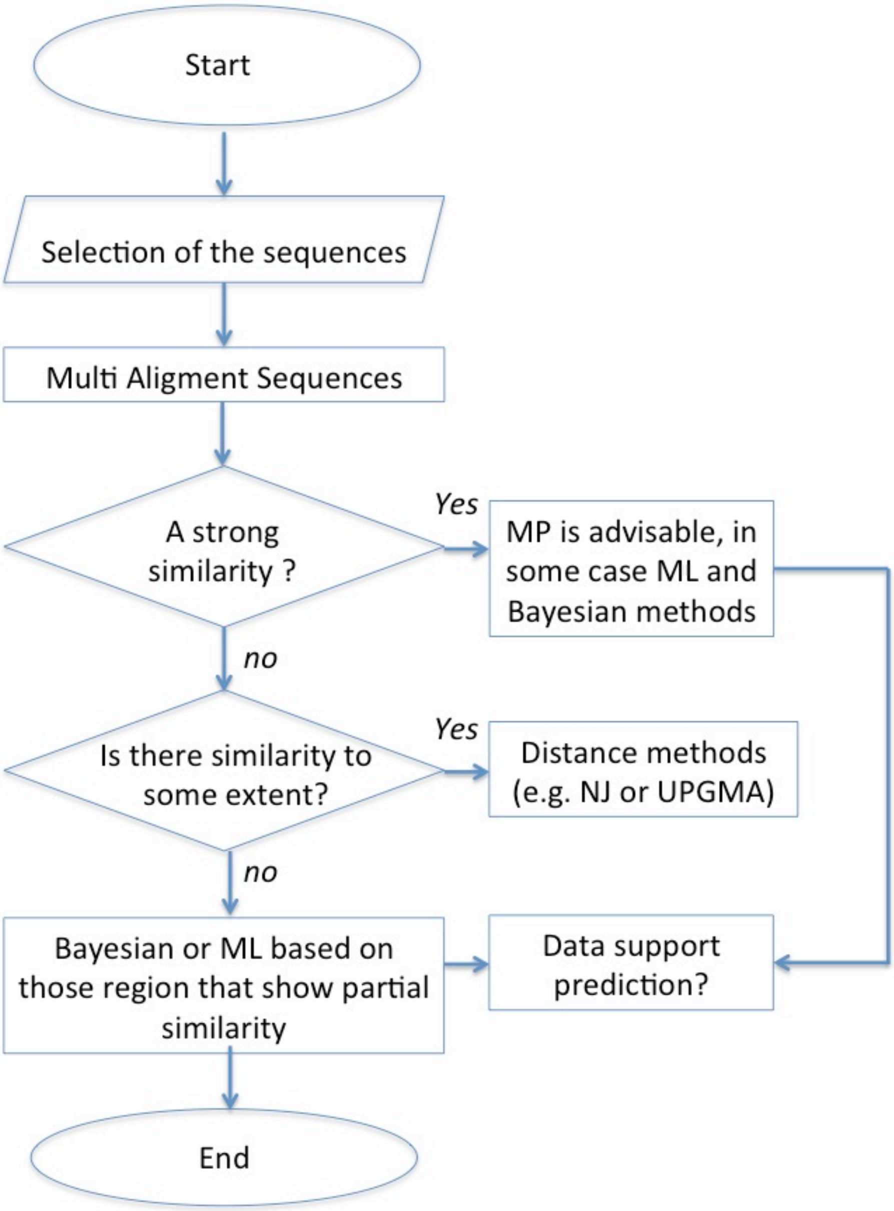

Fig. 3 How to choice a suitable method in phylogenetic analysis (modified from Mount, 2008)

MP method produces several trees; the method is advisable for about a dozen of sequences (Mount, 2008), since all the trees produced should be tested. In distance methods, the amount of variation between each pair of sequences in the alignment is measured as the fraction of aligned characters that change (the genetic distance). This method is not precise as MP to variation in the multiple alignments. Distance methods produce an evolutionary tree calculating the degree of difference among pairs of sequences in the multiple alignments. In this case, when the distances increase, the uncertainty of the alignments also increases, and correction or reassessment may be necessary. Distance methods can be used with a large number of sequences, not significantly affected by variations in rates of mutation over evolutionary time. If a considerable variation among the sequences is present, then the ML or Bayesian method are more suitable. Maybe based on similar regions of the multiple alignments. However, it is important to evidence that Bayesian and ML methods are computationally intense and complicated, with the increasing of the number of the selected sequences. Sometimes, these operations can take long time, even weeks or months, or the necessity of a super-computer.

1.5 GIS AND GEO-STATISTICS

1.5.1 Introduction

Geographic Information Systems (GIS) is a computer software programmed to collect, retrieve, transform, display, and analyse spatial data. It includes geo-referenced data producing maps and layers. Usually, a map layer or a theme is composed of only one type of data. GIS have the ability to import and manage data from different sources: mapped data, alphanumeric data, remotely sensed data. These types of data may then be combined to build a GIS database. Through this database, the user may create map outputs or display views relative to specific questions. These systems have recently improved their abilities to carry out spatial analyses, integrating new built-in functions, such as spatial interpolation, autocorrelation etc. However, while GIS has allowed researchers to work with spatially referenced data, the modelling of spatial patterns needs a set of statistical tools, such as those ones given by Geostatistics.

In entomology, geostatistic is used for quantifying and modelling the spatial correlation at a spectrum of spatial scales of insect, or to interpolate data between sample points by kriging[1] method and related procedures (Gilbert *et al.*, 2001). Nowadays, it is possible to identify three main areas where GIS has been applied in entomology: i) characterization and investigation of environmental susceptibility to outbreaks; ii) analyses of diversity for conservation planning; and iii) modelling

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[1] Kriging is a geostatistical method of interpolation for spatial data, based on regression of observed *x* values of correlated data points, weighted according to spatial covariance values. It is mainly used in prediction models.

the impact of climate change on species distribution. Outbreak frequency or aggregation maps of insects can be overlaid with bio-geo-climatic maps to determine how the flora and climate are related to outbreak frequency. In addition, characterization of particular areas can be used to predict when and where outbreaks will occur in the future. The application of GIS and geostatistic is useful also for quantifying spatial patterns in numerical data related to insects. For example, when a localized discontinuity (abundance or presence) is indicated, this may be typically due to either sampling errors or it may reflect spatial dependence that occurs at a spatial scale. In this case, it can reflect the phenomena in which insect aggregations at spatial scales exist. There are many instances in ecological research and pest management science where it is necessary to interpolate among spatially stratified samples.

1.5.2 Whiteflies and GIS

As previously said, whiteflies are one of the major threats for agriculture worldwide. To improve farm practice and Integrated Pest Management (IPM) programs, it is important to locate where aggregates of injurious whiteflies are localized, where they might arrive and where they may spread causing conspicuous damage. For this purpose, it is important to study their ecological features (*oikos*) (Ricklefs, 1990). During the last decades, the biology and life history of many whitefly species have been studied deeply under laboratory or controlled environmental conditions, such as screen houses (Noldus & van Lenteren, 1990; van Roermund *et al.*, 1997; Bosco & Caciagli, 1998; Hoddle *et al.*, 1998; Demichelis, 2005; Xu *et al.*, 2010; Parrella *et al.*, 2012). However, relatively scarce information is available on whiteflies ecology and life history under natural conditions. Damage caused by whiteflies in agriculture is mainly due to a relative restricted number of species, extremely polyphagous nature and incredible flexibility in host selection (Byrne *et al.*, 1990). Instead, in natural environments, whiteflies density is much lower due to the bioclimatic condition, resources competition, limited human intervention and natural enemies (Ricklefs, 1990; Stewart *et al.*, 2000).

GPS (Global Positioning System), GIS and Geostatistics are becoming important tools to study and analyze data from different environments and map whiteflies distribution. In fact, GIS can be adapted to any size operation and data can be incorporated at any scale, from a single field to an agricultural region; it allows storing a vast amount of data about the type of pest and where it has been spotted, providing information for tracking and predictive analysis. Several pest information like their presence in one area, their absence from others and their abundance, can be important for strategic pest management decisions, such as potential restrictions on trade or designing of pest surveys and domestic quarantines, so as trying to keep pest out from sensitive areas (Heesterbeek & Zadoks, 1987). Actually, there is a lack of information on the spatial heterogeneity and distribution of whitefly populations over the world. Spatial information is, therefore, needed not only to improve the understanding and monitoring of whiteflies but also to underpin the development of IPM programs.

1.5.3 Spatial analysis of insect diversity and distribution

Insect biodiversity is usually studied at the community level (ecosystem), species level or genetic level. If at species level, the unit to investigate is the species and it is surveyed for its absence or presence in a given area. As for the genetic diversity, the unit of diversity may be a phenotypic trait (e.g. gene expression) or a fragment of the DNA (Scheldeman & van Zonneveld, 2010), such as the cytochrome Oxidase I gene (COI) or the Barcoding region. The insect diversity investigated may include its relative subunit (if present), such as species, biotypes or genotypes. A direct measurement of diversity consists of counting the number of diversity unit observed (number of species, biotypes, alleles) in a given area. This is known as *richness*. Since the *richness* depends on the number of samples taken and on the distribution of the selected points, an appropriate sampling method is fundamental, as since such results are not always accurate (Hijmans *et al.*, 2000). If the level of error is not too high, this may be fixed by the rarefaction method, which is an elaborate analytical approach. In this manner, it is possible to recalculate the diversity and standardize an identical number of sampled points of the surveyed area (Petit *el al.*, 1998; Leberg, 2002). In the aim to respect the proportion of each unit (e.g. a species), *Shannon and Simpson* index can be used, which takes into consideration the *evenness*. In addition to studying the *alpha* diversity, as mentioned above, it is possible to calculate also the *beta* diversity, which takes into consideration the divergence in species or in genetic traits, between subunits of the study area. An example can be given by the study of an insect species along an altitudinal gradient in a given area. In this way it can be possible to understand how genetic diversity is spatially structured.

1.5.4 Assessment of a species complex along an altitudinal gradient

Recently, investigating the insects *oikos* along an altitudinal gradient has received much attention in ecological researches. Altitudinal gradient can influence the distribution of insects and reflect the life history and the ability of species to adapt to a specific habitat (Hodkinson, 2005). Elevation influences the spatial distribution of insect species. It also affects the environment in term of temperature, rainfall, host plants and natural enemies. All these factors influence obviously the multi-trophic interaction between insects, host plant and environment (Preszler & Boecklen, 1996). In fact, insects can be polyphagous not only for physiological but also for ecological reason (Bernays, 1999). Altitudinal gradient seems to be a strong ecological instrument for testing the response of a biota to geophysical influence (Körner, 2007). Given this function of "filter", studies regarding the spatial distribution of cryptic species along an altitudinal gradient can help researchers to collect additional information, which can be useful to distinguish species inside the group. Several parameters can be taken in consideration to distinguish cryptic species, such as:

- Temperature, radiation, precipitation, oxygen and wind turbulence
- Host plants, competitor species or parasitoids and predators
- Altitudinal trends of size; but this may be positive or negative, depending on their biology

- Life history: number of instars, developing efficiency at lower gradients, number of generations per year or reducing/extending the life cycle

An insect species may vary in its population response to altitude with different biotypes/cryptic species, showing increasing/declining or no altitudinal trends in abundance. As Körner (2007) in his paper wrote: "*there is no 'standard mountain', any data collected along altitudinal gradients will reflect the combined effect of regional peculiarities and general altitude phenomena*". This should be remembered when results from different geographical studies are compared.

1.5.5 Modelling the potential insect distribution

For most insect species, including many economically important pests, only a limited amount of information is currently available on their spatial distribution. Modelling the distribution of an entity can fill the gap of information on the natural distribution of a species (Guarino *et al*., 2002; Hernandez *et al*., 2006). Mapping the potential distribution of insect pests, identifying areas where they could potentially occur given the favourable environmental conditions, can play an important role in agriculture and forestry pest management (Baker *et. al*., 2000; Bosso *et al*., 2012). This method can be applied also to investigate intraspecific units of diversity, based on morphological or molecular markers. There are many cases of changes in outbreak frequencies among insect pests in response to the environmental modification by human activity or climate change. These data are often based on historical records or adequate knowledge of the insect's population dynamics (Simard *et al.*, 2006; Esper *et al.*, 2007). In addition, invasive pests can cause severe damage not only on food crops, stored products and ornamental plants, but also on the new ecosystems (Vitousek, 1990; Worner & Gevrey, 2006). In fact, recently alien pest invasions have been also identified as a threat for biodiversity since invasive species have been able to replace the native ones (Worner & Gevrey, 2006). For these reasons, any insect pest with high chance to invade a new area, given the environmental characteristics and susceptibility to invasion, needs to be examined (Worner, 2002). Zonneveld *et al.* (2011) in their paper synthetized the most recent knowledges in GIS and Eco-geographic distribution of biodiversity, giving a list of several software packages and tools for GIS, geostatistic and distribution modelling. These can be commercial software or open-source. Among the open-source, here are some:

- **DIVA-GIS**: user-friendly GIS software for biodiversity analysis, species distribution mapping and many other functions. The website provides also free spatial data for the whole world.
- **GRASS**: A free GIS, used for data management, image processing, graphics production, spatial modelling and visualization of many types of data.
- **QGIS**: A GIS software similar to others, but QGIS includes the possibility to be integrated with other software, including GRASS.
- **Maxent**: A software for the distribution modelling based on the maximum-entropy approach.
- **OpenModeller**: A powerful software providing several algorithms as plugins, such as GARP, Climate Space Model, Bioclimatic Envelopes, Support Vector Machines and others.

- **Biomapper**: A GIS software with several statistical tools designed to build distribution models and maps for any kind of animal or plant, based on the Ecological Niche Factor Analysis (ENFA).

Chapter 2: AIM AND OUTLINE OF THIS THESIS

Losses from whitefly pests can be serious in almost every region of the world. However, they are especially severe in Tropical and sub-Tropical regions. Moreover, environments affect whiteflies development, reproduction, survival and their ability to suppress crop productions. Intrinsic differences between the two environmental conditions are based mainly in the diversity of crops, agricultural systems and the harmfulness of the pest (the latter one mostly in relation to often deep differences in life cycles and phenology).

On the taxonomic point of view, these pests show a great diversity between Tropical and sub-Tropical areas, which is also motivated by their tropical origin. However, many whitefly species, though native to the tropics and restricted to those areas for an extreme long time, recently invaded other domains and nowadays they are common also in sub-Tropical and temperate regions. Many of these species have become now integral part of different biocenosis, in very different kind of ecosystems; thus many of their biological and ecological features are intriguing to be investigated comparatively in different regions.

Starting from these assumptions, the present study aimed at comparing some bio-ecological aspects related to the whitefly fauna living on herbaceous plants in a typical Tropical environment (close to the geographic area which is presently considered as the probable native area of whiteflies) and in a sub-Tropical – temperate one. Practical reasons led to choose the cassava growing areas of Tanzania, as a case study for a Tropical environment, and the whole territory of

Sicily (Italy), for its central position in the Mediterranean region and its role of crossroads between sub-Tropical and temperate climates.

Research questions, at the base of the study, were:

1. *Which whiteflies and parasitoids occur on herbaceous plants in a sub-Tropical to temperate environment?*

   In this study we investigated the whiteflies and parasitoids on herbaceous hosts (cultivated and wild) through surveys around the entire regional territory of mainland Sicily. The specimens collected were identified through both morphological approach and, when needed, molecular analyses. GIS and Geostatistics techniques were adopted in the aim to study the abundance and distribution of the whiteflies occurring in the area. The data will provide statement (including maps) of the current status of whiteflies and their parasitoids in cultivated and natural environments, carrying out a spatial analysis on pest/parasite diversity and distribution.

2. *Which whiteflies and parasitoids occur on herbaceous plants in a Tropical environment?*

   Given the existing relationship among the University of Catania and the International Institute of Tropical Agriculture of Tanzania (IITA-Tz), this country was selected as study area about tropical environment. In particular, Tanzania has been selected also as an interesting case study, due to the occurrence, in the north-western part of the country, of *Bemisia* *tabaci* super-abundant population associated with a disastrous viral disease, the cassava mosaic disease (CMD). Taking in consideration the programs of IITA-Tz and the scientific and logistic facilities given by the local institution, a survey was carried out in north-western part of Tanzania around cassava growing region. However, during the trip in the given area, many non-cassava plants were investigated (tomato, aubergine, zucchini, pumpkins, bitter tomato, potato, sweet potato, beans, several weeds, etc.). The survey methodology applied during the survey was the same of the one used in Sicily, in order to obtain comparable data.

3. *Are there differences between the common whitefly pests to both Tropical and sub-Tropical to temperate regions?*

   We investigated the differences and compared the data among the Tropical and sub-Tropical environments, in terms of host preference, abundance (on the same host-plant), cultivated or wild hosts and the harmfulness of the pest. In addition, in the aim to understand the major agricultural threat to both tropical and sub-tropical regions, under an ecological point of view, *Bemisia tabaci* was assessed along the elevation gradient.

Chapter 3: MATHERIALS AND METHODS

3.1 FIELD SURVEY: EXPLORATION FOR WHITEFLIES AND THEIR NATURAL ENEMIES

3.1.1 Survey methodology

Search for biodiversity includes establishing the number of insect species occurring within a geographic region as well as the mutual relationships among them and with their environment. Sampling methods should include knowledge on insect biology, habitats and life cycle. Many insects show change in population densities in different seasons or years (Hunter & Price, 1998). In Mediterranean and tropical climates, insects show a seasonal activity pattern (Tauber & Tauber, 1981). In Mediterranean regions these patterns are activated by photoperiod, temperature and humidity (van Asch & Visser, 2007). Apart from some particular case, such as the monsoon conditions, it is still unclear what exactly guide phenology in tropical environments (van Asch & Visser, 2007). At the equator, for example, photoperiod and temperature are almost the same all the year round. Designing an efficient sampling method (or choosing an existing one) is crucial to evaluate what the samples show us about the insect population as a whole.

Since this research covered broad areas (even entire regions), the first step was to divide the geographical area under consideration into quadrants of 20 km by 20 km so as to cover the total area in spatially balanced manner. In each quadrant a field or location was selected to conduct the observations. Since both agricultural and natural areas were investigated, the following two sampling strategies were adopted.

a) *Agricultural areas*. To obtain reliable results, it is important to collect samples from several points throughout the field. Nevertheless, inspecting every plant in the field and looking at all plant parts is obviously impossible. For this reason, in each field, 30 plants were randomly selected, along an "x" or "z" transect.
b) *Natural areas*. At each sampled site, a random sampling method based on slope and habitat suitability was adopted (Hirzel & Guisan, 2002). A grid of 50 m x 50 m was defined and at least 40 min of sampling was conducted inside this selected area. Five plants per species were randomly selected and inspected.

Then, for each plant sampled, the lower five leaves were detached, kept inside paper bags and preserved in a cool box until laboratory. Subsequently, host plants were identified and GPS co-ordinates were recorded at each location, using a handheld geographic positioning system (accuracy approximately 10 m). Given the extensive duration of each survey and the probable risk to damage the samples if kept for long time, there was the need to process the infested leaves daily. Nymphs were counted and pictures were taken using a netbook and a USB digital microscope (250x). Unparasitised and parasitized third/fourth instar nymphs were detached from the leaves, individually put in small glass vials, sealed with a small ball of cotton, and held for approximately one week, waiting for the parasitoid emergence. The percentage of parasitism was calculated by dividing the number of emerged parasitoids by the sum of 3rd and 4th instar whitefly nymphs collected. Emerged insects and their empty pupae were preserved in ethanol 90% and taken to the laboratory for further analysis.

3.1.2 Study Areas

3.1.2.1 Mediterranean environment (Sicily)

Sicily is the largest island in the Mediterranean, with a total area of about 25,000 km^2, extending in latitude between 36° and 38° North and longitude between 12° and 15° East. Despite the particular orographic status, with a unique morphological features, it is possible to subdivide the landscapes in three distinct main areas: the northern part, which extends from Capo Peloro to Capo Lilibeo; the Southern part, extending from Capo Lilibeo to Capo Passero; the eastern side, which extends from Capo Passero to Capo Peloro. The topography shows sharp contrasts between the northern portions of the island, which is mainly mountainous, respect to the south central and south western parts, that are essentially hilly and volcanic in nature.

According to the macroclimatic classification of Köppen, Sicily can be defined as a region of temperate-humid climate (type C) (average of the coldest month below 18 °C but higher than -3 °C) or, better, humid-subtropical, with dry summer, which is typical Mediterranean climate, characterized by an average temperature of the warmest month over 22 °C and precipitation regimes characterized by rainfall during the cold season (autumn-winter). However, this definition has only a macroclimatic value that serves to distinguish, for example, the Sicilian climate from that of the Middle East or Central Europe. According to Pinna (1978), it is possible to distinguish different subtypes: temperate subtropical, warm temperate, temperate sub-coastal, temperate sub-continental and temperate fresh.

Although the region is relatively small in area, it has a diverse agro-ecology dictated by the variability in topography and climatic conditions. A wide range of settings varying from alpine environments to semi-desert coastal dunes characterizes Sicilian landscape. Within the Sicilian territory, the richness of habitats, the diversity of geological substrates and the favourable climatic conditions concur to define a great number of environments and remarkable biodiversity. From a floral view, the island shows a significant phytogeographic correlation that clearly matches to the paleogeographic developments of the territory from Miocene onward (Martino & Raimondo, 1979; Nimis, 1984; Raimondo, 2004). The island's flora is characterized by a rich endemic proportion (which marks its geographic isolation) and by entities, which are quite rare or relict from the neighbouring Mediterranean countries.

About half of the Sicilian territory is affected by agro-ecosystems where a traditional and advanced agriculture is carried out. As in other Mediterranean countries like Spain, France, Greece and many others, also Sicily has been encountered severe whitefly infestations attacking protected crops and open field cultivations (Patti & Rapisarda, 1981).

To enhance the understanding of the whiteflies and their parasitoids fauna composition, during 2011-2012 a survey was carried out in Sicily. Sampling exclusively on herbaceous plants., several environments were investigated, such as natural areas (including protected areas) and cultivated areas (greenhouse and open field crops). A regular grid was designed and situated over the experimental area, resulting in 62 quadrants; around fifty were sampled, since cities and areas with high urban activity were excluded. Regarding the landscape, 40% was categorized as large agricultural fields, 25% as greenhouse agriculture, 33% as natural areas and 2% as kitchen garden. To improve the spatial analysis, 6 predictor maps were selected (fig.4). These maps described the environmental conditions of the studied area explaining the potential variability in insects sampling in terms of presence/absence or abundance. All maps were resampled using DIVA-GIS Version 7.5 (Hijmans *et al.,* 2001).

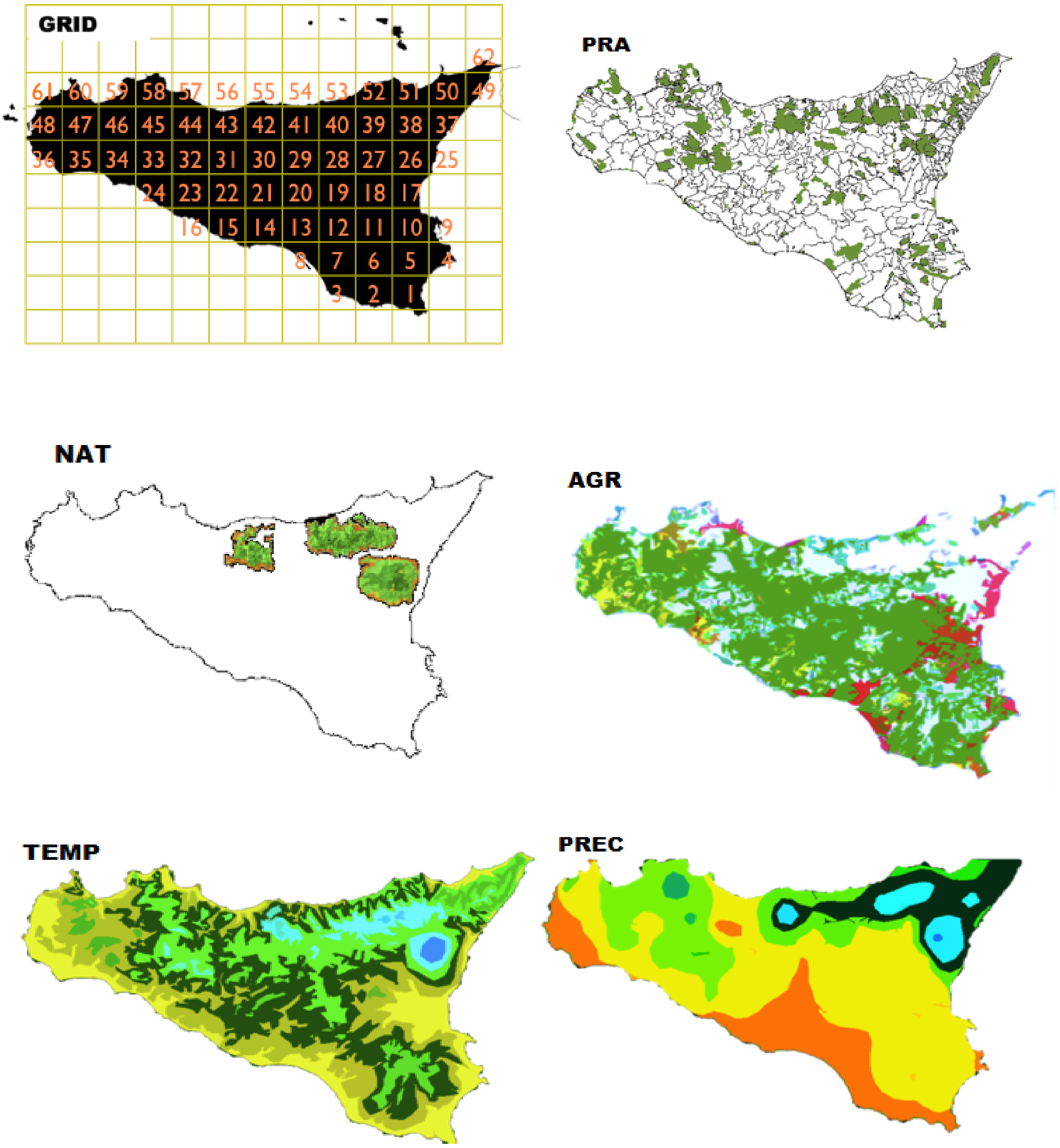


Fig.4 Maps used in survey design and spatial association. GRID; PRA: Protected areas (green); NAT: Natural parks (green). AGR: Agriculture (in red: high activity; yellow: medium; green: low); TEMP: Average annual temperature (yellow: 17-19 °C; deep green: 15-17 °C; bright green: 14-16 °C; azure: 11-13 °C; blu: ≤ 10 °C); PREC: average annual precipitation (orange: 300-500 mm; yellow: 501-700 mm; bright green: 701-900 mm; deep green: 901-1100 mm; blue: ≥ 1101 mm).

3.1.2.2 Tropical environment (Tanzania)

Tanzania is located in East Africa, on the Indian Ocean, bordered by Kenya to the north and Mozambique to the south. It has an area of 945,000 km2, which includes the three major coastal islands of Mafia, Pemba and Zanzibar, and a coastline that is about 800 km long. Its geography is characterized by plains along the coast, a central plateau, and highlands in the north and south. The northwest of the country encompasses approximately one-half of Lake Victoria, the second largest body of freshwater in the world, and the western and south-western borders about the comparably massive Lake Tanganyika and Lake Nyasa. Elevations range from the sea level to the highest point in Africa, the peak of Kilimanjaro at 5,895 m. Topography and climatic conditions, however, limit cultivated crops to only 4% of the land area. The climate ranges from tropical to temperate in the highlands. Average annual precipitation over the entire nation is 1,042 mm. Average temperatures range between 17 °C and 27 °C, depending on location. Natural hazards include both flooding and drought. Within the country, altitude plays a large role in determining rainfall patterns, with higher elevations receiving more precipitation. Only about half of the country receives more than 762 mm annually (Mwandosya *et al.*, 1998). Tanzania's precipitation is governed by two rainfall regimes. The bimodal rainfall, comprised of the long rains of Masika, between March-May, and short rains of Vuli, between October-December, is the pattern for much of the northeastern, northwestern (Lake Victoria basin) and the northern parts of the coastal belt. A unimodal rainfall pattern, with most of the rainfall during December-April, is more typical of most of the southern, central, western, and southeastern parts of the country.

Agriculture is clearly the most important sector of the Tanzanian economy. It comprised 45.1% of GDP in 2000 (World Bank, 2002). Up to 80% of the population of the country relies directly on agriculture of one sort or another for their livelihood. The most important crops are: maize, cassava, coffee and cotton – with maize and cassava being a major food staple, coffee a major cash crop grown in large plantations, while cotton is another cash crop grown largely by smallholder farmers. During the last decade, the production of cassava in East-Africa has been constrained and reduced by pests and diseases, of which cassava mosaic virus disease (CMD) is the most damaging, transmitted by the whitefly *B. tabaci* (Legg *et al.*, 2006; Legg, 2010). Since severe CMD associated with the pandemic and *super-abundant* whiteflies are known to be present in the north-western part of Tanzania, surveys were carried out especially in this area in 2012 and 2013. The survey areas encompassed parts of the three regions: Mwanza, Shinyanga and Tabora. These are all at mid-altitude elevations of between 1,100 and 1,500 metres above sea level. The study area was divided in four quadrants and appropriate coordinates defined the geographical positions. The north boundary was marked at 3°S, the south boundary at 5°S, the western boundary at 32°E and the eastern boundary at 34°E. In each quadrant, twenty fields were sampled for a total of 80 fields. To analyse the spatial pattern of whiteflies, 7 predictor maps were designed (Fig. 5-11). These maps describe some environmental conditions of the investigated area, helping to understand the potential variation in whiteflies and their parasitoids distribution.

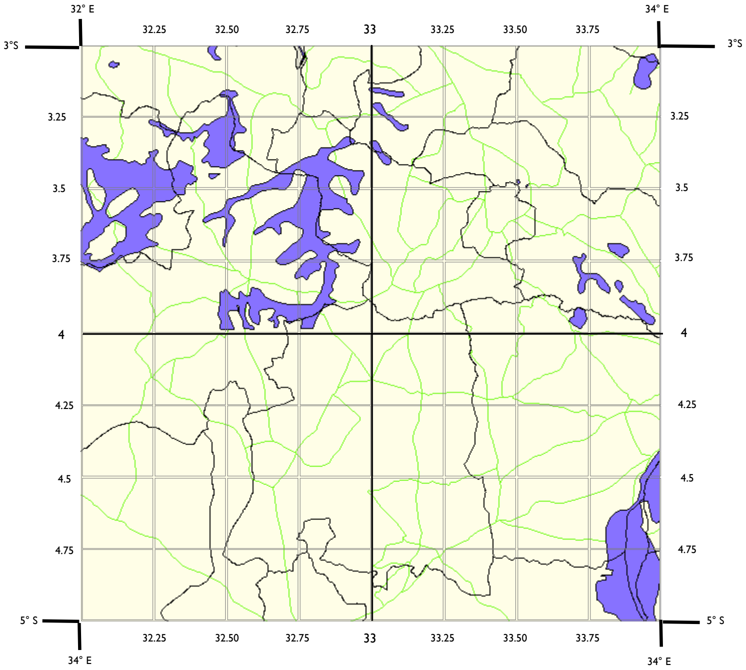


Fig. 5 GRID: Sampling area in north-western part of Tanzania. Four quadrants were defined and in each 20 fields.

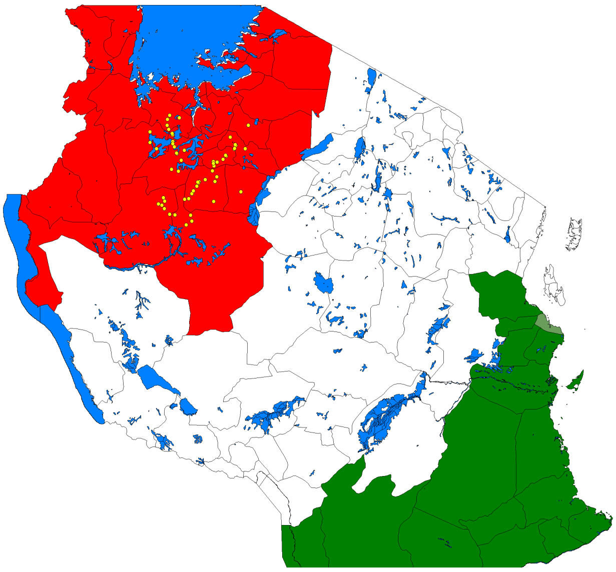


Fig. 6 Cassava growing regions of Tanzania. Green: region non-affected by super-abundant whiteflies; red: region affected by super-abundant whiteflies. Yellow dots indicate sampling sites.

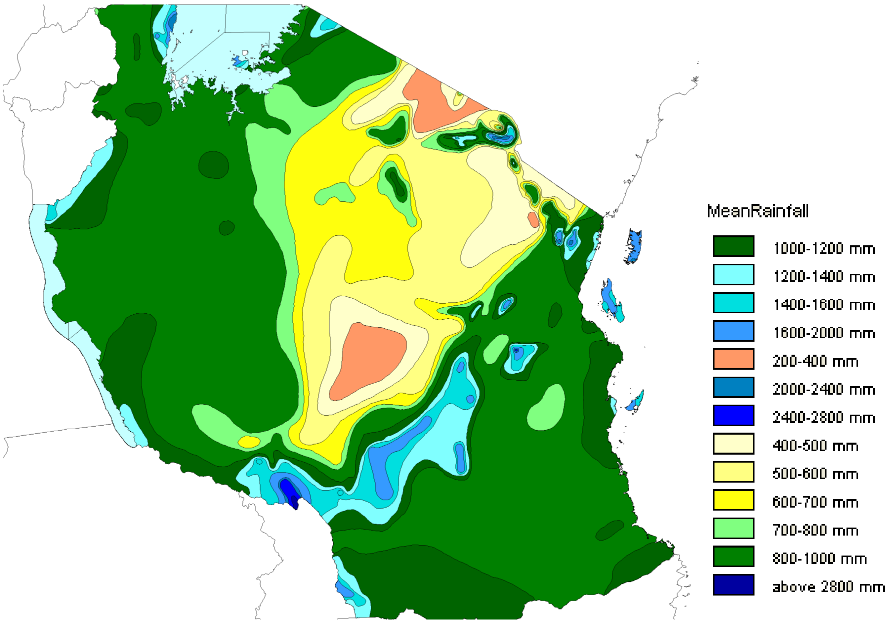


Fig. 7 Meaning yearly rainfall in Tanzania

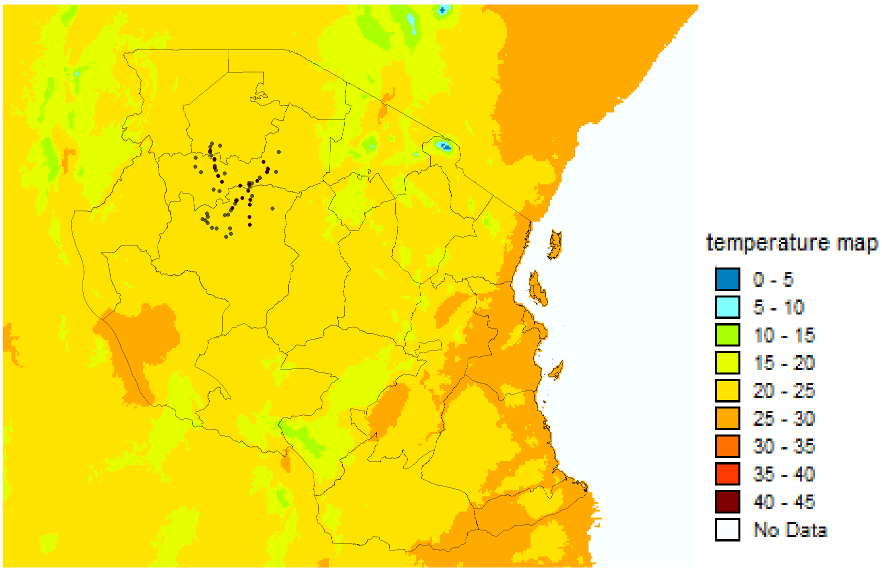


Fig. 8 Average yearly temperature in Tanzania. Black dots indicate sampling sites.

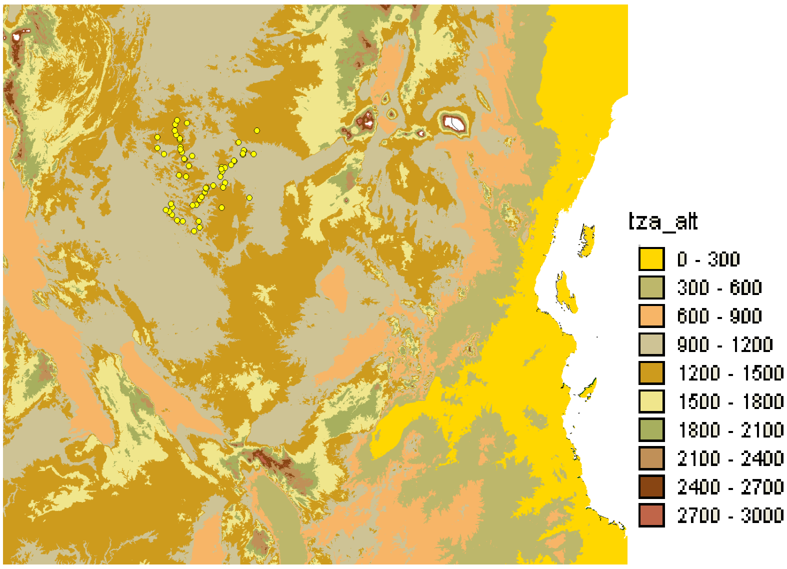


Fig. 9 Elevation in Tanzania. Yellow dots indicate sampling sites.

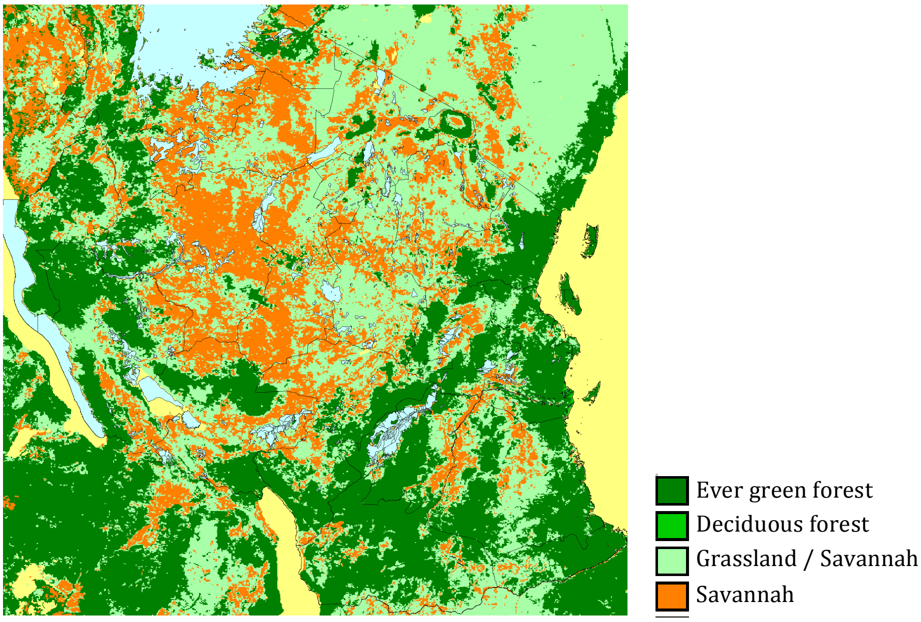


Fig. 10 Land cover in Tanzania

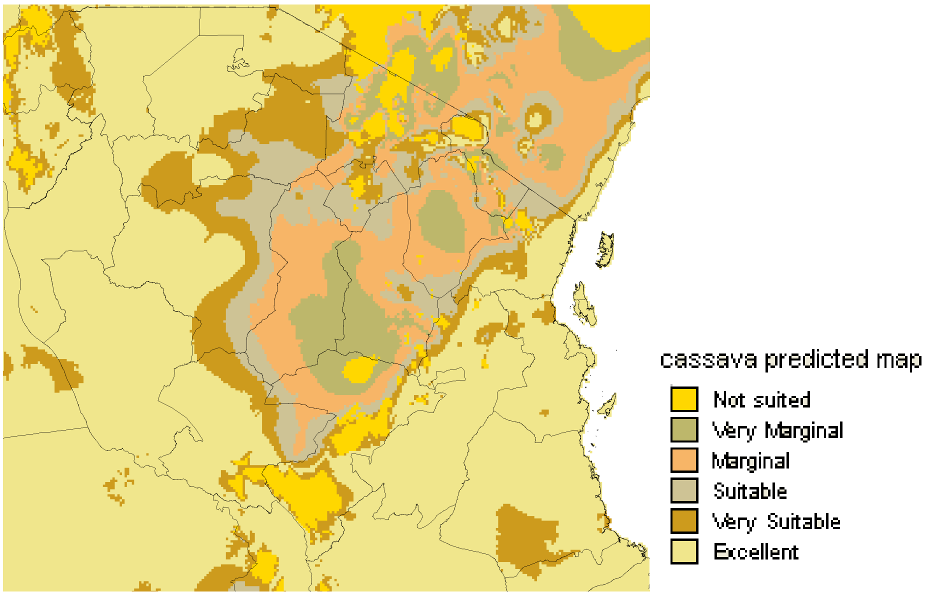


Fig. 11 Suitable areas for cassava cultivation in Tanzania.

Sampling was done in and around farmers' cassava fields. The herbaceous plant hosts occurring were: cassava [*Manihot esculenta* Crantz] (Euphorbiaceae) as a first objective, then: sweet potato [*Ipomoea batatas* (L.) Lam.] (Convolvulaceae); been [*Phaseolus vulgaris* L.] (Fabaceae); okra [*Abelmoschus esculentus* (L.) Moench] (Malvaceae); cowpea [*Vigna unguiculata* (L.) Walp.] (Fabaceae); tomato [*Solanum lycopersicum* L.] (Solanaceae); bitter tomato [*Solanum incanum* L.] (Solanaceae); pumpkin [*Cucurbita maxima* Duchesne] (Cucurbitaceae); peanut [*Arachis hypogaea* L.] (Fabaceae); Billy goat weed [*Agerutum conyzoides* L.] (Asteraceae); Broom weeds [*Sida* spp. L.] (Malvaceae); Capsicum [*Capsicum frutescens* L.] (Solanaceae); cassava wild [*Manihot glaziovii* Muell. Arg.] (Euphorbiaceae); [*Euphorbia heterophylla* L.] (Euphorbiaceae); cotton [*Gossypium herbaceum* L.] (Malvaceae); bottle ground [*Lagenaria siceraria* (Molina) Standl.] (Cucurbitaceae); cassia [*Cassia occidentalis* (L.) Link] (Caesalpiniaceae); double thorn [*Oxygonum* sp. Burch]

(Solanaceae); *Malvastrum* sp. A. Gray (Malvaceae); castor plant [*Ricinus communis* L.] (Euphorbiaceae).

3.2 TAXONOMY: MORPHOLOGICAL IDENTIFICATION

3.2.1 Aleyrodidae

Genera and species of whiteflies were defined based primarily on characters of their fourth instar nymph, also called puparia stage (Suh & Hodges, 2008), which has more useful morphological structures (fig.5) than the adult stage, making the whitefly taxa separation possible. Hodges & Evans (2005) described the important steps to follow for the correct identification of specimens. The morphological features taken in consideration can be diverse. These include the shape, colours, the wax filaments and their arrangement, spines, hairs, pores and many others (Hodges & Evans, 2005). Accurate whitefly species identifications is possible through a microscopic examination of a slide-mounted puparium or pupal case. However, sometimes the morphological identification of whiteflies can be intricate by phenotypic variations within the same species (Neal & Bentz, 1999). In other cases, difficulties may rise from the occurrence of cryptic species complexes, having no evident morphological difference allowing their mutual identification. For example, the *Bemisia tabaci* species complex includes more than 30 putative species; these species are impossible to distinguish by morphological characters (Bedford *et al.*, 1993; Calvert *et. al.*, 2001; Perring, 2001; Lee *et al.*, 2013). For this reason, when in need to have a precise identification, *B. tabaci* s.l. specimens must be also characterized molecularly.

In the research here reported, whitefly puparia were slide mounted according to the protocol proposed by Martin (1987, 1999) and identified using the taxonomic keys of Martin (1999), Martin *et al.* (2000) and Dooley (2011). As a final step, C. Rapisarda, from DiGeSA (University of Catania, Italy), confirmed the specimens from Mediterranean regions; and G.A. Evans, from the United States Department of Agriculture [(USDA-APHIS/NIS); Maryland, USA], confirmed whitefly species collected from the tropics.

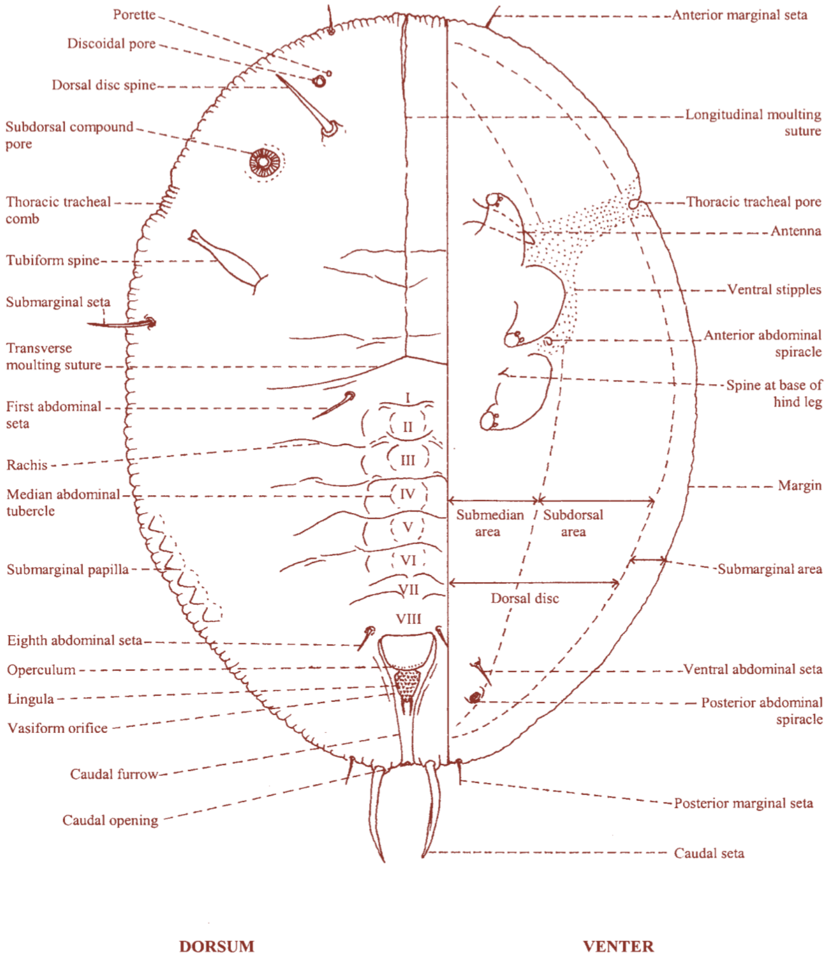


Fig.12 General morphology of a whitefly pupal case (adopted from OEPP/EPPO Bulletin 2004).

3.2.2 Aphelinidae

The correct identification of an entomophagous insect that is found in association with a target pest species is of equal importance to accurate pest determination, especially when planning biological control programs. In many cases, natural enemies, such as whitefly parasitoids, belong to groups of very minute wasps, which often are difficult to identify. In addition, the knowledge on some of their taxonomic group remains still incomplete. To date, approximately 30 genera of Hymenoptera are known to be either facultative or obligate parasitoids of whiteflies (Polaszek *et al.*, 1992). They belong to six families: Aphelinidae, Eulophidae, Pteromalidae, Signiphoridae and Encyrtidae, within the superfamily Chalcidoidea, and Platygastridae in the superfamily Platygastroidea (Polaszek *et al.*, 1992; Viggiani, 2000). Given the importance of the Hymenoptera in agriculture, such as biological control agents of pests, pollinator for food production, etc., their exact identification becomes fundamental (Seltmann, 2004).

Contrary to the whiteflies, during the research here exposed, the identification of parasitoids was based on the examination of adults (mainly females; fig.6) mounted in Hoyer's medium (Noyes, 1982). In the literature there are several morphological keys, which include keys of orders to families (Goulet & Huber, 1983; Charles & Norman, 2004), or the description of species belonging to particular genera (Viggiani, 1976; MacGown & Nebeker, 1978; Wolley, 1988; Mottern *et al.*, 2011). However, more often it is easier to work through a specific key related to a particular geographic area (Viggiani & Gurrieri, 1988; Evans, 1993; Hayat, 1998; Hernandez-Suarez *et al.* 2003, Otim *et al.*, 2006), or even regarding a specific field of study, such as biological control (Viggiani, 1994, 1997). The morphological keys used in this work were first selected considering the geographical areas where the specimens were collected from (Africa or Mediterranean basin), or from papers including latest review of a specific genus. In this scenario, the keys were adopted from Polaszek *et al.* (1992), Evans (1993), Zolnerowich & Rose (1998), Schmidt & Polaszek (2007) and Mottern *et al.* (2011). The morphological identification of the specimens collected in the Mediterranean region (Sicily) was particularly facilitated thanks to the huge amount of literary data available on whitefly parasitoids. In fact, the knowledge about parasitic wasps and their use in Italy goes back to the early 1990s, thanks to Antonio Berlese and Filippo Silvestri, who discovered and described several species. A considerable contribution has been given by Gennaro Viggiani, who described several new species of whitefly parasitoids and studied their ecology. Among the literature written by Viggiani, a huge amount of books and papers occur which include identification keys of Aphelinids which has been used in this work (Viggiani, 1967, 1975, 1977, 1980, 1994, 1997). More recently, following the example set by Viggiani, Paolo Pedata, using both morphological and molecular techniques, is keeping updated the knowledge on these whitefly biocontrol agents all over Italy (Pedata & Viggiani, 1991, 1993; Pedata *et al.*, 1995; Pedata & Polaszek, 2003). Therefore, using the above reported morphological keys, it was possible to identify most of the specimens collected during the surveys in the investigated areas.

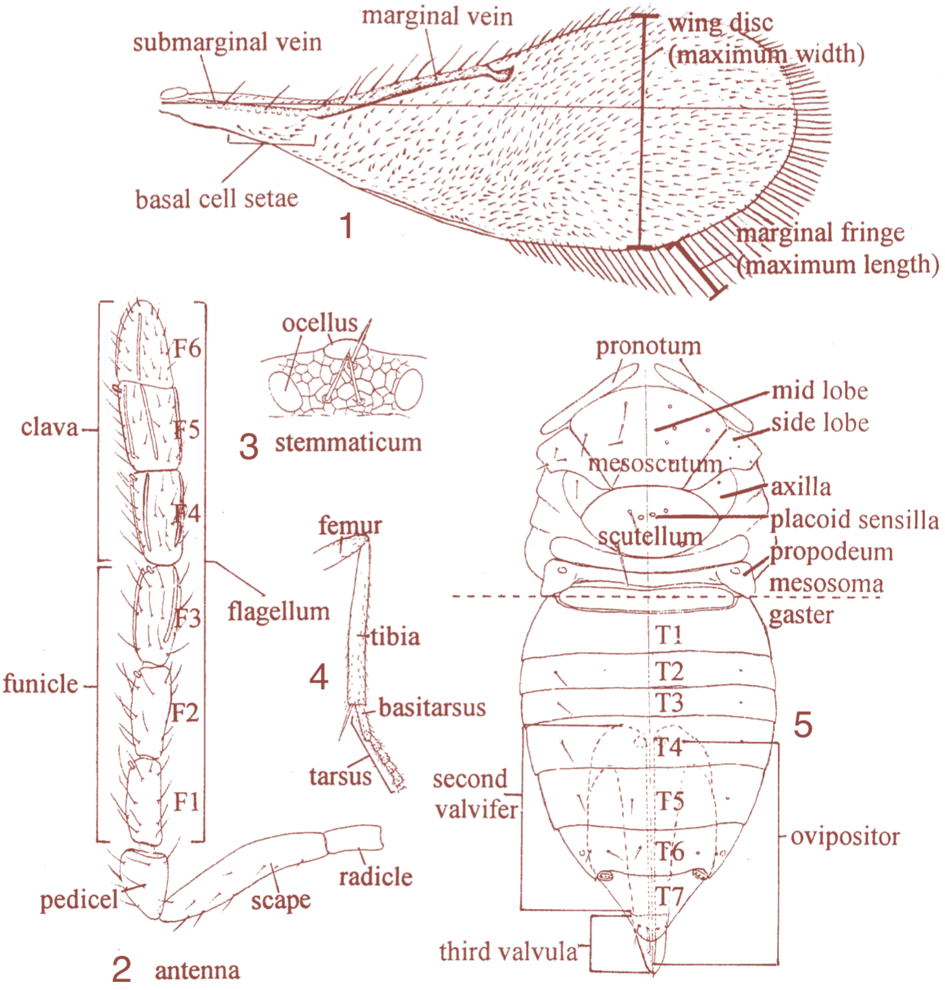


Fig. 13 General morphology of a parasitoids wasp (adopted from Polaszek *et al.,* 1999).

An exception has been done for the intricate genus *Eretmocerus*, which is often difficult to identify morphologically at species level. The specimens collected were divided into species-groups based on the number of pairs of setae on the midlobe of the mesoscutum (MS) and the length/width (L/W) ratio of the antennal club (www.fsca-dpi.org/homoptera_hemiptera/whitefly/whitefly_catalog.htm, 2002; Evans, pers. comm.). Successively, the representative specimens of each group were investigated molecularly.

To ensure the species identity, representative parasitoid specimens identified were sent for confirmation to Dr P. Pedata (especially species of the genus *Encarsia*) and Dr G.A. Evans (especially species of the genus *Eretmocerus* and tropical species of *Encarsia*).

The microphotographs were taken using Leica DFC420 and processed using Leica Application Suite (LAS V3.3.0). The pictures were enhanced using GIMP 2.8.4 (GNU Image Manipulation Program).

3.3 MOLECULAR METHODS IN INSECT TAXONOMY

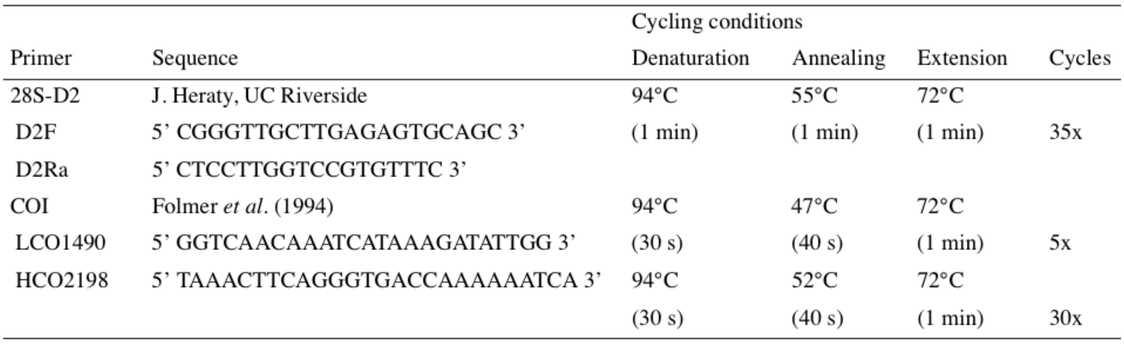
3.3.1 Whiteflies (the Bemisia tabaci species complex)

Since *Bemisia tabaci* is not a single but rather a complex of cryptic species (Bird, 1957; Costa & Brown, 1991; Bartlett & Gawel, 1993; Campbell *et al.,* 1993; Perring *et al.*, 1993; Brown *et al.*, 1995, Brown, 2000; De Barro *et al.*, 2005; Dinsdale *et al.*, 2010; De Barro *et al.*, 2011; Lee *et al.*, 2013), and populations of different geographic origins have been shown to be morphologically indistinguishable, molecular investigations were needed. The approaches used to identify the *B. tabaci* species occurring in the surveyed areas were the analysis of the Cytochrome Oxidase I gene (COI) (Simon *et al.*, 1994) and the restriction of the amplified fragment of the mtCOI with the enzyme *Tru9*. This approach was able to separate clearly the five *B. tabaci* species (previously known as biotypes B, Q, M, S, and T) reported until now in the Mediterranean Basin (Bosco *et al.*, 2006). For reconfirmation, genetic analysis of mtCOI gene was used to reconstruct a phylogenetic tree. On collected material, total DNA was extracted from single insect using protocol described by Walsh *et al.* (1991) and modified by De Barro & Driver (1997). The PCR reaction was conducted using the forward and reverse primers C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3'), respectively (Simon *et al.*, 1994). All PCR reactions were performed in 20 µl volumes with 0.85X of FailSafe™ PCR 2X premix F (Epicentre technologies), 0.5 µl of each primer 10 µM, 1.5 U of Taq Polymerase recombinant 5U (Life Technologies) and 2 µl of DNA template. The cycling conditions were: 96 °C for 5 min, then 35 cycles at 96 °C for 45 s, 45 °C for 1 min, 72 °C for 1 min, followed by final cycle at 72 °C for 10 min. All reactions and cycling conditions were conducted in an automated thermal cycler [Mastercycler®(Eppendorf)]. An aliquot of PCR products was digested by restriction enzyme *Tru*9 using protocol described by Bosco *et al.* (2006). PCR products were then sequenced at BMR Genomics sequencing service (Padua, Italy) or Macrogen Inc. (USA). The sequences were aligned using Cluster Omega algorithm as implemented in Seaview 4 (Gouy *et al.*, 2010) and cleaned manually. Furthermore, several mtCOI sequences were downloaded from GenBank and included in the analysis. Then, sequences were analysed by MrBayes version 3.2 (Huelsenbeck and Ronquist, 2001) employing Markov Chain Monte-Carlo (MCMC). The models selected were HKY + G and MrBayes was run for 10 million generations and trees were sampled every 1000 generations (Boykin *et al.*, 2007). The runs were stopped when reached a standard deviation of split frequencies closer to 0.001, and the potential scale reduction factor (PSRF) was close to 1. The trees obtained from the MrBayes 3.2 runs were visualized in Figtree 1.4 (Institute of Evolutionary Biology, University of Edinburgh, United Kingdom).

3.3.2 Parasitoids

Representative parasitoid specimens were examined genetically. DNA was extracted using a non-destructive extraction protocol (Walsh *et al.*, 1991; Burks *et al.*, 2011). Specimens were placed in 80 µL of 10% Chelex (Bio-Rad, Richmond, CA) solution containing 10 µL proteinase-K (SIGMA) and were incubated at 55°C for one hour and 100°C for 12 min. The parasitoid specimens were then removed from the lysis and the DNA extracts were preserved at -20°C until amplified. The parasitoids were then rinsed in distilled water and transferred to 90% ethanol where they remained until slide preparation. In this study, the barcoding region of the mitochondrial cytochrome oxidase I (COI) gene and the domain 2 region (D2) of the 28S rDNA gene were sequenced to characterise species at the molecular level. In addition, for the genus *Eretmocerus*, the ribosomal ITS-1 gene region was sequenced. PCR was used to amplify the gene regions. Amplification was performed using 1 µl of the DNA extract in a total reaction volume of 20µl containing 0.2µM primer mix, 0.2mM dNTPs, 2.5mM $MgCl_2$, 1x PCR reaction buffer and 0.3 units *Taq* DNA polymerase (Sigma). Primer sequences and temperatures are given in table 1.

Table 1 Primer sequences (adopted from Schmidt *et al.*, 2011).



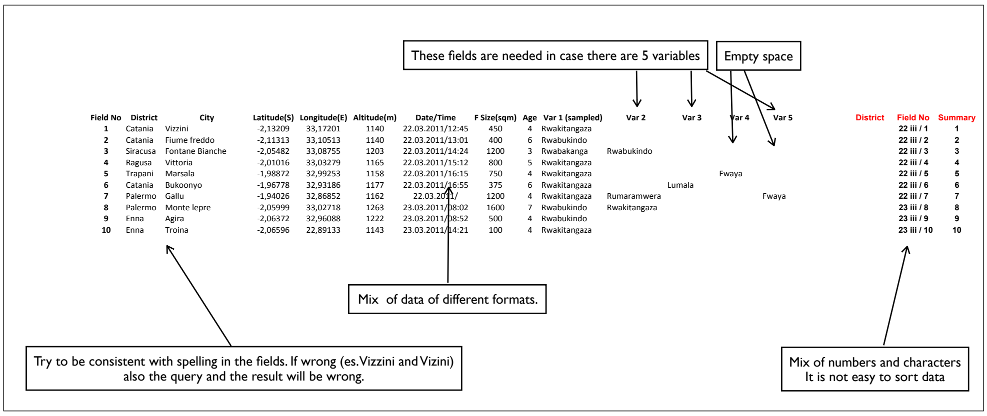
		Cycling conditions			
Primer	Sequence	Denaturation	Annealing	Extension	Cycles
28S-D2	J. Heraty, UC Riverside	94°C	55°C	72°C	
D2F	5' CGGGTTGCTTGAGAGTGCAGC 3'	(1 min)	(1 min)	(1 min)	35x
D2Ra	5' CTCCTTGGTCCGTGTTTC 3'				
COI	Folmer *et al*. (1994)	94°C	47°C	72°C	
LCO1490	5' GGTCAACAAATCATAAAGATATTGG 3'	(30 s)	(40 s)	(1 min)	5x
HCO2198	5' TAAACTTCAGGGTGACCAAAAAATCA 3'	94°C	52°C	72°C	
		(30 s)	(40 s)	(1 min)	30x

Aliquots of 5µl PCR products were separated on a 1% agarose gel and visualized under UV light using Gel Red. Where bands of expected base pair sizes were obtained, the respective PCR products were sent for sequencing at Macrogene Inc. (USA). The mtCOI, D2 and ITS-1 gene sequences were aligned using Clustal W

(Thompson *et al.* 1994). To study the phylogeny, maximum parsimony analysis was performed using PAUP 4.0b10 (Swofford, 2002) integrated in EPOS by Griebel *et al.* (2008). Tree–Bisection–Reconnection (TBR) was used.

3.4 DATA MANAGEMENT

Data storage was a crucial point in this work. Many errors can occur if the consistency, redundancy and safety are not well verified, and all data collection and results can be compromised. As data are the most valuable part, it was really important to keep them well organized and safe. Instead of storing data in paper sheets or extended and confusing spreadsheet files (fig.14) a well-defined Data Base (DB) was created, preserving safely all data and providing an easy and fast access to survey data. In this way, it was possible to produce less data errors by a simple user interface during the data entry. A relational model was chosen for this DB, and data were kept in multiple tables (fig.15). This model works with an identifying value called “key”. Every entry has a key by which it is identified uniquely. By means of this key, various tables can be combined. In the relational DB model, by the Structured Query Language (SQL), it was possible to query descriptive and geotechnical data and relate them to other predesigned information. The data were organized in a multi-user database with MySQL engine.



These fields are needed in case there are 5 variables

Empty space

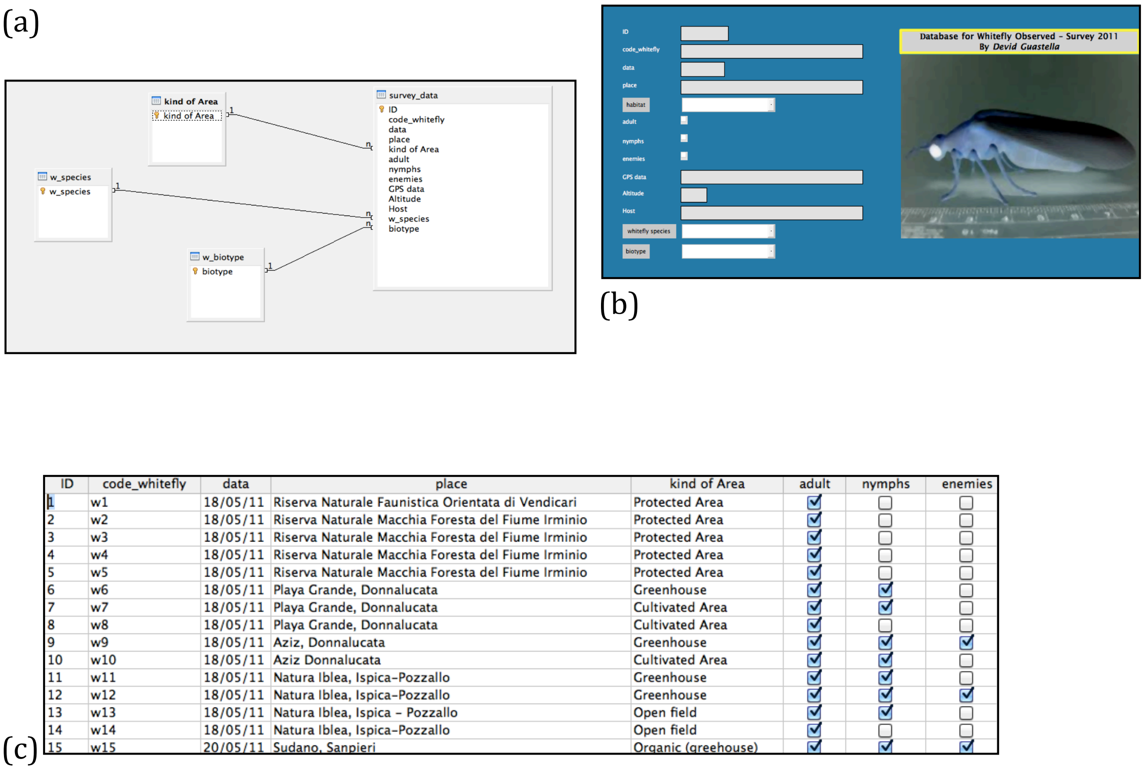
Field No	District	City	Latitude(S)	Longitude(E)	Altitude(m)	Date/Time	F Size(sqm)	Age	Var 1 (sampled)	Var 2	Var 3	Var 4	Var 5	District	Field No	Summary
1	Catania	Vizzini	-2,13209	33,17201	1140	22.03.2011/12:45	450	4	Rwakitangaza						**22 iii / 1**	**1**
2	Catania	Fiume freddo	-2,11313	33,10513	1140	22.03.2011/13:01	400	6	Rwabukindo						**22 iii / 2**	**2**
3	Siracusa	Fontane Bianche	-2,05482	33,08755	1203	22.03.2011/14:24	1200	3	Rwabakanga	Rwabukindo					**22 iii / 3**	**3**
4	Ragusa	Vittoria	-2,01016	33,03279	1165	22.03.2011/15:12	800	5	Rwakitangaza						**22 iii / 4**	**4**
5	Trapani	Marsala	-1,98872	32,99253	1158	22.03.2011/16:15	750	4	Rwakitangaza			Fwaya			**22 iii / 5**	**5**
6	Catania	Bukoonyo	-1,96778	32,93186	1177	22.03.2011/16:55	375	6	Rwakitangaza		Lumala				**22 iii / 6**	**6**
7	Palermo	Gallu	-1,94026	32,86852	1162	22.03.2011/	1200	4	Rwakitangaza	Rumaramwera			Fwaya		**22 iii / 7**	**7**
8	Palermo	Monte lepre	-2,05999	33,02718	1263	23.03.2011/08:02	1600	7	Rwabukindo	Rwakitangaza					**23 iii / 8**	**8**
9	Enna	Agira	-2,06372	32,96088	1222	23.03.2011/08:52	500	4	Rwabukindo						**23 iii / 9**	**9**
10	Enna	Troina	-2,06596	22,89133	1143	23.03.2011/14:21	100	4	Rwakitangaza						**23 iii / 10**	**10**

Mix of data of different formats.

Try to be consistent with spelling in the fields. If wrong (es.Vizzini and Vizini) also the query and the result will be wrong.

Mix of numbers and characters
It is not easy to sort data

Fig. 14 Example of the difficulties in spreadsheet. It can only show 1:1 relationship and need to include fields for *n* possibilities.



ID	code_whitefly	data	place	kind of Area	adult	nymphs	enemies
1	w1	18/05/11	Riserva Naturale Faunistica Orientata di Vendicari	Protected Area	☑	☐	☐
2	w2	18/05/11	Riserva Naturale Macchia Foresta del Fiume Irminio	Protected Area	☑	☐	☐
3	w3	18/05/11	Riserva Naturale Macchia Foresta del Fiume Irminio	Protected Area	☑	☐	☐
4	w4	18/05/11	Riserva Naturale Macchia Foresta del Fiume Irminio	Protected Area	☑	☐	☐
5	w5	18/05/11	Riserva Naturale Macchia Foresta del Fiume Irminio	Protected Area	☑	☐	☐
6	w6	18/05/11	Playa Grande, Donnalucata	Greenhouse	☑	☑	☐
7	w7	18/05/11	Playa Grande, Donnalucata	Cultivated Area	☑	☑	☐
8	w8	18/05/11	Playa Grande, Donnalucata	Cultivated Area	☑	☐	☐
9	w9	18/05/11	Aziz, Donnalucata	Greenhouse	☑	☑	☑
10	w10	18/05/11	Aziz Donnalucata	Cultivated Area	☑	☑	☐
11	w11	18/05/11	Natura Iblea, Ispica-Pozzallo	Greenhouse	☑	☑	☐
12	w12	18/05/11	Natura Iblea, Ispica-Pozzallo	Greenhouse	☑	☑	☑
13	w13	18/05/11	Natura Iblea, Ispica - Pozzallo	Open field	☑	☑	☐
14	w14	18/05/11	Natura Iblea, Ispica-Pozzallo	Open field	☑	☐	☐
15	w15	20/05/11	Sudano, Sanpieri	Organic (greehouse)	☑	☑	☑

Fig. 15 (a) A simple Relational Database Structure. Multiple tables for every 1-to-many relationship. (b) An easy windows can help users for data entry. (c) Allows new temporary tables (Queries) to be formed by linking separate 1-1 tables using their key fields.

3.5 GIS AND GEOSTATISTICS

In order to assess the spatial analysis and distribution of whiteflies and their parasitoids, the main points investigated were: (1) mapping the spatial distribution of whiteflies on herbaceous plants; (2) analyzing their spatial diversity; (3) identifying pest risk areas by a modeled distribution and (4) identifying priority zones for biocontrol agents *in situ* conservation. Free spatial data regarding the country administrative boundaries for use in GIS were taken from the Global Administrative Areas database (www.gadm.org); elevation data from CGIAR-Consortium for Spatial Information (http://srtm.csi.cgiar.org); and Bio-climatic data from WorldClim (www.worldclim.org; refer also to Hijmans *et al.*, 2005) and International Livestock Research Institute – GIS service (http://192.156.137.110/gis). In this case study, DIVA-GIS 7.5 (Hijmans *et al.* 2004) was used to develop climatic models for predicting the distribution of species. Maps of square 18 × 18 km (10 minutes degrees) grid cells were made and the species richness was calculated as the number of insect species in each quadrant. The distribution of numbers of individuals among species (evenness) is a measure of each species importance. Richness and evenness have been combined mathematically to calculate diversity indices based on the proportional abundances of a species (Magurran, 2004). The indices used in this study was Simpson's index, calculated as follow:

$$D = \sum_{i=1}^{n} (n_i(n_i - 1))/(N(N - 1))$$

Where n_i is the number of individuals in the ith species and N is the total number of individuals. Diversity decreases as D increases, so Simpson's index generally is expressed as 1 – D or 1/D. Once the number of species exceeds 10, the underlying rank-abundance pattern is important in determining the value of D. The Dominance (D_m) was obtained from the McNaughton & Wolff's index (McNaughton & Wolff, 1970), expressed as

$$D_m = Y_{1,2} / Y$$

Where $Y_{1,2}$ is the sum of individuals of the two most abundant species, while Y is the total number of the individuals.

To analyse if any spatial autocorrelation existed within the same species, Geary and Moran indexes were calculated for nearby geographical grids using the function implemented in DIVA-GIS based on the "Rookcase" software by Sawada (1999). Then, bioclim tool, as well implemented in DIVA-GIS, was used to model and examine whether the distributions of the main whitefly pest species in the surveyed regions were closely approximated by their predicted distribution based on bio-climatic variables (Peterson *et al.*, 1999; Raxworthy *et al.*, 2003). In addition, with the aim to understand the adaptive capacity of parasitoid species to different bioclimatic conditions and agro-ecosystems, the limits of the species niche was investigated, giving information about where these wasps were really occurring and where they could occur or not. To find this out, reserve selection algorithm developed by Rebelo & Siegfried (1992) was used in DIVA-GIS, which calculates the minimum number of quadrants (grid cells) necessary to conserve a given number of species (Hijmans *et al.*, 2001).

3.5.1.1 Additional Statistical Analysis

Data of collected whiteflies and their parasitoids were pooled and used for statistical analysis. The software used in this study were StatPlus:mac (2009) and Prism 6.0 (2013). Pearson's correlation analysis was calculated to determine if there were some relationships among altitude and abundance. ANOVA was used where elevation predicted the site-based average of the mean phylogenetic distance between the different sites. The phylogenetic distance among the specimens was calculated by Mega 5 (Tamura *et. al.*, 2011).

Chapter 4: RESULTS

4.1 WHITEFLIES AND THEIR PARASITOIDS ON HERBACEOUS PLANTS IN MEDITERRANEAN ENVIRONMENT

4.1.1 Introductory notes

For a long time, in the Mediterranean region, whiteflies have caused conspicuous damage to many crops, both in protected cultivations and in open fields (Patti & Rapisarda; 1981; Rapisarda, 1990; Gerling & Mayer, 1995; Sartor, 2008). Thanks to the favourable climatic and environmental conditions, as well as to the intense trade and movements of goods in the area, many whitefly species, not native from the Mediterranean, have been well established during the last century (Gomez-Menor, 1943; Avidov, 1956; Gerling, 1995). This was the case of several whitefly species coming from the tropics, which once established became serious agricultural pests (Martin *et al*., 2000). Among them, *Aleurodicus dispersus* Russell in Canary Island, *Bemisia tabaci* (Gennadius) species complex and *Trialeurodes vaporariorum* (Westwood) almost everywhere, are known as major pests within Mediterranean regions. But we can also mention many citrus feeding whiteflies of both Far-East Asia and neotropical origin (Rapisarda, 1990). Damage caused by whiteflies is still conspicuous in Mediterranean regions and their control involves great effort and attention. At present, a critical issue is represented by the lack of information regarding spatial distribution (presence/absence) of pests or their abundance, which is especially serious for whiteflies and may be of extreme strategic importance for pest management decisions, such as the application of restrictions to some infested area, the designing of pest surveys or pest surveillance and, last but not least, the application of protocols to keep injurious pests out from sensitive areas (Heesterbeek & Zadoks, 1987). In Sicily, there is a gap of detailed information on the spatial diversity and distribution of whitefly populations, especially other than agriculture under protected environment. The most recent comprehensive data published about the whitefly fauna in Sicily dates back more than 10 years ago (Patti & Rapisarda, 1981; Rapisarda, 1982; Rapisarda & Patti, 1983; Rapisarda, 1990; Martin *et al.*, 2000). Moreover, very scarce information is available about parasitoids attacking whiteflies on herbaceous plants in this region, apart from works that have been done mainly during the 1980s on biological control of *T. vaporariorum* in Sicilian greenhouse crops by introducing commercial parasitoids. Therefore, an updated spatial analysis of whitefly (and their parasitoids) diversity and distribution is strongly needed in this region, which will also facilitate the development of future IPM programs.

4.1.2 Whiteflies

4.1.2.1 Identification of the collected material

A total of 524 whitefly adults and 2123 nymphs were collected on several herbaceous plant species (tab.2) during this study. All specimens belonged to a total of 9 whitefly species: *Aleurolobus marlatti* (Quaintance) (0.7%), *Aleurothrixus floccosus* (Maskell) (6.5%), *Aleyrodes elevatus* Silvestri (11%), *Aleyrodes proletella* (Linnaeus) (12.5%), *Bemisia afer* (Priesner & Hosny) (8%), *Bemisia tabaci* (Gennadius) (33%), *Dialeurodes citri* (Ashmead) (2%), *Trialeurodes lauri* (Signoret) (1.3%) and *Trialeurodes vaporariorum* (Westwood) (25%). Identification of the *B. tabaci* putative species was carried out by molecular techniques (fig.16). Out of 430 samples of *B. tabaci***,** 67% were belonging to the putative species “Mediterranean” (Q biotype), 30% to “Middle East-Asia Minor 1” (B biotype) and 3% to “Italy” species (T biotype). Due to the large sampling effort, the survey method was tested by a regression analysis. The graph below (fig.17) confirms that there was a relationship between the number of samples considered and the number of species observed, with an R^2 = 0,7958. In fact, after almost one-third of the total inspected quadrants, the accumulation curve did not reach an asymptote and the number of whitefly species still grew but at a diminishing rate. This does not mean that the total species richness of whiteflies on herbaceous plants was sampled, but most likely the most common species occurring in the surveyed area have been collected.

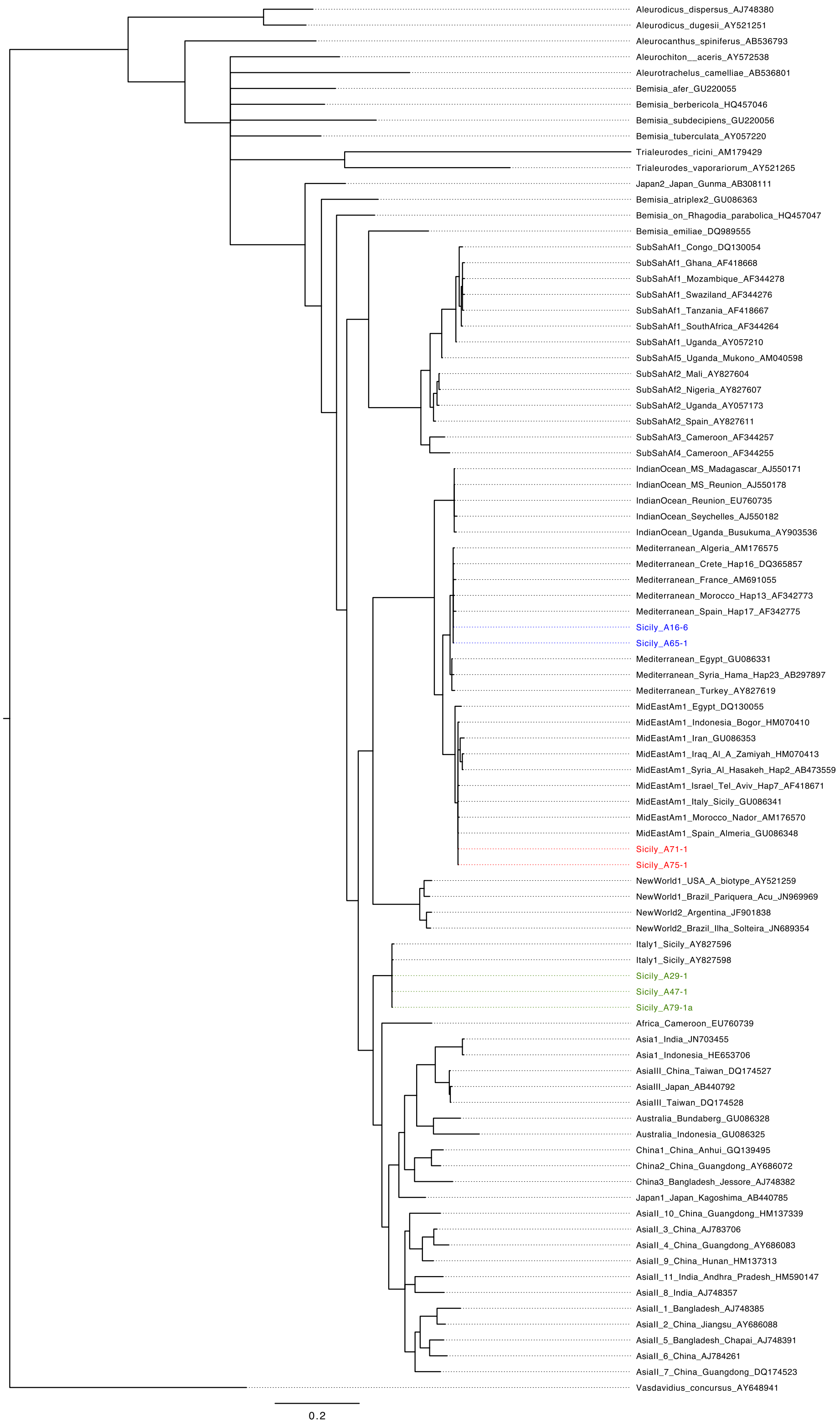


Fig. 16 Bayesian analyses of *Bemisia tabaci* COI sequences obtained from the survey and *Bemisia tabaci* global dataset (Released version 31_Dec_2012). In colour: (blue) Mediterranean; (red) Middle East-Asia Minor 1; (green) Italy.

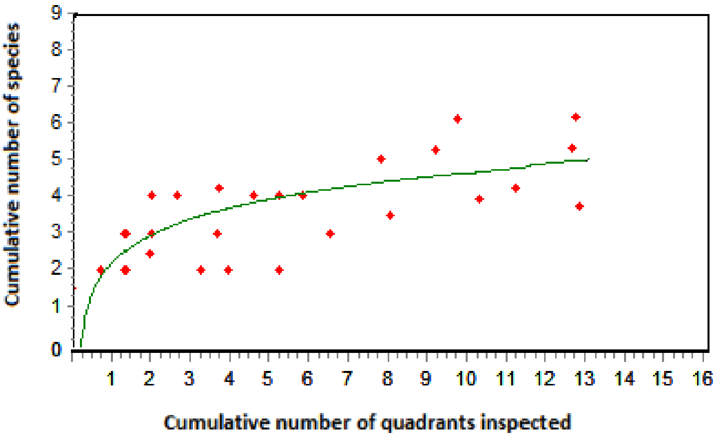


Fig. 17 Relation between the number of observations and species in cell.

Table 2 List of whitefly species recorded and their host plants.

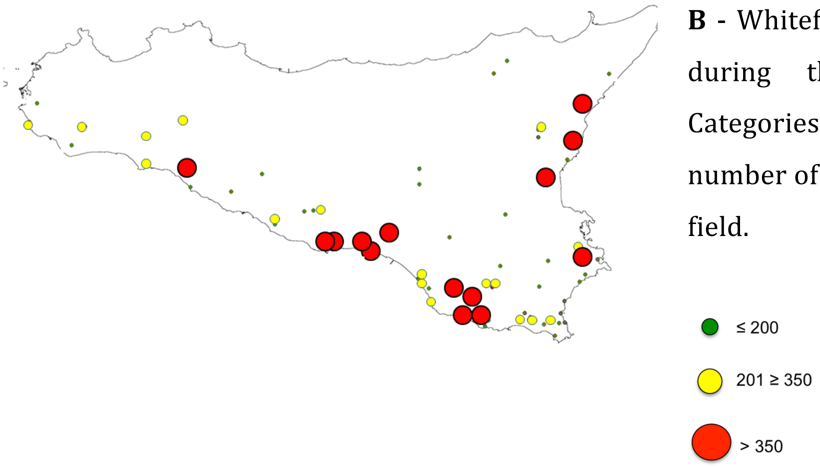
Whitefly species	Host plants
A. marlatti	*Capparis spinosa*
A. floccosus	*Parietaria officinalis* (*Citrus* growing areas)
A. elevatus	*Helianthus annus, Mercurialis annua, Parietaria officinalis, Urtica dioica*
A. proletella	*Euphorbia dendroides, Marrubium vulgare, Parietaria officinalis, Sinapis pubescens*
B. afer	*Hibiscus* sp., *Marrubium vulgare*, *Solanum nigrum*
B. tabaci Middle-East Asia Minor 1	*Chrozophora tinctoria, Citrullus lanatus, Cucumis melo, Cucurbita maxima, Cucurbita pepo, Ibiscus sp., Lycopersicon esculentum, Solanum melongena, Solanum nigrum, Solanum tuberosum, Urtica sp., Tarassacum sp.*
B. tabaci Mediterranean	*Acanthus mollis, Anagallis sp., Ballota nigra, Ballota* sp., *Cerinthe major, Clematis sp., Convolvulus arvensis, Cucumis melo, Cucurbita pepo, Ibiscus* sp., *Lycopersicon esculentum, Lycopis* sp., *Malva silvestris, Malva* sp., *Mentha* sp., *Phaseulus vulgaris, Philadelphus* sp., *Prunus* sp., *Ricinus communis, Suctellaria* sp., *Solanum melongena, Solanum nigrum, Solanum tuberosum, Tropaeolum majus, Tropaeolum* sp., *Vitalba* sp., *Lathyrus odoratus, Xanthum strumarium.*
B. tabaci Italy	*Acanthus mollis*, *Euphorbia characias*, *Lycopersicon esculentum*
T. lauri	*Laurus nobilis*
T. vaporariorum	*Cucumis melo*, *Cucurbita pepo*, *Lagenaria longissima*, *Lycopersicon esculentum*, *Mentha* sp., *Tropaeolum majus*

4.1.2.2 Species richness and abundance

Figure 18 depicts the whitefly species richness map. We used 18x18 km grid cell highlighted in different colors to indicate the number of species occurring in each quadrant. In addition, to assess diversity taking in consideration the respective proportion (evenness) of each whitefly species in each quadrants, the Simpson 'Index was calculated. In this case the value of the Simpson's index of Diversity (1-D) ranges between 0 and 1, where greater values indicate higher sample diversity. Finally, to calculate if values of nearby geographical objects were more or less similar (correlated) than those values further away, the autocorrelation implemented in DIVA-GIS based on the "Rookcase" (Sawada, 1999) was used.

The number of whitefly species was distinctly lower in the agricultural/cultivated areas compared to natural areas (tab.3). In natural environments, there was not a particular species dominating others; by contrast, in cultivated areas there were one or two species more abundant than others. The total whiteflies abundance (evenness) was significantly higher in agricultural areas, especially in greenhouse areas. Differences between species abundance were detected in population size and spatial autocorrelation. The most abundant species *B. tabaci* and *T. vaporariorum* were aggregated across landscape, but in different way (tab.4). While *B. tabaci* abundance had a spatial correlation both in and outside protected crops, *T. vaporariorum* abundance was correlated only in greenhouses. Other whitefly species were randomly distributed and, in some cases, there was not enough information for completing the test, or it was not significant.

A – Whiteflies species richness map in Sicily. The colors, light to heavy, represent the richness from the minimum to maximum value, while black color represents no data or not enough for data elaboration.



B - Whiteflies abundance recorded during the survey in Sicily. Categories represent the total number of whiteflies counted in the field.

Fig. 18 Maps of the species richness (A) and whitefly abundance (B) in Sicily.

Table 3 Measurement of diversity taking in consideration the whitefly richness and evenness. The Simpson's index of diversity, expressed as 1–*D*. The dominance (Dm) was calculated using the McNaughton & Wolff's index (1970). Dm = $Y_{1,2}$/Y, where $Y_{1,2}$ is the sum of individuals of the most abundant species and Y equals the total number of individuals. Dm values equal or closer to 1 indicate dominance by 1 or very few species, respectively.

Location	Species richness	Whiteflies abundance[2]	Simpson's index of diversity (1-D)	Dominance (D_m)	Kind of area
Gela (CL)	2	520	0,49	1	Cultivated area
Vittoria (RG)	2	246	0,64	1	Cultivated area
Ragusa (RG)	3	480	0,58	0,8	Cultivated area
Ragusa(RG)	6	360	0,78	0,55	Natural area
Scicli (RG)	3	158	0,59	0,79	Cultivated area
Ispica (RG)	7	335	0,8	0,49	Natural area
Pachino (SR)	2	273	0,46	1	Cultivated area
Vendicari (SR)	4	118	0,69	0,72	Natural area
Siracusa (SR)	2	500	0,46	1	Cultivated area
Catania (CT)	2	480	0,33	1	Cultivated area
Fiumefreddo (CT)	2	550	0,46	1	Cultivated area
Etna (CT)	5	175	0,71	0,44	Natural area
Marsala (TP)	3	478	0,64	0,89	Cultivated area
Platani (AG)	2	80	0,38	1	Natural area

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[2] Abundance number indicates field counts.

Table 4 Spatial autocorrelation of the two most abundant whitefly species in Sicily: *B. tabaci* and *T. vaporariorum*. If 0 c 1 [Geary] and i>0 [Moran], it means that data are clustered and autocorrelation exist; if c=1 and i=0 then data are independent, at random.

	Geary (c)	Moran (i)
B. tabaci		
(c) (i)	0.049	1.212
z-Normal	1.472	2.841
Var. Normal	0.417	0.189
z-Random	1.886	2.798
Var. Random	0.254	0.194
T.vaporariorum		
(greenhouses)		
(c) (i)	0.585	1.156
z-Normal	14.591	9.309
Var. Normal	0.098	0.015
z-Random	3.408	10.977
Var. Random	1.809	0.011

4.1.3 Modelling the potential distribution of whitefly pests

One of the main objectives of this study was to determine which bioclimatic variables could induce and/or explain modifications in the spatial distribution of whitefly pests. Hence, as a case study, the potential distribution of the two major whitefly pests, *B. tabaci* and *T. vaporariorum*, was modeled, by correlating both bioclimatic data, taken from www.worldclim.org (using 2.5-minute resolution data) and the sites where these species have been already observed. The model enabled to approximate the distribution range of both whitefly species, identifying those areas where probably the pests could occur in the region (fig.19). Similar approach could be applied to many other whitefly pests.

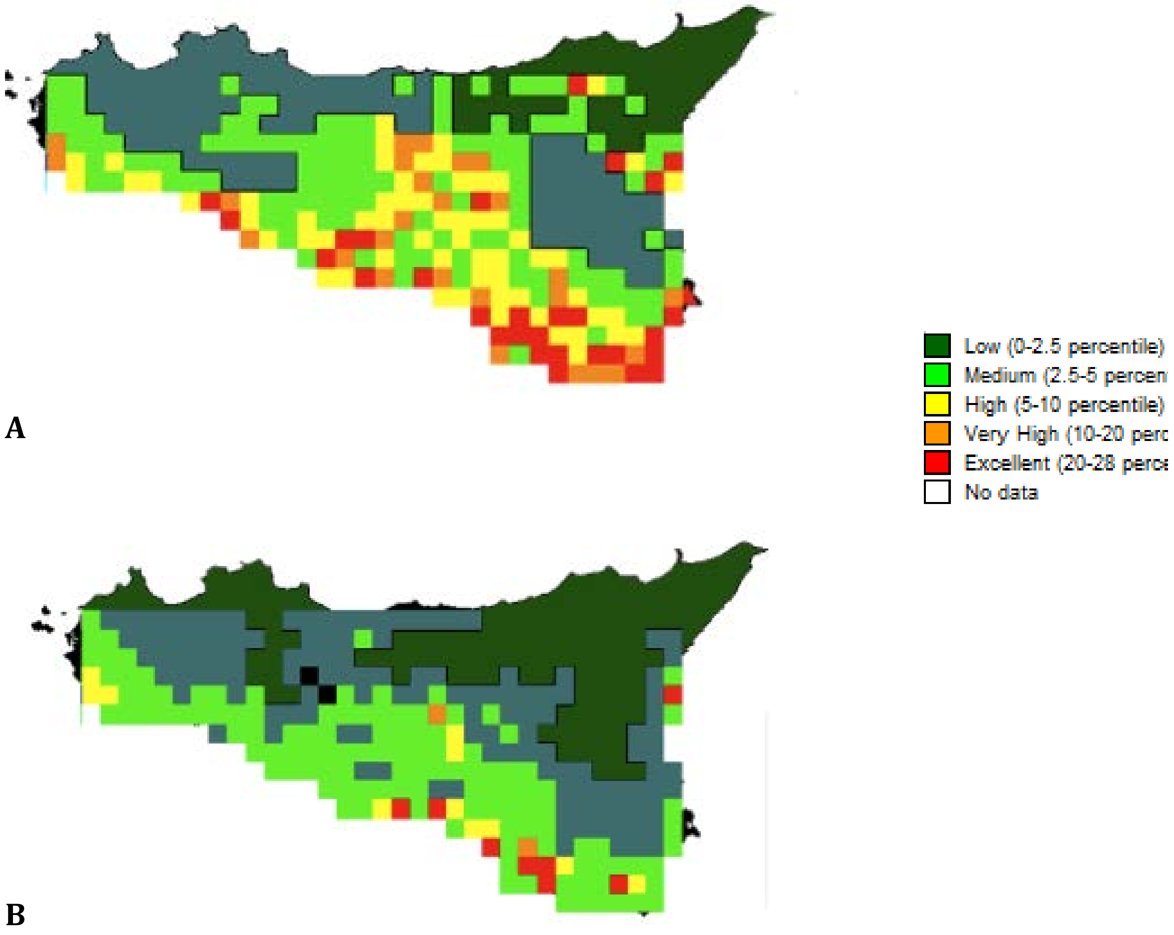

Fig. 19 **Potential distribution of *B. tabaci* (A) and *T. vaporariorium* (B). The colors suggest different levels of suitability based on the bioclimatic conditions that appear to be suitable for these whiteflies.**

4.1.4 Parasitoids

4.1.4.1 Who was parasitizing whom?

Eight species of parasitoids, representing 4 genera and 3 families, including 1 hyper-parasitoid, were recorded. No undescribed species were collected. *Encarsia pergandiella* Howard was found to parasitize *A. elevatus, A. proletella, B. tabaci,* and*, T. vaporariorum.* This species was the most widespread parasitoid in Sicily, occurring in all the environments surveyed. *Encarsia lutea* (Masi) parasitized *A. marlatti*, *A. proletella*, *B. tabaci* and *T. vaporariorum.* Despite the fact that this wasp seems to colonize several whitefly species, *B. tabaci* was the host on which it was frequently found. *Encarsia tricolor* Förster, a parasitoid of several whitefly pests, was found attacking *A. proletella, A. elevatus* and *B. afer*. This parasitoid was collected only in natural areas. *Encarsia inaron* (Walker) parasitized *A. proletella*, *A. elevatus* and, in a few cases and only in natural environment, *B. tabaci*. *Encarsia formosa* Gahan was recorded on *B. tabaci* and *T. vaporariorum*. *Cales noacki* Howard was found to parasitize mainly *A. floccosus* and occasionally *B. afer. Eretmocerus mundus* Mercet was recorded on *B. tabaci*, *B. afer* and *T. vaporariorum*. This parasitoid was widespread almost all over Sicily, occurring on *B. tabaci* in cultivated areas and on *B. afer* in natural areas. *Signiphora townsendi* Ashmead was rarely reared from *A. floccosus*.

4.1.4.2 Reserve selection of whitefly parasitoids

In the aim to reveal areas with the maximum amount of parasitoid fauna diversity, the “reserve selection” implemented in DIVA-GIS was used. This method identified sets of grid cells in the map that are complementary to each other. However, several problems were encountered. Parasitoid populations varied greatly in density and presence/absence among the locations. Therefore, areas with conspicuous populations and diversity were selected. The result of the analysis indicated that parasitoids are partially conserved in two main geographical areas (fig.20). A deeper observation at a smaller scale enabled to obtain more information. The satellite pictures (fig.21) show some of the areas contained in the reserve selection analysis, including natural areas surrounded by cultivated fields. Comparison of the reserve selection map with the regional map of protected areas revealed the role of natural areas as reserve of whitefly parasitoids. However, this was not the case for every protected area in Sicily. In fact, correlating the map of protected areas with the regional bioclimatic map, using De Martonne Index (fig.20-C), it was possible to explain the results obtained by the reserve selection. Parasitoids seem to occur mainly in semi-arid (in light-brown) and in warm-temperate areas (in orange) but not in arid areas. Finally, since a huge part of the southern and eastern coast is cultivated (especially by greenhouse crops), using previous data on environmental pollution (pesticides, fertilizers etc.) by agricultural activities, we tried to understand the reasons for the absence/low population of parasitoids in these areas. The map reported in fig. 20(D) shows that about 10% of the total cultivated areas in the region are susceptible to environmental pollution. This partial result can explain the absence of a stable parasitoid fauna in these areas, where intensive farming is carried out.

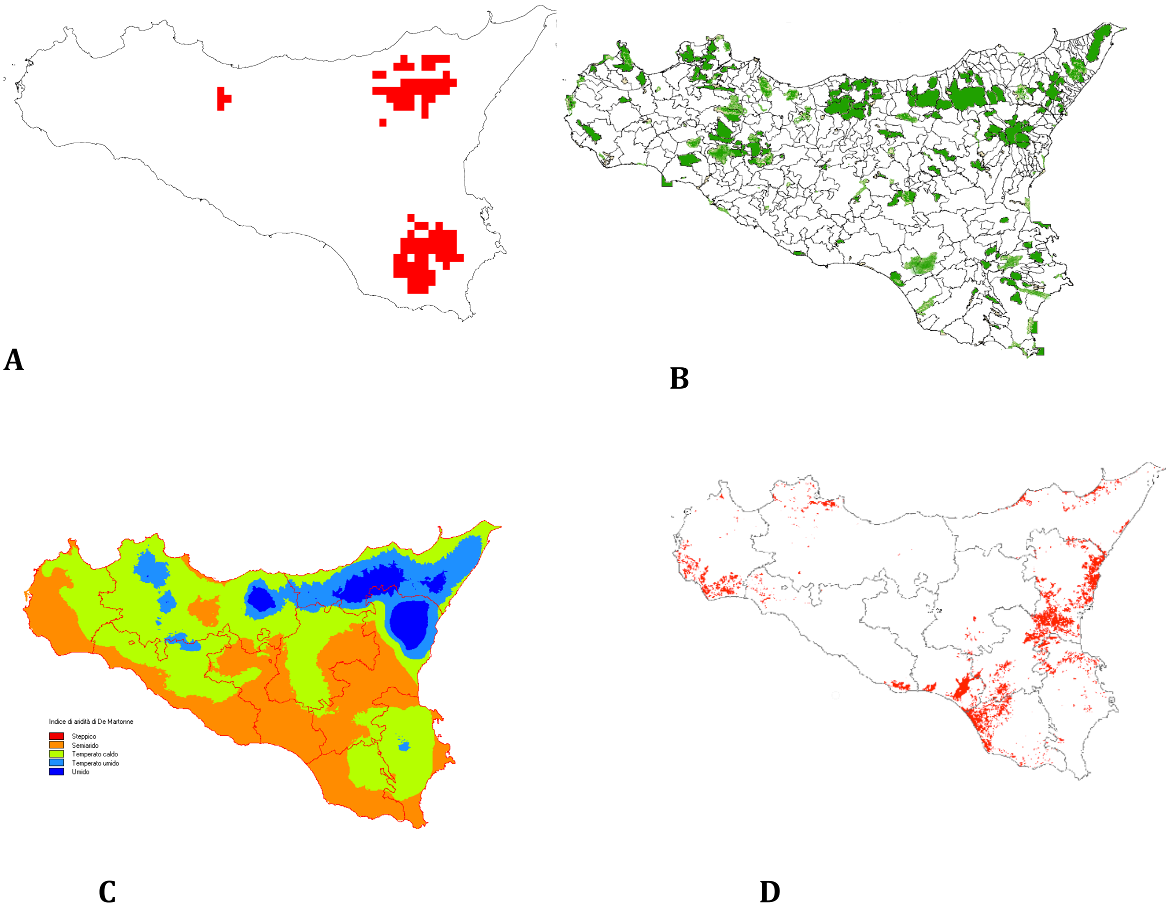

Fig. 20 Reserve selection map of the main conserved areas of whitefly parasitoids (A); Regional map of protected areas of Sicily (B); Bioclimatic map based on De Martonne Index (C) (adopted from Drago, 2005). Blue means humid climate, orange means semi-arid; green means warm-temperate; Map of Sicilian areas with high environmental pollution caused by intensive agricultural activities (D) (modified from Anzà *et al.*, 2005).

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Fig. 21 Examples of natural areas (yellow shaded area) influencing positively the neighboring cultivated lands; Above: the canyons of Cava d'Ispica (Forza's Park) (Ispica, SR); Below: Pantano Cuba (Pachino, RG) (modified from Google Earth, 2013).

4.1.5 Discussion

4.1.5.1 Whiteflies

The whitefly fauna in Mediterranean Basin comprises 56 species that are considered to be native or naturalized (Martin *et al.*, 2000). About thirty of these are present in Sicily, but only fifteen or so feed on herbaceous plants. In this study nine whitefly species were identified. Apart from *B. tabaci* and *T. vaporariorum*, the other whiteflies did not show a particular pattern in their geographic distribution. For example, *A. floccosus* or *D. citri*, both pests of citrus, were found occasionally on herbaceous plants near citrus fields. So their distribution is mainly correlated with the primary host. *A. elevatus*, *A. proletella* and *B. afer*, all polyphagous whiteflies, were recorded at low population level and in distant localities. In *B. tabaci* species complex, contrary to previous study by Cavalieri & Rapisarda (2008), reporting the *B. tabaci* Middle-East Asia Minor 1 as the predominant species, in this research this species was found to have been almost replaced by *B. tabaci* Mediterranean species, confirming recent findings from other countries by Teng *et al.* (2010). Moreover, here new host plants were identified for *B. tabaci* Italy species, such as the cultivated tomato, *Lycopersicon esculentum* (L.), and a naturally occurring wild plant, *Acanthus mollis*. L.

In this study, a measure of the diversity at geographical level was given through the species richness. In this way it was possible to map areas where high/low numbers of species were occurring and also to map the relative abundance. The results showed that whitefly species richness was higher in natural or nearby areas, where a less intensive agriculture was carried out. In these cases, none abundant population of a particular species were recorded. Opposite to that, where one or two whitefly species were recorded in one area, especially in cultivated lands, abundant population of *B. tabaci* and *T. vaporariorum* were observed. The result obtained in this study gave several useful information regarding the spatial distribution and abundance of whiteflies at regional level, which can be fundamental in pest surveillance program, especially in those areas where the natural equilibrium was broken by human interference. Aggregated spatial distribution for *B. tabaci* and *T. vaporariorum* were evidenced in cultivated lands along the southern and eastern coasts. However, these two species decreased in population size, sometimes reaching almost nil, in natural or semi-natural areas or nearby surroundings. Subsequently, the potential distribution of the two major injurious whitefly pests was modeled at regional scale, providing a picture of suitable areas for these species. Further studies and surveys should be directed to the areas identified in this study as potential locations for pests outbreak.

4.1.5.2 *Parasitoids*

Whitefly parasitoids are known for their importance as biological control agents. However, little is known about them in Sicily. In this study nine parasitoid species were recorded on several whitefly hosts. Some species like *Encarsia pergandiella*, *En. lutea* and *Er. mundus* were collected almost everywhere. On the contrary, other species such as *En. formosa*, often utilized as biological control agents against whitefly pests during the last decades, exhibited a narrow distribution range, being recorded only from some geographical points. It was the same for two other species, *En. inaron* and *En. tricolor*. Reserve selection model is an important approach for biodiversity conservation (*in situ*) (Jiang & Xue, 1996), and it was used here, permitting to identify two main areas that function as reserve for whitefly parasitoids. Comparing the reserve selection map with others describing environmental variables, it was possible to hypothesize the reason for the geographical isolation of parasitoids. Generally, parasitoids seem to be sensitive to environmental variations, such as temperature, humidity, vegetation and particularly to anthropogenic influence. From the bioclimatic and agricultural maps, it can be easily seen how agriculture (especially greenhouse crops) is mainly distributed along the coasts. However, these areas are classified as steppe climate, presenting a long dry and warm season and hence less plant species diversity or almost none, excluding the possibility of ecological corridors outside cultivated zones. This means that these areas are not that suitable for whitefly parasitoids. In addition, environmental pollution (pesticides, fertilizers etc.) seems to play an important role in the decreasing (or absence) of parasitoid population. Areas where abundant parasitoid populations have been noted include natural areas or nearby fields, where the environmental conditions were more suitable to them. These zones are characterized by a higher level of humidity (such as presence of a river, lake or dam), high plant species diversity in time and space and scattered cultivated fields (Altieri, 1999). In fact, such areas influenced positively the presence of parasitoids and their activity in cultivated fields in the immediate surroundings. Studies at smaller spatial scale and quantitative analysis are needed for each of the parasitoid species, in order to identify suitable areas and ecological corridors that can help the dispersion of natural enemies, consequently increasing their efficiency in whitefly control.

4.2 WHITEFLIES AND THEIR PARASITOIDS ON HERBACEOUS PLANTS IN TROPICAL ENVIRONMENT

4.2.1 Introductory notes

Cassava (*Manihot esculenta* Crantz) is one of the most important staple crops in sub-Saharan Africa. During the last decades, its production has been affected mainly by the whitefly *Bemisia tabaci* (Gennadius) which is the vector of cassava mosaic geminiviruses (CMGs), causing cassava mosaic disease (CMD) and cassava brown streak virus (CBSV), causing cassava brown streak disease (CBSD) (Storey, 1939; Bock, 1994; Legg, 1994; Maruthi *et al.*, 2005; Legg *et al.*, 2006). In addition, super-abundant *B. tabaci* populations have been reported in those African countries, which are considered pandemic-affected by the diseases mentioned above (Legg *et al.*, 2006; Legg, 2010).

In the aim to explore strategies for controlling *B. tabaci* and to upgrade the actual knowledge on whitefly and their parasitoid fauna in Tanzania, an extensive survey was carried out on cassava and non-cassava plants occurring in super-abundant whitefly areas of the country. in Tanzania, only a few whitefly species are known to occur on cassava. These are *B. tabaci* (Storey, 1939; Legg, 1994; Legg *et al.*, 2006), *Aleurodicus dispersus* Russell (Mware *et al.*, 2010), *Bemisia afer* (Priesner & Hosny) and in a few cases *Trialeurodes ricini* (Misra) (Pallangyo *et al.*, 2004; Sway and Slumpa, 2005). In addition, incomplete information is available regarding the parasitoid species attacking whiteflies in the country. Only few parasitoid species have been recorded and these include *Encarsia sophia* (Girault & Dodd) and *Eretmocerus* sp. Heldeman (Swai & Slumpa, 2005).

This study is valuable to identify which whitefly parasitoid species are occurring in cassava growing-region of Tanzania and the host range of each whitefly parasitoids. In addition, a model was done to identify the areas, which could be potentially suitable for the occurrence of super-abundant *B. tabaci* population. Results obtained during the survey are presented here, which permit to better understand the status of and enrich the list of whiteflies and their parasitoids in Tanzania.

4.2.2 Whiteflies

4.2.2.1 *Identification of the collected material*

The following ten whitefly species were identified in the studied area: *Aleurodicus dispersus* (Russell); *Aleurothrixus floccosus* (Maskell); *Bemisia tabaci* (Gennadius) species group [*B. tabaci* Sub-Saharan Africa 1, *B. tabaci* Mediterranean and *B. tabaci* Indian Ocean]; *Bemisia afer* (Priesner & Hosny), *Bemisia* sp. (formerly *Asterobemisia* sp.), *Dialeurodes citri* (Ashmead), *Paraleyrodes bondari* Peracchi and *Trialeurodes vaporariorum* (Westwood) (fig.22; tab.5). As expected, on cassava, *B. tabaci* was the predominant species followed by *B. afer*, with the exception of those areas where heavy populations of *A. dispersus* were detected. The remaining whitefly species were found sporadically and in low numbers. Similar levels of infestation were found for the whitefly species on the other herbaceous plants surrounding cassava fields.

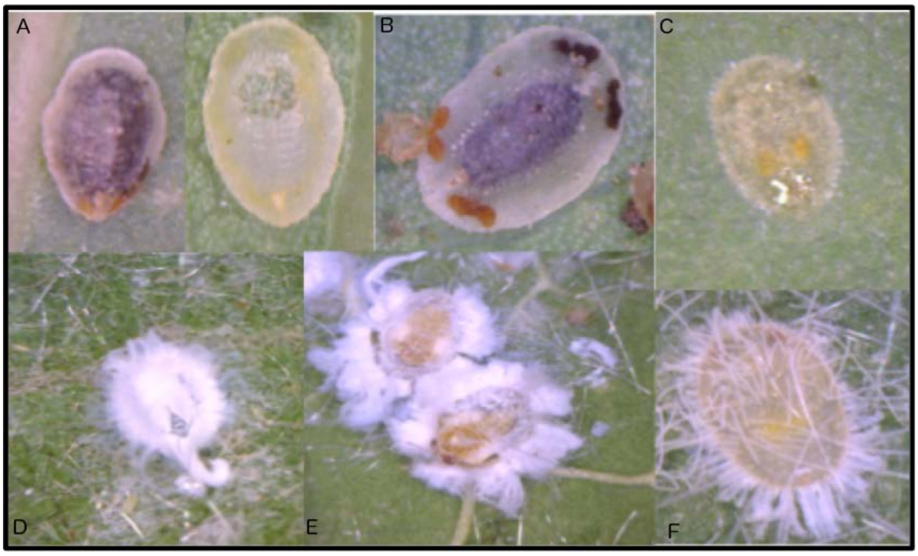

Fig. 22 Pupa cases of whiteflies collected during the surveys in Tanzania. (a) *Bemisia tabaci*; (b) *Bemisia afer*; (c) *Trialeurodes vaporariorum*; (d-e) *Aleurodicus dispersus*; (f) *Paraleyrodes bondari.*

Table 5 Host plants with 3rd-4th instar whitefly nymphs collected and identified.

Whitefly species	Host plant
A. dispersus	Billy goat weed, Broom weeds, *Capsicum* sp., cassava, castor plant, *Cucumis* sp., *Manihot glaziovii*, cowpea, *Euphorbia heterophylla*
A. floccosus	Sporadically on cassava near *Citrus* sp. trees
B. afer	Cassava, *M. glaziovii*, cotton, cowpea, been, bitter tomato, *E. heterophylla*, okra, pumpkins, sweet potato, tomato
B. tabaci SSA1	Cassava and *M. glaziovii*
B. tabaci Mediterranean	Been, bitter tomato, bottle gourd, broom weed, cowpea, *Cassia* sp., *Capsicum* sp., *Cucumber* sp., cotton, double thorn, *E. heterophylla*, *Malvastrum* sp., okra, pumpkins, sweet potato, tomato, peanuts
B. tabaci Indian Ocean	Pumpkins
Bemisia (ex *Asterobemisia*) sp.	Rarely cassava; however, it favours trees
D. citri	Sporadically on cassava near *Citrus* sp. trees
P. bondari	Sporadically on cassava and *M. glaziovii*; however it favours trees
T. vaporariorum	Cassava, castor plant, bitter tomato, tomato, sweet potato

4.2.3 Parasitoids

4.2.3.1 *Identification of the collected material: morphological approach*

Morphological based identification of the parasitoid species was the first approach used in this study. By this way, it was possible to identify *Cales noacki* Howard and the *Encarsia* specimens to the level of species or at least to place them in their appropriate species-group (fig.23). The species identified at this stage were: *En. sophia* (*strenua*-group), *Encarsia guadeloupae* Viggiani (*luteola*-group) and *Encarsia dispersa* Polaszek (*luteola*-group). Other specimens belonged to *parvella*-group, *lutea*-group and a few specimens have been identified as *Encarsia* sp. pr. *circumsculpturata* Viggiani (*aurantii*-group) (fig.24). A similar approach has been applied also to the parasitoid material belonging to the genus *Eretmocerus*, and the species-groups identified were: *mundus*-group, with 2 pairs of setae on the midlobe of the MS and L/W ratio >5x; *paulistis*-group, with 2 pairs of setae on the midlobe of the MS and L/W ratio 5x; *californicus*-group, with 3 pairs of setae on the midlobe of the MS and L/W ratio >5x. *Encarsia* and *Eretmocerus* specimens were, therefore, kept for subsequent molecular analysis, which are described in the next section.

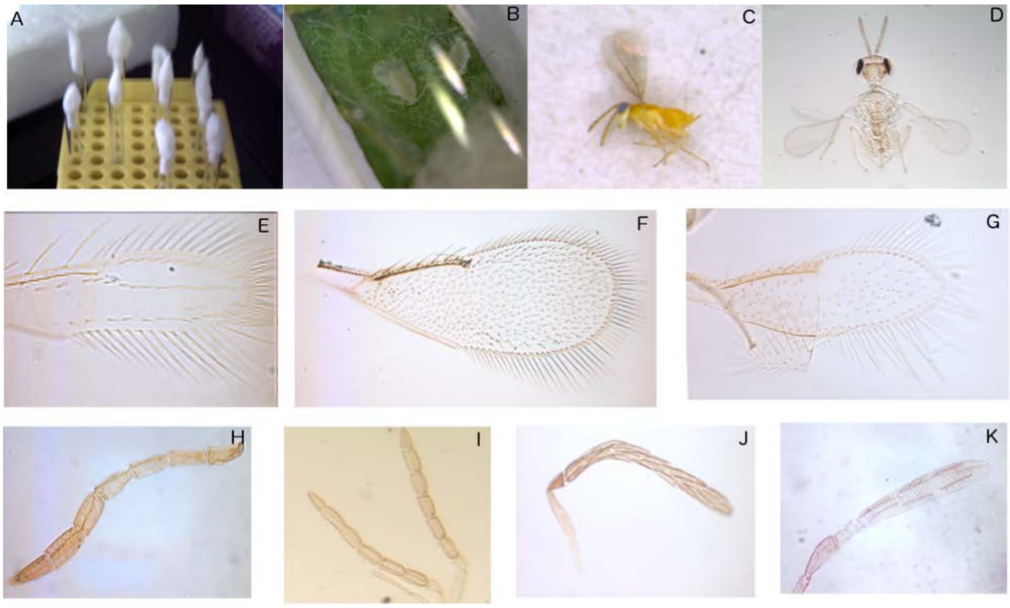

Fig. 23 **(A-B) Whitefly puparia placed inside glass vials and sealed with cotton. (C) Specimen of an *Encarsia* sp. after chemical treatment and before card mounting. (D) *Encarsia* sp. slide mounted. Wings: (E) *Cales noacki*; (F) *Encarsia* strenua-group; (G) *Encarsia* parvella-group. Antenna: (H) *Encarsia* strenua-group; (I) *Encarsia* luteola-group; (J) *Eretmocerus* sp. ♂; (K) *Eretmocerus* sp. ♀.**

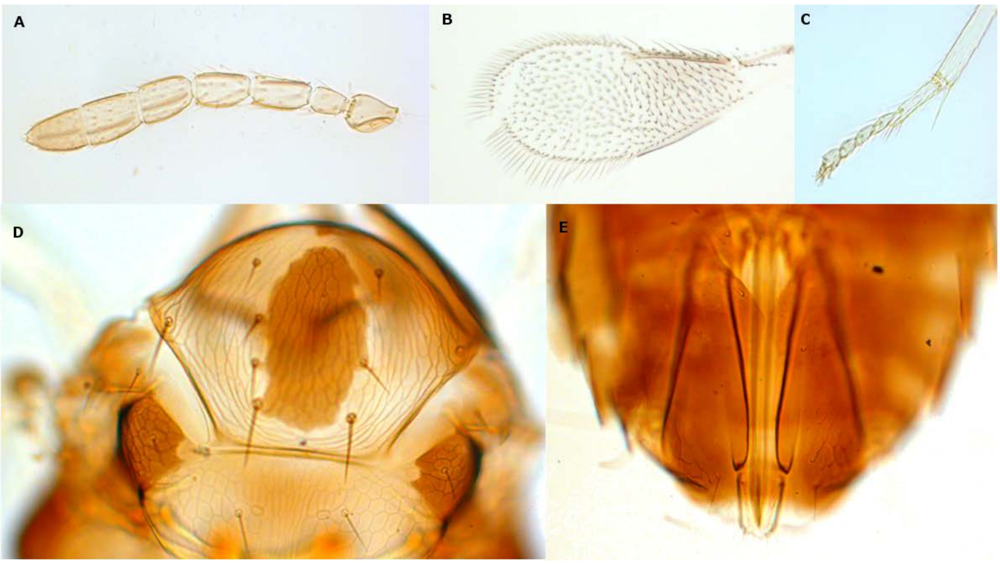

Fig. 24 ***Encarsia* sp. pr. *circumsculpturata* Viggiani (*aurantii*-group), female. (A) Antenna; (B) Fore wings with infuscation below the marginal vein; (C) Tarsa; (D) Mesoscutum with sculpture well defined; (E) Ovopositor not extruded.**

4.2.3.2 Molecular identification

As motioned above, the sub-Saharan African parasitoid fauna is less known and there are very few studies documenting this fauna. Due to this fact, further investigation using molecular approaches was required to confirm morphological identification of specimens or to identify specimens that were not identified morphologically to the species level. Results from the adopted molecular approaches will be discussed below according to the genera to which they have been applied.

4.2.3.2.1 Genus *Encarsia* Förster

Mitochondrial cytochrome oxidase I

Sequences between 750-800 bp were obtained and the aligned data set (including sequences from GenBank) was 620 bases. The analysis (fig.25) of the mitochondrial cytochrome oxidase I did not give enough information to confirm the identity of species collected in Tanzania, except for *Encarsia sophia*. The mean pairwise distances among the different clades were not more than 12-13%, except for *Encarsia* sp. pr. *circumsculpturata* from Tanzania (Tanzania_Encarsia_F13) and the Australian *Encarsia iris* (Girault), where COI sequences exhibited an overall mean distance of 50% in comparison to the other *Encarsia* clades. The other sequences were blasted in GenBank with blast results close most of the time to *En. sophia* or *Encarsia luteola* Howard but with a max identity between 100% and 85%.

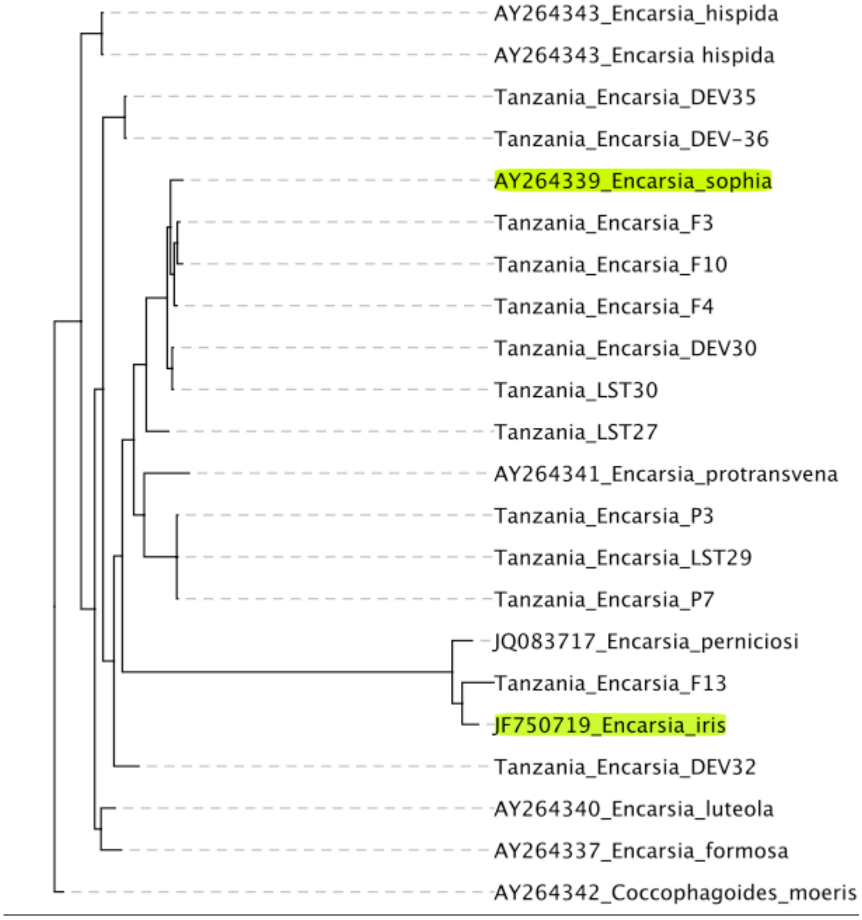

Fig. 25 Bayesian analysis of representative *Encarsia* COI sequences from Tanzania with additional reference sequences from GenBank. Since there were not sequences of *Coccophagoides fuscipennis* (Girault) available in GenBank, here *Coccophagoides moeris* (Walker) was used.

D2 expansion segment of the 28S rRNA

The aligned data set was 560 bases long, with sequences ranging from 580 to 640 bases. The sequences obtained, together with additional sequences retrieved from EMBL/GenBank (from Babcock & Heraty, 2000; Babcock *et al.*, 2001) were aligned and errors checked manually. A *Coccophagoides fuscipennis* (Girault) sequence was used as an out-group (Babcock *et al.*, 2001). Phylogenetic relationships derived from the 28S-D2 data set (fig.22) concur with the molecular analysis of *Encarsia* species presented by Babcock *et al.* (2001). In fact, comparing sequences from their previous results with this study, four *Encarsia* sequences matched: *En. guadeloupae* (1.8%), *En. sophia* (0.4%-1.1 %), *Encarsia lutea* (Masi) (1.2%) and *Encarsia mineoi* Viggiani (0.2%). The mean pairwise distances among the *Encarsia* species collected in Tanzania were: between *En. sophia* and *En. mineoi* was approximately 13%; between *En. sophia* and *En. lutea* around 11%; between *En. guadeloupae* and *En. sophia* around 16%.

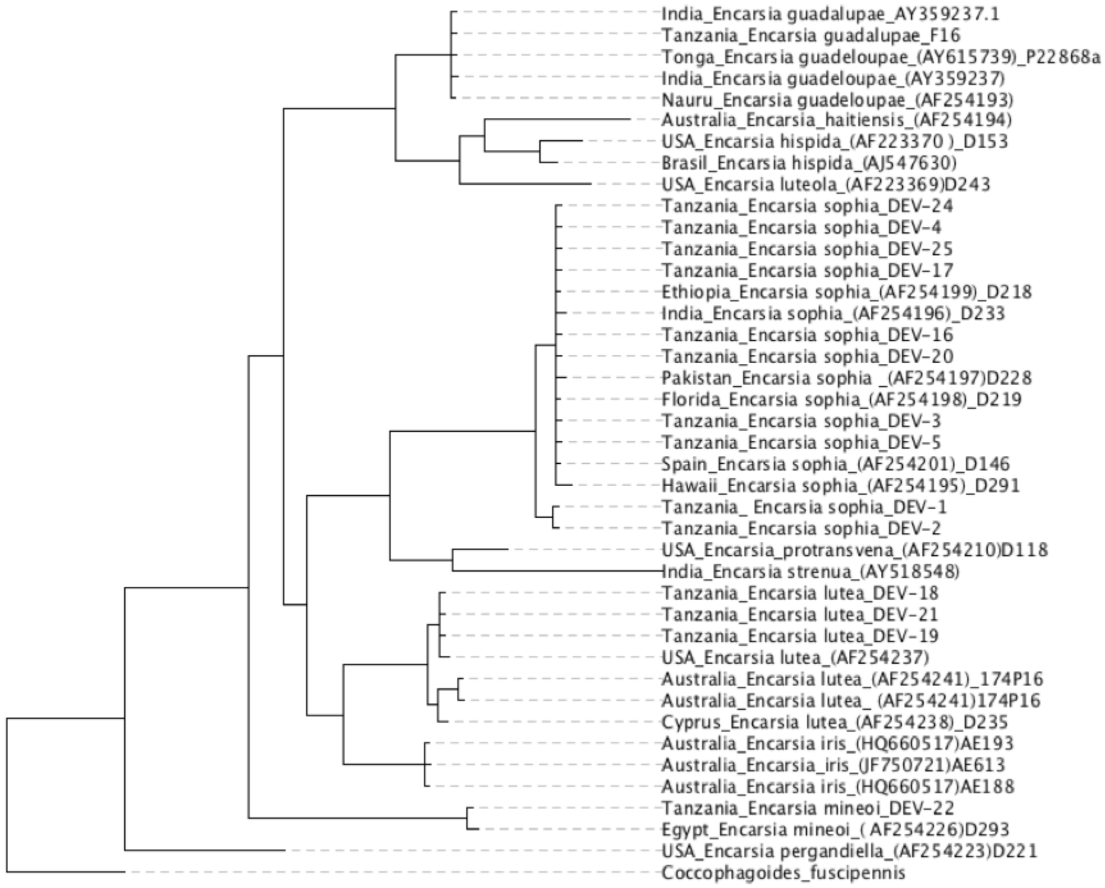

Fig. 26 Bayesian analysis of *Encarsia* based on 28S-D2 rDNA. *Coccophagoides fuscipennis* (Girault) was used as out-group.

4.2.3.2.2 Genus *Eretmocerus* Haldeman

Mitochondrial cytochrome oxidase I

Mitochondrial DNA analysis was used as first approach to determine how many different species (or group) of *Eretmocerus* were present. Sequences between 760-850 bp were obtained. The aligned data set was of 739 bp. Four *Eretmocerus* groups were noted (fig.27), among which it was possible to detect *Eretmocerus mundus* Mercet and *Eretmocerus hayati* Zolnerowich & Rose, the latter introduced into Tanzania from Australia by the International Institute of Tropical Agriculture in 2012 while this study was ongoing. The genetic distances within each of the different groups (represented by different colours) were less than 2% and between the groups > 3%. For example, the distance between Tanzania_P29 and Tanzania_D17 was 1.5%, between Tanzania_P29 and Tanzania_P86 was > 7% and between Tanzania_P29 and Tanzania_P71 was 7%. However, since the systematics of the genus *Eretmocerus* is highly unresolved and very few COI sequences are present in GenBank, using this gene it was not possible to identify the different species, except for *Er. mundus* (fig.27, green clade).

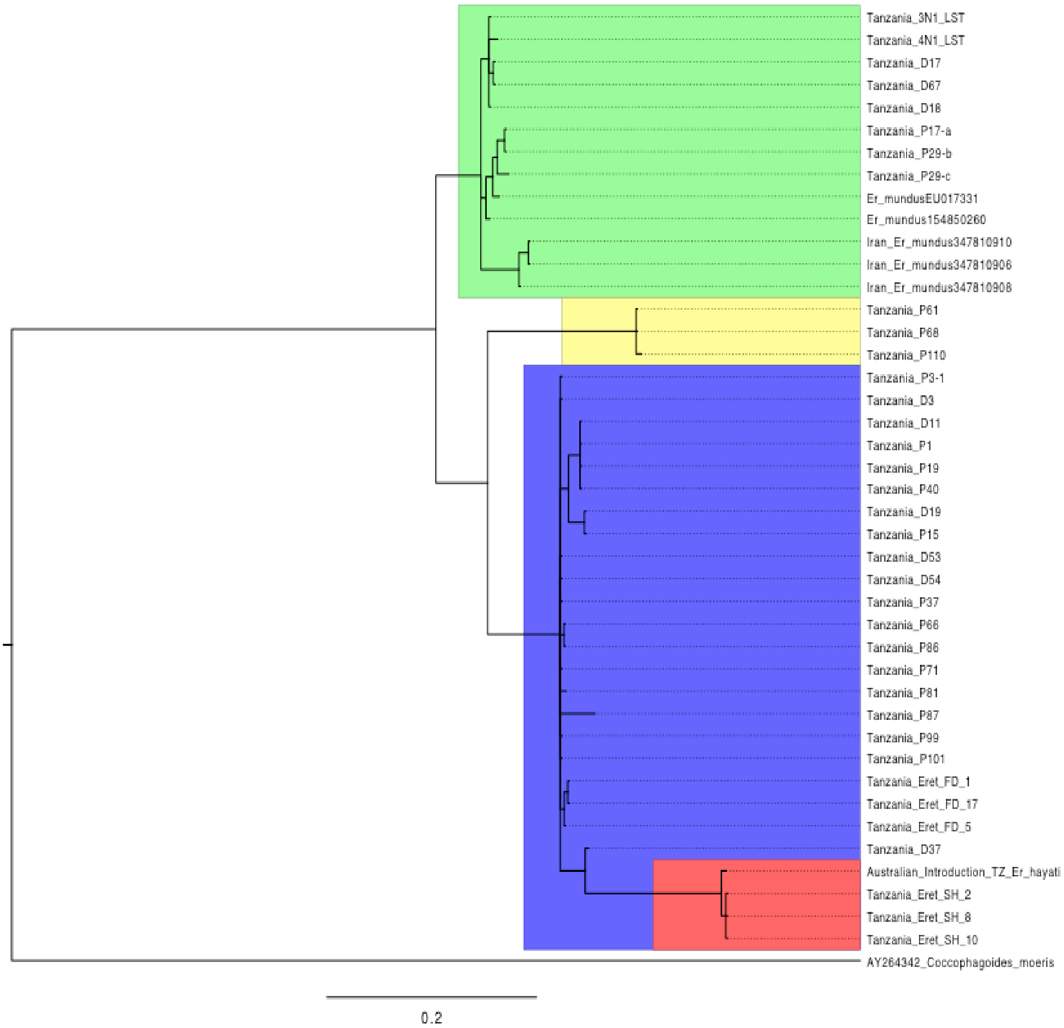

Fig. 27 Bayesian analysis of *Eretmocerus* based on COI. *Coccophagoides moeris* (Walker) was used as out-group.

D2 expansion segment of the 28S rRNA

The four *Eretmocerus* groups noted previously using COI were further investigated using 28S-D2 and sequenced. Sequences ranging 510-620 bp were obtained. The aligned data set was 498 bp. The unrooted network showed three supported monophyletic clades respect to the previous four species-group of *Eretmocerus* (Fig.28), maybe providing a less sensitive level of species separation respect to

COI. Bootstrap values were based on 1000 bootstrap replicates. There were 29 positions that varied among the 500 positions in the alignment of the sequences. This part of the study concurs with the previous work by De Barro *et al.* (2000). When the sequence of "Tanzania_SH" (SH= Screenhouse) was blasted in GenBank, it was found to be identical to *Eretmocerus* sp. Hong Kong isolate (AF273669) and *Eretmocerus queenslandensis* Naumann & Schmidt (AF273668). Unfortunately, all other sequences blasted were near to *Er. mundus* (eg. Tanzania_DEV-12 and Tanzania_DEV-27), whereas, based on this analysis (*D2*), they are clearly different species.

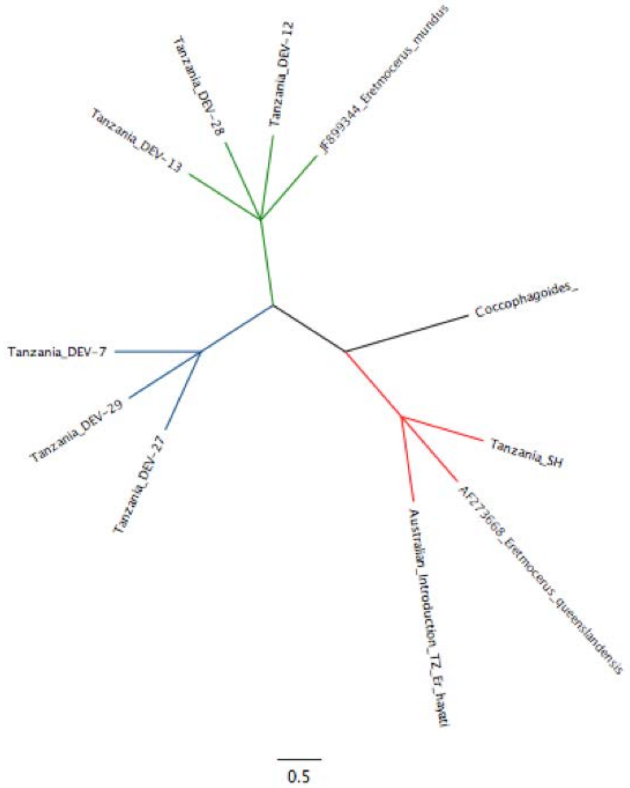

Fig. 28 Strict consensus most parsimonious unrooted network tree of the *28S-D2* data set of *Eretmocerus* spp. Each branch represents bootstrap support values for 100 iterations.

Ribosomal *ITS1*

Ribosomal *ITS1* of 18 specimens were sequenced for all the four groups of *Eretmocerus* investigated. The sequences obtained were aligned together with additional sequences retrieved from De Barro *et al.* (2000). The phylogenetic analysis did not give clear information about the different species that were identified from COI and D2 sequences (Fig.29). The variation in *ITS1* cluster sequences appeared to be more correlated with the geographical distribution of *Eretmocerus*. These results, in addition to the absence of useful morphological characters, did not provide sufficient criteria to distinguish the different species.

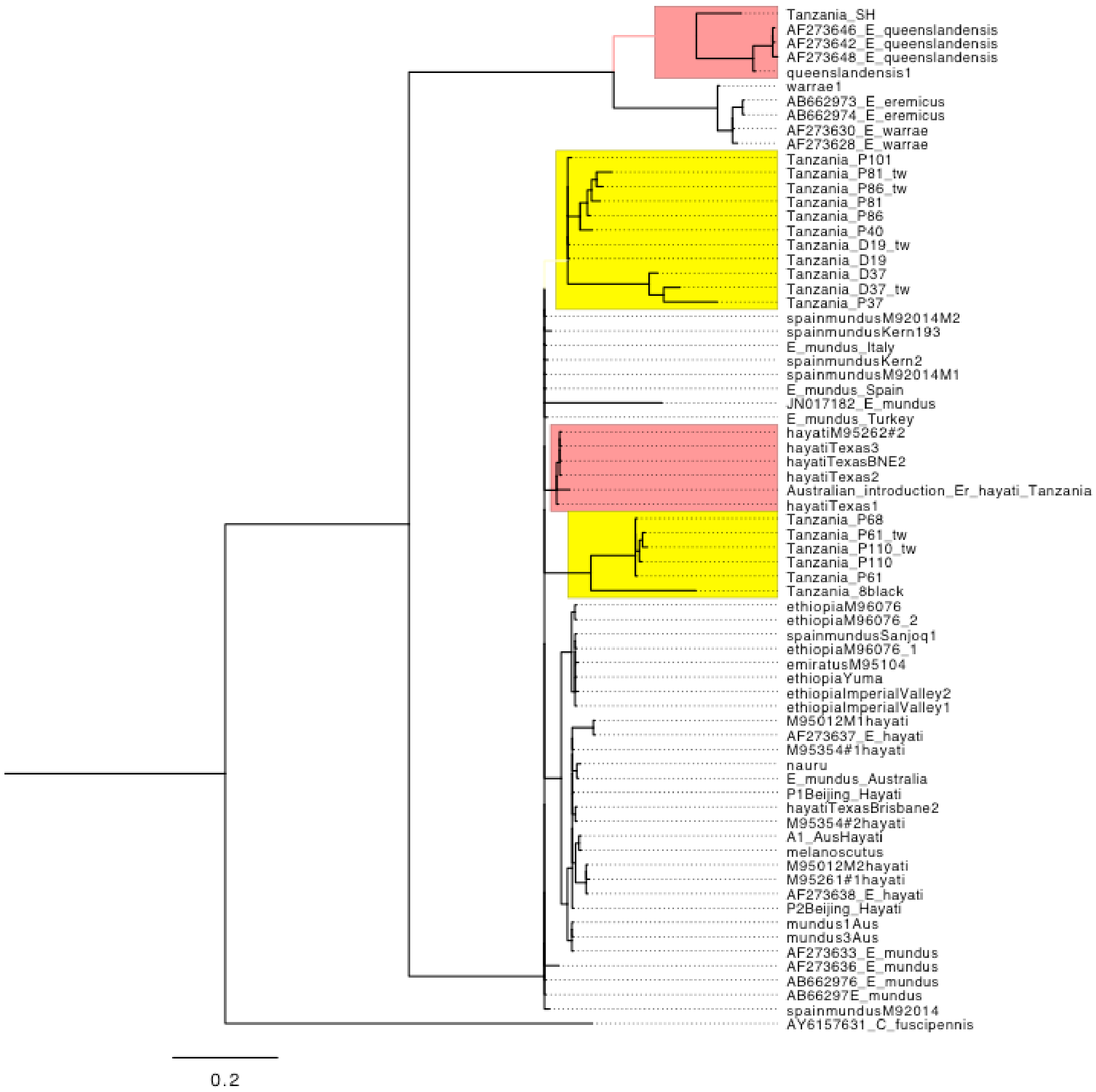

Fig. 29 Bayesian analysis of *Eretmocerus* based on *ITS1*. In yellow, the clades of parasitoids from Tanzania. In red, *Er. hayati* (introduced from Australia) and *Er. queenslandensis*.

4.2.3.3 Who was parasitizing whom?

Among the whiteflies collected on cassava, *B. tabaci* was parasitized by four *Encarsia* species, four species of *Eretmocerus* and one *Cales* species (tab.6). Within the *Encarsia* species, according to previous studies carried out in Uganda (Otim *et al.*, 2005; Semaganda *et al.*, 2005), *En. sophia* was the most common parasitoid on *B. tabaci,* followed by *En. lutea, En. mineoi* and *En. guadeloupae.* As for the last species, only males were reared. Morphological features for identifying *Eretmocerus* species can be ambiguous, due the intraspecific variability. In fact, the use of DNA analysis was fundamental to confirm the morphological data. The three *Eretmocerus* species identified in this study were: *Er. mundus*, *Er. hayati* and *Er. queenslandensis*. One species of *Eretmocerus* was not identifiable to the level of species and, in this work, it is referred as *Er*. sp.1.. During this study, *B. tabaci* was seldom parasitized by *C. noacki*, which is most often reared from *Aleurothrixus floccosus*. Parasitoids reared from *B. afer* were: *En. sophia*, *En. lutea, En. mineoi, Er. mundus* and *Er. queenslandensis.* Only in rare cases, *B. afer* was parasitized by *En. guadeloupae*. *Aleurodicus dispersus* was mainly parasitized by *En. dispersa* and *En. guadeloupae*. Moreover, the two species of *En. dispersa* (previously referred to as *En.* near *haitiensis*) and *En. guadeloupae* were introduced in Tanzania within the frame of the National Biological Control Programme (NBCP) to manage spiraling whitefly, which invaded the country in 2002. *Trialeurodes vaporariorum* was parasitized by *En. sophia* and *Er. mundus.* Bondar's nesting whitefly, *Paraleyrodes bondari*, was occasionally collected on cassava and parasitized by *En. dispersa.* The citrus whitefly, *Dialeurodes citri,* collected on cassava, was parasitized by *Encarsia* sp. pr. *circumsculpturata*. No parasitoids were collected from the few specimens of *Bemisia* sp. (formerly *Asterobemisia* sp.).

Table 6 Parasitoids associated with whiteflies in cassava growing regions of Tanzania.

	Whitefly parasitoids										
Whitefly species	*En. sophia*	*En. lutea*	*En. mineoi*	*En. dispersa*	*En. guadeloupae*	*En.* sp. pr. *circumsculpturata*	*Er. mundus*	*Er. queenslandensis*	*Er. hayati**	*Er.* sp *(1)*	*C. noacki*
A. dispersus				x	x						
B. tabaci	x	x	x		x		x	x	x	x	x
B. afer	x	x	x		x		x	x	x	x	x
Bemisia sp.											
D. citri		x				x					
P. bondari				x							
T. vaporariorum	x										

* Introduced from Australia

4.2.4 Modelling the potential distribution of whiteflies and their parasitoids

4.2.4.1 Whiteflies species hotspots based on richness

Figure 30 depicts the map of whitefly species richness in the investigated areas of Tanzania. The different grids are highlighted in different colours to show the richness. The highest richness values were found in the central and south-eastern part of the surveyed quadrant that covers part of Shinyanga and Tabora regions. Lower species richness was recorded from north-western side, where the presence of *B. tabaci* seems to be higher and almost exclusive not only on cassava but also on other cultivated plants and weeds, leading to hypothesize a sort of reduction in whiteflies diversity where *B. tabaci* occurs in invasive population. However, all the investigated areas did not show a high level of diversity, resulting in a minimum of two to a maximum of four whitefly species collected on the same host plant per each field inspected.

4.2.4.2 Modelling the potential distribution of B. tabaci in Tanzania

Since *B. tabaci* was the predominant whitefly pest species all over the surveyed areas, a potential distribution model based on “Climate envelope model” (BIOCLIM) was designed for this species (fig.31) Its prevalent feature is that the most suitable zones for whitefly presence were located mainly in the north-western side of the country, including part of the western side, and with a similar pattern until Zambia. Central and southern regions, on the other hand, were not potentially suitable. Comparing the potential distribution with other maps based on environmental variables, we could explain that the north-western side of Tanzania comes as the most suitable area for *B. tabaci* (in super-abundant status), not only for the presence of cassava in these areas, but also thanks to the favourable environmental conditions. The most suitable areas decline sharply, such as in the eastern-side, where very limited and scattered zones were predicted. However, suitable areas seem to be present at the south-western border of the country with Zambia and Malawi, identifying substantial and uninterrupted corridor of geographic distribution. Based on these results, we found that suitable ranges could increase in the future.

Based on the model, *B. tabaci* has a higher probability of occurrence in those areas characterised by a relatively high altitudes (1000–1300 m a.s.l.) and annual mean temperatures of 24-26 °C. Moreover, *B. tabaci*, as super-abundant population, seems to select areas with a range of precipitation values of > 150 mm in March or April and characterised by yearly average annual precipitation between 750-1000 mm (fig.32-35). In addition, it was possible to identify a particular broad area, which seems to be unsuitable for both *B. tabaci* and cassava. This area could function as a barrier for the spread of *B. tabaci* to other cassava growing areas in the eastern and/or south-eastern part of the country.

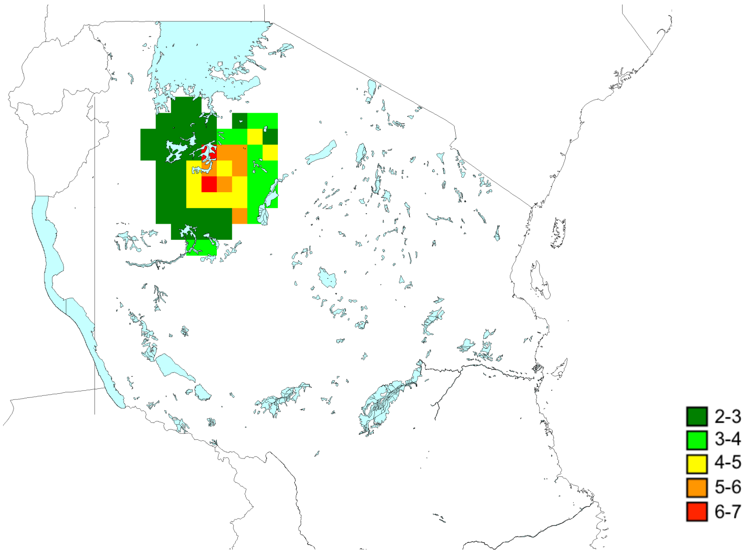

Fig. 30 Whiteflies richness recorded in the investigated quadrants of Tanzania. The colors, light to heavy, represent the richness from the minimum to maximum value, while white color represents no data.

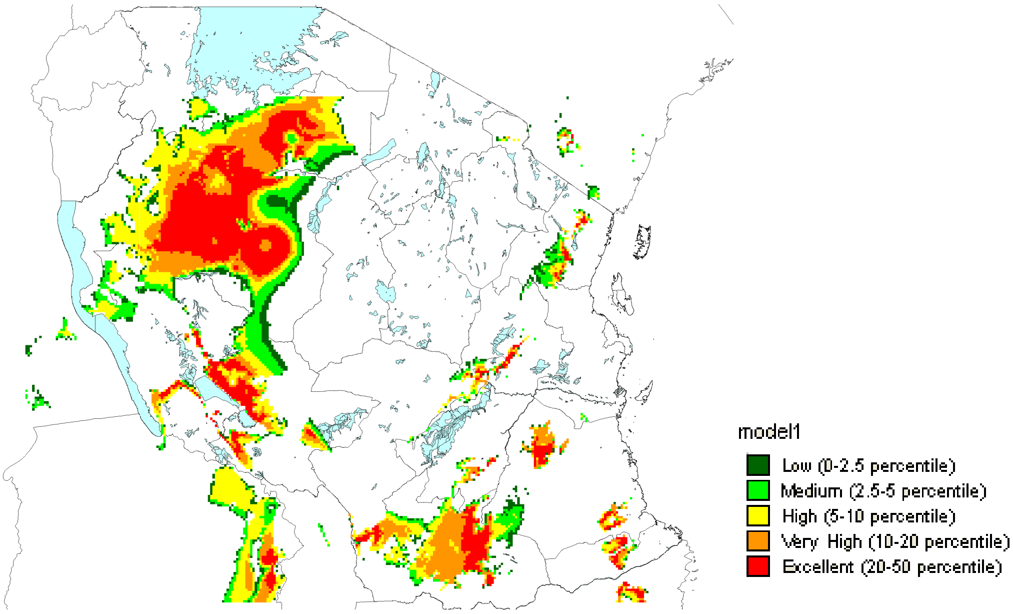

Fig. 31 le areas for *B. tabaci* in Tanzania. The colors suggest different levels of suitability based on the bioclimatic conditions that appear to be suitable for these whiteflies. White color represent no data or unsuitable areas.

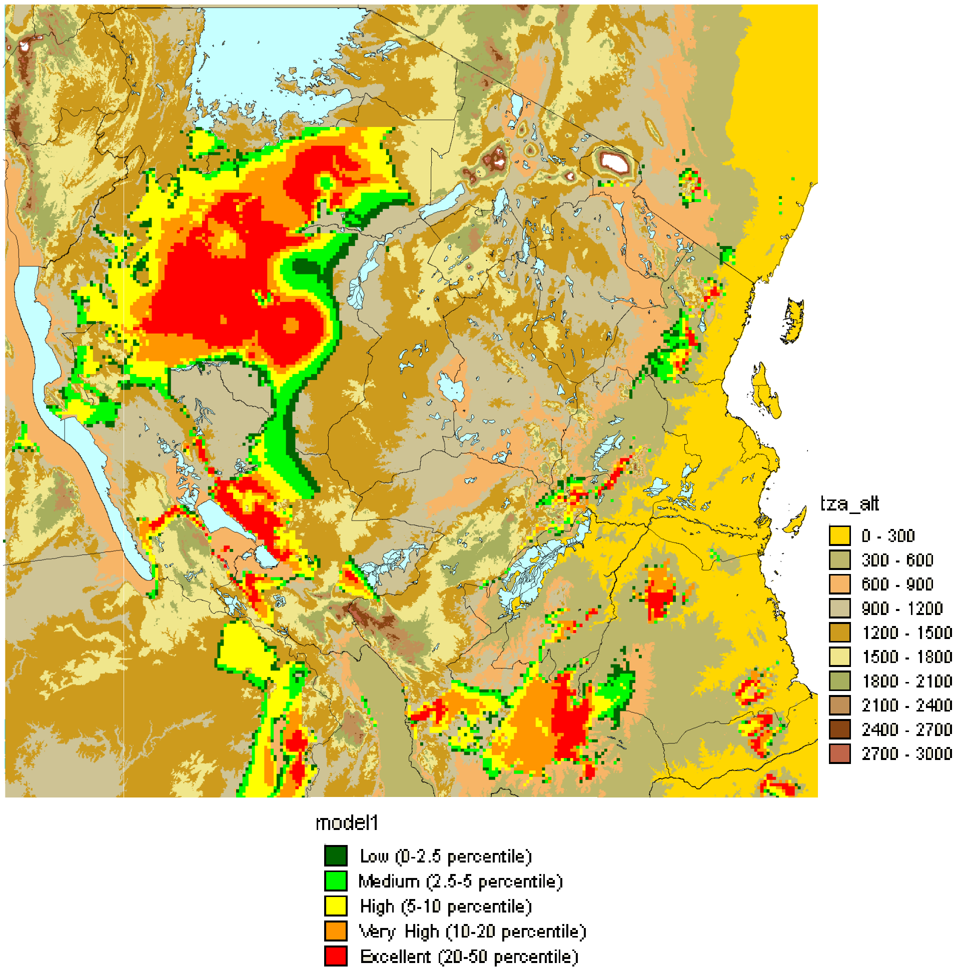

Fig. 32 Overlay between elevation (m.a.s.l.) and suitable areas for *B. tabaci* populations in Tanzania.

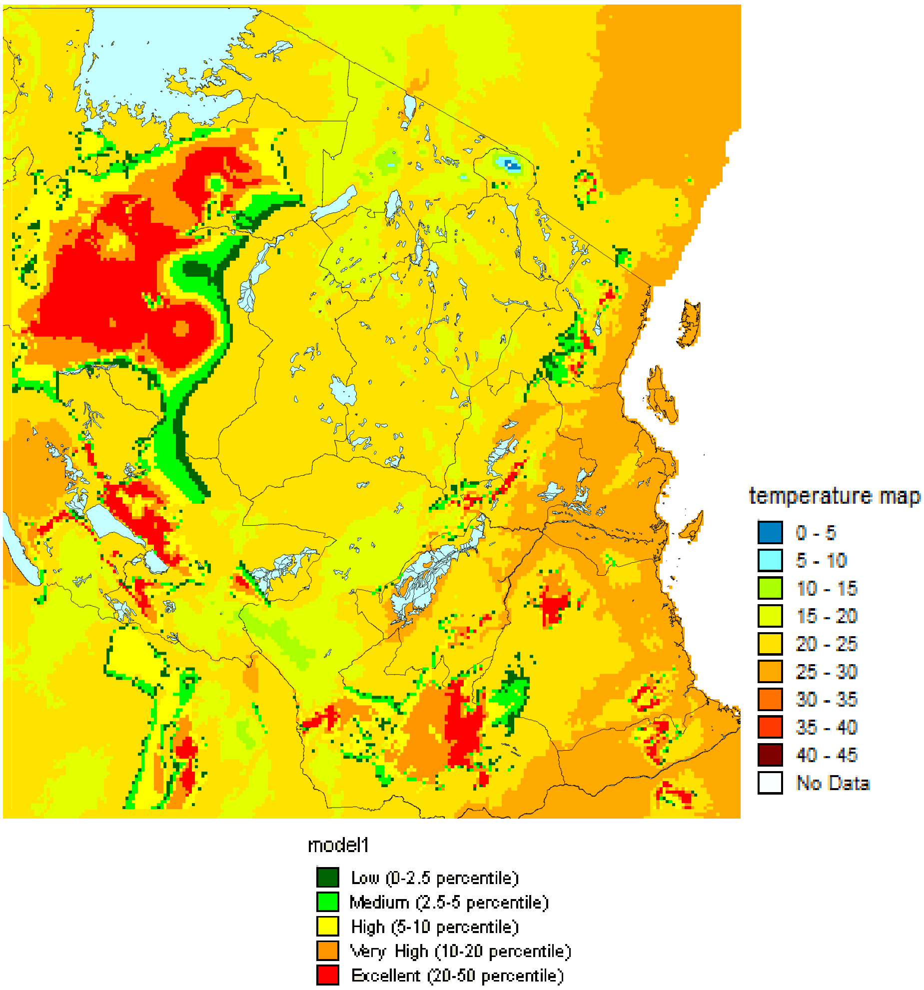

Fig. 33 Overlay between yearly average temperature (°C) and suitable areas for *B. tabaci* populations in Tanzania.

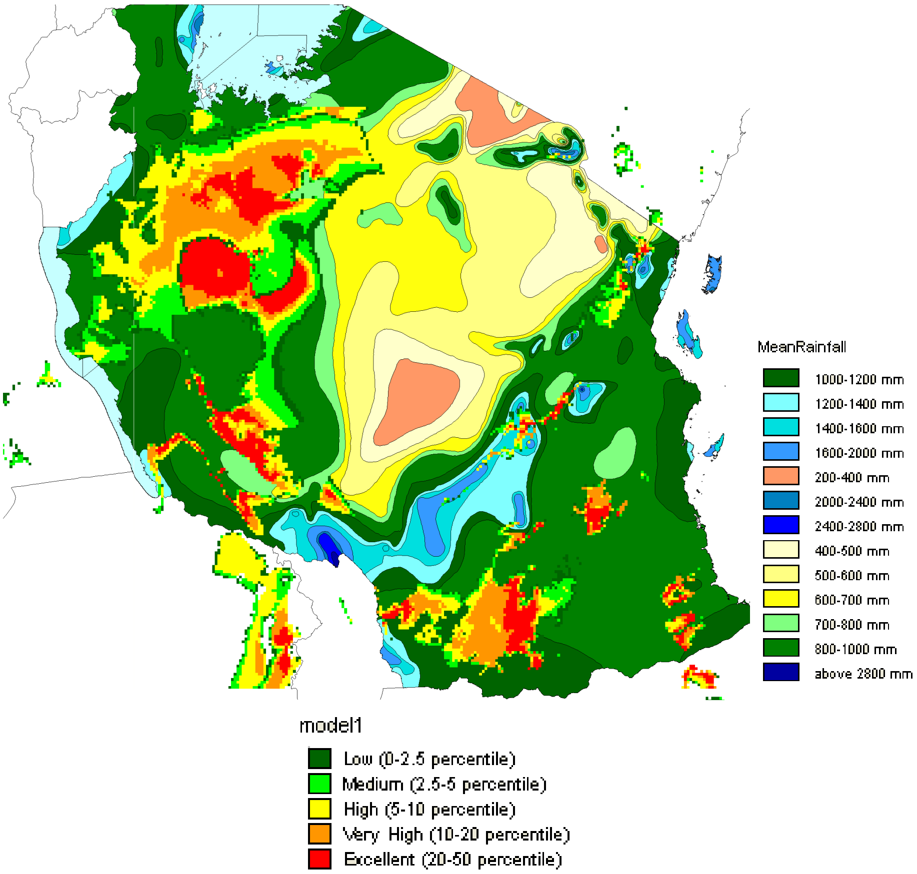

Fig. 34 **Overlay between annual mean rainfall (mm) and suitable areas for *B. tabaci* populations in Tanzania.**

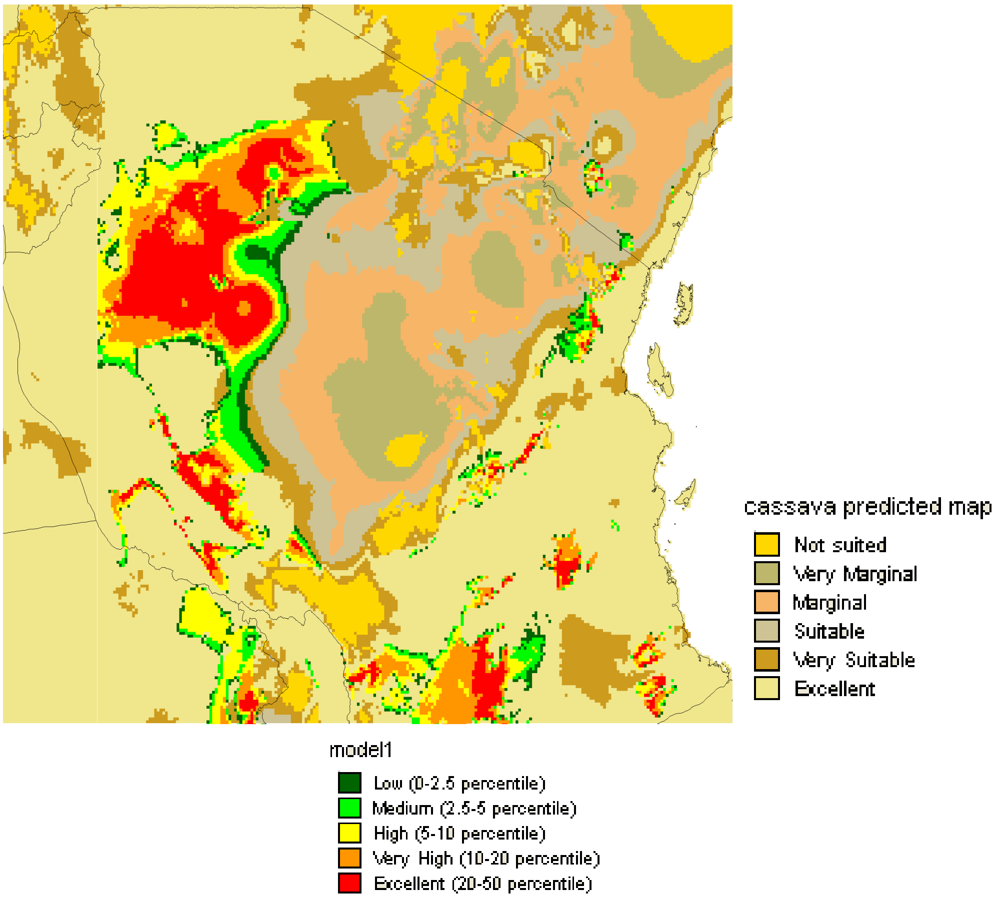

Fig. 35 Overlay between suitable cassava growing areas and suitable areas for *B. tabaci* populations in Tanzania and a broad area serving as an environmental barrier.

4.2.4.3 Parasitoids and rate of parasitism

With exclusive reference to the most important whitefly pest detected in the investigated areas of Tanzania, the highest rate of parasitism among the several host plants where *B. tabaci* was collected (tab.7) was found on weeds (20.8%), whereas the lowest rate of parasitism was found on cultivated crops, such as cassava (10%) or pumpkin (8.5%). This showed that the parasitism rate on *B. tabaci* could differ significantly on different host plants and cultural practices (P<0.05, F: 3.96, df: 4). Among the weed species, the highest parasitism rate was recorded on plant species ascribed to the family Lamiaceae. The occurrence of whitefly parasitoids in the highly infested areas of north-western Tanzania (fig.36) confirmed their movement with whiteflies; though parasitoids have been detected anyway at very low numbers showing the occurrence of a relatively scarce natural enemies complex.

Table 7 Rate of parasitism recorded for *B. tabaci* on several host plants in super-abundant whitefly area of Tanzania.

Host Plants (crops and weeds)	Mean nymph abundance (3^{rd}-4^{th} instar nymphs/5 leaves)	Parasitism average (%)	Parasitism range (Min.-Max.) (%)
Cassava	53.8	10.9	0-52.4
Sweet potato	13.3	17.68	0-60.3
Been	(-)	(-)	(-)
Okra	12.4	10.75	1-18.2
Tomato	(-)	(-)	(-)
Bitter tomato	(-)	(-)	(-)
Pumpkin	33	8.5	2-17.8
Peanut	(-)	(-)	(-)
Weeds (Lamiaceae and Brassicaceae)	31.2	20.8	2-66.8

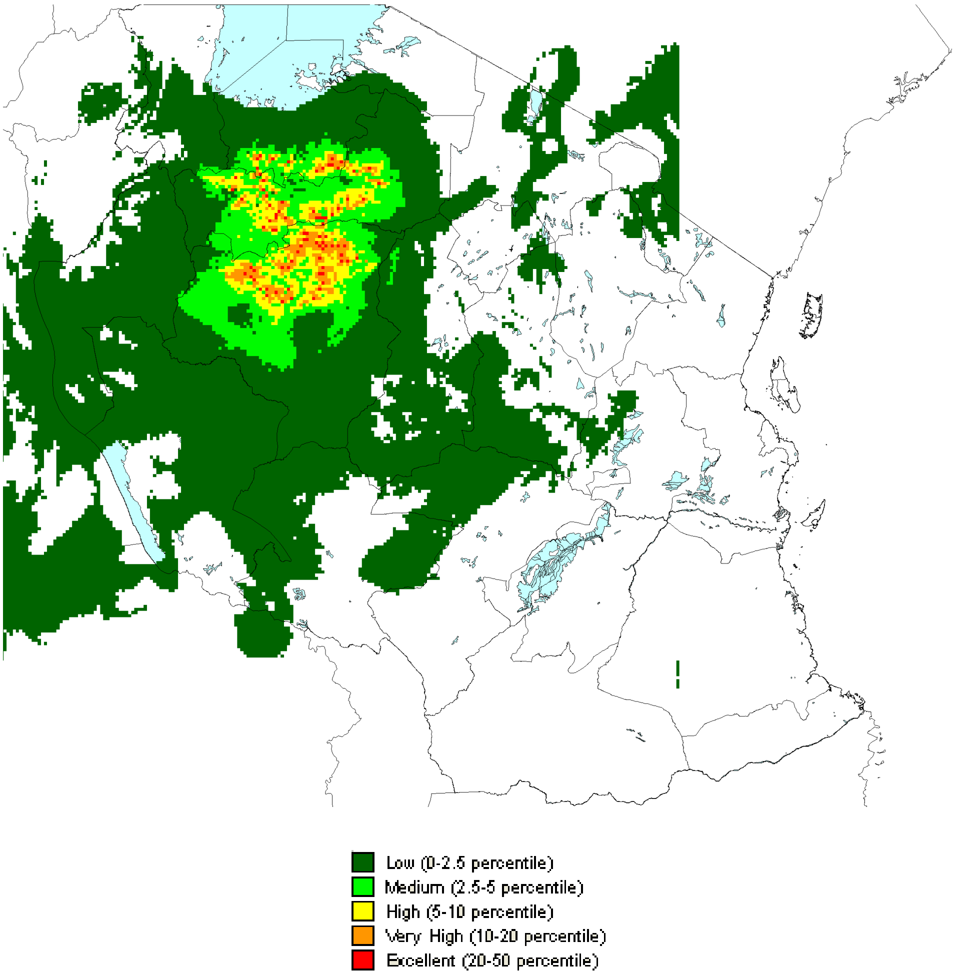

Fig. 36 Potential distribution of whitefly parasitoids in the north-western part of Tanzania based on bioclimatic data. White colour represents no data or not suitable areas.

4.2.5 Discussion

4.2.5.1 *Whiteflies and their parasitoids*

With this study, the diversity of whitefly species attacking cassava and other herbaceous host plants in cassava growing areas in Tanzania was investigated. Many of them are widely distributed species known from several regions of the world. Among the species identified, two of them, *A. dispersus* and *B. tabaci,* are still serious pests in Tanzania. A diverse community of parasitic Hymenoptera was found to attack *B. tabaci* in the surveyed locations of Tanzania. In previous studies (Gerling, 1985; Legg, 1994; Otim, 2005) only three parasitoid species were identified from East Africa. The work reported here added more species to the previous list of parasitoids present in East Africa and led to the discovery of perhaps new species of the genus *Eretmocerus.* As regards to the remaining whitefly species, no data on their parasitoids were available from Tanzania up to now. This study, however, provided useful information on parasitoids of the whitefly species occurring in the study area.

4.2.5.1.1 Genus *Encarsia* Förster

Undoubtedly, *Encarsia* is a widespread genus of parasitic wasps belonging to the family Chalcidoidea, with 344 described species, playing an important role as biological control agents (Heraty *et al.*, 2007). However, *Encarsia* is a taxonomically difficult genus that has been divided into 29 species-groups, some of which are defined ambiguously (Huang & Polaszek, 1998). Thanks to the huge amount of data available in the literature and based on the morphology, it was possible to place all the specimens collected in Tanzania in their appropriate species-group, and in most cases, identify the whole collected *Encarsia* material to the species level. Between the two different molecular approaches used in this study, the 28S-D2 region was most appropriate, as it was possible to compare sequences produced from this study with a number of exemplary sequences already available from other studies (Babcock *et al.*, 2001). Despite the large number of species in the genus *Encarsia*, there is still limited sequence data on COI, that, according to other authors, can be a useful marker in separating *Encarsia* species (Manzari *et al.*, 2002; Monti *et al.*, 2005). This study revealed the presence of six species of *Encarsia* collected on several hosts in Tanzania. Even if *En. sophia* was widely distributed and abundant in all the cassava growing regions, the low efficiency of this parasitoid with respect to the *super-abundant* status of *B. tabaci* highlights the need to augment the activity of the natural enemies and/or introduce exotic and more efficient parasitoids.

4.2.5.1.2 Genus *Eretmocerus* Haldeman

For about two decades, *Eretmocerus* species have been utilized in biocontrol programs against *B. tabaci* (Zolnerowich & Rose, 1998; De León *et al.*, 2010). The genus is known only to attack whiteflies, and several species have been reared from *B. tabaci.* Currently, more than seventy species have been described (Rose *et al.*, 1996). Despite the many studies that have been conducted on this genus, the identification of species often remains problematic (Noyes, 1998). In the absence of any good distinguishing morphological characters, it was impossible to identify the species collected in Tanzania based only on their morphology. Only by molecular analysis, it was possible to identify part of them. In this study, we employed and compared three different gene regions for the identification of the specimens in this genus. The species identified were: *Er. mundus, Er. queenslandensis, Er. hayati* (introduced from Australia) and *Er*. sp.1 (still unidentified) (fig.37). Based on the first approach (COI), it was possible to note four groups. This marker, however, was not able to identify all specimens to the species level and only *Er. mundus* was identified to this level. We were unable to differentiate the specimens by using the D2 expansion segment of the 28S rRNA. Nevertheless, blasting the sequences produced by amplification of this region on GenBank, the presence of *Er. mundus* and *Er. queenslandensis* was revealed. Unfortunately, very few sequence data is available in GenBank and hence it was not possible to identify all specimens using this region. As the third approach, *ITS1* region was used and sequences produced in this study were aligned with other sequences reported by De Barro *et al*. (2000) and Goolsby *et al.* (1999). This revealed in species-clades that were mixed, so it was also impossible to distinguish the different species using this gene region, as it was with the other two gene regions utilized. However, the variation in *ITS1* was correlated with geographical distribution, which is similar to that found in previous studies (Vogler & De Salle, 1994: De Barro *et al.*, 2000).

4.2.5.1.3 Genus *Cales* Howard

Cales is a small group of Chalcidoidea (Hymenoptera) that are parasitoids of whiteflies. The species *C. noacki* has been introduced from South America into citrus-growing regions of Africa for biological control against the woolly whitefly *A. floccosus* (Gruenhagen & Perring, 2001; Mottern *et al.*, 2011). In Tanzania, *C. noacki* has been found to parasitize *B. tabaci* and especially *B. afer,* but it does not have a major impact on their populations*.* In many cases, this happened in areas where cassava was grown close to citrus.

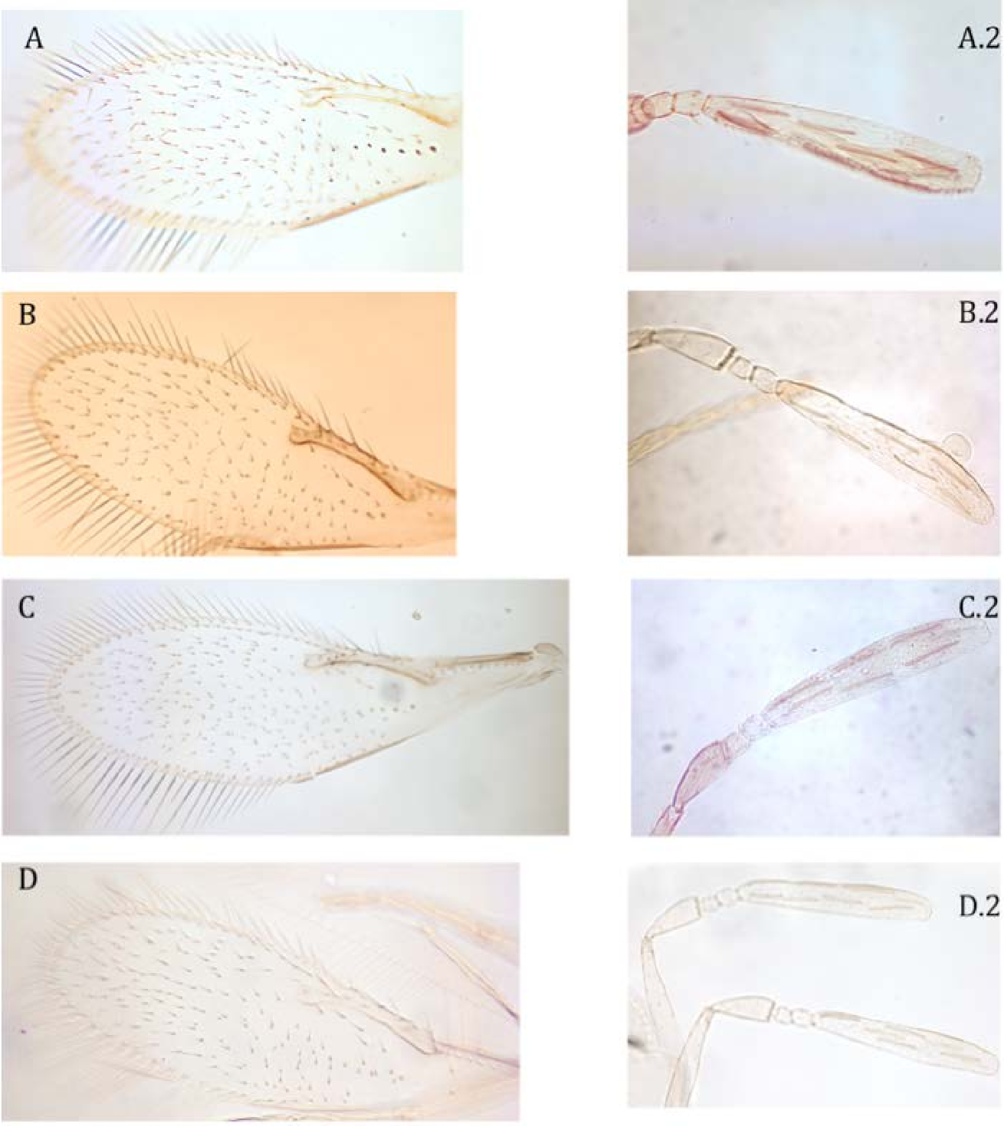

Fig. 37 Species of *Eretmocerus* collected in Tanzania and validated by molecular analysis. (A-A.2) *Er. mundus* forewing and antenna ♀; (B-B.2) *Er. hayati* ♀ (introduced from Australia); (C-C.2) *Er.* sp. Tanzania ♀; (D-D.2) *Er. queenslandensis* ♀.

4.2.5.2 Spatial analysis

During the last decades, a few studies have investigated climatic factors (mainly temperature and rainfall) influencing whitefly biology in Africa. In Tanzania, Storey (1936) observed higher whitefly activities at low land (coastal areas) respect to high land, due to the relative variation of temperature. Leuschener (1978) and Fauquet *et al.* (1988) reported temperature as the key factor in driving high population of *B. tabaci* in West Africa. On the contrary studies by Legg (1994) reported abundant whitefly populations over 1000 m a.s.l. in Uganda, Tanzania and neighbouring countries.

Generally, the effects of heavy rainfall have been reported in association with decline in *B. tabaci* population (Naranjo *et al.*, 2003; Naranjo & Ellsworth, 2005). Similarly in Africa, Fishpool *et al.* (1995) indicated a negative correlation between whitefly abundance and rainfall. On other hand previous studies by Robertson (1985) in Africa, reported high population of whiteflies associated with areas of high mean annual rainfall. However, Legg (1994) reported no relation between the factors. Nevertheless, ecological data on whitefly in Africa are scattered in the literature and out-dated.

Recently, pest management have been improved by exploring geographical data using GIS and Geostatistics. These techniques have been largely utilized for investigating harmful insects in Africa (Donnelly & Townson, 2000; Bayoh *et al.*, 2001; Lindsay *et al.*, 2013) and disease transmitted by pests (Brooker *et al.*, 2000; Simarro *et al.*, 2010; Bouwmeester *et al.*, 2012). However, almost no study specific to *B. tabaci* using GIS and geostatistic have been done.

In the study reported here, several parameters have been investigated, regarding one of the most affected areas where *B. tabaci* is occurring in Africa. The model designed here can provide maps of the potential distribution of *B. tabaci* in north-western part of Tanzania, identifying areas that may or may not be suitable for the occurrence of the pest.

The potential distribution map, in addition to the current area where super-abundant *B. tabaci* is already known, shows suitable areas in the western part of the country until the bordering countries. In part, it could confirm the recent spreads of super-abundant whitefly populations recorded in north-eastern part of Burundi (Legg, 2003) and in Zambia, where more recently a new spread of super-abundant whitefly has been detected (Chikoti *et al.*, 2013). In addition, it was possible to localize an area completely unsuitable to *B. tabaci* occurrence that seems to act as an ecological barrier between the super-abundant whitefly area, also known as pandemic area for cassava mosaic disease, and other cassava growing areas near to the coast.

Comparing these results with the previous ecological data reported for Tanzania, it could be possible to assume that:

- In Tanzania, super-abundant *Bemisia tabaci* populations occur at about 1000 m a.s.l., with rainfall ranging from 750 -1000 mm.
- The spread of whiteflies to the eastern and south-eastern part of the country is blocked by a large area that is characterized by low precipitation and less vegetation cover, hence serving as an environmental barrier.
- The relative variation of temperature between the suitable and unsuitable areas seems not to play an important role in whiteflies distribution.

- The spread of whiteflies to the western and north-western part of the country could be possible due to the bioclimatic suitability that extends further to the bordering countries.

Diversity and density of *B. tabaci* parasitoids were very low to draw any conclusion on species richness and their potential distribution in the investigated area. All data obtained through the present study revealed that parasitoid species were randomly distributed. However, trying to map the potential distribution of *B. tabaci* parasitoids and considering them as a whole (not treated as single species), it was possible to observe a potential distribution similar to *B. tabaci* in the north-western part of the country, but with a lower rate of suitability. Therefore, hypothesizing the parasitoid model as valid and taking into consideration the lower rate of bioclimatic suitability for the parasitoids compared to *B. tabaci*, there is a risk that the pest could continue to spread further from the super-abundant area, without being slowed down by the presently existing biological control agents.

Such information will be useful to design strategies in pest management. However, climatic conditions are not the only factor involved in pest occurrence, as the space-time variations in the density of host plants also play a very crucial role (Hirano *et al*., 1995). Moreover, agricultural, biological or climatic changes can lead to *B. tabaci* outbreaks in susceptible areas (Morales & Jones, 2004). In addition, the relatively low parasitism rate achieved by the actual parasitoid fauna suggests that there is the need to locate and select the most efficient *B. tabaci* parasitoids all over Africa and thus enhancing the current biological control in Tanzania.

4.3 ASSESSMENT OF *BEMISIA TABACI* SPECIES COMPLEX ALONG AN ALTITUDINAL GRADIENT IN SICILY AND NEW HOST RECORDS FOR *BEMISIA TABACI* SP. ITALY

4.3.1 Introductory notes

Recently, study of insects along an altitudinal gradient has received much attention in ecological research. Altitudinal gradient influence the distribution of insects and reflect the life history and the ability of species to adapt to a specific habitat (Hodkinson, 2005). As elevation influences the spatial distribution of insect species, it also affects the environment, such as temperature, rainfall, host plants and natural enemies. These factors influence the multi-trophic interaction between insects, host plants and environment (Preszler & Boecklen, 1996). Altitudinal gradient seems to be a strong ecological instrument for testing the response of a biota to geophysical influence (Körner, 2007). *Bemisia tabaci* (Gennadius) is a polyphagous insect that occurs on hundreds of different plant species, especially herbaceous plants (Martin *et al*., 2000). It has a global distribution and is considered as a pest both in greenhouses and open field (Gerling, 1996). This let us think that *B. tabaci* has an extensive altitudinal gradient and is not so much influenced from geophysical barrier, except for the numerous constituent species (previously called biotypes) identified within the species complex (Oliveira *et al.*, 2001, Dinsdale *et al*. 2010) which show unique patterns of geographic distribution. From long time *B. tabaci* is considered in Italy as an important pest in agriculture, especially after the introduction of several viruses like tomato yellow leaf curl geminiviruses (TYLCV) in Sicily (Rapisarda, 1990). Considering the data available from previous works (Bosco & Caciagli, 1998; Demichelis *et al.*, 2000; Cavalieri & Rapisarda, 2008), no study has been conducted to evaluate the distribution of *B. tabaci* species-complex along an altitudinal gradient and little is known about it. This whitefly is only expected to be prevalent in disturbed agricultural areas (Morales & Jones, 2004). However, in Italy this is true for two *B. tabaci* species, Mediterranean (ex Q-biotype) and Middle East-Asia Minor 1 (ex B-biotype). In 1999, for the first time in Italy, *B. tabaci* "T" biotype was collected on *Euphorbia characias* L. in Sicily and exactly in a limited area of Nebrodi-Peloritani mountains in the undergrowth of a pine forest and between 600-1000 m above sea level (Simón *et al.*, 2003). Demichelis *et al.* (2005) studied the biology of "T" biotype and established that it was restricted to a wild plant species growing in nature, *E. characias.*

Environmental conditions and *B. tabaci* species complex may vary along elevational gradients. In the following pages, the altitudinal distribution and abundance of each species of *B. tabaci* complex occurring in Sicily was investigated and the results are presented below.

4.3.2 RESULTS

4.3.2.1 Surveyed areas

Overall, a total sampling of 557 km in three separate altitudinal transects were carried out. The first transect of 250 km has covered all the south coast of the island (from 33m a.s.l. to 220m a.s.l.); a second transect has covered 120 km, passing through the inland (from 400m a.s.l. to 700m a.s.l.); the third one covered 187 km along the east coast, until Mount Etna (from 60m a.s.l. to 1700m a.s.l.). The species richness of host plants (cultivated plus weeds) of *B. tabaci* showed a peak between 300-500m a.s.l. (Fig.38).

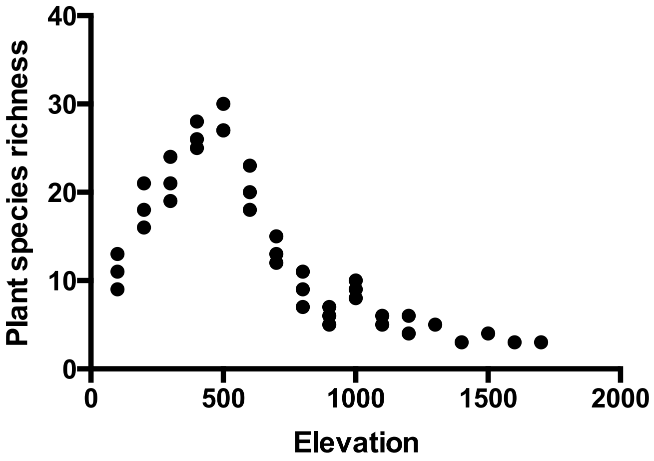

Fig. 38 Plant species richness (herbaceous only) along the elevation gradients of the investigated transects.

4.3.2.2 Species of the B. tabaci *complex and phylogeny*

In total, more than 400 whitefly nymphs were collected, belonging to three different species of *Bemisia tabaci* complex. The species found were: *B. tabaci* Mediterranean (ex-biotype Q), *B. tabaci* Middle East-Asia Minor 1 (ex-biotype B) and *B. tabaci* Italy (ex-biotype T) (Fig. 39). The first two species were more abundant at lowland where there are agricultural crops, especially horticulture (between 50-400m a.s.l.) (fig.40-A). In natural areas, *B. tabaci* abundance increased with plant richness with a peak at 400-500m a.s.l. (Fig.40-B). This is because the environment was too dry in the lowland along the coastal part of the island. In fact, at mid-altitude during the warm season it was still possible to find some green and wet areas. However, even if some of the host plant species growing at mid-altitude were present also at higher altitude, the insect abundance was much lower. In natural environment, the abundances of *B. tabaci* sp. Mediterranean and Italy were significantly correlated with the elevation gradient (Mediterranean: $p<0.0017$, $r=0.54$; Italy: $p<0.034$, $r=0.40$) (fig.41). *B. tabaci* sp. Italy was absent from low elevation areas, even if host plants were there. Sporadically, this species was observed around 400m a.s.l on *E. characias,* (which is the principal host plant of this species), *Ancanthus mollis* L. and in rare occasions on tomato [*Lycopersicon esculentum* (L.)] grown in open fields near natural areas. As the elevation gradient increases, *B. tabaci* sp. Italy reaches its peak in natural environments between 600-1000m a.s.l., colonizing mainly *E. characias* and *A. mollis*.

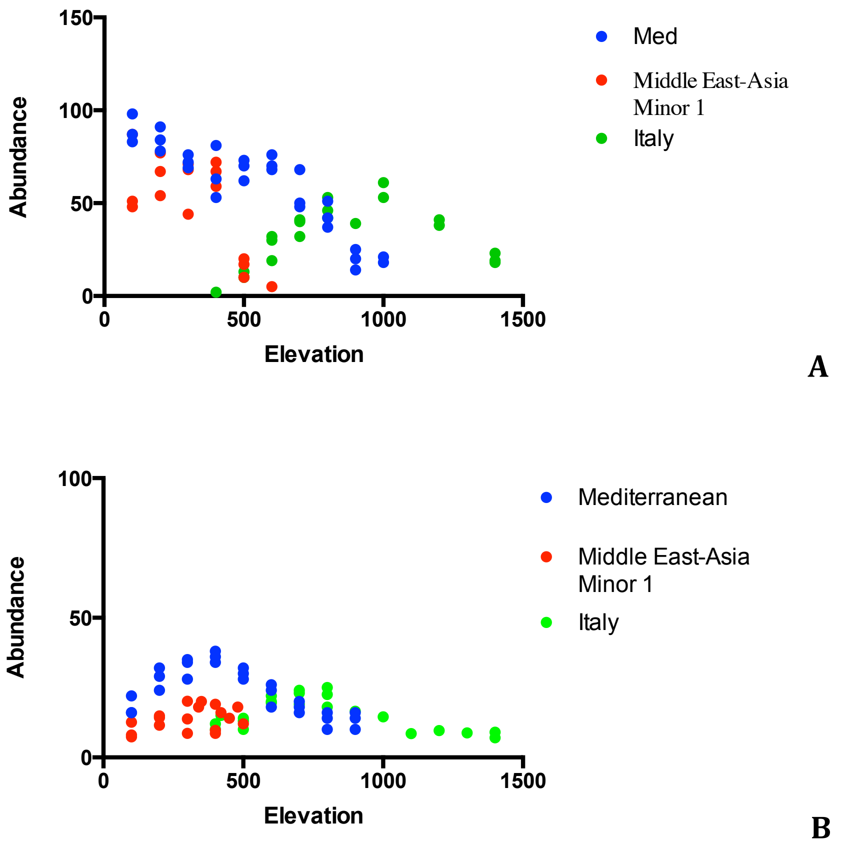

Fig. 39 Fig. 3 Abundance of species of the *B. tabaci* complex along an altitudinal gradient (m.a.s.l.). (A) Cultivated areas; (B) Natural areas.

The phylogenetic distances were correlated with the elevation gradient to investigate the phylogenetic community structure with altitude. Three main altitudinal categories were taken in consideration: low-land (<450m a.s.l.) dominated by *B. tabaci* Mediterranean and Middle East-Asia Minor 1; medium (451-600m a.s.l.) dominated by the *B. tabaci* Mediterranean group; and high (>600m a.s.l.) were the *B. tabaci* Italy group is well established. The phylogenetic distances of *B. tabaci* showed that communities at high altitudes were closely related, ranging from 0.10% to 0.30%, compared to those communities at mid and low elevation where the phylogenetic distances reached the 1.84%.

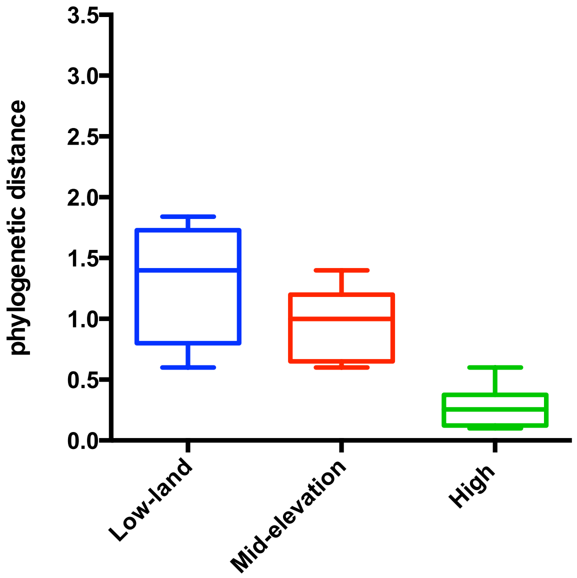

Fig. 40 Influence of the elevation gradient on the phylogenetic distances of Bemisia tabaci species complex between sites. The altitudinal categories were: low (<450m a.s.l.), medium (451-600m a.s.l.), high (>600). F= 9.61, p < 0,001.

4.3.3 DISCUSSION

The results of this study show how altitude can influence the species diversity and distribution of *Bemisia tabaci* species complex. The elevation gradient can be used as an environmental filter where insect species are filtered. These data can be combined with all ecological characters already known for most of the species of the *B.t.* group. As previously described by De Barro *et al.* (2011), *Bemisia tabaci* sp. Mediterranean (ex-biotype Q) has its origin in the countries bordering the Mediterranean Sea, including Sicily. *Bemisia tabaci* sp. Middle East-Asia Minor 1 (ex-biotype B) is an invasive species coming from the Middle East–Asia Minor region but at present found diffused over the world. *Bemisia tabaci* sp. Italy (ex-biotype T) seems to be an older species coming from the Middle East and established in Italy may be millions of years ago. Nowadays, *Bemisia tabaci* sp. Italy looks like to be concentrated predominantly in natural areas at medium-high altitude (600-1200m a.s.l.). The narrow phylogenetic distances within the *Bemisia* *tabaci* sp. Italy suggested a lower phylogenetic turnover respect the other two putative *Bemisia tabaci* species present in the island. Maybe, as for other cases (Hodkinson, 2005), the harsh and unfavourable environmental conditions, together with the isolated and fragmented habitats, pushed this group for a microevolution and environmental adaptation. In addition, we can also speculate that the *Bemisia tabaci* sp. Italy was outperformed by the other two *Bemisia tabaci* species and slowly restricted to its actual habitat.

However, even if mixed populations were found in a few areas, the probability of mating interaction is very low. In previous studies, Liu *et al.* (2012) clarified the pattern of reproductive interaction between *Bemisia tabaci* species. The Italy species may interact only with the Mediterranean group without obtaining hybrid female F1. The interbreeding with the Middle East-Asia Minor 1 seems to be impossible. Instead, the interbreeding between the Mediterranean and the Middle East-Asia Minor 1 groups look like to be possible obtaining, even if in a low number, F1 hybrid females (Liu *et al.,* 2012). Moreover, given the geographic vicinity with other Mediterranean regions and the intense commercial exchange of agricultural products among them, a major genetic turnover could be possible.

Our results showed also how, in natural environments, a higher host plants cover was correlated with the increase of general whitefly abundance. However, this was not completely true for *Bemisia tabaci* Middle East-Asia Minor 1, since this species has it altitudinal limit at 400-500m a.s.l., whereas the Mediterranean group showed an extended altitudinal gradient and was able to colonize a wide range of weeds. Nevertheless, the capacity is known of the Mediterranean species to outperform the Middle East-Asia Minor 1, given a wider host (Jiao *et al.*, 2013). In some case, the Mediterranean species was able to displace the Middle East-Asia Minor 1 (Horowitz *et al.,* 2003; Chu *et al*., 2010).

As for cultivated areas, as expected, both *Bemisia tabaci* species (Mediterranean and Middle East-Asia Minor 1) were more abundant at lowland, occurring both in greenhouses and outdoor. In fact, in such conditions a decline of natural host plants diversity, given by the dry and warm season, increases the pest abundance (Poveda *et al.,* 2012) or better, drives pests to concentrate in favourable areas, such as cultivated ones, where they can find green hosts and a wet environment.

Also in this case, the elevation gradient influenced the distribution of the Middle East-Asia Minor 1 as in natural environments.

However, these results cannot to be generalized to all agro-ecosystems. There are not full studies in the past to compare with our results. It could be interesting to compare these results with studies carried out in other Mediterranean regions. Therefore, 'Standard Mountain' doesn't exist; since data collected along elevation gradients exhibit the multi-effect of that particular geographic area and general altitude phenomena (Körner, 2007). This is essential when different studies from different areas are compared.

Finally, the combined ecological and molecular approaches show how elevation gradient can be an additional instrument to study cryptic-species and their environmental competitiveness. The effects of altitude have been under-explored within the framework of *Bemisia tabaci* species complex. Previous studies confirmed the importance of ecological tools in environmental and genetic control of insects (Bird & Hodkinson, 2005; Nufio *et al.*, 2010; Garibaldi *et al*., 2011; Hoiss *et al.*, 2012). Our results provide a first approach and clear evidence that variation in elevation gradient influence the distribution of the three *Bemisia tabaci* putative species occurring in Sicily.

Chapter 5: FINAL REMARKS

Whiteflies and their parasitoids have a worldwide distribution presently; however, according to the literature, they are tropical in origin. Due to natural factors and, especially, anthropogenic activities they spread from the tropics to sub-tropical regions and extending also to temperate areas.

Not all species ascribed to the family Aleyrodidae are considered pests. In fact, among the over 1,500 whitefly species described to date, only about fifty can be considered as a threat to cultivated and ornamental plants; and only a few species are known to be particularly dangerous, causing severe losses to agriculture. In some cases, they are not only a huge problem for the yield losses they cause and the consequent economical impact, but also because they can become a serious risk for the food-security of entire populations. This case is extremely frequent in Africa, where severe outbreaks of *Bemisia tabaci* (*s.l.*) in many cassava-growing countries are able to reduce the resource of food, as it is reported from Uganda, Kenya, Tanzania, Rwanda and Congo. The combination of *super-abundant* whitefly populations and the transmission of two groups of viruses (cassava mosaic geminiviruses and cassava brown streak viruses) causing devastating diseases has had dramatic effects on the largely agricultural economies of affected regions, resulting in scarce food resources for already vulnerable human populations. To date, in these areas, control measures against *B. tabaci*-transmitted viruses are still on-going.

In the tropics, another harmful whitefly pest is *Aleurodicus dispersus*, also known as the spiraling whitefly, which is a highly polyphagous pest having an extensive host-range. Specially, it affects citrus, avocado, guava, banana, coconuts and cassava by direct feeding and production of sooty mould. However, recent studies showed that it seems to be also related with the spread of certain viruses.

In sub-tropical countries, whitefly species such as *B. tabaci* and *Trialeurodes vaporariorum* have been among the major pests causing tremendous losses in both open field and protected crops. During the last century, both whiteflies have been well established around the Mediterranean region, severely affecting cotton, tomato, cucurbits, sweet pepper and many other crops by their large populations and several virus-related problems. Therefore, given the higher level of technical knowledge and, especially, the wider possibilities of economic investment in this part of the world, *B. tabaci* and *T. vaporariorum* have been usually controlled here by cultural, biological and chemical methods.

However, tropical and sub-tropical agriculture share also similar successful stories about harmful whiteflies. These are the cases of *Aleurothrixus floccossus*, *Dialeurodes citri*, and *Parabemisia mirycae* that have been a bottleneck for the citrus production over the world. These whitefly species have been successfully controlled by their natural enemies keeping the pest populations at low levels. This was possible through the introduction of exotic parasitoids in the whitefly-infested areas, as the indigenous parasitoid fauna was not efficient or able to adapt to the new alien pest. Another case that deserve to be mentioned concerns the introduction of *Siphoninus phillyreae* (known as the ash whitefly) into the New

World and the consequent biological control program which has been based on the introduction of exotic parasitoids and gave successful results in a very few years. Unfortunately, clear evidences of *B. tabaci* control by its natural enemies do not exist. The parasitoid fauna of *B. tabaci* includes several species but still only a few of them have been investigated. Some of them have been extensively studied inside laboratory or in a controlled environment; but studies regarding their behavior and biology in natural environment are comparatively scarce.

Other problems in studying whiteflies parasitoids derive from their frequent difficult identification at species-level. Experts on taxonomy of whitefly parasitoids are really few in the world and the systematics of several parasitoid groups need to be revised, under the light of the new techniques available to date. In addition, comparing studies based on only morphological or only molecular methods collides, thus creating a “Tower of Babel”. However, during the last decade, entomological researches are trying to combine different methods, such as morphological, molecular and biological, with the aim to characterize each species giving precise information.

Recently, new methods are available that are useful to analyze the spatial diversity and distribution of entities and understand the bio-climatic factors influencing their spread. These are Geographic Information System (GIS) and Geostatistics. These instruments can help in pest management programs, giving information on their presence/absence in one region and identifying suitable (sensitive) areas for further invasion. In the same way, it is possible to identify areas (ecological niches) where the bio-climatic conditions are suitable for the parasitoid fauna. This could be useful in cases like studies for biodiversity conservation or modeling of suitable areas as biological corridors.

In Tanzania, the research and studies reported in the present pages produced important data about the whitefly and parasitoid fauna occurring in cassava growing regions of the country. These data will be important for further biological control program on *B. tabaci*. Moreover, information regarding the potential distribution of *B. tabaci* population was also given. Several parasitoid species were collected and identified through this work. However, parasitoids have been detected at very low numbers, showing the occurrence of a relatively scarce natural enemies complex. The results of this study have given baseline information about the current status of parasitoids and their host-range, that may be crucial and helpful for designing further biological control strategies against whitefly pests, in addition to the ones already applied successfully in the past.

In Sicily, this study gave comprehensive and updated information regarding the diversity and distribution of the current whitefly and parasitoid fauna occurring on herbaceous plants. Environmental pollution and human activities were among the factors identified to affect the distribution and abundance of whiteflies and their parasitoids. The growth of crop monocultures correlated with the simplification of the biodiversity at the expense of the natural flora surrounding the cultivated lands seem to affect positively the development and increasing of whitefly pest complexes. On the contrary, this environmental simplification together with unfavorable bioclimatic factors affects negatively the distribution and abundance of whitefly parasitoids.

Moreover, natural areas that function as parasitoid reservoir were identified; and this can be of a great importance to develop strategies for using and maintaining the natural enemy fauna. Furthermore, dispersal of parasitoids from zones that function as reservoir may enhance pest control, which in turn will have a positive effect on biological control. At the moment, such areas act as unique resources not only to preserve but also to select parasitoids, which are useful for biological control of whiteflies, especially considering how the present European policy does not allow new introduction of exotic parasitoids.

Future line of works should focus on monitoring and follow up of areas already infested and or areas with high bio-ecological suitability for whiteflies and hence at risk of outbreaks, to design pest management strategies before harmful outbreaks or invasions. Integrated Pest Management strategies should involve the use of biological control combined with three methods adopted for this study (morphological, molecular and GIS) in the aim to assess three key issues for pest management and use of biocontrol agent resources: (1) spatial analyses at species level (at genetic level in the case of cryptic species) to elucidate the geographic diversity and distribution of pest and their natural enemies, (2) bio-climatic adaptation analysis for each species both whiteflies and parasitoids, and (3) prioritization modelling for biodiversity conservation. Such combined analysis can be used to support risk assessment of potential invasive species, conservation management and for selecting or prioritizing insect species for further studies.

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