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Alien Invasive Species in Europe: Three Case Studies

Director of the School:	Ch.mo Prof. Antonio Berti
Supervisor :	Prof.ssa. Giuseppina Pellizzari

PhD Student: Evangelia Chatzidimitriou

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ABSTRACT

The incidental introduction of alien phytophagous insects and mites has become quite a common event in the world owing to intensive commercial exchanges of plants and goods and ever-increasing tourist traffic. There is evidence that this phenomenon is increasing, in spite of the control measures of the EU phytosanitary system in order to minimize unintentional introductions.

The introduction of an alien species in a new ecosystem and the interaction between an alien species and the autochthonous species usually has many disadvantages. The alien species can dominate the invaded ecosystems and eventually become an invasive species due also to the absence or paucity of natural enemies. These invasions can affect the native species that become less common or threatened with extinction. Apart from the environmental impacts alien species are known for their economic and health impacts.

In this study were investigated mostly 3 recently introduced alien species in Italy, namely *Tuberocephalus (Trichosiphoniella) tianmushanensis* Zang (Hemiptera Aphididae), *Cydalima perspectalis* (=Glyphodes) (Walker, 1859) (Lepidoptera Crambideae), the box caterpillar and *Phenacoccus defectus* Ferris (Rhynchota Pseudococcidae).

The first chapter is a background of invasive ecology and presents with graphs the high number of alien species introduced in Europe the last years..

The second chapter is dealing with *Tuberocephalus (Trichosiphoniella) tianmushanensis* Zang, an Asiatic heteroecious species so far not recorded in Italy. This species was collected in the University Botanical Garden of Padova in spring 2012. On May 30, 2012 reddish-pink galls, with aphids inside, were observed on the leaves of two *Prunus subhirtella cv. pendula* trees (Rosaceae) (Weeping Higan Cherry), about 40 years old. Once mounted on slides the aphids were identified as *Tuberocephalus (Trichosiphoniella) tianmushanensis* Zang. The purposes of this study were to collect data on species distribution over the territory, by monitoring ornamental cherry trees in the Veneto region, to observe the phenology and biology of the Asiatic aphid, to study the life-cycle in screen houses and outdoors, to verify if its secondary host plant was an *Artemisia sp.*, as reported in bibliography. Another aspect

of the work was to provide an overview of the species belonging to the genus *Tuberocephalus* so far described, by consulting the available literature. It was made an effort to gather all the currently available information for each species, its distribution and information on their biology mainly regarding the first and secondary host plants. Results showed that *Tuberocephalus (T.) tianmushanensis*, is now considered acclimatized in our environment. The aphid is closely related to the presence of its primary host *Prunus subhirtella v. pendula* with pink flowers. The aphid can carry on two generations on *Prunus* and can induce two types of leaf galls. The gall A is induced by the fundatrix, while the gall B is induced by the fundatrigeniae. The trial of colonization on *Artemisia vulgaris*, failed for the second successive year so possibly *Artemisia vulgaris* is not the secondary host plant of the aphid, as reported in literature.

The third chapter concers *Cydalima perspectalis* (Lepidoptera, Crambiidae) (Walker, 1859), an asiatic pest of *Buxus*. It was reported for the first time in Europe in Germany in 2007. In Italy it was detected in 2011, in Lombardy, Como province. In a very short time it invaded the other northern regions and was recorded in Veneto in 2012. The larvae feed on leaves and shoots of the box trees and the infestations lead to defoliation of the plants. The objective of this study was to investigate the phenology of *C. perspectalis.* More precisely we examined the biological cycle of life, the number of molts and the overwintering stage. In addition host plants were monitored by regular samplings, from late winter to late fall to collect data on species distribution over the territory. The life-cycle was studied in screen houses and in the field, to investigate the role of potential predators and parasitoids. Additionally experiments were conducted with pheromones traps with the purpose of checking, monitoring and collecting data on species distribution over the territory, finding any possible differences based on climate, checking the potential differences between types of traps. According to the results of 2014 and 2015 in the Veneto Region *C. perspectalis* develops three generations / year. In 2014 the overwintering larvae started their activity early in February until mid-April while in 2015 one month later until end of April probably due to different climate conditions between these years. *C. perspectalis* overwinters in a silk cocoon in-between the leaves as a larva of 2nd instar and the number of larval instar is 5.

The number of captures from the sex pheromones traps was low. No differences were observed between the two types of pheromones. *C. perspectalis* has spread quickly in our environment proving that it has acclimatized. So far, it seems there has been no adaptation by indigenous natural enemies (parasitoids) to *C. persectalis*.

The next chapter is focued on the difficulty to separate the *Phenacoccus solani* Ferris and *P. defectus* Ferris (Hemiptera: Pseudococcidae). They are morphologically similar and the microscopic morphological characters of the adult female alone are not enough**.** In order to resolve their identity, a canonical variates morphological analysis of 199 specimens from different geographical origins and host plants and a molecular analysis of the CO1 and 28S genes were performed. The morphological analysis supported synonymy of the two species, as although the type specimens of the "species" are widely separated from each other in the canonical variates plot, they are all part of a continuous range of variation. The molecular analysis showed that *P. solani* and *P. defectus* are grouped in the same clade. On the basis of the morphological and molecular analyses, P. defectus is synonymized under the senior name *P. solani*, **syn. n**.

Finally a zoogeographic analysis of the Greek scale insects fauna (Hemiptera, Coccoidea) was carried out with the aim to highlight how many alien scale insects species are so far present in the Greek territory. According to the last data, the scale insect fauna of whole Greek territory comprehends 207 species; a total of 187 species are recorded in mainland Greece and minor islands, whereas only 87 scale species are known so far in the island of Crete. The most numerous families are the Diaspididae, with 86 species in total, followed by Coccidae, with 35 species and by Pseudococcidae, with 34 species. The results of a first zoogeographical analysis of scale insect fauna of mainland Greece and the island of Crete is also presented. Five scale species, respectively four in mainland Greece and one in Crete, are considered as endemic. This analysis demonstrated that alien scale insects, introduced and acclimatized a long time ago or recent invaders, make up 30% of the Greek scale insects fauna.

Chapter I

Introduction

Introduction

Invasion ecology has become an important topic among the scientific community. Lots of authors have attempted to give a more exhaustive definition of biological invasion and to clear out the distinction between the terms alien, exotic, non-indigenous, imported, introduced, nonnative, immigrant, colonizer, naturalized which are commonly used to advert the invasive species (Crawley *et al.,* 1996; Green 1997; Mack *et al*., 2000; Pimentel *et al.,* 2000; Kolar & Lodge 2001; Williamson & Fitter 1996; Lonsdale 1994; Davis *et al.,* 2000; Bazzaz 1986; Williamson 1996; Richardson *et al.,* 2000; Colautti & MacIsaac, 2004; Valery *et al.,*2008).

Some scientists supports that the definition of invasions species are related with economic and environmental impacts (Mack *et al.,* 2000; Colauti & MacIsaac, 2004) while others suggests that it should be related only with the biogeographic/ demographics status of a species without any kind of impact associated with (Richardson *et al.,* 2000).

A general definition of biological invasion is considered the spreading of a species out of its original distribution area and for the new environment the species is called exotic or alien species. Invasion also involves not only transport of organisms to a new location but also establishment and population increase in the invaded locality (Vitousek, 1990; Mack *et al.,* 2000; Shea & Chesson, 2002).

Valery *et al.,* (2008) revising most of these studies made an effort to give a more specific definition: “A biological invasion consists of a species’ acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation, which allows it to spread rapidly and to conquer novel areas within recipient ecosystems in which it becomes a dominant population”.

This incidental introduction of alien phytophagous insects and mites has become quite a common event in the world, owing to intensive commercial exchanges of plants and goods and ever-increasing tourist traffic. There is evidence that this phenomenon is increasing, in spite of the control measures of the EU phytosanitary system in order to minimize unintentional introductions.

The introduction of an alien species in a new ecosystem and the interaction between an alien species and the autochthonous species usually has many disadvantages. The alien species can dominate the invaded ecosystems and eventually become an invasive species due also to the absence or paucity of natural enemies. These invasions can affect the native species that become less common or threatened with extinction (Netwing & Josefsson, 2010). Apart from the environmental impacts, alien species are known for their economic and health impacts.

The DAISIE project (Delivering Alien Invasive Species Inventories for Europe) and its Handbook provide information on alien species in Europe (terrestrial, aquatic and marine, fungi, plants, animals). It includes approximately 11.000 alien species recorded in Europe and can be the basis for future scientific investigations, management and control of alien invasive species (Drake, 2009). According to DAISIE there are two types of alien species 1. Alien to Europe and 2. Alien in Europe. The first term includes species coming from areas out of Europe such as Africa, Asia or Australia and vice versa. The second term indicates species moving inside of Europe e.g. from Mediterranean areas to Central Europe (Nentwig & Jofenson, 2010).

With regard to Italy, its position in the central Mediterranean and its wide climatic range (from North to South) allow the incidental introduction and the establishments of a high number of species. More than 1146 alien Arthropods were incidentally introduced to Italy in the period between 1442 (the discovery of America) and 2013 (Zapparoli, 2014). Among them there are highly invasive species (i.e. *Paysandisia archon, Anoplophora glabripennis, Rhynchophorus ferrugineus*). The most numerous alien introduced insects are Hemiptera, Coleoptera, Lepidoptera. The majority of the introduced species have come from America, Asia, Africa, and Australia. In some cases Italy has been the first focus of an exotic pest in Europe (i.e. *Corythucha ciliata* Say, *Parectopa robiniella* Clemens and *Metcalfa pruinosa* Say) (Pellizzari & Dalla Montà, 1997). In other cases, some of the aliens have reached Italy from previously infested European countries: e.g. the moth *Cameraria ohridella* Asian ambrosia beetle *Xylosandrus crassiusculus* (a pest of forest trees), the oriental chestnut gall wasp *Dryocosmus kuriphilus*, the locust gall midge *Obolodiplosis robiniae*, the mealybug *Pseudococcus comstocki* (a pest of fruit trees), the oak lace bug *Corythucha arcuata* and the longhorn beetle *Anoplophora chinensis* (about 40 of those species unfortunately established in North Italy).

To indicate the year of introduction of an exotic species into a new environment usually it refers to the year in which it was first reported its presence with a publication in a scientific journal. However, it is clear that this year sometimes doesn’t respond to the reality as the species may be present in the area for some time but hasn’t found until then. Following the arrival of an alien species, there are three different possible acclimatized conditions (Pellizzari and Dalla Montà, 1997; Mack *et al.,* 2000; Roques, 2010):

- Species not acclimatized
- Species acclimatized and widely distributed throughout the territory
- Species acclimatized but in a limited way and often isolated territory

(A species is considering acclimatized when sufficient numerical strength of population persist indefinitely into new territory)

According to the empirical law of 10%, out of 100 species introduced only 10% of them survived and of these, another 10% (e.g 1/100) turns out to be invasive (Richardson *et al.,* 2000).

Origin and taxonomy of exotic arthropods in Europe

Observing the collected data in 2009 of the project DAISIE (Delivering Alien Invasive Species Inventories for Europe) it is obvious that the number of the alien terrestrial arthropods introduced to Europe, which have established until now, reaches a total of 1590 species. From these species 1364 are originated from other continents plus about 226 cosmopolitan species of uncertain origin (cryptogenic). Insects are considered the most often accidentally transported, among the organisms fairly since largely dominate this list, accounting for more than 87%, far above of mites (6.4%) but also there are other arthropods, fungi, bacteria, viruses and plant and animal species (Figure1.) (Roques *et al*., 2009).

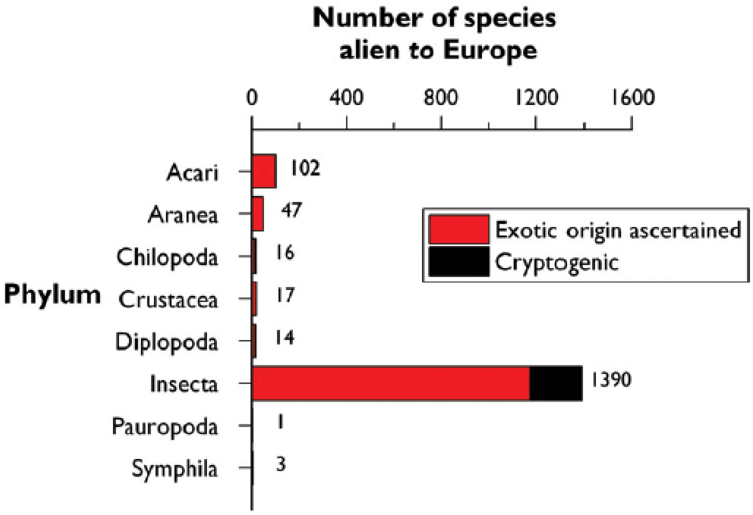
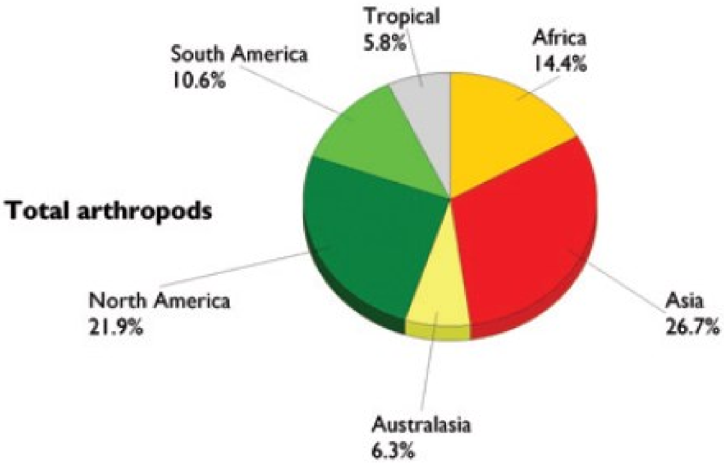


Figure 1 – Relative importance of the different phyla in the 1590 species of arthropods alien to Europe. Species of ascertained exotic origin and cryptogenic species are presented separately. The number to the right of each bar indicates the total number of alien species observed per phylum.

As for the origin of non-native species present in Europe, Asia was the major contributor not only of the alien arthropods with (26.7%) and North America (21.9%) but also for the phytophagous species with 30% for Asia and 26.7% for North America. The great contribution from these continents is mainly due to reasons of historical and climatic similarities that are observed in zones with same latitudes. The other continents contribute to a minor degree: 14.4% of the African originate species, 6.3% originates from Oceania, 10.6% from South America, while the remaining 5.8% of the species have tropical origin. For the cosmopolitan species (14.3% for arthropods and 0.7% for phytophagous species), cannot be given with certainty the geographical origin (Figure 2).

a)



b)

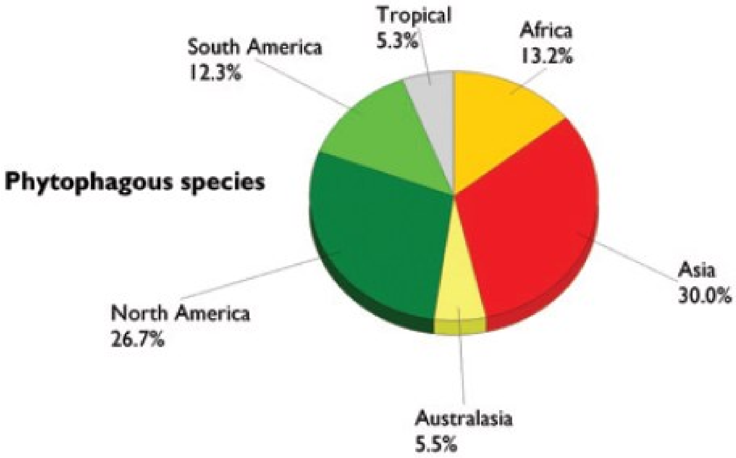


Figure 2 – Region of origin of the 1590 arthropod and phytophagus species alien to Europe. Total arthropods and breakdown per feeding regime are presented. Percentages of the total per category are shown under each region**.**

With regard to the taxonomy of exotic insects present in Europe, three are the orders that cover a 65% of total alien arthropods with Coleoptera (25%), followed by Hemiptera (20%) and Hymenoptera (18.7%). Other species belong to the orders Diptera (6.2%), Lepidoptera (6.1%), Thysanoptera (3.3%), Psocoptera (3.1%) while the other orders are present with smaller percentages (Figure 3).

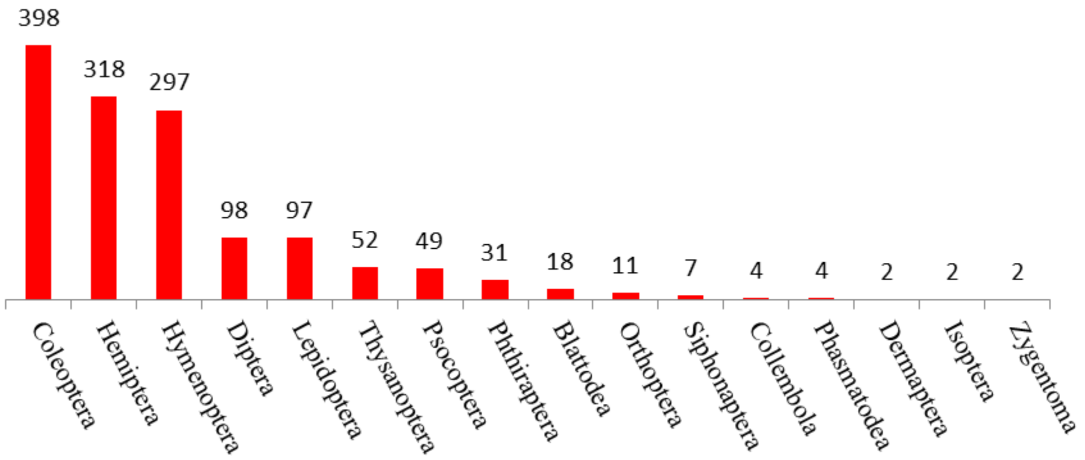


Figure 3 – Relative importance of each order of exotic insects present in Europe.

In particular, Hemiptera, is represented mainly by species belonging to the families Aphididae (102 species), Diaspididae (60) and Pseudococcidae (37). The prevalence of these families is very likely due to the small size of the individuals and their unique characteristic to localize/colonize in hidden parts of the plants. This means that individuals come to be unnoticed on the control of the plant material at the borders.

The introduction of the new exotic species in Europe has significantly changed the composition of the native fauna. Before the introductions, 30 families were not represented in the European fauna while in other families may alien species over-represented (usually for scales).

For example in the case of Diaspididae, almost the half (44.6%) of the total number of species belonging to the family and found in Europe are allochthonous (60 out of 130 species. Even in Coccidae (23 out of 70 species) and Pseudococcidae (37 out of 141 species) families, the percentage of the exotic species reaches 32.3% and 25.7% respectively.

Finally, the distribution of the alien arthropods species in Europe varies between countries. Italy has the highest number of non-native species, with 700 different species recorded, followed by France (690 species), Great Britain (533 species), Spain (486 species) and Germany (362 species).

The number of exotic species seems to depend from longitude since decreases significantly while the latitude seems to have no significant influence on it. Also there is a strong correlation between the number of exotic species (present in a country) and the volume trade of import, the density of the road network and the number of inhabitants of the country itself. This confirms the importance of trade in accidental introduction of exotic species.

Elton, (1958) was the first one that he mentioned that the introduction of alien species and its expansion can cause loss of biodiversity and have economic impact.

As refered previously biological invasions by exotic species can cause not only loss of biodiversity but also they can cause socio economic health impact and ecological losses (Kenis *et al*., 2009).

According to COM (2008) invasive species are one of the major threats to biodiversity and their impact on the ecology includes the competition with other organism food and habitat, predation on native organisms, changing ecosystem structures, hybridisation with native species, direct toxicity, being a reservoir for parasites or a vector for pathogens and disrupting pollination services.

Finally, resulting for the effects above, causing extinction of native species

As far as concern the economic impacts, invasive species can reduce yields from agriculture, forestry and fisheries. They can decrease water availability and to cause land degradation, to damage infrastructure due to burrowing or via their root systems, to obstruct transportation by blocking waterways.
Finally invasive species can cause a number of human health problems, such as allergies and skin problems.

A basic estimate of the costs in order to control and eradicate the invasive species is around 12.500 million per year (Kettunen *et al.,* 2009). This amount although can considered as underestimated since the real economic and environmental impacts are unknown for most of the alien species (Vilà *et al.,* 2009).

In this study 3 recently introduced alien species in Italy were investigated, namely *Tuberocephalus (Trichosiphoniella) tianmushanensis* Zang (Hemiptera Aphididae), *Cydalima perspectalis* (=*Glyphodes*) (Walker, 1859) (Lepidoptera Crambideae), the box caterpillar and *Phenacoccus defectus* Ferris (Rhynchota Pseudococcidae).

Moreover, a zoogeographic analysis of the Greek scale insects fauna (Hemiptera, Coccoidea) was carried out with the aim to highlight how many alien scale insects species are so far present in the Greek territory. This analysis demonstrated that alien scale insects, introduced and acclimatized a long time ago or recent invaders, make up 30% of the Greek scale insects fauna.

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Chapter 2

Tuberocephalus (Trichosiphoniella) tianmushanensis Zang (Hemiptera Aphididae)

Introduction

On May 30, 2012 reddish-pink galls, with aphids inside, were observed on the leaves of two *Prunus subhirtella* cv. *pendula* trees (Rosaceae) (Weeping Higan Cherry), about 40 years old in the University Botanical Garden of Padova.

Once mounted on slides the aphids were identified as *Tuberocephalus (Trichosiphoniella) tianmushanensis* Zang, an Asiatic heteroecious species not yet recorded in Italy (Pellizzari & Frigimelica, 2014). According to literature (Remaudière & Sorin, 1993) the primary host plant of *Tuberocephalus* species are different *Prunus* trees whereas the secondary host plants are *Artemisia sp*.

Botanic gardens offer a unique opportunity in detecting alien insects and mites, due to the presence of exotic plants introduced from different areas of the world. The regular surveys carried out in the Botanical Garden have led to the discovery of several alien insects and mites, previously unrecorded in Europe (Pellizzari *et al.,* 2010).

Basic information on galling aphids

Aphids are phloem–feeders. As a result of the subtraction of the sap and the transmission of saliva in plant tissues, the plant renders weak and cause deformation on the affected organs. The ingestion of the phloem sap permits the emission of honeydew, which fell on the leaves, and promotes the development of "sooty mold" (Fig. 1) or saprophytes fungi with result to reduce photosynthesis and the aesthetic value of ornamental plants. Moreover aphids can create malformations of the leaves and spots on fruit and plant tissues. Aphids can be also vectors for virus diseases from infected plants to healthy (Eastop, 1997).

Figure 4 – Example of "sooty mold" on the leaves.

A particular aspect is the formation of galls by aphids and for this reason aphids are defined as galling-aphids.

Their distribution is related to the presence of their host plant in the territory. Monophagus aphids of exotic origin (tropical and sub-tropical) are usually present in artificial habitats, such as nurseries, parks, gardens or greenhouses while other species which are harmful to agricultural crops, ornamentals plants and forestry can cause serious economic damage. It is estimated that from the 102 alien in Europe aphids species, 52 attack to agricultural and horticultural crops (Blackman & Eastop, 2000; Coeur d'Acier *et al.,* 2010).

Dioecious galling Aphids

Galls, with their characteristic shapes and colors, were a fascinating subject of study by entomologists, ecologists and naturalists (Inbar *et al.,* 2004; Pellizzari, 1988). Among the organisms that can induce these particular strains of formation on morphological plant organs, galling aphids are most remarkable, not only for their complex and unusual biological cycle which is closely related to the alternation of generations of different host plants but also for the formation of the gall (Forrest, 1971).

Galling species constitute a minority among aphids. Just a 10% of 4401 aphid species (Blackman & Eastop, 1994) are true gall formers, excluding “pseudogalls” and leaf rolls. They are phloem feeders and have complex life cycles, with alternating sexual and parthenogenetic generations as well as host alternation (Moran, 1988: 1992). Moreover galling aphids have unique properties something which make them interesting for ecological study and behavioral phenomena in nature, with evolutionary implications (Wool, 2004).

In fact for galling aphids, unlike with other gall organisms, the gall is not only a shelter but also a sort of "reproductive incubator" in which the aphids can reproduce parthenogenetically and have a microenvironment favorable for their development. The availability of food within the galls (Forrest, 1971) and pseudogalls (Kennedy, 1958) indirectly explains the high density of specimens that usually is found within them (Barbagallo, 1985).

Galling organisms, whether they are insects, mites or fungi, are strictly specific, meaning each species induces a characteristic, species-specific gall (Wool, 2004; Pellizzari, 1988). The gall has a characteristic shape and color, which make easy to identify the inducer species even when it has abandoned the gall, at the end of its development.

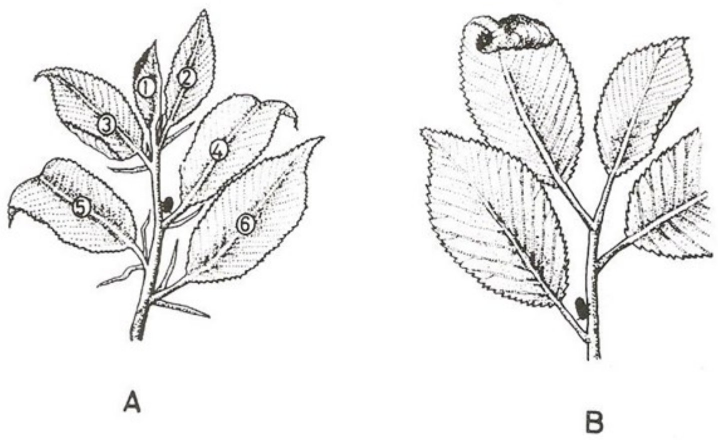


Figure 5 – Bud in development affected by the presence of a fundatrix that prick the tissue between the leaves 3 and 4 (A). Formation of a laef gall 2 (B) (From Forrest, 1987).

Morphology of the galls

Aphids can induce both pseudogalls and true galls on their host plants. Pseudogalls are usually found on leaves and appear as leaf folds, rolls, curls, or blisters while true galls are more diverse in shape, complex in structure and there are not located only on the leaves (Chen & Qiao, 2012).

The different shapes, size, colors and structure of galls are characteristics which can allow the identification of the species which induced them and can divided to three different types. The majority of galls induced by aphids they are formed by a simple rolling up or folding down of the leaf lamina. Among the numerous types of galls have been described, the mention below the most primitive (Mani, 1964; Akimoto, 1983; Pellizzari, 1988).

Gall like bag, invagination of the lamina leaf that creates the characteristic shape of a bag, the folding effect of the upper and lower leaf lamina along to the main vein. There is present a small hole called "ostiole" that usually is blocked. Within this gall lives and reproduces the cecidogenous. This type of leaf galls induced by *Forda formicaria* Von Heiden on *Pistacia palaestina.*

Another typology is the rolling or folding of the leaf margin. The tissues become swollen, enlarged and change color. The outer surface of the gall may appear wrinkled or curled, for example the *Dysaphis plantaginea* (Passerini) on apple trees, or it can be neat and smooth as in the case of *Myzus privet* (Mosley) on *Ligustrum sp* . and *Dysaphis devecta* (Walker) on apple. Except from aphids, these galls can also be formed eriophyids mites.

The wart-like galls. The cecidogenous initially is located in the outer surface of the leaf and induces the gall inserting its stylets at intervals around itself between the major veins. The plant tissues expand to swell out and a “pocket” is formed around aphid. The aphid continues to increase the “pocket” with its stylets until to enclose the aphid completely and to remain within the cavity that is formed. This deformation can be created by *P. filaginis* (Dixon, 1973).

The dioecious aphids are most numerous among species that create gall. The gall is always formed on the primary host plant and its induction is caused by the injection of saliva by the female fundatrix (Mani, 1964).

Origin and geographic distribution of galling aphids

Galling aphids are species known in every continent except Antarctica (Wool, 1984). According to Mani (1964) there are over 700 identified species of galling aphids and the half of these have Holarctic origin while only a small part of them have tropical origin. Different individuals of the same species have been described, over the years, on diverse host plants in various parts of the world. This has, as a result, that many of the most common species have numerous synonyms in the literature. The taxonomic classification of aphids, among the other reasons, is not simple because of their life cycle complexity. Additionally, there are still species not well known and that makes the estimated value of over 700 different species of gall aphids reported by Mani (1964) excessively overestimated (Wool, 1984).

As for the distribution of galling aphids, it turns out to be, incidentally or not, strongly influenced by human activities. The trade of ornamental plants and not only, between countries and continents have contributed to the transfer of aphids in areas where they had never been previously reported (Wool, 1984). The genus *Tetraneura*, probably originated from Asia, is present in all continents but its distribution seems to be almost not as a result of natural dispersion (Hille Ris Lambers, 1970).

It must be emphasized that the distribution of a species is linked to the distribution of the primary host. However aphids that live on the secondary host plant may have a larger distribution (Hille Ris Lambers, 1957) even when the primary host plant is absent, can survive without forming galls (anolociclo).

Dicotyledonous plants which are among the primary host plants, are those mainly affected by the formation of galls. Among the families with economic importance, the *Rosaceae, Rutaceae, Saxifragaceae* and *Solanaceae* are the majorly attacked (Forrest, 1987).

Life cycle of galling aphids

Galling aphids, as it was said before, have a complex life cycle that involves a sequence of generations that alternate between two host plants, mostly belonging to different botanically groups and are defined as primary and secondary host plants. This striking life history phenomena occurring in aphids is obligate seasonal switching between unrelated hosts known as host-plant alternation or heteroecy (Moran, 1988).

The life cycle is dioecious and is characterized by a thelytoky parthenogenetic generation (only female individuals) during the spring and summer period, followed by an amphigonic generation during autumn with a winter diapause in the egg stage.

Aphids usually overwinter in the egg stage on the primary host plant (usually a tree or shrub) near the buds. Winter egg is usually glossy black. After the egg hatching in spring, the wingless parthenogenetic female called fundatrix appears and induces its gall. Inside the gall parthenogenetic generations of females called fundatrigeniae develop. The winged fundatrigeniae (alatae) (migrants) move to the secondary host plant (usually herbaceous species) where they give birth to females called virginogeniae. In autumn among the virginogeniae appear the winged sexuparae gynoparae which return to the primary host plant to originate the oviparous females

Meanwhile, in the secondary host plant appear the apterous sexuparae androparae which give the winged males (re-immigrants), which return on the primary host plant in order to mate with the oviparous females. After mating oviparous females lay eggs on the bark to overwinter. Sexuparae females and males have no developed mouthparts, therefore do not feed; their purpose is reproduction (Wool, 2004).

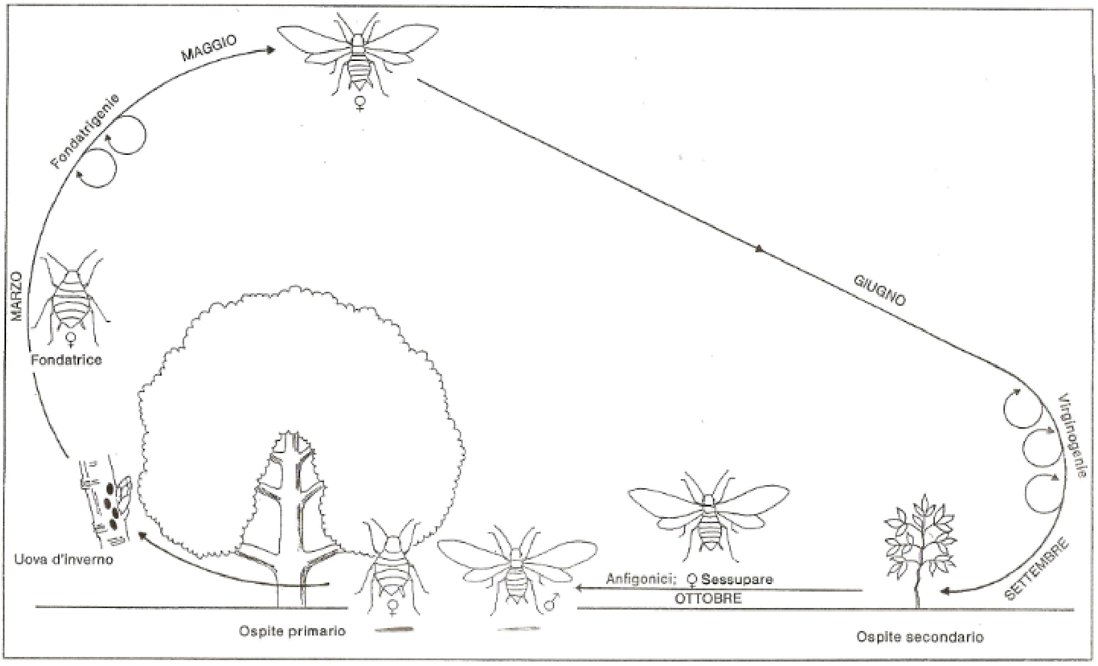


Figure 6 – Schematic presentation of holocycle dioecious

A biological cycle that takes place with completion of eterogonic generations and alternating parthenogenesis and amphigony (monoecious or dioecious) is defined as holocycle. The necessity of an aphid being dioecious arises from the biochemical composition of the primary host plant turns out to be no more suitable for the physiological needs of aphids. The transition from the primary to secondary host plant permits to aphids to have the food needed in order to continue the life cycle. During the summer, the primary host plant is characterized by vegetation stagnation of the while the secondary host (herbaceous) is under development.

Genus *Tuberocephalus* Shinji

The genus *Tuberocephalus* Shinji (1929) belongs to the subfamily Aphidinae and includes 13 different species distributed in Japan, Korea, China, Taiwan, Malaysia, Pakistan, India and East of Russia (Blackman & Eastop, 2000; Sorin & Remaudière, 1998).

The species belonging to the genus *Tuberocephalus* have a dioecious holocycle. They have as primary host woody plants of the genus *Prunus sp*. and as secondary host herbaceous plants of the genus *Artemisia sp*. or *Chrysanthemum sp.*, both belonging to the family *Asteraceae.*

On the primary host fundatrix create the galls that are different for each one of the species belonging to this genus (Fig. 4).

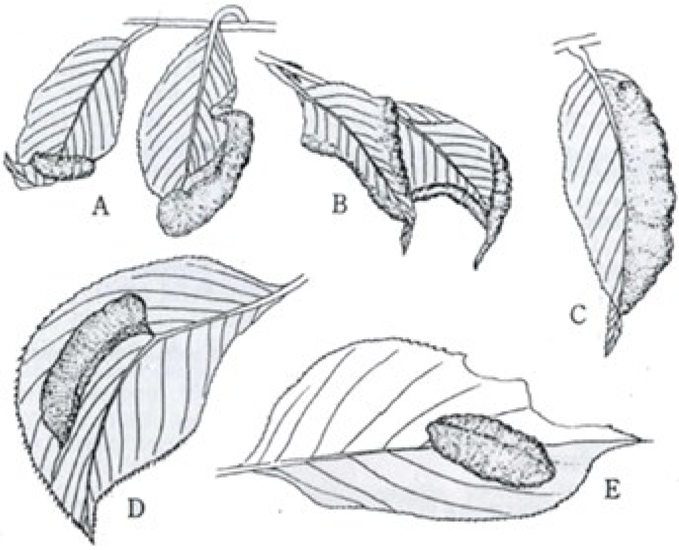


Figure 7 – Galls of *Tuberocephalus higansakurae hainnevilleae* Remaudière & Sorin (A and B), gall of *Tuberocephalus sp*. (C), gall *Tuberocephalus artemisiae* Shinji (D) and gall of *Tuberocephalus sasakii* (Matsumura) (E) (Moritsu & Hamasaki, 1983).

Since the description of the species of genus *Tuberocephalus* from Miyazaki (1971), many other species have been described from Japan, China and Pakistan (Chang & Zhong, 1976; Zhang & Zhong, 1980; Moritsu & Hamasaki, 1983; Remaudière & Sorin, 1993). For some of these species the biological cycle was further described and explained the following years (Sorin, 1993; 1994; Sorin & Imura, 1996; Sorin & Remaudière, 1998). A review of the genus *Tuberocephalus* by Sorin & Remaudière (1998) has provided new information on the biology of some species as well described new forms and new taxa. When Miyazaki in 1971 described as *Tuberocephalus* sp. the first species found on the roots of Artemisia, he realized that it didn't belong to the genus *Tuberocephalus* sensu stricto. Later in 1998, Sorin & Remaudière, thanks to new information on the life cycle of these aphids, separated *Tuberocephalus sakurae, Tuberocephalus laoningensis¸ Tuberocephalus higansakurae hainnevilleae* and *Tuberocephalus uwamizusakurae* in the sub-genus, *Trichosiphoniella* Shinji, 1929.

The species belonging to the genus *Tuberocephalus* Shinji sensu stricto colonize the underside of the leaves of the secondary host *Artemisia*, while species belonging to the subgenus *Trichosiphoniella* colonize on the roots of *Artemisia*. Below are the subdivisions of the two subgenera proposed by, Sorin & Remaudière (1998) and their characteristics:

Subgenus *Tuberocephalus* Shinji strict sense

Tuberocephalus (Tuberocephalus) artemisiae Shinji, 1929

Tuberocephalus (Tuberocephalus) naumanni Sorin & Remaudière, 1998

Tuberocephalus (Tuberocephalus) pakistanicus Remaudière & Sorin, 1993

Tuberocephalus (Tuberocephalus) sasakii (Matsumura, 1917)

Subgenus *Trichosiphoniella* Shinji

Tuberocephalus (T.) higansakurae hainnevilleae Remaudière & Sorin, 1993

Tuberocephalus (T.) higansakurae higansakurae (Monzen, 1927)

Tuberocephalus (T.) jinxiensis Zhang G. & Zhong, 1976

Tuberocephalus (T.) laoningensis Zhang G. & Zhong, 1976

Tuberocephalus (T.) misakurae Moritsu & Hamasaki, 1983

Tuberocephalus (T.) momonis (Matsumura, 1917)

Tuberocephalus (*T.*) *sakurae* (Matsumura, 1917)

Tuberocephalus (*T.*) *tianmushanensis* G. Zhang, 1980

Tuberocephalus (*T.*) *uwamizusakurae* Sorin & Remaudière, 1998

For some species of *Tuberocephalus* the secondary host plants are still unknown as well there is no information about their biology and life cycle. Based on the bibliography, an overview of the species described until now is presented. For each species is given the primary and secondary host plant, its distribution and some notes for their biology.

1. Subgenus *Tuberocephalus* Shinji strict sense.

***Tuberocephalus artemisiae* Shinji** (1929)

Primary host: *Prunus donarium var. spontaneous sub-var. speciosa, P. serrulata, P. yedoensis.*

Secondary host: *Artemisia sp., A. montana, A. princeps, A. stolonifera, A. vulgaris var. indica.*

Distribution: Japan, East of Russia.

Biological notes: galls are formed on the upper surface of the leaves of the primary host, along the grain side. The galls have a typical bag shape , often erected with irregular top and the bottom is sealed; their length, height and width are 27-33mm, 9,5-12,5mm, 6-7,5mm respectively. Initially, their color is yellow-green, but with exposure to the sun turns into reddish. The fundatrix appears in May and gives birth to the fundatrigeniae that will become winged viviparous (migrants); at the beginning of June, the migrants, shift to the secondary host. On the underside of the leaves of the secondary host the apterous viviparous females feed. The gynoparae appear between October and November and returning on the primary host to give birth to sexual females, while winged males return to the primary host in November where take place the deposition of the eggs. Eggs have shiny black color and oval shape.

***Tuberocephalus (T.) naumanni* Sorin & Remaudière** (1998)

Primary host: *Prunus cornuta*

Secondary host: unknown

Distribution: Pakistan

Biological notes: mature galls appear glossy, large, with a color which can vary from light green to white. Their length is around 2-2,5cm and the walls can be 3-4mm thick. They can contain up to three generations of aphids, but the third generation has just winged individuals and migrants and making their appearance only in May. All aphids have a color ranging from light green to bright green.

***Tuberocephalus (T.) pakistanicus* Remaudière & Sorin (1993)**

Primary host: *Prunus cornuta*

Secondary host: unknown

Distribution: Pakistan

Biological notes: mature galls are papery with color ranging from dark green to red, covered with red dots with a diameter of 1 mm, which often can attach to each other to form a single spot on the top of the surface. The galls have a length of 15-30 mm and a thickness of 2.5-3 mm. Each gall contains only a fundatrix and several nymphs fundatrigeniae winged. When the last stages arrive at maturity, the gall opens. In June, the infested leaves appear almost normal, even if they have some reddish spots, irregular border and slightly widened. The aphid population has red-brown and the migrants appear in May.

***Tuberocephalus (T.) sasakii* Matsumura (1917)**

Primary host: *Prunus sp., P. donarium, P. donarium var. spontaneous, P. itosakura var. ascendens, P. jamasakura, P. leveilleana, P. levelleana var. spontaneous, P. sachalinensis, P. serrulata var. glabra (= P. leveilleana var. spontaneous), P. serrulata var. spontaneous, P. sieboldii, P. tomentosa, P. yedoensis*.

Secondary host: *Artemisia sp.*, *A. capillaris*, *A. feddei*, *A. princeps*, *A. stolonifera*, *A. vulgaris*, *A. vulgaris* var. *indica*

Distribution: Pakistan, Japan, Taiwan, Malaysia, Russia.

Biological notes: the fundatrix creates a gall-shaped bag on the upper surface of the leaves of the primary host, along the grain side. The galls are very similar to those formed by *T. artemisia* Shinji. Apterous viviparous females have a whitish flattened body and live on the lower surface of the leaves of secondary host.

2. Subgenus Trichosiphoniella Shinji

***Tuberocephalus (T.) higansakurae hainnevilleae* Remaudière & Sorin, (1993)**

Primary host: *Prunus sp.*, *P. donarium* var. *spontanea, P. itosakura*, *P. pendula*, *P. pseudocerasus*, *P. subhirtella*, *P. subhirtella* var. *pendula*

Secondary host: *Artemisia sp., A. vulgaris var. indica.*

Distribution: Japan, China

Biological notes: According to Sorin & Remaudière (1998) this species forms two types of galls, a larger bag-shaped and a narrow elongated tubular shape, curled and wrinkled. The bag-shaped galls are induced by the fundatrix on the edge of the tender leaves. The fundatrix appears in May and gives birth to numerous individuals. The wingless fundatrigeniae create tubular galls, where inside them, give birth of nymphs - fundatrigeniae (winged migrants) which they appear in late May and early June, migrating to the secondary host to give birth to the nymphs. The juvenile virginogeniae lie in the ground of the secondary host, near the root system that use as a food source. The gynoparae emerge from late October to mid-November, returning on the host primary where females give birth to oviparous females; after mating eggs are laid on the buds to overwinter.

Tuberocephalus (T.) jinxiensis **G. & Zhang Zhong (1976)**

Primary host: *Prunus humilis, P. pseudocerasus*

Secondary host: unknown.

Distribution: China

Biological notes: the biology of this species has not yet been clarified. The comparison of this species with the other belonging to the same sub-genus appears to be difficult, given that the description only occurred on female fundatrigeniae.

Tuberocephalus (T.) laoningensis **G. & Zhang Zhong (1976)**

Primary host: *Prunus pseudocerasus*, *P. tomentosa, P. yedoensis*

Secondary host: *Artemisia vulgaris var. indica.*

Distribution: China, Japan

Biological notes: the galls are formed on the leaves of the primary host (*Prunus tomentosa*), are heavy and very curly. The galls reaching maturity resemble with a mosaic, as they are yellow-green with red-purple spots. The immigrants appear in mid-May and move to the secondary host. The virginogeniae lie in the ground of the secondary host, near the root system that use as a food source.

Tuberocephalus (T.) misakurae **Moritsu & Hamasaki (1983)**

Primary host: *Prunus pseudocerasus, P. pauciflora, P. takenakae, P. yedoensis*

Secondary host: *Chrysanthemum sp., C. morifolium var.*

Distribution: China, Japan

Biological notes: the fundatrix appears in early March and induce a bag shape gall on the margins leaf. The winged fundatrigeniae appear in May and immigrate to the secondary host. The virginogeniae are wingless throughout the summer and feed on the roots of the secondary host, causing deformation. The gynoparae appear between late October and early December, returning later to the primary host to give birth to oviparous females. The winged males return on the primary host from mid-November to early December and oviparous females lay egg near the buds.

Tuberocephalus (T.) momonis **Matsumura, (1917)**

Primary host: *Prunus sp., P. cerasus, P. davidiana, P. glandulosa, P. ishidoyana, P. itosakura, P. jamasakura, P. leveilleana, P. leveilleana var. spontaneous, P. mume, P. persica, P. pseudocerasus, P. serrulata, P. subhirtella, P. tomentosa, P. yedoensis*

Secondary host: *Artemisia princeps, Elsholtzia ciliata (?)*

Distribution: Japan, Taiwan, China, Korea

Biological notes: Only the fundatrigeniae apterous and the fundatrigeniae alatae have been described. The biology of the species is not well known yet.

Tuberocephalus (T.) sakurae **Matsumura (1917*)***

Primary host: *Malus formosana, Prunus sp., P. ansu, P. cerasus, P. donarium, P. ishidoyana, P. jamasakura, P. leveilleana, P. leveilleana var. spontaneous (= P. yamasakura), P. mume, P. pauciflora, P. persica, P. pseudocerasus, P. sachalinensis, P. sargentii, P. serrulata, P. serrulata var. spontaneous, P. subhirtella, P. tomentosa, P. yedoensis*

Secondary host: *Artemisia sp., A. montana, A. princeps, A. vulgaris var. indica, Elsholtzia ciliata (?)*

Distribution: Japan, China, Korea, Russia East

Biological notes: the fundatrix appears from early April to mid-May, inducing galls along to the main vein of the leaves, as result to form a wrinkled tubular holeon the underside of leaves.. Inside the galls the fundatrix gives birth to nymphs. The fundatrigeniae may be wingless or winged; the first ones create new galls, while the latter, appearing between early May and mid-June, immigrate to the secondary host and give birth to their offspring on the undersides of leaves. The first stage of virginogeniae moves to the ground on the roots. All the stages of virginogeniae are wingless. The winged males and the gynoparae appear in mid-October which they return to the primary host and give birth to oviparous females.

Tuberocephalus (T.) tianmushanensis **G. Zhang (1980)**

Primary host: *Prunus sp.*

Secondary host: unknown

Distribution: China, Japan

Biological notes: the biology of this species has not yet been clarified.

Tuberocephalus (T.) uwamizusakurae **Sorin & Remaudière (1998)**

Primary host: *Prunus grayana*

Secondary host: *Artemisia vulgaris var. indica*

Distribution: Japan

Biological notes: The fundatrix has green dark color and appears in early April. The fundatrix forms galls green-yellow or greenish-brown, along the leaf margin of the primary host. Their shape is elongate and similar to a pouch which is closed on the lower side, often the galls can be as large as half of the leaf surface; at maturity, the galls are 50-60 mm long and 10 mm wide and closed on the bottom. The second generation appears from late April to early May, immigrating on the secondary host plant. The immigrant females give birth to the next generation on the undersides of the leaves and stems of the secondary host. Virginigeniae move on the roots causing malformations. In late November oviparous females lay egg on buds of the primary host.

***Tuberocephalus (Trichosiphoniella) tianmushanensis* Zang, 1980**

Order: Hemiptera

Family: Aphididae

Syn.: Tuberocephalus (T.) higansakurae hainnevilleae Remaudière and Sorin, 1993)

Tuberocephalus (T.) tianmushanensis Zhang was detected for the first time in Italy May 30, 2012, in the Botanical Garden of the University of Padua (Pellizzari & Frigimelica, 2014). Reddish-pink galls were observed on the leaves of the *Prunus subhirtella cv. pendula* trees (Rosaceae) (Weeping Higan Cherry). The galls were formed on the edge of the leaves, folded inward the underside, with a typical bag shape, curled and wrinkled and their distribution on the tree canopy was almost homogenous.

The morphs present inside the galls, at the collecting date, were few nymphs, nymphs with wing pads, alatae fundatrigeniae (emigrants).

The species was identified as *Tuberocephalus (T.) tianmushanensis* Zhang, (Hemiptera Aphididae), an Asiatic heteroecious species known so far in Japan and China (Su *et al.,* 2010) and not yet recorded in Italy (Pellizzari & Frigimelica, 2014).

A *Tuberocephalus* species had been reported for the first time in Europe by Remaudière & Sorin in France in 1993. These authors recognized this species as new and described it as *Tuberocephalus (T.) higansakurae hainnevilleae* Remaudière and Sorin, 1993).

In the fall of 1990, about one thousand trees of *Prunus subhirtella cv. pendula* were imported from Japan and planted in a nursery at Putanges-Pont-Ecrepin, a small town in the Orne département of north-western Normandy, France. In the spring of the following year, specimens of aphids were observed on cherry trees which were heavily infested with reddish leaf galls on the lead margin. The infested leaves were carefully removed and chemical treatment on *Prunu*s performed (Remaudière & Sorin, 1993). During the chemical treatment, the authors observed that the winged migrants had left the galls to reach the secondary host, probably a species of *Artemisia*. Subsequently, Remaudière & Sorin attempted to breed the fundatrigeniae alatae on *Artemisia vulgaris* in controlled conditions but no new generation observed.

In the spring of 1992 no galls were observed in any *Prunus* plant and the species was considered eradicated in France. Since then the aphid was never reported again in Europe (Coeur d'Acier *et al.,* 2010).

A revision of the genus *Tuberocephalus* by Remaudière & Sorin in 1993 has suggested the possibility that *Tuberocephalus (T.) higansakurae hainnevilleae* Remaudière & Sorin could be considered as synonymous with *Tuberochepalus (T.) tianmushanensis* Zhang given the remarkable similarity between the two species.

Later Su *et al.* (2010), after comparing *Tuberocephalus (T.) tianmushanensis* with the original description and drawings of *Tuberocephalus (T.) higansakurae hainnevilleae* Remaudière and Sorin confirmed the synonymy between the two species. This synonymy is currently accepted in Aphid Species File Database (Favret, 2013).

The first record of *Tuberocephalus (T.) tianmushanensis* after France was in Italy in 2012 (Pellizzari & Frigimelica, 2014).

According to Sorin & Remaudiere (1998), this species forms two types of galls on the leaves of *Prunus subhirtella*. The first type gall is induced by the fundatrix while the second type is induced by apterous fundatrigeniae. The characteristic to separate fundatrix by apterous fundatrigeniae is the body size (bigger for the fundatrix and more restrain for the fundatrigeniae).

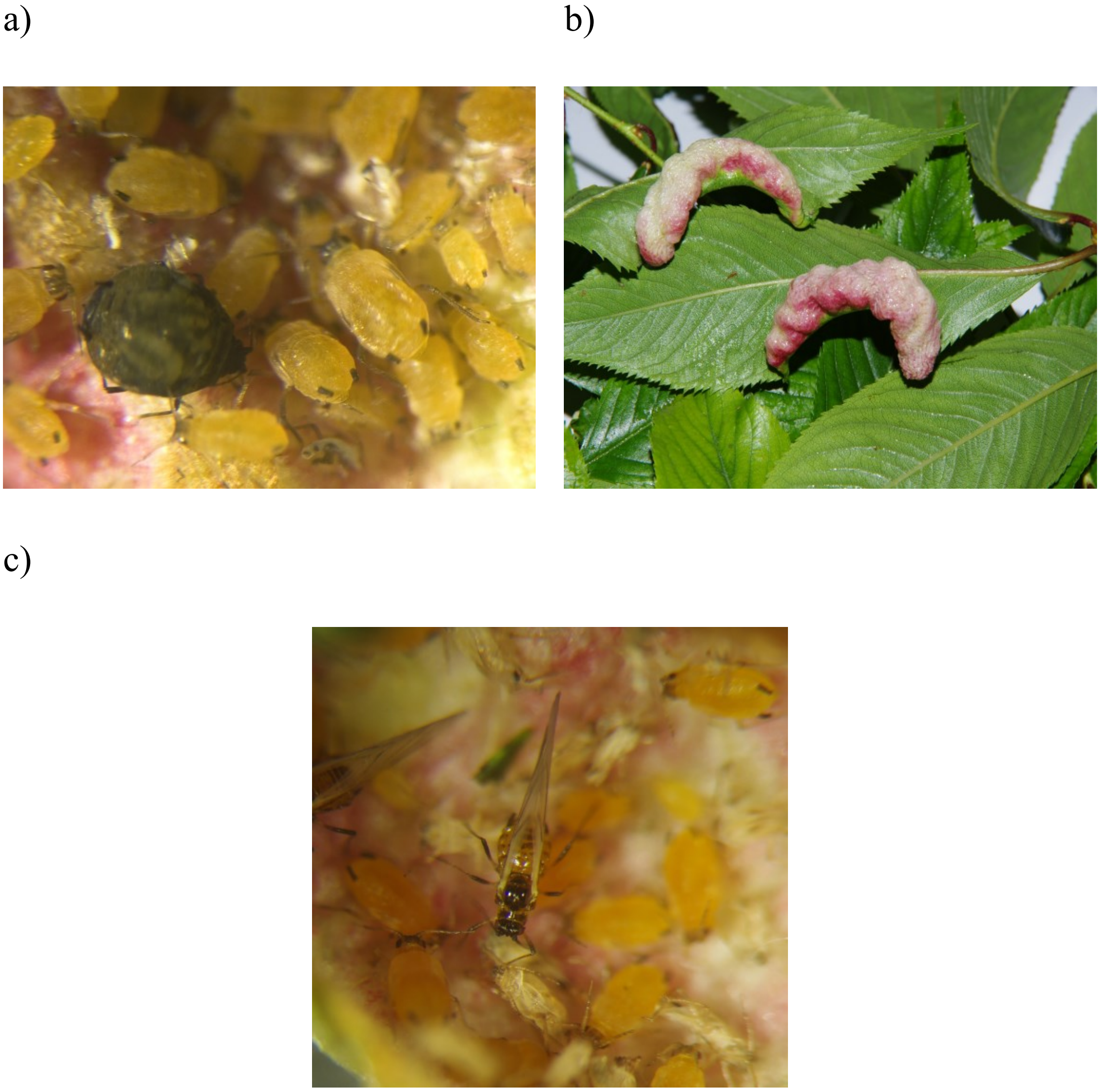


Figure 8 – *Tuberocephalus (T.) tianmushanensis*: fundatrix on the left top, gall with fundatrigeniae alatae and apterous on the right top, the characteristics galls type A and B on the right below, fundatrigeniae????

Objectives of the study

The purposes of this study were:

1) To collect data on species distribution over the territory, by monitoring ornamental cherry trees in the Veneto region.

2) To observe the phenology and biology of the Asiatic aphid *Tuberocephalus (Trichosiphoniella) tianmushanensis* Zhang.

3) To study the life-cycle in screen houses and outdoors.

4) To verify if its secondary host plant was an *Artemisia* sp., as reported in bibliography.

Another aspect of the work was to provide an overview of the species belonging to the genus *Tuberocephalus* so far described, by consulting the available literature. It was made an effort to gather all the currently available information for each species, its distribution and information on their biology mainly regarding the first and secondary host plants.

Material and Methods

The research of infested primary host plants

The primary host plant of *Tuberocephalus tianshanensis* is the Japanese flowering cherry *Prunus subhirtella cv. pendula*, tree with white or pink flowers. *Prunus subhirtella* is an ornamental plant present in private gardens, public parks and nurseries

Preliminary research were carried out in order to identify the presence of any other infested primary host plants besides the *Prunus subhirtella cv. pendula* of the Botanical Garden of the University of Padua. The observation started in April-May 2013 and involved also other varieties of *Prunus* besides the *Prunus subhirtella cv. pendula.* The research took place in the city of Padua (Public gardens, avenues) and in the neighbouring villages of Legnaro, Saonara and Peraga di Vigonza, where nurseries of ornamental plants are widespread and plants of *Prunus pendula* are cultivated for ornamental purposes. These surveys consisted in visual observation of the Japanese cherry trees, trying to detect the presence of leaf galls.

After these preliminary observations, the monitoring of the life cycle of the aphid was made on the two cherry trees present in the Botanical Garden and in the nursery "Bordin" of Saonara (PD). The two *Prunus* of botanical Garden took the code *Prunus* A (in front of the screenhouse) and *Prunus* B (at the entrance)

In 2014 monitoring continued in the Botanical Garden and in the nursery “Bordin” in Saonara. The observations started at the beginning of March and finished in June of 2014.

Research of winter eggs

For identifying the presence of the overwintering egg on the primary host, young branches were cut from the two *Prunus subhirtella cv. pendula* A and B of Botanical Garden of Padua during autumn and winter. The first sampling was carried out at the end of October in 2012 and a total of 3100 cm of branches were collected. The second sampling was carried out in mid-February, 2013. This time, were collected 970 cm of branches from Botanical Granden and 1566 cm from *Prunus* in nursery. The branches were examined under the stereoscope to search for winter eggs.

The branches with winter eggs were kept in the dark and cold to allow the continuity of overwintering phase. In the meantime 2 plants of *Prunus subhirtella* were placed close to the screen house of Agripolis (experimental area of Entomological Department) and in March, the collected branches with winter eggs were placed on these cherry trees. The monitoring of the plants was daily with the purpose to notice any changes on the vegetation.

A third sample was carried out in the mid of March in 2013, the time that *Prunus* started flowering. In this sampling about 3900 cm of young branches collected in the Botanical Garden were examined under stereoscope.

Figure 9 – Branches with overwinter egg placed on *Prunus* in the screen-house in 2013.

Monitoring of galls

Sampling was started in May 2013 when the presence of leaf galls was observed on *Prunus subhirtella cv. pendula,* both in the Botanical Gardens and in the nursery "Bordin". It was decided to check respectively a) the size of the whole population that the aphid develops within a gall, b) the phenology of the different stages present inside the gall, the time of appearance of winged migrants and the time of immigration to the secondary host.

a) Population size inside a gall

In order to define the population size of the aphids inside a gall, at the beginning of May 2013 some leaf galls were inserted in tulle bags on *Prunus* of Botanical. Every tulle bag contained a single gall. This way when the aphids reached the alatae stage could not have the possibility to escape from the bag. The number of tulle bags was 6 on *Prunus* A and 8 on *Prunus* B. The number of galls inserted in the bags was low in order of not reducing excessively the population and allowing the prosecution of the cycle on the secondary host. Galls were chosen randomly in the tree canopy at different high. At mid-June when all the individuals inside the tulle bag were dead, the bags were collected and transferred in the laboratory and all the individuals of each gall counted. All the aphids obtained were preserved in tubes with 70% ethanol, assigning a code for each gall.

In 2014 the same procedure was followed. Leaf galls were inserted in tulle bags in the nursery. This time a total of 10 tulle bags with a single gall inside was placed on the *Prunus* in the nursery while other 10 tulle bags had inside a gall and healthy leaves.

In the first week of June, when all the individuals inside the tulle bag were dead, the bags were collected and transferred in the laboratory.

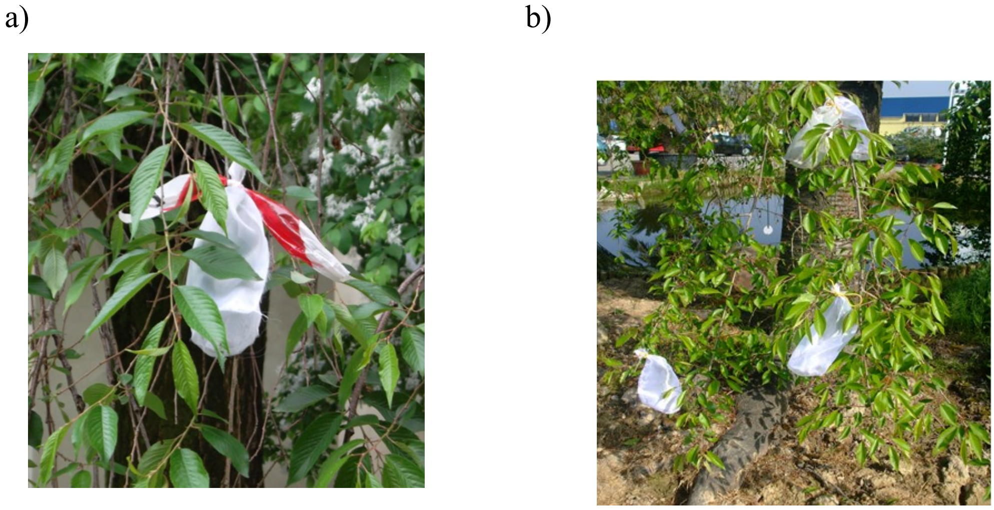


Figure 10 – Tulle bags with galls placed on *Prunus subhirtella cv. Pendula in* a) 2013 and b) 2014.

b) Phenology of the different stages inside the gall

Leaf galls of *Prunus subhirtella cv. pendula* from the nursery were collected to follow the development of the individuals during the spring season. Weekly sampling was made and leaf galls were transferred in the laboratory and examined under stereoscope the same day of collecting.

c) Trial of colonization of Artemisia plants

As *Artemisia sp.* plants are indicated as the secondary host plant, of *Tuberocephalus* spp. a trial of colonization by transfer alatae migrants on *Artemisia* plants was made with the aim of verifying if *Artemisia* was the secondary host.

During May 2013 8 pots with *Artemisia* plants were prepared and placed in cages outdoors. On these plant were transferred the fundatrigeniae alatae collected during the sampling.

At the beginning of May 2014 in order to obtain a high numbers of alatae a number of galls of the *Prunus* A and B were enveloped into tulle bags. In *Prunus* A were placed 20 tulle bags of which 10 with only a galled leaf inside and 10 with a galled leaf plus healthy leaves inside; on *Prunus* B 12 tulle bags (6 with one gall inside and 6 with a gall plus healthy leaves) were placed at the end of May. The same operation was made on the *Prunus* tree of the nursery (8 tulle bags with one leaf gall inside)

Also in the following year alatae migrant were collected and transferred weekly in the *Artemisia* plants with the same methodology.

Because of the different growth time between the *Prunus* A and B, the leaf galls developed at different times. Every week the alatae were transferred on potted *'Artemisia vulgaris* in Agripolis. The tulle bags were replaced with others, to be able to monitor always the same galls. At the end of June all 32 tulle bags were collected to count the remained population within the galls and observe the possibility of development of secondary galls.

Figure 11 – Tulle bags on *Prunus* A of Botanical garden 2014.

Figure 12 – Gall type A on the left and gall type B on the right, 2013.

a) b)

Figure 13 – a) Pots with *Artemisia vulgaris* on the left 2013-2014. b) Individuals alatae inside the tulle bag 2014.

Samplings in the secondary host plant

During the summer sampling started in order to verify the presence of *Tuberocephalus (T.) tianmushanensis* on the secondary host. From the second half of July until the beginnig of October 2013, samples of soil with Artemisia roots were collected every ten days and observed under magnification.

The same sampling was carried on in August 2014

The aphids which were found were preserved and stored in tubes with 70% ethanol and later mounted on slides.

Observations on the presence of predators of aphids

During the monitoring, inside the galls the presence of predators was noted: they were identified as some of the most common predators of aphids.

Results

Research of primary host plants (distribution over the territory)

During the preliminary research to find other infested cherry trees over the territory no signs of leaf galls on the canopy in any of *Prunus subhirtella cv. pendula* trees were observed nor in Padova nor in Legnaro e Peraga di Vigonza .

A few infested plants were recorded in the Nursery Bordin in of Saonara. They consisted in one 20-years tree in the middle of the nursery garden, with its canopy well- infested with galls, and in 3 young, potted *Prunus* ready for selling.

Moreover, an infested old *Prunus* was observed in Rossano Veneto (VI) but the tree was suffering and on its canopy were present only few old, empty galls.

Phenology and biology

In 2014 the growing season of the two *Prunus* (A, B) of Botanical garden was delayed due to bad weather conditions and low temperature. Leaf galls were observed only in May 2014. On *Prunus* A the presence of galls was noted on 5 May 2014 while *Prunus* B, galls started to develop on 20 May 2014, mainly in the apical sunny part. On these two different types of galls were observed; *Prunus* A in the beginning of May showed both types of gall, the gall type A in major number, with a bright red color, compared to the gall type B smaller in size and green-red color. The *Prunus* B, at the end of May showed mainly galls type B and fewer galls type A (Fig.).

The development of fundatrigeniae alatae ended on 17 June 2014 when the galls on the canopy of *Prunus* were abandoned by the aphids.

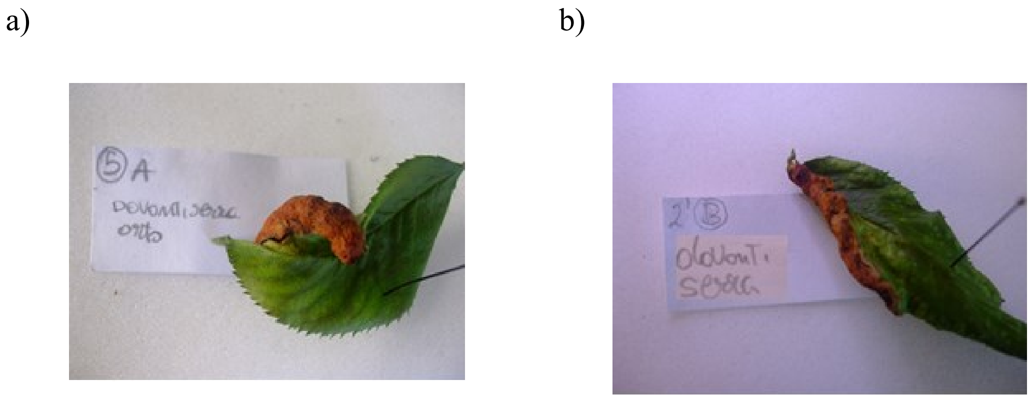


Figure 14 – a) Gall type A and b) gall type B in 2014.

At early April, the *Prunus* of the nursery presented only the gall type A and only at the end of the month were present some galls type B.

The last ten days of May all the individuals inside the tulle bags were dead.

Winter eggs

The winter eggs on the primary host were found for the first time in the mid of February 2013. The presence of the overwinter egg was confirmed in both *Prunus* of Botanical garden (27 in totals) and on *Prunus* of the nursery (13). Winter eggs were present on the *Prunus* of Botanical Garden until the last ten days of March 2013. The data of samplings for winter eggs are presented below (Table 1.)

Table 1 – Branches observed in cm and the number of overwinter egg found 2013.

Sampling date	***Prunus subhirtella* cv. *Pendula***	**Branches observed (cm)**	**N° eggs found**
17.10.2012	Orto Botanico PD – *Prunus* A	1420	0
	Orto Botanico PD – *Prunus* B	1681	0
14.02.2013	Orto Botanico PD – *Prunus* A	408,5	25
	Orto Botanico PD – *Prunus* B	561,5	2
	Garden nursery Saonara (PD)	1197	13
20.03.2013	Orto Botanico PD – *Prunus* A	2509	0
	Orto Botanico PD – *Prunus* B	1405	1

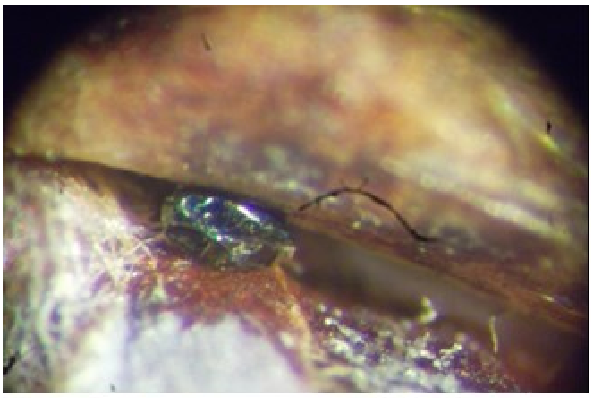


Figure 15 – Winter egg 2013.

Additionally the number of infested leaves was calculated in order to have approximately the level of infestation. From *Prunus* A of Botanical garden 208cm of young branches were randomly collected, with a total of 83 leaves. Only 7 (8,4%) of the leaves had the characteristics galls.

Trials on the primary host *Prunus subhirtella* outdoors

The branches with winter eggs kept in a cold place and transferred on March on potted *Prunus subhirtella* trees in the screenhouse of Agripolis. During the spring period, they were checked every 2 days but no galls were observed on their leaves.

Monitoring of galls

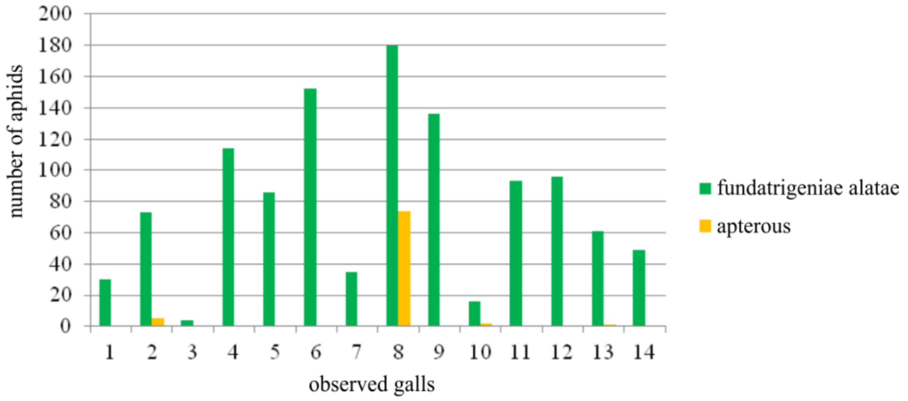
Population size inside a gall

The tulle bags were collected from the *Prunus* on 17.6.2013 and transferred in the laboratory with all the individuals dead. The majority of individuals present in the galls were fundatrigeniae alatae (migrants). Moreover, fundatrix were present and only in a few galls were present wingless individuals.

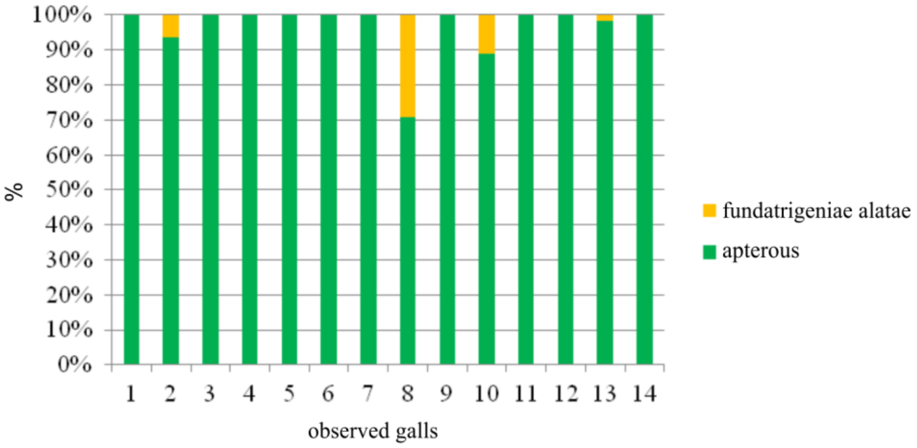
The minimum number of fundatrigeniae alatae inside a gall was 16 while the maximum number was 180. The average number of fundatrigeniae alatae for each gall was 80,4. Only in three cases were found few aphids wingless, with an average 2.6/gall and only in one case (bag No. 8) the presence of wingless aphids was high (74individuals - 29%) of total aphids present Graph1.

Also the presence of presence of parasitoids was observed. In the cases of gall n° 1, n° 5 and n° 10 Syrphid larvae were noticed.

a)



b)

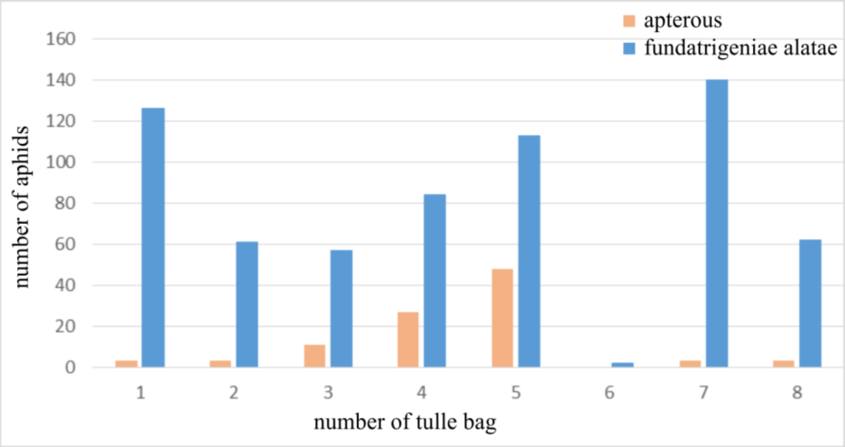


Graph 1 – Number and percentage of fundatrigeniae alatae and apterous inside a gall 2013 in a) and b), respectively.

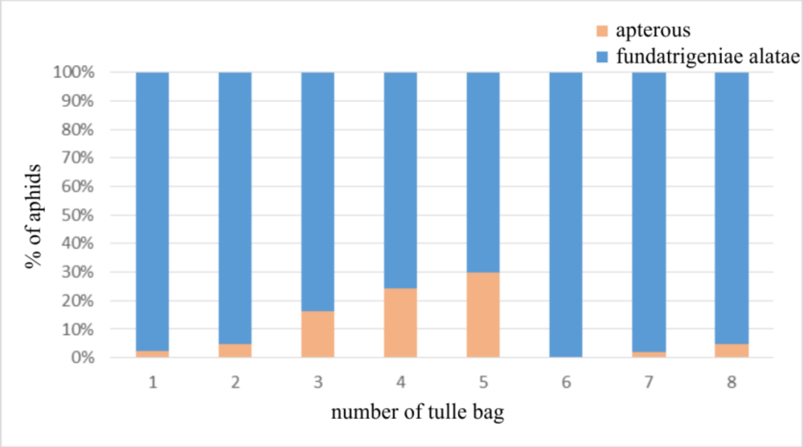
For the year of 2014 on June 24rth, 32 tulle bags placed in *Prunus* A and B were collected. Given that the alatae were transferred on *Artemisia* plants the number of individuals inside a gall is not noteworthy. On the other hand on 3rd of June 20 tulle bags placed in *Prunus* of nursery were collected and counted the number of individuals which they developed within each gall.

The Graph 2 shows the number of alatae and apterous in each gall. The gall 6 presents minimum numbers for both individuals (alatae and apterous), while the gall 7 has the highest number of alatae.

a)



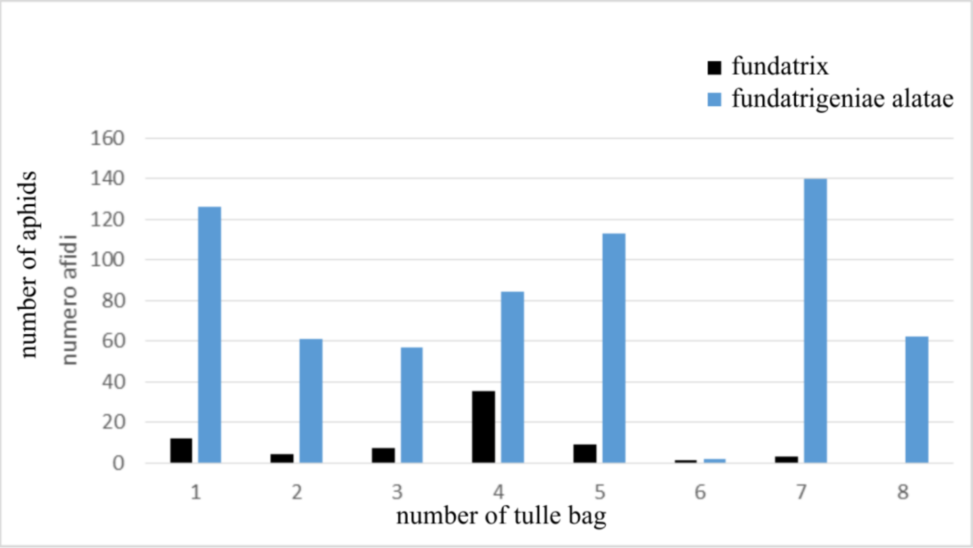
b)



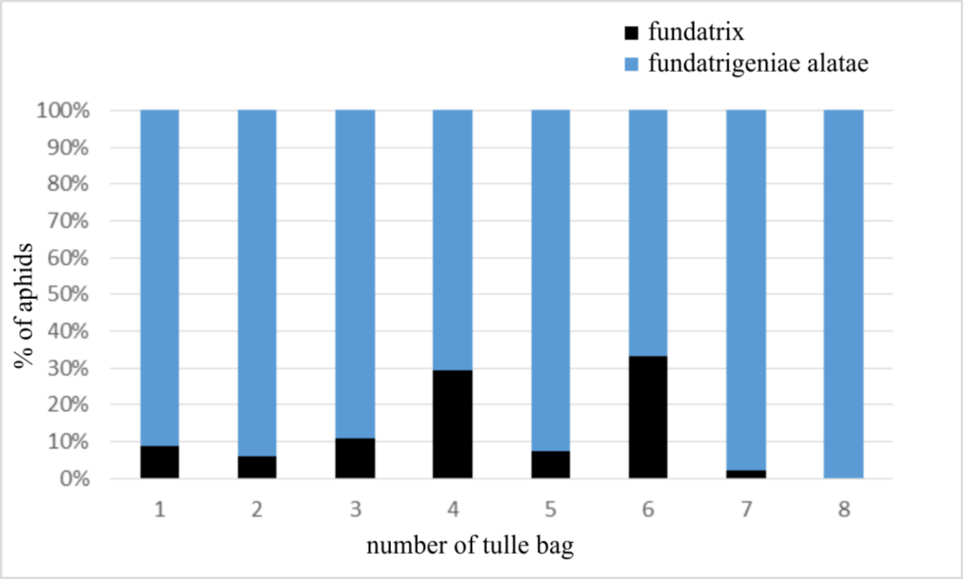
Graph 2 – Number and percentage of alatae and apterous individuals in each gall on 5/06/14 in a) and b), respectively.

The Graph 3 presents the number of alatae and the fundatrix in each gall and their percentage. The gall 6 and 4 has the lowest and highest number of fundatrix respectively.

a)



b)



Graph 3 – Number and percentage of alatae and fundatrix present in each gall on 5/06/14 in a) and b) respectively.

Phenology of different stages inside a gall

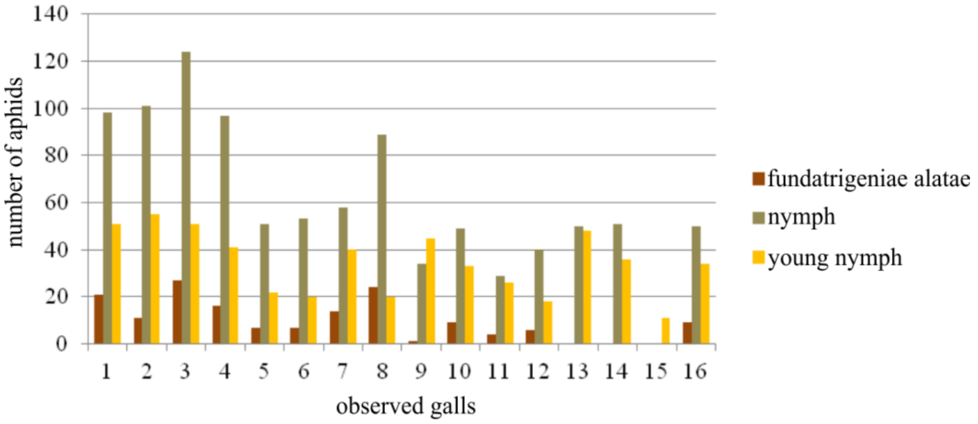
Inside 16 galls collected on May 7, 2013 from *Prunus subhirtella cv. pendula* of the nursery Bordin at Saonara the following stages were observed: fundatrix, apterous and alatae fundatrigeniae.

Two different forms of female fundatrix were noticed: with; one with a sub-triangular body shape and dark green color while the other with a more rounded shape and two dark lines on the abdomen.

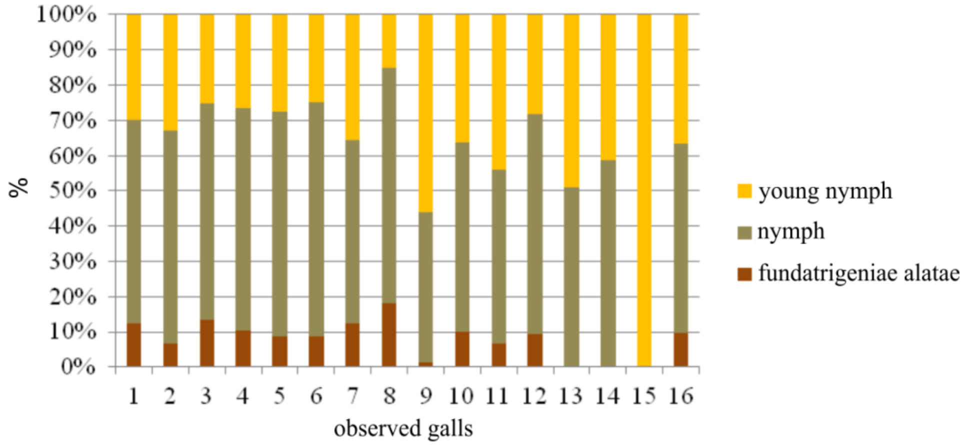
Neanids and nymphs have a bright yellow color with black siphons while the alatae have the head and thorax black and abdomen yellow with dark spots.

The Graph 4 presents all the different stages of aphis inside a single gall and their percentage. The minimum and maximum number of alatae is 0 and 27 respectively with an average of 9,75%. The nymphs have a minimum 0 and maximum 124 individuals that give an average of 60,9% nymphs/ gall. Finally for neanids, the minimum and maximum number inside a gall is 11 and 55 with an average of 34.4 individuals/gall.

a)

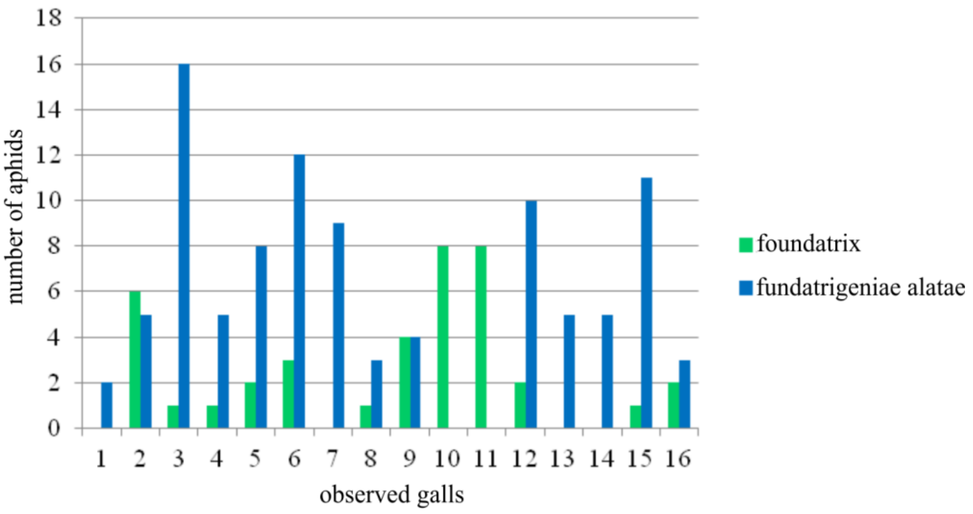


b)



Graph 4 – Different stages of aphids inside an each gall in 07.05.2013 (number and percentage in a) and b), respectively).

In the leaf galls (16) collected in May 14, 2013 from *Prunus* (nursery) of Saonara it was observed the presence of fundatrix and fundatrigeniae alatae (migrants), except for two galls in which there were no winged individuals. The average number of fundatrigeniae alatae was 6.2 /gall. The Graph 5 shows the results of the sampling.



Graph 5 – Different stages present inside each gall in 14.05.2013.

In the same *Prunus* in 21 May 2013 some galls smaller and closed on the leaf blade were observed for the first time. They were different from those observed until then. A total of 23 leaves were collected randomly. Some of them had more than one gall (total no of galls=30). Each gall was classified according to its shape and its appearance. A total of 13 galls similar to a bag shape (type A) were observed , plus 17 galls of smaller size and narrow (type B).

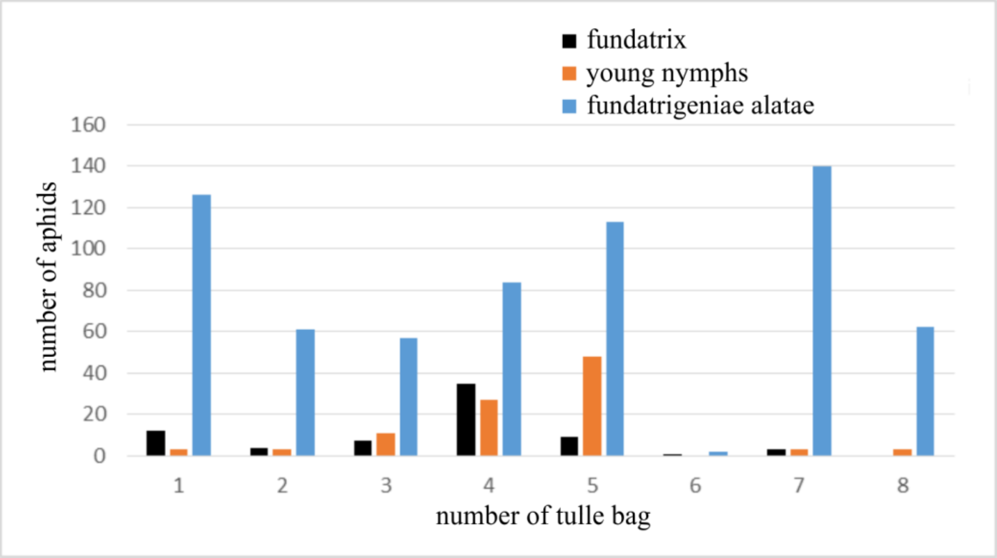
Comparing the data collected in the two types of galls, it was observed that in the galls type A the number of neanids was very low (2-3 individuals per gall), while in the galls type B the number of neanids was definitely higher (10-30 individuals per gall) with an average number of 14.9 individuals for each gall.

As far concerns the number of alatae in the galls type A, were relatively low (2 to 3 individuals per afloat). Only in one case the number of alatae reached the 15 individuals while in the galls type B the alatae stage wasn't present.

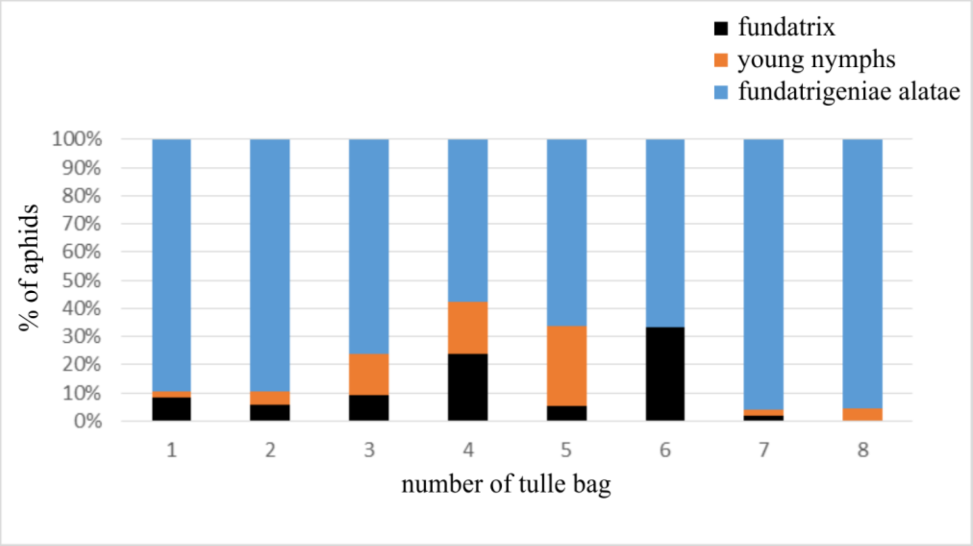
The sampling carried out May 27, 2013 confirmed the end of the first part of the cycle, on the primary host since the galls present on *Prunus* of Botanical Garden were empty and the individuals inside the tulle bags were dead. On the other hand the galls of *Prunus* in the nursery still had few alatae and apterous fundatrigeniae alive.

The Graph 6 shows the number of each phenological stage of aphids in each gall and their percentage.

a)



b)



Graph 6 – Different stages of individuals in each gall on 5/06/14 presented in absolute values and percentage in a) and b), respectively.

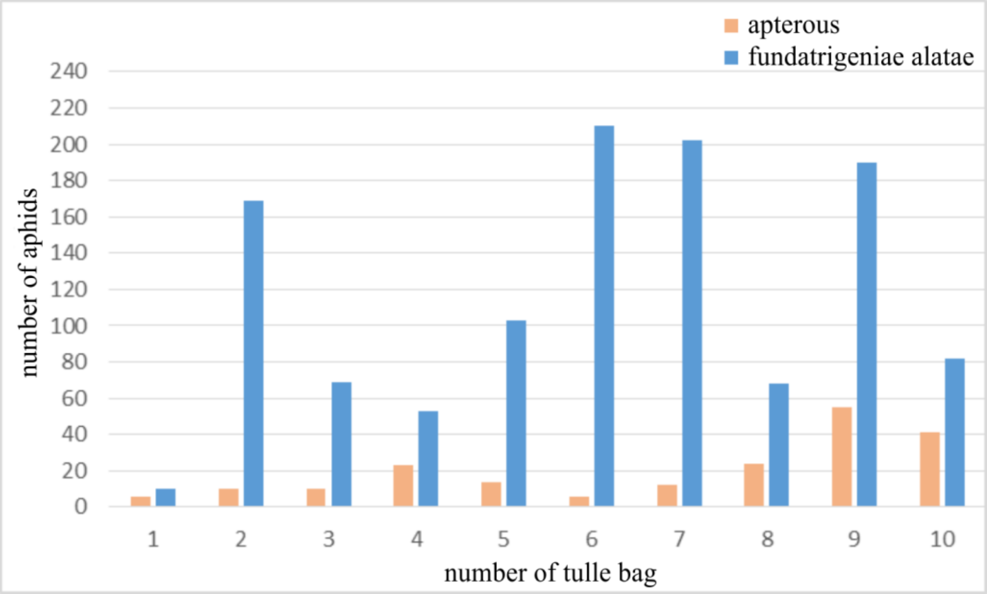
In the Table 2 below are presented the tulle bags with galls and healthy leaves.it is interesting to notice that in four cases (No 1,4,5 and 10) the gall type B was developed.

Table 2 – Number of individuals inside in each gall type A and A+B 2014.

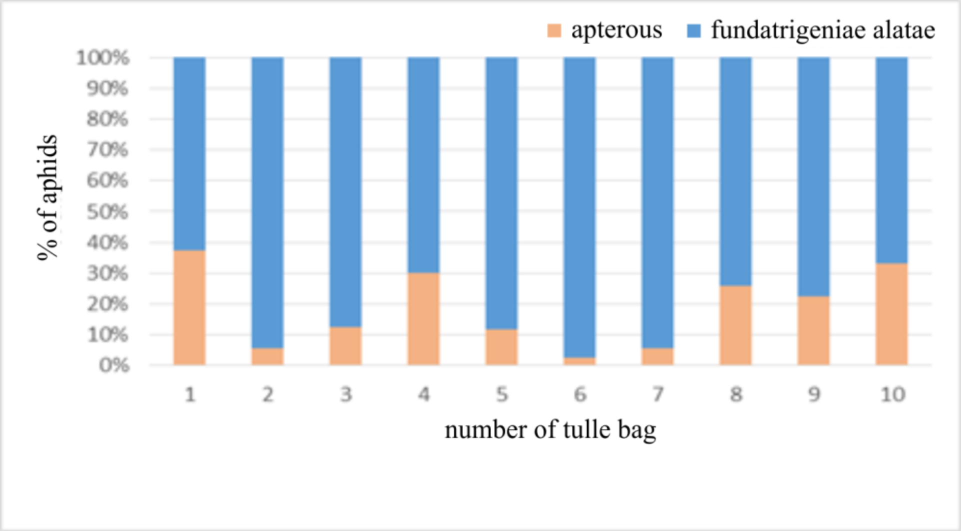
N° bag	**Gall type**	**N° fundatrix**	**N° neanids/nymph**	**N° alatae migrants**	**Predators**
1	A+B	4	6	10	
2	A	10	10	169	
3	A	4	10	69	Coccinellid
4	A+B	6	23	53	
5	A+B	6	14	103	
6	A	4	6	210	
7	A	9	12	202	
8	A	2	24	68	
9	A	7	55	190	
10	A+B	5	41	82	
Avarage of aphid/gall		5,7	20,1	115,6	

The Graph 7 presents the number of alatae and apterous in each gall and their percentages.

a)



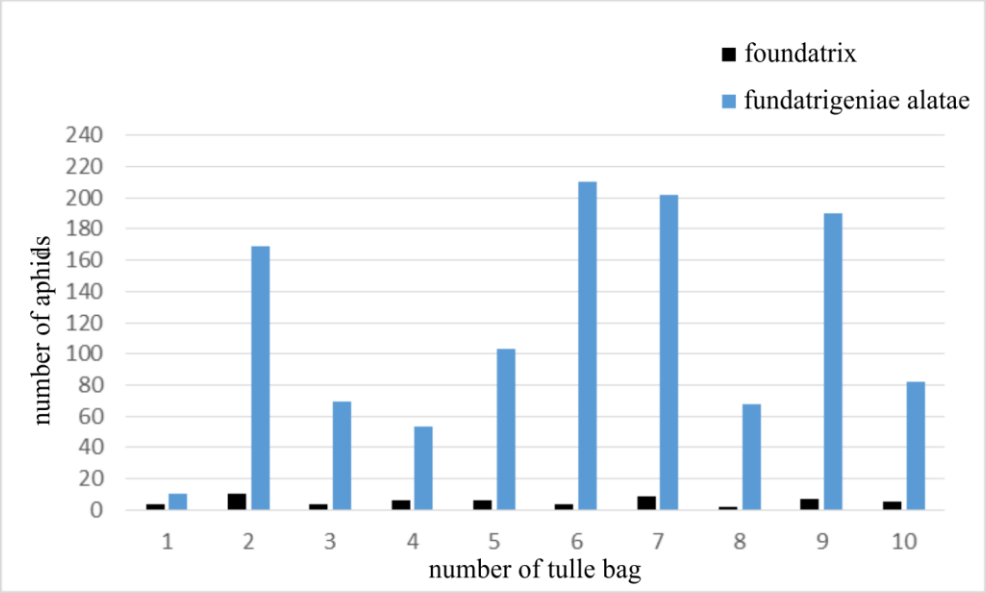
b)



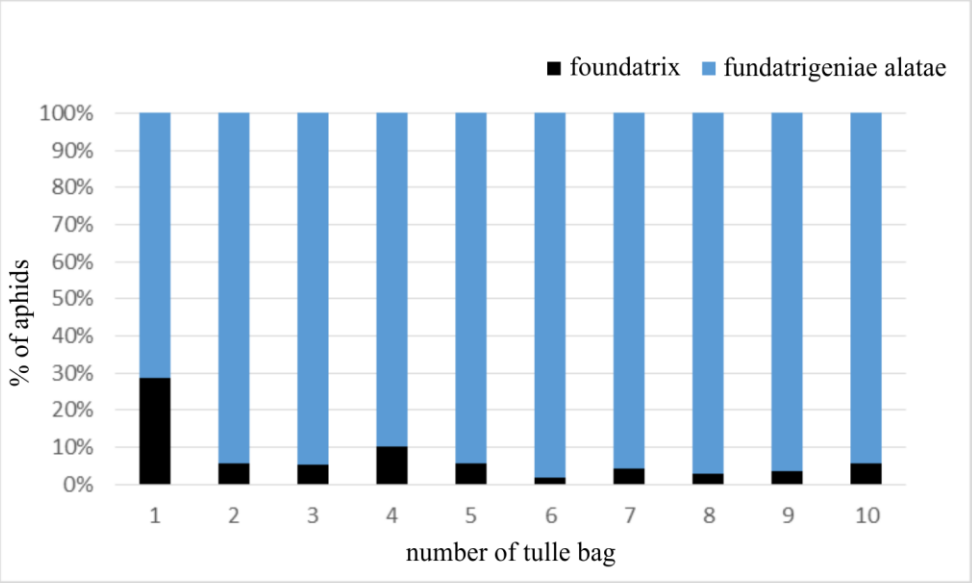
Graph 7 – Number of individuals alatae and apterous in each gall 2014 14 presented in absolute values and percentage in a) and b), respectively.

In the Graph 8 is presented the number of alatae and fundatrix in each gall. The gall 1 and 2 had the minimum and maximum number of alatae and fundatrix respectively.

a)



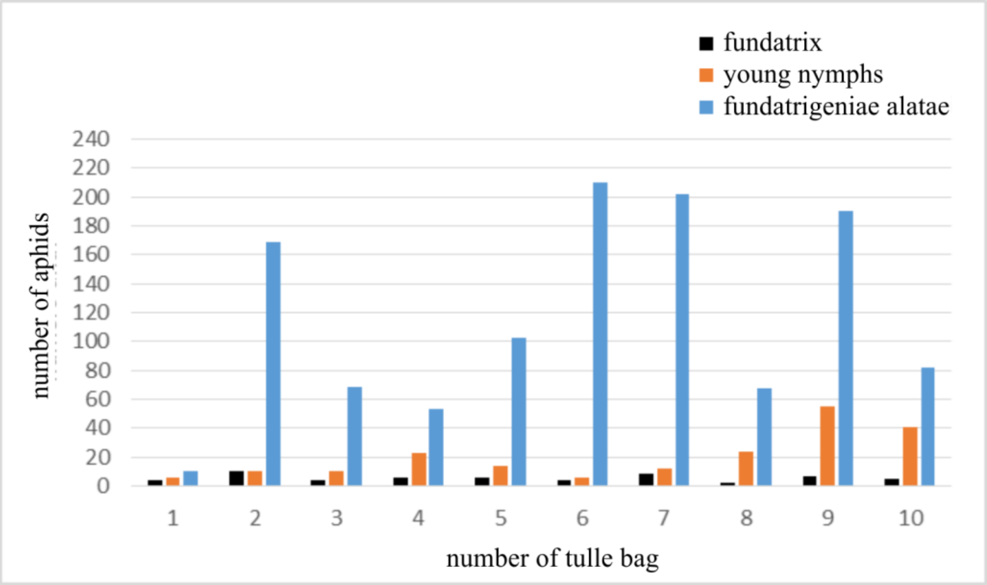
b)



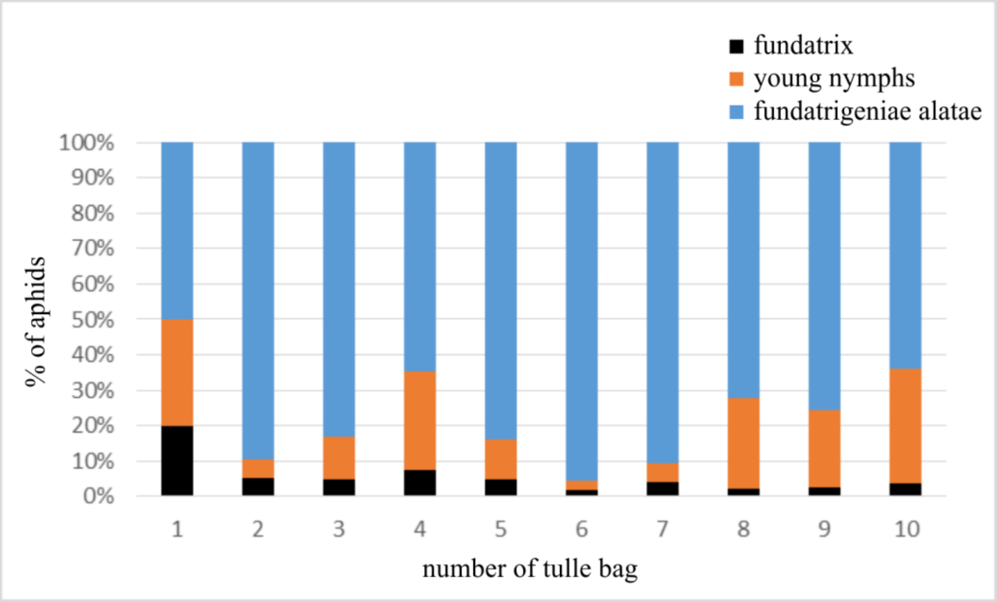
Graph 8 – Number of alatae and fundatrix individuals present in each gall on 5/06/14 presented in absolute values and percentage in a) and b), respectively.

The Graph 9 shows the number of each stage of the aphid in each gall and their percentage.

a)



b)



Graph 9 – Different stages in each gall on 5/06/14 presented in absolute values and percentage in a) and b), respectively.

Trial of colonization on *Artemisia* plants

The trials of colonization of *Tuberocephalus tianmushanensis* on the potted secondary host *Artemisia vulgaris* failed in 2013 as no specimens of *Tuberocephalus (T.) tianshanensis* was recorded on the roots of the secondary host plant *Artemisia vulgaris*.

Some aphids collected by sampling Artemisia roots were identified as *Macrosiphoniella artemisiae* BDF, a very common species on Artemisia, *Neotoxoptera violae* (Perg.), a cosmopolitan species, mostly reported on *Viola odorata* ,and *Pleotrichophorus glandulosus* (Kalt.) also very common on the leaves of *Artemisa vulgaris.*

The trial of colonization by *T. tianmushanensis* fundatrigeniae alatae carried out again in 2014 on the potted secondary host *Artemisia vulgaris* failed for the second consecutive year.

Presence of predators 2014

The presence of a very few natural enemies inside the galls was also noticed. Most of them were Coleoptera Coccinellidae (*Adalia bipunctata* (L.) and *Adalia 22-punctata* (L.)) and a few larvae of Diptera - Syrphidae

Discussion

The discovery of overwinter egg and the presence of the characteristics leaf galls for three consecutive years, allow us to say that *Tuberocephalus (T.) tianshanensis* Zhang, is probably acclimatized in our environment. Since it was able to complete its holocycle, this indicates that the aphid population could persist into the new territory in which it was introduced.

Tuberocephalus (T.) tianmushanensis is closely related to the presence of its primary host *Prunus subhirtella v. pendula,* moreover it was recorded only on the variety of *Prunus* with pink flowers. In the nursery of Saonara (PD) it was present also another *Prunus subhirtella cv. pendula,* which differed from the previous ones only by the color of flowers (white instead of pink). However this *Prunu*s with white flowers was not infested. This suggests that the aphid colonizes only on *Prunus subhirtella pendula* with pink flowers. The presence of Japanese ornamental cherry tree is limited and fragmented in our territory.

The observations during samplings confirmed that the aphid can carry on two generations on *Prunus*, as reported by Remaudière & Sorin (1993) and can induce two types of leaf galls (Sorin, 1993). The gall A is induced by the fundatrix, while the gall B is induced by the fundatrigeniae apterae. However this aspect of the biology needs further confirmation.

Fundatrigeniae alatae abandon the galls from late May until mid of June and dispersed in the territory in search of the secondary host plant.

The trial of colonization on *Artemisia vulgaris*, failed for the second successive year so possibly *Artemisia vulgaris* is not the secondary host plant of the aphid, as reported in literature (Sorin & Remaudière, 1998). Although even if the secondary host plant has not been yet identified, its presence is sure both in the surrounding of the Botanical Garden and in the nursery, because leaf galls were found for three consecutive years. In fact, the aphids re-immigrate from the secondary host to the primary host in order to lay the winter eggs: this indicates clearly that the dioecious holocycle is completed.

Inside the galls on *Prunus* have been found occasionally predators of aphids, but the number was not sufficient in order to control the aphid population.

The presence of Asiatic *Tuberocephalus (T.) tianshanensis* in the Botanical Garden and mainly in the nursery, confirms once again that the international trade of ornamental plants is linked with the accidental introduction of alien species. In Botanical Garden there are many plant species that over the years have been introduced from different parts of the world lots of exotic insects. Additionally the presence of the species in the nursery shows the important role that the nurseries of ornamental plants have in introduction and spread of exotic species in the new environment.

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Chapter 3

Cydalima perspectalis (=*Glyphodes*) (Walker, 1859)

(Lepidoptera, Pyraloidea, Crambidae)

Superfamily of Pyraloidea – Family of Crambidae

One of the largest superfamilies of Lepidoptera is Pyraloidea which is currently divided in two families of Pyralidae and Crambidae (Regier *et al.,* 2012) and includes about 16.000 species worldwide including important pests, biological control agents. (Solis, 2007; Regier *et al.,* 2012).

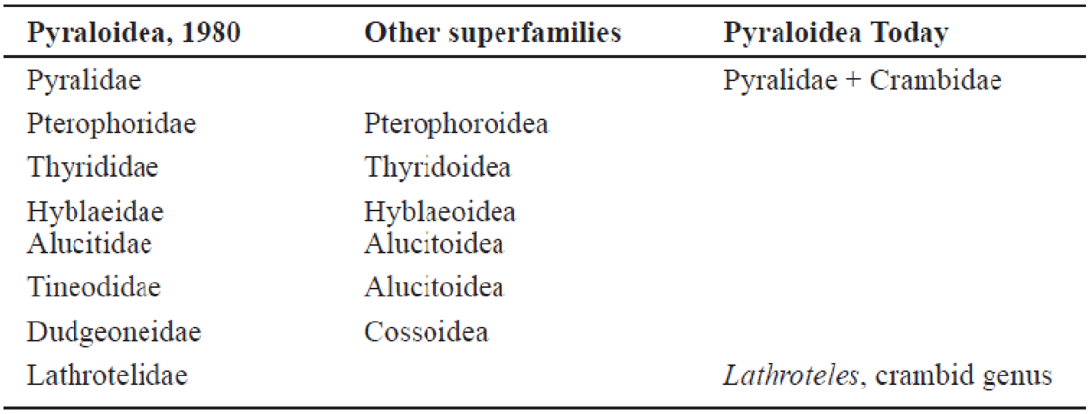
The main morphological characteristic of the superfamily is defined by a basally scaled proboscis and the presence of abdominal tympanal organs, paired tympanal chambers on sternite 2, each with a tympanum and a conjunctiva, a maxillary palpus that is usually present, veins R3 and R4 of the forewing stalked or fused, and Sc+R1 and Rs of the hindwing anastomosed distad of the discal cell (Solis. 2007; Regier *et al.,* 2012).

According to Solis (2007) (Table 1) the distinction between the families Pyralidae and Crambidae is based on two distinct tympanal organ types of the adult abdomen and some characters of the larvae. The Pyralidae family has a tympanal case almost closed; conjunctiva and tympanum are in the same plane while the praecinctorium is absent. The Crambidae family has a tympanal case open with a wide anteromedial aperture, conjunctiva and tympanum are in a different plane and meet at a distinct angle while the praecinctorium is present. Also Solis (2007) refers that Börner (1925) was the first to recognize the difference between the two families, and Munroe (1972, 1973, 1976) proposed the informal groups, Pyraliformes and Crambiformes based on the major differences between the two types of tympanal organs. Minet (1983) subsequently elevated Munroe's groups to the Pyralidae and Crambidae based on an extensive study of tympanal organs in Lepidoptera.

The larvae of many species are economically important pests of crops and stored products (Solis, 2007). Pyraloid larvae are hidden feeders which create holes or mines inside stems or fruits, or live in webs or leaf rolls. Crambidae' larvae basically feed on living plants and their damages depend on which subfamilies they belong to (Tabesh *et al.,* 2015). According to van Nieukerken *et al.,* 2011 this family contains 9655 species classified into 1020 genera and consists of thirteen subfamilies.

The moths of Crambidae family are mainly medium-sized. The adults fly in groups in the middle and late summer. Usually they are active during the night but sometimes can be seen during the day. The common habitats of the adults are meadows, steppes, biotopes while the larvae can grow on gramineae, on mosses and on remnants of vegetative origin (Shodotova, 2008).

Table 3 - Higher-level Pyraloidea taxa from Solis 2007.



Pyraloidea, 1980	Other superfamilies	Pyraloidea Today
Pyralidae		Pyralidae + Crambidae
Pterophoridae	Pterophoroidea	
Thyrididae	Thyridoidea	
Hyblaeidae	Hyblaeoidea	
Alucitidae	Alucitoidea	
Tineodidae	Alucitoidea	
Dudgeoneidae	Cossoidea	
Lathrotelidae		*Lathroteles*, crambid genus

The host plant Genus *Buxus*

The genus *Buxus* is comprehensive of evergreen shrubs belonging to the family of Buxaceae (Cocker, 1991). It is a genus of about 100 species divided in two centers of diversity: Caribbean-Latin America and East Asia and a minor one in Africa. The Asian - Eubuxus includes the "Mediterranean" taxa as western representatives (*Buxus sempervirens* L. and *Buxus balearica* Lam.). Usually only three boxwood species and hybrids of these species are used in the nursery and landscape trades: Littleleaf Boxwood (*B. microphylla*), Common Box (*B. sempervirens*), and Korean Boxwood (*B. sinica var. insularis*) (Niemiera, 2012).

Buxus is commonly known as "boxwood, "man's oldest garden ornamental" or box according to the American Boxwood Society. Its botanical name is derived from the Greek word 'puxos', which means "hard" with Aristotle and Theophrastus using this name for *B. sempervirens*. In latin "sempervirens" means "evergreen" indicating its green in all seasons (Colak, 2003; Colak *et al.,* 2012).

It is native in the woods and rocky hills in southern Europe, North Africa, West Asia and it is spontaneous in many thermophilous forests of central and northern Italy especially on calcareous, poor or stony substrates (Loru *et al.,* 2000).

Morphology

According to Cervato *et al.* (1966) *Buxus* normally is presented as evergreen shrub of 0.3-3 m high, sometimes as bush-ascending prostrate and rare as tree of 2-8 m (typically arborescence). The shrub is densely branched and its average high can reach up to 6.5 meters. Sometimes can become a tree and reach 10 to 16 meters high. It has an irregular, bushy, very dense shape, especially when is young (Figure 1).

Stem and bark: the stem is low, sinuous, usually divided and branched from the base; forms a large canopy which cans expand rather low and regular. The color of bark is rough and ocher. In young woody plants becomes hazelnut - brownish, while in the older ones presents reliefs and ribs.

Leaves, gems and branches: the leaf is persistent, simple, with a rounded blade, ovate - elliptical or lanceolate (depending on the different varieties), with rounded apex or emarginated. The margin is entire and slightly bent towards the underside (revolute).

The leaves have strongly leathery texture with dark green shiny color on the upper side and light green-yellowish below, shortly petiole 1-3cm long.

It is a monoecious plant with unisexual, apetalous flowers. The inflorescence is formed by a female flower in the center, surrounded by male flowers, producing three-horned, capsule-like fruits. View from the top the three protuberances are placed in the shape of a triangle. These flowers are very small and placed along the twigs leaf axils. Inconspicuous, apetalous flowers in axillary clusters are pale green to yellow to creamy white. The flowering occurs between April and May (Cervato *et al.,* 1966; Cocker, 1991; Rice, 2011).

Figure 16 – On the top left a *Buxus* tree, on top right a *Buxus* hedge, while down a shrub.

Distribution and habitat

According to Di Domenico *et al*., (2012) *Buxus* has a very long history in Europe and there is evidence of its presence as fossil record since the Miocene until Early Pleistocene and Holocene history.

B. sempervirens L. has an abundant distribution (Tutin *et al.,* 1968) all around Europe. It is present in the Pyrenees, French Prealps, all around the Jura Mts, Swiss Alpine forelands, in the southern European Peninsulas (Iberian, Italian, and Balkan), France, Corsica, Massif Central, United Kingdom, Ireland, Austria, Belgium, Luxembourg, Switzerland, Germany, Greece, Italy, Portugal, Spain, Balearic Islands (Minorca), Mallorca, Adriatic Coast, Slovenia, Croatia , Montenegro, F.Y.R.O.M., Albania, Serbia, Turkey, Montenegro, Kosovo. Also it is present in some parts of Africa like Morocco and Algeria and also in Asia in Georgia, Iran, Kazakhstan, Azerbaijan, Russia (Di Domenico *et al.,* 2012; Kenis *et al.,* 2013) (Figure 2).

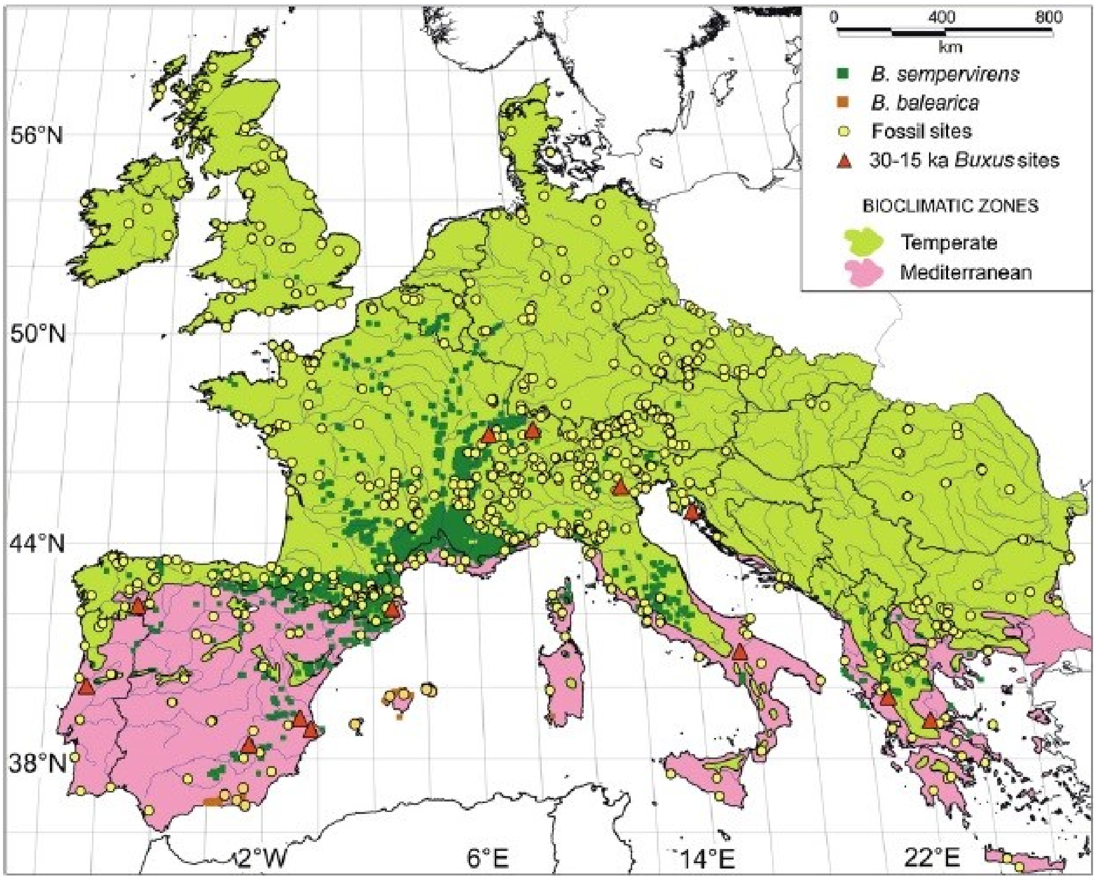


Figure 17 – Modern distribution of *Buxus* in Europe: *B. sempervirens* is represented in dark green, and *B. balearica* in orange. The yellow dots represent the reviewed fossil sites. Red triangles represent fossil records between 30 and 15 ka cal BP. The Mediterranean (pink) and Temperate (pale green) bioclimatic zones follow Di Domenico *et al*,. 2012.

Di Domenico *et al.* (2011), based on modern distribution data associated with past distributions of box plants, showed that *Buxus sempervirens* in Italy has a fragmented distribution and it shows a progressive population growth in different times from region to region and it is located at latitudes between 41°N and 43°Nl. *Buxus* populations are mainly present in N. Italy because of the favourable climate and human activities (for its ornamental value) and in Sardinia while in S. Italy *Buxus* is absent in Sicily, Calabria and Apulia (Figure3).

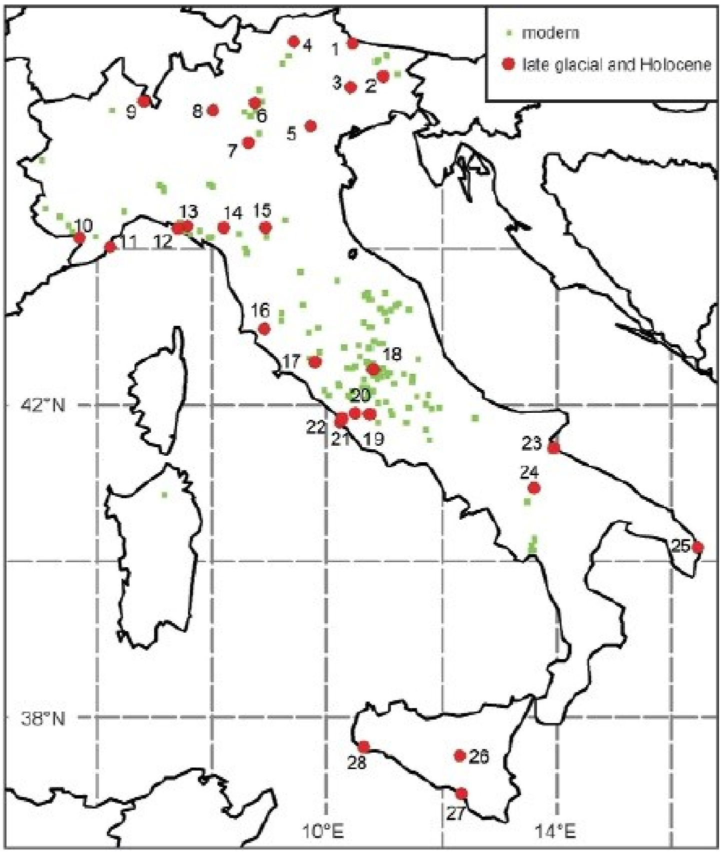


Figure 18 – Location of Italian sites where pollen of *Buxus* was found (red dots), compared to its modern distribution (green squares) (Di Domenico *et al.,* 2011).

Buxus sempervirens lives in deciduous forests on cliffs and rocks. It is encountered in the calcareous *Quercus*- *Fraxinus* forests, in calcicolous sub-mountain of Acer and thickets of *Quercus ilex*, *Laurus* and *Pistacia lentiscus* but above all it is often found in *Fagus* forest. Also it is associated with other thermophilic species such as cherry canine, the cotoneaster, the apple and wild pear, the broom, the blackthorn (*Prunus*) and medlar (*Mespilus*). Usually it grows as wild on dry, base-rich soils and rocky plains to the mountain. In the mountain and sub-mountain in northern Italy and Sardinia it can be found to 800 meters while in Pyrenees reaches to 1,050 meters and in Olympus can reach almost to 2000 meters. It is indifferent to the nature of the soil, but prefers soils loosely compacted, permeable with medium deep and not too dry, usually with calcareous composition. *Buxus* loves warm and sunny locations with dry ground until to moist; does not tolerate stagnant water, but can withstand frost (Ferrari & Doctors, 1996; Di Domenico *et al.,* 2011).

Importance of *Buxus*

The importance of genus *Buxus* has been pointed out in several studies due to its enclosing scientific, cultural, economic and ecologic interest (Zimmermann *et al.,* 2010; Wegmüller, 1984; Raven & Axelrod, 1974; ur-Rahman *et al.,* 1991; Loru *et al.,* 2000; Leporatti & Ghedira, 2009; Record, 1921; Batdorf, 2006; Köhler, 2007; Di Domenico *et al.,* 2011; Kenis *et al.,* 2013).

The boxwood is considered an important plant since ancient times for many reasons. In Greece it was sacred to the goddess Cybele and Hades that he was god of the underworld and protector of evergreens plants, as symbol of life that continues in the darkness of winter (Baumann, 1993; Brosse, 2004; Cattabiani, 2006).

According to Guidi (1996) and Eskin & Tamir (2005) it was also an important plant for the folk medice. The extracts of *Buxus* could be used for the cure of syphilis, epilepsy, rheumatism, gout and malaria, including the treatment of HIV.

From the ancient times the fine wood had various uses, including the production of everyday objects such as whips, combs, flutes and writing tablets, cultural items and containers for storing medicines (Colak *et al.,* 2012). As plants alone or in combination with other plant material also, they can be used as a foundation for homes and public buildings (Relf *et al.,* 2001).

Boxwood plants (*Buxus* species) are historically popular for their extensive use in landscape trades, home gardens, and public grounds because they have a wide range in potential size and rate of growth and there have several variations in size of foliage, colour, and texture characteristics (Relf *et al.,* 2001).

As an ornamental shrub or small tree, *Buxus* is a fundamental element for the gardens since the Romans and mainly with the formal Renaissance until nowadays. *Buxus sempervirens* L. is by far the most suitable plant for topiary and shaping responds very well on pruning and its litter tends to improve the soil. In fact, for the Italian gardens boxwood is one of the great glories since with the topiary art they could emphasize and define detail (Del Bene *et al.,* 1996). Boxwood had played an essential role through the centuries in the landscape of European gardens, historical villas and parks (Kenis *et al.,* 2013).

Ornamental varieties

Buxus balearica Lam: very similar to *B. sempervirens*, but glabrous or soon glabrescent shoots stouter and more stiffy erect; leaves 25-40x9-18mm with leathery, bright green elliptical. Inflorescence c. 10mm in diameter, with suborbicular, obtuse bracteoles; male flowers pedicellate; anthers c. 2-5mm; styles of mature capsule 5-7mm, arcuate. (Tutin *et al.,* 1968; Ferrell, 1994) (Figure 4).

Figure 19 – *Buxus balearica*: on the left, leaves; on the right conformation of the shrub.

Buxus bodinieri: evergreen with broad leaf reaching on average, 4.5 meters high (Ferrell, 1994) (Figure 5).

Figure 20 – Shrub of *Buxus bodinieri.*

Buxus harlandii: with opposite, simple, leathery, shiny and dark green leaves; the spring flowers are inconspicuous yellow-cream (Ferrell, 1994) (Figure 6).

Figure 21 – On the left young plant and on the right particular leaves of *Buxus harlandii.*

Buxus microphylla: evergreen with oval bright green leaves, with rounded or notched tip (Ferrell, 1994) (Figure 7).

Figure 22 – Shrub of *Buxus microphylla.*

Buxus sinica var. insularis: with small elliptical bright green leaves which turned to be bronze when autumn arrives (Ferrell, 1994) (Figure 8).

Figure 23 – Particular color of the leaves of *Buxus sinica var. insularis.*

Buxus wallichiana L.: native to the mountains of Nepal and the Himalayas, with gray branches and narrow bark, lance-shaped leaves (Ferrell, 1994) (Figure 9).

Figure 24 – On the left, shape of *Buxus wallichiana*; on the right the leaves.

Phytophagous species and pathogens of *Buxus*

A few years ago the box trees were mainly attacked by few native pests, either insects or mites. The infestation level from those pests was quite high but relatively infrequent and controllable. In 2007 in Germany the accidental introduction of the asiatic moth *Cydalima perspectalis,* monophagous pest of boxwood, after the intensive and repeated attacks on *Buxus* plants had led to complete defoliation, desiccation and finally the death of plants.

Monarthropalpus buxi (Laboulbène), 1873 (Diptera, Cecidomyiidae)

Distribution

Monarthropalpus buxi commonly known as box leaf midge is widespread in Europe and the United States. It is present in Austria, Chech Rebublic, Slovakia, Denmark, England, Germany, France, Hungary, Italy, Poland, Romania, Sweden, Switzerland, the Netherlands, ex. Soviet Union, Turkey and Greece (Vamvakas *et al.,* 2005; Skuhrava *et al.,* 2005).

Host plants and damages

M. buxi is a leaf miner which causes blister-like galls on *Buxus* spp. The main host plant is *Buxus sempervirens* but also has been recorded to other species of boxwood as *B. balearica, B. variegata, B. variegata argentea, B. variegata aurea, B. augustifolia, B. var. hansworthi, B. suffruticosa, B. arborescens, B. rotundifolia.* The attacks in the later species are less commonly due to some kind of resistance as toughness of the leaves (Brewer & Skuhravy, 1980; Brewer *et al.,* 1984; Vamvakas *et al.,* 2005).

The larvae (Figure 10) form an oval, water-soaked swelling (gall) on the lower leaf surface (Brewer *et al.,* 1984). In case of heavy infestation the *M. buxi* creates yellowish and reddish galls on the boxwood and it can cause considerable defoliation, deterioration (dieback) and sometimes death of plants, especially when the attacks are continued for several consecutive years (Baker, 1972).

Life cycle

Adults (Figure 10) are yellowish – orange, about 3 mm long, appearing in early May or a bit later and the have only a few days life (Barnes, 1948). Females lay a total of 150-250 eggs in the underside of the leaf surface below the upper epidermis (mesophyll) (Del Bene *et al.,* 1996). In each leaf they can lay from 1 to 15 eggs. The incubation takes from 12-18 days but most of the larvae which born died in the very early stages. During the first age, which lasts more or less one month the larva remains immobile within the leaf cavity already occupied from the egg. After the first age the insect makes a second molt to overwinter in the third stage of development. In spring it starts its activity reached the fourth age in mid-March.

In the second half of April, the larvae become pupae and after about twenty days, at the beginning of May adults appear. The *M. buxi* completes only one generation per year (Baker, 1972; Brewer & Skuhravy, 1980; Brewer *et al.,* 1984; Soporan *et al.,* 2015).

Methods of control

M. buxi can be combated mechanically by pruning the plants or removing and burning the infested parts of the plant, before the larvae become pupae or adult. Chemically it can be Figureht during the summer when the adults are present using pyrethroids (Fernandes, 1987; Brewer *et al.,* 1984).

Figure 25 – On the left adult of *Monarthropalpus buxi,* on the right larvae of *Monarthropalpus buxi.*

Psylla (*Asphagidella) buxi* (Linnaeus, 1758) (Hemiptera, Psyllidae)

Distribution

It is widespread in North America and throughout the Europe (GBIF, accessed 2016). Austria, Belgium, Bulgaria, Czech Republic, Denmark, France, Germany, Great Britain, Ireland Italy, Moldova, Netherlands, Norway, Poland, Romania, Slovakia, Spain, Sweden, Switzerland, Ukraine (O'Connor & Malumphy, 2011; European Fauna accessed, 2016).

Host plants and damages

This psyllid lives exclusively on boxwood plants (*Buxus sempervirens*). It attacks the leaves of sprouts causing the characteristic gall-like malformations of apical shoots while the plant growths very slow (Richards & Davies, 2013). The larvae are covered with a white waxy coat and they prick and suck out dry young shoots as result to become like spoon-shaped curly shoots (http://www.buxuscare.com/en/pests-and-diseases).

Infestations mainly concern the plants used for hedges and borders, where the insect can have favorable conditions for its development because of presence of young shoots and shadow.

Life cycle

P. buxi (Figure 11) overwinters as larva beneath the chorion of the egg from which it has emerged in autumn. There is period of true diapause from September until December of initiated by unknown cues. The next stage is the reactivation phase that leads up to the molt to second instar in March (Hodkinson, 2009).

During this first phase of the cycle the insect moves in the distal part of the shoots and remains there protected by a white waxy swab. The adults appears in the second half of May or later, before summer. After mating, the females lay their eggs inside the outer dormant buds scales. These eggs remain in diapause throughout the summer and at the end of October the embryonic development ends.

Methods of control

Psyllid's infestations can be prevented through agronomic practices: for example, pruning which can provide better circulation of light and air for the plants and avoid prolonged stagnation of moisture in vegetation. When the plants are pruned at the end of the year, a part of the damage is removed in a natural way (http://www.buxuscare.com/en/pests-and-diseases).

Figure 26 – On the left the damage created by *Asphagidella buxi* and on the right the adult of *Asphagidella buxi.*

*Eurytetranychus buxi (*Garman, 1935) (Prostigmata: Tetranychidae)

Distribution

Boxwood spider mite is widespread in many European countries as Belgium, France, Hungary, Ireland, Italy, Poland, Portugal and England (Bolland *et al.,* 1967). It is also present to Iran (Bolland *et al.,* 1967) and it occurs also in United States (New York, Connecticut, Michigan, Ohio, Virginia, Georgia, north Carolina, Oregon) (Jeppson *et al.,* 1975).

Host plants and damages

Eurytetranychus buxi (Figure 12) is monophagous on *B. semrervirens L*., feeding on boxwood leaves and causing spots, followed by discoloration (yellow and brown) near the mid vein of under leaf surfaces while in the upper surface small comma-shaped spots are appeared. Excessive infestations make the leaves bronze and lead to complete defoliation, leaving the plant almost naked. During heavy infestations all stages of this mite can be found on both leaf surfaces (Jeppson *et al*., 1975).

Life cycle

Overwintering eggs are laid in September-October. In early April to May the eggs hatched. The 3 development stages are completed in 2-4 days, 2-3 days and 3-7 days respectively. A female can lay 25-35 eggs (3-4/day) during her lifetime. The eggs are lemon yellow, diamond-shaped and hatch in 6-10 days into yellowish green larvae. The nymphal instars are deep green and green to yellowish brown. Since the duration of life cycle lasts 18-21 days, possibly eight generations per year occur (Jeppson *et al.,* 1975).

The favorable conditions for mite development are high temperatures and low humidities because they prefer to feed on tender shoots (Jeppson *et al.,* 1975).

Methods of control

Eurytetranychus buxi has a numerous natural enemies which can control satisfactory the infestation of *E. buxi* like green lacewings, lady beetles, harvestmen, and spiders (Stewart et al. 2002). It needs to be treated with a specialised acaricide starting in May, spray when the weather is warm with the active ingredients like tebufenpyrad, bifenazaat (http://www.buxuscare.com/en/pests-and-diseases).

Figure 27 – Adult male of *Eurytetranychus buxi.*

Aceria unguiculata (Canestrini, 1891) (Acari, Eriophyoidea)

Aceria unguiculata attacks on the gems of boxwood. The gems swell up dramatically, create a globular shape and they can reach 1cm in diameter which make them visible on the branches of boxwood from which lean out.

According to Fauna Europea (accessed, 2015) it is officially recorded only in Italy and Croatia.

The colony of eriophyid lives inside the buds. Apart from the striking galling of the buds, the leaves show small blister-like swellings (Figure 13). Sometimes the colony is accompanied by other species of eriophyid, considered as lodgers.

In early May the eriophyids abandon the gall to move to the new buds to continue the infestation.

The eriophyids inside the galls can be often attacked by predators as Hymenoptera larvae of Calcidid *Tetrastichus eriophyes* Taylor, whose adults swarm from galls during May in northern Italy (Pellizzari, 1988).

Figure 28 – *Buxus sempervirens* on the left, opened gall with mites on the right. http://www.bladmineerders.nl/gallen/acari/aceria/unguiculata/unguiculata.htm.

Volutella blight (DC.) Berk. 1850

The damage starts with the leaves turning brown. After that they dry out but stay attached to the plant. At the bottom of the leaves there are traces of a pale pink fungus. Brown, dry leaves and twigs mar the plant. When the damage is severe the plant can die. It needs to be treated with specialized fungicides. Extra attention needs to be paid after pruning or after damage from frost (Figure 14) (Dodge, 1944). (http://www.buxuscare.com/en/pests-and-diseases).

Figure 29 – *Volutella blight*.

Puccinia buxi DC., 1815

Small, dark brown pustules of spores appear under the surfaces of the leaves. It occurs mostly in shadowy, wild boxwood populations. It is quite rare in gardens.

Damage: brown pustules of spores affect the appearance of the plant (Figure 15).

Treatment: If necessary, it should be treated with fungicides including the active ingredients tetraconazole, tebucanozole + trifloxystrobine.
(http://www.buxuscare.com/en/pests-and-diseases).

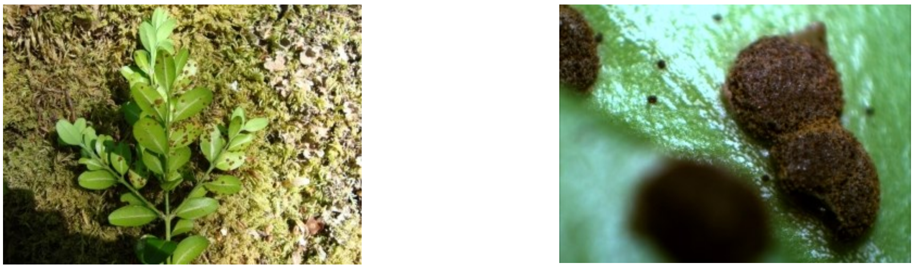


Figure 30 – Boxwood rust.

Cylindrocladium buxicola Henricot 2002

Cylindrocladium buxicola is a fungus pathogen commonly known as *Cylindrocladium* boxwood blight. It is a leaf and twig blight disease of *Buxus* spp and is a new species of *Cylindrocladium* (Henricot & Culham, 2002; Henricot *et al.,* 2000) (Figure 16).

First time the new blight disease appeared in 1994 in a nursery of UK. There was no other report until 1997 when an outbreak was noticed (Henricot *et al.,* 2000).

Leaves have dark brown spots and black streaks on the stems that cause severe defoliation (Henricot & Culham, 2002).

The pathogen has spread throughout Europe, in Belgium (Crepel & Inghelbrecht, 2003), the Netherlands (CABI, 2007; Henricot *et al.,* 2000), Germany (Brand, 2005), France (Saurat, 2012), Ireland (CABI, 2007; & LeBude, 2011), Italy (Saracchi, 2008), Switzerland (Vincent, 2008), Slovenia (Benko Beloglavec, 2009), Spain (Varela *et al.,* 2009), Croatia (Cech *et al.,* 2010), Austria (EPPO, 2010; Ivors & LeBude, 2011), the Czech Republic (EPPO, 2012), Denmark (Groen & Zieleman, 2012), the Republic of Georgia (Gorgiladze, 2011) and Turkey (Akilli *et al.,* 2012).
In 2011 it was reported also in the United States and Canada (Dart *et al.,* 2011; Ivors & LeBude, 2011; Milius, 2012).

Figure 31 – *Cylindrocladium buxicola.*

Cydalima perspectalis (Walker, 1859) ((Lepidoptera, Crambidae)

Cydalima perspectalis (Walker) (Lepidoptera Crambidae), known as Box-tree moth belongs to the family of Crambidae. It is originally native to East - Asia (Japan, China, Korea, India and eastern Russia) (Inoue, 1982; Leraut, 2012; Wan *et al.,* 2014). It was previous placed with the genus *Phakellura, Neoglyphodes, Diaphania* or *Glyphodes* (Mally & Nuss, 2010). It is pest of *Buxus* spp. and can cause severe damages to the plants.

It was included in the EPPO Alert List for more than 3 years (EPPO, accessed 2013). It was detected in Italy in 2011, in Lombardy, Como province (EPPO, 2011). In a very short time it invaded the other northern regions and was recorded in Veneto in 2012.

Distribution

Cydalima perspectalis was reported in Europe for the first time in 2007 in south-east Germany (Krüger, 2008; van de Straten & Muus, 2010; Reinhold & Schumacher, 2013) and the Netherlands (Muus *et al.,* 2009; van de Straten & Muus, 2010;) probably due to trade of the plants for planting from Asia (Krüger, 2008).

Cydalima perspectalis spread very fast throughout Europe and not only. Based on CABI and bibliography *C. perspectalis* is reported in Switzerland (Kappeli, 2008), UK (England and Wales) and Ireland (Mitchell, 2009), Austria (Rodeland, 2009; Perny, 2010; van de Straten & Muus, 2010), Slovakia (Slamka, 2010), Belgium (Casteels *et al.,* 2011), Hungary (Sáfián & Horváth, 2011), Romania (Szekely *et al.,* 2011), Liechtenstein (CABI/EPPO, 2012), France (mainland) (CABI/EPPO, 2012), Czech Republic (CABI/EPPO, 2012), Slovenia (Seljak, 2012), Croatia (Koren & Crne, 2012; Matosevic, 2013), Turkey (Hizal *et al.,* 2012), Denmark (Hobern, 2013), Russian Federation (Russian Far East and Southern Russia) (EPPO, 2012; Kirpichnikova, 2005; EPPO,2014), Serbia (Glavendekić, 2014), Greece (Strachinis *et al.,* 2015), Bulgaria (Beshkov *et al.,* 2015), Bosnia-Hercegovina (Ostojic *et al.,* 2015) and Georgia (Kenis *et al.,* 2013).

In 2011 it was reported in Italy, in Como province (Lombardy) (Griffo *et al.,* 2012; Tantardini *et al.,* 2012); probably it came through Switzerland and spread in the northern Lombardy (in the provinces of Lecco and Varese) (APFV, 2012) and in 2012 arrived in the Veneto region, in the provinces of Padua and Vicenza (Bella, 2013).

In 2012 it has been found in Friuli Venezia Giulia, in the province of Pordenone (Governatori, 2013), later in Emilia Romagna (Reggio Emilia and Ravenna provinces) (Fei, 2012), in Tuscany (Pistoia province) in (Cespevi, 2012), in Marches, (Urbino province) (Fei, 2013) and also in Sicily, Catania province (Bella, 2013).

Host plants

In Europe as host plant are considered the *Buxus* spp. species but mainly *Buxus microphylla*, *B. microphylla* var. *insularis*, *Buxus sempervirens* and *Buxus sinica*.

In Asia except from genus *Buxus,* it was reported as host plants also *Ilex purpurea, Euonymus japonicus Euonymus alata* (Korycinska & Eyre, 2009).

Morphology and life cycle

The eggs are translucent laid in groups of 5-20 eggs (Figure 17) slightly overlapping each other on the lower leaf surface, are covered by a translucent jelly (Leuthardt & Baur, 2013). When first laid, eggs are pale yellow but as they mature, they develop a black spot where each larval head capsule is forming (Korycinska & Eyre, 2009). Leuthardt & Baur (2013) showed that females prefer ovipositing on the variety of *Buxus sempervirens* ‘Rotundifolia.

When the eggs hatch, the larvae that emerge are greenish-yellow, with shiny black head. The newborn larvae can spread over an area of 20-25 cm diameter on the branches of the shrub until pupation (Leuthardt and Baur, 2013) (Figure 17). The larvae start scraping the surface of the underside of the leaf and later start tying leaves together. As they grow the head capsule remains black and the body develops dark brown stripes along the entire length of the body. Mature larvae retain their color but develop thin black stripes with white dots and hairs along the body with white dots along the dorsal side. The fully grown caterpillar is about 4 cm long (van der Straten & Muss, 2010).

Pupae are about 2 cm and are initially green with black stripes along the ridge. As they approach maturation they become light brown with a dark brown (brown wing borders) while the pupal skin itself is transparent (Figure 17). They are well hidden between the leaves, in silken cocoons and they are rarely visible (Korycinska & Eyre, 2009).

Adults have a medium size, with a wingspan of about 4 cm. Their wings have a white-silver iridescent color with a drown border at the outer margin. Except for the normal white form there is also a rare melanic (brown) form with the wings completely brown (van der Straten & Muss, 2010) (Figure 17). Both forms have a characteristic white spot on the forewing, in the discoidal cell (Mally and Nuss, 2010). Adults are good flyers and can live up to two weeks. During daytime, they tend to rest on the box trees or on other surrounding plants. Also adults considered as a hitchhiker since the main introduction pathway of *C. perspectalis* from Asia to Europe is the international trade of *Buxus* plants (CABI accessed 2015).

C. perspectalis diapause lasts 6-8 weeks (Nacambo *et al.,* 2013). Two generations per year usually occurs in central Europe but the biology of *C. perspectalis* in not fully known yet. In its native range, may vary from one to four generations per year. According to Maruyama & Shinkaji (1987) and Nacambo *et al,* (2013) threshold temperatures for the development of eggs, larvae and pupae vary between 8°C and 12°C, depending on factors such as the geographical location of the investigated population.

Damages

The larvae of *C. perspectalis* feed on the leaves and shoots of box trees and also can attack the bark, causing them to dry out and die (Leuthardt and Baur, 2013). The larvae feed on the leaves in a different way depending on their stage. The newly hatched larvae feed on the undersides of leaves, leaving the upper surface intact while the mature larvae feed on the entire leaf edges, with sometimes only leaf skeletons remaining. Webbing of the branches with frass and residues of moulting such as, black head capsules of different sizes are attendant symptoms. Heavy damage or repeated attacks lead to total defoliation of the shrubs, the subsequent attack of the bark causing the death of the plant (Korycinska & Eyre, 2009).

Often the attack of the borer is accompanied by the fungus pathogen *Cylindrocladium buxicola* Henricot, which burdens the health of the plant, increasing the process of desiccation of the leaf (Blackwell, 2014; Kenis *et al.,* 2013)

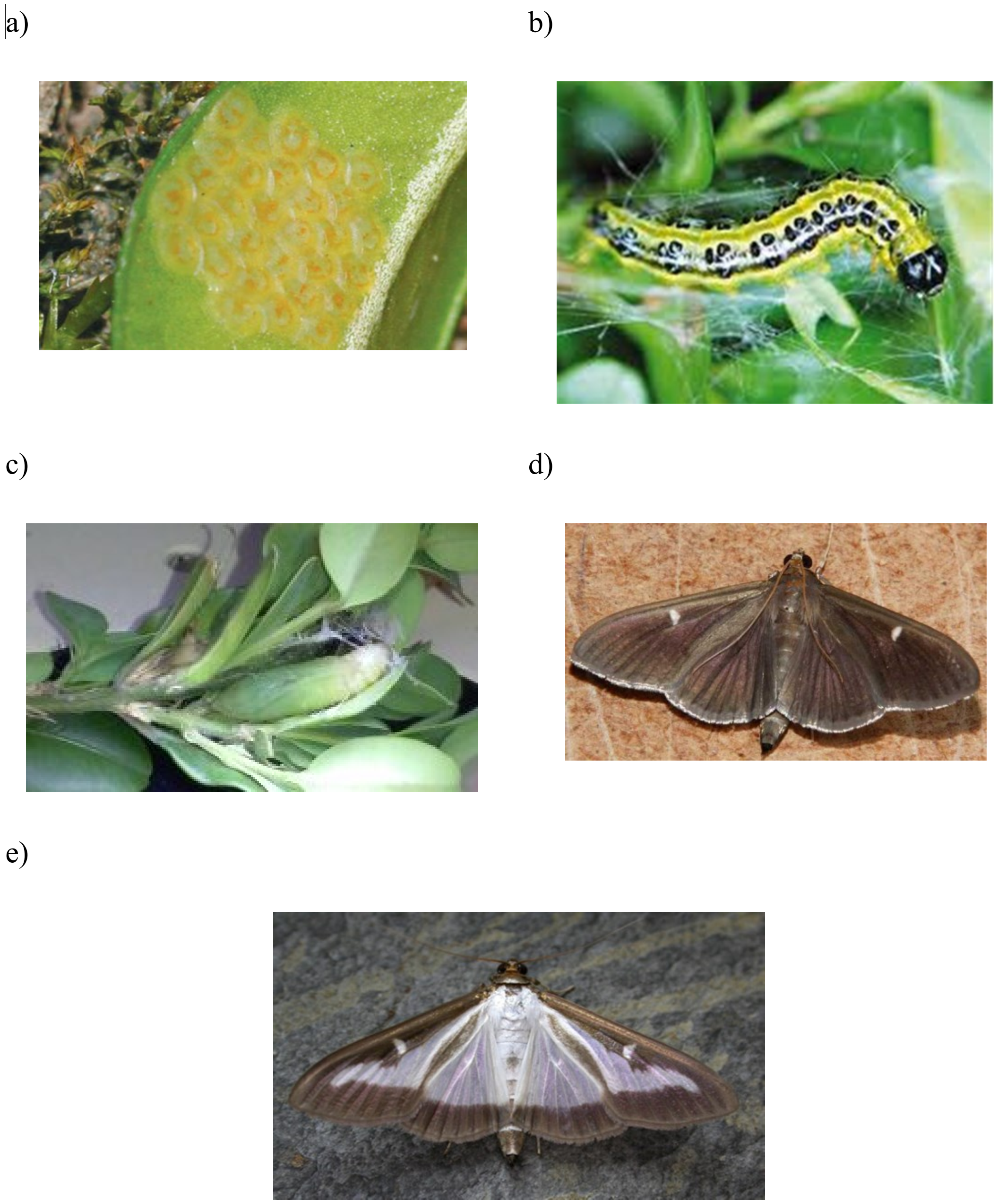


Figure 32 – Different stages of *C. perspectalis:* a) eggs, b) larvae, c) pupa and d) e) the two forms of adults.

Aims of the project

The aim of this thesis was to clarify some aspects of the biology and phenology of *Cydalima perspectalis* that were still unclear in northern Italy, in order to control the infestations of *Buxus*. In particular, it was necessary to determine the following traits:

1. Overwintering stage.

2. Number of larval instars.

3. Sex ratio

4. Number of generations per year in our environment.

Additionally, in 2015, experiments were conducted with sex pheromones traps as an effort to check, monitor and collect data on species distribution over the territory, to find any possible differences based on climate and to check the potential differences between types of traps.

Material and Methods

The biology and phenology of *C. perspectalis* were assessed both in field on box plants placed outdoors in cages and in laboratory, following the procedures described below.

Sampling sites

In order to assess the biological cycle, in particular when larvae come out from diapause five samples were made in five different places and at different times for two consecutive years 2013/2014 and 2014/2015.

The first sampling was carried out in the first week of December of 2013 in Montecchio Maggiore (province of Vicenza), in the hilly area of the Castles of Romeo and Juliet. Approximately 50 branches of boxwood were checked and 62 larvae were collected. A second sampling was performed the last week of December at Padova. The number of collected larvae was 26. A third sampling took place at the Parco dei Faggi, in Voltabarozzo (Padova), in the first ten days of January 2014. Branches of boxwood were collected with 40 overwintering larvae. The fourth sampling carried out in the mid of January at Legnaro (Padova) and 15 overwintering larvae were found. The last sampling was made near Montecchio Maggiore (Vicenza), in the first ten days of February, in an urban area. The larvae found were 17. A total of 160 overwintering larvae were collected.

From December 2014 until January 2015 the same procedure was followed and a total of 143 larvae collected.

Overwintering stage

After collection, the larvae were immediately transferred in the campus of Agripolis and kept outdoors in cages and regularly checked (i.e. every 2 days)

When the overwintering larvae started their activity they were transferred to the laboratory an additional sampling of 33 branches of infested boxwood was made in Montecchio Maggiore (Vicenza). All the samples were transferred to the laboratory.

With the help of a stereoscope with eye - ruler the width of the head capsules was measured. This process served to identify the age of overwintering larvae.

Monitoring of life cycle

After winter diapause the larvae were transferred on the box plants outdoors in the cages with the purpose to obtain adults and eggs to start rearing in the laboratory.

Once eggs were laid, the leaves with eggs were placed separately in petri dishes, each having inside a paper towel soaked in water to keep the humidity up. Since eggs hatched (119) we placed each larva inside a petri dish in order to check the duration of each larval stage, the number of larval instars, to measure the width of head capsule of each stage, the oviposition period (Figure 18).

In each Petri dish was placed an absorbent paper to maintain the humidity, plus a small box branch to allow the nutrition and the development of the larvae. With the help of a pipette and hydrophobic cotton the leaves were kept fresh. Each Petri dish was closed and the date of birth and serial number of the larvae noted.

Monitoring was daily, changing the box-leaves and collecting the head capsules which each larva left after molt. The head capsules of each larva were preserved individually in tubes containing 70% alcohol and they used later to calculate the number of larval instars and for the measurements of the width of head capsule through stereoscope eye - ruler.

Finally the date of birth of each larva, the date of pupation, the adult's emerge and their longevity and the sex ratio were noted.

This process was carried out for the first and the second generation of the moth.

By comparison of measurements of the width head capsule of overwintering larvae and those from larvae in the laboratory it was possible to define the age of overwintering stage.

Figure 33 – On the left petri dish for rearing in the laboratory and on the right 119 samples.

Longevity of adults

To assess the longevity of adults, once swarmed they were placed individually in tulle cages (Figure 19). Fresh branches of *B. sempervirens* partly immersed in water were provided as oviposition substrate. In the bottom of the cages a cotton soaked in a sugary substance (90% water and 10% of honey) was provided as food source. Moths, after their death, were collected individually and preserved in test tubes containing 90% alcohol, for the genitals examinations.

Pupae were collected from the field (Galzignano Terme) during 2015 and transferred in the laboratory. When the adults swarmed, two individuals of opposite sex were placed in tulle cages. The method that followed was the same with the longevity of adults.

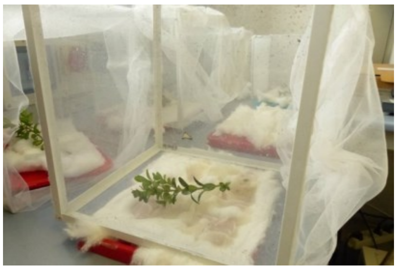


Figure 34 – Cage with adult in the laboratory**.**

Sex pheromones traps

During 2015 trials of male trapping were carried out in 9 locations characterized by different altitude and climate: Trento 421m, Terlago 438m, Vigolo Vattaro 707m, Cerna 683m, Castagne 580m; Limana 319m; Legnaro 8m, Padova 12m, Galzignano Terme 25m (Figure 20).

On May 10, 2015 two different types of pheromone traps, DeltaTrap (Koppert) and Funnel (Novepher, S.a.s) were placed. The sex pheromone was supplied by Koppert Pherodis *Glyphodes perspectalis* and by Novapher S.a.s which its composition was: Z11-16:Ald (80%) + E11-16:Ald (20%). Pheromones were changed every one month. Traps removed on October 30, 2015.

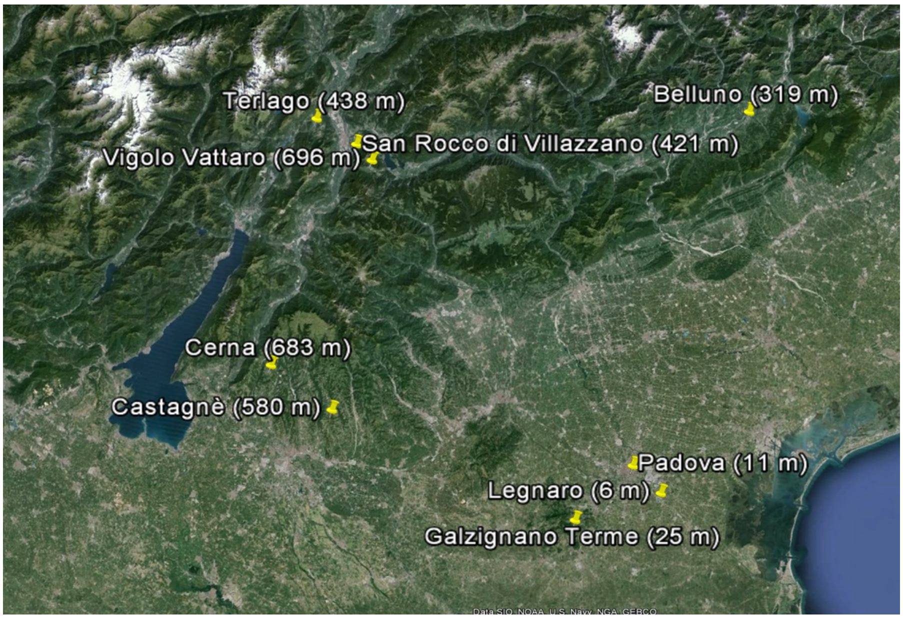


Figure 35 – Map of the localities where the sex pheromones traps were placed.

Potential parasitoids

An effort to find possible parasitoids was carried out. Eggs, larvae of different stages and pupae were collected in the field, transferred in the laboratory and placed individually in petri dishes with wet absorbent paper and fresh food. Their development was daily checked.

6. Results

In this chapter the results of observations on phenology and life cycle of C. *perspectalis* carried out in the field and laboratory are presented.

6.1 Observations on the end of diapause in 2014 and 2015

In 2014 the overwintering larvae started their activity in early February and were recorded until the mid of April 2014.

In 2015 the overwintering larvae started their activity at the end of March, one month later with respect to 2014 and were recorded until end of April.

6.2 Life cycle in laboratory 23°C

In 2014, 3rd of June eggs were collected and transferred in the laboratory. A total of 302 eggs were collected. The Figure below (Figure 21) shows the average length of each stage respectively of the first and second generation. The dark cycles are the presents the average number of each stage while the pale cycles the standard deviation. For the first generation incubation period was about 4 days, the larval stage lasted 28 days, the pupal stage lasted 10 days and the adult longevity was about 7 days. In the second generation incubation period was about 5 days, the larval stage lasted 21 days, the pupal stage lasted 7 days and the adult longevity was about 7 days. The time required in order to complete the life cycle of the first generation was approximately 50 days while for the second was 40.

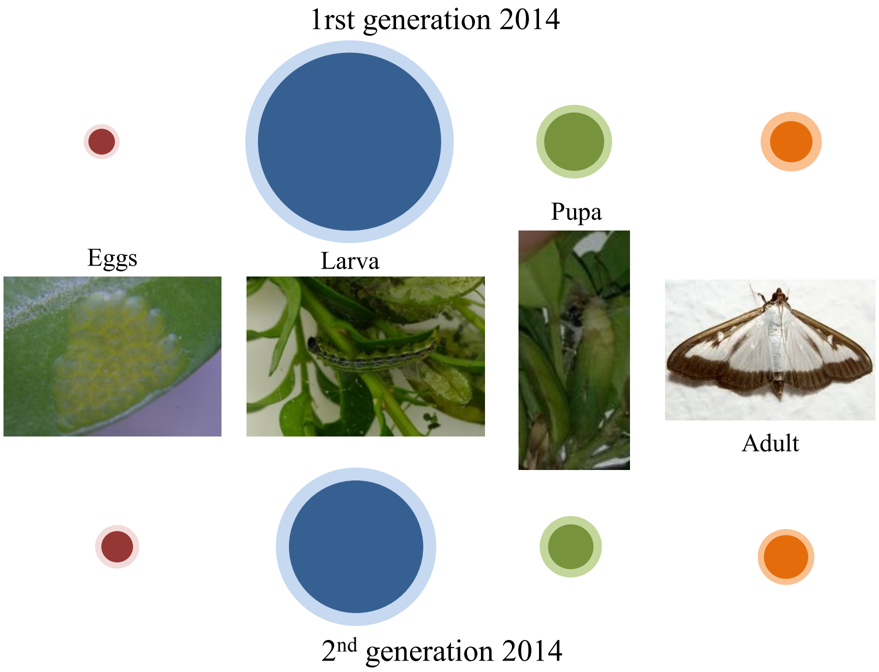
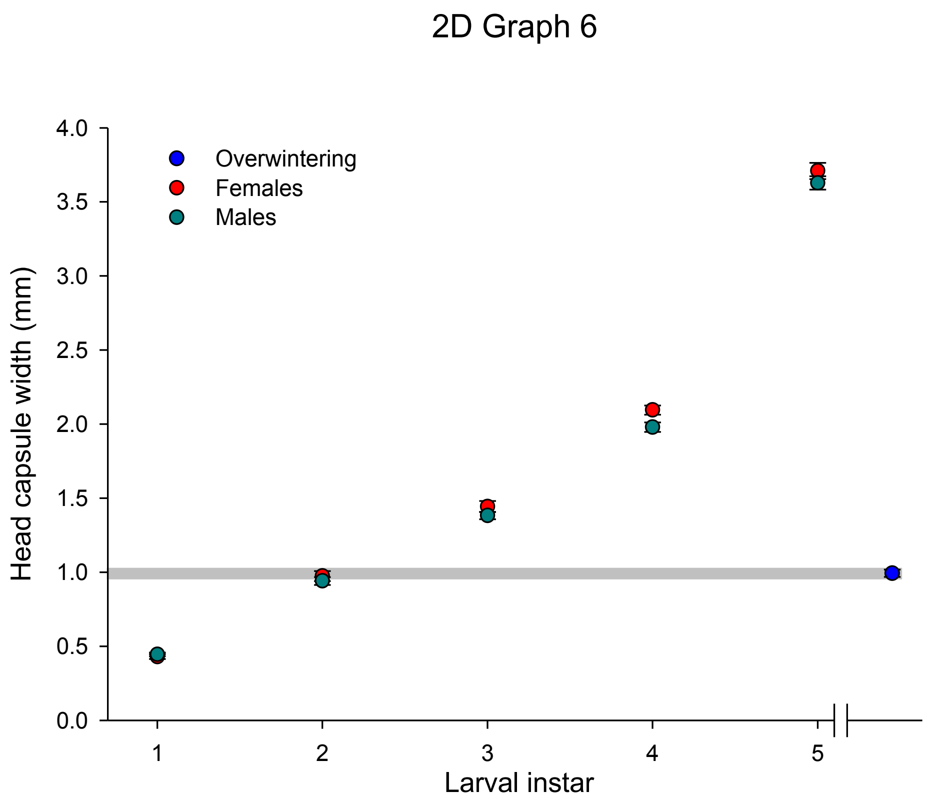


Figure 36 – Average length of each stage in the first and second generation.

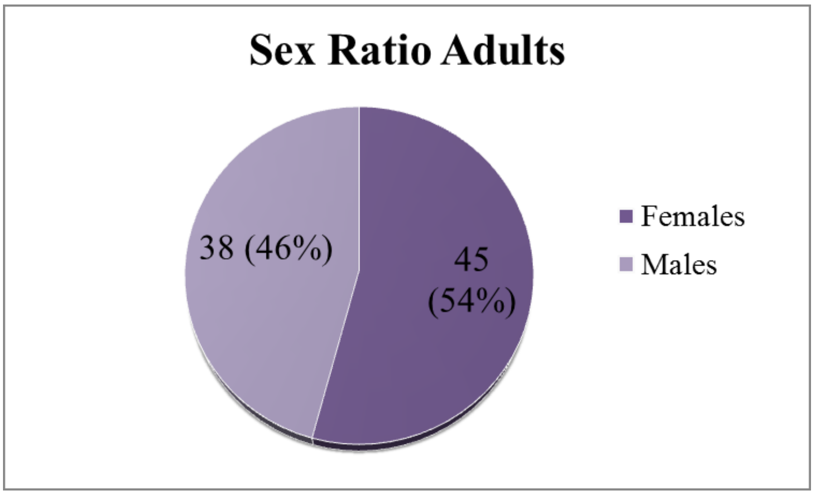
The Graph 1 is presente the number of larval instars and the average width of head capsule in each larval stage. The red and green dots present the average of the head capsule width of the females and males respectively while the blue dot presents the average of the head capsule width of overwintering larvae. Error bars are standard errors and when they are not visible is because they fall within the symbol size. *C. perspectalis* has 5 instars and overwinters mostly as larvae of the 2nd instar.



Graph 1 – Number of larval instar and the average width of head capsule foe each larval stage.

6.3 Sex Ratio

The examination of genitals of the first generation adults bred in laboratory gave a 54% of females and 46% of males (Graph 2).

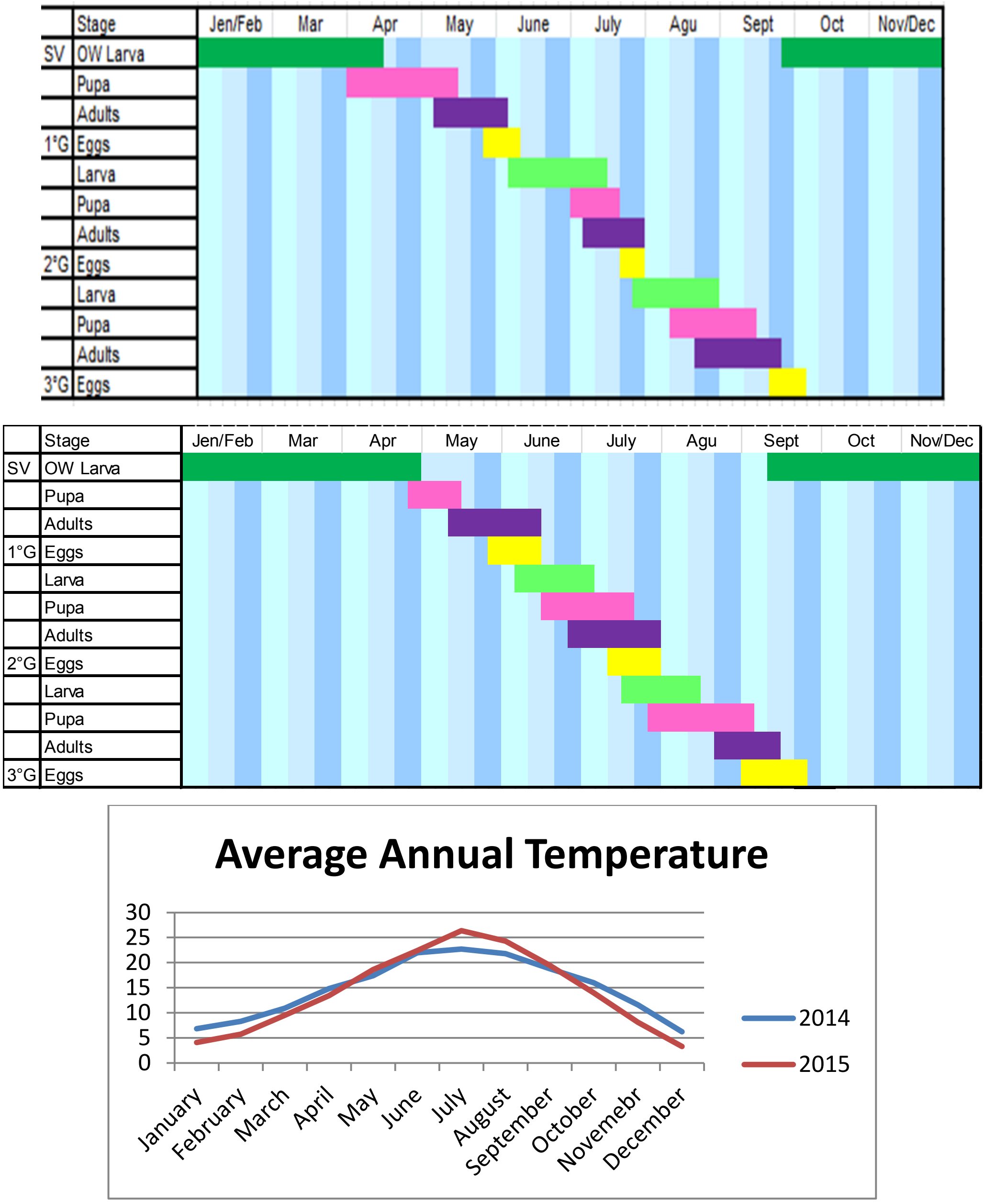


Graph 210 – Percentage of females and males in the first lab generation.

6.4 Number of generations in 2014 and 2015

In 2014 overwintering larvae started their activity in early February. The larvae were active until mid-April while in 2015 overwintering larvae started their activity at the end of March until end of April.

First and second generation larvae are present in June-July and August while the overwintering larvae (third generation) are those born in September. The adults fly in May, in July and in August until September. Three generations per year occurred in 2014 (Graph 3).



Graph 3 – Number of generations in 2014 (top) and 2015 (middle) and the annual average temperature for 2014 and 2015 (bottom)

Potential parasitoids

Unfortunately no parasitoids were found.

Sex pheromone traps

a) The total number of captures was 123 (DeltaTrap: 64 and Funnel: 59). The efficacy of the two types of traps was compared and no differences were found (T-test, P > 0,05).

b) As far concerns the distribution of *C. perspectalis* in North Italy the captures of the traps confirmed that *C. perspectalis* is present in all the localities where traps were placed except from Vigolo Vattaro (707m). The gradation of the color for each pin presents the total number of captures. As darker the color more captures, lighter less.

Galzignano Terme (GAL) was the site with the highest number of captures (38) while in Vigolo Vattaro (VIG) the numbers of captures was 0. In Cerna (CER) and Castagne (CAS) were 10 for both places. In Trento (TN) and in Agripolis (LEG) the number of captures was 25 and 24 respectively. In Botanical Garden (PD) were 7, in Terlago (TER) was 5 while in Limana (LIM) there was only one capture during the season with 4 adults (Figure 30). Among the captures the melanic form of the moth was found in Trento and Castagne.

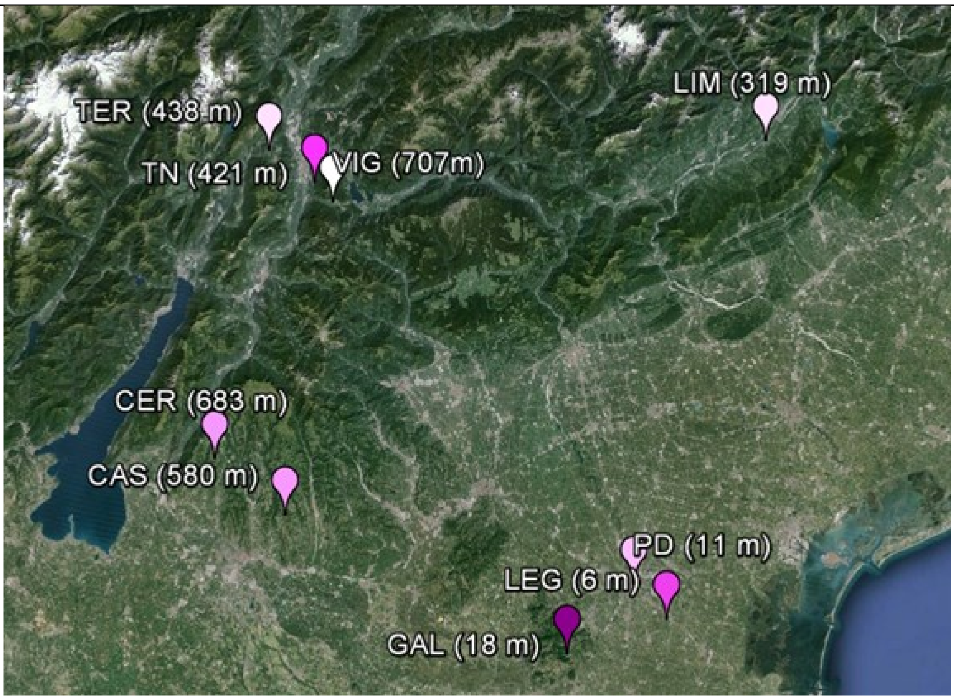
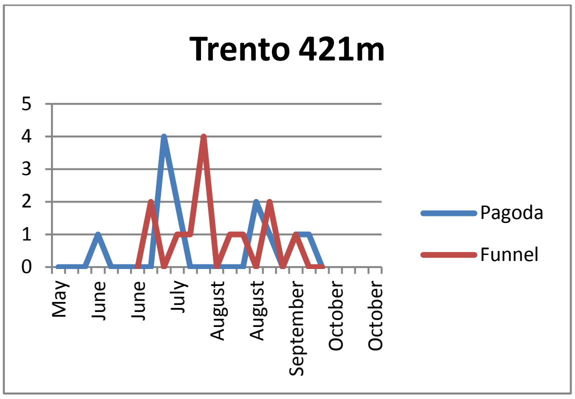
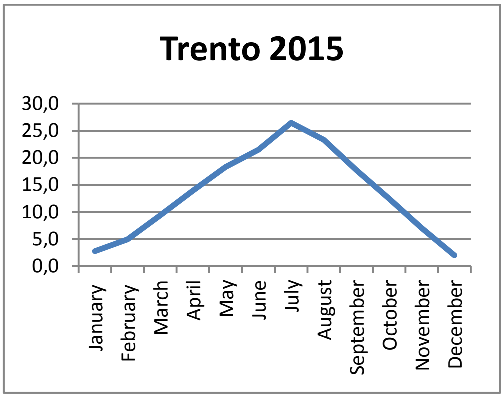
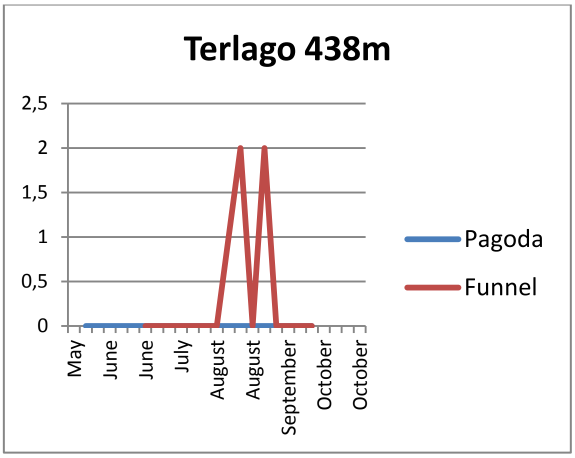


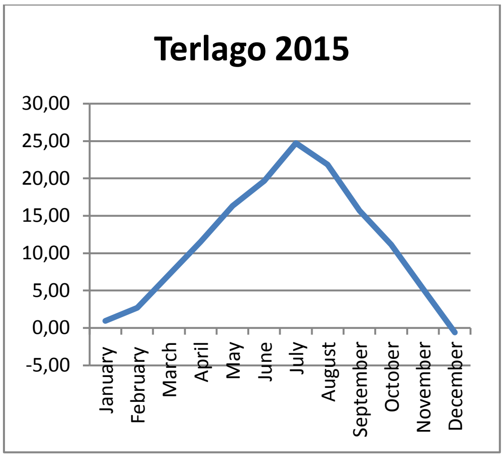
Figure 37 – Distribution of *C. perspectalis* in North Italy. The gradation of the color for each pin presents the total number of captures. As darker the color more captures, lighter less.

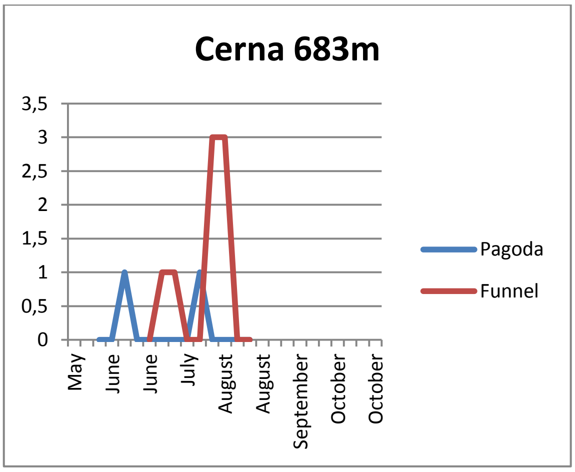
c) Captures / trap in the different localities

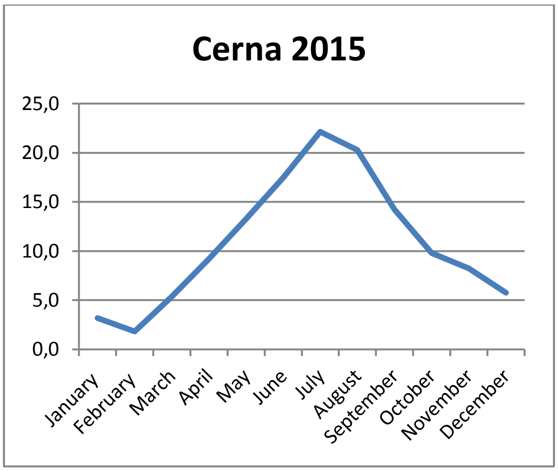


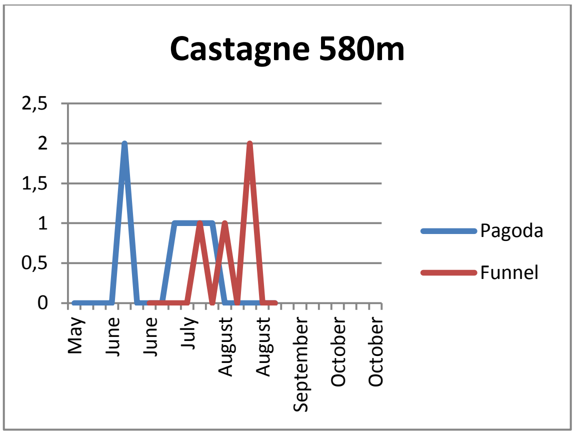











Castagne 580m
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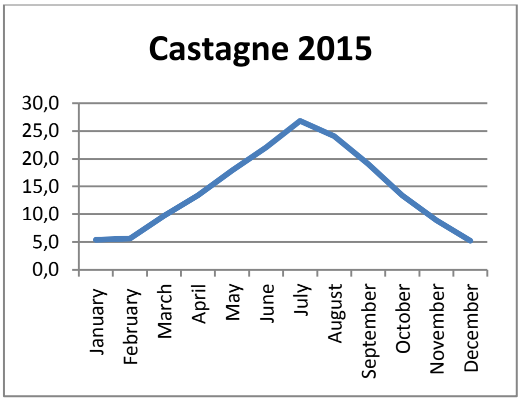
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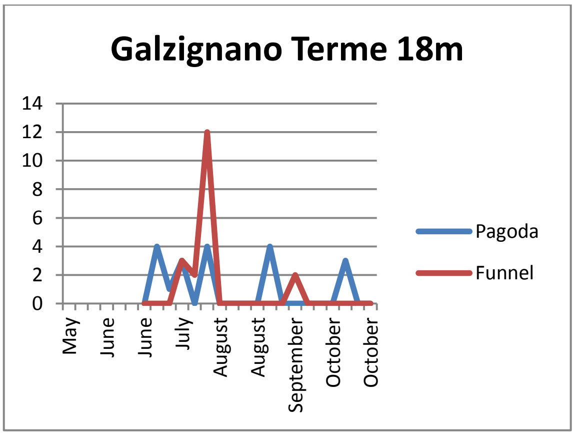
Galzignano Terme 18m
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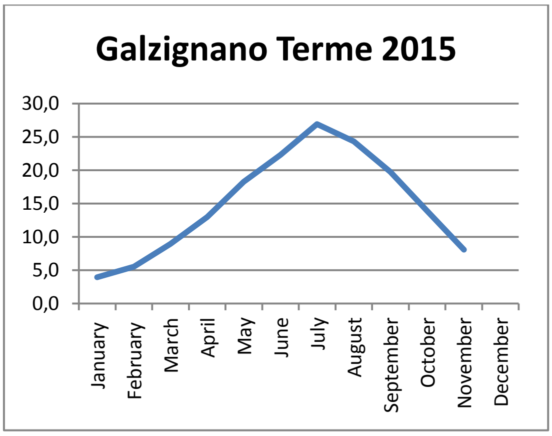
Galzignano Terme 2015
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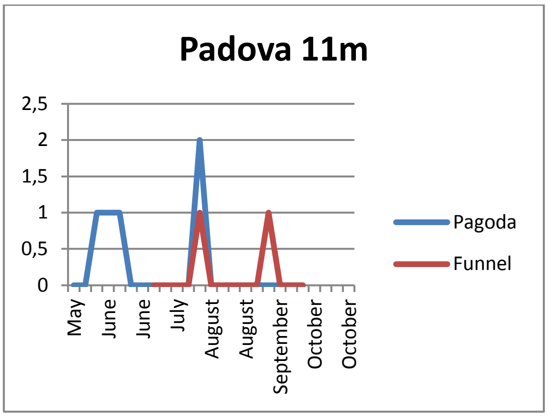
Padova 11m
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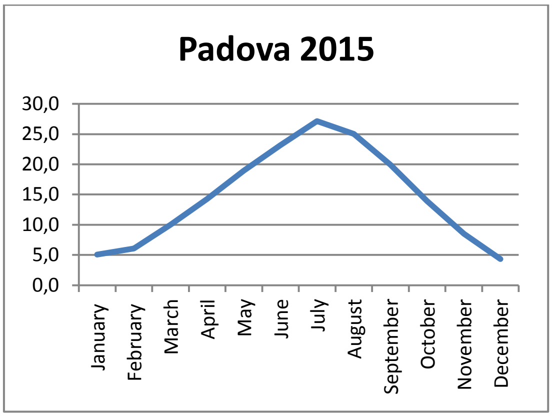
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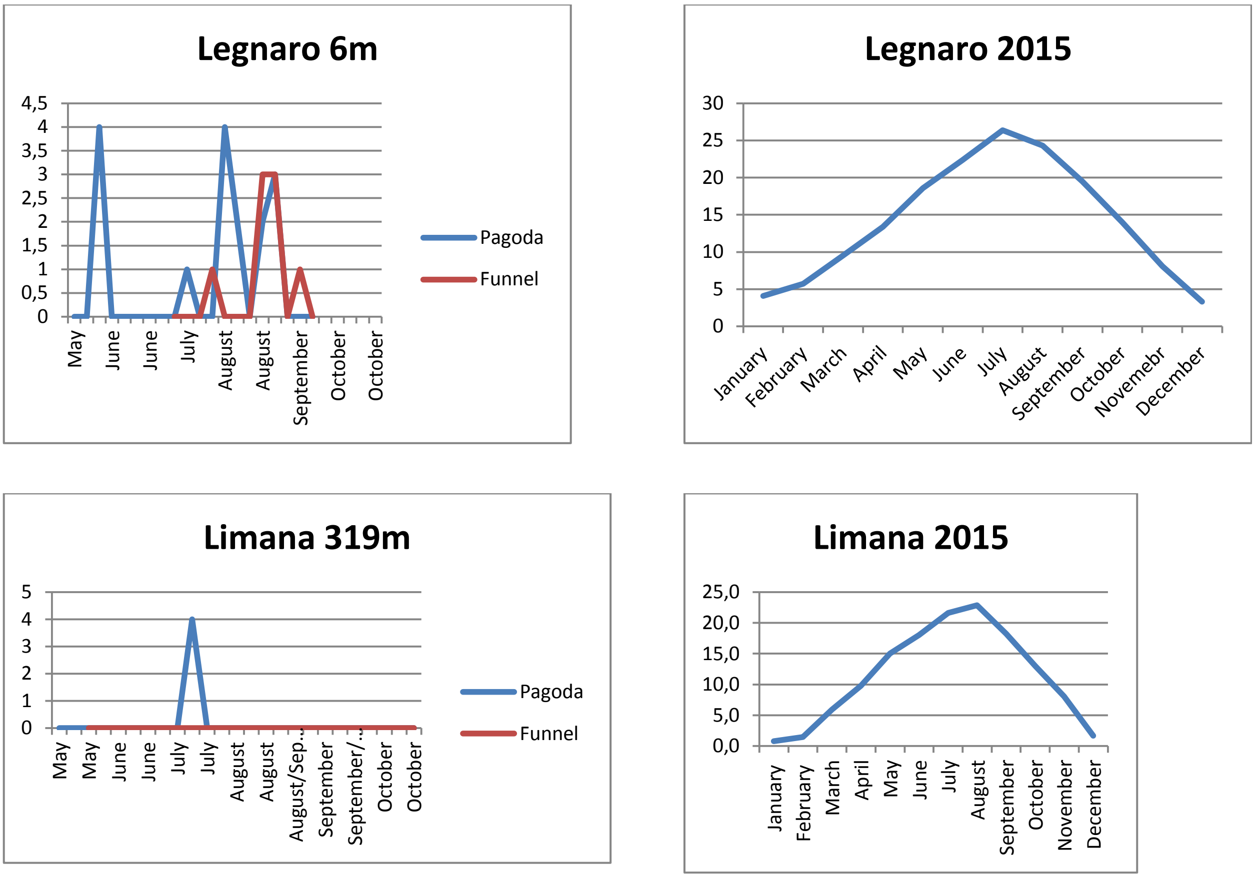

Graph 4 – Captures for each trap in each place (left column) and the annual average temperature / each place for 2015 (right column).

Conclusions – Discussion

According to the results of 2014 and 2015 in the Veneto Region *C. perspectalis* develops three generations / year. Santi *et al.* (2015) performed experiments in Bologna and also found found that *C. perspectalis* occurs 3 generations. Due to small distance between the localities, we could consider the simiral climatic conditions.

According to Nacambo *et al.* (2013) *Cydalima perspectalis* develops 2-3 generations per year in Central Europe while in China can complete 3 to 5 generations, depending on the climatic conditions (Wan *et al.,* 2014).

In 2014 the overwintering larvae started their activity early in February until mid-April while in 2015 one month later until end of April probably due to different climate conditions between these years.

C. perspectalis overwinters in a silk cocoon in-between the leaves as a larva of 2nd instar while in Switzerland overwinters as a larva of 3rd instar (Nacambo *et al,* 2013).

The number of larval instar is 5, while in the native area, it seems that this species complete 6 or 7 larval instars dependently always from the climate conditions (Shinkaji and Maruyama, 1991).

In 2015 the number of captures from the sex pheromones traps was low. No differences were observed between the two types of pheromones. A possible explanation could be that in 2014, when the population of the moth was high, lots of chemicals treatments were performed to control the moth.

C. perspectalis has spread quickly in our environment proving that it has acclimatized. Its damage is considerable noticed to the box plants in parks, historical villas, gardens and urban areas. So far it, seems there has been no adaptation by indigenous natural enemies (parasitoids) to *C. persectalis*. It is not excluded, however, that this will happen in the next few years, as happened before with other alien species (eg. the leafminer of locust *Parectopa robiniella* or the American caterpillar *Hyphantria cunea*).

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Chapter 4

Are *Phenacoccus solani* Ferris and *P. defectus* Ferris (Hemiptera: Pseudococcidae) distinct species?

EVANGELIA CHATZIDIMITRIOU, MAURO SIMONATO, GILLIAN W. WATSON, ISABEL MARTINEZ-SAÑUDO, HIROTAKA TANAKA, JING ZHAO AND GIUSEPPINA PELLIZZARI

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INTRODUCTION

Among the Nearctic species of *Phenacoccus* (Hemiptera: Pseudococcidae), *P. defectus* Ferris, *P. solani* Ferris and *P. solenopsis* Tinsley are unusual in lacking quinquelocular and dorsal multilocular disc pores, and in possessing 18 pairs of cerarii. *Phenacoccus solenopsis* and *P. solani* have spread to Asia, the Mediterranean basin including North Africa, and beyond, and are having a strong economic impact on several important crops (Hodgson *et al*. 2008; Wang *et al.* 2010; Hemba *et al.* 2012). *Phenacoccus defectus* also has been found outside the Nearctic, usually on ornamental plants grown under glass.

Phenacoccus defectus, *P. solani* and *P. solenopsis* are morphologically similar and it can be difficult to separate them using microscopic morphological characters alone, as pointed out in several studies (McKenzie 1967; Williams & Granara de Willink 1992; Culik & Gullan 2005; Hodgson *et al*. 2008). The diagnostic characters used by Ferris and McKenzie (from McKenzie 1967) to separate these species are summarized in Table 1.

Table 1 – Diagnostic characters used by Ferris and McKenzie (in McKenzie, 1967) to separate *Phenacoccus solenopsis*, *P. solani* and *P. defectus*.

Species	**Multilocular disc pores on abdomen**	**No. of antennal segments**	**Circulus**	**Body length in mm**	**Trilocular pores associated with anal lobe cerarius**
Phenacoccus solenopsis	40+, on segments IX to VI, most concentrated around the vulva	9, occasionally 8	moderately large, flaccid, situated between segments IV and III	1.5-4.3	most numerous and concentrated
Phenacoccus solani	"considerable numbers", on segments IX to IV, occasionally only as far forward as segment V	8, sometimes seg. 8 partially divided or with 9 segments	quite small, circular to slightly oval, not divided, sometimes situated above intersegmental line	2.5-3.2	fewer and less concentrated than *P. solenopsis*, more so than *P. defectus*
Phenacoccus defectus	20 or fewer, on segments VIII and VII, rarely 1 pore on VI	9, occasionally 8	oval, divided, situated between segments IV and III	2.2-3.5	relatively few, least concentrated

Phenacoccus solenopsis has a higher number of multilocular disc-pores, more widely distributed on the body, and usually has a larger circulus than *P. defectus* and *P. solani* (Williams & Granara de Willink 1992; Hodgson *et al*. 2008).

Zhao *et al.* (2014) gave molecular and morphological evidence that *P. solenopsis* is distinct from *P. solani*, and separated them using morphological characters alone. However, the morphological boundary between *P. solani* and *P. defectus* remains much less clear. The present knowledge of the distribution, host plants and biology of *P. defectus* and *P. solani* are summarized below, based on the literature.

Phenacoccus solani Ferris, 1918

Phenacoccus solani was described from specimens collected on roots of *Hemizonia rudis* (Asteraceae) in California, Santa Clara County, Palo Alto (Ferris 1918) and later recorded from several other U.S. states (McKenzie 1967); currently it is almost cosmopolitan, having been recorded from the Nearctic, Neotropical, Palaearctic, Afrotropical, Oriental and Australasian Regions (Ben-Dov *et al.* 2015). It is highly polyphagous, most commonly found on Solanaceae, and can cause significant damage to cultivated sweet pepper (*Capsicum annuum*) and many ornamentals.

The first incursion of *P. solani* in the Mediterranean Region was in Sicily (Mazzeo *et al.* 1999); later it was recorded from Israel, Turkey and Spain (Ben-Dov 2005; Kaydan *et al.* 2008; Beltrà & Soto 2011). Lloyd (1952) showed that *P. solani* is a parthenogenetic, thelyotokous species, (confirmed by Ben-Dov 2005) and McKenzie (1967) said it is viviparous; however, at that time workers did not distinguish between viviparity and ovoviviparity. True viviparity is rare in insects so we consider *P. solani* to be ovoviviparous. No males have been recorded for *P. solani* so it is likely that it reproduces parthenognetically.

Phenacoccus defectus Ferris, 1950

Phenacoccus defectus was described from specimens collected on *Eriophyllum confertiflorum* (Asteraceae) in California, Santa Clara County, Permanente Creek (Ferris 1950). Permanente Creek is just 3.2 miles from Palo Alto, the type locality of *P. solani*. *Phenacoccus defectus* was subsequently recorded from several other localities in California (McKenzie 1967) and Mexico (Williams and Granara de Willink 1992). The first European record of *P. defectus* was from Great Britain in 1997, indoors, on *Echeveria* and other succulent plants (Malumphy 1997). In 2006 it was recorded from southern France (Germain & Matile Ferrero 2006) and from 2009 onward, Italy

(Pellizzari & Porcelli 2013). In 2012 it was reported from the Ryukyu Islands of Japan (Tanaka & Uesato 2012).

Phenacoccus defectus develops on plants belonging to the Asteraceae, Chenopodiaceae, Crassulaceae, Euphorbiaceae, Fabaceae, Hydrophyllaceae, Lamiaceae, Poaceae and Polygonaceae (Ben-Dov *et al.*, 2015). In Crassulaceae, it has been recorded on *Aeonium arboreum*, *Crassula portulacea, Echeveria* sp., *E. craigiana*, *E. longissima*, *E. lurida*, *E. recurvata*, *E. sessiliflora*, *Sedum palmeri* and *Sempervivum tectorum* (McKenzie 1967; Williams & Granara da Willink 1992; Malumphy 1997; Pellizzari & Porcelli 2013). The species is parthenogenetic and ovoviviparous (Malumphy 1997; Pellizzari & Porcelli 2013).

Comparison of the host ranges of *P. solani* and *P. defectus* shows that they share the same host families (including Crassulaceae) (Ji & Suh 2012) except for Hydrophyllaceae, on which only *P. defectus* has been recorded (McKenzie 1967).

The aim of this study was to examine whether *P. defectus* and *P. solani* are distinct species, and if so, to discover a reliable means of separating them. A morphological analysis of adult females of the two species was performed to see whether these nominal species form separate populations, and to identify any consistent morphological differences between them. Williams & Granara de Willink (1992) used circulus size and shape, and the number of antennal segments to help separate *P. solenopsis* from *P. solani*, but Hodgson *et al.* (2008) found that both these characters were too variable to be reliable for diagnosis. Neither of these unreliable characters have been used in the past to distinguish between *P. defectus* and *P. solani,* so they were not used in this analysis.

A molecular analysis using the mitochondrial CO1 and nuclear 28S genes was carried out on specimens from different parts of the world to determine whether *P. defectus* and *P. solani* are distinct species.

MATERIALS AND METHODS

Material examined for the morphological analysis

Slide-mounted adult females of the two species were studied and notes on their morphology made by G.W. using a Zeiss compound light microscope with phase contrast illumination and magnifications of x20 to x800. The characters scored are listed under Morphological analysis below. The material examined (199 specimens) is listed under the original determinations below, in order of the depository and country of origin. The number given after the collection date is the number of specimens examined from that sample.

Abbreviations of depositories: UCD = Bohart Museum of Entomology, University of California, Davis; CSCA = California State Collection of Arthropods, California Department of Food and Agriculture, Plant Pest Diagnostic Center, Sacramento, California, U.S.A.; DAFNAE = Department of University of Padova, Italy; RUMF = Ryukyu University Museum, Fujukan, Japan.

Specimens from some of the samples used for molecular analysis were included in the morphological analysis (indicated by * in the listing below).

Phenacoccus defectus

Material at UCD:

Type material: holotype, U.S.A., California, Santa Clara Co., Permanente Creek, on roots of *Eriophyllum confortiflorum*, coll. G.F. Ferris, 5.v.1917. Paratypes, same data as holotype, 5; and California, Santa Clara Co., Stevens Cr., on *Monardella* sp., coll. G.F. Ferris, 26.viii.1917, 1.

Material at CSCA:

U.S.A., California:- Alameda County, Berkeley, on *Echeveria rubella*, coll. G.B. Laing, 24.i.1949, 3; Los Angeles County, Huntington Gardens, on *Echeveria carnicolor*, coll. A. Wiens, 8.iii.1993, 7; on *Centaurea diluta*, coll. T.C. Fuller, 27.vi.1963, 4; Sacramento County, Sacramento, on *Hibiscus* sp., coll. K. Casanave, 21.ii.2001, 4; San Diego County, Ramona, on *Echeveria* sp., coll. G.L. Hill, 1.ii.1957, 3; Vista, on *Echeveria* sp., coll. Mariscal, 5.x.1981, 4; Ramona, on *Echeveria* sp., coll. G.L. Hill, 16.i.1957, 4; Siskyu County, Etna, on *Sempervirens* sp., coll. W. Ferlatte, 8.ix.1995, 3; Ventura County, Oxnard, on *Ophiopogon japonicus*, coll. E. Kragh, 5.iv.1995, 4; Nevada; Orovada, on *Atriplex confertifolia*, coll. B.L. & K., 26.viii.1959, 4; ex Nevada, Las Vegas, intercepted at California, Yermo Inspection Station, on *Solanum lycopersicum*, coll. Martin, 24.viii.2009, 10.

Canada, British Columbia, White Lake, on *Centaurea diffusa* with ants, coll. T.P. Cuda, 25.iv. 1989, 1.

Material at RUMF:

Japan, Okinawa, collected by H. Tanaka: Ginowan, Ganeko, on *Ruellia brittoniana*, 30.viii.2011, 5; Naha, Shikina garden, on *Ruellia brittoniana*, 9.iii.2011, 12; Naha, Tomari, on *R. brittoniana*, 30.vii.2014, 2; on *Sphagneticola trilobata*, 30.vii.2014, 10; Nishihara, Sakata, on *Ruellia brittoniana*, 9.iii.2011, 12.

Material at DAFNAE (from the cultures from which the DNA samples were taken)**:**

Italy, material reared at the DAFNAE laboratory, collected by G. Pellizzari: on *Sempervivum* sp., 7.vii.2010, 2; on *Echeveria* sp.*, 30.ix.2013, 2; on *Echeveria* sp.*, 11.vii.2014, 2; on *Solanum lycopersicum*, 7.x.2013, 5; on *S. tuberosum* sprouts, 30.ix.2013, 4; on *S. tuberosum* sprouts, 26.vi.2014, 1; on *Sedum* sp., 2.ix.2011, 1.

Phenacoccus solani

Material at UCD:

Type material: holotype, U.S.A., California, Santa Clara County, Palo Alto, Stanford University campus, on *Hemizonia rudis*, coll. G.F. Ferris, 21.x.1916. One paratype on same slide as holotype.

Non-type material identified by G.F. Ferris: **U.S.A.**, California:- Glenn County, Orland, on roots of pigweed, coll. H.S. Smith, 15.x.1922, 3; Los Angeles County, Montebello, on *Eucharis amazonica*, coll. L.E. Meyers, 21.vii.1942, 2; Orange County, between Orange and Olive, on nutgrass, coll. R.J. Bumgardner, 11.x.1956, 2; Riverside County, Riverside, on injured tomato fruit, coll. Mr. French, ix.1924, 2; 3.4 mi. N.E. of Gilman Springs, on *Artemisia absinthum*, coll. D.R. Miller, 9.iii.1963, 2; Ventura County, on purslane, coll. A.H. Call, 1924, 2; Yolo County, Davis, on *Sida hereaceae*, coll. M.F. Benson, D.R. Miller & H.L. McKenzie, 2.vii.1964, 2; Florida, Alachua County, Gainesville, on *Ambrosia actinifolia*, coll. C.Q. Drake, vii.1918, 2.

Material at CSCA:

U.S.A., Arizona, Navajo County, Show Low, host not recorded, coll. D.M. Tuttle, 24.vii.1964, 1; California:- Fresno County, Fresno, on *Lycopersicon* sp., coll. G. Gaffney, 19.viii.2004, 3; Imperial County, Salton City, on *Mangifera indica*, coll. J. Barcinas, 9.i.1994, 3; Inyo County, Independence, on *Ambrosia* sp., coll. R.P. Allen, 28.vii.1965, 2; Lassen County, Milford, on *Nicotiana* sp., coll. Ogden, Pfeiffer & Rulofson, 15.vii.1992, 6; Modoc County, Cedarville, on roots of composite, coll. T.R. Haig, 21.v.1962, 1; Monterey County, King City, Oasis Road, on *Vitis vinifera*, coll. L. Bettiga, 19.v.2008, 9; Florida, Broward County, Fort Lauderdale, host not recorded, coll. B. Steinberg, 5.vi.1988, 3; Ex Texas, intercepted at California, Needles Inspection Station, on *Gossypium hirsutum*, coll. K.R. Hansen, 16.viii.1985, 1; Utah, Tooele

County, Knolls, on roots of *Hermazoni*a sp., coll. R.F. Wilkey, 10.vii.1964, 1; Washington, Walla Walla County, Walla Walla, on *Achillea* sp., coll. S. Nakahara, 29.v.1966, 4.

Canada, British Columbia, White Lake, on *Centaurea diffusa*, coll. T.R. Cuda, 25.ix.1989, 2.

Guam, Tamuning, on spider lily, coll. R. Quitugua, 18.xii.2006, 1.

Israel*, Jordan valley, Patza'el, 32°02′39″N, 35°26′57″E, 270 m, on *Capsicum annuum*, coll. Z. Mendel, viii.2013, 3. Bet-Dagan, host not specified, coll. unknown, 15.vii.2007, 1; ?ex Israel via U.S.A., New York, intercepted at California, San Francisco County, San Francisco, on *Artemisia dracunculus*, coll. D. Fulford, 22.x.2007, 1.

Iran, Shiraz, on *Vitis vinifera*, coll. V. Roumi, viii.2009, 5.

Brazil, Rio de Janeiro, on *Citrus* sp., coll. M. Rose, 13.iii.1973, 3.

American Samoa*, Tutuila I., Ottoville, Tafuna, on *Hymenocallis littoralis*, coll. M. Schmaedick, 22.xi.2013, 6.

Ex **Australia** via U.S.A., Oregon, intercepted at California, Hornbrook Inspection Station, on *Citrus sinensis* fruit, coll. Pastell, 16.viii.2000, 1.

Japan*, Okinawa, Ginowan, Ganeko, on *Ruellia brittoniana*, coll. H. Tanaka, 30.viii.2011, 5.

Turkey*, Adana, originally collected from Reşatbey on *Gazania rigens*, coll. A.F. Çalışkan, 27.ix.2012, subsequently laboratory reared on *Solanum tuberosum,* 2 measured specimens killed 19.vii.2013.

Material at RUMF:

Japan, Honshu, Yamaguti Pref., Yamaguti, reared on *Solanum melongena*, coll. Y. Higashiura, ?.i.2011, 3.

Morphological analysis

We used eight morphological characters, based on those used for the separation of *P. defectus* and *P. solani* by Hodgson *et al*. (2008) with a reported success rate of 90%:

1. Number of multilocular disc pores on posterior margin of abdominal segment VII.
2. Number of multilocular disc pores on anterior margin of abdominal segment VII.
3. Number of multilocular disc pores on posterior margin of abdominal segment VI.
4. Number of multilocular disc pores on posterior margin of abdominal segment V.
5. Number of multilocular disc pores on posterior margin of abdominal segment IV.
6. Total number of multilocular disc pores on segment III.

7. Number of multilocular disc pores on the submargins of the abdominal segments (not included in the pore counts above).

8. Total number of abdominal segments bearing multilocular disc pores.

Prior to analysis, the raw character scores each had 0.5 added to the value (to eliminate zero values) and were logarithmically converted to satisfy the prerequisite conditions of PCA, such as variables' continuity and homoscedasticity. These standardized data were then used for Principal component analysis (PCA), which was conducted by H.T. using the "princomp" function in R 3.1.1 (R Core Team 2014).

Origins of the samples used in molecular analysis

The samples of adult female *P. solenopsis*, *P. solani* and *P. defectus* analyzed in this study, previously identified based on their morphology, were collected from Europe (Italy), Asia (Turkey, Israel, Japan) and U.S.A. (Arizona, American Samoa) (Table 2).

Table 2 – Origins and host plants of the specimens sequenced for the CO1 and 28S genes. All the specimens are named using the determinations provided by the systematists who supplied them; their identification was confirmed by the present authors, based on the literature currently available. Specimens from some of the samples used for molecular analysis were included in the morphological analysis (indicated by *).

No.	Species	Locality	Host plant	CO1	28s	Collector
1	*P. defectus*	Italy*, Padova	*Echeveria* sp. (Crassulaceae)	KT369519	KT369510	G. Pellizzari
2	*P. defectus*	U.S.A., Arizona	*Euphorbia sp.* (Euphorbiaceae)	KT369520	KT369511	G. Watson
3	*P. solani*	Israel*, Jordan valley	*Capsicum annum* (Solanaceae)	KT369521	KT369518	Z. Mendel
4	*P. solani*	Turkey*, Adana	Reared on *Solanum tuberosum* (Solanaceae)	KT369528	KT369514	B. Kaydan
5	*P. solani*	Japan, Okinawa	*Ruellia brittoniana* (Acanthaceae)	KT369522	KT369517	G. Pellizzari
6	*P. solani*	American Samoa*	*Hymenocallis littoralis* (Amaryllidaceae)	KJ620517	KT734863	G. Watson
7	*P. solenopsis*	Japan, Kyushu, Hukuoka	*Ambrosia artemisiifolia* (Asteraceae)	AB858432		H. Tanaka
8	*P. solenopsis*	Turkey, Adana	*Hibiscus syriacus* (Malvaceae)	KT369525	KT369512	B. Kaydan
9	*P. solenopsis*	Turkey, Adana	*Hibiscus rosasinensis (*Malvaceae)	KT369527		B. Kaydan
10	*P. solenopsis*	Turkey, Adana	*Mirabillis jalapa* (Nyctaginaceae)	KT369523	KT369516	B. Kaydan
11	*P. solenopsis*	Turkey, Adana	*Solanum lycopersicum* (Solanaceae)	KT369524	KT369513	B. Kaydan
12	*P. solenopsis*	Israel, Arava valley	*Hibiscus sinensis* (Malvaceae)	KT369526	KT369515	Z. Mendel

Molecular analysis

All the specimens subjected to molecular analysis were first identified by the systematists who supplied them; their identification was confirmed by the present authors, based on the literature currently available (see Table 2). All the specimens were preserved in 100% ethanol and stored at -20° C. The DNA extraction was performed by the CTAB protocol (Henrion *et al.* 1994) with slight modifications. At least three specimens from each sample were processed. To assess the quality of the DNA, aliquots from extracted samples were separated in a 1% agarose gel and viewed under UV light after staining with SYBR Safe DNA gel stain (Invitrogen).

To study the phylogenetic relationships among specimens, two gene fragments were amplified: a fragment containing the mitochondrial cytochrome oxidase subunit I (COI) gene and the D2 and D3 region of the large subunit of ribosomal RNA gene. All PCR reactions were performed in a 20 µl volume containing 2 µl of DNA extract, 4 µl PCR Buffer 5X GoTaq Flexi Buffer (Promega), 2.5 mM MgCl2, 100 µM dNTPs, 0.5 µM of each primer and 1 U of GoTaq Flexi DNA polymerase (Promega).

The COI gene fragment was amplified using for the 5’ region the forward primer LCO-M-2dF (5’-ATAACTATACCTATYATTATTGGAAG-3’) (Malausa *et al.* 2011) and the reverse primer HCO2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer *et al.* 1994) and for the COI 3’ region the forward primer C1-J-2195 (5’ -TTGATTTTTTGGTCATCCAGAAGT-3’) and the reverse primer TL2-N-3014 (5’-TCCAATGCACTAATCTGCCATATTA -3’) from Simon *et al.* (1994). Additionally, a fragment of the 28S gene was amplified using the forward primer S3660 (5' - GAG AGT TMA ASA GTA CGT GAA AC - 3') (Dowton & Austin 1998) and the reverse primer A335 (5' - TCG GAR GGA ACC AGC TAC TA - 3') (Whiting *et al.* 1997).

The cycling programs were carried out in an Eppendorf Mastercycler Gradient. The PCR protocol for both COI regions consisted of a first step at 95°C for 3 min followed by 35 cycles with a denaturation step of 96°C for 1 min., an annealing step ranging 50°C for 45 sec and an extension step of 72°C for 1 min 15 sec, followed by a final extension at 72°C for 5 min.

The standard thermal profile for the 28S gene was: 94ºC for 4 min followed by 35 cycles of 94ºC for 1 min, 55ºC for 1 min, and 72ºC for 1 min 30 sec with a final extension at 72ºC for 4 min. The amplified products were separated in a 1% agarose gel and viewed under UV with SYBR Safe (Invitrogen) staining. PCR products were purified using Exonuclease and Antarctic phosphatase (GE Healthcare) before sequencing. Sequencing was performed at the BMR Genomics service (Padova, Italy).

The sequences generated were inspected and aligned using MEGA 6.0.6b3 (Tamura *et al.* 2013). A BLASTN GenBank analysis of the sequences obtained was run through the NCBI website (www.ncbi.nlm.nih.gov) to assess the identity of the sequences obtained. A sequence from the mealybug *Planococcus citri* (Risso) was also included in the analysis as an out-group for the COI (GenBank Accession number: FJ786963) and 28S (JQ651165) data sets.

Phylogenetic analysis

All COI sequences were translated with Transeq (EMBOSS: http://www.ebi.ac.uk/Tools/emboss/transeq/index.html) to exclude the presence of stop codons in the coding sequences. Moreover, in order to test for nucleotide composition bias among taxa, the program STATIO (Rzhetsky & Nei 1995) was used on both COI and 28S genes. A partition homogeneity test (ILD test of Farris *et al.* 1995) was performed for the COI and the 28S gene trees using PAUP*4b10 (Swofford 2002) in order to combine the different gene regions for the phylogenetic analysis. The test confirmed that these regions contained homogeneous signals ($p=0.56$), allowing data to be pooled for further analyses.

Phylogenetic relationships among sequences of insects with the combined COI and 28S data sets were estimated using two methods: approximate maximum-likelihood (ML) and Bayesian inference (BI) analysis. The ML tree was obtained using PHYML v2.4.4 software (Guindon & Gascuel 2003), with neighbor-joining starting trees and 1000 bootstrap replications using a GTR + I + G model. Clades were considered statistically significant with bootstrap probabilities (Bp) values of 70% (Hillis & Bull 1993). For the BI analysis the software MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) was used applying a GTR + I + G model. The 50% majority rule consensus tree and Bayesian posterior probabilities were obtained by discarding the first 25% of sampled generation. Convergence between runs was assessed with Tracer v1.5 (Rambaut & Drummond 2009) checking for both estimated sample size (ESS) and the stabilisation of log-likelihood scores (lnL). Posterior probabilities (Pp) values equal or higher than 95% were considered statistically significant (Huelsenbeck & Rannala 2004). Pairwise genetic distances between species were calculated for COI using the Kimura 2-parameter in MEGA 6.06b3.

RESULTS

Morphological analysis

The full collection data and character values for the type material of *P. defectus* and *P. solani* are given in Table 3.

Table 3 – Morphological character scores for type specimens of *Phenacoccus defectus* and *P. solani* examined in this study. Abbreviations: no. = number of; mldp = multilocular disc pore; post. = on posterior margin of; ant. = on anterior margin of; abd. seg. = abdominal segment.

Identity and data	**No. mldps post. abd. seg. VII**	**No. mldps ant. abd. seg. VII**	**No. mldps post. abd. seg. VI**	**No. mldps ant. abd. seg. VI**	**No. mldps post. abd. seg. V**	**No. mldps ant. abd. seg. V**	**No. mldps post. abd. seg. IV**	**No. mldps ant. abd. seg. IV**	**No. mldps abd. seg. III**	**No. mldps on submargins of abd. segs**	**Total mldps counted here**	**No. abd. segs with mldps on**
P. defectus holotype: California, Santa Clara Co., Permanente Creek, on roots of *Eriophyllum confortiflorum*, coll. G.F. Ferris, 5.v.1917	6	0	0	0	0	0	0	0	0	0	6	1
P. defectus paratype: data as holotype; row of 3, smallest	10	0	0	0	0	0	0	0	0	0	10	1
P. defectus paratype: data as holotype; row of 3, middle	6	0	0	0	0	0	0	0	0	0	6	1
P. defectus paratype: data as holotype; row of 3, larger at end	7	0	0	0	0	0	0	0	0	0	7	1
P. defectus paratype: data as holotype; row of 2, smaller	4	0	0	0	0	0	0	0	0	0	4	1
P. defectus paratype: data as holotype; row of 2, larger	5	0	0	0	0	0	0	0	0	0	5	1
P. defectus paratype: California, Santa Clara Co., Stevens Cr., on *Monardella* sp., coll. G.F. Ferris, 26.viii.1917	6	0	0	0	0	0	0	0	0	0	6	1
P. solani holotype: USA, Santa Clara County, Palo Alto, Stanford University campus, on *Hemizonia rudis*, coll. G.F. Ferris, 21.x.1916	23	0	21	0	11	0	3	0	0	0	58	4
P. solani paratype: on same slide as holotype	25	0	20	0	16	0	5	0	0	6	72	4

Multivariate analysis of the morphology

A scatter plot of the first vs second principal component scores (PC1 vs PC2) from the PCA of *P. defectus* and *P. solani* specimens is shown in Figure 1. The proportion of variance and principal component loadings of each character on the first and second PCs are given in Table 4. The cumulative contribution ratio of PC1 + PC2 is over 73% of the variance, so Figure1 represents a significant portion of the morphological variation in the material analyzed. The plot of PC1 vs PC2 scores for each individual (Figure 1) forms a continuous scatter, in which it is difficult to recognize any distinct grouping.

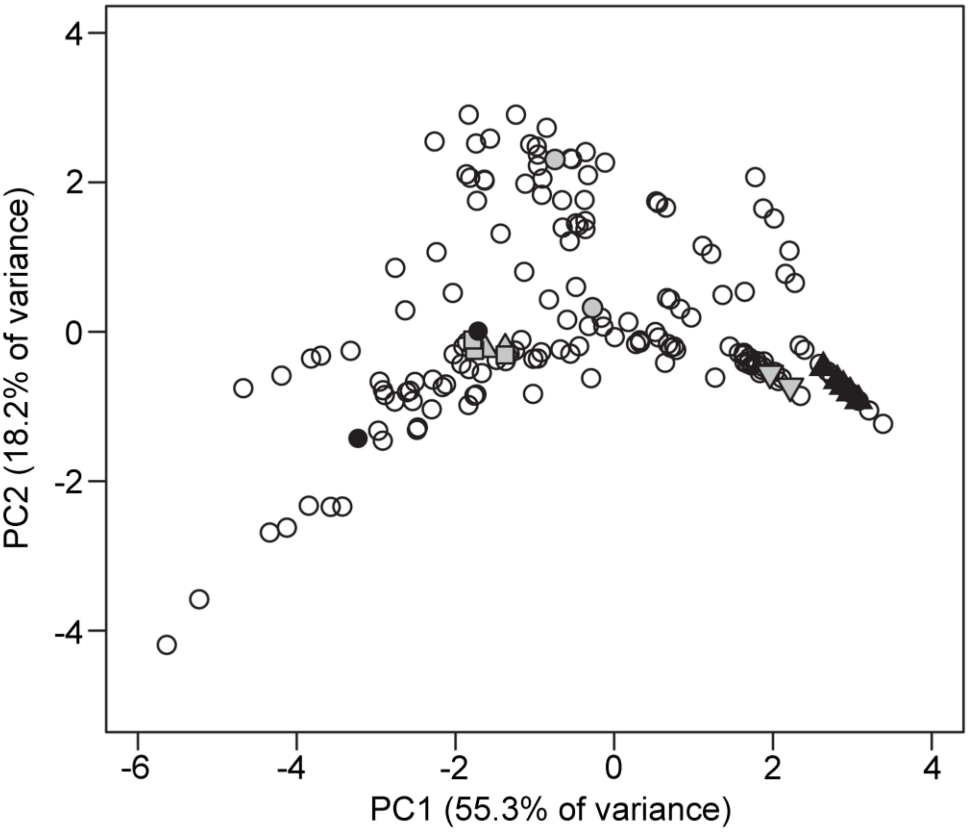

Figure 1. A scatter plot of the first two principal component (PC) scores from the Principal Component Analysis of 199 specimens. Solid black circles = *Phenacoccus solani* type specimens; solid black triangles = *P. defectus* type specimens; grey-filled circles = voucher specimens of *P. solani* from Japan from the population used for DNA extraction; grey-filled upwards-pointing triangles = voucher specimens of *P. solani* from Turkey from the population used for DNA extraction; grey-filled downward-pointing triangles = voucher specimens of *P. defectus* from Italy from the population used for DNA extraction; grey-filled squares = voucher specimens of *P. solani* from Israel from the population used for DNA extraction; and open circles = other non-type specimens of either *P. defectus* or *P. solani*.

Table 4 – Principal component loadings of each character and cumulative proportion of variance on the first and second principal components (PC) of the Principal Component Analysis.

Character	**PC1**	**PC2**
Number of multilocular disc pores on anterior margins of abdominal segments VII	-0.365	0.365
Number of multilocular disc pores on posterior margins of abdominal segments VII	-0.116	0.704
Number of multilocular disc pores on posterior margins of abdominal segments VI	-0.415	0.190
Number of multilocular disc pores on posterior margins of abdominal segments VI	-0.442	-0.039
Number of multilocular disc pores on posterior margins of abdominal segments IV	-0.390	-0.256
Total number of multilocular disc pores on segment III	-0.210	-0.373
Number of multilocular disc pores on the submargins of the abdominal segments	-0.320	-0.357
Total number of abdominal segments bearing multilocular disc pores	-0.432	0.037
Proportion of variance	0.553	0.182

Molecular analysis

Sequences of the COI gene 620 bp long were obtained for all 12 samples analyzed. The presence of stop codons in the coding sequences was excluded by Transeq. All the substitutions were found to be synonymous thus not affecting the amino acid sequence. For the D2 and D3 expansion regions of the 28S gene, a portion of 684 bp long was obtained for 11 out of the 12 samples. For one sample of *P. solenopsis* from Adana (Turkey) (Code 9 Table 1) it was not possible to amplify this region successfully. The analysis of nucleotide composition bias among taxa showed that the null hypothesis of DNA composition stationarity could not be rejected for both the COI and 28S genes. By combination of the two datasets, the resulting concatenated data was 1304 bp long (Figure 2).
The trees inferred separately from each gene were concordant between ML and BI analyses; both methods yielded similar topologies with different statistical supports for branch nodes. Moreover, the 28S and COI trees were topologically similar (Figs. S1 and S2).

The phylogenetic tree (Figure 2) distinguished between two highly supported clades. One of them grouped all the *P. solenopsis* samples together, while the other contained all the specimens of *P. solani* and *P. defectus*. Within the latter clade, the two sequences obtained from *P. defectus* specimens (collected in Italy and U.S.A.) were clustered in a highly resolved subclade while the relationships among the *P. solani* specimens were not clear, although the two specimens of *P. solani* from Israel and from USA formed a strongly supported group based on COI (Figure S1) but not on 28S (Figure S2).

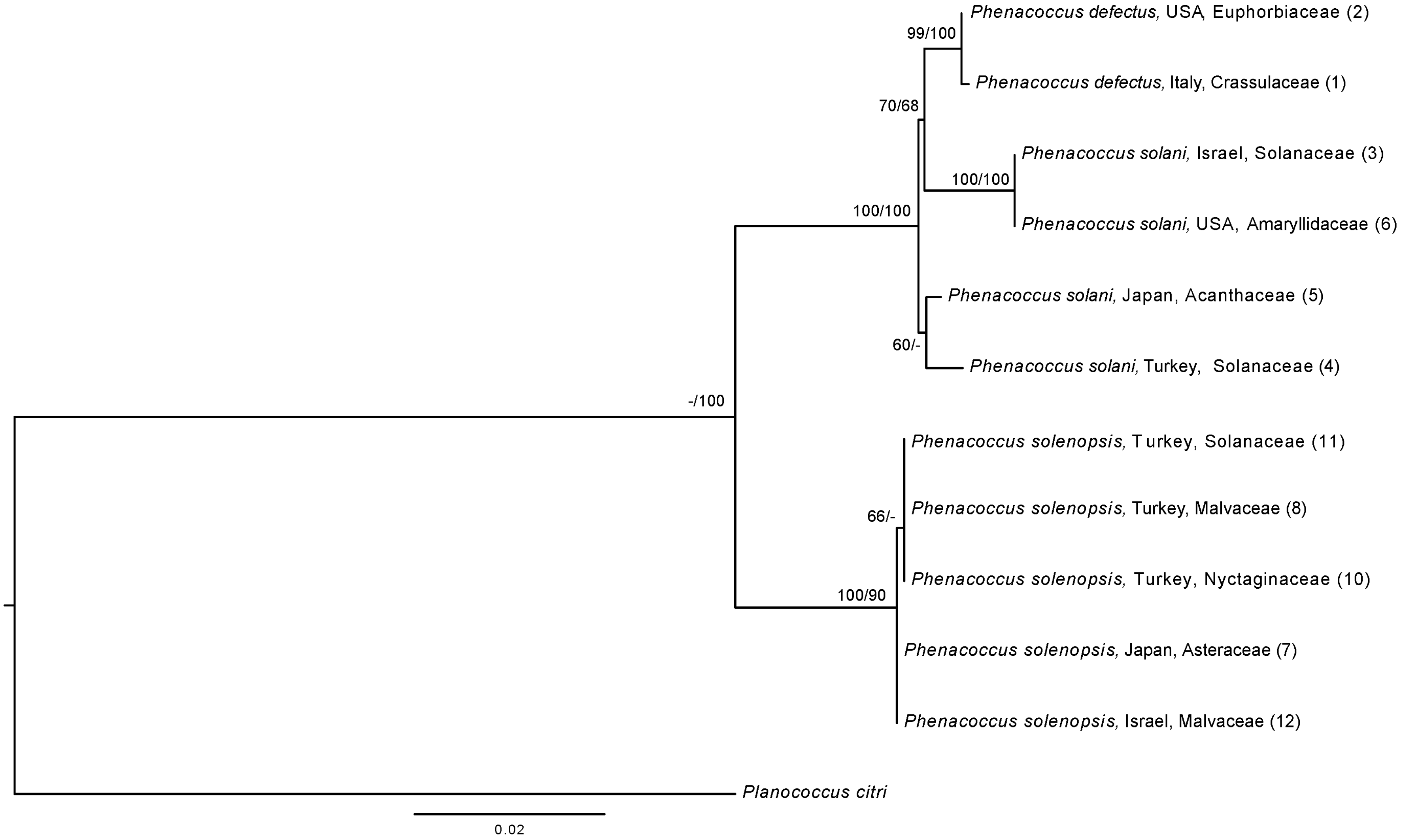

Figure 2. Maximum likelihood tree for *P. defectus, P. solani* and *P. solenopsis* based on CO1 and 28S sequences. Numbers on the nodes refer to ML bootstrap and Bayesian posterior probability values. The data for the outgroup was obtained by using the Genbank sequences for CO1 (FJ786963) and 28S (JQ651165) for *Planococcus citri*.

High genetic distances were found between *P. solenopsis* and *P. solani* (6.9%), and *P. solenopsis* and *P. defectus* (7.8%) for COI. In contrast, only slight divergence was shown between *P. defectus* and *P. solani* (2.1%) even when splitting *P. solani* into the two subgroups found in the phylogenetic tree (2.3% between *P. defectus* and *P. solani* from USA and Israel and 1.7% between *P. defectus* and *P. solani* from Turkey and Japan). In addition, a similar distance was found when considering the two latter *P. solani* subgroups (2.2%), while a low genetic distance was found between the two sequences of *P. defectus* from Italy and the U.S.A. (0.1%).

Discussion

Morphological analysis

The morphological analysis supports the synonymy of *P. defectus* and *P. solani*, because although the type specimens of the two "species" are widely separated from each other in the canonical variates plot (Figure 1), they are all part of a continuous range of morphological variation (i.e. there is no division of the specimens into two discrete populations on the basis of morphology). The spread of the points reflects morphological variation, whereas each small cluster probably represents specimens belonging to one sample (as shown by the placement of the type specimens of *P. defectus*).

Previous work on morphological variation in Pseudococcidae by Cox (1983) and Charles *et al.* (2000) and in Putoidae by Marotta & Tranfaglia (1995) showed that morphological characters such as the number of multilocular disc pores and tubular ducts are subject to variation induced by environmental factors like ambient temperatures during development. Generally, specimens that develop in higher temperatures tend to have fewer pores and ducts (Marotta & Tranfaglia 1995). It was the amount of morphological variation in *P. defectus* and *P. solani*, resulting in difficulties in separation of these nominal species, that led to the present study. The cross-breeding method used by Charles *et al.* (2000) to investigate morphological variation could not be applied in the present work because *P. defectus* and *P. solani* are both asexual.

As indicated in Figure 1, the morphological data from 199 specimens of *P. defectus* and *P. solani* collected from different parts of the world showed a considerable range of morphological variation. For example, the total number of multilocular disc pores per specimen varied from two to 114, and the number of abdominal segments bearing multilocular disc pores varied from one to five (the latter being highest in specimens with the highest pore counts).

Part of the observed morphological variation is likely to reflect the climatic conditions (such as temperature and humidity) experienced by the insects during their development. However, the relationship between pore count and environmental conditions in *P. defectus* and *P. solani* is not simple. For example, the specimen with the highest pore count was collected in USA, California, Lassen County, at about 1287 m altitude and 40º 10' 17" N in July (with a wide daily temperature range including cool nights, at fairly low humidity and long days), whereas the specimen with the lowest pore count was collected in USA, California, at about 436 m altitude at around 33º 2' 9" N in February (with a narrower daily temperature range and mild nights, in fairly low humidity and shorter days). A striking climatic difference between these sites is that Milford experiences freezing conditions in the winter, whereas Ramona does not. On the other hand, the specimen with the next highest pore count was collected from USA, California, Imperial County, Salton City, at -38 m altitude in January (with a wide daily temperature range with cool nights, at fairly low humidity), and the specimen with the next lowest pore count was collected from USA, California, Alameda County, Berkeley, below 535 m altitude in January (with a narrower daily temperature range with mild nights, at more moderate humidity); in this case, neither locality has freezing winters but Salton City experiences summer temperatures over 41º C, whereas summer temperatures in Berkeley rarely exceed 24º C. Both these comparisons differ from the pattern proposed by Cox (1983) and Marotta & Tranfaglia (1995), in that the locations with the widest temperature ranges (sometimes including freezing winters or extremely high summer temperatures) appear to be correlated with high pore counts, whereas low pore counts appear to be correlated with more moderate temperature ranges and milder winters.

Molecular analysis

Several DNA-based phylogenetic studies have been carried out to elucidate the classification of the family Pseudococcidae. Some of these studies (Malausa *et al.* 2011; Zhao *et al.* 2014) used the COI region, due to importance of this region in the 'barcoding of life". Other studies used the 28S nuclear gene for fine-scale analysis of cryptic species complexes and delimitation of species (Downie & Gullan 2004; Hardy *et al.* 2008). We therefore selected both the COI region and D2 and D3 expansion regions of the 28S nuclear gene to discriminate the three closely related taxa in this study. We have confirmed that *P. solenopsis* is a species separate from *P. solani*, as demonstrated by Zhao *et al.* (2014) and Chatzidimitriou et al. (2014), and is also clearly distinct from *P. defectus*. Inside the *P. solani* clade, *P. defectus* appears as a distinct subgroup (Figure2), suggesting that the morphological differentiation observed might not be related to environmental factors as inferred by

Hodgson *et al.* (2008), but may reflect a genetic differentiation. This supports our deductions based on the morphological variation observed (discussed above).

The molecular analysis found that the average genetic distance at the COI 5' region between *P. solani* and *P. defectus* was 2.1%. Since the deepest intraspecific differences observed by Park *et al.* (2011) among mealybugs were 2.5–5.8%, this suggests that the *P. solani* and *P. defectus* groups pertain to the same species. However, the authors did not exclude that these differences could reflect the presence of cryptic species not considered by current taxonomic treatments.

The fact that both *P. solani* and *P. defectus* reproduce parthenogenetically complicates assessment of the taxonomic status of these taxa, as cross-breeding experiments are not possible. It would be interesting to determine whether the parthenogenesis in *P. solani* and *P. defectus* is constitutive or induced by endosymbionts such as *Wolbachia* (Stouthamer *et al.* 1999). Although the number of specimens in the molecular analysis was small, apparently neither host plant or geographical origin were determinant in the *P. solani* and *P. defectus* clade differentiation, as neither of these factors was linked with the phylogenetic clades found.

Based on the morphological and molecular analyses, *P. defectus* Ferris, 1950 is here synonymized under the senior name *P. solani* Ferris, 1918 **syn. n.** The nomenclature for *P. solani* is:

Pseudococcus solani Essig, 1909: 36. Misidentification.
Phenacoccus solani Ferris, 1918: 60.
Phenacoccus herbarum Lindinger, 1942: 115.
Phenacoccus defectus Ferris, 1950: 137, **syn. n.**

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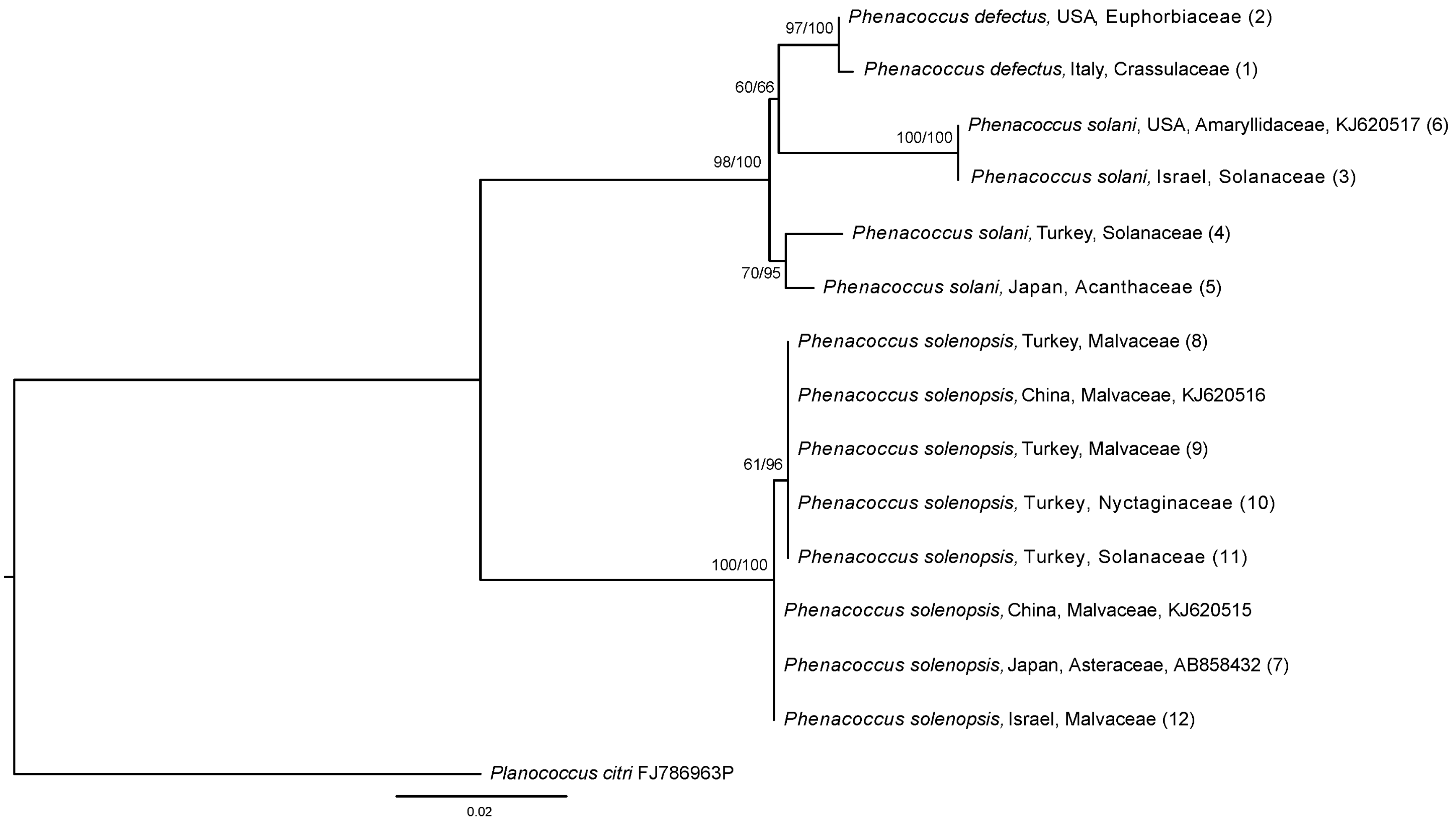

Figure S1. Maximum likelihood tree for *Phenacoccus defectus*, *P. solani* and *P. solenopsis* based on CO1 sequences. Numbers on the nodes refer to ML bootstrap and Bayesian posterior probability values.

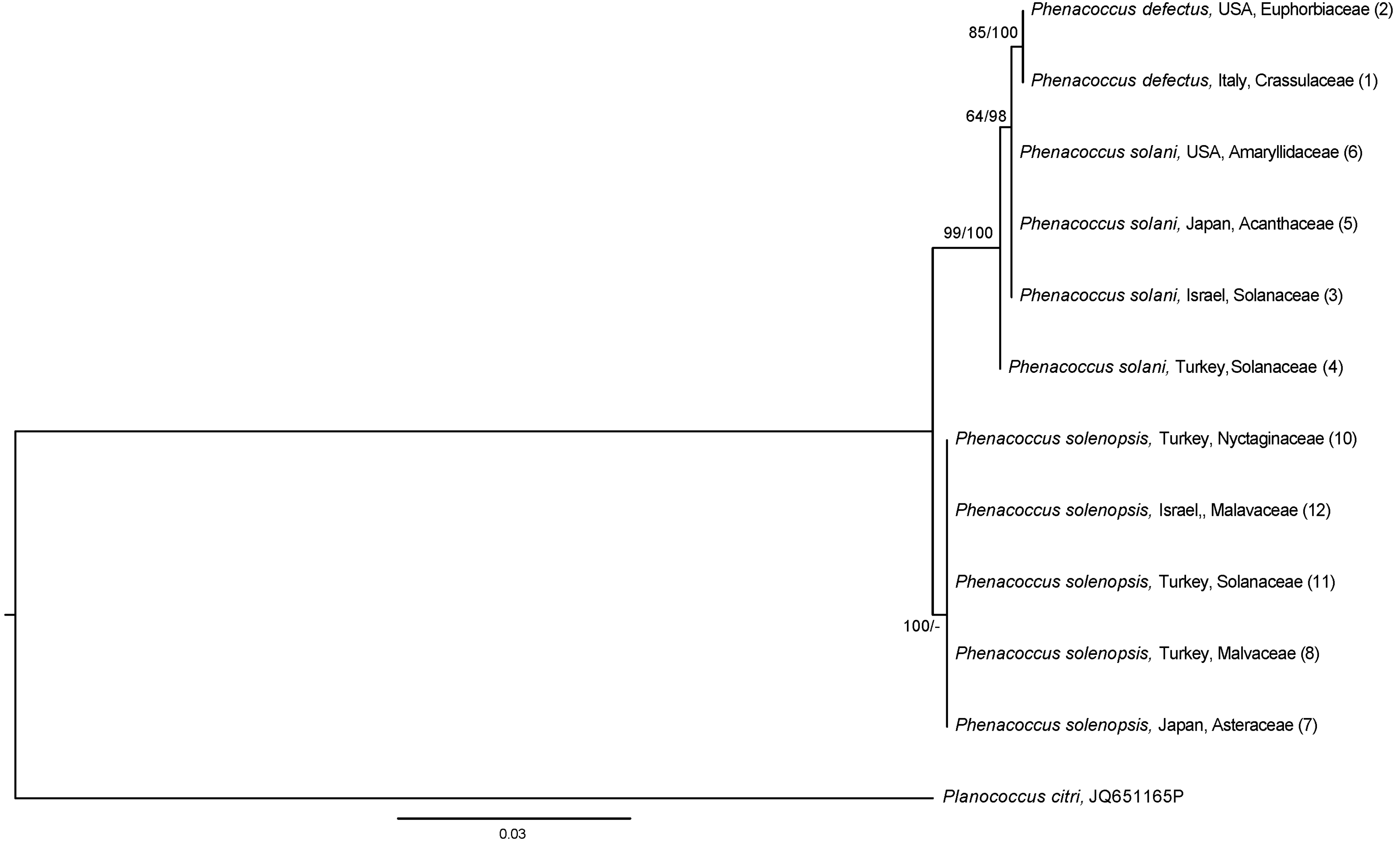
Phenacoccus defectus, USA, Euphorbiaceae (2)
85/100
Phenacoccus defectus, Italy, Crassulaceae (1)
64/98
Phenacoccus solani, USA, Amaryllidaceae (6)
99/100
Phenacoccus solani, Japan, Acanthaceae (5)
Phenacoccus solani, Israel, Solanaceae (3)
Phenacoccus solani, Turkey,Solanaceae (4)
Phenacoccus solenopsis, Turkey, Nyctaginaceae (10)
Phenacoccus solenopsis, Israel,, Malavaceae (12)
Phenacoccus solenopsis, Turkey, Solanaceae (11)
100/-
Phenacoccus solenopsis, Turkey, Malvaceae (8)
Phenacoccus solenopsis, Japan, Asteraceae (7)
Planococcus citri, JQ651165P
0.03

Figure S2. Maximum likelihood tree for *Phenacoccus defectus*, *P. solani* and *P. solenopsis* based on 28S sequences. Numbers on the nodes refer to ML bootstrap and Bayesian posterior probability values.

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Chapter 5

Check list and first zoogeographical analysis of scale insects fauna (Hemiptera, Coccoidea) of Greece

GIUSEPPINA PELLIZZARI, EVANGELIA CHADZIDIMITRIOU, PANAGIOTIS MILONAS, GEORGE J. STATHAS & FERENC KOZAR

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Introduction

The compilation of faunistic check lists provides a deeper knowledge on local distribution of autochthonous and alien species and gives additional information on the history of a territory and on environmental changes.

With regard to W-Palaearctic scale insects fauna, recent check lists of the following countries are presently available: France (Foldi 2001) Italy (Pellizzari & Russo 2005), Croatia (Masten Milek & Simala 2008), Slovenia (Selijak 2010), Portugal (Franco *et al.* 2011), Israel (Ben-Dov 2012), Hungary (Kozár *et al.* 2013), Iran (Moghaddam 2013), Malta (Mifsud *et al.* 2014).

The scale insects fauna of Greece has been studied in the past by Lindinger (1912), Koroneos (1934), Paloukis (1979), Argyriou (1983), Argyriou & Kourmadas (1980). Faunistic researches throughout the country have been carried out by Kozár (1985, 1991) and Kozár *et al.* (1991), etc..

A first check list of the scale insects recorded in Greece until 2006 was presented by Milonas *et al.* (2008). According to this paper, at that date, the number of scale insects known in Greece reached 168 species. This number is comprehensive of species occurring both in mainland Greece and Crete.

Additional faunistic papers published in the last years, added several new records or deal with description of new species (Ben-Dov 2006; Jansen *et al.* 2010; Stathas & Kozar 2010; Pellizzari *et al.* 2011; Kozar *et al.* 2012; Milonas *et al.* 2013; Stathas *et al.* 2013 a, b; Kaydan *et al.* 2014).

The above mentioned reasons suggested to check and gather all the known distributional data to have an updated checklist of scale insects recorded in mainland Greece and Crete. Moreover this stimulated a first approach to a zoogeographical analysis of Greek scale insect fauna and a comparison with the faunas of other Mediterranean countries.

Material and Methods

The previous lists of scale insects faunas of Greece and Crete (Milonas *et al.* 2008; Pellizzari *et al.* 2011) have been checked and updated by adding the new records occurred from 2008 onward. The references reported in ScaleNet (Ben-Dov *et al.* 2015) referring to species recorded in Greece or Crete have been checked by reading the original paper and, if erroneous, correctly reported in the present list.

By a biogeographic point of view, the flora and fauna of major islands are studied separately by mainland flora and fauna as they host endemic species. For this reason the species recorded in Greece have been assessed in two different columns according to species recorded in mainland Greece and species recorded in Crete (tab. 1). The very few records in minor islands have been included in mainland Greece column. With regard to literature, each species is listed together with the reference of its first record for mainland Greece and/or Crete. Some recent or relevant references have been also added.

The synonymies proposed by Kozár *et al*. (2013) for Acanthococcidae (= Eriococcidae), and reported in ScaleNet (Ben-Dov *et al*. 2015) for species of the other families, have been accepted.

In the present paper we adopted the chorotype classification proposed by Vigna-Taglianti *et al*. (1999) for the Western Palaearctic fauna and assigned to each scale species a chorotype based on its distributional pattern, according to distributional data reported in ScaleNet (Ben-Dov *et al*. 2015) and on faunistic papers and check-lists. The distributional data reported through the text devoid of citation are taken from ScaleNet (Ben-Dov *et al*., 2015).

According to Vigna Taglianti *et al*. (1999), the Western Palaearctic fauna is comprehensive of the following major groups of chorotypes:

- Chorotypes of species widely spread in the Holarctic Region
- Chorotypes of species widely spread in Europe
- Chorotypes of species widely spread in the Mediterranean countries
- Chorotypes of species widely spread in the Paleotropics and extending to Mediterranean countries

Each of the above major groups gathers several different chorotypes having a more restricted distributional pattern. The many chorotypes present in scale insect fauna of mainland Greece and Crete are reported in the Tables 2–7, grouped according to their main chorotype of pertinence

Species incidentally introduced by human activity in remote or recent years, presently acclimatized and largely distributed in the world, are grouped as Cosmopolitan or Cultural immigrants (Bodenheimer, 1935) or listed according to their origin (i.e. Australasian, Asiatic, Nearctic and so on). These alien species have no zoogeographic interest.

The species known so far only in restricted areas of mainland Greece or Crete are considered as Endemic species.

Data and comments are presented as in the previous zoogeographical analysis on Italian and Sicilian scale insect faunas by Longo *et al*. (1999) and Mazzeo *et al*. (2011).

Results

According to the present revised census, the scale insect fauna of whole Greek territory comprehends 207 species, with an increase of 39 species with respect to the previous check-list (Milonas *et al.* 2008); a total of 187 species are recorded in mainland Greece and minor islands, whereas 87 scale species are known so far in the island of Crete. More specifically, species from 16 families are recorded in the whole Greek territory. All of them are represented in mainland Greece and 12 in Crete. Most species belong to the family Diaspididae, with 86 species in total of which 77 for mainland Greece and 39 for the island of Crete. The second most numerous family is the family Coccidae with 35 species, followed by the Pseudococcidae with 34 species. In mainland Greece 33 Coccidae species have been recorded so far and only 16 in Crete. Six families are represented by just one species (Aclerdidae, Lecanodiaspididae, Marchalinidae, Margarodidae, Matsucoccidae and Putoideae).

Zoogeographical analysis of the scale insect fauna of mainland Greece and Crete

Mainland Greece is characterized by the presence of different climatic zones: the Mediterranean and temperate climates in Southern, Central, Eastern Greece and the Alpine climate, mostly in the mountainous Western Greece. Its territory comprehends different natural environments and this reflects on flora and fauna.

The island of Crete straddles two climatic zones, the Mediterranean and the North African: northwest Crete is characterized constant high humidity compared to the dry climate of the rest of the island. Crete has a high number of endemic plants and animals; the number of the endemic animals is unknown but it should be of the order of 1000 (Legakis & Kypriotakis 1994). Unfortunately only 87 scale insect species have been recorded so far in the island and this low number does not allow a reliable zoogeographical analysis, nevertheless, the data of Crete are here reported.

The main chorotypes present in scale insect fauna of mainland Greece and Crete are reported in the Tables 2-7 and scale species are grouped in each Table according to their chorotype of pertinence. Comments are reported below.

Species widely spread in the Holarctic Region (Table 2)

Mainland Greece

As many as 11 chorotypes are comprised in this large group of 82 species. Of these, only 4 (2% of the total number of species recorded in mainland Greece) are holarctic species, whereas Palaearctic and w-Palaearctic elements are 19 (10% of the total number of species); a group of 8 species (4%) have a Centralasiatic-Mediterranean distribution (i.e. *Salicicola kermanensis* and *Chionaspis etrusca*, linked to Tamaricaceae or *Salicicola kermanensis* of Salicaceae).

Several species have been referred to the Turanian-Mediterranean (13 species), Turanian-European-Mediterranean (13 species) and Turanian-European (11 species) chorotypes. All together these species constitute about the 20% of the whole species number recorded in mainland Greece. Their distribution pattern covers, besides the Mediterranean or European region also Middle East, Anatolia, Caucasus, Iran and W Turkestan (i.e. *Anapulvinaria pistaciae*, *Suturaspis pistaciae*, *Lepidosaphes granati*).

Crete

This group comprehends 39 species of which 11 (12,5%) belong to the Palaearctic and w-Palaearctic chorotype and is the most numerous.

Interesting records are *Phenacoccus abditus* and *Trionymus multivorus* of the Central Asiatic European chorotype, and the Turanian-Mediterranean *Getulaspis bupleuri* (Diaspididae), whose known distribution covers only Sardinia (Pellizzari & Porcelli 2014), some North African countries and Saudi Arabia (Matile-Ferrero 1984).

Species widely spread in Europe (Table 3)

Mainland Greece

European and S-European elements are 21 (11%). Most of them are linked to *Quercus*, Coniferae or Poaceae. A recent new interesting addition is *Rhodania occulta* (Milonas *et al*. 2013).

Crete

European elements are only 3 (3,5%): *Parthenolecanium rufulum* and *Physokermes piceae* (Coccidae), very common all over Europe, respectively on *Quercus* and *Picea,* and *Poaspis intermedia* of Poaceae.

3. Species widely spread in the Mediterranean countries (Table 4)

Mainland Greece

The Mediterranean elements number 22 species (12%); of them, 17 species distributed throughout the Mediterranean basin and develop on Mediterranean trees or shrubs, i.e. *Pollinia pollini* and *Filippia follicularis* of olive trees, *Lecanodiaspis sardoa* of *Cistus* sp., *Kermes vermilio* and *Gonaspidiotus minimus* of *Quercus ilex*.

Five species belong to the East-Mediterranean chorotype. Among them, there are *Ortheziola viti*, recently described on specimens from Greece and Turkey, *Marchalina hellenica*, a scale insect of great importance to apiculture; its original distributional area cover Greece and Turkey only, but it was voluntarily disseminated to enhance honey production; *Matsucoccus josephi*, present also in Cyprus, Turkey, Israel, Lebanon and Jordan (Mendel 1998) and *Kermes echinatus*, recorded in mainland Greece in 2013 (Stathas *et al*. 2013b) and known before only in Israel.

Crete

The species referred to this chorotype number 16 (18%), of which 10 pertain to the Mediterranean chorotype and 6 to the East-Mediterranean chorotype. Of them, three are not reported so far in mainland Greece: *Dynaspidiotus greeni,* (Diaspididae) present also in Cyprus, *Kermes greeni* (Kermesidae) recently recorded in the island with *K. echinatus* (Pellizzari *et al*. 2011, Porcelli & Pellizzari 2014) and known before only in Israel, and *Ripersiella palestinae* (Rhizoecidae), also known in Israel and Syria (Ben-Dov 2012).

Species widely spread in the Paleotropics and extending to Mediterranean countries (Table 5)

Mainland Greece

This group comprehends *Ceroplastes rusci*, very common on fig, and possibly *Saissetia privigna*. It is doubtful if *S. privigna* should be considered a true Afrotropical element or a Cultural immigrant from Africa. In the Mediterranean region it has been recorded also in Egypt, Israel and Turkey (Ben-Dov *et al*. 2015).

Crete

This group comprehends only the widespread *Ceroplastes rusci*.

Cosmopolitan or subcosmopolitan species (Cultural Immigrants) (Table 6)

Mainland Greece

Alien species, introduced and acclimatized far away or recent invaders, constitute a large group of 56 species and the 30% of the whole Greek fauna. They are mostly pest of agricultural or ornamental plants and their incidental introduction is due to human activity. Several species in this group have been considered as Cosmopolitan because of their present distribution even if their native area is well known (i.e. *Ulheria araucariae*, *Icerya purchasi* or *Diaspidiotus perniciosus*). Some species are restricted to greenhouses or are merely interceptions, with scarce possibility to survive outdoors. Recent invaders are the Nearctic *Phenacoccus madeirensis* and *P. peruvianus*, that are still spreading through the country (Stathas *et al*. 2015).

Crete

Alien species are 26, that is the 30% of the species recorded in the island and the same percentage of mainland Greece.

Endemic species (Table 7)

Mainland Greece

So far 4 species (2%) have been described from mainland Greece and are recorded only over there. Of these, *Anophococcus kotejai* and *Kawekia hellenica* (Acanthococcidae) are linked to Poaceae and it is possible that they have a wider distribution; *Physokermes hellenicus* (Coccidae) is linked to the Greek fir *Abies cephalonica*, that has a restricted distributional area; *Eumyrmococcus corinthiacus* (Rhizoecidae) collected near Corinth, carried in the mandibles of a swarming queen ant *Plagiolepsis* sp. (Williams 1993) is a very interesting record as it is the only species in the genus *Eumyrmococcus* found in the W Palaearctic area.

Crete

Possibly *Aonidiella yehudithae* (Diaspididae) is an endemic species. It was described in 2006 from specimens collected off *Hedera helix* at Avgeniki, (Ben-Dov 2006) and was collected again a few years later at Imbros Gorge on the same host plant (Jansen *et al*. 2010).

Comments

The number of species recorded in mainland Greece and Crete appears low in comparison with the Mediterranean countries whose scale insect fauna has been more intensively studied.

With regard to mainland Greece the record of 187 species is lower than expected if compared, for instance, with the number of Coccoidea species recorded in France (381 species) (Foldi 2001), Italy (390 species) (Pellizzari & Russo 2005) and Turkey (359) (Kaydan *et al*. 2013). Moreover, the comparison with the checklists of other European and Mediterranean countries highlights many distributional gaps: it is apparent that several common species have been not yet recorded in Greece, in spite of the fact that their distributional pattern covers also the Greek territory. For instance, the following species are widely distributed but not yet recorded Greece: *Acanthococcus roboris* (Goux) and *Diaspidiotus distinctus* (Leonardi), common on *Quercus, Planchonia arabidis* Signoret*, Trabutina mannipara* (Hemprich & Ehrenberg) linked to Tamaricaceae, *Acanthococcus aceris* Signoret, etc. We consider that these gaps will be filled by more intensive collecting.

The Mediterranean species are only 12% of the whole Greek scale fauna. In Italy they reach 15% and in Sicily 19.5 (Longo *et al*. 1999, Mazzeo *et al*. 2011). Some common Mediterranean species as, for instance, *Saharaspis ceardi* (Balachowsky) of *Pistacia* and plants of Mediterranean maquis, or the polyphagous *Targionia nigra* Signoret have been not yet detected in Greece. Performing faunistic researches will surely result in an increasing number of Mediterranean species.

The comparison with the zoogeographycal analysis of scale fauna of Italy (Longo *et al*. 1999) and Sicily (Mazzeo *et al*. 2011) highlights that the Greek scale insect fauna has a higher number of species whose distribution includes the Turanian region, as it was predicTable, due to its East-Mediterranean geographical position.

Both mainland Greece and Crete have a 30% of alien species in their scale faunas. It is clear that scales pests of cultivate and ornamental plants have been studied much more than scales of autochthonous wild plants due to their economic impact on agriculture. A comparison with the scale fauna of Italy highlights the same percentage of alien species on data referring to 1999 (Longo *et al.* 1999), whereas more recent analysis on scale insect fauna of other Mediterranean countries indicate that alien species reach 40% in the island of Sicily (Mazzeo *et al.* 2011) and 42% in Israel (Ben-Dov 2012). However, in spite of the many alien species recorded in Greece, possibly they are much more. In fact some alien species, already recorded in many European and Mediterranean countries have not yet been detected so far in Greece, for instance *Pulvinaria innumerabilis* (Rathvon) of grape, *Phoenicococcus marlatti* Cockerell of Arecaceae, *Ovaticoccus agavium* (Douglas) of Agaves, common invasive plants in Greek landscapes, or scales living on bamboos, probably overlooked because present at low population level.

Whereas the knowledge on scale insect fauna of mainland Greece has had a significant increase in the last years, the present knowledge on scales of Crete (87 species) is largely incomplete, in spite of recent additions (Jansen *et al.* 2010; Pellizzari *et al.* 2011). In comparison, the scale insect fauna of Sicily reaches 169 species (Mazzeo *et al.* 2011) whereas both Corsica and Sardinia have a number of recorded species near to Crete (respectively 99 and 111 species) (Foldi 2003, Pellizzari & Porcelli 2014). The scale fauna of these large Mediterranean islands deserves more intensive collections, taking into account that the presence of endemic species has been already recorded over there.

It is clear that the scale insect fauna of Greece and mostly in Crete is largely unexplored nevertheless these data improve our knowledge on the biogeography of scale insects. Moreover, the reported distributional data may be useful as indicators of biodiversity in different territories and also provide an overview to climate changes whose effects reflect also on scale insect species distribution.

TABLES

Table 1 – Check list of scale insect species recorded in mainland Greece and the island of Crete.

Family	**Species**	**Mainland Greece**	**Crete**	**Validation**
Acanthococcidae	*Anophococcus agropyri* (Borchsenius, 1949)	*	*	Kozár et al. 1991
	Anophococcus evelinae (Kozár, 1983)	*		Kozár 1985
	Anophococcus formicicola (Newstead, 1897)	*	*	Kozár 1985; Kozár et al 1991
	Anophococcus herbaceus (Danzig, 1962)	*		Kozár, 1985
	Anophococcus kotejai Kozár & Kaydan, 2013	*		Kozár et al. 2013
	Eriococcus buxi (Boyer de Fonscolombe, 1834)	*		Kozár et al. 1991
	Eriococcus williamsi Danzig, 1987	*		Kozár et al. 2013
	Gossyparia spuria (Modeer, 1778)	*		Kozár et al. 1991
	Kaweckia hellenica (Kozár , 1999)	*		Pellizzari & Kozαr 1999
	Pseudochermes fraxini (Kaltenbach, 1860)	*		Kohler 1998
	Rhizococcus baldonensis (Rasina, 1966)	*		Kozár et al. 2013
	Rhizococcus coccineus (Cockerell, 1894)	*		Stathas 2004
	Rhizococcus desertus (Matesova, 1957)		*	Pellizzari et al. 2011
	Rhizococcus greeni Newstead, 1898	*		Kozár et al. 1991
	Rhizococcus istresianus (Goux, 1989)	*		Kozár et al. 2013
	Rhizococcus munroi (Boratynski, 1962)	*	*	Kozár et al. 1991
	Rhizococcus reynei (Schmutterer, 1952)	*		Kozár et al., 2013
	Rhizococcus thymelaeae Newstead, 1897)	*		Hodgson & Trenkeva 2008
	Uhleria araucariae (Maskell, 1879)	*		Hoy, 1963
Aclerdidae	*Aclerda berlesii* Buffa, 1897	*		Santas 1989
Asterolecaniidae	*Asterodiaspis bella* (Russell, 1941)	*		Russell 1941
	Asterodiaspis ilicicola (Targioni Tozzetti, 1888)	*		Stathas et al. 2013a
	Asterodiaspis quercicola (Bouche, 1851)	*		Bodenheimer 1928

	Asterodiaspis repugnans (Russell, 1941)	*		Russell 1941
	Asterodiaspis variolosa (Ratzeburg, 1970)	*		Milonas et al. 2008a
	Pollinia pollini (A. Costa, 1857)	*	*	Bodenheimer 1928; Alexandrakis 1980a
Cerococcidae	*Cerococcus cistarum* Balachowsky, 1927		*	Kozár & Nagy 1998
	Cerococcus longipilosus (Archangelskaya, 1830)	*		Kozár 1985
Coccidae	*Anapulvinaria pistaciae* (Bodenheimer, 1926)	*		Argyriou 1983
	Ceroplastes cirripediformis Comstock, 1881	*		Argyriou 1983
	Ceroplastes floridensis Comstock, 1881	*	*	Argyriou 1979a; Argyriou & Kourmadas 1980a; Pellizzari et al. 2011
	Ceroplastes rusci (Linnaeus, 1758)	*	*	Bodenheimer, 1928; Ayoutantis, 1940
	Ceroplastes sinensis Del Guercio, 1900	*	*	Pellizzari et al. 2011; Argyriou et al. 1976
	Coccus hesperidum Linnaeus, 1758	*	*	Bodenheimer 1928; Podsialdo 1983; Ayoutantis, 1940
	Coccus pseudomagnoliarum (Kuwana, 1914)	*		De Lotto 1973
	Eulecanium ciliatum (Douglas, 1891)	*		Kozár et al. 1991
	Eulecanium sericeum (Lindinger, 1906)	*		Argyriou 1983
	Eulecanium tiliae (Linnaeus, 1758)	*		Bodenheimer 1928; Argyriou 1983
	Filippia follicularis (Targioni Tozzetti, 1867)	*	*	Bodenheimer 1928; Argyriou 1967; Pellizzari et al. 2011
	Lecanopsis formicarum Newstead, 1893		*	Pellizzari et al. 2011
	Lecanopsis turcica (Bodenheimer, 1951)	*		Milonas et al. 2008a
	Lichtensia viburni Signoret, 1873	*	*	Argyriou 1983; Argyriou et al. 1976
	Luzulaspis dactylis Green, 1928	*		Kozár 1985
	Nemolecanium graniforme (Wünn, 1921)	*		Stathas 2001
	Parthenolecanium corni (Bouché, 1844)	*		Argyriou 1983; Kozár, 1985; Santas 1985
	Parthenolecanium persicae (Fabricius, 1776)	*		Kozár 1985
	Parthenolecanium rufulum (Cockerell, 1903)	*	*	Kozár et al. 1991
	Physokermes hellenicus Kozár & Gounari, 2012	*		Kozár et al. 2012
	Physokermes hemicryphus (Dalman, 1826)	*		Argyriou 1983

	Physokermes inopinatus Danzig & Kozár, 1973	*		Stathas & Kozár 2010
	Physokermes piceae (Schrank, 1801)	*	*	Santas 1989
	Poaspis intermedia (Goux, 1939)		*	Kozár & Nagy 1998
	Poaspis jahandiezi (Balachowsky, 1932)	*		Koteja 1979
	Protopulvinaria pyriformis (Cockerell, 1894)	*	*	Ben-Dov et al. 2003; Jansen et al. 2011
	Pulvinaria floccifera (Westwood, 1870)	*		Bodenhaimer 1928; Argyriou & Mourikis 1983
	Pulvinaria horii Kuwana, 1902	*		Canard, 1994
	Pulvinaria vitis (Linnaeus, 1758)	*	*	Argyriou 1983
	Pulvinariella mesembryanthemi (Vallot, 1830)	*	*	Argyriou 1983
	Saissetia coffeae (Walker, 1852)	*	*	Argyriou, 1983; Ben-Dov 1993
	Saissetia oleae (Olivier, 1791)	*	*	Ayoutantis 1940; Argyriou 1963
	Saissetia privigna De Lotto, 1965	*		De Lotto 1976; Argyriou 1983
	Scythia festuceti (Šulc, 1941)	*		Kozár et al., 1991
	Sphaerolecanium prunastri (Boyer de Fonscolombe, 1834)	*	*	Argyriou & Paloukis 1976
Diaspididae	*Abgrallaspis cyanophylli* (Signoret, 1869)	*	*	Kozár et al. 1991
	Acanthomytilus intermittens (Hall, 1924)		*	Kozár et al. 1991
	Acanthomytilus jablonowskii Kozár & Matile-Ferrero, 1975	*		Kozár et al. 1991
	Adiscodiaspis ericicola (Marchal, 1909)		*	Pellizzari et al. 2011
	Aonidia lauri (Bouché, 1833)	*	*	Bodenheimer 1928; Koroneos 1934; Pellizzari et al. 2011
	Aonidia maroccana Balachowsky, 1949	*		Kozár et al. 1991
	Aonidia mediterranea (Lindinger, 1910)	*	*	Koroneos 1934; Pellizzari et al. 2011
	Aonidiella aurantii (Maskell, 1879)	*	*	Bodenheimer 1928; Ayoutantis 1940
	Aonidiella citrina (Coquillet)	*		Kozár et al. 1991
	Aonidiella yehudithae Ben-Dov, 2006		*	Ben-Dov 2006
	Aspidiotus hedericola Leonardi, 1920	*		Koroneos 1934
	Aspidiotus nerii Bouché, 1833	*	*	Bodenheimer 1928; Ayoutantis 1940

Aulacaspis rosae (Bouché, 1833)	*	*	Koroneos 1934; Kozar et al. 1991; Pellizzari et al. 2011
Carulaspis juniperi (Bouché, 1851)	*		Koroneos 1934
Carulaspis minima (Signoret, 1869)	*	*	Kozár 1985; Pellizzari et al. 2011
Carulaspis visci (Schrank, 1781)	*		Koroneos 1934
Chionaspis etrusca Leonardi, 1908	*		Danzig & Pellizzari 1998
Chionaspis lepineyi Balachowsky, 1928	*		Milonas et al. 2008a
Chionaspis salicis (Linnaeus, 1758)	*		Koroneos, 1934
Chrysomphalus aonidum (Linnaeus, 1758)	*		Koroneos 1934; Argyriou et al. 1976; Stathas & Kozár 2005-2006
Chrysomphalus dictyospermi (Morgan, 1889)	*	*	Ayoutantis 1940; Bodenheimer 1928
Diaspidiotus cecconii (Leonardi, 1908)	*		Koroneos 1934
Diaspidiotus degeneratus (Leonardi , 1896)	*		Koroneos 1934
Diaspidiotus gigas (Thiem & Gerneck, 1934)	*		Argyriou 1983
Diaspidiotus labiatarum (Marchal, 1909)	*		Lindinger 1909; Koroneos 1934
Diaspidiotus lenticularis (Lindinger, 1912)	*	*	Koroneos 1934; Pellizzari et al. 2011
Diaspidiotus osborni (Newell & Cockerell, 1898)		*	Pellizzari et al. 2011
Diaspidiotus ostreaeformis (Curtis, 1843)	*		Koroneos 1934
Diaspidiotus perniciosus (Comstock, 1881)	*		Paloukis 1979
Diaspidiotus pyri (Lichtenstein, 1881)	*		Paloukis 1979
Diaspidiotus thymbrae (Koroneos, 1934)	*		Koroneos 1934
Diaspidiotus wuenni (Lindinger, 1923)	*		Trencheva et al. 2009
Diaspidiotus zonatus (Frauenfeld, 1868)	*		Bodenheimer 1928; Koroneos 1934
Diaspis echinocacti (Bouché, 1833)	*		Argyriou et al. 1976
Diaspis syriaca Lindinger, 1912	*		Koroneos 1934
Discodiaspis salicorniae (Gómez-Menor Ortega, 1928)	*		Koroneos 1934
Duplachionaspis berlesii (Leonardi, 1898)	*	*	Pellizzari et al. 201; Koroneos 1934
Duplachionaspis natalensis (Maskell, 1896)	*	*	Kozár et al. 1991; Milonas et al. 2008; Pellizzari et al. 2011

Dynaspidiotus abieticola (Koroneos, 1934)	*		Koroneos 1934
Dynaspidiotus abietis (Schrank, 1776)	*		Koroneos 1934; Stathas 2007-2008
Dynaspidiotus britannicus (Newstead, 1898)	*	*	Koroneos 1934; Pellizzari et al. 2011
Dynaspidiotus ephedrarum (Lindinger, 1912)	*		Koroneos, 1934
Dynaspidiotus greeni (Balachowsky, 1951)		*	Kozár et al. 1991
Epidiaspis gennadii (Leonardi, 1898)	*		Leonardi 1898; Koroneos 1934
Epidiaspis leperii (Signoret, 1869)	*		Koroneos 1934; Milonas et al. 2008a
Fiorinia fioriniae (Targioni Tozzetti, 1867)	*		Koroneos 1934
Getulaspis bupleuri (Marchal, 1904)		*	Pellizzari et al. 2011
Gonaspidiotus minimus (Leonardi, 1896)	*	*	Koroneos 1934; Jansen et al. 2011
Hemiberlesia lataniae (Signoret, 1869)	*	*	Bodenheimer 1928; Koroneos 1934; Rosen & DeBach 1979
Hemiberlesia rapax (Comstock, 1881)	*		Koroneos 1934
Koroneaspis aegilopos (Koroneos, 1934)	*	*	Koroneos 1934; Pellizzari et al. 2011
Lepidosaphes beckii (Newman, 1869)	*	*	Hall 1922; Pellizzari et al. 2011
Lepidosaphes conchiformis (Gmelin, 1789)	*	*	Bodenheimer 1928; Koroneos 1934; Kozár et al. 1991
Lepidosaphes flava (Signoret, 1870)	*	*	Koroneos 1934; Pellizzari et al. 2011
Lepidosaphes gloverii (Packard, 1869)	*		Stathas 2003-2004
Lepidosaphes granati Koroneos, 1934	*		Koroneos 1934
Lepidosaphes juniperi Lindinger, 1912	*		Bodenheimer 1928
Lepidosaphes malicola Borchsenius, 1947	*		Kozár et al. 1991
Lepidosaphes pinnaeformis (Bouché, 1851)	*		Koroneos 1934
Lepidosaphes pistaciae Archangelskaya, 1930	*		Katsoyannos & Stathas 1995a
Lepidosaphes ulmi (Linnaeus, 1758)	*	*	Koroneos 1934; Katsoyannos & Stathas 1995b; Pellizzari et al. 2011
Leucaspis loewi Colvée, 1882	*	*	Koroneos 1934; Pellizzari et al. 2011
Leucaspis pini (Hartig, 1839)	*		Bodenheimer 1928
Leucaspis pusilla Löw, 1883	*	*	Bodenheimer 1928; Podsiadlo 1983

	Leucaspis riccae Targioni Tozzetti, 1881	*	*	Koroneos 1934; Pellizzari et al. 2011
	Lineaspis striata (Newstead, 1897)	*	*	Koroneos, 1934; Kozár et al. 1991; Panis 1981; Pellizzari et al. 2011
	Lopholeucaspis cockerelli (Grandpré & Charmoy, 1899)	*		Nakahara 1982
	Lopholeucaspis japonica (Cockerell, 1897)	*		Kozár 1985
	Melanaspis inopinata (Leonardi, 1913)	*		Koroneos 1934
	Mercetaspis halli (Green, 1923)	*	*	Koroneos 1934; Podsiadlo 1983;
	Mohelnaspis massiliensis (Goux, 1937)	*		Kozár et al. 1991
	Oceanaspidiotus spinosus (Comstock, 1883)		*	Pellizzari et al. 2011
	Odonaspis ruthae Kotinsky, 1915	*	*	Kozár 1985; Pellizzari et al. 2011
	Parlatoria oleae (Colvée, 1880)	*	*	Bodenheimer 1928; Koroneos 1934; Ayoutantis 1940
	Parlatoria parlatoriae (Šulc, 1895)		*	Jansen et al. 2011
	Parlatoria pergandii Comstock, 1881	*		Koroneos 1934
	Parlatoria theae Cockerell, 1896	*		Balachowsky 1953
	Parlatoria ziziphi (Lucas, 1853)	*	*	Koroneos 1934; Ayoutantis 1940
	Poliaspis media Maskell, 1880	*		Milonas et al. 2008a
	Prodiaspis tamaricicola (Malenotti, 1916)	*	*	Koroneos 1934; Pellizzari et al. 2011
	Pseudaulacaspis pentagona (Targioni Tozzetti, 1886)	*	*	Balachowsky 1954; Paloukis 1979
	Rhizaspidiotus donacis (Leonardi, 1920)		*	Kozár et al. 1991
	Salicicola kermanensis (Lindinger, 1905)	*		Kozár 1985
	Suturaspis pistaciae (Lindinger, 1906)	*		Koroneos 1934
	Targionia vitis (Signoret, 1876)	*		Koroneos 1934
	Unaspis euonymi (Comstock, 1881)	*	*	Koroneos 1934; Pellizzari et al. 2011
Kermesidae	*Kermes echinatus* Balachowsky, 1953	*	*	Stathas et al. 2013b
	Kermes greeni Bodenheimer, 1931		*	Pellizzari et al. 2011
	Kermes ilicis (Linnaeus, 1758)	*		Argyriou 1983; Kozár et al 1991
	Kermes quercus (Linnaeus, 1758)	*		Santas 1983; Kozár et al. 1991

	Kermes vermilio Planchon, 1864	*	*	Lindinger 1912; Hoy 1963; Argyriou 1983
Lecanodiaspididae	*Lecanodiaspis sardoa* Targioni Tozzetti, 1869	*		Argyriou et al. 1976
Marchalinidae	*Marchalina hellenica* (Gennadius, 1883)	*	*	Gennadius 1883; Ferris 1925; Argyriou 1983
Margarodidae	*Dimargarodes mediterraneus* (Silvestri, 1906)	*		Jakubsky 1965
Matsucoccidae	*Matsucoccus josephi* Bodenheimer & Harpaz {	*	*	Mendel & Schiller 1993; Gounari et al. 2010
Monophlebidae	*Gueriniella serratulae* (Fabricius, 1755)	*	*	Milonas et al. 2008; Podsiadlo 1983
	Icerya purchasi Maskell, 1879	*	*	Ayoutantis 1927; Ayoutantis 1940
	Palaeococcus fuscipennis (Burmeister, 1835)	*		Milonas et al. 2008a
Ortheziidae	*Newsteadia susannae* Kozár & Foldi, 2001	*		Milonas et al. 2008
	Orthezia arenariae Vayssière, 1924	*		Kozár 2004
	Orthezia urticae (Linnaeus, 1758)	*	*	Morrison 1925; Kozár 2004; Pellizzari et al. 2011
	Orthezia yashushii Kuwana, 1923	*		Milonas et al. 2008a
	Ortheziola britannica Kozár & Miller, 2000	*		Kaydan et al. 2014
	Ortheziola marottai Kaydan & Szita, 2014	*		Kaydan et al. 2014
	Ortheziola viti Szita & Konczné Benedicty, 2014	*		Kaydan et al. 2014
Pseudococcidae	*Antoninella parkeri* (Balachowsky, 1936)	*		Milonas et al. 2008
	Atrococcus arakelianae (Ter-Grigorian, 1964)		*	Kozár & Nagy 1998
	Balanococcus orientalis Danzig & Ivanova, 1976	*		Kozár et al. 1991
	Chaetococcus phragmitis (Marchal, 1909)	*	*	Kozár 1985; Kozár et al. 1991
	Chorizococcus rostellum (Lobdell, 1930)		*	Pellizzari et al. 2011
	Dysmicoccus brevipes (Cockerell, 1893)	*		Roditakis & Milonas 2013
	Heliococcus bohemicus Šulc, 1912	*	*	Milonas & Kozár 2008; Jansen et al. 2011
	Heterococcus nudus (Green, 1926)	*	*	Milonas & Kozar 2008; Pellizzari et al. 2011
	Hypogeococcus pungens Granara de Willink, 1981	*		Ben-Dov et al., 2002
	Mirococcopsis elongata Borchsenius, 1948	*		Kozár et al. 1991
	Mirococcus inermis (Hall, 1925)	*		Kozár 1985

	Peliococcopsis priesneri (Laing, 1936)	*	*	Kozár 1985
	Peliococcus kimmericus (Kiritshenko, 1940)		*	Kozár et al. 1991
	Peliococcus turanicus (Kiritshenko, 1932)	*		Kozár 1985
	Pellizzaricoccus gabrielis Kozár, 1991	*		Kozár 1991
	Phenacoccus abditus Borchsenius, 1949		*	Kozár et al. 1991
	Phenacoccus hordei (Lindeman, 1886)	*		Milonas & Kozár 2008
	Phenacoccus interruptus Green, 1923	*		Kozár 1985
	Phenacoccus madeirensis Green, 1923	*	*	Jansen et al. 2011; Papadopoulou & Chryssohoides 2012
	Phenacoccus peruvianus Granara de Willink, 2007	*		Gkounti & Milonas 2013
	Phenacoccus yerushalmi Ben-Dov, 1985	*		Ben-Dov et al. 2006
	Planococcus citri (Risso, 1813)	*	*	Ayoutantis 1940, Argyriou 1969
	Planococcus ficus (Signoret, 1875)	*	*	Argyriou 1983; Ezzat & McConnell 1956
	Planococcus vovae (Nasonov, 1908)	*	*	Cox 1989; Williams & Moghaddam 2000
	Pseudococcus calceolariae (Maskell, 1879)	*		Kozár et al. 1991
	Pseudococcus longispinus (Targioni Tozzetti, 1867)	*	*	Argyriou 1983
	Pseudococcus viburni (Signoret, 1875)	*		Kozár et al. 1991
	Rhodania occulta Schmutterer, 1952	*		Milonas et al. 2013
	Ritsemia pupifera Lichtenstein, 1879	*		Savopoulou et al. 1997
	Spilococcus halli (McKenzie & Williams, 1965)	*	*	Kozár 1985; Pellizzari et al. 2011
	Trionymus aberrans Goux, 1938	*	*	Kozár et al., 1991
	Trionymus cynodontis (Kiritshenko, 1932)	*		Kozár, 1985
	Trionymus multivorus (Kiritchenko, 1936)		*	Pellizzari et al., 2011
	Vryburgia amaryllidis (Bouché, 1837)	*		Argyriou, 1983
Putoideae	*Puto superbus* (Leonardi, 1907)	*		Bodenheimer 1928; Argyriou 1983
Rhizoecidae	*Eumyrmococcus corinthiacus* Williams, 1993	*		Williams 1993
	Rhizoecus albidus Goux, 1936	*	*	Kozár 1985; Pellizzari et al. 2011

	Rhizoecus cacticans (Hambleton, 1946)	*		Kozár & Konczne Benedicty 2007
	Ripersiella palestineae Hambleton, 1946		*	Hambleton 1979

Table 2 – Elements widely distributed in the Holarctic region.

Chorotype	Family	Species		Mainland Greece	Crete
Holarctic	Acanthococcidae	*Heterococcus*	*nudus*		*
		Rhizococcus	*greeni*	*	
	Coccidae	*Eulecanium*	*tiliae*	*	
	Diaspididae	*Dynaspidiotus*	*abietis*	*	
	Pseudococcidae	*Heterococcus*	*nudus*	*	
Palaearctic	Acanthococcidae	*Anophococcus*	*agropyri*	*	*
	Coccidae	*Eulecanium*	*ciliatum*	*	
		Parthenolecanium	*persicae*	*	
		Pulvinaria	*vitis*	*	*
	Diaspididae	*Aulacaspis*	*rosae*	*	*
		Carulaspis	*minima*	*	*
		Chionaspis	*salicis*	*	
		Diaspidiotus	*gigas*	*	
		Diaspidiotus	*ostreaeformis*	*	
		Lepidosaphes	*conchiformis*	*	*
		Lepidosaphes	*ulmi*	*	*
	Ortheziidae	*Orthezia*	*urticae*	*	*
	Pseudococcidae	*Planococcus*	*vovae*	*	*
w-Palaearctic	Acanthococcidae	*Rhizococcus*	*reynei*	*	
	Coccidae	*Lichtensia*	*viburni*	*	*
	Diaspididae	*Diaspidiotus*	*zonatus*	*	
		Dynaspidiotus	*britannicus*	*	*
		Leucaspis	*loewi*	*	*
	Ortheziidae	*Ortheziola*	*britannica*	*	
Sibiric-European	Acanthococcidae	*Pseudochermes*	*fraxini*	*	
		Rhizococcus	*munroi*	*	*
	Coccidae	*Lecanopsis*	*formicarum*		*
Asiatic-European	Acanthococcidae	*Rhizococcus*	*baldonensis*	*	
		Rhizococcus	*desertus*		*
	Diaspididae	*Parlatoria*	*parlatoriae*		*
	Kermesidae	*Kermes*	*quercus*	*	
	Pseudococcidae	*Balanococcus*	*orientalis*	*	
		Heliococcus	*bohemicus*	*	*
		Phenacoccus	*interruptus*	*	
		Trionymus	*aberrans*	*	*
	Rhizoecidae	*Rhizoecus*	*albidus*	*	*
Central Asiatic-European	Coccidae	*Lecanopsis*	*turcica*	*	
	Pseudococcidae	*Phenacoccus*	*abditus*		*
		Trionymus	*multivorus*		*
Central Asiatic-Mediterranean	Cerococcidae	*Cerococcus*	*longipilosus*	*	
	Diaspididae	*Chionaspis*	*etrusca*	*	

		Lepidosaphes	*malicola*	*	
		Lepidosaphes	*pistaciae*	*	
		Mercetaspis	*halli*	*	*
		Prodiaspis	*tamaricicola*	*	*
		Salicicola	*kermanensis*	*	
	Pseudococcidae	*Mirococcus*	*inermis*	*	
		Peliococcus	*kimmericus*		*
Turanian-European-Mediterranean	Acanthococcidae	*Anophococcus*	*formicicola*	*	*
	Asterolecaniidae	*Asterodiaspis*	*bella*	*	
	Diaspididae	*Chionaspis*	*lepineyi*	*	
		Diaspidiotus	*pyri*	*	
		Epidiaspis	*leperii*	*	
		Leucaspis	*pusilla*	*	*
		Leucaspis	*riccae*	*	*
		Targionia	*vitis*	*	
	Margarodidae	*Dimargarodes*	*mediterraneus*	*	
	Pseudococcidae	*Chaetococcus*	*phragmitis*	*	*
		Phenacoccus	*yerushalmi*	*	
		Spilococcus	*halli*	*	*
	Putoideae	*Puto*	*superbus*	*	
Turanian-European	Acanthococcidae	*Eriococcus*	*buxi*	*	
	Coccidae	*Scythia*	*festuceti*	*	
		Sphaerolecanium	*prunastri*	*	*
	Diaspididae	*Diaspidiotus*	*wuenni*	*	
		Epidiaspis	*gennadii*	*	
		Lepidosaphes	*juniperi*	*	
		Mohelnaspis	*massiliensis*	*	
	Ortheziidae	*Ortheziola*	*marottai*	*	
	Pseudococcidae	*Atrococcus*	*arakelianae*		*
		Mirococcopsis	*elongata*	*	
		Peliococcus	*turanicus*	*	
		Ritsemia	*pupifera*	*	
Turanian-Mediterranean	Coccidae	*Anapulvinaria*	*pistaciae*	*	
	Diaspididae	*Acanthomytilus*	*intermittens*		*
		Diaspidiotus	*cecconii*	*	
		Diaspis	*syriaca*	*	
		Dynaspidiotus	*abieticola*	*	
		Dynaspidiotus	*ephedrarum*	*	
		Getulaspis	*bupleuri*		*
		Koroneaspis	*aegilopos*	*	*
		Lepidosaphes	*flava*	*	*
		Lepidosaphes	*granati*	*	
		Lineaspis	*striata*	*	*
		Melanaspis	*inopinata*	*	
		Parlatoria	*oleae*	*	*

		Suturaspis	*pistaciae*	*	
	Pseudococcidae	*Peliococcopsis*	*priesneri*	*	*
European-Mediterranean	Diaspididae	*Diaspidiotus*	*labiatarum*	*	
		Diaspidiotus	*lenticularis*	*	*
	Monophlebidae	*Palaeococcus*	*fuscipennis*	*	
	Ortheziidae	*Orthezia*	*arenariae*	*	

Table 3 – Elements widely distributed in Europe.

Chorotype	**Family**	***Species***		Mainland Greece	Crete
European	Acanthococcidae	*Anophococcus*	*herbaceus*	*	
	Asterolecaniidae	*Asterodiaspis*	*quercicola*	*	
	Coccidae	*Eulecanium*	*sericeum*	*	
		Luzulaspis	*dactylis*	*	
		Nemolecanium	*graniforme*	*	
		Parthenolecanium	*rufulum*	*	*
		Physokermes	*hemicryphus*	*	
		Physokermes	*inopinatus*	*	
		Physokermes	*piceae*	*	*
		Poaspis	*intermedia*		*
		Poaspis	*jahandiezi*	*	
	Diaspididae	*Carulaspis*	*visci*	*	
		Leucaspis	*pini*	*	
	Pseudococcidae	*Phenacoccus*	*hordei*	*	
		Rhodania	*occulta*	*	
s-European	Acanthococcidae	*Rhizococcus*	*istresianus*	*	
	Asterolecaniidae	*Asterodiaspis*	*repugnans*	*	
	Diaspididae	*Acanthomytilus*	*jablonowskii*	*	
		Diaspidiotus	*thymbrae*	*	
	Ortheziidae	*Newsteadia*	*susannae*	*	
	Pseudococcidae	*Antoninella*	*parkeri*	*	
		Pellizzaricoccus	*gabrielis*	*	

Table 4 – Elements widely distributed in the Mediterranean Basin.

Chorotype	**Family**	**Species**		**Mainland Greece**	**Crete**
Mediterranean	Acanthococcidae	*Eriococcus*	*williamsi*	*	
		Rhizococcus	*thymelaeae*	*	
	Aclerdidae	*Aclerda*	*berlesii*	*	
	Asterolecaniidae	*Asterodiaspis*	*ilicicola*	*	
		Pollinia	*pollini*	*	*
	Coccidae	*Filippia*	*follicularis*	*	*
	Diaspididae	*Adiscodiaspis*	*ericicola*		*
		Aonidia	*lauri*	*	*
		Aonidia	*maroccana*	*	
		Aonidia	*mediterranea*	*	*
		Aspidiotus	*hedericola*	*	
		Discodiaspis	*salicorniae*	*	
		Duplachionaspis	*berlesii*	*	*
		Gonaspidiotus	*minimus*	*	*
		Rhizaspidiotus	*donacis*		*
	Kermesidae	*Kermes*	*ilicis*	*	
		Kermes	*vermilio*	*	*
	Lecanodiaspididae	*Lecanodiaspis*	*sardoa*	*	
	Monophlebidae	*Gueriniella*	*serratulae*	*	*
e-Mediterranean	Acanthococcidae	*Anophococcus*	*evelinae*	*	
	Diaspididae	*Dynaspidiotus*	*greeni*		*
	Kermesidae	*Kermes*	*echinatus*	*	*
		Kermes	*greeni*		*
	Marchalinidae	*Marchalina*	*hellenica*	*	*
	Matsucoccidae	*Matsucoccus*	*josephi*	*	*
	Ortheziidae	*Ortheziola*	*viti*	*	
	Rhizoecidae	*Rhizoecus*	*palestinae*		*

Table 5 – Afrotropical or oriental elements also present in the Mediterranean area.

Chorotype	**Family**	***Species***		**Mainland Greece**	**Crete**
Afrotropical-Mediterranean	Coccidae	*Ceroplastes*	*rusci*	*	*
		Saissetia	*privigna*	*	

Table 6 – Cosmopolitan or subcosmopolitan species (Cultural Immigrants).

Chorotype	**Family**	***Species***		**Mainland Greece**	**Crete**
Cosmopolitan	Acanthococcidae	*Gossyparia*	*spuria*	*	
		Uhleria	*araucariae*	*	
	Asterolecaniidae	*Asterodiaspis*	*variolosa*	*	
	Coccidae	*Ceroplastes*	*sinensis*	*	*
		Coccus	*hesperidum*	*	*
		Parthenolecanium	*corni*	*	
		Pulvinaria	*floccifera*	*	
		Saissetia	*oleae*	*	*
	Diaspididae	*Aonidiella*	*aurantii*	*	*
		Aonidiella	*citrina*	*	
		Aspidiotus	*nerii*	*	*
		Carulaspis	*juniperi*	*	
		Diaspidiotus	*perniciosus*	*	
		Duplachionaspis	*natalensis*	*	*
		Hemiberlesia	*rapax*	*	
		Lepidosaphes	*beckii*	*	*
		Lepidosaphes	*gloverii*	*	
		Lopholeucaspis	*cockerelli*	*	
		Lopholeucaspis	*japonica*	*	
		Odonaspis	*ruthae*	*	*
		Parlatoria	*pergandii*	*	
		Parlatoria	*theae*	*	
		Parlatoria	*ziziphi*	*	*
		Pseudaulacaspis	*pentagona*	*	*
		Unaspis	*euonymi*	*	*
	Monophlebidae	*Icerya*	*purchasi*	*	*
	Pseudococcidae	*Chorizococcus*	*rostellum*		*
		Planococcus	*citri*	*	*
		Planococcus	*ficus*	*	*
		Pseudococcus	*calceolariae*	*	
		Pseudococcus	*longispinus*	*	*
		Pseudococcus	*viburni*	*	
		Trionymus	*cynodontis*	*	
Pantropical	Acanthococcidae	*Rhizococcus*	*coccineus*	*	
	Coccidae	*Ceroplastes*	*cirripediformis*	*	
		Ceroplastes	*floridensis*	*	*
		Protopulvinaria	*pyriformis*	*	*
		Saissetia	*coffeae*	*	*
	Diaspididae	*Abgrallaspis*	*cyanophylli*	*	*
		Chrysomphalus	*aonidum*	*	
		Chrysomphalus	*dictyospermi*	*	*

		Fiorinia	*fioriniae*	*	
		Hemiberlesia	*lataniae*	*	*
		Lepidosaphes	*pinnaeformis*	*	
		Oceanaspidiotus	*spinosus*		*
	Pseudococcidae	*Dysmicoccus*	*brevipes*	*	
		Hypogeococcus	*pungens*	*	
		Phenacoccus	*madeirensis*	*	*
	Rhizoecidae	*Rhizoecus*	*cacticans*	*	
Afrotropical	Coccidae	*Pulvinariella*	*mesembryanthemi*	*	*
Asiatic	Coccidae	*Coccus*	*pseudomagnoliarum*	*	
Australasian	Diaspididae	*Poliaspis*	*media*	*	
Neartic	Diaspididae	*Diaspidiotus*	*osborni*		*
	Diaspididae	*Diaspis*	*echinocacti*	*	
Neotropical	Pseudococcidae	*Phenacoccus*	*peruvianus*	*	
	Pseudococcidae	*Vryburgia*	*amaryllidis*	*	
e-Palaearctic	Ortheziidae	*Orthezia*	*yashushii*	*	
Oriental	Coccidae	*Pulvinaria*	*horii*	*	
	Diaspididae	*Diaspidiotus*	*degeneratus*	*	

Table 7 – Endemic elements.

Chorotype	**Family**	***Species***		Mainland Greece	Crete
Endemic	Acanthococcidae	*Anophococcus*	*kotejai*	*	
		Kaweckia	*hellenica*	*	
	Coccidae	*Physokermes*	*hellenicus*	*	
	Diaspididae	*Aonidiella*	*yehudithae*	*	*
	Rhizoecidae	*Eumyrmococcus*	*corinthiacus*	*	

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