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First record of two leafhoppers, *Euscelis ohausi* and *Euscelidius variegatus*, for the island of Crete, Greece (Hemiptera: Cicadellidae)

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Abstract
The island of Crete, due to the favorable temperate environmental conditions, is known for its high insect species biodiversity. In this contribution, we report the occurrence of two leafhoppers in Crete, Greece, *Euscelis ohausi* (Wagner) and *Euscelidius variegatus* (Kirschbaum) (Hemiptera: Cicadellidae), collected during systematic surveys for Auchenorrhyncha performed from 2017 to 2019. This represents the first record of these leafhopper species for the island of Crete. Data on their distribution and bioecology on the island are included.

Keywords: *Euscelis ohausi*, *Euscelidius variegatus*, Cicadellidae, Leafhoppers, Crete, Greece.

Introduction

The Auchenorrhyncha (leafhoppers, planthoppers, sharpshooters, cicadas) are a diverse and species-rich group (Nickel 2003) consisting of approx. 42,000 described species worldwide, having adopted various life habits (Lariviere et al. 2010) and often, in temperate grasslands, being the most abundant insect herbivores (Hollier et al. 2005; Waloff & Thompson 1980). Auchenorrhyncha may cause both direct damage by their feeding activity in phloem vessels, and indirect damage through the production of honeydew and wax, transmission of phytopathogenic agents and in particular phytoplasmas. The family Cicadellidae is one of the largest insect families of Auchenorrhyncha, containing some 22,000 known species worldwide (Dietrich 2004), distributed in 40 subfamilies (Oman et al. 1990). Cicadellidae transmit phytoplasmas (originally called mycoplasma-like organisms), which are important insect-transmitted pathogenic agents causing more than 700 diseases, many of which are lethal, in hundreds of plant species (Weintraub & Beanland 2006) or associated with. Phytoplasmas occur in hundreds of commercial and native plants, causing minor to extensive damage.

Insect vectors, primarily leafhoppers, planthoppers, and psyllids, have been identified for relatively few phytoplasma diseases, limiting the capacity of managers to make informed decisions to protect crops and endangered indigenous plants. In the past two decades our knowledge of insect vector-phytoplasma interactions has increased dramatically, allowing researchers to make more accurate predictions about the nature and epidemiology of phytoplasma diseases. These better-characterized systems also may provide clues to the identity of insect vectors of other phytoplasma-associated diseases. We review the literature addressing the ecology of insect vectors, phytoplasma-insect ecological and molecular interactions, vector movement and dispersal, and possible management strategies with an emphasis on research from the past 20 years. The majority of leafhopper pests belong to the species-rich subfamily Deltocephalinae (38 tribes, 923 genera and 6683 valid species) (Zahniser & Dietrich 2013). Many of these species are very important in agriculture, since they are efficient vectors of plant patho-

genic viruses or bacteria (Purcell & Frazier 1985; Nault & Ammar 1989; Pinedo-Escatel & Moya-Raygoza 2018).

Certain cicadellid members of the genus *Euscelis* Brulle can be vectors of several important plant diseases. The leafhopper *Euscelis incisus* Kirschbaum, 1858 (Hemiptera: Cicadellidae: Deltocephalinae) is a vector of several phytoplasmas belonging to multiple ribosomal groups and subgroups (Jakovljevic et al. 2020) 53 (10%, such as the 16SrI-B phytoplasma causing Chrysanthemum yellows disease of marguerites in Italian Riviera (Conti et al. 1988), the 16SrI-C phytoplasma to white clover in Lithuania (Ivanauskas et al. 2014)16SrIII, 16SrV and 16SrXII have been found in Lithuania, but their insect vectors in the country have not been determined. 'Candidatus Phytoplasma asteris' and 'Ca. Phytoplasma pruni'-related phytoplasma strains were identified in five leafhopper species and three spittlebug species occurring in Lithuania. The occurrence in Lithuania of *Anaceratagallia ribauti*, reported as a vector of stolbur phytoplasma ('Ca. Phytoplasma solani', subgroup 16SrXII-A and the 16SrIII-B phytoplasma to *Cirsium arvense* L. (Asterales : Asteraceae) in Serbia. Additionally, *E. incisus* is of epidemiological importance because of spreading multiple inflorescence disease in creeping thistle (Jakovljevic et al. 2015) and might be potential natural vector. Another important leafhopper, *Euscelis lineolatus* Brulle, 1832 (Hemiptera: Cicadellidae), was associated to aster yellow phytoplasma (16SrI-C, 16SrI-B subgroups) and stolbur phytoplasma (16SrXII-A subgroup; Vergilbungskrankheit type I [VK-I]) in vineyard agroecosystems of the Marche region of Italy (Landi et al. 2013). Furthermore, *E. lineolatus* has been found to carry/host the phytopathogen *Xylella fastidiosa* (Wells et al. 1987) in olive orchards of Puglia province of southern Italy (Elbeaino et al. 2014). Hemiptera insects were collected from October to December, 2013, in olive orchards with high incidences of *X. fastidiosa* associated with "rapid decline" symptoms. The study focused on species in the Auchenorrhyncha (sharpshooter, leafhoppers and froghoppers or spittlebugs); however, epidemiological experiments have not yet proven its ability to transmit the pathogen from plant to plant.

Euscelidius Ribaut species are also known to act as vectors of various disease microorganisms which are responsible for important economic damage in crops (Brcak 1979; Nielson 1979). *Euscelidius variegatus* is considered a vector species of many diseases of wild and cultivated plants, such as the Chrysanthemum yellows (CY) phytoplasma (Palermo et al. 2001), the corn stunt spiroplasma (CSS) (Alivizatos 1987) reaching titres of over 1x106 colony forming units (cfu, Aster yellows MLO) (Severin 1947), the Clover Phyllody disease (Giannotti 1969) and Western X-disease virus (WXV) (Jensen 1969). In addition, it has been shown to be able to infect grapevine with Grapevine Flavescence Doree (FD) (Caudwell et al. 1970), which is a serious yellows disease of grapevine (*Vitis vinifera* L.) in Europe (Bressan et al. 2005). Additionally, *E. variegatus*

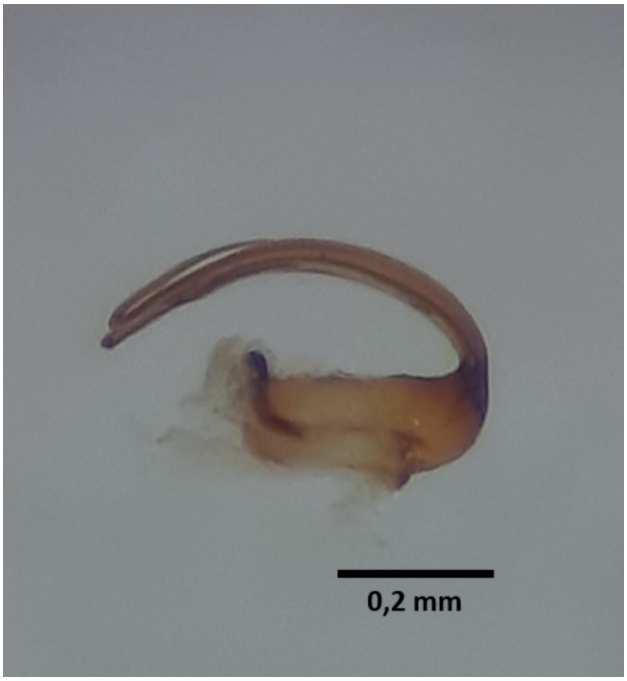

Fig. 1 – Male genitalia of *Euscelis ohausi*.

Fig. 2 – Ventral view of male and female *Euscelis ohausi*.

is an alternative vector of 'Candidatus Phytoplasma solani' to grapevines causing Bois noir (Quaglino et al. 2019), the most widespread disease of the grapevine yellows complex worldwide (Pierro et al. 2020).

Olive oil and wine production in Greece, and especially in the island of Crete, is of great cultural, social and economic importance. Thus, any potential threat to olive- and/or viticulture should be studied thoroughly to avoid pest population outbreaks and yield losses. The palearctic leafhoppers *E. ohausi* Wagner and *E. variegatus* Kirschbaum (Hemiptera: Cicadellidae) are widely distributed in Europe

Fig. 3 – Dorsal view of male and female *Euscelis ohausi*.

Fig. 4 – Male genitalia of *Euscelidius variegatus*.

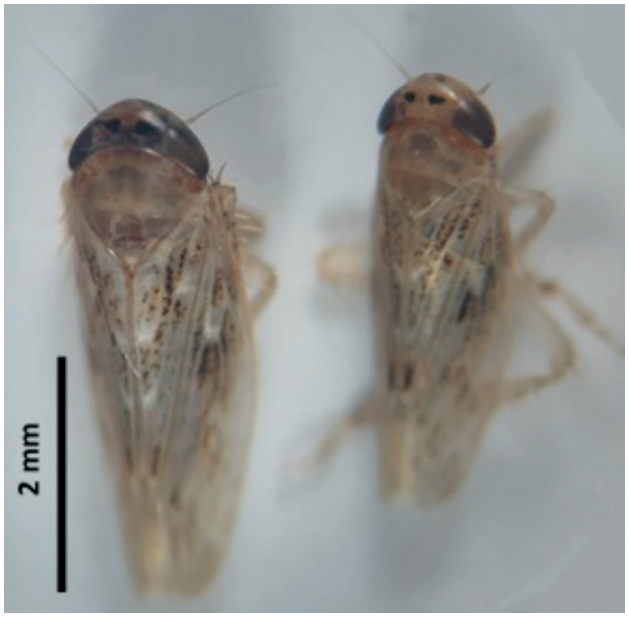

Fig. 5 – Dorsal view of male and female *Euscelidius variegatus*.

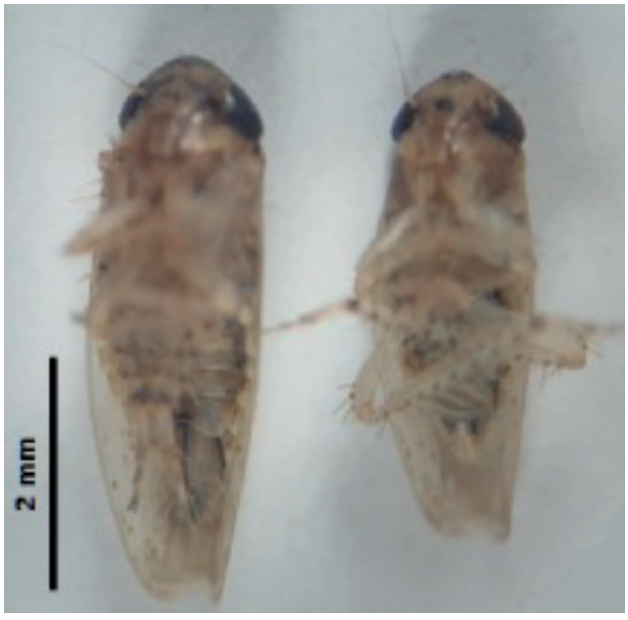

Fig. 6 – Ventral view of male and female *Euscelidius variegatus*.

(Hoebeke & Wheeler 2010) and recently found in several regions of the Greek mainland (Thanou et al. 2018). Moreover, *E. variegatus* has been found in North America including Canada (Parent et al. 2019), Asia and northern Africa (Nielson 1968). Hoebeke & Wheeler (2010) have also confirmed the establishment of *E. ohausi* in North America. Species of the genera *Euscelis* Brulle and *Euscelidius* Ribaut are morphologically similar, however they can be differentiated by male aedeagal characters (Remane 1967; Hoebeke & Wheeler 2010). This study records the presence of the species *E. ohausi* and *E. variegatus* for the first time in the island of Crete and underlines the need for further studies, aiming to understand the bioecology of these leafhoppers and their role as potential vectors of phytopathogens.

Material and methods

Specimens of adult individuals were collected on oat plants from the herbaceous cover of organic olive groves in Crete, Greece, with the use of a sweep net (38 cm di-

ameter), during systematic samplings of Auchenorrhyncha from 2017 to 2019. Specimens were retained in 70% alcohol and then species identification was performed. Insect identification was based on both external shape and male genitalia morphology according to the available literature (Le Quesne 1969; Biedermann & Niedringhaus 2009; Thanou et al. 2018). Habitus and genitalic photographs were taken using a Nikon D3300 DLR camera with a 65 mm macro lens. For identifying the specimens into species level, male genitalia were examined under binocular stereomicroscope (Konus Crystal – 45, Italy) and binocular microscope (Olympus BH – 2, Japan). Male genitalia were prepared before microscope inspection by soaking of the abdomen for 24 h in 10% KOH solution, then rinsing several times in distilled water and storing in glycerin. The collected specimens are preserved in the private collection of the first author as pinned dry specimens.

Results and discussion

Due to favorable temperate environmental conditions, the Greek mainland, as well as the island of Crete, is known for its high insect biodiversity. During systematic surveys of Auchenorrhyncha from 2017 to 2019 in the region of Crete, specimens of *E. ohausi* and *E. variegatus* were found on the herbaceous cover of olive orchards in altitudes ranging from 10 m, in coastal, to 500 m, in mountainous areas. *Euscelidius variegatus* is a polyphagous species (Ottati et al. 2020), in contrast to *E. ohausi*, which has been found mostly on Fabaceae host plants (Nickel & Remane 2002; Hoebeke & Wheeler 2010). In our study however, both species were swept from wild oat (*Avena sativa* L.) plants.

***Euscelis ohausi* Wagner, 1939**

Material examined. **GREECE: (CRETE),** CHANIA, Zymvragou, 35°26’27.1”N 23°45’25.0”E, 300 m, 15 November 2017 (0♂, 1♀), 23 May 2018 (5♂, 3♀), 29 October 2018 (0♂, 1♀); Nerokourou, 35°29’12.8”N 24°01’28.0”E, 10m, 09 May 2018 (2♂, 0♀), 03 September 2018 (2♂, 1♀), 17 September 2018 (1♂, 3♀); Pyrgos, 35°29’31.7”N 23°38’42.0”E, 30 m, 23 May 2018 (3♂, 2♀); Lousakies, 35°28’34.3”N 23°38’02.7”E, 150 m, 03 September 2018 (2♂, 2♀); Zounaki, 35°28’55.3”N 23°49’45.8”E, 180 m, 24 May 2019 (0♂, 1♀); Souda, 35°29’38.9”N 24°02’49.8”E, 7 m, 9 May 2019 (1♂, 0♀), 27 November 2019 (1♂, 1♀). RETHYMNO, Episkopi, 35°20’17.0”N 24°20’12.0”E, 66 m, 12 April 2019 (1♂, 0♀). HERAKLION, Fodele, 35°22’44.5”N 24°57’37.2”E, 45 m, 01 July 2019 (1♂, 1♀); Agia Varvara, 35°09’15.9”N 25°00’43.6”E, 519 m, 21May 2020 (1♂, 0♀).

Diagnosis. The genus *Euscelis* Brulle, belonging to the subfamily Deltocephalinae and tribe Athysanini, comprises more than 50 species (and subspecies) with most of them from the Palearctic Region (McKamey 2001; Hoebeke & Wheeler 2010). Specimens of *E. ohausi* collected from Crete were identified morphologically using identification keys and scientific publications (Thanou et al. 2018; Biedermann & Niedringhaus 2009 & Le Quesne 1969). *Euscelis ohausi* can be readily distinguished either from *E. obsoleta* (Kirschbaum, 1858) or from other similar-appearing Nearctic leafhoppers of the genera *Euscelidius* and *Streptanus,* by the distinct male aedeagus (Hoebeke & Wheeler 2010).

The base colour of adults is yellowish brown and the overall body length ranges from 3.4 to 4.7 mm in males, while in females from 4.1 to 5.5 mm. Regarding males, the aedeagus (Fig. 1) is broad with stout recurrent appendages from apex to below level of gonopore (Le Quesne 1969; Biedermann & Niedringhaus 2009). The vertex has a light brownish colour with two curved blackish streaks near anterior margin and several blackish spots, including two large ones posteriorly. The pronotum has a light brownish colour with some dark spots, sometimes forming longitudinal streaks. The forewings are generally longer than the abdomen in male, and slightly shorter in female. The face (Fig. 3) has a light brownish colour with variable dark markings with dark transverse stripes on frontoclypeus, sometimes fused into longitudinal median patch. Sometimes, there is a dark patch on anteclypeus and dark areas around base of antenna. Scutellum (Fig. 4) has a light brownish colour, often with dark markings. Furthermore, there are highly variable dark mottling of forewings, typically evenly distributed on wing (Le Quesne 1969; Ossiannilsson 1983; Hoebeke & Wheeler 2010).

Distribution. Euscelis ohausi is a Palearctic leafhopper widely distributed in Western Europe; it has been recorded in Belgium, Britain I., Danish mainland, French mainland, Germany, Poland, Portuguese mainland, Switzerland, The Netherlands (de Jong et al. 2014) and Luxemburg (Niedringhaus et al. 2010). In Greece, *E. ohausi* has been recorded by Thanou et al. (2018) with the use of Malaise traps and sweep net in several regions such as Mantoudi (Euboea), Ancient Corinth (Corinth), Elaea (Kiparissia, Messinia), Athens (Attica), Pelekanada (Messinia) and in different habitats such as olive groves, citrus orchards, vineyards, alfalfa, green pepper and fresh tomato. Recently, it was reported as new species to North America, in the United States of America (Oregon and Washington) and in Canada (British Columbia) (Hoebeke & Wheeler 2010). Despite of being found in several areas of the Greek mainland, its presence in the Greek islands is still unknown. This study confirms the presence of *E. ohausi* in several areas of Crete in a wide range of altitudinal spectrum from 10 m up to 519 m. Individuals *E. ohausi* were sampled in relatively high numbers from the herbaceous cover of olive groves in Crete.

Bio-ecology. It is found mostly in sunny, humid to moderately dry sites (Nickel 2003). Studies performed in Germany classified *E. ohausi* in vulnerable conservation status and show it overwinters at the egg stage, having one generation per year, as an oligophagous insect restricted to one plant family (Fabaceae), specifically to *Cytisus scoparius* and *Genista anglica* (Remane et al. 1998; Nickel & Remane 2002). Moreover, there are two forms of *E. ohausi* in central Europe with different host plant range, coloration, body size and distribution. The form "typica" lives on *Genista anglica* near the coast of the North Sea and the Baltic Sea in Germany, while the form "singeri" feeds on *Cytisus scoparius* in central and southern Germany and Luxemburg (Nickel 2003; Niedringhaus et al. 2010). Since both forms can interbreed and produce fertile offspring some authors consider them as synonyms at species level while others as distinct subspecies (Nickel 2003). According to the literature, as regards their occurrence to different host plants, the two forms are associated with *Genista anglica* and *Cytisus scoparius*. In our study, *E. ohausi* adults were observed on Gramineae plants (several grasses), particularly wild oats (*Avena sativa*) from May to October. The two previously recognized host plants, *G. anglica* and *C. scoparius,* were not present in the study area.

***Euscelidius variegatus* (Kirschbaum, 1858)**

Material examined. **GREECE: (CRETE),** CHANIA, Zymvragou, 35°26'27.1"N 23°45'25.0"E, 300 m, 03 September 2018 (0♂, 1♀); Nerokourou, 35°29'12.8"N 24°01'28.0"E, 10 m, 11 June 2018 (5♂, 2♀); Zounaki, 35°28'55.3"N 23°49'45.8"E, 100 m, 24 May 2019 (0♂, 1♀), 23 July 2018 (1♂, 1♀); Souda, 35°29'38.9"N 24°02'49.8"E, 7 m, 27 November 2018 (2♂, 2♀). HERAKLION, Fodele, 35°22'44.5"N 24°57'37.2"E, 45 m, 01 July 2019 (2♂, 1♀).

Diagnosis. In Europe, the genus *Euscelidius* Ribaut, 1972 includes three species (*E. variegatus* Kirschbaum, 1858; *E. schenckii* Kirschbaum, 1868; *E. mundus* Haupt, 1927) which have been also recorded in Greece (Drosopoulos et al. 1986; Thanou et al. 2018). Specimens of *E. variegatus* from Crete were identified morphologically using identification keys and relevant literature (Le Quesne 1969; Biedermann & Niedringhaus 2009; Thanou et al. 2018).

The base colour of adults is greyish yellow and the overall body length ranges from 3.7 to 4.7 mm in males while in females from 4.4 to 5.3 mm. Regarding males, the aedeagus, in side view, has hook-like apical appendages (Fig. 2) (Le Quesne, 1969; Biedermann and Niedringhaus, 2009). The vertex (Fig. 5) has a light brownish colour with two blackish wedge-shaped marks right behind the ocelli and other more or less extensive dark markings, sometimes fused with these. At the frontoclypeus (Fig. 6) there are two large dark patches near the upper margin, widely marked with dark transverse streaks below, which may be partly fused in lower part in darkly marked individuals. Anteclypeous has a few dark dots or a dark streak. Pronotum and scutellum has a light brownish colour with several brown or black markings. Fore wings are longer than abdomen with a light brownish colour and with more or less well-developed pattern of more or less fused irregular darker dots (Le Quesne 1969).

Distribution. Euscelidius variegatus is widely distributed in Europe, also known to occur in western (California, Washington, Oregon and Utah of USA) and eastern (Ontario, Canada).

North America (Young 1955; Hamilton 1983; Parent et al. 2019)62 were erroneously recorded previously although 12 of the European species subsequently have been found in North America.

Two North American species have been introduced into Europe, and one European species has been found in South America; North America has received 61 species from Europe, 3 from Asia, and 1 from Africa. One pan-tropical species is probably a recent introduction into the Caribbean from the Old World. There are 30 transboreal leafhoppers, 5 transarctic species inhabiting the tundra and alpine meadows, and 9 inhabiting temperate regions of both Eurasia and North America.

Specifically, according to de Jong et al. (2014), this species has been recorded in Austria, Azores Is., Balearic Is., Belgium, Britain I., Bulgaria, Canary is., Czech Republic, French mainland, Germany, Italian mainland, Moldova, Near East, North Africa, Poland, Portuguese mainland, Sardinia, Sicily, Spanish mainland, Switzerland, The Netherlands, Ukraine and Yugoslavia. Distributed in the western Palearctic Region and in North America, *E. variegatus* is a great colonizer. Interestingly, it is capable to establish in continental islands such as Great Britain, Sardinia, Sicily and Balearic Islands (Reis and Aguin-Pombo 2003). In Greece, *E. variegatus* was recorded for the first time in Athens (Attica) in olive and citrus orchards with Malaise trap and sweep netting (Thanou et al. 2018). Nevertheless, information about its presence in the Greek islands is pending. In our study, we found *E. variegatus* in two prefectures (Chania and Heraklion) of Crete, occurring from 10 m up to 300 m of altitude; like in Madeira, Azores and the Canary Islands, it was found in both dry and coastal areas of the island of Crete and in agricultural fields (Lindberg 1941; Sergel & Baez 1990; Reis & Aguin-Pombo 2003).

Bio-ecology. In our study, adults were observed from April to September, but they were more abundant in June. *Euscelidius variegatus* is considered a polyphagous species (Reis & Aguin-Pombo 2003) feeding on Fabaceae (*Trifolium repens* var. repens), Apiaceae (*Apium nodiflorum*), Chenopodiaceae, Lamiaceae, Malvaceae, Solanaceae and Vitaceae (Alma et al. 1988; Cardoso 1974; DeLong & Severin 1947; Quartau 1980). In the present study

however, adults were only found on Gramineae plants, specifically *A. sativa* in olive groves. Likewise, *E. variegatus* was found in Athens (Attica) in herbaceous vegetation growing within olive groves (Thanou et al. 2018).

Conclusions

This contribution illustrates the presence of the two leafhoppers *Euscelidius ohausi* and *E. variegatus* for the first time in the island of Crete. Moreover, this work could be evidence of the maybe recent and fast dispersal of the two leafhoppers from the Greek mainland to the Aegean islands, since older faunistic studies in Greece (Drosopoulos1980; Drosopoulos et al. 1986) did not report these two leafhopper species. Only recently however, Thanou et al. (2018) reported specimens of the two leafhoppers for the first time in areas of Central Greece suggesting they have been established recently in Greece. Both leafhopper species were found in several olive grove environments in Crete and in a wide range of altitude. Further research should investigate the presence of the two species in other Greek islands, in order to fully understand their distribution. Last but not least, their bioecology as well as their association with symbiotic bacteria and vector-borne diseases should be investigated.

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