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Rapid Communication

Uninvited pests of an unwelcomed tree: a survey on alien chalcidoid wasps (Hymenoptera: Chalcidoidea) associated with *Eucalyptus* trees in Cyprus

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Abstract

A five-month survey on *Eucalyptus* spp., one of the most commonly planted trees in Cyprus, was undertaken in Limassol and Akrotiri in urban, rural and protected habitats. Two alien gall-inducing *Eucalyptus* wasps: *Leptocybe invasa* Fisher and La Salle, 2004 and *Ophelimus maskelli* (Ashmead, 1900) (Hymenoptera: Eulophidae) were recorded for the first time from Cyprus. In addition, three new alien parasitoids: *Stethynium ophelimi* (Huber, 2006) (Hymenoptera: Mymaridae), *Closterocerus chamaeleon* (Girault, 1922) (Hymenoptera: Eulophidae) and *Megastigmus lawsoni* Doğanlar and Hassan, 2010 (Hymenoptera: Megastigmidae), were reared from *O. maskelli* and *L. invasa* galls. The distribution, introduction and management actions for *Eucalyptus* spp. and their alien Chalcidoidea associates are discussed.

Key words: biological invasions, *Ophelimus maskelli*, *Stethynium ophelimi*, *Closterocerus chamaeleon*, *Leptocybe invasa*, *Megastigmus lawsoni*, first records

Introduction

Native to Australasia, representatives of the genus *Eucalyptus* L'Hér were historically introduced around the world as ornamental trees, to dry up *Anopheles* mosquito habitats like marshes during anti-malarial campaigns, as well as for the production of pulp and timber (Cocquempot and Lindelöw 2010; Mifsud et al. 2010; Bayle 2019). In Cyprus, since the early colonization of the island up to the end of the Ottoman Empire, the island's once vast forests gradually degraded and shrunk due to unsustainable practices including but not limited to agriculture, population growth, mining and ship building (Harris 2007; Chatzikyriakou 2017). Shortly after the British occupation in 1878, native and alien species were planted for the reforestation of extensive areas in an attempt to mitigate the

deforestation (Ciesla 2004). *Eucalyptus globolus* Labill. seeds were transported from Tanzania to Nicosia and Larnaca (Harris 2007). Additionally, *Eucalyptus* seeds of 15 different species were introduced by a government gardener and planted in Famagusta, despite concerns among experts and the public regarding the import and usage of *Eucalyptus* spp. at the time (Baker 1879; Wild 1879; Harris 2007; Pescott et al. 2018). Although they struggled to survive during the first years of introduction, continuous tree planting eventually led to successful establishment of *Eucalyptus* spp. on the island (Harris 2007). Until this day, *Eucalyptus* spp. have been planted as ornamental in a diverse range of urban, semi-urban, rural, agricultural and natural habitats including protected areas throughout the island. This has led to the inevitable unintentional introduction and establishment of alien insects such as the *Eucalyptus* longhorn beetles *Phoracantha recurva* Newman, 1840 and *P. semipunctata* (Fabricius, 1775) (Alziar and Lemaire 2008), as well as the invasive alien red gum lerp psyllid *Glycaspis brimblecombei* Moore, 1964 and its Encyrtid parasitoid *Psyllaephagus bliteus* Riek, 1962 (Karaca et al. 2017).

The alien species of Cyprus are catalogued in the Cyprus online Database of Alien Species – CyDAS (www.ris-ky.info) (Martinou et al. 2020). Horizon scanning programmes have been implemented to predict the establishment and adverse impacts of invasive alien species (IAS) currently absent but capable of reaching Cyprus (Peyton et al. 2019, 2020). The alien entomofauna of Cyprus has recently been updated and currently holds approximately 350 species of alien, cryptogenic and questionable (i.e., in need of further research to establish their nativity or introduction) status (Martinou et al. 2020; Demetriou 2021; Demetriou et al. *in prep*). Public participation in the recording of alien species is currently very much encouraged, to address the necessity for networks of citizen scientists working jointly with the scientific community in biodiversity monitoring. The present work focuses on alien Chalcidoid insects on *Eucalyptus* spp.

Materials and methods

Samplings in urban, rural, and protected areas hosting individuals or populations of *Eucalyptus* spp. were performed from December 2020 to June 2021 (Supplementary material Table S1). Four locations at Akrotiri and two at Limassol were sampled weekly from February to June 2021, supplemented by opportunistic samplings in the western part of the island (Paphos district) and Nicosia. Collection sites and data of reared and observed material are presented in Figure 1 and Table S1. Some *Eucalyptus* leaves were observed to be abnormally swollen and deformed in the midrib and petiole area, thus indicating the presence of *L. invasa* (Mendel et al. 2004) (Figure 2A). Furthermore, the leaf lamina of various leaves was filled with galls indicating the presence of a representative of the genus *Ophelimus*

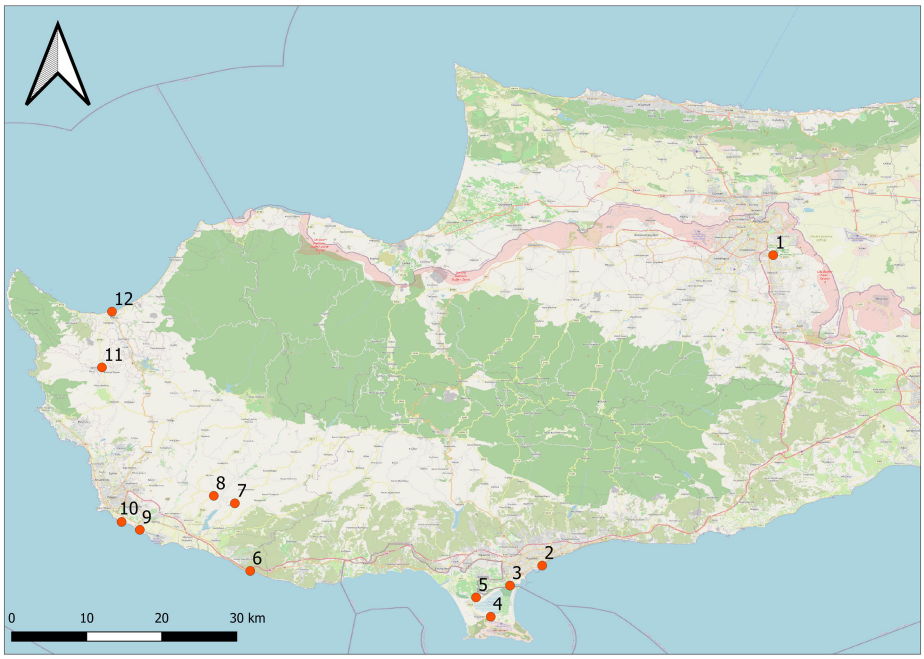

Figure 1. Map of sampled localities. For details see Table S1.

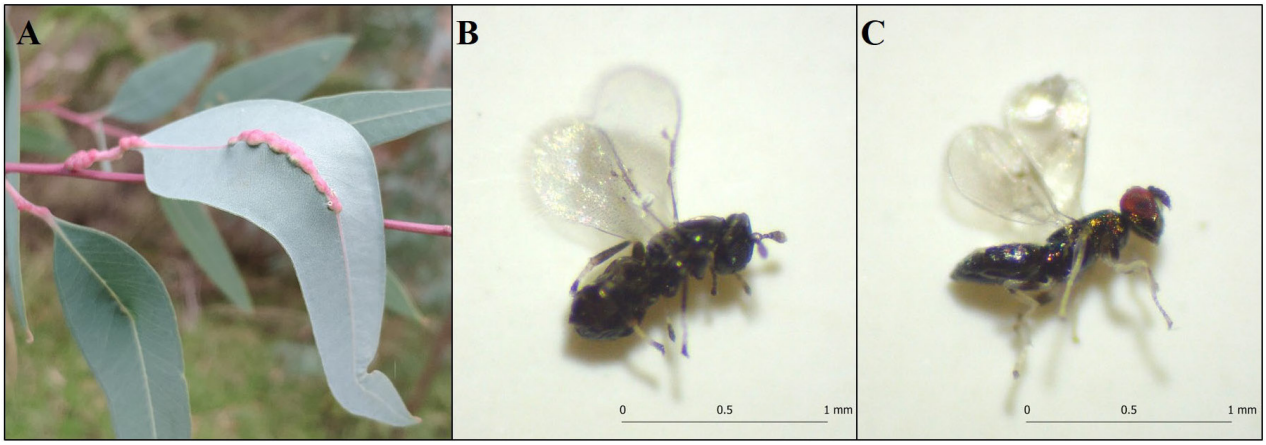

Figure 2. *Leptocybe invasa* galls (A), *Ophelimus maskelli* (B) and *Closterocerus chamaeleon* (C). Photographs by Jakovos Demetriou.

Haliday, 1844 (Protasov et al. 2007b). Collection methods followed Protasov et al. (2007b); infested leaves were cut and stored dry in sealed polyethylene bags. This method enabled the emergence of adult individuals from *Ophelimus* galls, but not rearing of *Leptocybe invasa* individuals. Therefore, data presented herein correspond to observed *L. invasa* galls and not reared specimens. Reared Chalcidoidea specimens were stored in 70% ethanol for further identification under a stereomicroscope. Species identification was performed using the identification keys and species descriptions of Noyes and Hayat (1984), Mendel et al. (2004), Huber et al. (2006), Berry (2007), Protasov et al. (2007a), Doğanlar and Hassan (2010), Borowiec et al. (2019) and Samková et al. (2020). Collected specimens will be deposited in the Department of Ecology and Systematics, Faculty of Biology, National and Kapodistrian University of Athens as well as the Joint Services Health Unit, Akrotiri, Cyprus as part of the first author's MSc Thesis. Maps in figures were created using QGIS Version 3.18.

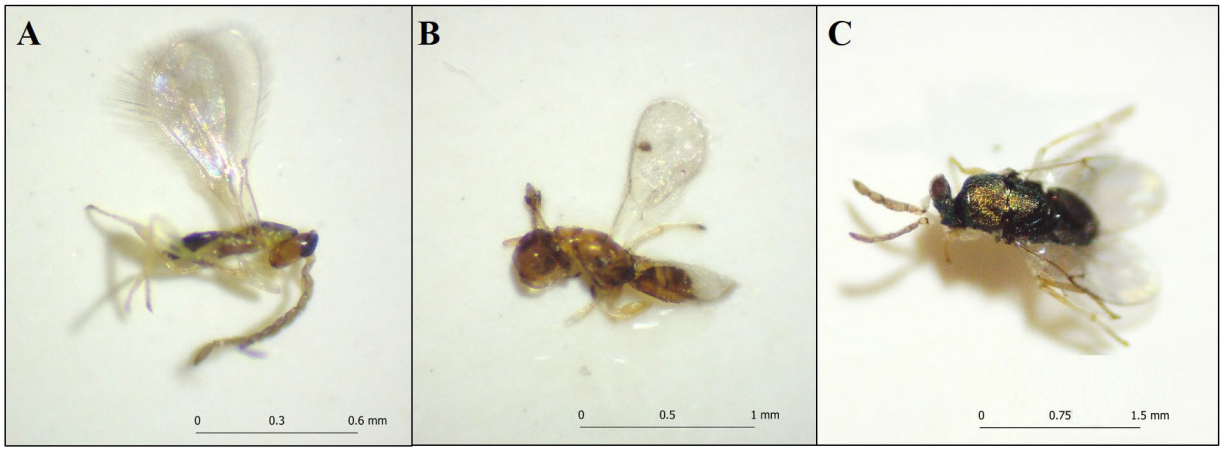

Figure 3. *Stethynium ophelimi* (A), *Megastigmus lawsoni* (B) and *Psyllaephagus bliteus* (C). Photographs by Jakovos Demetriou.

Results

Specimens of six alien Chalcidoidea species have been observed, collected or reared along the southern and western parts of Cyprus stretching up to the capital city of Nicosia (Figure 1; Table S1). In particular, new records for this country/island concern two *Eucalyptus* gallers *Leptocybe invasa* Fisher and La Saller, 2004 (Figure 2A) and *Ophelimus maskelli* (Ashmead, 1900) (Figure 2B) as well as their parasitoids *Closterocerus chamaeleon* (Girault, 1922) (Figure 2C) and *Stethynium ophelimi* Huber, 2006 (Figure 3A) for *O. maskelli*, and *Megastigmus lawsoni* Doganlar and Hassan, 2010 (Figure 3B) for *L. invasa*. In addition, the previously recorded *Psyllaephagus bliteus* (Figure 3C), was reared from lerps of its known host *Glycaspis brimblecombei* (Karaca et al. 2017).

Localities referring to reared parasitoids of *O. maskelli*, *C. chamaeleon* and *S. ophelimi*, also correspond to observed galls of *O. maskelli*, as its presence is required for *C. chamaeleon* and *S. ophelimi* to prosper (Figure 1; Table S1). Localities corresponding to *L. invasa* concern only observed galls. Both leaf gallers, *O. maskelli* and *L. invasa* seem to be widely distributed on the island. The former is predominantly parasitized by *C. chamaeleon* as evidenced by the number of reared specimens; particularly, out of 1714 chalcid wasps reared from *O. maskelli* galls, 65% (1120 individuals) were *C. chamaeleon* and only 14% (237 individuals) were *S. ophelimi*. The remaining 21% (357 individuals) of specimens were identified as *O. maskelli*, indicating that only about one fifth of galls were not parasitized and the galler emerged successfully. Most adults of *O. maskelli* (approx. 73% – 260 individuals) were reared from leaves collected on the 24th of March 2021. However, even in that case, 35% of reared individuals were parasitoids of *O. maskelli*. *Closterocerus chamaeleon* was reared from December to March but *O. maskelli* and *S. ophelimi* were reared only during February and March. Rearing *L. invasa* proved unsuccessful and only one specimen of its parasitoid *M. lawsoni* was reared from locality 2 (Table S1). *Psyllaephagus bliteus* was reared from two localities (2 and 4) (Table S1) although its host psyllid *G. brimblecombei* is widely distributed.

Discussion

All three aforementioned Australian eulophid species seem to have well established populations on the island, as dried and burst galls on older leaves indicate infestations from previous years. The wide distribution of *L. invasa* (Figure 2A) and *O. maskelli* (Figure 2B) on the island is likely to be a consequence of their transport as contaminants of infested host-plant material and possible unaided dispersal due to intensive flight periods, as reported from neighbouring countries, during the summer months (Protasov et al. 2007b). Their presence in urban and semi-urban habitats is common in Europe; parks and gardens have been found to hold the largest number of alien arthropods amongst invaded habitats (Lopez-Vaamonde et al. 2010). *Closterocerus chamaeleon* (Figure 2C), a parasitoid of *O. maskelli*, is renowned for its high dispersal potential in many Mediterranean countries utilizing both wind currents and human-mediated transportations (Doğanlar and Mendel 2007; Branco et al. 2009; Lo Verde et al. 2010; Caleca et al. 2011).

Eucalyptus gallers such as *O. maskelli* have been identified as affecting good quality of human life, with clouds of wasps reported as a public nuisance in heavily infested urban areas, e.g. schools, during periods of mass emergence (Protasov et al. 2007b). Their excessive numbers in agricultural areas have been also associated with the reduced market value of selected crops, i.e. lettuce (Protasov et al. 2007b), highlighting some of the socioeconomic impacts of the species recorded herein. Given the wide distribution of *Eucalyptus* spp. in Cyprus, both in urban areas like ornamental foliage and in agricultural areas as extensive windbreak rows, the documented socioeconomic impacts of Chalcidoidea associated with these trees should be further investigated and consideration given to mitigate adverse effects on the island. In order to assess the potential threats of *Eucalyptus* gallers and their parasitoids towards public well-being and agriculture, sampling in agricultural lands and urban areas need to be undertaken. Additionally, there is a need to consult farmers, school personnel and students on their perceptions of these species.

The finding of *S. ophelimi* (Figure 3A) is of considerable interest. This species constitutes only the third Mymaridae species known for Cyprus' the others are *Stephanodes similis* (Förster, 1847) and *Lymaenon litoralis* (Haliday, 1833) (Graham 1982; Triapitsyn and Berezovskiy 2002; Noyes 2019). Native to Australia, this alien fairyfly was introduced to neighbouring Israel together with *C. chamaeleon* as biological control agents of *O. maskelli* (Huber et al. 2006; Mendel et al. 2007). Unintentional introduction from neighbouring countries such as Israel and Turkey (Çikaran and Avci 2019) is possible but it is also likely that the species has been introduced to Cyprus from Australia together with its hosts but due to its small size remained undetected.

The applied rearing method used here did not yield any specimens of *L. invasa*. According to Mendel et al. (2004), adult emergence in Israel is observed during April and May. However, no specimens were reared during our study. In a zip-bag containing *L. invasa* galls collected on March 4th 2021 at Limassol marina (Molos) from various *Eucalyptus* individuals, a single male of its Megastigmid parasitoid *M. lawsoni* (Figure 3B) was reared. Taking into consideration the fact that *M. lawsoni* was imported from Australia to Israel (Doğanlar and Hassan 2010), its unintentional introduction from the Levantine coast could be a possibility. The specimen we found was about three kilometres from Limassol Port, the largest commercial harbour of Cyprus, which could indicate the importation of the species in infested plant material through ship cargo. Alternatively, as in the case of all aforementioned species, *M. lawsoni* could have been present on the island since the unintentional introduction of its host. It is currently unknown whether the species has formed established populations although it is certain that its presence in the sampled site reflects a recent range expansion, as the ornamental foliage in the area was planted during the last decade. Knowledge on the origin of the planted *Eucalyptus* stands could help determine the invasion history and potential origin of *M. lawsoni* in Cyprus.

Psyllaephagus bliteus (Figure 3C), a parasitoid of the red gum lerp psyllid, was reared from *G. brimblecombei* individuals collected from Limassol marina (Molos) and Akrotiri salt-lake. This species was first reported from Cyprus by Karaca et al. (2017). The presented data depict a wider distribution of both the parasitoid and its host psyllid in the island, reaching the southern coast of Limassol. Despite the parasitism of *G. brimblecombei* by *P. bliteus*, the former was frequently observed in high numbers in most sampling sites, especially during May and June. Therefore, *P. bliteus* does not seem to provide adequate biological control of the psyllid in the island, as determined for other Mediterranean countries such as Portugal (Boavida et al. 2016).

In summary, current records of alien Chalcidoidea as well as the extensive distribution of representatives of the genus *Eucalyptus* in both peri-urban and natural habitats, suggest that there is justification for further monitoring and surveillance of alien insect species in protected areas and conservation sites. Alien chalcid wasps associated with *Eucalyptus* spp. were collected from Athalassa Natural Forest Park (protected by the Cyprus Forestry Legislation), Akrotirio Aspro – Petra Romiou (NATURA 2000/CY5000005), Akrotiri Wetlands and Akrotiri salt-lake (Ramsar Wetland). Consequently, this survey supplements our knowledge on the presence of alien species within protected areas of the island.

Despite the minuscule size of Chalcidoidea associated with *Eucalyptus* spp., future research could involve citizen science approaches in mapping the

distribution of alien species such as *L. invasa* and *O. maskelli* because they both form easily detected characteristic galls on their host-plant. Additionally, citizens can alert municipalities and scientists about the socioeconomic impacts associated with *Eucalyptus* pests, such as clouds of wasps presenting a public nuisance (Protasov et al. 2007b) or obnoxious galls and alien psyllid lerps undermining the aesthetics of nature (Demetriou 2021). Reported sites of infested *Eucalyptus* spp. can be subsequently sampled for the identification of alien species anticipated to be found, such as alien insects reported from neighbouring Israel (Mendel and Protasov 2019).

Regarding the usage of *S. ophelimi* and *C. chamaeleon* as biological control agents, some important details need to be mentioned. The release of seven alien parasitoid agents against *Eucalyptus* gall wasps in Israel resulted in the successful control of the target species (Mendel et al. 2007; Mendel et al. 2014). However, their impacts on non-target native species have not been meticulously studied and native species were also found parasitizing the invading gallers (Mendel et al. 2007, 2014). Although no indigenous species were reared during our study, the importation of additional biological control agents is discouraged. A more rational approach to the problem could be the environmental education of people in order to raise awareness about the adverse impacts of *Eucalyptus* spp. in the island. As a second step, we would suggest planting urban and semi-urban sites with native species, e.g., *Ceratonia siliqua* L., *Cupressus sempervirens* L., *Olea europaea* L., *Pistacia terebinthus* L.

The question however arises, whether these Chalcidoid wasps should be considered as uninvited pests of an unwelcomed host. Further investigation is required to assess the population-level effects of the newly recorded parasitoids on *O. maskelli* and *L. invasa*. Alien *Eucalyptus* spp. have long been cause for controversy among forestry experts, governmental agencies and the public (Baker 1879; Wild 1879; Harris 2007; Pescott et al. 2018). *Eucalyptus* forests such as those in Akrotiri peninsula (Pescott et al. 2018), Paphos airport area and Athalassa Natural Forest Park may serve as a "green lung" to the continuously expanding city centres while, simultaneously, parks provide relaxation and a number of recreational activities to visitors and residents. In this context, the premature shedding and reduced performance caused by *Eucalyptus* gall-inducers (Protasov et al. 2007b), poses an important negative socioeconomic impact of the newly recorded Chalcidoid parasitoids. Nevertheless, *Eucalyptus* spp. in the Mediterranean basin have been associated with the alteration of terrestrial and freshwater communities leading to low abundances of ground arthropods (Zahn et al. 2009) and plant community species richness (Becerra et al. 2017). They have also been linked with reduced herbivory and availability of habitats for invertebrates and fish (Graça et al. 2002). Although in Spain mature *Eucalyptus* stands may provide a suitable nesting site for certain birds such as *Accipiter gentilis* (Linnaeus, 1758)

(García-Salgado et al. 2018) or an important food resource for flower-visiting birds (Calviño-Cancela and Neumann 2015), they have been also found to host the poorest avifauna compared to pine and oak stands (de la Hera et al. 2013; Calviño-Cancela 2013). Furthermore, *Eucalyptus* trees affect soil properties by increasing soil hydrophobicity, reducing both stream debris and nutrient contents as well as leading to soil loss (Graça et al. 2002). Therefore, this alien plant seems to cause adverse ecological impacts on both native biodiversity and ecosystems.

The gradual replacement of *Eucalyptus* spp. forests with native trees, historically known to grow in the target areas, poses an important step towards ecological restoration. Management actions should also take into consideration the protection of natural vegetation remnants and the creation of stands of different ages and tree species (Brockerhoff et al. 2013). Finally, the adverse impacts of non-native species in Cyprus have to be addressed in the following years. The continuous planting of *Eucalyptus* trees and other non-native species, even in the context of EU subsidised governmental projects (EU Rural Development Program: under action 2.4), underlines the necessity for training not only the public but also governmental agencies, on the pivotal ecological threats of biological invasions in the island.

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Ethics and permits

Arthropod collection permits were received by the Department of Environment, Ministry of Agriculture, Rural Development and Environment of Cyprus.

Authors' contribution

Research conceptualization: JD; CR; HER; MA; AFM. Sample design and methodology: JD; CR; HER; MA; AFM. Investigation and data collection: JD; EK; AFM. Data analysis and interpretation: JD; EK; CR; HER; MA; AFM. Ethics approval: AFM. Funding provision: AFM. Writing – original draft: JD. Writing – review and editing: JD; EK; CR; HER; MA; AFM.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Localities and metadata of observed and collected material.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2022/Supplements/BIR_2022_Demetriou_etal_SupplementaryMaterial.xlsx