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Rapid Communication

First record and DNA barcoding of the fig gall wasp *Josephiella microcarpae* (Hymenoptera: Chalcidoidea: Pteromalidae) in Greece

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Abstract

The Chinese banyan, *Ficus microcarpa* is an ornamental fig tree, widely planted throughout Southern Greece. Native to Asian – Australasian regions, it can be found in plazas, parks, roadsides as well as private gardens. Along with its host, various insect species have managed to follow and establish themselves outside of their native range. In Rhodes, the mass defoliation of an emblematic fig tree, led to the subsequent finding of *Josephiella microcarpae* infestation. Repetitive samplings in the following years confirmed the establishment of the species on the island, which constitutes the first record of this species in Greece. Collected specimens from Rhodes and Cyprus were sequenced for the COI DNA barcoding region. Further investigation of fig trees throughout various locations in Southern Greece, proved fruitless. Based on our findings, the distribution of *J. microcarpae* in Greece seems restricted in the city of Rhodes.

Key words: alien species, biological invasions, *Ficus microcarpa*, fig wasps, Epichrysomallinae, cytb

Introduction

Globalization and international trade have led to a continuously rising number of translocated species across the globe (Hulme 2009), with insects representing one of the most numerous groups of alien species in Europe (Roques et al. 2009). Hundreds of non-native insects have been reported from Greece over the last centuries – a total of 469 species according to the most up-to-date estimate (Demetriou et al. 2021). Several of these have been translocated as a result of the international movement of horticultural plants and may have adverse impacts on both native and introduced flora, e.g. the sisal weevil *Scyphophorus acupunctatus* Gyllenhal, 1838 (Kontodimas and Kallinikou 2010), the lantana plume moth, *Lantanophaga pusillidactylus*

(Walker, 1864) (Lepidoptera: Pterophoridae) (Demetriou et al. 2020), and the box-tree moth *Cydalima perspectalis* (Walker, 1859) (Kazilas et al. 2021).

DNA barcoding has gradually emerged as an important tool in entomology assisting not only the identification of species but also revealing their hosts, trophic relationships, crypticity in species complexes or relationships between morphologically variable individuals in cases of different castes or sexual dimorphism (Jinbo et al. 2011; Gariepy et al. 2019; Polaszek et al. 2022). These advances have been utilized in the field of Invasion Biology both in the identification of various developmental stages of alien insects (Pieterse et al. 2010; Jinbo et al. 2011), as well as in quarantine inspections (Kang et al. 2019; Madden et al. 2019).

Known by many names, the Chinese or Malayan banyan, *Ficus microcarpa* L. is a well-known ornamental tree originating from Southeast Asia (Berg et al. 2005; van Noort et al. 2013). Like many other representatives of the genus *Ficus* L., it has been introduced outside its native range and planted in urban and semi-urban areas (house gardens, squares, parks etc.) (Compton et al. 2020). *Ficus microcarpa* has been extensively planted throughout many Mediterranean coastal areas, including islands such as Cyprus, Rhodes, and Symi (Wang 2014; Compton et al. 2020).

Following the extended translocation of *F. microcarpa* throughout the world, a series of fig wasps belonging to the Chalcidoidea (Insecta: Hymenoptera), have been accidentally introduced along with their host plant to Greece (Compton 1989; Wang 2014; Wang et al. 2015a). These fig wasps are associated with figs either being putative phytophages [i.e. *Eupristina verticillata* Waterston, 1921 (Chalcidoidea: Agaonidae); *Meselatus bicolor* Chen, 1999; *Odontofroggatia gallili* Wiebes, 1980; *O. ishii* Wiebes, 1980; and *Walkerella microcarpae* Bouček, 1993 (Chalcidoidea: Pteromalidae)], or parasitoids [i.e. *Sycophila maculafacies* Chen, 1999 (Chalcidoidea: Eurytomidae) and *Philotrypesis emeryi* Grandi, 1926 (Chalcidoidea: Pteromalidae)] (Compton 1989; Wang 2014; Wang et al. 2015b).

Native to Southeast Asia, *Josephiella microcarpae* Beardsley and Rasplus 2001 has managed to reach Hawaii, where it was originally described, presumably through the transportation of infested host-plant material (Beardsley and Rasplus 2001). While *J. microcarpae* has not been found within its potential range of origin yet, it is believed to be already widespread in the Mediterranean basin (Compton et al. 2020), where it has already been recorded from Spain (including the Canary Islands; Beardsley and Rasplus 2001; Rodrigo Santamalia et al. 2017), Italy (Lo Verde 2002), Malta (Mifsud et al. 2012), Cyprus (Compton et al. 2020) and Portugal (Madeira; Bella and Aguiar 2020).

Unlike other fig wasps associated with *F. microcarpa*, whose biological cycle is related to figs, *J. microcarpae* is known to be a leaf galler and probably reproducing parthenogenetically (Beardsley and Rasplus 2001; Compton et al. 2020). Apart from *J. microcarpae*, only *Josephiella malabarensis* Narendran,

1993 has been described as a fig leaf-galler, but on *F. benghalensis* (Narendran and Sheela 1993; Beardsley and Rasplus 2001). Except from fig leaf-gallers, *Leeuweniella ficophila* Ferriere, 1929 is known to induce galls on twigs of *F. recurva* Blanco (Ferriere 1929; Beardsley and Rasplus 2001). Recently, investigations on fig trees in Honolulu led to the discovery of an undescribed *Josephiella* species, developing galls on young stems (HDOA 2012; Bhandari and Cheng 2016). A similar shoot-and-branch-infecting *Josephiella* species was reported from Italy (NPPO of Italy 2015). While both specimens were labeled as a different species from *J. microcarpae*, whether they belong to one or more species has not been determined yet (NPPO of Italy 2015).

So far, there has been no published record of *J. microcarpae* in Greece, whilst indications of its presence in Rhodes can be traced back to 2019, when the extended foliage loss of an emblematic fig tree at the Rhodes town center led to an examination of the infestation by the Benaki Phytopathological Institute (Athens, Greece). While a phytotoxin was deemed the source of the foliage loss, galls were observed on the collected leaf material, presumably induced by *J. microcarpae*. Despite the extended media coverage (Rodiaki 2022), as well as public awareness raised from this incident, these findings were not published in a scientific journal.

In this publication, material sampling confirms the presence of an established population of *J. microcarpae* in Rhodes using morphological criteria, while DNA barcoding of samples from Rhodes and Cyprus is conducted to aid future endeavors in resolving the interspecific relationships of *Josephiella* species.

Materials and methods

Study area, sampling and identification

Chinese-banyan leaves were examined for galls indicating the presence of *J. microcarpae* in various locations in southern Greece throughout 2021 (Figure 1, Supplementary material Table S1). Infested leaves (Figure 2) from Rhodes and Paphos (Cyprus) were identified following the methodology of Beardsley and Rasplus (2001), collected and stored dry in sealed polyethylene bags. Reared individuals of *J. microcarpae* were subsequently stored in 70% and 90% ethanol for further examination under a stereomicroscope. Identification was confirmed based on the following diagnostic characters: body dark brown, head subglobose, bilobed clypeus, antennae inserted above ocular line, tarsi four-segmented, and marginal vein of forewing at an angle of 90° (Beardsley and Rasplus 2001, van Noort and Rasplus 2021). From the material collected, one specimen from each location was used for DNA extraction, while several specimens were deposited at the Museum of Zoology of the National and Kapodistrian University of Athens (ZMUA). Hence our vouchers are secondary vouchers (specimens from the same population that were not extracted).

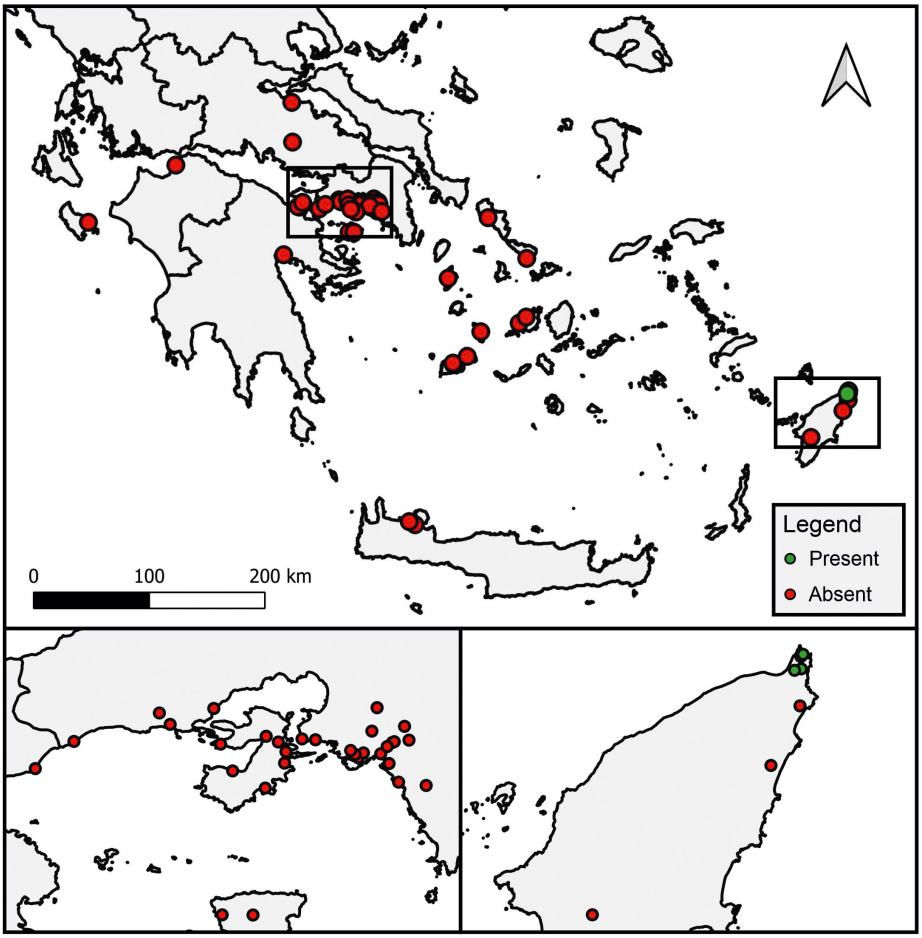

Figure 1. Sampling localities of *Ficus microcarpa* L. in Greece. Localities where *Josephiella microcarpae* Beardsley and Rasplus 2001 was observed are indicated with green, while its absence is shown in red.

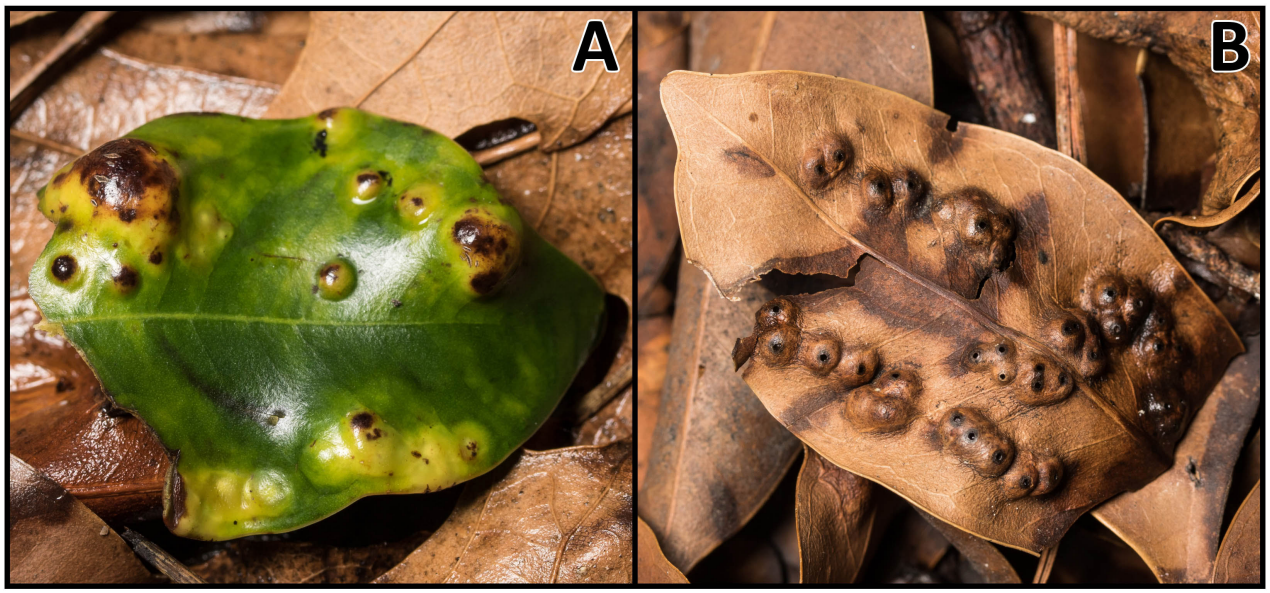

Figure 2. Galls of *Josephiella microcarpae* Beardsley and Rasplus 2001 on leaves of *Ficus microcarpa* L. in Rhodes Greece. (A) Leaf bearing fresh (unemerged) galls. (B) Emergence holes on dried leaf. Photos by KK.

Molecular analysis

Total genomic DNA was extracted from each individual separately in the Laboratory of Forest Entomology (Forest Research Institute, Hellenic Agricultural Organization Demeter), using PureLink® Genomic DNA kit (Invitrogen) and following the manufacturers' instructions, whereas tissues were grounded using polypropylene pestles due to the minute size of samples. Polymerase Chain Reaction (PCR) was run in 25 μl volumes with primers LCO1490/HCO2198 that amplify a 658bp-fragment of the mitochondrial cytochrome oxidase subunit I gene (COI) (Folmer et al. 1994). PCR conditions included an initial denaturation stage at 95 °C (3 min), followed by 5 cycles of 20 s at 95 °C (denaturation), 20 s at 47 °C (annealing), and 30 s at 72 °C (extension), and then by 40 cycles of 20 s at 95 °C (denaturation), 20 s at 52 °C (annealing), and 30 s at 72 °C (extension). The final extension period was carried out at 72 °C over 5 min. PCR products were purified with PureLink® PCR Purification Kit (Invitrogen) following the manufacturer's protocol. Purified products were sequenced in the automated sequencer ABI3730XL of CeMIA Company (Larisa, Greece), using the aforementioned primers. Finally, sequences were initially visualized with Chromas Lite version 2.01, and deposited in the NCBI GenBank database.

Results

Material examined

5♀ Greece, Dodecanese, Rhodes, Rimini sq. (36.446855, 28.225077), 01.III.2021, K. Kalaentzis leg., 1 ♀ deposited in the collection of Museum of Zoology of the National and Kapodistrian University of Athens, Greece (ZMUA, voucher code: HYM00000262) [GenBank OL753682].

3♀ Greece, Dodecanese, Rhodes, Rodini (36.42704, 28.221232), 01.III.2021, K. Kalaentzis leg.

4♀ Greece, Dodecanese, Rhodes, Ioanninon street (36.425465, 28.209638), 01.III.2021, K. Kalaentzis leg.

15♀ Cyprus, Paphos, port area (34.756289, 32.409806), 14.I.2021, J. Demetriou leg., 8♀ deposited in the collection of Museum of Zoology of the National and Kapodistrian University of Athens, Greece (ZMUA, voucher codes: HYM00000254–HYM00000261) [GenBank OL753683].

Molecular analysis

The results of DNA barcoding generated a 592 bp-long fragment of the cytochrome oxidase I (COI) of *J. microcarpae*, which was previously unknown. In addition, the haplotype assignment revealed that both individuals, collected from Greece and Cyprus, belonged to a single haplotype.

Discussion

Widely planted in Southern Greece, *F. microcarpa* serves as a host for a rising number of introduced species (EK *unpublished data*). While species like *Gynaikothrips ficorum* (Marchal, 1908) (Thysanoptera: Phlaeothripidae) and *Macrohomotoma gladiata* Kuwayama, 1908 (Hemiptera: Homotomidae) are well distributed throughout Southern Greece (EK *unpublished data*), the distribution of *J. microcarpae* seems to be restricted in Rhodes. Given the extensive sampling that was employed in this study, it is presumed that the fig gall wasp has still a limited distribution in the country, which may point towards a recent establishment. The introduction pathways of the species in the country, as well as its potential dispersal in other parts of Greece where *F. microcarpa* is present, are still to be determined in the future.

Infestations caused by *J. microcarpae* are known to significantly reduce the attractiveness of fig trees (Compton et al. 2020). Combined with the impact of already known, introduced in Greece phloem-eating species such as *G. ficorum* (Marchal, 1908) causing deformities to foliage (Antonatos et al. 2015) and *M. gladiata* Kuwayama, 1908 responsible for large patches of woolly waxy secretions (Mifsud and Porcelli 2012), alien insects of *F. microcarpa* could potentially undermine the aesthetics of the urban landscape with possible socioeconomic impacts (Kueffer and Kull 2017), especially in Rhodes, where *F. microcarpa* is highly abundant.

Although no immediate threat exists for the host plant, efforts to control infestations through systemic insecticides has somewhat been proved to provide protection for up to 14 months post treatment when trunk injection of emamectin benzoate was utilized (Bhandari and Cheng 2016). Mechanical control by pruning and discarding infested leaves may help reduce the local population but may also attract females wishing to lay eggs in the newly formed leaves of the new growth (Dreistadt 2016). Additional research is necessary to determine whether pest control is required to prevent the further spread of *J. microcarpa* in the island of Rhodes.

DNA barcoding revealed identical haplotypes for the specimens collected from both Greece and Cyprus, which can be partially explained due to the species reproducing by thelytokous parthenogenesis and the populations on both islands deriving from the same source. Since DNA sequences of this species are generated for the first time, no genetic comparisons can be made at this time. However, the DNA barcode generated in this study is expected to aid future endeavours in resolving the taxonomic relationships of the genus and perhaps elucidate its introduction pathways.

Recent discoveries of cryptic species of *Josephiella* not associated with leaves indicate the need of further investigation of fig trees in the areas where they are introduced, as well as their putative areas of origin, where both species' presence remains unknown. Efficient control measures and an early warning system assisting the mapping of the species' distribution

are crucial, even in Greece, where *J. microcarpae* appears to have a restricted distribution. The involvement of citizen scientists in monitoring alien insects is already encouraged by Alientoma in Greece (Kalaentzis et al. 2021) and is expected to aid future efforts in accurately mapping the range of fig gall wasps.

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Authors' contribution

Research conceptualization: EK, JD; sample design and methodology: KK, EK, JD, CK, DNA, CG; investigation and data collection: KK, JD, EK; data analysis and interpretation: DNA; ethics approval: CG, DNA; funding provision: CG; writing – original draft: CK, KK, JD, EK; writing – review: CK, KK, JD, EK, DNA, CG; editing: KK, CK, CG, DNA.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Localities where *Ficus microcarpa* L. foliage was examined for galls of *Josephiella microcarpae* Beardsley and Rasplus 2001.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Kalaentzis_etal_SupplementaryMaterial.xlsx