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SHORT COMMUNICATION

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Zizyphus fruit fly (*Carpomya incompleta* (Becker), Diptera: Tephritidae) is expanding its range in Europe

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Abstract

Aim of study: To identify a new pest of jujube reported by the farmers in Spain.

Area of study: The Iberian Peninsula (Spain).

Material and methods: The insects were identified according to the mitochondrial cytochrome C oxidase subunit I (COI) gene and the main morphological features of this tephritid species, including the ocellar seta, the mesonotum and apical crossband in winds.

Main results: Based on morphological characterization the insects were identified as *Carpomya incompleta* (Becker) (Diptera: Tephritidae), which was confirmed by the phylogenetic analysis with more than 94% of identity. Besides, the distance analysis showed very low intraspecific divergence in *C. incompleta* sequences.

Research highlights: We report the presence of the zizyphus fruit fly in Spain for the first time. This presence has been reported to the Early Warning Systems on Alien Invasive Species of the Andalusian Government (Spain).

Additional key words: Tephritid; COI gene; identification; morphology; detection.

Abbreviations used: COI (cytochrome C oxidase subunit I); K2P (Kimura 2-parameter).

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Introduction

The zizyphus fruit fly (*Carpomya incompleta* (Becker), Diptera: Tephritidae) is a monophagous pest of jujube (*Ziziphus* spp., Rhamnaceae), with two to five annual generations between spring and autumn (Morsy, 1971; Al-Masudey & Al-Yousuf, 2013). Adult flies lay the eggs on fruit at the onset of ripening, and the carpophagous larvae, which go through three instars, dig a tunnel inside the fruit. They subsequently develop, with prepupariating third instars dropping to the soil to pupate, where enters diapause by the end of April (Morsy, 1971). Larval feeding activity promotes the decomposition of plant tissue, leads to bitter fruits, fruit rot and drop, but sometimes both eggs and larvae are disappearing inside the fruit (White & Elson-Harris, 1992; Rizk *et al.,* 2014). *C. incompleta* may produce a low yield and poor quality of fruits, which in many instances can exceed 60% of infested trees (Al-Masudey & Al-Yousuf, 2013).

This species has been recorded in Burkina Faso, Egypt, Eritrea, Ethiopia, Morocco, Iraq, Israel, Kenya, Libya, Ni-

ger, Oman, Saudi Arabia, Sudan, United Arab Emirates and Yemen. In Europe, its presence has been reported in France and Italy (El Harym & Belqat, 2017; Korneyev *et al.*, 2017; CABI, 2022). *C. incompleta* is distributed worldwide from approximately 46°S to 50°N, as well as the Ber fruit fly (*Carpomya vesuviana* Costa), that could be located from 50°S to 60°N according to climate predictions on temperature and rainfall. In these predictions, Spain has been included in the pest risk analysis (PRA) area due to the historical climate and natural rainfall (Guo *et al.*, 2019; EPPO, 2022). In addition, clusters of countries that have the most similar fruit fly species show the possibility of the introduction of *C. incompleta* in Spain (Qin *et al.*, 2015).

One of the current molecular methods to identify fruit flies is DNA barcoding. It focuses on specific genes, such as COI ITS, and 18S (Barr, 2009; Jiang *et al*., 2018). For insects, the barcoding is based on a partial sequence of the COI gene that allows the discrimination of most fruit flies, although in recent years new markers have been developed for simultaneous broad detection during quarantine inspections since not all species can be detected using the current methods (Jiang *et al.*, 2018). Herein, data from the first finding in Spain of *C. incompleta* are presented. Specifically, individuals collected in traps and inside jujube fruits in an organic farm in southern Spain were identified with molecular and morphological features.

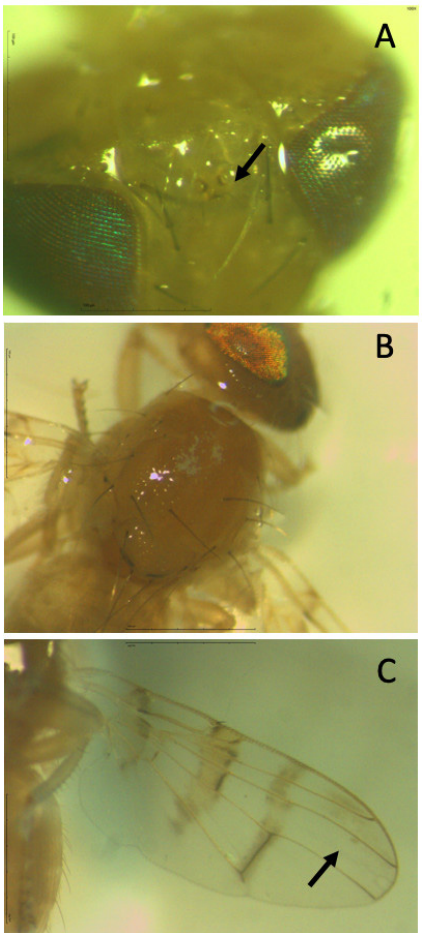

Figure 1. Taxonomic details for identification of *Carpomya incompleta* adults: A) ocellar seta, B) reddish yellow mesonotum, C) apical crossband absent.

Material and methods

The insects were collected in July 2020 in a field located in Ecija, Seville (37° 29'N, 5° 16'W) on a six-year-old organic jujube (*Ziziphus jujuba* Mill., Rhamnaceae) orchard as part of a polyculture of 12500 m^2. The farmer placed 10 traps with *Ceratitis capitata* pheromone (Econex S.L., Murcia, Spain) in the field on May 1st, and in light of the captures, the number of traps was increased up to 90 on July 1st. The number of collected unknown flies ranged between 30 and 40 per trap per week during July and August. In September 2020, the farmer brought nine adults and one larva found inside one fruit to the laboratory of the AGR 163 Agricultural Entomology research group of the Agronomy Department of the University of Córdoba (Spain). The species were identified by observing morphological features with a Moticam 10+ camera (Motic Spain SL, Barcelona, Spain) connected to an SMZ800 stereomicroscope (Nikon Corporation, Tokyo, Japan). An identification key of the Carpomyini tribe was used (Pollini & Cravedi, 2014; Korneyev *et al.*, 2017).

The insects were also molecularly identified using the standard protocol of DNA barcoding published by the European and Mediterranean Plant Protection Organization (EPPO, 2016) with the following modifications. Insects were disrupted in FastPrep®-24 (M.P. Biomedicals, Santa Ana, CA, USA) and DNA was extracted using the Quick-DNA™ Tissue/Insect Microprep Kit (Zymo Research, USA) following the manufacturer's instructions. A 709 bp fragment spanning the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with the following primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The total reaction volume was 25.0 µL, and it contained 2.0 µL of genomic DNA, 5.0 µL of MyTaq® Red 5X (Bioline GmbH, Germany), 0.5 µL of each primer (10 mM), and 1.0 µL of MyTaq® Red DNA Polymerase (Bioline GmbH, Germany). The PCR products and a 100-bp molecular weight standard (Solis Biodyne, Tartu, Estonia) were electrophoresed on 1% agarose gels buffered with 1X TAE and stained with SYBR® Safe (Invitrogen, Paisley, UK), purified from the agarose gels using Quantum Prep Freeze 'N Squeeze DNA Gel Extraction Spin Columns (Bio-Rad, Hercules, CA, USA), and sequenced by STAB Vida Lda. (Caparica, Portugal). The NCBI-BLAST was used to analyze the sequence homologies (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch), and available *C. incompleta* sequences were retrieved from the BOLD database (https://

Table 1. Kimura two-parameter distances between and within genera

	***Rhagoletis* spp.**	***Bactrocera* spp.**	***Carpomya vesuviana***	***Carpomya incompleta***
Rhagoletis spp.	**0.050**			
Bactrocera spp.	0.168	**0.061**		
C. vesuviana	0.099	0.167	**0.021**	
C. incompleta	0.105	0.172	0.058	**0.001**

Numbers in boldface indicate the distance within genera or species.

www.boldsystems.org/index.php/IDS_OpenIdEngine). The sequences were analysed, and the alignment between these sequences and those of nearby species from the same region (Table S1 [suppl]) was performed using the MegAlign program (DNASTAR package, London, UK). The phylogenetic analysis was carried out using the MEGA 11 program (Kumar *et al.*, 2018; Stecher *et al.*, 2020). The consensus tree was obtained with the maximum likelihood method and Kimura 2-parameter (K2P) model (Kimura, 1980). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). Bootstrap values lower than 50% are not shown. The distances between and within groups (genera) were estimated using the K2P distance implemented in MEGA 11.

Results and discussion

The present work shows the first detection of at least ten individuals of *Carpomya incompleta* in Spain.

In the morphological identification, the adults showed the short ocellar seta (Fig. 1A), pale reddish yellow mesonotum (Fig. 1B), and apical crossband absent (Fig. 1C). According to the identifications, the presence of this invasive species in Spain was reported to the Early Warning Systems on Alien Invasive Species of the Andalusian Government (https://www.juntadeandalucia.es/medioambiente/portal/areas-tematicas/biodiversidad-y-vegetacion/especies-exoticas-invasoras/red-de-alerta-temprana-andaluza-de-especies-exoticas-invasoras) on May 10th, 2021. The adults of this species usually emerge in June when the jujube fruits are receptive to the biting, but in 2021 only two adults were observed in yellow sticky traps, probably due to the mass trapping performed in 2020 and the total collection of fruits. However, the farmer estimated yield losses over 80% in 2020. The dramatic reduction in the collected insects in 2021 could have been reached because of the control measure established following decisions in Italy (the nearest country) for both species that attack to jujube tree, *C. incompleta* and C. *vesuviana.* In previous works, yellow sticky traps are recommended for early detection, population monitoring and mass trapping (Pollini & Cravedi, 2014; Radonjić *et al.*, 2019). In addition, jar traps baited with 3% diammonium phosphate or 10% hydrolysed protein should be changed every 7 days for both population monitoring and control (Pollini, 2012). Fourteen insects were collected from June to August 2022, when control measures were still applied. Other control measures applied to control *C. incompleta* are the spraying with chemical insecticides (carbamates, pyrethoids and spinosad), the use of the parasitoids *Opius concolor* Szépligeti (Hymenoptera: Braconidae) and *Biosteres* (=*Chilotricha*) *persulcatus* (Hymenoptera, Braconidae, Opiinae) and the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) (Jamil *et al.,* 2004; Al-Masudey & Al-Yousuf, 2013; Abdel-Galil *et al.,* 2014, 2019; Pollini & Cravedi, 2014; Rizk *et al.,* 2014).

The sequences obtained from the nine adults and the larva did not show differences in their nucleotide string and they ranged between 637 and 644 bp. Only the sequence of 644 bp (named I4) was used as the type-sequence and deposited in the Genbank database with accession number OK147923. Sequences that had homology to our type-sequence were identified by BLAST and BOLD searches. The type-sequence showed 99.84% with 100.0% query coverage to *C. incompleta* sequences and 94.8-95.2% identity with 100.0-95.0% query coverage to previously reported *C. vesuviana* sequences in the BLAST database and 94.7-95.2% similarity in the BOLD database (Table S1 [suppl]). In addition, the phylogenetic tree showed that the obtained sequence was closely related to the *C. incompleta* sequences in a separate clade from the three clades of *C. vesuviana* sequences (Fig. 2). The DNA barcoding performed in this work should provide a successful identification for further investigations, since *C. incompleta* sequences were not previously included in DNA barcodes analysis. However, the sequences of the closer species *C. vesuviana* have shown high divergence in previous analysis probably due to different infections of the endosymbiont Wolbachia, with has specific strains in *C. vesuviana* specimens (Karimi & Darsouei, 2014; Kunprom & Pramual, 2019). These authors detected a divergence in *C. vesuviana* species, where the insects collected in Thailand were clustered in a different clade than those collected in Iran, India and China. In the present work, the specimens collected in Thailand grouped together in the

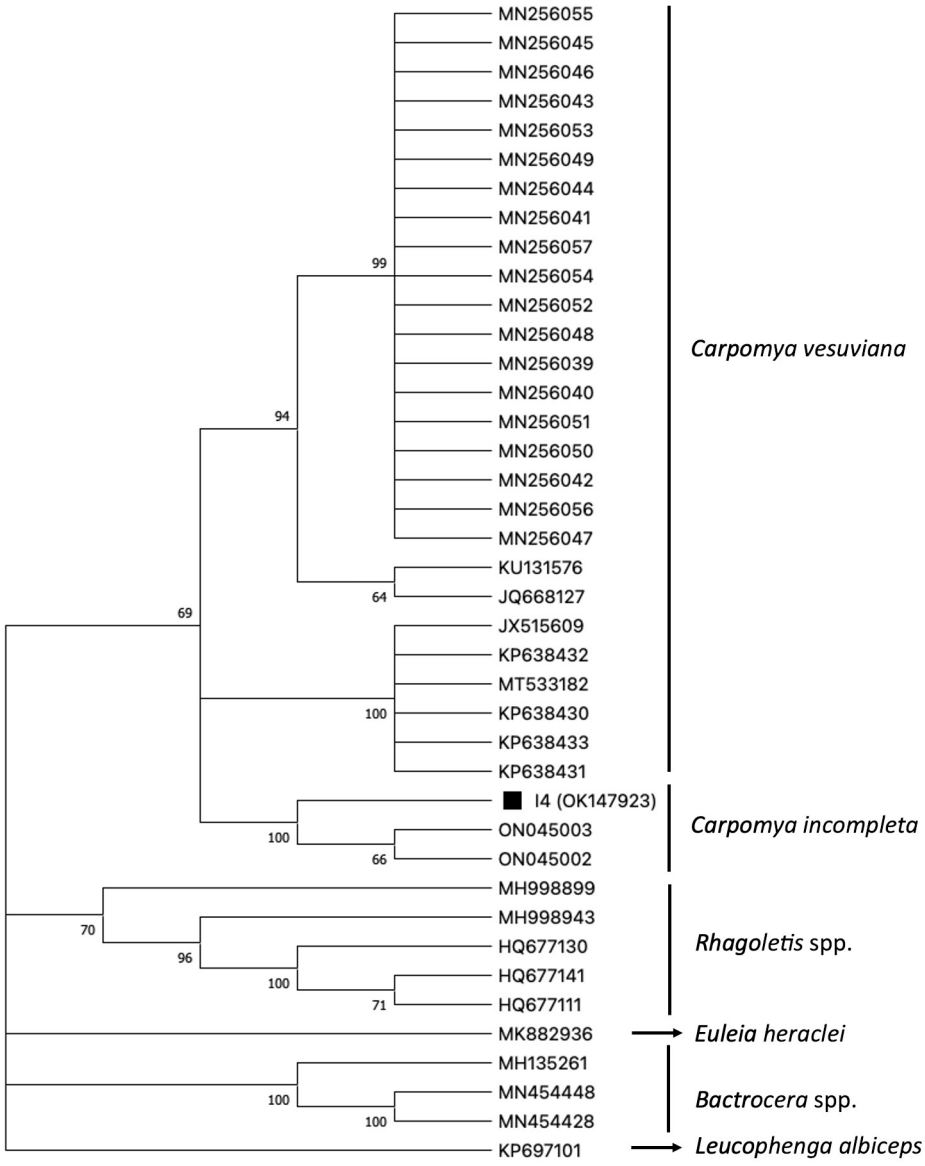

Figure 2. Maximum likelihood tree based on the Kimura 2-parameter model with LCO1490 and HCO2198 primers from COI gene.

same clade, but the other two clades were not related to the country of origin. In fact, the distance analysis showed intraspecific genetic distances slightly higher than 2% in *C. vesuviana,* while Kunprom & Pramual (2019) reported a deep divergence in specimens from Thailand (4.8% of genetic distance). In the present study, the distance between *C. incompleta* and *C. vesuviana* was 0.058 and within *C. incompleta* 0.001, showing differences between the two species but very low intraspecific divergence in *C. incompleta* (Table 1). Although the genetic distances between species within *Rhagoletis* and *Bactrocera* genera were small (0.050 and 0.061), the phylogenetic tree could distinguish all the included specimens clearly.

The possible source of the introduction is uncertain. Most likely this species has been present in Spain since a long time ago and came from Italy, but its presence, like those of other tephritids, may go undetected. Lack of detection, along with the fact that the jujube tree is now a marginal crop in Spain, could support this assumption. The detection of *C. incompleta* on jujube in Spain contributes to improving our knowledge of its expansion in Europe. This paper will provide valuable information for its identification and further decision-making of this pest in Spain.

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Writing – review & editing: I. Garrido-Jurado, E. Quesada-Moraga, M. Yousef-Yousef.

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