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Encounter with *Encarsia cubensis*, a new natural enemy of invasive woolly whitefly *Aleurothrixus floccosus* in Kerala, India: Implications of the findings for horticultural crops

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Abstract

This study documents the first occurrence of *Encarsia cubensis* Gahan (Hymenoptera: Aphelinidae) as a parasitoid of the woolly whitefly, *Aleurothrixus floccosus* (Maskell), in India, with a special emphasis on horticultural crops, particularly guava. Species identification was made using morphological characterization and analysis of the mitochondrial *COI* gene. Surveys conducted in the field revealed that the parasitism level exceeded 70 per cent; therefore, *E. cubensis* could be utilised as a biological control agent for the woolly whitefly. This discovery is crucial for the integrated pest management of guava and other horticultural crops, promoting sustainable production.

Key words: Woolly whitefly, *Encarsia cubensis*, parasitism, biological control, India.

Introduction

Whiteflies fall among the most destructive polyphagous insect pests of crops globally, which significantly impact horticultural production. Their feeding mechanism involves sucking plant sap, excreting honeydew that promotes sooty mold, and they are also vectors of viral diseases, making pest control necessary. The whiteflies are highly invasive and can tolerate a wide range of environmental conditions, hence they have spread across countries, affecting both food and cash crops. The woolly whitefly, *Aleurothrixus floccosus* (Maskell) falls under the family of Aleyrodidae is one of the most destructive pests to horticultural crops, which include guava (*Psidium guajava* L.). This pest has a waxy appearance and is native to the Neotropical area, but has spread to warmer climates worldwide. It was recently reported as an invasive pest on guava in the Kozhikode district of Kerala, India (Sundararaj *et al.*, 2020). The subsequent transmission to other regions of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, and the Lakshadweep islands underlines its danger to the horticultural industry (Sundararaj *et al.*, 2021).

Control of woolly whiteflies in horticultural crops has been difficult, mainly because of the abrasive wax exteriors and insecticide resistance (Belay *et al.*, 2011). This requires other approaches, such as biological control. One identified potential control method is the use of the parasitic wasp called *Encarsia cubensis* because its parasitic nature offers excellent whitefly control. This work documents the initial report of *E. cubensis* from India and assesses its biological control efficiency in suppressing the impact of woolly whitefly on horticultural crops.

Materials and methods

Survey, collection, and preservation: Purposive surveys were conducted across Kerala from May 2021 to March 2023 to document natural enemies associated with the woolly whitefly, *A. floccossus,* on guava. During the survey, we made a significant discovery- parasitized puparium of *A. floccossus* was observed on guava leaves at five locations (Fig. 1). The infested leaves with various life stages of whiteflies were carefully collected in polyethene bags, labeled with locality details, secured with rubber bands, and brought to the laboratory.

In the laboratory, the whitefly-infested leaves collected from different localities were carefully examined under a microscope (Leica EZ4HD) for the symptoms of parasitism. The parasitized puparium showed blackish discolouration, and the exuvium of the puparium, where the parasitoid emerged, showed a round exit hole (Fig. 2). The unparasitized puparium remained bright

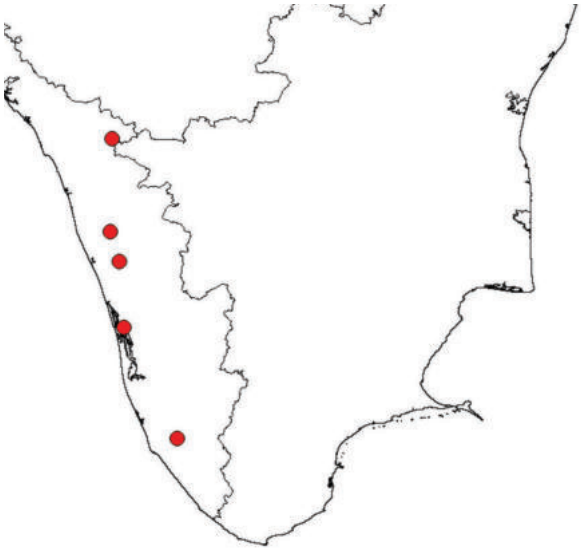

Fig. 1. Distribution of the parasitoid *Encarsia cubensis* on the woolly whitefly *Aleurothrixus floccossus* in Kerala, India.

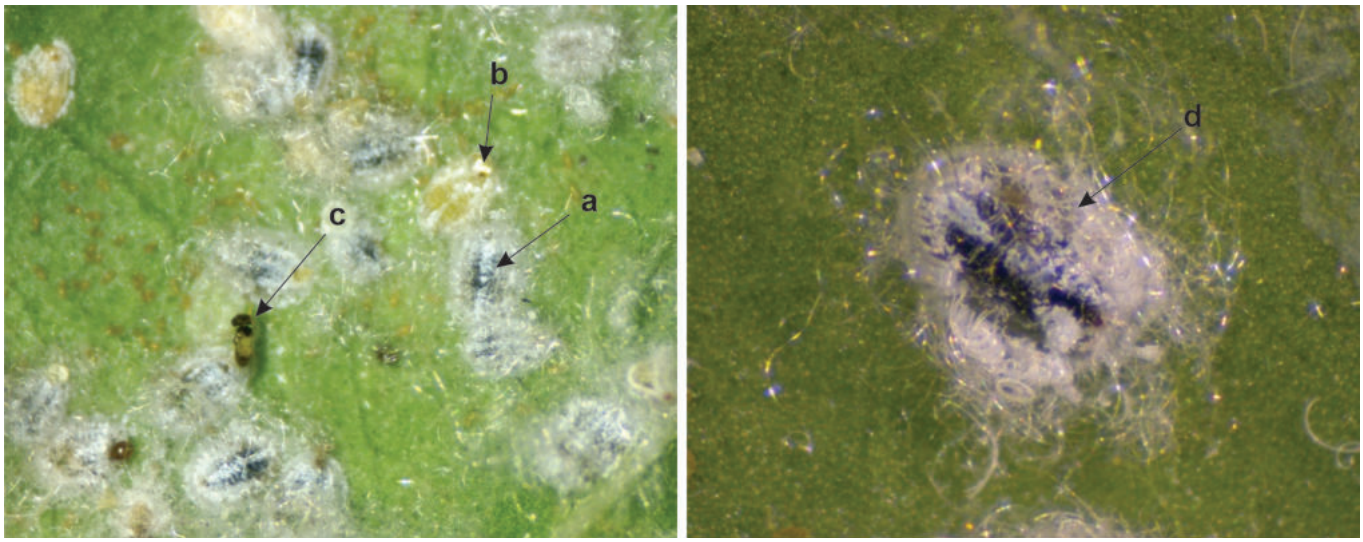

Fig. 2. Colony of *Aleurothrixus floccossus* with parasitized puparium (a), unparasitized puparium (b), *Encarsia cubensis* (c) and magnified image of parasitized puparium (d)

yellow, and the exuvium was transparent with a T-shaped exit hole. The total number of pupariums (live and exuviae) and the number of parasitized pupariums on each leaf were recorded, and the percent parasitism was computed. Infested leaves with parasitized puparia were cut into small pieces and placed in glass vials with mouths covered with muslin cloth for the emergence of parasitoids. The emerged parasitoids were preserved in 70 and 95 percent ethyl alcohol, respectively, for morphological and molecular identification of the species. This finding, alongside subsequent investigations, promises to significantly advance our understanding of biological control strategies in agriculture.

Morphological characterization: The *Encarsia* parasitoids were mounted on rectangular cards and microscope slides with strict adherence to the protocol provided by Noyes in 1982. The species of *Encarsia* parasitoids were identified using the original description and the following literature (Myartseva and Evans, 2007; Dozier, 1933; Schauff and Evans, 1996; Evans and Polaszek, 1997). Identified specimens were then deposited at the National Zoological Collection (NZC), Zoological Survey of India, Kolkata, West Bengal, India.

Molecular characterization: The adult parasitoids were used to characterize the partial (658 bp) mitochondrial cytochrome c oxidase I (*COI*) gene. Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel), following the manufacturer's protocol. The quality of the DNA isolated was checked using agarose gel electrophoresis. The PCR amplification of the 5' terminus of the *COI* gene was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems), using universal primers LCO 1490 5'-GGT CAA CAA ATC ATA AAGATA TTG G-3' and HCO 2198 5'-TAA ACT TCA GGGTGA CCA AAA AAT CA-3' (Folmer *et al.,* 1994). The PCR products were checked in 1.2% agarose gel prepared in 0.5 X TBE buffer containing 0.5µg/mL ethidium bromides. The molecular standard used was a 2-log DNA ladder (NEB). The gel was visualized in a UV transilluminator (Genei), and the image was captured under UV light using a gel documentation system (Bio-Rad). The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) following manufacturer's protocol at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out (Table 1).using Geneious Pro v5.1 (Drummond *et al.*, 2010).

Table 1. Summary of experimental workflow and conditions for mitochondrial *COI* gene characterization of *Encarsia cubensis*

Protocol step	Method or equipment used	Specific parameters or conditions
DNA isolation	NucleoSpin® Tissue Kit (Macherey-Nagel)	Followed the manufacturer's protocol.
PCR amplification	PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems)	Universal primers used: LCO 1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO 2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3').
Gel electro-phoresis	1.2% agarose gel in 0.5X TBE buffer	Gel contained 0.5 µg/mL ethidium bromide. Molecular standard: 2-log DNA ladder (NEB). Visualized with UV transilluminator (Genei) and imaged with Gel documentation system (Bio-Rad).

Results and discussion

Morphological characterization: Based on morphological characteristics, the research identified *Encarsia cubensis* Gahan, a species within the order Hymenoptera and family Aphelinidae. Initially described by Gahan in 1931, the syntype female specimen was collected from Santiago de Las Vegas, Cuba, and reared from the woolly whitefly, *Aleurothrixus howardi* (now *floccosus*). The specimen, cataloged as type N 43530, was examined and was housed in the USNM collection. The male of this species remained unidentified. Subsequent studies, including Dozier (1933) and Evans and Polaszek (1998), referenced this species, with the former listing it as *Trichoporus cubensis* and the latter providing a revision of the species group. *Encarsia cubensis* is a parasitoid of several host species, including *Aleurothrixus floccosus*, *Aleurotrachelus trachoides*, *Bemisia tuberculata* (Noyes, 2022; Myartseva and Evans, 2007), and *Aleurotrachelus atratus* (Borowiec *et al.*, 2010; Selvaraj *et al.*, 2023). This research contributed to the understanding of its host range and morphological identification. The species is distributed in Brazil,

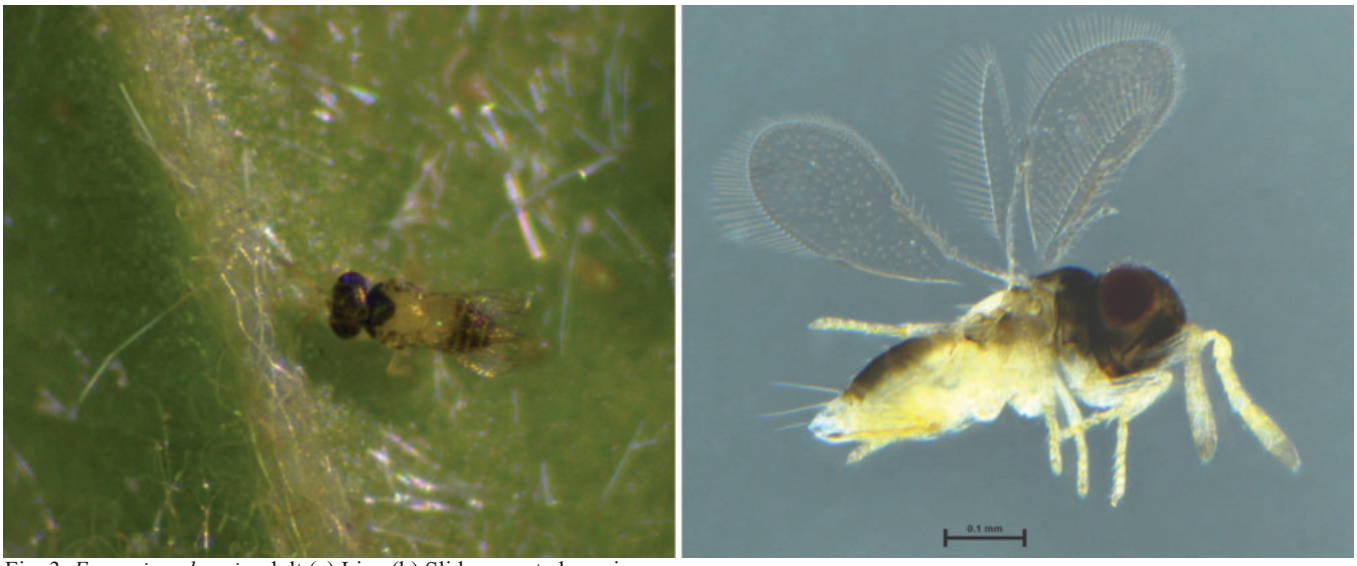

Fig. 3. *Encarsia cubensis* adult (a) Live (b) Slide mounted specimen

Costa Rica, Cuba, Dominican Republic, Guadeloupe, Haiti, Mexico, Puerto Rico, USA (Noyes, 2022; Myartseva and Evans, 2007; Schauff and Evans 1996) and India (Selvaraj *et al.*, 2023).

Brief diagnosis: Body mostly dark coloured except for the scutellum, the lateral margins of the mid-lobe of the mesoscutum and the central portion of metasomal tergites I and II which are yellow; 2nd funicle segment of the antenna is longer than the 3rd; fore wing broad with a large asetose area under the stigma vein; mid-tarsus with 4 segmented (Fig. 3).

Material examined: Among the different localities surveyed in Kerala, parasitized puparia of *A. floccosus* were collected from Ambalavayal, Wayanad; Pattambi, Malappuram; Vellanikkara, Thrissur; Kottarakkara, Kollam and Vyttila, Ernakulam (Table 2).

Parasitism: Laboratory observation of the whitefly-infested guava leaves revealed a high level of natural parasitism, exceeding 70 per cent. The different localities recorded parasitism of 83. 28 (Ambalavayal), 69.06 (Pattambi), 79.54 (Vellanikkara), 75.07 (Kottarakkara) and 81.95 percent (Vyttila) (Table 2). This high level of natural parasitism reveals the potential of the parasitoid in the sustainable management of the invasive woolly whitefly on guava.

Table 2. Natural parasitism of woolly whitefly *Aleurothrixus floccossus* on guava by *Encarsia cubensis* in Kerala, South India

Accession number	Locality of collection	GPS coordinates	Percent parasitism
$GVA_{16}AVL_2$	Ambalavayal, Wayanad	Latitude:11061'75" Longitude:76021'22"	83.28
GVA_{18} PTB_1	Pattambi, Malappuram	Latitude:10080'66" Longitude:76017'12"	68.06
GVA_7VKA_1	Vellanikkara, Thrissur	Latitude: 76016'56" Longitude:10032'52"	79.52
$GVA_{12}KRA_1$	Kottarakkara, Kollam	Latitude: 8.98'22" Longitude: 76.81'30"	75.07
$GVA_{14}VTA_1$	Vyttila, Ernakulam	Latitude: 76019'11" Longitude: 9055'37"	81.95

Molecular characterization: The mitochondrial cytochrome c oxidase I (*COI*) gene fragment of *Encarsia cubensis* was meticulously amplified and sequenced, ensuring the accuracy and reliability of our findings. The sequence was then carefully deposited in the GenBank database under accession number PP479680.1. The *COI* sequence demonstrated a 99.85 percent similarity to *E. cubensis* (GenBank accession number: ON881119.1) from an earlier submission from Bangalore, India, with zero E value.

The dendrogram (Fig. 4) revealed the evolutionary relationships

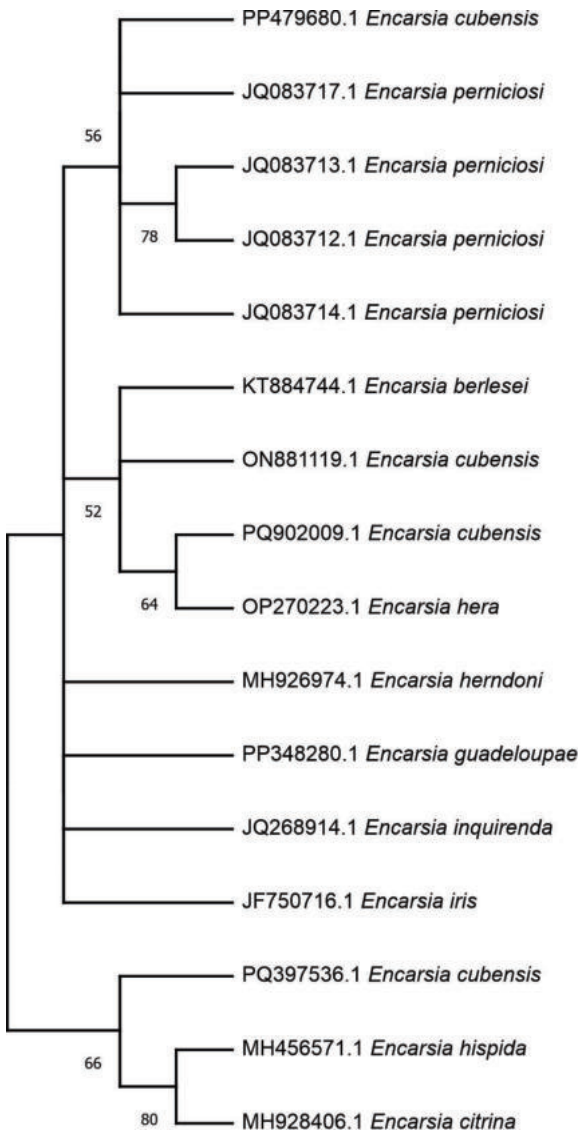

Fig. 4. Phylogenetic analysis of *Encarsia cubensis* and related *Encarsia* species

among different *Encarsia* species. The *E. cubensis* sequences are distributed across different clades, indicating genetic diversity within the species. *E. cubensis* sequences are clustered closely with *E. perniciosi, E. berlesei and E. hera,* suggesting varying degrees of genetic similarity. The bootstrap values with higher values indicate stronger support, providing confidence in these relationships. This distribution suggests possible evolutionary divergence within *E. cubensis*.

Selvaraj *et al.* (2023) reported this parasitoid earlier in India as the primary parasitoid of the invasive palm-infesting whitefly, *Aleurotrachelus atratus* Hempel, on coconut and other palm species. However, our research is particularly intriguing because this is the first report of *E. cubensis* on the woolly whitefly *A. floccosus* from India. This discovery significantly adds to the understanding of this species.

The potential of *E. cubensis* as a natural enemy of the invasive woolly whitefly offers an opportunity for natural pest regulation. Exploring mass-rearing opportunities of *E. cubensis* for commercial use in controlling woolly whitefly infestations could can significantly reduces reliance on chemical pesticides.

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