The First Record in Turkey of Pachycrepoideus vindemmiae as a parasitoid of Ceratitis capitata: Molecular identification

Gülsevim Tiring (glsvm.trng@gmail.com)
Cukurova Technocity
Adnan Tusun
Çukurova University
Çağlar Kalkan
Çukurova University
Serdar Satar
Çukurova University

Short Report

Keywords: Medfly, new record, PCR

Posted Date: April 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1551875/v1

Abstract

Ceratitis capitata Wied. (Diptera: Tephritidae) is a polyphagous species and a serious pest worldwide. In the present study, surveys for associated *C. capitata* parasitoids were conducted in the Adana province of Turkey. Our finding represents the first record in Turkey of *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) as a parasitoid of *C. capitata*.

Introduction

Tephritid flies, also known as fruit flies, are one of the most damaging groups of insect pests that infest economically significant fruit and vegetable. Among fruit flies, *Ceratitis capitata* (Diptera: Tephritidae) infest more than 300 hosts, with worldwide losses amounting to several billion USD (Liquido et al., 1990; Szyniszewska & Tatem, 2014; Satar & Tireng, 2016; Tiring & Satar, 2017; 2021).

C. capitata control management in Turkey is most of based on the use of insecticides. Nevertheless, safe eco-friendly strategies such as mass trapping are being applied. Other eco-friendly control systems like Biological Control have been conducted the several studies. However, biological control potential has been inspected in only a few species (Hepdurgun et al., 2009). Here we report the first record of a pupae parasitoid from *C. capitata* in Turkey.

Materials And Methods

Sample collection and DNA isolation

Surveys for associated medfly parasitoids were carried out between June and July in 2021 in Adana (37°03' 98.21"N, 35°36' 05.73"E), Turkey. These specimens were collected from loquat fruits. Surveys for *C. capitata* parasitoids were conducted on both infested fruits and pupae that dropped into the soil. The infested fruits and pupae samples were transferred to the Citrus Pest Laboratory at Cukurova University. Infested fruits were maintained in plastic trays at 24 ± 1°C and 65–80% RH until the emergence of adult *C. capitata* and parasitoids. The emerged parasitoids were stored in 96% ethanol for preservation. DNA was isolated from *C. capitata* parasitoids using the standard extraction kit and protocol provided by DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany).

Pcr Amplification

Approximately 630 bp of the barcoding region of the mtCOI gene was amplified using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al., 1994). Amplification was conducted in 50 µl with the containing 2x Master Mix (containing Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM of each dNTP, Taq DNA Polymerase), 10 µM primer, 50 ng DNA template and added PCR grade H_2O to a final volume. The thermocycler conditions were: 5 min at 94°C for pre-denaturation, followed by 35 cycles at 94°C for 1 min,

50°C for 1 min, at 72°C for 1 min, and a final period at 72°C for 7 min. All the PCR products were confirmed electrophoretically using 1% agarose gel, stained with ethidium bromide, and viewed with a gel imaging system before two-way sequencing by a commercial company (Macrogen, Amsterdam, Netherlands).

Data analysis

The sequences were edited using FinchTV (version 1.4.0) and aligned by CLUSTAL W that had been integrated with MEGA7 software (Tamura et al., 2013). The MEGA 7 software was also used to create maximum likelihood (ML) trees, with 1000 bootstrap replicates performed to assess branch support. The sequences were compared to the closest available reference sequences in the NCBI nucleotide collection in GenBank (http://blast.ncbi.nlm.nih.gov/Blast). To root the phylogenetic tree, the sequence of *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae) was used as the out-group.

Results

A total number of 4500 pupae of *C. capitata* was collected from both infested fruits and pupae that dropped into the soil. The 10 *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) emerged from pupae (Fig. 1). Morphological taxonomic identification results were consistent with molecular findings.

The universal primer set used in this study (LCO1490 and HCO2198) targets the mitochondrial COI gene. It yielded efficient amplification in the parasitoid strain of *C. capitata*. The mean size of the amplified fragment was 630bp. The nucleotide sequences are available in GenBank under the accession number OM956372.

For the COI sequences, used the Maximum Likelihood model Tamura-3-parameter tree. Tree topology showed *P. vindemmiae* was highly distanced from *D. longicaudata* and other *P. vindemmiae* strains (Fig. 2).

Discussion

This study has examined to the presence of *C. capitata* parasitoids in Turkey (Adana). Our finding represents the first record in the Turkey of *P. vindemmiae* as a parasitoid of *C. capitata*. Kaçar (2020) indicated that *P. vindemmiae* attacks the pupae of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) and can effectively parasitize in the laboratory. Our study, *P. vindemmiae* ADN-01 was on a different branch from a clone from Turkey, Düzce (MK813907). *P. vindemmiae* individuals from *D. suzukii* and *C. capitata* were clearly discriminated by nucleotide sequences of COI with high bootstrap values.

Previously, *Spalangia cameroni* Perkins Rondani (Hymenoptera: Pteromalidae) and *P.vindemmiae* had been found as native pupal parasitoids of the medfly in Spain (Falco et al., 2006). Harbi et al. (2015) was

detected that *P. vindemmiae* parasitizing pupae of *C. capitata* in Tunus. *P. vindemmiae* is known to attack pupae of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Wang & Messıng, 2004; Zhao et al., 2013).

Declarations

Acknowledgments

The authors are greatly indebted to assoc. prof. Gül SATAR and Mahmut Mete KARACA for helping with molecular analysis.

Authors' contributions

GT, AT and SS conceived and designed the study and collected the data.GT and AT wrote the initial draft and analyzed the data, and all authors edited and contributed to subsequent drafts.

Funding: This work was supported by the Citrus Pest Laboratory at Cukurova University**.**

Confict of interest The authors declare that there are no interests to declare.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Falcó, J. V., Garzón-Luque, E., Pérez-Hinarejos, M., Tarazona, I., Malagón, J., & Beitia, F. J. (2006). Two native pupal parasitoids of *Ceratitis capitata* (Diptera, Tephritidae) found in Spain. In *International Conference on Integrated Control in Citrus Fruit Crops* (pp. 71–74). IOBC/WPRS.
2. Harbi, A., Beitia, F., Sabater-Muñoz, B., Falcó, J. V., & Chermiti, B. (2015). First record of *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) parasitizing pupae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Tunisia. Afr. Entomol. 23 (2), 514–518. http://dx.doi.org/10.4001/003.023.0212
3. Hepdurgun, B., Turanli, T., & Zümreoğlu, A. (2009). Parasitism rate and sex ratio of *Psyttalia (= Opius) concolor* (Hymenoptera: Braconidae) reared on irradiated *Ceratitis capitata* larvae (Diptera: Tephritidae). Biocontrol Science and Technology, 19(sup1), 157–165. https://doi.org/10.1080/09583150903090479.
4. Kaçar, G. (2020). New records of the parasitoids of *Drosophila suzukii* (Matsumura, 1931)(Diptera: Drosophilidae) in newly invaded areas in Turkey: molecular identification. Turkish Journal of Entomology, 44(1), 71–79. https://doi.org/10.16970/entoted.607415
5. Liquido, N. J., Cunningham, R. T., & Nakagawa, S. (1990). Host plants of Mediterranean fruit fly (Diptera: Tephritidae) on the Island of Hawaii (1949–1985 survey). Journal of Economic Entomology, 83(5), 1863–1878.

6. Tiring, G., & Satar, S. (2017). Population fluctuation of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in some fruit orchards. Entomology Bulletin of Turkey,, 7(3), 239–247. https://doi.org/10.16969/entoteb.381234
7. Tiring, G., & Satar, S. (2021). Annual population fluctuations of Mediterranean fruit fly in the Eastern Mediterranean Region of Turkey; Problem of non-marketing fruit. Phytoparasitica, 49(5), 807–817. https://10.1007/s12600-021-00932-w
8. Satar, S., & Tireng, G. (2016). Determined of effectiveness of the use of traps against the *Ceratitis capitata* Wied. (Diptera: Tephritidae) in Okitsu wase mandarin and relationship between fruit pomological characteristics and infestation. Derim, 33(2), 221–236. https://doi.org/10.16882/derim.2016.267904
9. Szyniszewska, A. M., & Tatem, A. J. (2014). Global assessment of seasonal potential distribution of Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). PLOS one, 9(11), e111582. https://doi.org/10.1371/journal.pone.0111582
10. Wang, X. & Messing R.M. (2004). The ectoparasitic pupal parasitoid, *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), attacks other primary tephritid fruit fly parasitoids: host expansion and potential non-target impact. – Biological Control 31: 227–236.
11. Zhao, H.Y., Zeng, L., Xu, Y., Lu, Y.Y. & Liang G.W. (2013). Effects of host age on the parasitism of *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), an ectoparasitic pupal parasitoid of *Bactrocera cucurbitae* (Diptera: Tephritidae). – Florida Entomologist 96: 451–457.

Figures

Figure 1

Pachycrepoideus vindemmiae

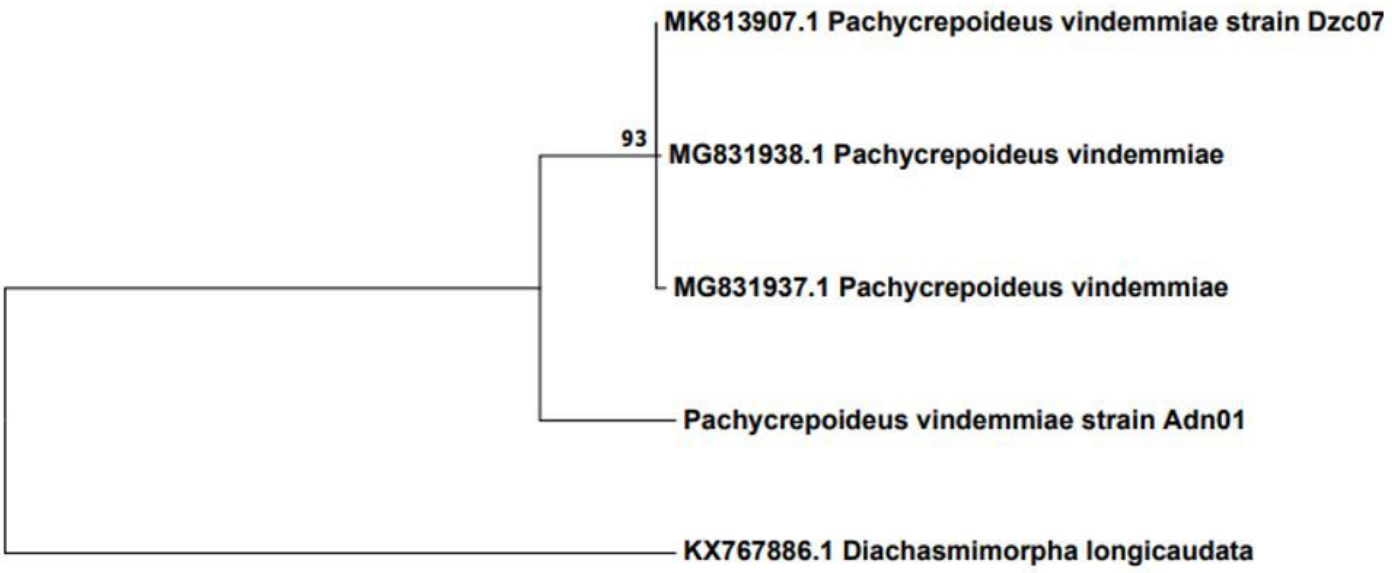

Figure 2

Phylogeny of the *Pachycrepoideus vindemmiae* associated with *Diachasmimorpha longicaudata* based on the COI locus (the out-group). The bootstrap values (percentage, based on 1000 replicates) are shown on the branches.