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ORIGINAL ARTICLE

MORPHOLOGICAL AND MOLECULAR STUDY OF TWO SPECIES OF *CARPOMYA* COSTA, 1854 (DIPTERA, TEPHRITIDAE) INFESTING SIDR FRUITS IN BAGHDAD, IRAQ

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ABSTRACT

Sidr trees are exposed to infestation by various insects, including the fruit fly species that belongs to the genus *Carpomya* Costa, 1854 (Diptera, Tephritidae), which comprises different species, some specialized in infesting sidr fruits. Therefore, this study aimed to diagnose the species of this genus infesting fruits of various species of *Ziziphus* Mill., 1754 cultivars. using distinct morphological features and molecular analysis (PCR). The paper also included constructing a phylogenetic tree for the genetically sequenced species using the COI gene and the sequence data were then compared with available data in GenBank.

The survey was conducted from December 2024 to May 2025, when a total of 25 kg of infested sidr fruits were collected from various locations in Baghdad Province, central of Iraq. This study molecularly recorded two species within this genus, representing the first molecular records for each: *Carpomya vesuviana* Costa,1854 which is the first molecular record in Iraq, and *Carpomya incompleta* (Becker, 1903), which is the first molecular record in Baghdad. The sequences were registered in GenBank under accession numbers PV809906 and PV810821, respectively.

Keywords: *Carpomya*, Fruit fly, Molecular identification, PCR, *Ziziphus*.

INTRODUCTION

Sidr trees, belonging to the genus *Ziziphus* Mill., 1754, are notable for their considerable diversity across various environmental systems, including arid, semi-arid, saline, and desert environments. Their distribution is widespread across the globe (Muhammad *et al*., 2022). Sidr trees are valued for their high nutritional fruit content, utilization of by-products, medicinal applications, and adaptability to harsh environmental conditions (Liu *et al*., 2020; Hasan and Zainulabdeen, 2022; Mulyono, 2023; Al-Zaydi and Al-Jibouri, 2025; Jia *et al*., 2025). Sidr trees, like any other plants, are exposed to infestations by insect pests, like in

India. More than 130 insect pest species have been recorded as infesting sidr trees (Haldhar *et al*., 2016). Among these numerous insect pests, the Fruit Flies, *Carpomya vesuviana* Costa, 1854 and *Carpomya incompleta* (Becker, 1903), are considered the most significant, causing direct yield losses (Garrido-Jurado *et al*., 2022; Kavin *et al*., 2024). These two pests are monophagous, which specialize in sidr fruits trees and cause substantial reductions in the productivity and quality of Sidr fruits (Kavin *et al*., 2024). *C. incompleta* can lead to crop losses ranging from 40-60% in uncontrolled areas (Al-Masudey and Al-Yousuf, 2012). *C. vesuviana*, on the other hand, can cause severe infestations that might result in yield reductions of up to 80% (Vadivelu, 2014). In both species, adult females lay their eggs inside the fruits, and the hatched larvae feed internally, creating tunnels that contain feces. This leads to fruit decay, secondary pathogen growth, an undesirable taste, fruit deformation, delayed development, or premature fruit drop (White and Elson-Harris, 1992; Jabbar, 1996).

Our investigation aims at identifying the main morphological characterization of *Carpomya* species infesting sidr fruits in Baghdad Province, Iraq, by identifying key diagnostic features that facilitate their differentiation. and provide the first molecular record of *C. vesuviana* in Iraq, as no previous molecular records of *C. vesuviana* have been established in Iraq and, building on the previous work by Tahir and Alyousuf (2023) who were the first to molecularly record *C. incompleta* in Basrah Province, this research will provide the first molecular record for *C. incompleta* specifically in Baghdad Province, utilizing PCR technology with the mitochondrial cytochrome oxidase subunit I (COI) gene and submit the obtained genetic sequences to the global GenBank database to establish a reliable genetic reference for the studied species and construct a phylogenetic tree using the submitted genetic sequences and compare the degree of genetic relatedness between the local Iraqi samples and those previously recorded in global databases like GenBank, or those from neighboring countries, to determine their genetic proximity.

MATERIALS AND METHODS

Study area: The specimens of infested sidr fruits were collected from different orchards belonging to both Radwaniya and Abu Ghraib Districts in Baghdad Province in a sampling which was conducted over a six-month period, from December 2024 to May 2025. During this time, approximately 25 kg of infested fruits were gathered from two distinct species of sidr trees: *Ziziphus mauritiana* Lam., 1789 and *Ziziphus spina-christi* (L.) Desf., 1798 Following collection, the infested sidr fruits were transferred to plastic containers. Each container was prepared with a 2 cm layer of soil sterilized in an oven at 170°C for 1 hour and each container was covered with a piece of tulle cloth, which was secured with an elastic band. The containers were then kept in the laboratory at a temperature ranging from 25 to 30°C until the emergence of adult insects.

Sampling, preservation, and photography of specimens: Once the adult specimens of two *Carpomya* species emerged, some were preserved, and stored in a freezer at approximately -20°C for a molecular identification study, while others were preserved in test tubes containing 70% ethyl alcohol to be identified morphologically using taxonomic keys (Korneyev *et al*.,

2017). To ensure accuracy, some of the specimens were also sent to the Iraq Natural History Research Center and Museum- University of Baghdad for identification confirmation.

The specimens were examined under a light microscope (Olympus) and photographed using Sony camera model ILCE-7RM4A and an advanced imaging system depending on photo stalking. Scale bars were provided for all the captures achieved by placing a calibrated ruler next to the insect specimens to be photographed. After capturing the image with a camera, the photo was processed using the ImageJ software. Within this software, the pixels of the ruler in the digital photo were measured for every 1 mm. After determining the number of pixels per millimeter in the image, a 1 mm scale bar was drawn next to the insect specimen using the ImageJ software. The image of the ruler was then cropped out using the same software.

Molecular study of adult *Carpomya* species using PCR technology: Molecular identification of the species were conducted through DNA analysis using the PCR (Polymerase chain reaction). This procedure was carried out at AL-Musaib Bridge CO. for Scientific and LAB. Equipment in Baghdad.

DNA Extraction and Molecular Identification: Genomic DNA was extracted from a single pooled sample containing eight adult individuals of *C. incompleta* (Becker, 1903) and a single pooled sample containing eight adult individuals of *C. vesuviana* Costa, 1854 using the GSYNC™ DNA Extraction Kit Quick Protocol (Geneaid, south Korea), following the manufacturer's instructions. Whole insect bodies were utilized for the DNA extraction process to ensure a sufficient quantity and quality of genomic DNA, as the small body size of these species.

Amplification and Sequencing of COI Gene: Polymerase Chain Reaction (PCR) device from Bioneer, South Korea was used to amplify approximately 710 bp of the target Mitochondrial cytochrome oxidase I (COI) gene. Using the primer set, LCO1490 and HC02198 produced by Bioneer, south Korea, was utilized for the amplification process depending on (Folmer *et al*., 1994).

Following PCR amplification, products were visualized by electrophoresis on a 1.5% agarose gel alongside a 100 bp DNA Ladder (Geneaid, South Korea) to confirm successful amplification and product size. Purified PCR products were subsequently submitted to Macrogen (South Korea) for Sanger sequencing.

Phylogenetic analysis: Raw nucleotide sequences were evaluated and edited for quality control using BioEdit and MEGA12 software. The resulting sequences were then aligned and compared with homologous sequences retrieved from the NCBI GenBank database using the BLAST search alignment tool. Subsequently, the sequences for both *Carpomya* species were submitted and registered in the NCBI GenBank database.

Morphological and molecular study

A phylogenetic tree was constructed to illustrate the genetic relationships between the *Carpomya* species investigated in this study and homologous *Carpomya* sequences available in the NCBI GenBank database. The Neighbor-Joining (NJ) method was employed as implemented in MEGA12, with 10,000 bootstrap replicates to assess nodal support. The Kimura 2-parameter (K2P) model was selected as the best-fit evolutionary model for the dataset, and sequence alignment was performed using Clustal W *Rhagoletis completa* Cresson, 1929 (MH998953.1) was utilized as a designated outgroup for comparative analysis and tree rooting.

RESULTS

Morphological study

In this study, the adults emerged from infested sidr fruits. These infested fruits were collected from both sidr tree species, *Z. mauritiana* Lam., 1789 and *Z. spina-christi* (L.) Desf., 1798. The two emerged species are *C. incompleta* (Becker, 1903) and *C. vesuviana* Costa, 1854. The distinguishing morphological characteristics used to differentiate between them will be described below:

Carpomya incompleta (Becker, 1903): A small-bodied species, length 3.3-3.5 mm body colour uniform, pale reddish-yellow, particularly on the mesonotum from a dorsal view (Pl. 1A), but it has two black spots located underneath the scutellum, which are not visible from above (Pl. 3A). Wing has three faint transverse bands and lacks an apical crossband, costal bristle at the anterior margin of the wing length 0.08-0.09 mm is noticeably shorter (Pl. 4A) than that of *C. vesuviana*. Ocellar setae length 0.06-0.07 mm appears shorter when compared to *C. vesuviana* (Pl. 2A).

Carpomya vesuviana Costa, 1854: A small-bodied species, length 4.5-5.5 mm body colour yellow/fulvous. (Pl. 1B); scutum with a specific pattern of 13 black spots (eight laterally, four sub-medially, and one medial spot extending to the scutellum) (Pl. 3B). Wing with four distinct yellow/orange transverse bands and longitudinal bands, costal bristle at the anterior margin of the wing length 0.12-0.125 mm is noticeably longer than that of *C. incompleta* (Pl. 4 B). Ocellar setae length 0.12-0.13 mm appears longer when compared to *C. incomplete* (Pl. 2 B).

Molecular study

The results from Polymerase Chain Reaction (PCR) analyzed by agarose gel electrophoresis of the two pooled samples revealed a single successful DNA band for each species, approximately 710 base pairs (bp) in size from the adults of *C. incompleta* and *C. vesuviana,* which is the expected target band of the COI gene (Pl. 5). The Raw nucleotide sequences came from Macrogen, a Korean company, the sequences were evaluated and edited for quality control. The sequences became 620 (bp) for *C. incompleta* and 679 (bp) for *C. vesuviana*. Those sequences were used to compare with previously identified and registered species in NCBI. The COI gene sequences of *C. incompleta* showed 100% similarity per identity and a maximum score of 1025 with the *C. incompleta* specimen isolated from Iraq, Basrah (Accession number ON045002.1). This confirmed that the sequences amplified by

PCR belong to this species so the sequence of *C. incompleta* registered in NCBI got (Accession number PV810821.1). On the other hand, the COI gene sequences of *C. vesuviana* showed 100% similarity per identity and a maximum score of 1254 with the *C. vesuviana* specimen isolate from Iran (Accession number NC 071721.1) and with the *C. vesuviana* specimen isolate from China (Accession number MT121231.1). This confirmed that the sequences amplified by PCR belong to this species so the sequence of *C. vesuviana* registered in NCBI got the (accession number PV809906.1).

Phylogenetic Relationships

The Neighbor-Joining (NJ) phylogenetic analysis, robustly supported by high bootstrap values, revealed two distinct major clades within the genus *Carpomya* (Diag. 1). These clades demonstrated strong statistical support, clearly separating *C. vesuviana* and *C. incompleta*.

C. vesuviana Clade: All *C. vesuviana* accessions analyzed, including the representative sample from Baghdad, Iraq (PV809906.1), clustered into a single, homogeneous clade. This clade received strong statistical support with a bootstrap value of 99%. Within this robust cluster, the Iraqi accession (PV809906.1) exhibited a particularly close genetic relationship with *C. vesuviana* samples from China (MT121231.1) and Iran (NC 071721.1), supported by a high bootstrap value of 95%. This suggests a remarkable genetic similarity among these geographically disparate populations. Additionally, it is grouped with *C. vesuviana* from India (PQ198005.1) and Iran (JQ668127.1), confirming its conspecificity.

C. incompleta Clade: Similarly, all *C. incompleta* accessions, including the representative sample from Baghdad, Iraq (PV810821.1), formed another distinct clade, also strongly supported by a 99% bootstrap value. Within this clade, the Iraqi accession (PV810821.1) showed strong genetic affinity with *C. incompleta* samples from Italy (NC 071720.1) and Basrah, Iraq (ON045002.1), with bootstrap support values of 97% and 99%, respectively. This close association with the Basrah sample is expected given their geographical proximity.

Outgroup: *Rhagoletis completa* Cresson, 1929 (MH998953.1) was appropriately positioned as the outgroup, confirming its more distant evolutionary relationship to the *Carpomya* species and accurately rooting the phylogenetic tree.

DISCUSSION

This study reports the presence of two fruit fly species, *C. incompleta* (Becker, 1903) and *C. vesuviana* Costa, 1854, infesting *Z. mauritiana* Lam., 1789 and *Z. spina-christi* (L.) Desf., 1798 fruits in Baghdad Province. *C. incompleta* was first mentioned in Iraq by Hussain (1963) on sider fruits. Its presence was subsequently confirmed by Derwesh (1965) and also cited by Abdul-Rassoul (1976). However, the first documented morphological taxonomic record for this species was established by Al-Saffar (2011). This fly is characterized by a small body length, a pale-colored body, and faint, incomplete, narrow transverse bands on the wing. Similarly, *C. vesuviana* was first mentioned in Iraq by Khalaf and Al-Omar (1974) on *Z. spina-christi* fruits. Yet, the first documented morphological taxonomic record for this species was also established by Al-Saffar (2011). This species is distinguishable by its small body

length, dark spots on the mesonotum, and transverse bands on the wing. Our study focuses on distinguishing the morphological characteristics of both *C. incompleta* and *C. vesuviana*, especially since both species infest the fruits of the same host plants, *Z. mauritiana* and *Z. spina-christi*.

Recent organism identification now relies on both morphological and molecular analyses, with gene barcoding proving particularly valuable. In Iraq, molecular identification studies for the genus *Carpomya* are limited. Previously, only one such study, by Tahir and Alyousuf (2023), focused on *C. incompleta* in Basrah. Notably, there has been no prior molecular-level study of *C. vesuviana* anywhere in Iraq. While both *C. incompleta* and *C. vesuviana* have existing morphological records in Iraq, this research fills a critical gap by providing the first molecular identification of *C. vesuviana* in Iraq. This establishes a vital genetic baseline for this species in the region. Furthermore, our phylogenetic analysis revealed a notable genetic homogeneity among geographically disparate populations of *C. vesuviana*, with the Iraqi sample clustering closely with accessions from Iran and China. These findings suggest that the Iraqi *C. vesuviana* population may share at the same ancestry with these geographical regions, despite the long distance. Dispersal could have taken place with the advancement of human means of transportation and through trading and transferring infested fruits between these areas and Iraq. Consequently, this finding ought to be considered in pest management, especially in the agricultural quarantine, to reduce the odds of other pest dispersal.

In the same way, although *C. incompleta* was previously recorded in Basrah at a molecular level, our study marks the first molecular record of *C. incompleta* in Baghdad. This genetic record for the area is important for the study of the species geographical distribution and possible genetic diversity in the country. The genetic similarity between our Baghdad sample and the Basrah sample is to be expected, given their geographical proximity: Baghdad is roughly 549 km north of Basrah. However, the similarity between the current sample and the samples from Italy, which are thousands of kilometers away, is striking and suggests a different dispersal pathway that is likely human-mediated.

Our use of DNA barcoding, targeting the cytochrome c oxidase subunit I (COI) gene, not only confirmed the morphological identifications but also established a reliable genetic reference for both species. This technique is particularly valuable when morphological identification of insect specimen is challenging. Such difficulty can arise during the larval stages and even, sometimes in adult stages, where precise species identification can be problematic, especially if parts of the adult specimen are damaged (Wells *et al*., 2001). Submitting these sequences to GenBank makes our work publicly accessible and provides a genetic tool for future researchers to use in identifying and tracking these species nationally and regionally.

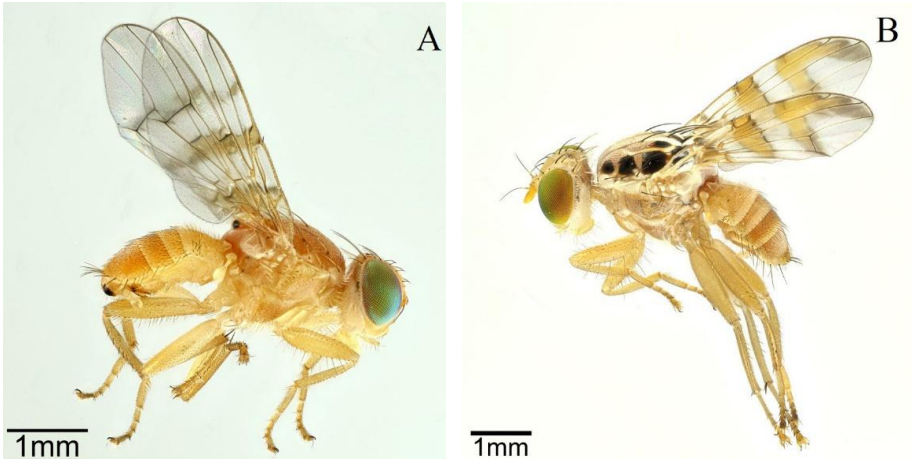

Plate (1): Lateral view of male; (A) *C. incompleta*, (B) *C. vesuviana*.

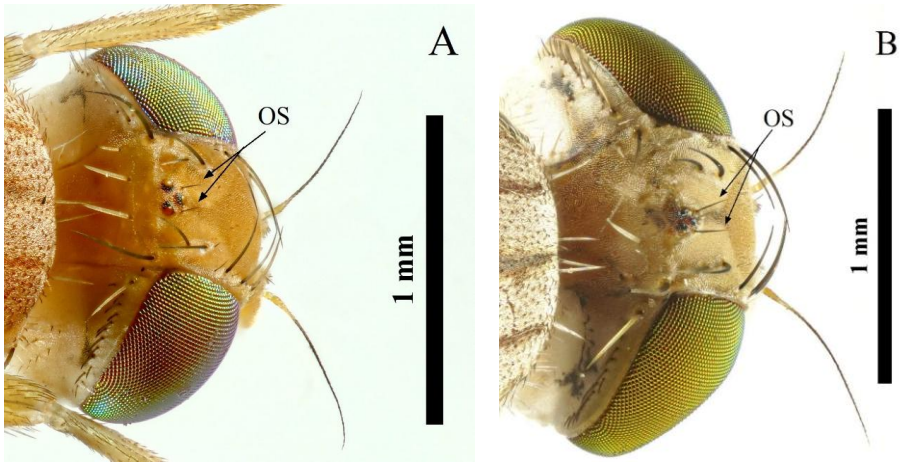

Plate (2): Dorsal view of head in male; (A) *C. incompleta*, (B) *C. vesuviana*. [OS: ocellar setae].

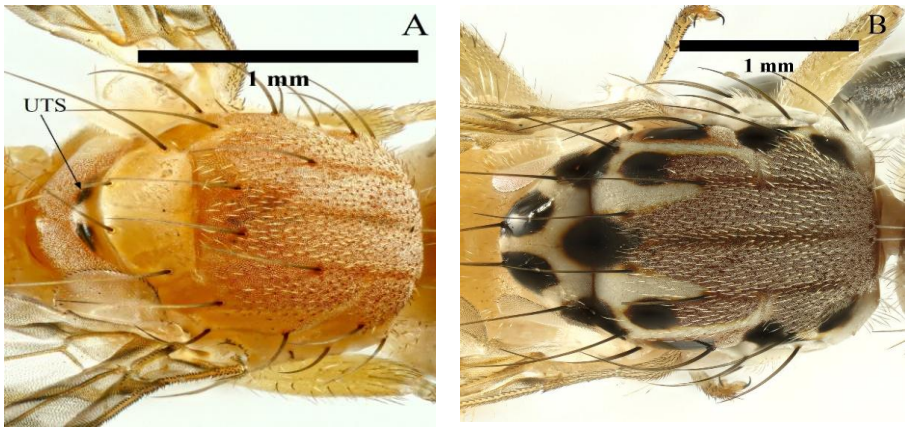

Plate (3): Dorsal view of thorax in male; (A) *C. incompleta*, (B) *C. vesuviana*. [UTS: underneath the scutellum].

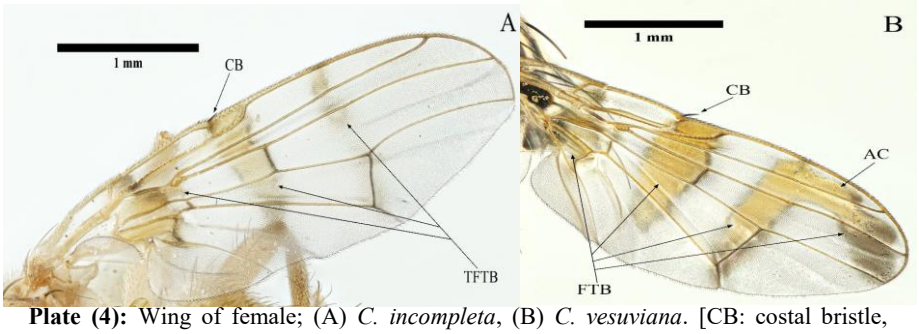

Plate (4): Wing of female; (A) *C. incompleta*, (B) *C. vesuviana*. [CB: costal bristle, T+FTB: three faint transverse bands, AC: apical crossband, FTB: four transverse bands].

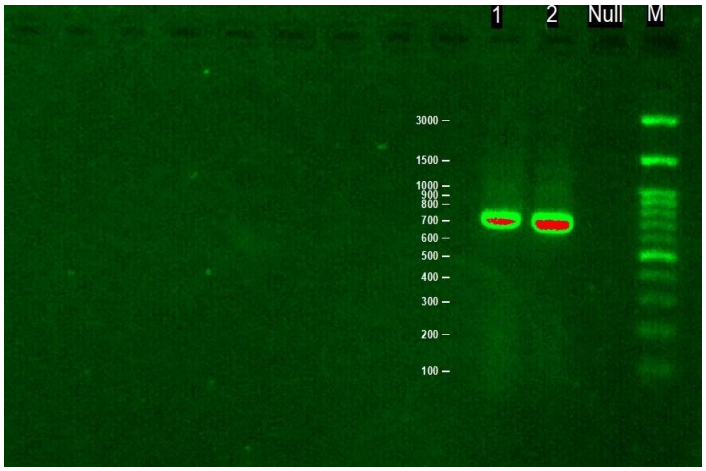

Plate (5): Electrophoresis of PCR amplification products for the COI gene in the studied species.

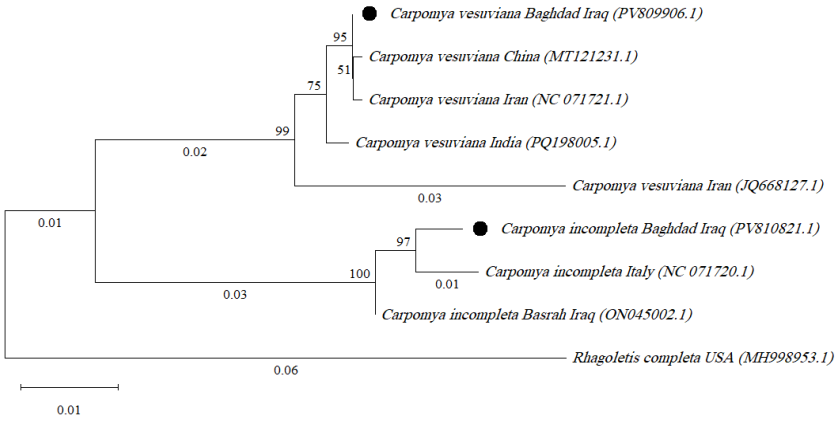

Diagram (1): The Phylogenetic tree of *C. incompleta* and *C. vesuviana* from Baghdad-Iraq based on COI gene Sequence.

CONCLUSIONS

Species within the genus *Carpomya* that infest sidr fruits possess distinct morphological characteristics, facilitating their relatively straightforward differentiation. Molecular identification, particularly through the utilization of COI gene sequences, offers a modern, rapid, and reliable approach that significantly enhances the confirmation of morphological identifications and aids in distinguishing among various *Carpomya* species.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST STATEMENT

The results of the present study are part of the requirements of Ph.D. in Insects, Department of Plant Protection, College of Agriculture Engineering Sciences-University of Baghdad for the first author. As well, we are the authors of this manuscript, declare and confirm that there is no significant financial or other relationship with any official institution.

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دراسة مظهرية وجزيئية لنوعين من ذباب فاكهة من جنس *Carpomya* Costa, 1854 (Diptera, Tephritidae) التي تصيب ثمار السدر في بغداد، العراق

إياس ياسين الجبوري و فريال بهجت هرمز

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الاستلام: 2025/8/11، المراجعة: 2025/10/12، القبول 2025/10/16، النشر: 2025/12/20

الخلاصة

تتعرض أشجار السدر للإصابة من قبل حشرات مختلفة، بما في ذلك أنواع من ذباب الفاكهة التي تنتمي الى جنس (Diptera, Tephritidae) *Carpomya* Costa,1854، الذي يضم أنواعًا متخصصة في إصابة ثمار السدر. لذلك، هدفت هذه الدراسة إلى تشخيص الانواع التي تصيب ثمار أصناف مختلفة من أشجار جنس *Ziziphus* Mill., 1754، وذلك بالاعتماد على الخصائص المظهرية المميزة والتحليل الجزيئي (PCR). كما تضمنت الدراسة إنشاء شجرة وراثية للأنواع التي تم تسلسلها جينيًا باستخدام جينCOI ، ومن ثم مقارنة بيانات التسلسل مع البيانات المتوفرة في بنك الجينات GenBank.

أُجري المسح من شهر كانون الأول 2024 إلى أيار 2025. تم جمع ما مجموعه 25 كغم من ثمار السدر المصابة من مواقع مختلفة في محافظة بغداد، وسط العراق. سجلت الدراسة نوعين ضمن هذا الجنس، ويعد اول تسجيل جزيئي لكل منهما: النوع *Carpomya vesuviana* Costa,1854 اول تسجيل جزيئي في العراق، والنوع *Carpomya incompleta* (Becker, 1903) اول تسجل جزيئي في بغداد. تم تسجيل التسلسلات الجينية في بنك الجينات بأرقام الوصولPV809906 و PV810821، على التوالي.