First morphological and molecular identification of mealybug *Cadra cautella* (Lepidoptera: Pyralidae) in Karbala, Iraq

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Abstract

Cadra cautella (Walker, 1863) (Lepidoptera: Pyralidae) is a significant pest insect of dried products that has become widespread in storge products of Karbala Province. Integrating molecular data using Next Generation sequencing alongside morphological data from a comprehensive collection of live specimens was applied to identify *C. cautella* in Karbala, Iraq. The morphemic identification of the almond moth common in Karbala is *C. cautella*. This identification was confirmed molecularly. In addition, this insect's mitochondrial genes mtCOX1 were recorded in GenBank with accession numbers PP916775 and PP921921, with 657 and 651 bp long, respectively Another two genes of this insect were sequenced successfully: the hydroxymethylglutaryl-CoA lyase and vitellogenin genes deposited in GenBank under accession numbers PP928485 and PP928484. The results showed that for the first time, the identification of *C. cautella* and its genes was reported based on molecular biology in Karbala, Iraq. The research results help identify and distinguish the almond moth species using NGS and are beneficial for the better choice and application of control strategies against it.

Keywords: *Cadra cautella*, endosymbionts, almond moth, Next Generation sequencing.

Introduction

Cadra cautella (Walker, 1864) synonym of *Ephestia cautella* (Walker, 1863) is considered one of the most known stored product insects of the subfamily Pyralidae, and among them stands out the Pyralidae species *C. cautella* [1, 2]. This cosmopolitan moth species was initially distributed in the Balearic region, mainly around the Mediterranean [3]. However, it was spread because of human activity to other areas of the world utilizing the merchandise transportation of stored products [4]. This unlucky event is of great importance because the mentioned Lepidoptera species is one of the most serious stored product pests that can take place. Indeed, *Cadra cautella* can be found in domestic and industrial situations, feeding on a large range of stored products, from raw ones such as grain, dried fruit, and nuts to processed foods [4].

A biological study of its bioecology under abiotic factors typical of warehouses and homes was carried out to increase the bioecology information from other authors in the past [5]. The main objective of this scientific research was to know in depth about this

damaging species to prevent or face its populations, keep them under control, and reduce the economic consequences of its natural life cycle. However, this insect has not been investigated, and its distribution in other Iraqi provinces has not been confirmed. The morphology of numerous insect species is typically impacted by environmental variables, leading to substantial phenotypic diversity even among individuals of the same species. This variability presents challenges and ambiguities in species classification, which can be addressed by applying molecular information obtained through new molecular techniques and an integrative approach to taxonomy [6]. Recurrently complex species, including biotypes, can be distinct based on behaviour, morphological characteristics, pesticide resistance, and the mtDNA COI sequence [7,8]. The evolution of gene rate could, by examination, reveal that cox1 and atp6 exhibit the lowest and highest gene replacement rates, respectively, and other genes [9]. Recognizing the insect is imperative for any successfully integrated pest management program (IPM). The accuracy of defining organisms is vital in identifying and discovering their species. Inappropriately, classifying insects and counting parasitoids is often tricky and is assumed to contain many complex species [10,11,12]. Herbert and his team [13] introduced DNA barcoding by amplifying the 680bp mtCO1 gene. Molecular identification using mtDNA (Barcoding DNA) might assistance classify complex insects that are unidentified morphologically [14]. Primary and secondary endosymbiont bacteria are vital for terrestrial insect biology and ecology [15].

One of these new molecular techniques is next-generation sequencing (NGS), which has revolutionized the identification and study of insects by supplying rapid, complete, and cost-effective analyses of insect genomes. This advanced technology empowers scientists to sequence entire insect genomes or specific gene regions on a large scale, aiding in species identification and detecting cryptic species that may appear similar morphologically but possess distinct genetic traits [16,17]. Moreover, NGS facilitates the exploration of insect transcriptomes, providing valuable insights into gene expression patterns across different environmental contexts and developmental phases. Furthermore, NGS permits the examination of insect microbiomes and viruses, unveiling intricate relationships with symbiotic organisms and pathogens. The wealth of genetic data obtained through NGS supports various applications such as biodiversity assessment, pest management strategies, and conservation initiatives, positioning NGS as an essential instrument in contemporary entomological investigations [18].

Endosymbiont bacteria have been informed to affect many aspects of their host biology (insects) that might affect genetic diversity and other environmental factors. Endosymbionts and mtDNA are vertically transmitted and linked to their host's evolutionary history. Therefore, they might show evolutionary developments linking both sides of endosymbiosis [19].

Due to a lack of information regarding the cotton mealybug, *Phenacoccus solenopsis*, with its associated endosymbionts in Karbala Province, this study aimed to identify this insect morphologically and molecularly with its endosymbionts in Karbala province, Iraq.

Materials and Methods

Insect Collection

Numerous adults of *C. cautella* individuals used for morphological analysis were collected in 2023 from various ornamental trees at the Holy Shrine of Hussein's nurseries and the gardens of Martyr Ahmed Zaini in Holy Karbala Province (32°27′N 43°48′E), Iraq.

Morphological identification

The adults of *C. cautella* were examined using a stereo microscope 60x for identification using taxonomy characteristics described by Subramanyam (1995) [20]. The adult of *C. cautella* is predominantly light brown, with smaller, typically grey hind wings. When extended, its wingspan ranges from 14 to 22 mm. The back edges of the wings are lined with a short fringe [20].

DNA extraction, Next-Generation Sequencing, and Bioinformatic analysis

The whole genomic DNA of insects was extracted from these insects using the AccuPrep® Genomic DNA Extraction Kit (Bioneer/South Korea) following the manufacturer's instructions. The gDNA extracted was exploited to make paired-end shotgun libraries via the TruSeq Nano DNA Kit (Illumina, California, US). These libraries were sequenced at Macrogen company (Seoul, South Korea) operating the NovaSeq 6000 Illumina platform to produce 151 bp paired-end raw reads [21]. The FastQC package was used to approve the quality of raw reads obtained (Babraham Bioinformatics, Cambridge, UK). The raw reads were then aligned to the reference sequences of the five chromosomes of *C. cautella* (GCA_009761765.1) and the sequence of the mitochondrial cytochrome c oxidase subunit I (COX1) gene (MZ542528.1), operating the Bowtie2 (V. 2.4.5) tool [22]. The unaligned reads were consequently exposed to De Novo assembly via the SPAdes (V3.15.4) tool [23] and compared against the GenBank database of endosymbionts acquired from the NCBI using BLASTn and BLASTp. Additionally, the draft sequences of the COX1 gene and the endosymbionts detected were BLASTn against the NCBI nucleotide collection (nr/nt) database to verify their presence and confirm the detection. After that, all these sequences were submitted to NCBI-GenBank. Furthermore, the phylogenetic trees were built with the multiple alignments in the neighbour-joining tree under bootstrap analysis (1000 replicates) using the distance tree of NCBI-BLASTn results [24].

Results and Discussion

Morphological Confirmation of the *C. cautella*

The specimens were identified using the guide provided by Subramanyam (1995) [20]. The adult of *C. cautella* (Figure 1) is predominantly light brown. The adult wingspan ranges from 14 to 22 mm when extended.

Figure (1): Morphology of *C. cautella;* A. Dorsal view; B. Ventral view.

Molecular identification of *C. cautella*

Molecular confirmation of the *C. cautella* dispersed in Karbala Province using barcode mitochondrial mtCOX marker was applied. The results showed variation within the sequences of *C. cautella* collected from Karbala province (Figure 2). However, the mtCOX sequences of *C. cautella* displayed a very high similarity (100%) with various global isolates *C. cautella*, particularly the isolates (Figure 3). Thus, the mtCOX sequences received unique accession numbers in GenBank databases PP916775 and PP921921 with *C. cautella* isolate Karbala-1. The phylogenetic analysis also revealed a close relationship between those isolates of *C. cautella* and those in the same clade (Figure 3). Two genes of *C. cautella* hydroxymethylglutaryl-CoA lyase and vitellogenin genes were deposited in GenBank under accession numbers PP928485 and PP928484. Thus, these sequences were submitted to GeneBank as a first draft of the whole genome of the Iraqi *C. cautella* (Figure 4 and 5).

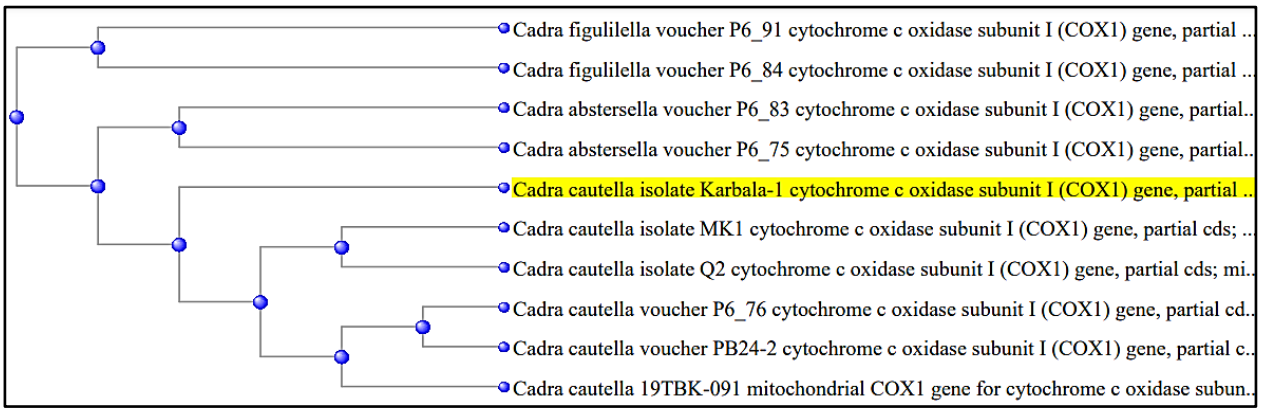

Figure (2): Using the Neighbour-joining method, the phylogenetic tree of Iraqi *Cadra cautella* isolate Karbala-1 based on COX1 gene sequences.

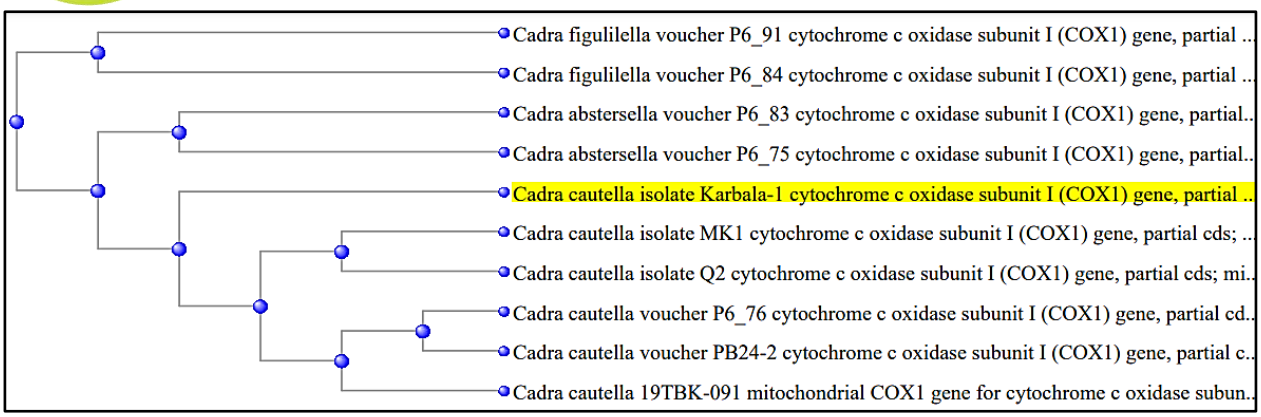

Figure (3): Using the Neighbour-joining method, the phylogenetic tree of Iraqi *Cadra cautella* isolate Karbala-1 based on COX1 gene sequences.

Two genes of *C. cautella* isolate Karbala-1 hydroxymethylglutaryl-CoA lyase and vitellogenin genes were sequenced successfully and recorded in GenBank under accession numbers PP928485 and PP928484. The phylogenetic trees of these genes confirmed this identification by showing a close relationship with bacteria in the same species (Figure 4 and 6).

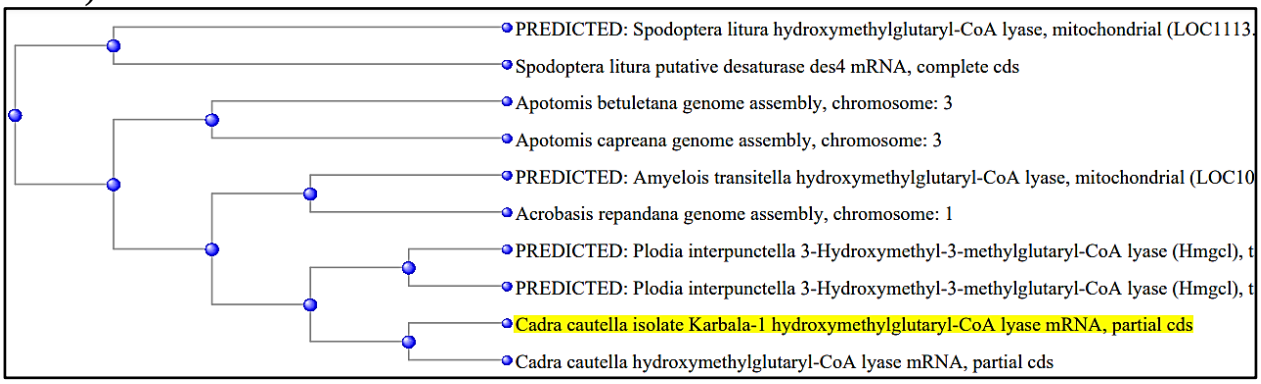

Figure (4): The phylogenetic tree of the gene hydroxymethylglutaryl-CoA lyase isolate Karbala-1 (highlighted in yellow), which was constructed based on the similarity of its nitrogenous base sequences with the sequences of the global strains of bacteria commensal inside insects obtained from the GenBank data repository. The genetic distances were calculated using the neighbor-joining method.

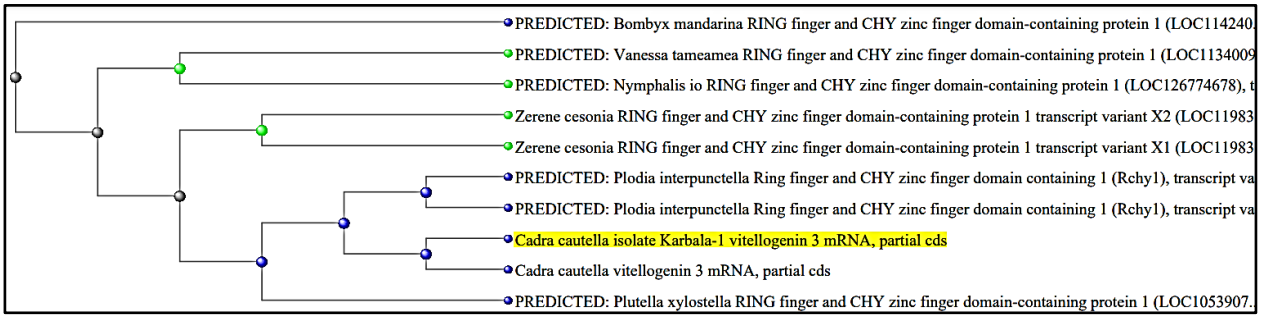

Figure (5): The phylogenetic tree of the gene vitellogenin isolate Karbala-1 (highlighted in yellow), which was constructed based on the similarity of its nitrogenous base sequences with the sequences of the global strains of bacteria commensal inside insects

obtained from the GenBank data repository. The genetic distances were calculated using the neighbor-joining method.

Cadra cautella is an essential stored insect that causes massive storage production damage by feeding on them. Presently, *Cadra cautella* has become a severe pest in Karbala storages. This research aimed to identify the C. cautella in Karbala molecularly. The mtCOI and mtCOX genes were used for identification and recorded in GenBank. Five samples from different locations in Karbala City were sequenced using the next-generation sequencing method to identify the insects and their symbionts. As a result, two sequences of *C. cautella* were recorded in GenBank, and two other genes, hydroxymethylglutaryl-CoA lyase and vitellogenin, were recorded in GenBank under accession numbers PP928485 and PP928484. This is the first time molecularly recognized *C. cautella*. This considers as first study about this pest using molecular techniques and is similar to several studies of insects in Karbala [7,8]. However, more studies are needed to find the link between the insect and its adaptation.

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