Research Paper (Survey: Insects) بحوث (حصر: حشرات)

Morphology and Molecular Identification of Mealybug, *Phenacoccus solenopsis* and its Endosymbionts in Karbala, Iraq

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Abstract

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The cotton mealybug, *Phenacoccus solenopsis* (Hemiptera, Pseudococcidae), is a significantly important insect pest of cotton and ornamental plants that has become widely spread in most nurseries of Karbala province. Integrating molecular data using next generation sequencing (NGS) alongside morphological data from a comprehensive collection of live specimens was applied to identify the *Phenacoccus solenopsis* and their endosymbionts in Karbala, Iraq. The morphological identification of the cotton mealybug common in Karbala suggested that it is *Phenacoccus solenopsis*. Identification was confirmed molecularly by obtaining the five chromosome sequences of the Iraqi *Ph. solenopsis* with pairwise identity of 90-97.8%. In addition, this insect's mitochondrial genes mtCOX1 (two sequences) were registered in GenBank under accession numbers PP768170 and PP768171. Furthermore, four endosymbiont bacteria were reported (*Candidatus Syntrophocurvum alkaliphilum*, *Enterobacteriaceae endosymbiont*, *Buchnera aphidicola* and *Candidatus Tremblaya endosymbiont)* associated with this Iraqi *Ph. solenopsis*. All these bacteria were registered in the GenBank under accession numbers PP897797, PP897798, PP897799 and PP882706, respectively. This is the first report on the identification of *Ph. solenopsis* with its endosymbiont bacteria, based on morphology and molecular biology, in Karbala, Iraq. The research results helped to identify and distinguish the species of cotton mealybug using NGS and will prove useful in selecting control strategies to control this pest.

Keywords: *Phenacoccus solenopsis*, endosymbionts, mealybug, next generation sequencing.

Introduction

Phenacoccus solenopsis (Girault, 1915) (Hemiptera, Pseudococcidae) is an important insect pest of crops and ornamental plants. This insect is polyphagous and is known to feed on more than 219 host plant species from different families (Ben-Dov *et al.,* 2017), including economically important crops such as cotton and various horticultural plants (Afzal *et al.,* 2009; Hodgson *et al.,* 2008; Wang *et al.,* 2009; Yi-Yong *et al.,* 2011). *Phenacoccus solenopsis* causes massive yield loss, decreasing cotton yield by around 50% (Jhala *et al.,* 2008). So far, *Ph. solenopsis* has been reported from 37 countries worldwide (Ben-Dov *et al.,* 2017). It was reported on the ornamental plant *Lantana camara* (Verbeneceae) in Baghdad, Iraq, in 2014 (Abdul-Rassoul *et al.,* 2015). However, this insect has not been intensively 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 (Li *et al.,* 2019). Recurring complex species, including biotypes, can be distinct based on behaviour, morphological characteristics, pesticide resistance, and the mtDNA COI sequence (Abd *et al.,* 2021; Kareem *et al.,* 2020). The evolution of genomic organization revealed that cox1 and atp6 exhibit the lowest and highest gene replacement rates, respectively (Choudhary *et al.,* 2015). Recognizing the insect is imperative for any successful integrated pest management program (IPM). The accuracy of characterizing organisms is vital in identifying species. Inappropriate classification of insects and their parasitoids is often difficult because of the presence of many complex species (Abbas *et al.,* 2019; Aljaafari, 2022; Smith *et al.,* 2006). Hebert *et al.* (2003) introduced DNA barcoding by amplifying the 680bp mtCOI gene. Molecular identification using mtDNA (Barcoding DNA) might assist in classifying complex insects that are unidentified morphologically (Park *et al.,* 2011). Primary and secondary endosymbiont bacteria are vital for terrestrial insect biology and ecology (Cass *et al.,* 2014).

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 the detection of cryptic species that may appear morphologically similar but possess distinct genetic traits (Abass & Lahuf, 2023; Papanicolaou *et al.,* 2016). 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

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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 (Salman & Lahuf, 2022).

Endosymbiont bacteria have been known to affect many aspects of insect biology that might affect genetic diversity. 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 (Kapantaidaki *et al.,* 2015).

Due to a lack of information regarding the cotton mealybug, *Phenacoccus solenopsis,* with its associated endosymbionts in Karbala Province, Iraq, 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 *Ph. solenopsis* 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 *Ph. solenopsis*were examinedusinga stereo microscope at 60x magnification for identification using taxonomy characteristics described earlier (Abdul-Rassoul *et al.,* 2015; Williams, 2004). The samples of *Ph. solenopsis* were also sent to Dr. Gillian Watson, Natural History Museum, London (NHML), for confirmation (Khudhair, *et a*l., 2025).

DNA extraction, next-generation sequencing, and bioinformatic analysis

The whole genomic DNA of insects was extracted 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, USA). These libraries were sequenced at Macrogen company (Seoul, South Korea) operating the NovaSeq 6000 Illumina platform to produce 151 bp paired-end raw reads (Abass & Lahuf, 2022). 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 *Ph. solenopsis* (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 (Ye *et al.,* 2022). The unaligned reads were consequently exposed to De Novo assembly via the SPAdes (V3.15.4) tool (Mohammed & Lahuf, 2023) 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 (Lahuf, 2021).

Results and Discussion

Morphological Confirmation of the *Ph. solenopsis*

The specimens were identified using the guide provided by Williams (2004). An adult female of *Ph. solenopsis* (Figure 1) was flat, covered with powdery wax and wingless. It has a lengthened oval body, around 3 mm long and 1.5 mm wide. *Ph. solenopsis* was yellowish-green and furnished with 18 pairs of white lateral short-waxy filaments around the margin of the body. The first feature was wingless. Secondly, the face included two spots, one on each antenna. Thirdly, the dorsal view of the thorax had a lateral yellow stripe on each side. Finally, the abdomen typically included dark symbols on tergite III, except on tergite.

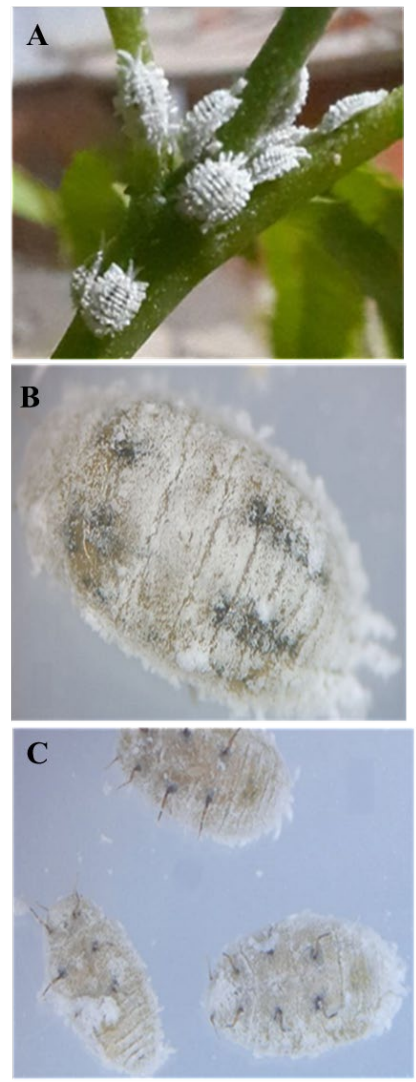

Figure 1. Morphology of *Ph. solenopsis.*(A) Female feeding on the host, (B) Female dorsal view, (C) Female ventral view.

Molecular identification of *Ph. solenopsis*

Molecular confirmation of the *Ph. solenopsis* distributed in Karbala Province was made by using the barcode mitochondrial mtCOX marker. The results showed variation within the sequences of *Ph. solenopsis* collected from Karbala province (Figure 2). However, the mtCOX sequences of *Ph. solenopsis* displayed a very high similarity (100%) with various global *Ph. solenopsis* isolates, particularly the isolates MZ542528.1, AB858432.1 and KR001884.1 (Figure 3). Thus, the mtCOX sequences received unique accession numbers in GenBank databases PP768170 and PP768171 with the name *Phenacoccus solenopsis* isolate Karbala-1. The phylogenetic analysis also revealed a close relationship between those isolates of *Ph. solenopsis* and those in the same clade (Figure 3).

Furthermore, this identification was confirmed by obtaining the five chromosome sequences of the Iraqi *Ph. solenopsis* with pairwise identity of 90-97.8%. The raw reads were assembled to the reference sequences of the five chromosomes, with coverage extended from 83 to 99.8%. Thus, these sequences were submitted to GenBank as a first draft of the whole genome of the Iraqi *Ph. solenopsis* (Figure 4).

Furthermore, four endosymbionts' bacteria were reported (Candidatus *Syntrophocurvum alkaliphilum*, Enterobacteriaceae endosymbiont, *Buchnera aphidicola* and *Candidatus Tremblaya*). They were found to be associated with the Iraqi *Ph. solenopsis,* and they were registered in the GenBank under accession numbers: PP897797, PP897798, PP897799 and PP882706, respectively. The phylogenetic trees of these endosymbionts bacteria confirmed their identification by showing a close relationship with bacteria of the same species (Figure 5).

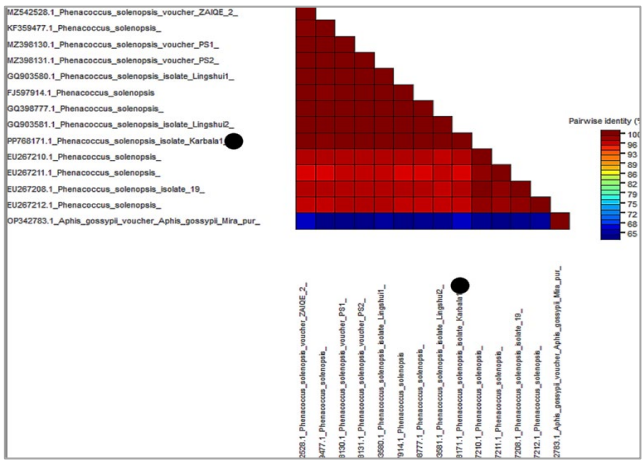

Figure 2. The pairwise identity of Iraqi *Phenacoccus solenopsis* isolate Karbala-1 with other universal isolates of the same insect.

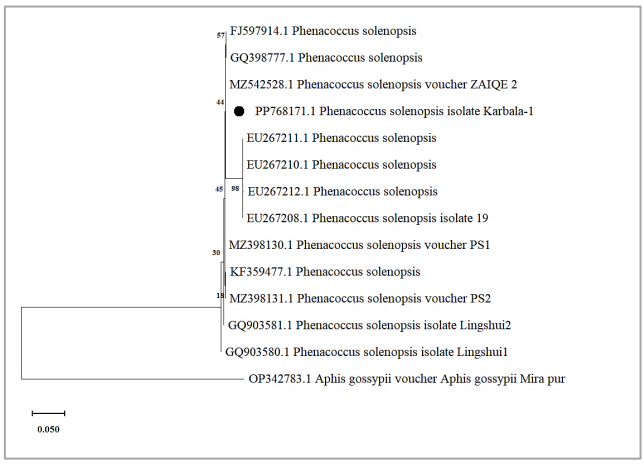

Figure 3. The phylogenetic tree of Iraqi *Phenacoccus solenopsis* isolates Karbala-1 based on COI gene sequences, using the neighbour-joining method.

Figure 4. The coverage and pairwise identity of the five chromosomes of *Phenacoccus solenopsis*.

*Ph. solenopsis*is is an oriental plant pest that causes serious damage in nursery production. Presently, *Ph. solenopsis* has become a severe pest in Karbala. This research aimed to molecularly identify the *Ph. solenopsis* and their symbionts in Karbala plant nurseries. The mtCOI and mtCOX genes were used for identification. Five samples from different locations in Karbala region were sequenced using the next-generation sequencing method to identify the insects and their symbionts. As a result, two sequences of *Ph. solenopsis* were registered in GenBank, and four other endosymbionts, *Candidatus Syntrophocurvum alkaliphilum*, *Enterobacteriaceae endosymbiont*, *Buchnera aphidicola* and Candidatus *Tremblaya endosymbiont*, of *Ph. solenopsis* were identified in Karbala for the first time. Though more studies are needed to find the link between the insect and its symbionts and their adaptation to the local environment in Iraq.

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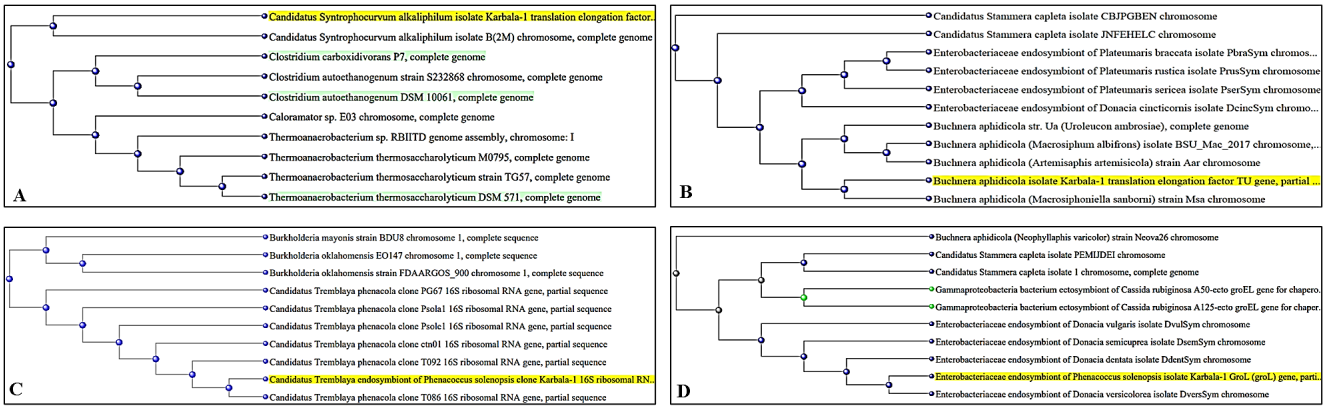

Figure 5. The phylogenetic tree of the bacterium (A) Candidatus *Syntrophocurvum alkaliphilum* isolate Karbala-1 (highlighted in yellow) based on its nitrogenous base sequences, (B) the bacterium *Buchnera aphidicola* based on its nitrogenous base sequences, (C) the Candidatus *Tremblaya endosymbiont* based on the similarity of its N-terminal sequences, (D) the *Enterobacteriaceae endosymbiont* based on the similarity of its N-terminal sequences, which was constructed based on the similarity 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.

الملخص

عباس، أحمد حسن خضير وعلي عبد الحسين كريم. 2026. التشخيص الجزيئي لحشرة بق القطن الدقيقي (*Phenacoccus solenopsis*) والبكتيريا المتعايشة معها داخلياً في محافظة كربلاء، العراق. مجلة وقاية النبات العربية، 44(2):183–188. https://doi.org/10.22268/AJPP-001386

تعدّ حشرة بقّ القطن الدقيقي (*Phenacoccus solenopsis*) (Hemiptera: Pseudococcidae) من الآفات الحشرية المهمة التي تصيب القطن ونباتات الزينة، والتي انتشرت على نطاق واسع في معظم مشاتل محافظة كربلاء. تم التشخيص الجزيئي للحشرة باستخدام تسلسل الجيل التالي جنباً إلى جنب مع التشخيص المظهري اعتماداً على كاملات حشرة بقّ القطن الدقيقي والبكتيريا المتعايشة معها داخلياً في محافظة كربلاء، العراق. تم تأكيد التشخيص المظهري لحشرة بقّ القطن الدقيقي المنتشرة في كربلاء بكونها *Phenacoccus solenopsis*، وكذلك تمّ تأكيد التشخيص جزيئياً من خلال الحصول على تسلسلات الكروموسومات الخمسة للحشرة بنسبة 90.0–97.8%. بالإضافة إلى ذلك، تم تسجيل تسلسلين لجينات الميتوكوندريا mtCOX1 لهذه الحشرة في GenBank تحت الأرقام PP768170 و PP768171. وعلاوةً على ذلك، تمّ تسجيل تسلسلات جينية لأربعة أنواع بكتيريا متعايشة *Candidatus Syntrophocurvum alkaliphilum*، *Enterobacteriaceae endosymbiont*، *Buchnera aphidicola* و *Candidatus Tremblaya endosymbiont* مرتبطة بحشرة البق الدقيقي في العراق. سُجّلت جميع هذه البكتيريا في بنك المورثات GenBank تحت الأرقام التسلسلية PP897797، PP897798، PP897799 و PP882706، على التوالي. تعدّ هذه أول دراسة في التشخيص الجزيئي لحشرة البقّ الدقيقي والبكتيريا المتعايشة معها داخلياً في كربلاء، العراق. ساعدت نتائج البحث في تحديد وتشخيص أنواع حشرة البق الدقيقي القطنية باستخدام الجيل التالي من الجينوم وستثبت فائدتها في اختيار استراتيجيات المكافحة لمكافحة هذه الآفة.

كلمات مفتاحية: تعايش داخلي، بقّ دقيقي، تسلسل الجيل التالي، *Phenacoccus solenopsis*.

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