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Original Article

Molecular Detection of *Varroa* spp. Mite Infesting Honey Bees in Apiaries of Al-Diwaniyah Province, Iraq

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Abstract

Introduction: The honey bee (*Apis mellifera*) is a hymenopteran insect of high economic importance to humans, primarily due to its ecological role. Like other living organisms, this beneficial insect is susceptible to various pests and diseases, some of which severely affect colony activity, leading to increased mortality and weakening or loss of colonies. Among the most destructive pests is the *Varroa* mite, which infests larvae, pupae, and adult bees. The danger of *Varroa* lies in its rapid reproduction and widespread dissemination, ultimately resulting in colony collapse. This study aimed to molecularly identify *Varroa* mite collected from apiaries in Al-Diwaniyah province, determine their genetic sequence, construct a phylogenetic tree, and analyze the nucleotide sequence diversity of local isolates (*Varroa* sp. IQD). The sequences were then compared with other reference strains from NCBI using the mitochondrial cytochrome c oxidase subunit 1 (COX1) gene.

Methods: DNA was extracted from *Varroa* isolates, and its quality was verified on agarose gel. Polymerase Chain Reaction (PCR) was employed to amplify an 821 bp fragment of the COX1 gene. The amplified products were sequenced and aligned using ClustalW. Phylogenetic trees were constructed in MEGA 6.0 using the UPGMA and Maximum Likelihood methods to compare local isolates with reference strains.

Results: The findings revealed a high genetic similarity between the local isolates (*Varroa* sp. IQD.1–IQD.5) and the reference strain *Varroa destructor* (GQ379069.1), with a homology of 99.48%.

Conclusion: The study concludes that the *Varroa* mite prevalent in Iraq is *Varroa destructor*, a parasitic mite globally known for its destructive impact on honey bee colonies.

Keywords: Honey bee (*Apis mellifera*), Cox1 gene, *Varroa* mite, Diwaniyah / Iraq

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Introduction

The honey bee (*Apis mellifera*) is one of the most economically important insects due to its significant role in providing many important food and medicinal products, such as honey, wax, and propolis, as well as its importance in pollinating plants and preserving biodiversity in the environment (1, 2), However, it faces numerous threats, among which the *Varroa destructor* mite is considered one of the most dangerous pests worldwide. It parasitizes all developmental stages of the bee, weakening colonies by consuming hemolymph and transmitting serious viruses, such as the Deformed Wing Virus (DWV) (3, 4). Traditional methods of identifying *Varroa* mites are insufficient for species determination or genetic diversity analysis. Molecular techniques, particularly PCR, have therefore become precise tools for detecting the parasite and characterizing its genetic structure (5.6). Such research is particularly crucial in developing countries, including Iraq, where molecular-level studies on *Varroa* remain limited, despite its devastating impact on local apiaries.

Accordingly, this study aimed to perform a molecular survey of *Varroa* species infesting apiaries in Al-Diwaniyah province, Iraq, and to document their genetic diversity, thereby contributing to the establishment of scientific strategies for managing this parasite and mitigating its adverse effects on beekeeping.

Materials and Methods

Study Sites and Sample Collection

The study was conducted across ten apiaries in different regions, including Preparatory Agriculture Phase Station, Al-Furat district, Al-Saniya, Nifer, Al-Daghara, Al-Bdeir, Soumer, and Al-Hamza Al-Sharqi. Each apiary was visited three times per month during the study period from October 2024 - April 2025, with regular monitoring of temperature and humidity. Preliminary data were also collected via questionnaires directed to beekeepers. Five honey bee colonies were examined in each apiary for *Varroa* infestation using two main approaches:

Adult bee inspection

- Direct examination under magnifying lenses.

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- Powdered sugar method according to (7): bees were coated with sugar in a mesh-covered jar, shaken to dislodge mites, which were then collected for analysis.

Brood examination

The detection process relied on examining the pupae, especially the drones, as *Varroa* mites can be clearly seen on the white pupal surface. The closed brood was opened using forceps or a capping scratcher at an angle that would not damage the pupae (8). Ten brood samples were randomly examined from each side of the frame. In the absence of drone brood, infested worker bee brood was examined instead. The collected pupae were placed in test tubes containing 95% ethyl alcohol for preservation, with the apiary data, collection date, and location recorded.

Molecular Diagnosis of Varroa Using the PCR Technique

DNA Extraction

DNA was extracted from 50 *Varroa* mite samples, and its concentration and purity were examined using a Nanodrop device (THERMO, USA), which measures concentration (ng/µL) and purity at absorbance (260/280 nm).

Primer Design

Primers specific to the *Varroa* mite were designed based on the report in (9). These primers were manufactured by Scientific Researcher Company Limited - Iraq, as shown in Table 1.

PCR Procedure

PCR reactions were prepared using a mixture of template DNA, primers (forward and reverse), dNTPs, Taq polymerase, and buffer. The reaction was performed in a thermal cycler (Thermal Cycler, USA). The COX1 gene (821 bp) was amplified under standard amplification conditions according to the following program:

Initial activation for 5 minutes at 95°C, cycled at 35°C, denaturation at 95°C for 30 seconds, annealing according to the melting temperature of the primers for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. PCR products were separated by electrophoresis on a 1.5% agarose gel in TBE buffer. 3 µL of ethidium bromide was added, the gel was poured into the designated wells, the comb was attached, and the gel was allowed to solidify. Samples (10 µL of PCR product) were placed in the wells, with a 100bp ladder in the first well. Electrophoresis was performed for 1 hour at 100 V and 80 mA. The products were imaged using an ultraviolet device to document the size and identification of the gene bands.

Table 1. Primers used in the study

Primer name	Sequence (5′–3′)	Product size (bp)
Vd-F	5′-ATG GCA GTT TGG TAA GGT G-3′	458 pb
Vd-R	5′-CCT CTG TTC CAA CCT CAG T-3′	458 pb

Results

Molecular diagnosis of the *Varroa* parasite was performed by extracting mitochondrial DNA from five samples isolated and previously morphologically diagnosed as *Varroa* mites. The concentration and purity of the DNA were measured as previously mentioned and ranged from 5–50 ng/µl, while purity ranged from 1.8–2.5, within the accepted standard limits for DNA concentration and purity, indicating the success of the extraction process. Amplification was performed using PCR targeting the first subunit of mitochondrial cytochrome c oxidase (COX1). All samples were positive, as the targeted diagnostic gene was identified at 821 base pairs, as evidenced by the clear appearance of the expected bands at this molecular weight on agarose gel electrophoresis, as shown in Figure 1. Multiple Sequence Alignment (MSA) results for local *Varroa* sp. isolates. IQD.1–IQD.5 for COX1 gene products showed high genetic similarity with the global strain Varroa destructor, registered in NCBI GenBank under sequence number GQ379069.1 (Figure 2). The genetic similarity percentage reached 99.48%, and the total genetic distance ranged from 0.01% to 0.04%. Substitution mutations were also observed in the nitrogenous base sequence of this gene between local isolates and some other global strains registered in the database, indicating limited genetic variation (Figure 2).

These relationships were confirmed by constructing a phylogenetic tree using the UPGMA method and the Maximum Composite Likelihood algorithm in MEGA 6.0. The tree showed that the local isolates are closely related to the global isolate *V. destructor* (GQ379069.1) and form a single evolutionary branch with slight differences. From the above, the genetic similarity rate and genetic distance indicate that the local isolates of the Varroa parasite in Iraqi apiaries belong genetically to the global strain *Varroa destructor*, as shown in Figure 3 and Table 2, which display the similarity rates between the local isolates and the international reference isolates.

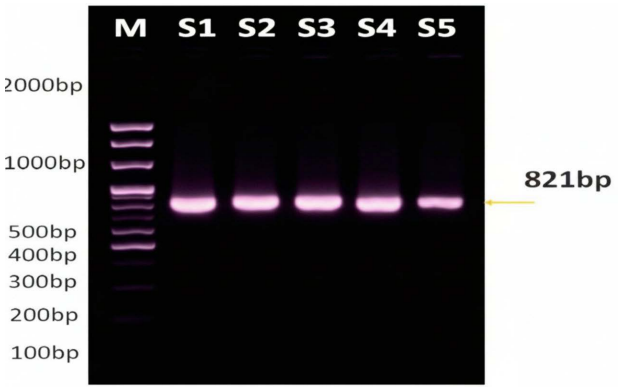

Figure 1. Gel electrophoresis analysis of PCR products targeting the COX1 mitochondrial gene (821 bp) for *Varroa* sp. isolates.

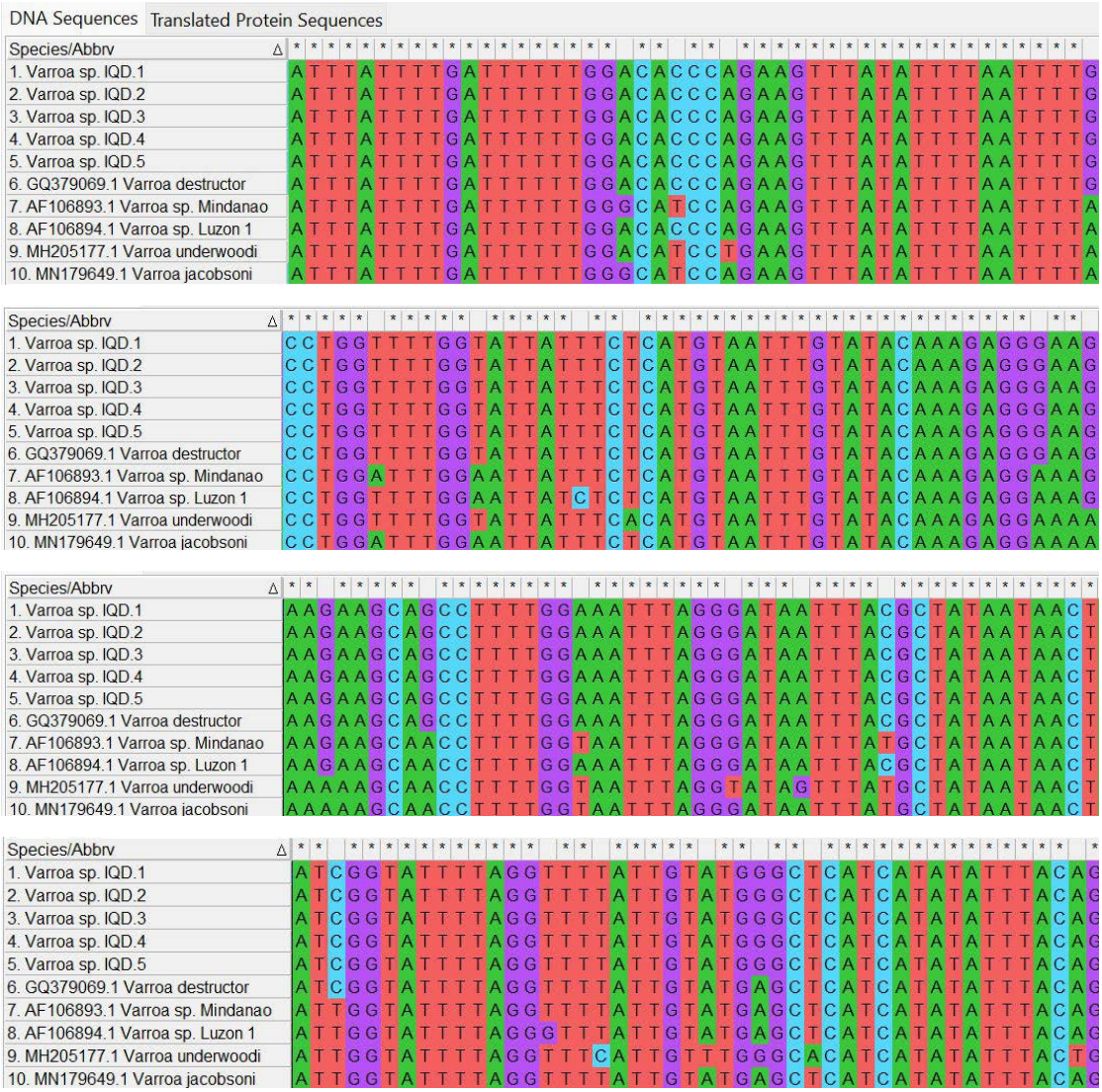

Figure 2. Multiple sequence alignment analysis of the mitochondrial COX1 subunit 1 gene in local *Varroa* isolates IQD and *Varroa* species registered in GenBank International (NCBI). Multiple alignment analysis was performed using the ClustalW alignment tool. This alignment analysis showed similarity in the nitrogenous bases alignment and the presence of substitution mutations in the COX1 gene among the isolates.

Discussion

Results of DNA analysis indicate that the local Varroa isolates in Iraq clearly belong to the global strain, Varroa destructor. This is confirmed by the high similarity percentage (99.48%) and by the presence of the targeted gene at the expected size of 821 base pairs in all samples. The acceptable values for DNA concentration and purity support the reliability of the molecular data obtained and indicate the success of the adopted DNA extraction methods.

The close relationship between the local isolates and the reference strain GQ379069.1 indicates that the parasite prevalent in Iraqi apiaries is the same global parasite known for its destructive impact on *Apis mellifera* colonies worldwide, as confirmed by (10) and (11). However, the occurrence of substitution mutations in the COX1 gene suggests genetic variation that may result from environmental differences, adaptation to local conditions, or the introduction of infected bee colonies from other countries. These results are consistent with those of (12) and (13). The small genetic distance (0.01–0.04%) observed between local isolates and the reference strain indicates high genetic homogeneity. These results are consistent with the findings of (14) and (15), who reported that low mitochondrial diversity in some invasive populations of *V. destructor* may indicate a recent introduction of the parasite followed by rapid expansion. This scenario is likely in Iraq due to the introduction of new bee colonies through international trade, as confirmed by (16) and (17) regarding the role of bee transport in the global spread of the parasite.

The small genetic distances (0.01–0.04%) between local Iraqi isolates and the global reference isolate indicate high genetic similarity among the isolates. These results are consistent with the findings of (13 and 14), who reported that low mitochondrial diversity in some *V. destructor* populations may indicate a recent, rapid expansion of

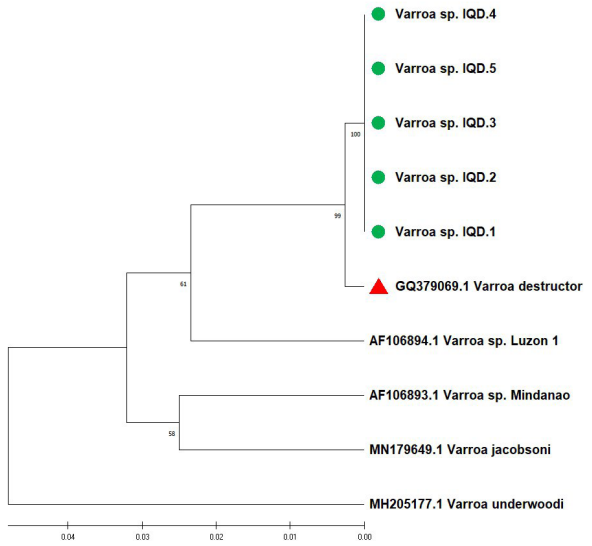

Figure 3. Phylogenetic tree constructed using UPGMA and Maximum Composite Likelihood methods based on COX1 gene sequences of local *Varroa* isolates compared with global reference strains

the parasite. This conclusion is highly likely in Iraq, given the introduction of *Varroa*-infected bee colonies through international trade and the import of various bee species for production. This is consistent with the findings of (15) and (16).

The results of the current study are also consistent with the findings of (17) and (18), which discussed the importance of the COX1 gene as a powerful molecular marker for resolving taxonomic problems in the classification of organisms, including the *Varroa* mite. Furthermore, the use of analysis tools such as ClustalW and MEGA 6.0 is compatible with global applications in molecular studies, as confirmed by (19). These results underscore the importance of genetic characterization of local *V. destructor* isolates to understand their spread dynamics and reduce their negative impact on beekeeping, as indicated by (5) and (20).

Finally, the identification of mutations in the COX1 gene facilitates future research to elucidate the functional implications of these alterations and correlate them with the parasite's capacity to acquire resistance to *Varroa* mite pesticides, posing a significant challenge for the management of this parasite, as emphasized by (21) and (22).

Conclusion

Based on the results of this study, we conclude that the Varroa mite prevalent in Iraq is Varroa destructor, a parasitic pest known for its devastating impact on honeybee (*Apis mellifera*) populations worldwide.

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Table 2. Homology sequence identity between local Varroa isolates and reference strain (GQ379069.1)

Local Isolate	Reference Strain (NCBI accession)	Identity (%)
IQD.No.1	*Varroa destructor* (GQ379069.1)	99.48%
IQD.No.2	*Varroa destructor* (GQ379069.1)	99.48%
IQD.No.3	*Varroa destructor* (GQ379069.1)	99.48%
IQD.No.4	*Varroa destructor* (GQ379069.1)	99.48%
IQD.No.5	*Varroa destructor* (GQ379069.1)	99.48%

Authors' Contribution

Conceptualization: Hadi M. Al-Mayali, Reyam J. Khadim, Abbas K. Hamza.
Data curation: Hadi M. Al-Mayali, Reyam J. Khadim.
Formal analysis: Hadi M. Al-Mayali.
Investigation: Reyam J. Khadim, Abbas K. Hamza.
Methodology: Hadi M. Al-Mayali, Abbas K. Hamza.
Project administration: Hadi M. Al-Mayali, Abbas K. Hamza.
Resources: College of Education, University of Al-Qadisiyah.
Software: Hadi M. Al-Mayali, Reyam J. Khadim, Abbas K. Hamza.
Supervision: Hadi M. Al-Mayali, Abbas K. Hamza.
Validation: Hadi M. Al-Mayali, Reyam J. Khadim.
Visualization: Reyam J. Khadim.
Writing–original draft: Reyam J. Khadim.
Writing–review & editing: Hadi M. Al-Mayali.

Competing Interests

The authors declare that they have no competing interests that could have influenced the outcome of this study.

Consent

Consent was obtained from all beekeepers who participated in this study. Each participant was informed about the objectives and procedures of the investigation, and verbal consent was given before sample collection.

Ethical Approval

This study was approved by the Scientific Committee of the College of Education at the University of Al-Qadisiyah, Iraq. All sampling procedures were performed in accordance with ethical guidelines for research involving animals and the Declaration of Helsinki (1964) and its later amendments. Beekeepers were informed about the study's purpose and assured that all data and locations would remain confidential.

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