



MOLECULAR DIAGNOSIS AND GENETIC DIVERSITY OF THE GREAT WAX MOTH *GALLERIA MELLONELLA* (L)

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ABSTRACT

This study includes molecular identification of greater wax *Galleria mellonella* (L) from Iraq utilizing mitochondrial CO1 gene sequencing. Samples were collected from infested honey bee colonies in six provinces: Babylon, Karbala, Najaf, Wasit, Maysan and Basrah. DNA was extracted and PCR amplified a 700 bp fragments, and all samples were confirmed as *G. mellonella* with high genetic similarity of 98.46-100%. Phylogenetic analysis revealed two genetic clusters: the main cluster included most Iraqi samples, while the Maysan isolate formed a separate group, suggesting possible geographic isolation. The Basrah sample had 100% identity, whereas the Wasit sample had the lowest (98.46%). The sequences were submitted to Gen Bank (LC852680- LC852685).

Key words: *Galleria mellonella*, mtCO1, molecular identification, phylogenetic analysis, Iraq, Babylon, Karbala, Najaf, Wasit, Maysan, Basrah, population variation, genetic diversity

The greater wax moth *Galleria mellonella* (L) is a destructive pest of honey bee colonies, causing significant economic losses globally (Roversi et al, 2008). Its larvae damage hives by consuming wax, producing silk, and disrupting brood chambers (El-Gendy, 2021). In the U.S., annual losses exceed \$8 million (Szabo and Heikel, 1987) while infestation rates in Iraq range from 21.3 to 45.28% (Al-Yasiri, 1977). Effective management requires deeper morphological, physiological, and molecular studies (Ellis and Mortensen, 2013). Traditional identification relies on morphology (Gulati and Kaushik, 2004), but molecular methods like mitochondrial CO1 (mtCO1) gene analysis provide higher resolution for phylogenetic studies (Liu et al., 2016; Yang et al., 2020). Recent research reveals genetic diversity in *G. mellonella* populations, such as distinct strains in India and low diversity in Thailand (Sopaladawan, 2025). Despite its sequenced mitochondrial genome (Lange et al., 2018), regional genetic variations remain understudied (Roh et al, 2020). In Iraq, molecular tools have identified pathogens like *Oscheius tipulae* in *G. mellonella* (Ahmed, 2024), highlighting their importance for pest control. This study provides the details of molecular characterization of *G. mellonella* in central and southern Iraq using mtCO1, establishing genetic baselines for future monitoring and targeted management.

MATERIALS AND METHODS

Samples of *G. mellonella* were collected from infested honey bee colonies across central and southern Iraq, encompassing the governorates: Babylon (N, 32°41'28.8" E" 44°25'01.6"), Karbala (N, 32°38'54.0" E" 43°58'28.9" E"), Najaf (N, 32°00'40.6 E" 44°21'12.3", Wasit (N, 32°33'39.0" E" 45°48'20.9"), Maysan (N, 31°51'02.2" E" 47°06'58.9") and Basrah (N, 30°25'58.6" E 30.4329, 47.8829). Samples were collected from 18 March to 11 April 2023 and placed on infested wax combs containing various developmental stages. These were transported to the Insect Laboratory at the Department of Plant Protection, College of Agriculture, University of Basrah. Representative specimens were submitted to the Natural History Museum in Basrah for taxonomic verification and species identification. Infested wax combs containing *G. mellonella* were maintained in rearing cages (30x 19x 19 cm) with old wax frames under controlled environmental conditions (28 ± 2°C, 70 ± 10% RH). Each cage was labeled with the governorate of origin, apiary name, collection date, and GPS coordinates. Upon adult emergence, five individuals were isolated in 9 cm dia petri dishes for further processing. To minimize potential genetic material contamination from abdominal enzymes, the head, wings, and abdominal sections were carefully removed from each *G. mellonella* specimen. The remaining legs and thoracic

regions, which contain sufficient and less contaminated tissue, were preserved in 2 ml Eppendorf tubes for subsequent molecular analysis.

DNA extraction was performed using the Genaid extraction kit, strictly following the manufacturer's protocol, at the Molecular Genetics Laboratory, Department of Animal Production, College of Agriculture, University of Basrah. Polymerase chain reaction (PCR) amplification of mtCO1 gene was performed using standard PCR protocols with specific primer pairs. The reaction was designed to target a 700 bp fragment. The reaction mixture was prepared with 25 microliters of reaction components which included 12.5 microliters of Master Max and 3 microliters of DNA template and 1 microliter of each primer and complete the mixture to 25 microliters using deionized water (Vrijenhoek, 1994). The amplification was carried out using a thermal cycler according to the conditions, Initial Denaturation stage took 4 min and a temperature of 95 °C, denaturation stage took 30 sec and a temperature of 95 °C, The annealing stage took 45 sec and a temperature of 48 °C, while extension phase took 1 min and the temperature was 72 °C with final extension stage of 7 min at 72 °C.

RESULTS AND DISCUSSION

Electrophoresis of the PCR products confirmed the successful amplification of the target 700 bp COX1 gene fragment from all *G. mellonella* samples. As depicted in Fig. 1, a distinct band corresponding to the expected 700 bp size was observed for each sample on the 1.5% agarose gel, run at 70 V and 65 mA. A 100-3000 bp DNA ladder (from Geneaid) was used to accurately determine the amplicon size. Initial diagnostic results from the Natural History Museum in Basrah confirmed the presence of a single species of greater wax moth, *G. mellonella*, across all collected samples from the studied governorates. To further validate these morphological identifications, a molecular comparison of the extracted

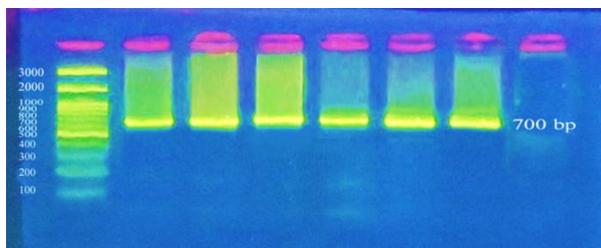


Fig. 1. Agarose gel electrophoresis showing the 700 bp COX1 gene amplicon from *G. mellonella* samples. A 100-3000 bp DNA ladder was used as a molecular weight marker

mtDNA sequences was performed against registered species in the National Center for Biotechnology Information (NCBI) GenBank BLASTn analysis confirmed that all samples exhibited high identity to *G. mellonella* reference sequences. The maximum score (Max Score) for all samples was consistently greater than 965, indicating a strong and significant match with the reference sequences. Furthermore, a 100% query coverage was observed for all samples, signifying that the entire submitted sequence was compared against the database. The E-value, a measure of statistical accuracy, was very low, indicating a negligible probability of the observed matches occurring by chance (Wang et al., 2011).

The estimate of genetic identity with the reference *G. mellonella* species ranged from 98.46% to 100%, unequivocally confirming that all collected samples belong to *G. mellonella* (Fig. 2). Notably, the Basrah sample displayed the highest genetic identity (100%), while the Wasit sample exhibited the lowest (98.46%). This observed variation suggests that the Wasit population might possess greater genetic diversity compared to the other sampled populations. Such genetic differences can be attributed to several factors, including geographic isolation, which can lead to the accumulation of distinct mutations due to restricted gene flow between populations (Branchicela et al., 2017). Environmental pressures, such as variations in temperature or humidity, may also influence reproductive success or survival preferences, thereby contributing to genetic divergence. Despite the presence of less than 100% identity, these minor genetic mutations do not alter the species classification, as identities above 98% are generally considered indicative of belonging to the same species.

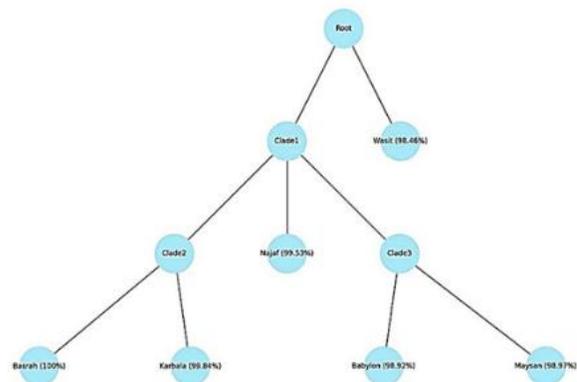


Fig. 2. The genetic tree of *G. mellonella* sample from the provinces of Iraq, drawn based on the similarity percentages (identity%) between the samples.

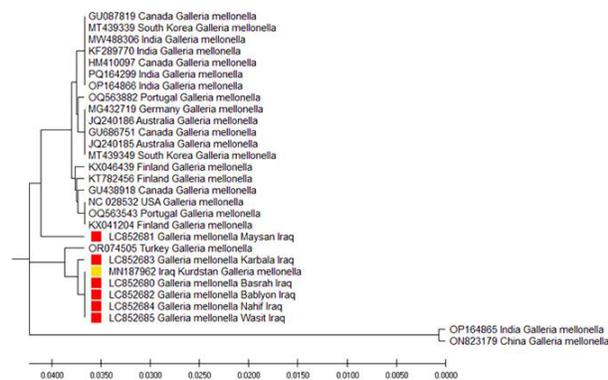


Fig. 3. Evolutionary history of *G. mellonella* samples using the UPGMA method

Phylogenetic analysis of the extracted DNA sequences, as illustrated in Fig. 3, revealed the evolutionary relationships among the *G. mellonella* samples. The analysis showed that the *G. mellonella* populations from the studied regions in Iraq clustered into two main groups. The first cluster exclusively contained the Maysan Governorate sample (LC852681). The second, larger cluster comprised samples from Babylon, Wasit, Basrah, Karbala, and Najaf governorates. This clustering indicates a clear geographical pattern and a high degree of genetic similarity among the populations within the second cluster, suggesting the presence of a homogeneous local strain or recent gene flow among these regions. The genetic distances between samples from the governorates within the second cluster were very small, ranging from 0.0000 to 0.0100, indicating a low mutation rate and significant genetic flow. In contrast, the Maysan Governorate sample (LC852681) showed a notable genetic divergence, separating from the rest of the Iraqi governorates by a distance of 0.0250. This higher genetic distance for the Maysan isolate suggests an emerging differentiation, potentially due to localized selective pressures, a degree of genetic isolation, genetic drift, or the presence of a unique mutation. Such differentiation, if sustained over time and coupled with persistent geographical barriers and environmental factors, could potentially lead to the emergence of a distinct sublineage within the Iraqi *G. mellonella* populations.

This study represents the first molecular characterization of *G. mellonella* populations in Iraq through COX1 gene sequencing. Our findings confirm the widespread presence of *G. mellonella* across six Iraqi provinces, exhibiting 98.46–100% genetic similarity to global strains. Phylogenetic analysis revealed distinct clustering patterns, with the Maysan population (LC852681) forming a separate cluster,

suggesting potential geographic isolation or localized genetic divergence. The newly deposited sequences (LC852680–LC852685) establish a valuable genetic baseline for future studies aimed at understanding *G. mellonella* population dynamics and developing effective pest management strategies in Iraq.

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AUTHOR CONTRIBUTION STATEMENT

R.M.K. and M.A.A. conceived and designed the research. R.M.K. conducted the experiments and performed data collection. M.A.A. supervised the project and provided critical intellectual input. R.M.K. and M.A.A. analyzed the data. R.M.K. wrote the initial draft of the manuscript. M.A.A. reviewed and edited the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

No conflict of interest

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