Molecular Diagnosis of the Cigarette Beetle *Lasioderma serricorne* (F.) in Erbil-Province –Iraq

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Abstract

The cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), is an important pest in the food and tobacco industries in many regions of the world. The present study provides molecular identification of *Lasioderma serricorne* (F.) using samples collected from a variety of small local grocery stores and herbal shops in traditional markets across different localities of Erbil province from June 2024 to August 2025. The partial cytochrome oxidase subunit I (COI) gene sequence obtained from the samples was subjected to BLAST analysis against the NCBI GenBank database to confirm its molecular identity. *Lasioderma serricorne* (PV839858) showed 100% identification and query coverage with the GenBank reference sequence (PV248645).

Keywords: Cytochrome oxidase, Molecular diagnostics, Stored Products insects, Phylogenetic analysis.

1. Introduction:

The Cigarette beetle, Lasioderma serricorne (F.) (Coleoptera: Anobiidae), is regarded as the most significant pest of stored products [5]. The genus Lasioderma includes more than 50 species found worldwide [1]. Taxonomically, L. serricorne belongs to the tribe Lasiodermini, subfamily Xyletininae, within the family Anobiidae [10]. L. serricorne was first described in France in 1792 from specimens collected in America; however, archaeological evidence shows its presence in Egypt, where remains were discovered in the tomb of Tutankhamun, indicating its long-standing connection with stored products [23]. The cigarette beetle, L. serricorne, is considered one of the most serious economic insect pests of stored grain products [18]. herbarium specimens, foodstuffs, stored tobacco, cocoa, ground chili, paprika, cayenne pepper, and spices [16]. as well as turmeric [20]. In addition, this insect infests and damages fenugreek, coriander, cumin, fennel, celery, carom, and dried red chili [6]. It is one of the most common insects damaging stored products, including dried and processed animal products [4.[

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Larvae are primarily responsible for damage, feeding internally within stored products, reducing their commercial value through weight loss, contamination with frass, cast skins, pupal cases, and dead insects, and, in severe cases, rendering products unfit for consumption [8]. Infestation levels are influenced by environmental factors [21]. In addition to direct consumption losses, *L. serricorne* adults are classified as "true penetrators" of packaging materials, thereby further contaminating processed goods [3].

Beyond economic losses, *L. serricorne* has also been associated with public health risks. Cases of intestinal canthariasis, an illness caused by ingesting contaminated food with beetle larvae or adults, have been reported in China and Malaysia. Symptoms include gastrointestinal disturbances, ocular irritation, and general malaise [17].

The taxonomic study revealed significant morphological variation within the species, which complicates the identification of insects, especially stored pests [26]. A novel approach, DNA barcoding, has the potential to ease the challenges of identifying insect pests [25].

In recent years, DNA-based methods such as PCR have become important for analyzing genetically modified organisms (GMOs) and identifying pest species, including insects. These methods provide an excellent means for diagnosing and identifying all stages of insect life cycles, from immature to mature forms, and even for detecting latent species [7]. A short fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene is widely used as a barcode sequence for animals. It is also frequently employed in applied entomology [13]. The COI gene is chosen for barcoding because it is the largest of the three mitochondrial genes encoding cytochrome oxidase subunits and features a high rate of insertions and deletions. Its high nucleotide variability helps in identifying cryptic species. Additionally, universal COI primers are highly durable. Mitochondrial gene sequences have been used in the phylogenetic and population-genetic studies to reconstruct the evolutionary history of related insect species [24]. Species-specific primers have already been successfully used to identify various storage pests [29]. Numerous studies have developed molecular methods to identify arthropod species that commonly infest stored products [2]. Some of these methods are specifically designed to detect a particular species in the grain samples and to quantify infestation levels [19].

Varadínová, et. al., [28] established a new rapid and accurate cytochrome c oxidase subunit-based system for molecular identification of five common stored-product *Cryptolestes* species. Five species-specific primer pairs for a traditional Multiplex polymerase chain reaction assay are described. Devi, et. al., [9] evaluated the utility of mitochondrial cytochrome oxidase I sequences for quick and accurate species diagnosis of the two Indian populations of the rice weevil *Sitophilus oryzae* (L.) and *S. zeamais* (Motschulsky) (Coleoptera: Curculionidae). The phylogenetic analyses revealed. In Erbil province, Khdir et. al. [14] designed specific primers for each of the three insects on dried fruits.; red flour beetle (*Tribolium castaneum* Herbst), khapra beetle (*Trogoderma granarium* Everts), and sawtoothed grain beetle (*Oryzaephilus surinamensis* (L.) with the use of the PCR technique. Aslam, et.al., [2] identified three stored grain insect pests, *Sitophilus oryzae*, *Callosobruchus chinensis,* and *Oryzaephilus surinamensis* based on mitochondrial cytochrome C oxidase subunit I (COI) gene sequences

Solà, et.al., [27] developed a multiplex PCR method to simultaneously detect and identify the five most common species of internally developing pests in stored grains. In contrast, Pava-Ripoll, et.al., [22] developed singleplex and multiplex PCR methods to detect some insect contaminants in food with three sets of group-specific primers. However, even if these

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DNA-based methods can detect all life stages, they use specific primers, which limit the detection of all arthropod pest species potentially present in stored cereals in a single PCR reaction. In Iraq, Hamdan, and Kareem [11] detection the early infestation of insect pests that infect stored grains by using the Multiplex-PCR (Polymerase Chain Reaction) technique. The technique included two experiments conducted using specific primers for each species. In the first experiment, an internally infected species, *Rhyzopertha dominica*, and two externally infected species, *Tribolium castaneum* and *Oryzaephilus mercator*, were identified in a wheat grain sample. In the second experiment, an internally infected species, *Trogoderma granarium*, and an externally infected species, *Latheticus oryzae*, were identified in a red bean sample.

However, in the Kurdistan region of Iraq, no comprehensive research has been conducted on the molecular diagnosis of cigarette beetles; therefore, this study aims to survey, characterize, and identify cigarette beetles in stored products by creating a DNA barcode dataset. The main objectives of this work are to confirm previous morphological identifications using molecular methods for stored-grain insect pests, thereby developing more effective management strategies and supporting further research.

Methodology:

Survey and Sample Collection:

This investigation relied on more than 50 specimens representing the insect's adult stages. The specimens were acquired from a variety of small local grocery stores and herbal shops located in traditional markets and shopping centers within the Erbil Governorate from June 2024 to August 2025. Adult specimens of *L. serricorne* were collected from infested store spices, turmeric *Curcuma longa* (Fig.1a) belongs to the family Zingiberaceae, fenugreek, *Trigonella foenum-graecum* (Fig.1b), which belongs to the family Fabaceae, as well as homemade mixing species utilized for flavoring pizza and culinary dishes (Fig. 1c). For further analysis, all experimental procedures were conducted in the Entomology Laboratory of the Plant Protection Department at Salahaddin University in Erbil's College of Agricultural Engineering Sciences. All samples were

Fig. 1: A. Infested Fenugreek, *Trigonella foenum-graecum*, **B.** Turmeric: *Curcuma longa*, **C.** Homemade mixing specie
(Photo: Chnar M.Hawla, 2024, Plant Protection Department, College of Agricultural -Engineering Sciences, Salahaddin University)

maintained at approximately 30 ± 2 °C and 60 ± 2% relative humidity prior to molecular processing.

Molecular identification

DNA Extraction and Amplification

Fifteen preserved adult individuals were selected, and approximately 20-30 mg of tissue from each insect was separated and placed individually in a small 1.5 ml centrifuge tube for DNA extraction. The genomic DNA was extracted using the ZYMO Quick-DNA Tissue/Insect Microprep Kit (No. D6015, USA), following the manufacturer's recommendations. The quality and quantity of DNA yields were assessed using a NanoDrop spectrophotometer (Thermo Scientific, UK). The extracted DNA was stored at −20°C for later downstream applications. The partial sequences of the cytochrome c oxidase subunit I (COI) gene were amplified from each extracted DNA. The polymerase chain reaction (PCR) was performed in a 50 µl reaction mixture. The mixture contained 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S, Stenhuggervej 22), 10 picomoles (pmol.) of the forward primer (C1-J-1718: 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′) 10 picomoles (pmol) of the reverse primer (HCO2198;5′TAAACTTCAGGGTGACCAAAAAATCA-3′) DNase-free water, and template DNA were used with the Bioresearch PTC-200 Gradient thermocycler (Table 1). The amplification program consisted of an initial denaturation for 5 min at 95 °C, then 30 cycles of denaturation for 1 min at 95 °C, primer annealing for 1 min at 50 °C, and extension for 1 min at 72 °C. The final step included an additional extension at 5 min for 72 °C [12]. The PCR products were stored at -20 °C for later electrophoresis and subsequent analysis. The selected primers generated a 550 bp band of the PCR products. Amplified DNA was run on a 1.5 % agarose gel for electrophoresis, after which the samples were stored at -20 °C for subsequent analysis .

Table 1: COI PCR Amplification Reagents

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	20 Pmol	3
3	Reverse Primer	20 Pmol	3
4	DNase free Water	-	15
5	Template DNA	50ng/µl	4
Total			50

Agarose Gel Electrophoresis Preparation and Visualization of DNA Fragments

The amplified COI gene products were run on a 1.5% agarose gel for confirmation. This included preparing agarose gels,

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electrophoresis of PCR products, and visualization of DNA fragments. For agarose gel preparation, 1.5 grams of agarose was dissolved in 100 me of 1X TBE, heated until clear, and cooled to 37–45°C. After adding 5–10 µL ethidium bromide to 50 µL of melted agarose, the agarose solution was poured into the casting tray and placed in the electrophoresis chamber. After being loaded 5 µl of DNA samples mixed with 2–3 µl of 6X loading buffer into the wells, the DNA ladder (1Kbp) was added to a separate well, and the products were run at a voltage of 50–100 V. The PCR products were visualized using an ultraviolet transilluminator (Biostep-UST-20M-8K) and photographed using a digital camera (Canon, Japan).

Sequencing of DNA

The PCR products of the partial COI gene were sequenced using the ABI Prism Terminator Sequencing Kit (Applied Biosystems) at the Microgene Center in Korea. Chromatograms of the COI gene were edited, and base calls were checked using the FinchTV program. The sequences were aligned, edited, and compared with previously deposited sequences in the National Center for Biotechnology Information (NCBI).

Sequence Alignment and Submission

The cytochrome oxidase I (COI) gene sequence was analyzed using the Basic Local Alignment Search Tool (BLAST), which employs sequence alignment methods (https://blast.ncbi.nlm.nih.gov/Blast.cgi), available on the National Center for Biotechnology Information (NCBI) website.

Results and Discussion

Genomic DNA isolated

The 1% agarose gel image (Fig. 2) confirms successful extraction of high-quality genomic DNA from five insect specimens. The genomic DNA from all five specimens (Lanes 1–5) shows a distinct, sharp band. The absence of smearing in all lanes indicates minimal DNA degradation and sample purity. This purity is essential for the efficiency of subsequent enzymatic reactions. The negative control (NC) lane shows no band, confirming the absence of contamination during extraction. Specifically, Lane 4 corresponds to the cigarette beetle, *L. serricorne*, which exhibits a clear, strong DNA band, similar in intensity and position to those of the other samples. This demonstrates the effectiveness of the extraction procedure for obtaining pure, amplifiable genomic DNA from *L. serricorne.* The quality of the DNA from this sample ensured reliable PCR amplification of the COI

gene fragment, which later yielded 100% sequence identity with *L. serricorne* reference sequences in GenBank.

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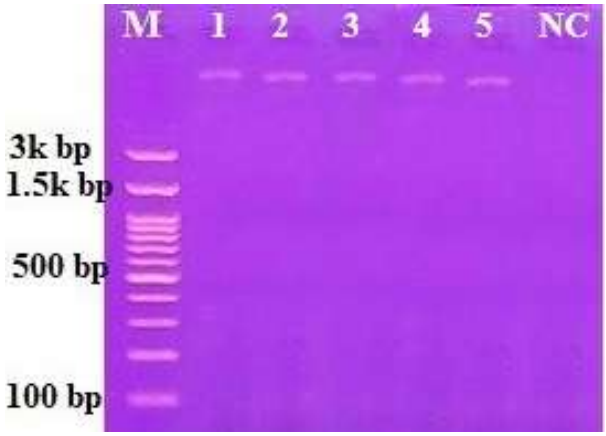

Fig. 2: Genomic DNA isolated from *L. serricorne, Callosobruchus maculatus. Carpophilus obsoletus, Sitophilus oryzae*, and *Plodia interpunctella,*

The gel image shows successful, specific amplification of the target COI gene fragment (Fig. 3). A distinct, single, crisp band of the expected size, approximately 550 bp, is visible in each of the five sample lanes (Lanes 1-5). The PCR reaction is efficient, and the template DNA from the specimen is of sufficient quality for amplification. The absence of smearing or non-specific products in each lane indicates that the primers annealed specifically to the target COI gene region. This high specificity is crucial for obtaining clean sequences downstream. The band aligns with the 550 bp marker on the DNA ladder, providing empirical confirmation of the amplicon size and validating the in silico primer design. The absence of a band in the Negative Control (NC) lane confirms that the PCR reagents were not contaminated with extraneous DNA or amplicons from previous reactions, ensuring that the bands in Lanes 1-5 are genuine products from the intended insect DNA templates.

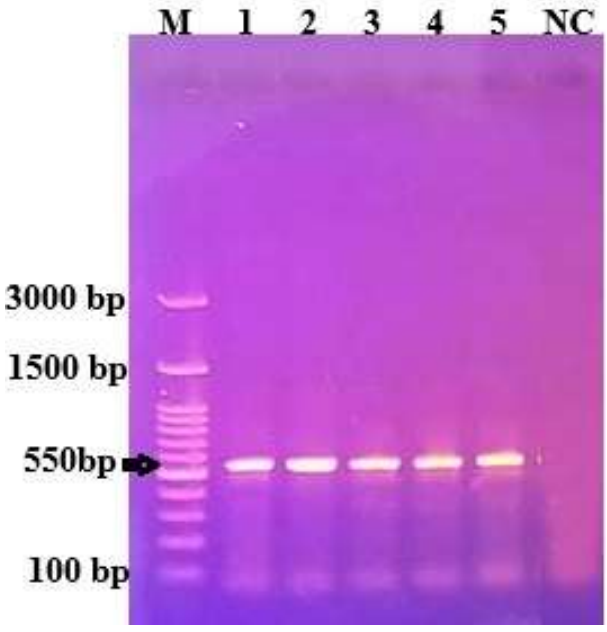

Fig. 3: PCR amplification of partial cytochrome C Oxidase I gene from insects. M; indicate: ladder (3000 bp-100 bp), lane 1- 5: 550 bp of PCR products of from insects and NC is negative control.

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Sequence alignment and submission of the gene to GenBank

DNA sequencing was performed separately on an ABI 3130X genetic analyzer (Applied Biosystems) using only the forward primer C1-J-1718 (GGAGGATTTGGAAATTGATTAGTTCC) and the reverse primer HCO 2198 (TAAACTTCAGGGTGACCAAAAAATCA) . The sequence appears as a clean, repeated string (TACCCCTTTTACTCTTTTTATTTTCCAG AGTATTGTAGAAATGGGCAGGAACT), suggesting a high-quality chromatogram. In a real chromatogram, this would be represented by sharp, single-peak signals for each nucleotide, indicating a lack of ambiguity in base-calling and the absence of mixed signals that would suggest contamination or heteroplasmy [11] .

Gene sequence data were checked for quality using BioEdit v.7.0.5 software. Homology, insertions, deletions, stop codons, and frameshifts were evaluated with NCBI-BLAST. The sequences were compared and aligned with other biological sequences to identify similarities and nucleotide variations. BankIt, a web-based submission tool with wizards to guide the process, was used. The GenBank database was intended for new sequence data that was determined and annotated by the submitter. sequence was uploaded to GenBank. The BLAST results confirmed that the query sequence showed high similarity to insect identification records in the NCBI Genbank (Table 2).

Table 2: Sequences of the partial COI gene in NCBI and aligned with the same sequence after submissions.

Isect samples Accession Number	Query Covers %	Identic Number %	Genbank Accession Number	Genbank Identification
PV839858	100	100	MG458968	*Lasioderma sericorne*
	100	100	MW728042	
	100	100	OM698838	
	100	100	KJ680546	
	100	100	PV248645	

Phylogenetic inferences:

A phylogenetic tree (Fig. 4) is a hypothesis of evolutionary relationships among a set of stored-product insects. Branches indicate ancestry, and nodes (junction points) represent common ancestors. The closer two species are on the tree, the more recently they diverged from a common ancestor. The tree was constructed using the Maximum Likelihood method in MEGA11 under the Tamura-Nei model, with 100 bootstrap replicates. Partial DNA sequences of the concatenated COI mitochondrial gene were used as input. The

tree shows that *L. serricorne* forms a strongly supported monophyletic group (bootstrap = 100%). The *L. serricorne* clade is the closest relative of *Callosobruchus maculatus*. This relationship has strong bootstrap support (96%), indicating a close evolutionary connection between these two beetle pests. Although *Lasioderma* belongs to the family Anobiidae (subfamily Ptininae) and *Callosobruchus* to Chrysomelidae (subfamily Bruchinae), their phylogenetic proximity may reflect the conservation of mitochondrial markers. Similar patterns have been reported in stored-product Coleoptera phylogenies. Other insect pests, such as *Carpophilus obsoletus, Sitophilus oryzae*, and *Plodia interpunctella*, formed distinct, well-supported clades, highlighting their genetic divergence from *L. serricorne*. *Sitophilus oryzae* (family Curculionidae) grouped independently with moderate support (bootstrap = 75%), while *Plodia interpunctella* (family Pyralidae) was resolved in separate lineages. In summary, the phylogenetic placement of *Lasioderma sericorne* confirms its genetic uniqueness and indicates a closer evolutionary connection with *Callosobruchus maculatus*. These findings improve understanding of the molecular relationships among economically important stored-product pests. Yang et al. [31] constructed a phylogenetic tree of *L. serricorne* using 13 PCGs and 14 other beetles. They showed that *L. serricorne* is closely related to *Stegobium paniceum*, which agrees with the conventional taxonomy of both stored pests.

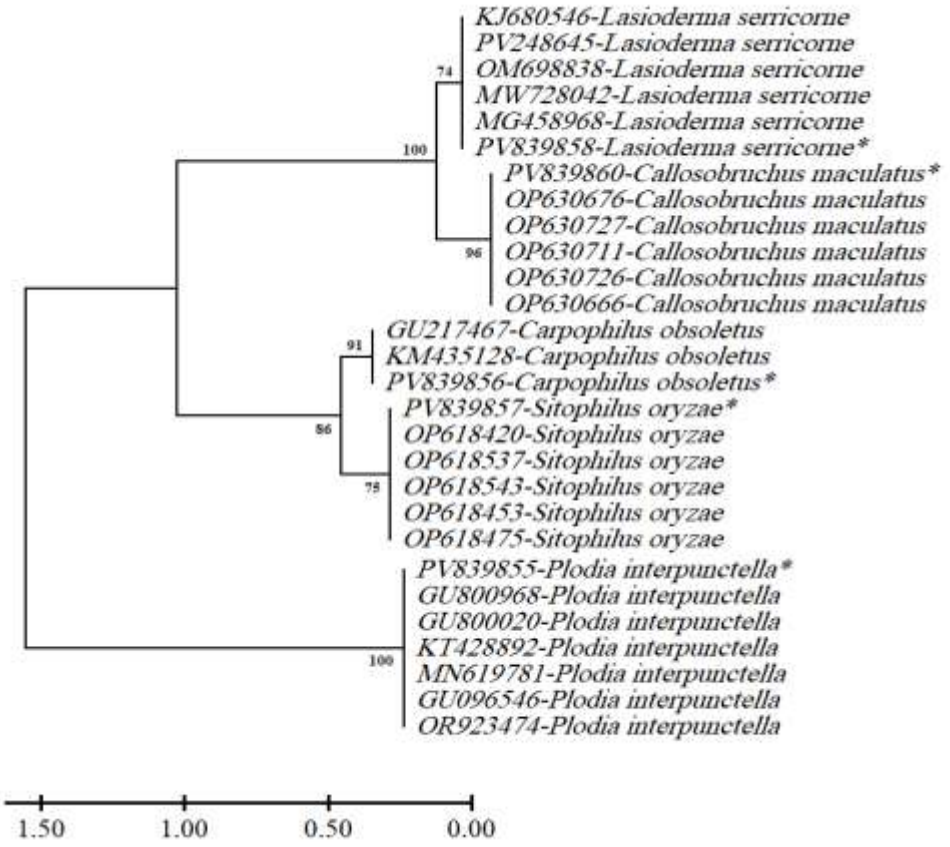

Fig. 4: Phylogenetic tree of five stored insect species in Erbil Province, Iraq

Conclusion

The molecular identification of *L. serricorne* aligns seamlessly with morphological observations, which include its distinctive brown, oval body and serrated antennae. These findings confirm that combining morphological examination with DNA barcoding is a reliable method for identifying stored-product beetle species. This barcode can be used to quickly and precisely identify this species in future infestations, even from fragmented specimens or early life stages where morphological identification is difficult. The BLAST analysis conclusively verifies that the insect specimen with GenBank accession PV839858 is the Cigarette Beetle, *L. serricorne*. This molecular identification offers strong, definitive confirmation, essential for accurate pest status assessment, biosecurity measures, and future phylogenetic studies. Regarding other species, it is noteworthy that the *L. serricorne* sample achieved a perfect 100% match, while some other species listed (e.g., Plodia interpunctella) showed matches ranging from 99.75% to 99.77%. This slight variation (often 1-2 nucleotides) likely reflects normal genetic diversity within a species. The consistent 100% match for *L. serricorne* highlights a high level of sequence consistency in the sampled population for this particular gene fragment.

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