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Morphological and molecular identification of grape leaf worm larvae *Hippotion celerio* L.(Lepidoptera: Sphingidae)

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ABSTRACT

This study was conducted to identify the grapevine leafworm, both phenotypically using morphological characteristics of the larvae and molecularly using PCR techniques on larvae isolated from grape orchards in Al-Alam District, Salah al-Din Governorate, Iraq. The study revealed that the collected larvae were *Hippotion celerio*, based on morphological characteristics observed by the researcher in the laboratory. The mitochondrial cytochrome oxidase (COX I) gene was used for molecular identification.. The resulting amplification base pairs identified the insect as *Hippotion celerio*, with a 99.81% similarity to the sample identified in the United States. The record is registered under the global number KP720072.1. While collecting larvae from orchards, the researcher noticed that some larvae had been preyed upon by the red wasp *Vespa orientalis* L. Predation of the insect larvae by adult red wasps *Vespa orientalis* L. was observed with remarkable intensity. To date, no scientific sources have reported any association between these species, making this a new record of predation on grapevine leafworm larvae.

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التحديد المورفولوجي والجزيئي ليرقات دودة ورق العنب *Hippotion celerio* L.

عواد جاسم
قسم وقاية النبات ، كلية الزراعة ، جامعة تكريت ، العراق

الخلاصة
أجريت هذه الدراسة للتعرف على دودة ورق العنب ، سواء من الناحية الظاهرية باستخدام الخصائص المورفولوجية لليرقات أو جزيئيا باستخدام تقنيات تفاعل البوليميراز المتسلسل على اليرقات المعزولة من بساتين العنب في قضاء العلم ، محافظة صلاح الدين ، العراق. وكشفت الدراسة أن اليرقات التي تم جمعها كانت *Hippotion celerio* ، استنادا إلى الخصائص المورفولوجية التي لاحظها الباحث في المختبر. تم استخدام جين أوكسيديز السيتوكروم الميتوكوندريا (COX I) للتعرف الجزيئي.. حددت أزواج قاعدة التضخيم الناتجة الحشرة على أنها *Hippotion celerio* ، مع تشابه بنسبة 99.81 ٪ مع العينة المحددة في الولايات المتحدة. يتم تسجيل السجل تحت الرقم العالمي KP720072.1. أثناء جمع اليرقات من البساتين ، لاحظنا أن بعض اليرقات قد افترسها الزنبور الأحمر فيسبا أورينتاليس ل.لوحظ افتراس يرقات الحشرات بواسطة الدبابير الحمراء البالغة فيسبا أورينتاليس ل. بكثافة ملحوظة. حتى الآن ، لم تبلغ أي مصادر علمية عن أي ارتباط بين هذه الأنواع ، مما يجعل هذا رقما قياسيا جديدا للافتراس على يرقات دودة أوراق العنب.

INTRODUCTION

The grapevine leafworm, *Hippotion celerio* L., belongs to the order Lepidoptera and the family Sphingidae. Several common names, such as the silver-striped hawkmoth or taro moth, are widely recognized. This insect undergoes complete metamorphosis, progressing through egg, larval, pupal, and adult stages. A distinct caudal horn characterizes the larval stage. At the same time, adults possess slender forewings, comparatively shorter hindwings, streamlined bodies, rapid flight, and an elongated proboscis that enables them to feed on nectar-bearing flowers. As nocturnal pollinators, members of the Sphingidae play a crucial ecological role by contributing to the reproduction of a variety of tropical plants. Suelo *et al.* (2023).

Although *H. celerio* supports ecosystem balance as both a pollinator and herbivore, its larvae occasionally act as destructive pests. They have been documented feeding on a range of host plants, including *Caladium, Colocasia, Amaranthus, Mirabilis jalapa, lettuce,* and several root crops such as sugar beet *(Beta vulgaris)* and sweet potato *(Ipomoea batatas).* (Stoeckel& Kelber. 2019) Severe infestations can cause complete defoliation, leaving only the leaf veins, thus affecting crop vigor and yield. Grape leaves are among the preferred hosts, and heavy larval feeding may result in significant vineyard damage. Despite this potential, population outbreaks remain infrequent due to natural mortality factors such as climatic extremes and predation. Jeenkoed *et al.* (2016).

Given the dual importance of *H. celerio* as both a pollinator of ecological significance and an occasional agricultural pest, accurate species identification is essential. Morphological examination alone may sometimes be insufficient because of similarities with related species. Therefore, molecular techniques, particularly those targeting the mitochondrial cytochrome oxidase subunit I (COI) gene, have been increasingly employed to provide reliable and precise species-level identification (Kareem, *et al.,* 2020). This study was conducted to diagnose *H. celerio* larvae collected from grape orchards in Salah al-Din Province, Iraq, using both morphological features and molecular characterization through PCR-based methods.

MATERIAL AND METHODS

Location and Date of Specimen Collection.

Larvae of the target insect were collected on 15 October 2023 at 10:30 a.m. from vineyards located in Al-Alam District, Salah al-Din Province, Iraq, under partially cloudy conditions with an ambient temperature of 29 °C. Morphological identification was first performed at the Department of Plant Protection, College of Agriculture, Tikrit University, using larval taxonomic keys including the Proleg key, Chaetotaxy (setal pattern) key, and Head capsule morphology key. For molecular characterization, the specimens were subsequently transferred to the laboratories of Al-Dawliya Company, Baghdad.

DNA Extraction

Genomic DNA was obtained from larval tissue using a commercial extraction kit (Intron Biotechnology, G-spin DNA Extraction Kit, Cat. No. 17045). The manufacturer's protocol was followed, which involves enzymatic digestion with Proteinase K and RNase A, followed by purification through silica column binding and elution in buffer. The procedure ensured removal of protein and RNA contaminants, yielding DNA suitable for downstream analysis (Park *et al* 2017) .

Agarose Gel Electrophoresis

The quality and integrity of the extracted DNA were assessed by agarose gel electrophoresis. A 1% gel prepared in TBE buffer was stained with RedSafe dye, and electrophoresis was performed at a constant voltage 80-100 V. until the DNA fragments were clearly resolved (Sharp *et al*1973).

PCR Amplification

The mitochondrial COI gene was chosen as the molecular marker for species identification. Universal primers described by Folmer et al. (1994) were used:

- Forward primer: 5'-GGTCAACAAATCATAAAGATATTG-3'

- Reverse primer: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

PCR reactions were carried out in 20 µl volumes using Maxime PCR PreMix (i-Taq, Cat. No. 25025). Each reaction contained 1 µL of each primer, 1.5 µL of DNA template, 5 µL of PreMix, and nuclease-free water to bring the volume to 10 µL. Amplification was performed with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 58 °C for 45 s, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 7 min.

Gel Verification of PCR Products

Amplified fragments were visualized on 2% agarose gels in TBE buffer at 5 V/cm for approximately 90 min. A 100 bp DNA ladder was used to confirm the expected fragment size. (~720 bp) Sambrook, & Russell, (2001).

DNA Sequencing and Analysis

Successful PCR products were purified and submitted to a commercial sequencing facility (Pioneer, South Korea). The resulting nucleotide sequences were compared to reference sequences in the NCBI GenBank database using BLAST analysis (Camacho *et al* 2009). Species-level confirmation was considered valid when sequence similarity exceeded 99% with registered *Hippotion celerio* entries.

RESULTS AND DISCUSSION

Phenotypic diagnosis

The morphological diagnosis of the larvae revealed that they belong to the insect Hippotion celerio L. and were diagnosed by Professor Dr. Mohammed Shaker Mansour, Professor of Economic Entomology and Assistant.Prof.Dr. Safaa Zakaria Baker , in the Department of Plant Protection, College of Agriculture, Tikrit University, identified the species through its clear morphological characteristics and reliance on taxonomic keys. These results are This result is in agreement with the findings reported by the researcher Suelo *et al*. 2023 reported when studying the insect's developmental stages, providing a detailed description of each stage. Larvae of the family Sphingidae can be easily recognized by the presence of a caudal horn or button and by 6-8 annulets on each body segments (Pittaway & Kitching, 2020).

Molecular diagnosis

Sequences of nucleotide sequences of the product of the gene polymerase chain reaction (cytochrome oxidase sub unit 1 –in the mitochondria) of insects were determined to diagnose the product of the PCR reaction

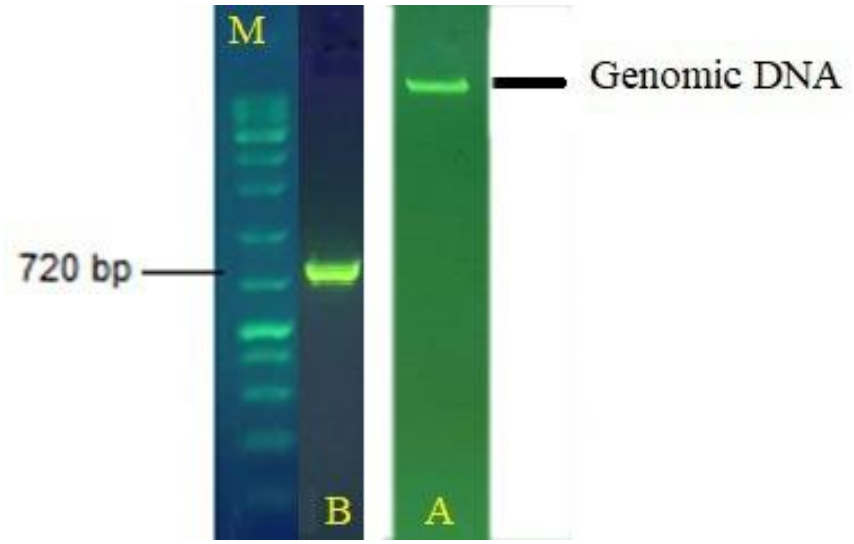

Figure 1. (A) Electrophoretic profile of genomic DNA isolated from Hippotion celerio. The DNA was separated on a 1% agarose gel at a voltage of 5 V/cm for approximately 75 minutes. (B) Amplified PCR fragment showing a band of ~720 bp, resolved on a 2% agarose gel using 1× TBE buffer under 5 V/cm for 90 minutes. Lane(M): 100 bp DNA ladder.

Determination of nucleotide sequences and diagnostics to the species level

The sequences obtained from the Korean company Pioneer were analyzed using the global website of the National Center for Biotechnology Information (NCBI) within the Blast sub-window, The secondary Nucleotide blast sub-window was selected, the alignment and matching process was performed with the strains registered in the global gene bank, and then the Iraqi isolation was recorded in the Genebank under the world number according to (Table1) Gaduaa, A. A., & Kareem, A. A. (2023).

Table (1) Molecular diagnostics of an insect based on the percentage correspondence of cytochrome c oxidase subunit I (COX1) gene sequences with the highest matching insect registered in the world genetic bank

The world registration number of the insect diagnosed in this study	The insect strain diagnosed in this study	Similarity ratio %	Region	Accession number	The species and strain of the insect most closely match
OP776813.1	*Hippotion celerio* isolate Aw.J-2	99.81	USA	KP720072.1	*Hippotion celerio* voucher Hippo_1

Final diagnosis:

Hippotion celerio

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Bombycoidea; Sphingidae; Macroglossinae; Macroglossini; Hippotion.

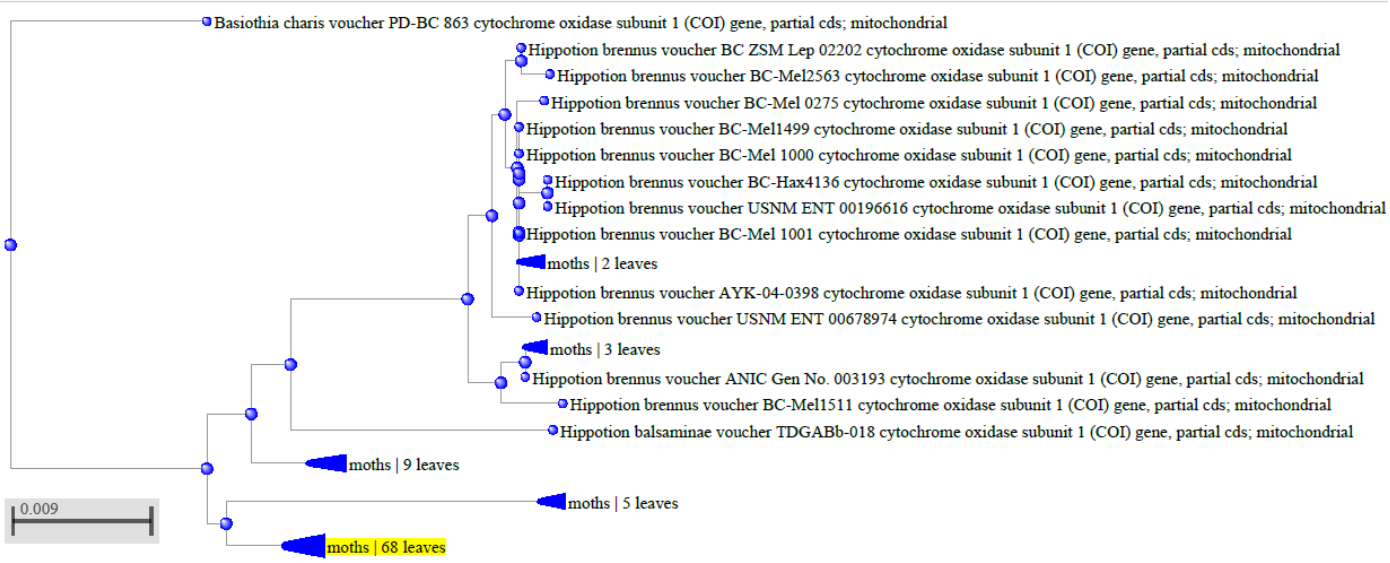

Figure (2) Genetic tree of Hippotion celerio and its relationship to some species recorded on the NCBI site

Some field views of the pest

During the larval survey, the study revealed that these caterpillars feed on grape leaves in vineyards. and also repeatedly watched a red wasp *Vespa orientalis* L., attacking some of the larvae; Figures (3) illustrate this behavior, which is believed to be the first report of its kind. To

investigate further, throughout the manuscript, avoid presenting the result in the first person pronoun collected ten larvae and placed them near a beehive. Although red wasps are regarded as honeybee pests and are well-documented as such, the wasps ignored the bees and began to attack the larvae. Within about 45 minutes, it had consumed them all. This suggests that the wasp prefers larvae over bees, perhaps because the caterpillars contain higher levels of protein and fat and lack the defensive mechanisms that bees possess.

Figure (3) Predation of larvae by the red wasp *Vespa orientalis* L.

No references have reported or documented predation by the red wasp on these larvae; however, some studies have indicated that certain wasps show a preference for leaf-feeding caterpillar's ، - Prezoto *et al.* (2019) reported in a study conducted in Brazil that it is necessary to search for control alternatives in order to reduce the environmental impact caused by insecticides. This review presents a description of the use of social wasps as agents of biological control, focusing on the perspectives of their use in small farms and urban gardens, and to discuss the benefits of using this method. Studies have shown that 90–95% of the prey captured by wasps in small crops is made of leaf-eating caterpillars.

CONCLUSION

We concluded from this study that Molecular diagnostic approaches facilitate the identification of insect species by employing techniques such as using polymerase chain reaction. The results revealed that the collected larvae matched the species *Hypothion celerio*, with a genetic similarity of 99.81% to the recorded specimens from the United States. The study highlights the ecological importance of this insect, as it acts both as a pollinator and as a pest affecting agricultural crops, reflecting its balance of benefits and harms in the ecosystem.

REFERENCES

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. BMC Bioinformatics, 10, 421. https://doi.org/10.1186/1471-2105-10-421

Carroll, S. B., & Greider, C. W. (1984). DNA extraction techniques.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3(5), 294–299.

Gaduaa, A. A., & Kareem, A. A. (2023). Molecular identification of peach fruit fly *Bactrocera zonata* and its symbionts in Karbala Province, Iraq. IOP Conference Series: Earth and Environmental Science, 1214, 012045. DOI:10.1088/1755-1315/1214/1/012045

Jeenkoed, R., Bumroongsook, S., & Tigvattananont, S. (2016). Biology and Host Plants of Hippotion celerio (Lepidoptera: Sphingidae). International Journal of Agricultural Technology,12(7.2),2089-2094.

Park, S. H., Lee, J. H., & Lee, H. S. (2017). Genetic diversity analysis of insect populations using mitochondrial DNA markers. *Journal of Asia-Pacific Entomology, 20*(3), 789–795. https://doi.org/10.1016/j.aspen.2017.05.002

Pittaway, A. R., & Kitching, I. J. (2020). *Sphingidae of the Western Palaearctic*. Retrieved from https://tpittaway.tripod.com

Prezoto, F., Maciel, T. T., Detoni, M., Mayorquin, A. Z., & Barbosa, B. C. (2019). Pest control potential of social wasps in small farms and urban gardens. *Insects, 10*(9), 276. https://doi.org/10.3390/insects10090276.

Sambrook , J. , Et al. (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press.

Sharp, P. A., Sugden, B., & Sambrook, J. (1973). Detection of two restriction endonuclease activities in Haemophilus parainfluenzae using agarose gel electrophoresis. Biochemistry, 12(16), 3055–3063.

Stoeckel, E. L., & Kelber, E. (2019). The visual environment of nocturnal moths. Vision research, 155, 1-10.

Suelo, M. S., Dela Cruz, R. Y., Luceño, A. J. M., Lituañas, C. R. M., Toledo, J. M. S., Viernes, R. M. P., & Mohagan, A. B. (2023). Metamorphosis of silver-striped hawkmoth (*Hippotion celerio* L.) (Lepidoptera: Sphingidae) collected and reared in Bukidnon, Philippines. *Species*, 24(73), 285–293. https://discoveryjournals.org/Species/current_issue/2023/v24/n73/e4s1004.pdf.