Molecular identification and characterization of gall wasp, *Diastrophus mayri*, and its impacts on oak trees in Erbil province, Iraq

Nawzad B. Kadir [1]

[1] **Department of Plant Protection, College of Agricultural Engineering Sciences, Salahaddin University-Erbil/Iraq**

***Corresponding author e-mail: nawzad.kadir@su.edu.krd**

https://doi.org/10.59658/jkas.v12i4.5165

Received: Aug. 25, 2025

Accepted: Nov. 02, 2025

Published: Dec. 25, 2025

Abstract

The identification of insect pests has become importance as assess the biological impacts of global climate change and try to protect species diversity in the face of accelerating habitat destruction. This study focuses on the morphometric and molecular identification of the gall wasp *Diastrophus mayri* (Hymenoptera: Cynipidae) on oak trees in Erbil Province, Iraq. Twenty oak stem galls were collected from November 2024 to April 2025. Genomic DNA was extracted from adult wasps, and a partial cytochrome c oxidase subunit I (COI) gene (~500 bp) was amplified by using PCR with universal primers. Sequencing and BLAST analysis confirmed 100% identity of the sampled wasps with *D. mayri* (Accession Number: DQ012639), distinguishing them from related species like *D. turgidus* (92.63% identity) and *D. rubi* (91.94%). Phylogenetic analysis using maximum likelihood methods further validated the species identification. This work represents the first molecular confirmation of *D. mayri* (Accession Number: PV299164) in Iraq's Kurdistan region and underscores the efficiency of DNA barcoding for accurate cynipid identification, overcoming limitations of morphological methods.

Keywords: Cynipidae, *Diastrophus mayri*, Gall wasps, Molecular identification, Oak trees.

Introduction

Cynipidae is part of the superfamily Cynipoidea within the Hymenopteran order, which encompasses around three thousand identified species [1]. Since nearly all cynipoids are parasitoids except for members of the Cynipidae family and a group of gall-dwelling inquiline genera within the Figitidae, it is most likely that cynipid gall wasps evolved from parasitoid ancestors [1,2]. Cynipid gall wasps (Hymenoptera: Cynipidae) are responsible for producing a number of the most visually impressive and structurally intricate plant galls found globally.

The majority of the approximately 1,400 identified species within the Cynipidae family are known to induce galls. However, approximately 180 species categorized into nine genera, exist as inquilines within the galls created by other cynipid species [3,4].

In addition to some morphological characteristics, this categorization also considers variations in biology and host plant relationships. All 185 species of cynipid gall wasps

are members of the Synergini tribe and are known as cynipid inquilines. They are phytophagous and can induce the formation of nutritive tissues within galls, but they don't appear to be able to create their own galls from scratch [5]. One of the largest families of gall-forming insects, cynipid gall wasps are found throughout Europe, North Africa, and Asia Minor, with over 1400 species [3,6].

This group of hymenopteran insects is almost completely phytophagous, with their larvae developing within plant galls. They are divided into eight tribes and involve both gall inducers, which cause the abnormal plant growths to form, and inquilines, which stay in galls formed by other species [4]. According to [1], six of these tribes were previously proposed: the "Aylacini," Diplolepidini, Eschatocerini, Pediaspidini, Synergini, and Cynipini. Woody rosid gallers, herb gallers, and inquilines are the three clades into which the Cynipidae may be separated according to host plant associations, as suggested by [7].

According to [8], the genus *Diastrophus* Hartig, 1840 (tribe Aylacini) currently includes 18 species, a count also supported by [9].

No one has identified *Diastrophus mayri* on oak trees in Iraq, particularly in Erbil, using molecular techniques, nor have any authors documented the target gall wasp on oak trees. Thus, the primary objectives of this work are to register the gall wasp in Erbil City's Gene Reference (NCBI) and to record *D. mayri* on oak trees identifying it using mitochondrial DNA.

Materials and Methods

Sampling

A survey was conducted to collect stem galls on oak trees in various oak forests near Erbil province, Iraq. The oak tree twigs with galls were cut using a special cutter tool (garden scissor) and brought to the laboratory, then twenty galls were randomly selected for measurements (length and width of galls) by using a Vernier caliper. After this, the galls were cut to describe the internal side morphologically and collect wasp larvae; The cut galls containing larvae were placed inside a plastic container for adult emergence. For molecular identification, a total number of 100 samples of wasps were collected from twenty oak stem galls in Erbil between November 2024 and April 2025 for sequencing. The galls were stored at room temperature in plastic bags following sampling. Gall tissue was dissected to remove the insect material, allowing for future DNA extraction. Adults that erupted from the same gall were included in a single tube sample. Additional adult DNA extractions were carried out.

Molecular study

Insect species have been identified and diagnosed utilizing molecular techniques. For the molecular identification of various organisms, including insects, the Cytochrome c oxidase I (COI) gene is commonly used as a conserved region in molecular biology. PCR techniques were employed to amplify the COI gene of the stem gall wasp, *D. mayri*, utilizing a universal primer [10].

1- Extraction of DNA

The Genomic DNA was isolated from adult individuals of the stem gall wasps, *D. mayri*. Each specimen was extracted using the ZYMO Quick DNA Tissue/Insect Microprep Kit, manufactured in the USA. D6015 follows manufacture's protocol [11]. Then, isolated DNA was visualized in 1.5% agarose gel.

Polymerase Chain Reaction (PCR) Amplification Cytochrome Oxidase c subunit I (COI):

The partial Cytochrome Oxidase c subunit I (COI) gene was amplified via Polymerase Chain Reaction (PCR) using a 50 µl reaction volume. This mixture contained 2x Taq DNA Polymerase Master Mix (supplied by AMPLIQON A/S, Stenhuggervej 22), 10 pmol each of the forward primer C1-J-1718 (sequence: GGAGGATTTGGAAATTGATTAGTTCC) and the reverse primer C1-J-1718 (sequence: ACTGTAAATATATGATGAGCTCA) [12], DNase-free water, and the DNA template, as listed in Table 1. PCR was conducted using a Bioresearch PTC-200 Gradient thermocycler.

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

The temperature profile consists of several steps: the first step involves an initial denaturation at 95°C for 5 minutes. This is followed by 35 cycles, which include a denaturation step at 95°C for 40 seconds, a primer annealing step at 60°C for 40 seconds, and an extension stap at 72°C for 1 minute. The final step includes an additional extension at 72°C for 10 minutes.

2- Visualization of DNA fragments

An intercalating dye, Ethidium bromide, is incorporated into 1.5% melted agarose gel within 1X TAE buffer after 30 minutes in the electric field of electrophoresis. The positions of the bands are identified by observing the gel under a UV trans-illuminator.

3- Sequencing of DNA

The amplified COI gene fragments were sequenced using the ABI Prism Terminator Sequencing Kit (Applied Biosystems) at the Microgene Center, Korea. Resulting chromatograms were processed and base sequences were confirmed with the Finch TV software.

4- Submitting aligned sequences

The COI gene sequence was used in the Basic Local Alignment Search Tool (BLAST), a search tool that employs the sequence alignment method (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This tool is accessible on the NCBI (National

Center for Biotechnology Information) website, allowing for the comparison and alignment of laboratory or query sequences with other biological sequences for identifying the similarities with other targets.

IBM SPSS software was used to conduct a statistical data analysis, version 25.0 [13]. Descriptive statistics were calculated for the major parameters, with each locality analyzed independently. Statistical significance ($p<0.05$) among various levels was analyzed using analysis of variance (ANOVA). A Completely Randomized Design (CRD) was used to estimate significant effects, and the Duncan test was used to determine variation between treatment means. Excel software was used to calculate the correlation analysis and draw a straight-line equator for different values.

Results and Discussion

Nature and impact of gall insects on the trees:

This wasp insect belongs to the Hymenopteran order and the family Cynipidae; its scientific name is *D. mayri* (Figure 1-A). The larvae are legless and cream-white in color arched to some extent after reaching the full growth, they are remaining in their own specific chamber under the bark of the tree twigs or stems especially young twigs until pupation to overwinter and then the adults emerge when the weather condition is suitable from the emergence holes which are visible on the galls (Figure 2-b). The galls appeared elongated and swollen stem galls (Figure1-b), the gall causes a swelling along the stem as a reaction to larval activity. Gall length and width (Table 2) varied from one to another, they ranging from 4 to 11 cm. They averaged 7.8 ± 0.67, the length and width ranged from 0.6 to 1.5 cm and averaged 0.99 ± 0.08 cm, with a warty surface. Each gall consists of multiple chamber (Figure 2-A) and is formed within a woody tissue, and becomes corky as the gall matures. It was noticed that there was only one larva in each chamber inside the stem gall.

Figure (1): Adult of gall wasp (*D. mayri*) (10x) and galls induced by it

Figure (2): Cross section of a gall and emergence holes of adult gall wasp

Table 2: Measurements of stem gall induced by the gall wasp on oak tree twigs

Particulars of the stem gall	Diameters of stem gall centimetres		
	Min.	Max.	Mean± SE
Length of gall	4	11	7.8 ± 0.67
Width of gall	0.6	1.5	0.99 ± 0.08

The results of this study regarding the characteristics of the larvae of the gall wasp, *D. mayri* agree with those of [14], who described the larvae as cream-white, and legless, developing to maturity within galls where they remain in a specific chamber until pupation and overwintering.

The outcomes of this study on the structure of galls induced by gall wasps are consistent with those of [15], who reported that the gall wasp on plant stems are abnormal growths caused by the insect's larvae, typically showing as elongated, swollen, and sometimes knotty formations.

The findings of this study on the length and width of stem gall induced by the gall wasp are in disagreement with those of [16], who showed that stem galls caused by the gall wasp, *D. mayri* are irregular, spindle-shaped swellings on the stem of the plant, measuring (1–3 cm) in length.

Molecular identification

Partial COI gene amplification by PCR

Gene-specific primers for the mitochondrion were developed utilizing the sequences of cytochrome c oxidase subunit I, which were synthesized by Micro-gene Company in South Korea. These primers are capable of producing a band approximately 500 bp in size. The PCR product underwent electrophoresis and was visualized using a 1.5% agarose gel (Figure 3).

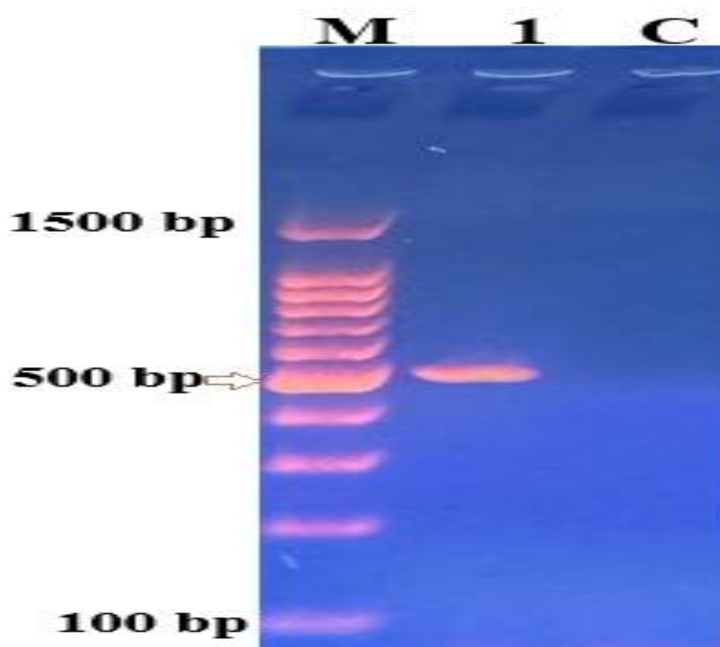

Figure (3): PCR amplification of the partial cytochrome C Oxidase I gene from insects. M: indicate: ladder (1500-100bp), lane 1: 500bp of PCR products from insects and C is the negative control.

COI Gene partial (Cytochrome c Oxidase Subunit I)

DNA sequencing was conducted utilizing solely the forward primer C1-J-1718, executed independently by the ABI 3130X genetic analyzer (Applied Bio-system). The PCR products from the twenty-five specimens were utilized as DNA templates for targeted sequence-specific PCR amplification.

Molecular Identification of Genus and Species of Insect

COI sequence samples of the stem gall wasp, *D. mayri*, with a size of 500bp, were analyzed using the program BLAST from GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to contrast our amplified sequences with other wasp species sequences maintained in the database. The findings of BLAST analysis revealed that the query sequence showed the highest similarity to the first record in the NCBI GeneBank for insect identification. These findings suggest the submission of our query sequences to the NCBI GenBank, and corresponding accession numbers as provided below (Table 3).

Table (3): Partial COX Subunit I Gene Sequences in NCBI and aligned with identical Sequences after Submission

Specimen Identified	Accession Numbers	Query Cover %	Identic Number %	Accession No. of BLAST Identification	Compression with other species of GenBank
Diastrophus mayri	PV299164	100	100	DQ012639	*Diastrophus mayri*
		100	92.63	AY368913	*Diastrophus turgidus*
		100	91.94	DQ012640	*Diastrophus rubi*

		100	91.01	AY368914	*Diastrophus potentillae*
		81	90.60	KR408439	*Diastrophus*

Phylogenetic inferences

Phylogenetic analysis utilizing the COX1 nucleotide sequence demonstrated the predicted categorization of the four sample species under investigation. The data on sequence divergence and the designed phylogeny indicated that the sample within their corresponding genera was closely related to one another (Figure 4).

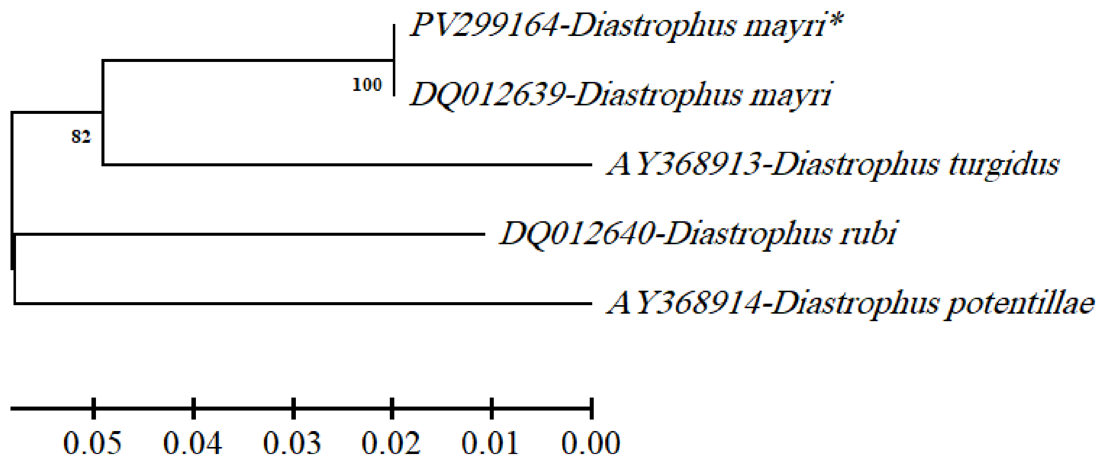

Figure (4): phylogenetic tree of insect species samples collected from the Kurdistan region of Iraq. This phylogenetic tree was developed using the maximum likelihood method, following the Tamura-Nei model, within the MEGA11 software, and included bootstrap analysis with 100 resampling's. The input data comprised partial DNA sequences of the concatenated partial COI mitochondrial gene.

Globally, an estimated 25.169 species of Hymenopteran have been barcoded yet between 150.000 described species [17]. In addition, [18] recently performed a study on DNA barcoding to distinguish agriculturally significant insect pests. Different predators, parasitoids, and other pest species were collected from various areas across the country for this purpose. Characteristics essential to insect pests, for example their vast diversity, role in biological control and the economic & epidemiological importance of some groups, have positioned them as the primary target of DNA barcoding studies. This standardized database serves as a valuable resource for studies in agriculture, ecology, phylogeny, taxonomy and the conservation of different organism groups [19]. Numerous studies and several contributions using mitochondrial COI for molecular identification have been effective in and useful in the detection of cryptic insect species.

The stem gall wasp, *D. mayri*, is a remarkably diverse group of insects, according to molecular diagnostics of wasp items. Many introduced pest species, including some that are commercially significant and may be advantageous in some places, like forest trees, were eaten by stem gall wasps.

Molecular analysis of gut contents is increasingly being used for identifying various invertebrate species [20,21,22]. This approach is particularly valuable for uncovering data that are difficult or time-intensive to obtain through traditional methods like

rearing or gut dissection, such as determining the host range of parasitoids [20,21] or identifying vertebrate hosts of ticks [22].

Additionally, visual identification methods often fail to reflect the diversity and abundance of insect taxa accurately; molecular approaches provide a substantial advantage over morphology-based identification for wasps [23].

Identifications based on morphology are less accurate than the method shown here. The genus and species were determined in this investigation. This contrasts with identifying the same wasp species just by morphology [24]. However, the correctness and accessibility of sequences in molecular databases are crucial for molecular technique identifications.

Using molecular identifications also has the important benefit of allowing for future taxonomic identifications of sequences (e.g., adding lower levels of taxonomic information) or identifications to be modified. Morphological identifications cannot do this, especially as samples are frequently thrown away at the conclusion of the study. As a result, molecular identifications offer a continuous and perhaps cooperative resource (e.g., [17]) that can significantly enhance our understanding of a given species or dive rsity at particular sites.

These findings show that our query sequences were submitted to NCBI GenBank, where we received the accession number PV299164. It is widely recognized that insect pest identification can be done using different approaches, including both morphological and molecular techniques. This research used both methods for the accurate identification of the insect samples. It began with a morphometric analysis of the adult body parts, involving measurements of different anatomical features of the entire bodies of adult specimens.

This study successfully identified the stem gall wasp, *D. mayri* on oak trees in Erbil Province through molecular techniques targeting the COI gene. The results confirmed the presence of *D. mayri* with 100% sequence identity to known reference sequences, supported by phylogenetic analysis. Compared to morphological methods, molecular identification proved more precise and reliable, especially in distinguishing closely related species. The findings emphasize the utility of DNA barcoding in entomological taxonomy and biodiversity assessment, especially for cryptic or morphologically similar species. Moreover, these findings underscore the importance of molecular tools in monitoring forest pests and contribute critical data for the biodiversity inventory of gall wasps in Iraq and Western Asia.

References

1) Ronquist, F. (1999). Phylogeny, classification and evolution of the Cynipoidea. *Zoologica scripta*, 28(1-2), 139-164.
2) Ronquist, F. (1994). Evolution of parasitism among closely related species: phylogenetic relationships and the origin of inquilinism in gall wasps (Hymenoptera, Cynipidae). *Evolution*, 48(2), 241-266.
3) CSóKA, G.; Stone, G. and Melika, G. (2005). Biology, ecology, and evolution of gall-inducing Cynipidae.

4) Nieves-Aldrey, J. L.; Rodríguez, P. A. and Medianero, E. (2013). Description of a new species of *Diastrophus* (Hymenoptera: Cynipidae:"Aylacini") from Colombia: the first herb gall wasp native to the Neotropical region. *Annals of the Entomological Society of America*, 106(6), 719-728.
5) Brooks, S. E. and Shorthouse, J. D. (1998). Developmental morphology of stem galls of *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and those modified by the inquiline *Periclistus pirata* (Hymenoptera: Cynipidae) on *Rosa blanda* (Rosaceae). *Canadian Journal of Botany*, 76(3), 365-381.
6) Melika, G. and Abrahamson, W. G. (2000). Review of the cynipid gall wasps of the genus loxaulus Mayr (Hymenoptera: Cynipidae) with descriptions of new species. *Proceedings of the Entomological Society of Washington*, 102(1), 198-211.
7) Liljeblad, J. and Ronquist, F. (1998). A phylogenetic analysis of higher-level gall wasp relationships (Hymenoptera: Cynipidae). *Systematic Entomology*, 23(3), 229-252.
8) Melika, G. and Klymenko, S. (2005). New species of aylacine gall wasps (Hymenoptera, Cynipidae, Aylacini) from Ukraine. *Vestnik zoologii*, 39(2), 23-30.
9) Schick, K.; Zhiwei, L. and Goldstein, P. (2003). Phylogeny, historical biogeography, and macroevolution of host use among *Diastrophus* gall wasps (Hymenoptera: Cynipidae). *Proceedings of the Entomological Society of Washington*, 105(3), 715-732.
10) Mozhaitseva, K. (2023). *Genetic basis of gall formation and modes of reproduction in gall wasps (Hymenoptera, Cynipidae).* Université Paris-Saclay.
11) Wei, S.; Levy, B.; Hoffman, N.; Cujar, C.; Rodney-Sandy, R.; Wapner, R.; D'Alton, M. and Williams, Z. (2020). A rapid and simple bead-bashing-based method for genomic DNA extraction from mammalian tissue. *Biotechniques*, 68(5), 240-244.
12) Barcenas, N. M., Unruh, T. R., and Neven, L. G. (2005). DNA diagnostics to identify internal feeders (Lepidoptera: Tortricidae) of pome fruits of quarantine importance. Journal of Economic Entomology, 98(2), 299-306.
13) Corp, I.B.M., (2017). IBM SPSS Stastisticsfor Macintosh, Version 25.0.
14) Gordinier, H. (2003). Biology of *Diastrophus nebulosus* (Hymenoptera: Cynipidae) and its parasitoid/inquiline complex in galls on Rubus flagellaris (Rosaceae). *The Great Lakes Entomologist*, 36, 5.
15) Glasgow, B. (2020). Gall Inducing and Gall Associated Arthropods and Plant Hosts in Great Smoky Mountains National Park. *Journal of the North Carolina Academy of Science*, 136, 21-31.
16) Nastasi, L.F. and Davis, C.K., (2022). Field guide to the herb and bramble gall wasps of North America.
17) Alex Smith, M.; Fernández-Triana, J.; Eveleigh, E.; Gómez, J.; Guclu, C.; Hallwachs, W.; Hebert, P. D.; Hrcek, J.; Huber, J. and Janzen, D. (2013). DNA barcoding and the taxonomy of M icrogastrinae wasps (H ymenoptera, B raconidae): impacts after 8 years and nearly 20 000 sequences. *Molecular Ecology Resources,* 13(2), 168-176.

18) Jalali, S.K., Ojha, R., Venkatesan, T., and Chakravarthy, A.K. (2015). DNA Barcoding for Identification of Agriculturally Important Insects. New Horizons in Insect Science: Towards Sustainable Pest Management: Springer, India, pp. 13-23.

19) Jinbo U., Kato T., & Ito M. (2011). Current progress in DNA barcoding and future implications for entomology. Entomological Science, 14, 107–124.

20) Rougerie, R.; Smith, M. A.; Fernandez-Triana, J.; Lopez-Vaamonde, C.; Ratnasingham, S. and Hebert, P. D. (2011). Molecular analysis of parasitoid linkages (MAPL): gut contents of adult parasitoid wasps reveal larval host. *Molecular Ecology,* 20(1), 179-186.

21) Derocles, S. A.; Plantegenest, M.; Simon, J. C.; Taberlet, P. and Le Ralec, A. (2012). A universal method for the detection and identification of Aphidiinae parasitoids within their aphid hosts. *Molecular Ecology Resources,* 12(4), 634-645.

22) Gariepy, T.; Lindsay, R.; Ogden, N. and Gregory, T. (2012). Identifying the last supper: utility of the DNA barcode library for bloodmeal identification in ticks. *Molecular ecology resources*, 12(4), 646-652.

23) Kasper, M. L.; Reeson, A. F.; Cooper, S. J.; Perry, K. D. and Austin, A. D. (2004). Assessment of prey overlap between a native (Polistes humilis) and an introduced (*Vespula germanica*) social wasp using morphology and phylogenetic analyses of 16S rDNA. *Molecular Ecology*, 13(7), 2037-2048.

24) Clapperton, B. K. (1999). Abundance of wasps and prey consumption of paper wasps (Hymenoptera, Vespidae: Polistinae) in Northland, New Zealand. *New Zealand Journal of Ecology*, 23(1), 11-19.