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Morphological description and Molecular identification of species Phyllotreta striolata (Coleoptera: Chrysomelidae: Alticinae) in Erbil, Iraq

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ABSTRACT

The detail description of the genus Phyllotreta striolata (Coleoptera: Chrysomelidae: Alticinae) and a Molecules have been identified in the Erbil city. Samples were gathered by using an Arial insect net to collect from the Capparis spinosa plant during period from 1/October/2024 till 22/ November/2024. The important characters of species are, mandibles apical parts consist of four tentacles, maxillary palp consists of four segments. The Antennae pale brown- brown consist of 11th antennomers, 1st-5th antennomer pale brown, 5th antennomer 2 times as long as the 6th antennomer, swollen,11th antennomer oval elongated. The elytra dark brown, middle part pale yellow. Speculum gaster Y-shaped. Aedeagus pale brown, tubular-shaped. Pictures of the key parts were included. They show where each sample was found, what plants they were on, and the date they were collected.

In order to create and differentiate genetic evolutionary tree, the molecular test showed a 550 base pair band from the mitochondrial gene. Cytochrome c oxidase I, which was amplified using PCR from Phyllotreta striolata. In order to compare the sequenced nucleotides with those of other Phyllotreta species, the Cytochrome c oxidase I gene sequences of the insect species were aligned. The search was performed within the National Center for Biotechnology Information's GenBank database using tools based on the Basic Local Alignment Search Tool algorithm.

P. striolata sequences were acquired using the mitochondrial Cytochrome c oxidase I gene, as shown in the result illustration. P. striolata's sequence Cytochrome c oxidase I was uploaded to GenBank under accession PV 123233.

***Keywords*:** Morphological, Molecular, Phyllotreta striolata, Chrysomelidae.

INTRODUCTION

Phyllotreta Chevrolat, 1836, is a large genus of flea beetles in the family Chrysomelidae. It belongs to the group Chevrolat, Coleoptera, Chrysomelidae. This genus includes about 155 species found in the Palearctic region. There are also more than 250 species spread across the world. [1,2]. Members of this group are herbivores that mainly eat plants from the Brassicaceae family. They also feed on related plant groups like Resedaceae, Cleomaceae, Limnanthaceae, Capparaceae, and Tropaeolaceae. [3]. Most species in this genus are known as pests that damage crops. P. striolata (Fabricius) is one of the serious pests directly and indirectly both immature and matures eat the leaves of the host plant and create tiny, spherical, or uneven openings in the leaf surface and indirectly transmitted diseases The insect is multivoltine, and the larvae of the species are typically root feeders cited by [3]. [4] revised the Palearctic species of Phyllotreta. [5,6] categorized Phyllotreta species into two primary categories: those with yellow or reddish patterns on the top side, those with elytra that are completely or nearly yellow, and those with a top side that is uniformly black or black with metallic reflections. These authors also divided species with a uniform black top side into two groups based on whether the center of the frons is punctured [4].

The presence of molecular primers capable of identifying alterations in the base sequence of species specific DNA, like mt-DNA genes, facilitates the effective genetic variation of specific species. MT- CO1 is fully function with maintained of introns in organisms, facilitating the development of primers applicable across many groups and the

sequences of resultant DNA sequences used in population genetics and evolutionary relationship analyses [7,8]. The invention of Polymerase Chain Reaction primers that are able to amplify the CO1 barcode region from a wide variety of organisms has made it possible for this region to be utilized a great deal for the purpose of species discrimination, including the identification of arthropods [8]. The aims of this study detail description of this species; as well as, molecular identification.

Materials And Method

First: Sample collection

In this study more than 70 specimens of adult stages P. striolata male (40) and female (30) were collected from various locations in Erbil province Kurdistan Region- Iraq (Qushtapa, Grdarasha, Sami-Abdulrahman park, Iskan, and Hnara) from Capparis spinose plant during period from 1/October/2024 and 22/ November/2024.The specimens were collected by hand picking.

Second: Specimens examination and Dissection

The morphology of adults' specimens have been examined by using dissecting binocular microscope (Human Scope Sterer/Human/ Germant), The tiny pieces were examined by the preparation of microscopic slides, were hydrated and relaxed by placing them in middle hole of small square of cork pieces and floating them inside a beaker 100 ml with 50 ml water and covered by suitable size petri dish and warmed to the boiling temperature for 5 minutes to soften their parts depending on the size specimens and to avoid them from crashing.

For slides that were used in microscopic inspections, using two disposable insulin syringes, the adults were dissected with fine pins (NO, 0.01) and small forceps, then the required parts (head, thorax and abdomen) were separated and soaked in a beaker 50 ml contained 20 ml of KOH 5% solution and placed on hotplate with shaking for 3-5 minutes (the duration depending on the size of specimens) for dissolving lipids from the body and destroy the muscles, after that water was used to wash the item for 2 to 5 minutes to lessen the impact of alkali. While, head and abdomen were placed in glass petri dish containing an amount of ethyl alcohol 25% and dissected beneath microscope to attain selected parts. Then the parts moved to a series of concentration of ethyl alcohol %50, %75, %96 and %100 accordingly for two minutes for dehydration of aqueous, then they placed in Xylol for 2 minutes to obtain translucency, then placed on glass slides with a drop of DPX and covered by cover slides [9,10,11]. Then the placed on hotplate at 50 ^{0}C for 5 minutes and transferred to the plastic plat under dry atmosphere for 24 hours to prepare for microscope examination.

Third: Molecular identification

The molecular procedural study involves the following steps are [12]:

1. Extraction of DNA:

Adult subjects were used to isolate mtDNA, and each material was extracted using a ZYMO Quick-DNA Tissue/Insect Microprep Kit manufactured in the United States of America followed by [13].

2. Amplification of Cytochrome Oxidase c subunit I using Polymerase Chain Reaction:

PCR amplification for COI partial gene was done in 50 µl of mixture of reactions that includes 2x Taq DNA Polymerase Master Mixes (AMPLIQON A/S Stenhuggervej 22) 20 Picomol (pmol) of forward primer C1-J-1718 (GGAGGA TTTGGAAATTGATTAGTTCC), 20 pmol reverse primer HCO2198 (TAAACTTCAGGGTGACCAAAAAAT), (Simon et al.,1994). Table 1 shows template DNA and DNase-free water using a Bioresearch PTC-200 Gradient thermocycler. beginning denaturation at 95°C for 5 minutes is step one of the temperature profile. Step two is followed by 35 denaturing cycles at 95°C for 40 seconds, beginning annealing at 60°C for 40 seconds, an extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes [14].

Table (1): COI PCR Amplification Reagents

No.	PCR constituents	Concentration	Volume (µl)
1	Master Mix	2x	25
2	F Primer	10 Pmol	3
3	R Primer	10 Pmol	3
4	DNase free Water	-	15
5	Genetic material	50ng/µl	4
	Total		50

3. Visualizations of DNA fragment

1.5% melted agarose gel in 1X TAE buffer is treated with an intercalating dye of ethidium bromide inside the electrophoresis electric field After 30 minutes. DNA band were visualized by UV illumination on a UV transilluminator, photographs were taken with a digital photographic camera.

4. Sequencing DNA Testing

Polymerase Chan Reaction produced partial gene Cytochrome c oxidase I The ABI Prism Terminators Sequence Kit was used to sequence the samples. (Applied Biosystems) at the Micro-Gene Centers in Korea, utilizing Finch TV software for programming. Base calls were confirmed, and the Cytochrome c oxidase I gene chromatograms were examined.

5. Sequence submission and Alignment

In order to find high similarity with other targets, the Cytochrome C Oxidase subunite I gene sequences were used in the Basic Local Alignment Search Tool, a search engine that employs alignment techniques (https: //blatncbinlmnihgov/Blastcgi) and is available at the National Biotechnology Information Centers (NCBI).

Results And Discussion

First: Morphological Description of adult P. striolata (Fabricius)

Body: Oval elongate, yellow-brown, length 1.8-2.2 mm, width 0.6-0.9 mm, [9] indicated that the body is 1.8-2.7 mm long and 1.1-1.4 mm wide. Head: spherical, brown, length 0.2-0.4 mm, surface with low moderate puncture, eyes prominent, brown, lateral ommatida smaller than the middle, length 0.1-0.2 mm, which have three ocelli. Antenna pale brown- brown color, length 1.0-1.6 mm, consist of eleven antennomers, 7^{th} – 11^{th} antennomer brown, 5^{th} antennomer 2 times as long as the 6^{th} antennomer, swollen, 5^{th} antennomer of female cylindrical shaped, 11^{th} antennomer oval elongated, apical tapered. [9] studied that the Antennomer 5 of male antenna strongly swollen and longest; relative lengths of antennomers 3-11 about 1.0: 1.0: 1.5: 0.9: 1.0: 1.2: 1.1: 1.1: 1.4; female antenna similar but antennomer 5 slender. Mouth parts: pale brown-brown, labrum semi-rounded, pale brown- brown, length 0.1-0.15 mm, anterior part low concave. Mandible brown, length 0.1-0.25 mm, sclerotized, apical part which have four tentacles, inner tentacle smaller than the outer, molar area with low short brown setae. Maxilla pale brown-brown, length 0.2-0.25 mm, distal lacinia nearly tubular, maxillary palp consist of four maxillary palps, First segment of maxillary palp almost rectangular, second cup-shaped, 1.2 times longer than first, fourth oval-elongated, times longer than third

, lateral and apical bears short-long brown setae. Labium pale brown, length 0.3-0.35 mm, prementum nearly spherical, labial palp consist of three segments, 3^{rd} segment of labial palp oval elongated, 1.2 times longer the 2^{nd} segment.

Thorax: Pale brown-brown color, nearly trapezoid shaped, low convex, length 0.5-0.7 mm, surface with moderate dense puncture and low short brown setae, anterior margin low concave, posterior margin semi-rounded, anterior part rounded, posterior part acute. Procoxal cavity open, anterior margin of prosternal straight, Prosternal process acute at the middle. Scutellum dark brown, semi-rounded. Elytra dark brown, elongated oval, middle part pale yellow, length 1.5- 1.7 mm, lateral margin dark brown and with one straight puncture, surface with high density of fine puncture and high density short brown setae. Hind wing pale brown. [9] point out that the elytrons have a yellow stripe with a little notch next to the shoulder hump and a deep outside notch in the center. occasionally two positions. The central black stripe has parallel edges and is merely narrowed at both ends.

Legs pale brown-brown, fore and middle femur cylindrical, hind femur oval expanded, length 0.8-0.9 mm. tibia tubular ¼ of apical expanded, lateral bears high long brown spines, apical bears single brown spurs, tarsus consist of four segments, The first segment has simple, hook-shaped claws and is 2.5 times longer than the second.

Abdomen: Brown, oval elongated, abdomen in dorsal view visible of 7^{th} tergite, anterior part of 6^{th} tergite straight, posterior part emarginated at the middle. abdomen in ventral view visible 8^{th} sternites, anterior part of 7^{th} sternite slightly straight posterior part low concave. speculum gaster pale brown, Y-shaped, length 0.4-0.5 mm.

Male Genitalia: Aedeagus pale brown, length 0.6-0.8 mm, tubular shaped, apical part acute, basal hood slightly sclerotized, nearly semi-rounded. Tegmen Y-shaped. [9] mention that the Tectum short and slender; middle dorsal surface with short, transverse, and parallel grooves from tectum apex to basal piece apex; internal sac weakly sclerotized, with one pair of lateral slender sclerites; male aedeagus straight, with pointed apex, firmly and ventrally curled.

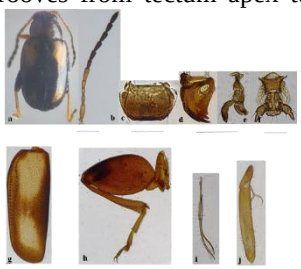

Figure (1) a. Adult Phylloterta striolata 27X; b. Antenna; c. Labrum; d. Mandible; e. Maxilla; f. Labium; g.Elytra; h. Hind leg; i. Spiculum gaster; j. Male genitalia

Scale bar (b,h,j= 0.25mm; c=0.12 mm; d=0.16 mm; e= 0.22 mm; f,i= 0.15 mm; g= 0.5 mm)

Second: Molecular Identifications

The PCR product was electrophoresed and visualized by 1.5% Agarose gel. The amplification of COI region produced a uniform fragment size ~550 bp band (Figure 2).

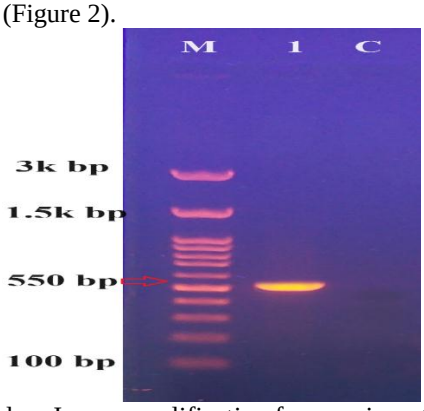

Figure (2) Partial cytochrome C oxidase I gene amplification from an insect using PCR. M; shows: ladder 100-3000 bp, lane 1: 550 bp of insect PCR products, and C is the negative control.

Partial cytochrome c oxidase I gene sequences

DNA sequencing was carried out independently using the ABI 3130X genetic analyzer (Applied Biosystem) and solely forward primer C1-J-1718. The DNA template for sequence-specific PCR amplification was obtained from the sample's PCR results (Figure 3).

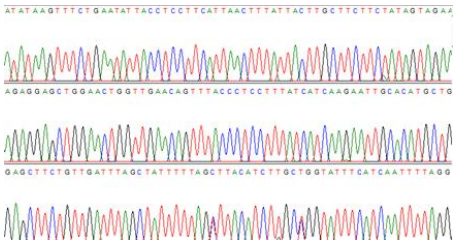

Figure (3): The partial sequence of the chromatogram for mitochondrial cytochrome c subunit I of P. striolata

DNA Barcoding of genus P. striolata (Fabricius)

Gen Bank's BLAST software (http://blast.ncbi.nlm.nih.gov/) yields the 550 bp COI sequence of P. striolata was employed to contrast our amplified sequences with those of other flea beetle species that were kept. The NCBI gene bank's initial record for insect identification had the highest identity query sequence, according to the BLAST results. These alignments demonstrate that we have uploaded our query sequences to the NCBI Genbank; table 2 below lists the accession numbers. The efficiency of DNA barcoding as a molecular identification method for P. striolata was validated by our investigation. The accuracy of the DNA barcoding identification process was 100%. Our findings are consistent with those of [15], who found that, using COI gene sequences, DNA barcoding is a simple and accurate method of identifying thirteen Phyllotreta and five Chaetocnema species.

Table (2) partial COX sub unit I gene sequences in NCBI and alimented with same sequences after submission

Insect Identified	Accession Numbers	Query Cover %	Identic Number %	Accession Number of	BLAST Identification
		100	100	OL631390	Phyllotreta striolata
		100	100	MZ033023	Phyllotreta striolata
		100	100	PQ066180	Phyllotreta striolata

Phyllotreta striolata	PV 123233	100	100	OL631367	Phyllotreta striolata
		100	100	OL631431	Phyllotreta striolata
		100	100	OL631370	Phyllotreta striolata
		100	100	OL631362	Phyllotreta striolata
		97	83.70	KJ962496	Phyllotreta armoraciae
		97	82.73	MH118682	Phyllotreta armoraciae
		91	81.27	KR483768	Phyllotreta albionica
		91	81.27	KM847124	Phyllotreta albionica

Phylogenetic inferences

Phyllotreta striolata species under investigation were grouped according to predicted lines by phylogenetic analysis based on the COXI nucleotide sequence. The evolutionary development and sequencing diverse similarity data showed that species from the relevant genera were near to one another. Our query sample is grouped in one cluster with high similarity of OL631390, are located to same branch of cluster but the other species have located to other branch of groups of phylogenic (Figure 4). Geographical barrier may act as evolutionary tool for the sequence divergence Thus, the present study revealed the importance of DNA barcoding in the identification of species.
In a similar study of [16] Based on the phylogenetic analysis of divergent genera in Chrysomelidae, C. confinis is closely related to Phyllotreta striolata of Alticinae.

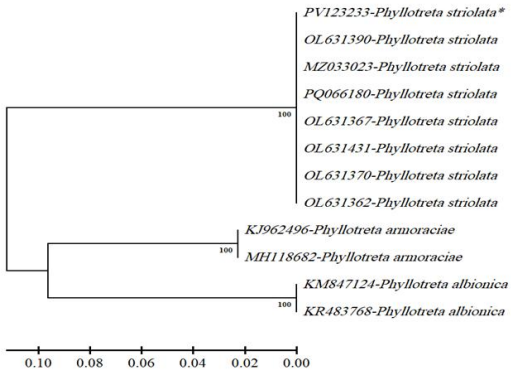

Figure (4) The Genetic evolutionary tree of the Kurdistan area of Iraq's Phyllotreta striolataon sample (*). Employ the MLE, the Genetic evolutionary tree was built using bootstrap analysis with 100 re samplings and the Tamura-Nei model in MEGA11 software. The input data consisted of concatenated partial COI Mt-DNA sequences.

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الوصف المورفولوجي والتعرف الجزيئي على نوع Phyllotreta striolata (Coleoptera: Chrysomelidae: Alticinae)في أربيل، العراق

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الخلاصة

الوصف التفصيلي للجنس *Phyllotreta striolata* ، *(Coleoptera: Chrysomelidae: Alticinae)*و والتعريف الجزيئي للجنس المذكور من محافظة اربيل . تم جمع العينات بواسطة شبكة حشرات هوائية من نبات الكاباريس *Capparis spinosa* خلال الفترة من 1/تشرين الاول /2024 الى 22/تشرين الثاني/2024 . من اهم صفات او سمات الأنواع هي أن الأجزاء القمية من الفك السفلي تتكون من أربعة مجسات، وملامس الفك العلوي تتكون من أربعة أجزاء. قرون الاستشعار بنية باهتة ، تتكون من احد عشر عقلة ، من العقلة الأولى إلى الخامسة بني باهت، العقلة الخامسة أطول بمرتين من العقلة السادسة ، منتفخة، العقلة الحادية عشر بيضاوية الشكل. الأجنحة الغمدية بنية داكنة، الجزء الأوسط أصفر باهت.

مَنظارُ الجَاسِر *(Speculum gaster)* على شكل حرف *Y.* ال *Aedeagus)* العضو التناسلي الذكري) بنيّ باهت ، أنبوبيّ الشكل. وثقت صورٌ للأجزاء المهمة. حُدِّدت المواقع، والنباتات المُضيفة، وتواريخ الجمع.

للتشخيص الجزيئي ب *Phylotreta striolata* بالاضافة الى التشخيص المظهري، تم تشخيص الجزيئي لهذه الحشرة *Phyllotreta striolata* و ايجاد العلاقات الوراثية التطورية بين هذا النوع العملي و الانواع المسجلة في الانحاء المختلفة في العالم. حيث تم الاستخدام الحامض النووي للمايتوكوندريا*MtDNA* و استعملت تقانة تفاعل البلمرة المتسلل *PCR* لتضخيم قطعة في الجين.

اذا اظهرت النتائج طول القطعة مورثة الجين كان 550 زوج القاعدي و بلغت نسبة تطابق هذا النوع مع الانواع المسجلة في الانحاء المختلفة في العالم ب 100% و تم تسجيله في بانق الجينات برقم *PV*123233

الكلمات المفتاحية: مظهرية ، الجزيئي ، *Phyllotreta striolata*، *Chrysomelidae*.