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### ORIGINAL ARTICLE

## MOLECULAR INVESTIGATION OF *PENTODON ALGERINUS* (FÜESSLY, 1778) (COLEOPTERA, SCARABAEIDAE), WITH DESCRIPTION OF THE LAST INSTAR GRUB

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### ABSTRACT

In this study, the species *Pentodon algerinus* (Füessly, 1778) (Coleoptera, Scarabaeidae) was genetically recorded in the NCBI GenBank database using the mitochondrial partial gene (COI) through a molecular technique. Then the third instar grub of this species was morphologically described using light microscopy. Throughout the study, 24 grubs were collected from different localities in Erbil Province. Most characteristics of the last instar grub were illustrated; the description was based on the reared grub to adults under field conditions to confirm identification. Although many research attempts have focused on the adult stage, no work has been conveyed on the immature stage that feeds in the root area of lawns in this province.

Keywords: Erbil, *COI*, Description, *Pentodon*, White Grub.

### INTRODUCTION

The Scarabaeidae family, belonging to the order Coleoptera, includes a large number of beetles. Numerous species of their larvae feed on decaying wood in forested areas, thereby playing an essential role in the recycling of organic materials (Rodrigues *et al.*, 2017). The adult stage of some species in this family acts as a pollinator of wild plant flowers (Coutinho *et al.*, 2011; Maia *et al.*, 2013), while most species may be pests of agricultural plants (Rodrigues *et al.*, 2011; Rodrigues *et al.*, 2012; Garcia *et al.*, 2013). Pentodontini of the family Scarabaeidae is the main tribe in the subfamily Dynastinae, and includes more than 500 species distributed worldwide (Krell *et al.*, 2016). The distribution of *Pentodon* Hope, 1837 is limited to the Palaearctic and oriental regions, and it includes 14 species and 18 subspecies (López-García *et al.*, 2015; Jang and Kim, 2019). Species of Pentodontini in the Neotropical region also attack the roots of economic crops such as sugarcane, cereals, corn, and tobacco (Gentry, 1965).

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Adult beetles are habitually active from September to December. After recognition between males and females, they begin to mate. Then females deposit their eggs in the soil, and the complete life span development requires approximately one year (Rani *et al.*, 2021). The distinction between male and female is difficult in the morphology of immature stages, whereas in adults it is easy and can be distinguished by the end of the abdomen, genitalia and length of the tarsomeres (Ferreira *et al.*, 2018). White grubs are characterized by their 'C-shaped' bodies and sclerotized head capsules, while the more variable adults generally have an ovoid body shape and lamellate antennae (Ritcher, 1966). The grub stage period differs from one to three years, depending on the ecological conditions and the species' life span (King, 1984).

The immature taxonomy of Scarabaeidae is insufficient, especially in identifying the species, and efficient mitochondrial DNA *COI* based methods for the definition and documentation of species belonging to the Scarabaeidae family have been reported (Dirk *et al.*, 2007). Therefore, this study aimed to use a molecular technique based on the mitochondrial partial gene (*COI*), and larval description precisely as a modern method for identifying and dealing with *Pentodon algerinus* (Füessly, 1778), which is a dangerous lawn pest in gardens and grasslands in Erbil Province, Iraq.

## MATERIALS AND METHODS

**Specimens collection and identification:** Grubs and adult beetles were collected from different localities in Erbil Province, Kurdistan Region, Iraq. A light trap was used to catch adults, while grubs were collected randomly and by hand picking from underground depths of around 15-20 cm. Specimens were collected during May, June and July of 2024. Some of the grubs were placed in plastic pots for rearing; where slices of apple are potato were offered for feeding and covered with moist soil in order to allow the grow as adult beetles for the easy confirmation of species identification. Grub specimens were preserved in glass vials containing 70% alcohol and kept under refrigeration for morphological description. The specimens were examined under a dissecting microscope (Huma scope- Germany), and the complex digital microscope (Huma scope quality attached LCD camera- Germany) was used to display micro-characteristics. The studied features were measured and scaled using a stage micrometre. The illustrations were edited and organized using computer software (Adobe Photoshop 2023). The specimens were identified by the Iraq Natural History Research Center and Museum, University of Baghdad (Document No. 13 on 9 February 2025), and confirmed via a molecular technique based on the mitochondrial partial gene (*COI*).

**Extraction of Genomic DNA:** Genomic DNA was extracted from the body of the grub. ZYMO Quick-DNA Tissue/Insect Micro Preparation Kit (USA, No. D6015) was used. 25 mg of cleaned insect body tissue was homogenized in in a 1.5 ml Eppendorf tube in 400 µl of lysis buffer-TE (Tris-EDTA-pH 8), with crushed tissues to lyse the cells. Then 12µl of proteinase K (20mg/ml) was added to the tube mixing well using a vortex. The substrate was incubated at 80 °C for 5-10 minutes. The sample was cooled at room temperature. The aqueous layer was prudently transported into a sterilized empty tube without disturbing the protein stratum at the interphase. 500 µl of isopropanol was mixed to this layer and incubated

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overnight, then centrifuged at 7000 rpm for 10 minutes. The supernatant was detached, and the pellet was cleaned with 70% alcohol. After this step, evaporated of allowed to dry, the pellet was dried and thawed in 30 µl of TE-I and then preserved at -20 °C. Then, it was inspected over 1% agarose gel and envisaged after staining with ethidium bromide (Dirk *et al.*, 2011; Murthy, 2020).

**Quantification of DNA, PCR amplification and COI gene fragment sequencing:** The isolated DNA samples were quantified to obtain the DNA amount; a NanoDrop 1000 spectrophotometer (Thermo Scientific) was used. The absorbance was measured at 260 nm as the nitrogenous bases in DNA would show strong absorpti on at this wavelength. at M/S. PCR amplification for the COI partial gene was performed in a 50 µl reaction mixture containing 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), 10 Picomol of forward primer C1-J-1718 (GGAGGA TTTGGAAATTGATTAGTTCC), with 10 picomol of reverse primer HCO 2198 (TAAACTTCAGGGTGACCAAAAATCA), DNase-free water and template DNA using a Bioresarch PTC-200 Gradient thermocycler.

**Sequence analysis and data interpretation:** The DNA sequencing was produced by the ABI 3130X Genetic Analyzer (Applied Biosystem). Using the forward primer C1-J-1718. The PCR products of the samples were used as a source of DNA template for sequence-specific PCR amplification (Binneck *et al.*, 2004). The ambiguous bases of Chromatograms were discarded, and revised sequences were allied using the Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI). The sequence data were submitted to NCBI, and accession numbers were attained (EF487736). Clustal W (version. 1.83) was used for multiple alignment of consensus *COI* partial gene sequences (Thompson *et al.*, 1994; Ditttrich-Schröder *et al.*, 2006).

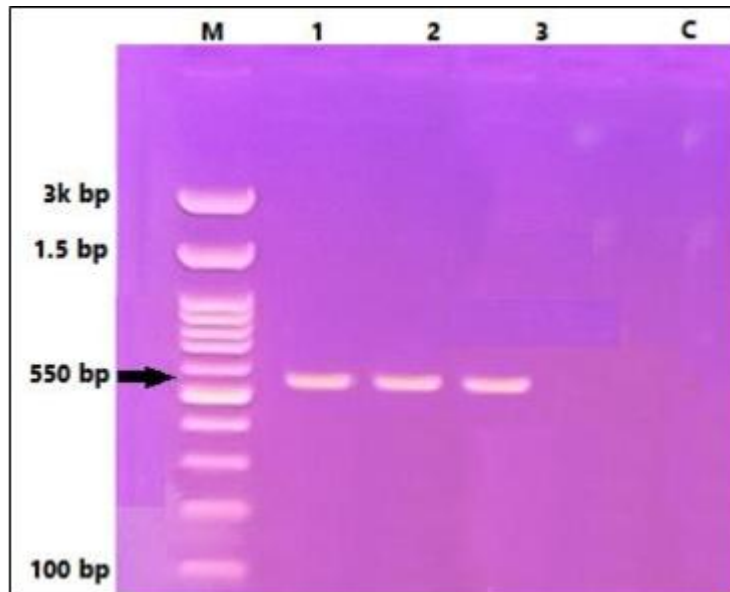
## RESULTS AND DISCUSSION

### Molecular identification

In this study, the isolated genomic DNA was characterized through the *COI* gene fragment (550 bp (Pl. 1), and it was significantly sequenced for *Pentodon algerinus* species, and the arrangement of its sequence was measured to lack any insertion or deletion. The species was sequenced across the *COI* regions, and a BLAST was completed using the NCBI database to determine the identity of Scarabaeidae species in Erbil Province, Iraq, as given in Table (1). Molecular sequence evidence from NCBI accurately exposed empathy as accurately as revealed by their morphological investigation and taxonomic characteristics.

**Table (1):** Percentage distribution of *Pentodon algerinus* according to the BLAST result in NCBI, and GenBank registration.

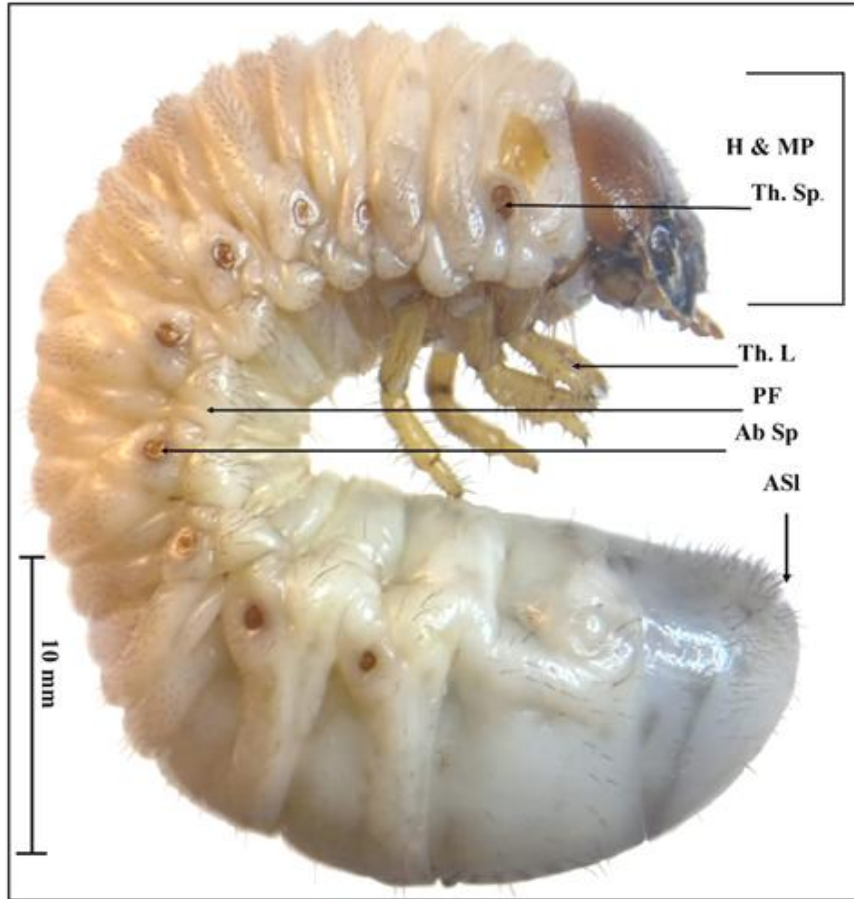
Gene used	Specimen accession number	Query cover %	Identic number %	GenBank accession number	GenBank identification of the species
COXI	PV628254	100	100	EF487736	<i>Pentodon algerinus</i>

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**Plate (1):** PCR amplification of the mitochondrial partial gene *COI* from the studied species *Pentodon algerinus* which M is ladder (3k bp-100 bp), lanes 1, 2 and 3 are the replicated samples of PCR products from the species with a molecular weight of 550 bp and C is a negative control.

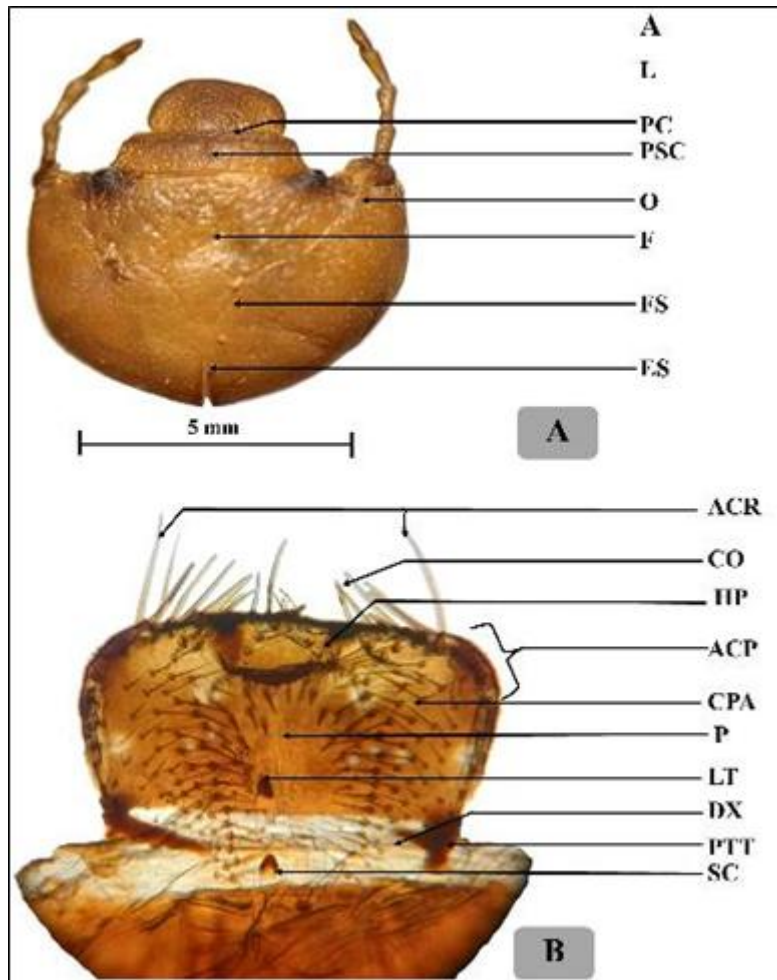
### Morphology

Description of the third instar grub: Grub body length about 36-46 mm, C-shaped, and somewhat thick and creamy-white, the end of abdomen suddenly becomes swollen, especially the anal segment, which bears numerous long setae. Head brown and completely visible. Plural folds well developed (Pl. 2). Cranium (Pl. 3 A); width of head capsule 6.1-7.8 mm with pale brown colour. Surface of vertex moderately punctate, with numerous long and tiny and scattered setae; epicranium less densely punctate; post-clypeus and pre-clypeus rugose. Epicranial and frontal sutures well distinct; surface of frons has numerous setae and two posterior setae on each side. Epicranium has 3-4 epicranial setae on the dorsal side with irregularly distributed hairs; ocelli absent; clypeus trapezoid with arched sides, its surface having two anterior setae posteriorly, and two external setae on each margin.



**Plate (2):** Habitus of the third instar grub of *P. algerinus*, lateral view. [H, head; MP, mouthpart; Th. Sp, Thoracic spiracle; Th. L, Thoracic legs; PF, Pleural folds; Ab. Sp., Abdominal spiracle; Asl, Anal slit]

Labrum (Pl. 3 A) subquadrate shaped with its surface closely equal, and anterior margin rounded, anterolateral margin of each side with five setae, and two lateral setae. Epi-pharynx with unique lateral margin (Pl. 3 B), and slightly angulated in the middle. Haptomeral process irregularly forms three sharp structures. Chaetoparia has 5-6 columns of micro-spinules, each column contained 10-11 micro-spinules. Acroparia includes relatively long and hooked setae. Corypha bears numerous long, and thick setae. Acanthoparia provided with several bent and short spinules. Pedium longer than wide and smooth. Dextiotorma widely elongated and slightly sclerotized. Laetorma shorter than dextiotorma. Pternotorma plumped and well sclerotized. Haptolachus has 6-8 microspinules. Sensory cone on the left nesium rounded and well defined, crepis absent.

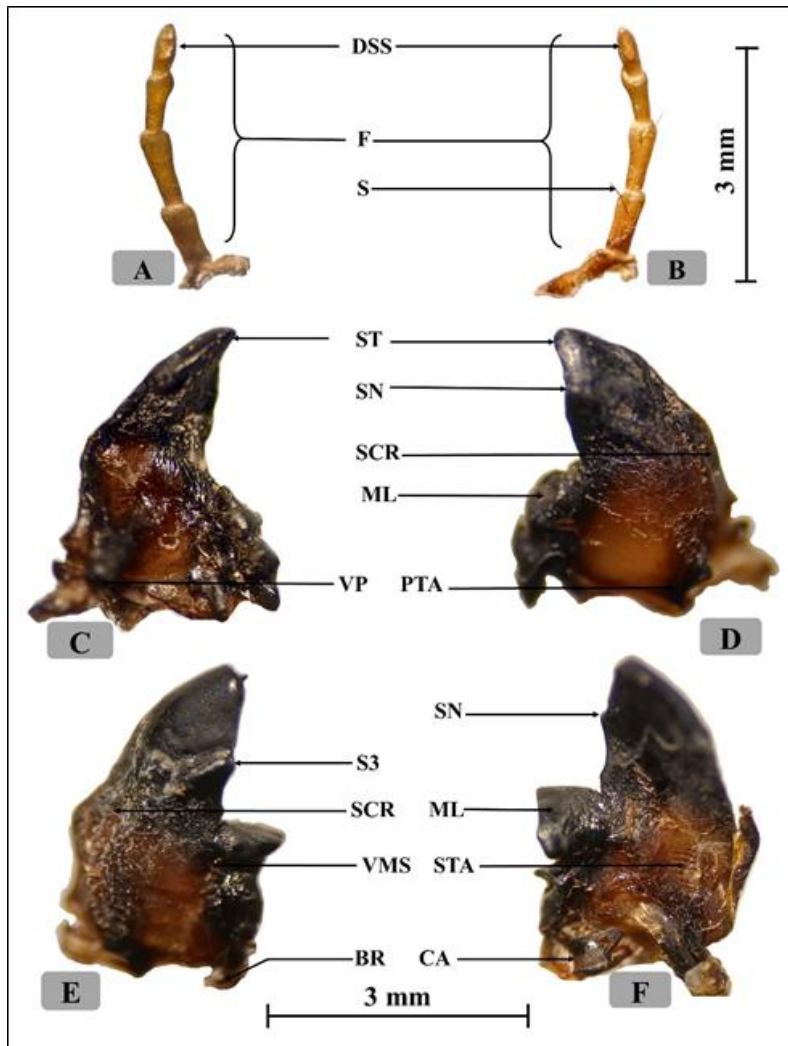
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**Plate (3):** Third instar grub of *P. algerinus*; (A) Head, frontal view, (B) Epipharynx. [A, antenna; L, labrum; PC, pre-clypeus; PSC, post-clypeus; O, ocellus; F, frons; FS, frontal suture; ES, epicranial suture; ACR, acroparia; CO, coryphe; HP, haptomeral- process; ACP, acanthoparia; CPA, chaetoparia; P, pedium; LT, laetotorma; DX, dextotorma; PTT, pternotorma; SC, sense cone].

Antenna (Pl. 4 A, B): five segmented, basal segments thicker than others, segments 2 and 3 are slightly equal in dimension with providing a terminal bristle. The fourth segment is sub-conical, and smaller than the previous segments. The apical segment is about half the length of segments 3<sup>rd</sup> and 4<sup>th</sup>, and carries one prominent sensory spot on the dorsal side.

Mandibles (Pl. 4 C-F): Form falcate, triangular and curved inward with strongly sclerotized. Cissorial region has three apical teeth; first and second teeth joined, while third separated by scissorial notch. Scrobis bears thick setae are longitudinally arranged. Acia acute and

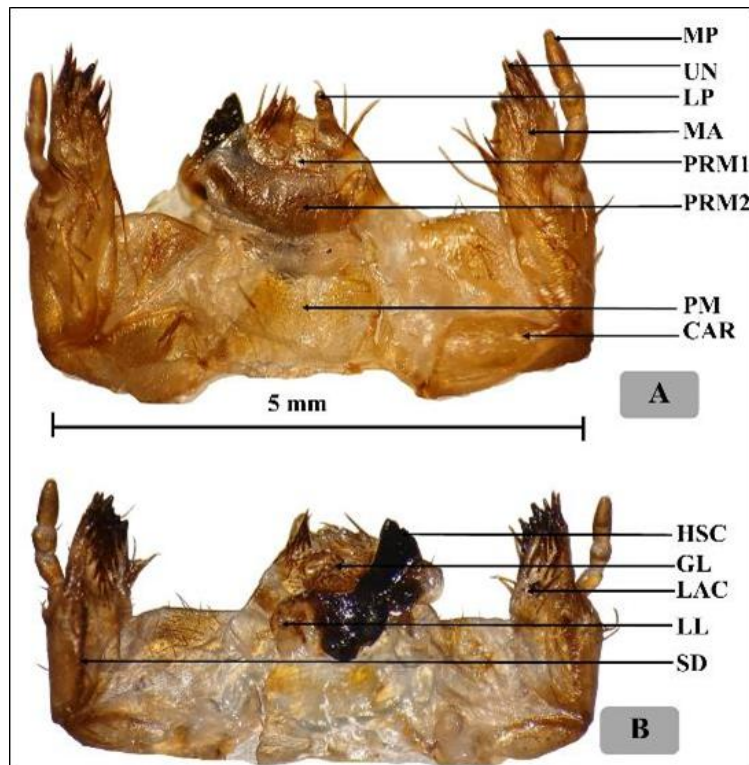
completely developed, with two basolateral setae. Stridulatory area barely separated by subparallel ridges on the ventral surface. Molar lobe prominent and expanded, with a clump of molar setae. Preartis concave and separate. Ventral process broad. Postartis medium-sized and globular shaped.



**Plate (4):** Antenna and mandible of the third instar grub of *P. algerinus*; (A, B) Antenna, left and right [DSS, dorsal sensory spot; F, flagellum; S, seta], (C, D) Left and right Mandible, in which C, Ventral view, (D, E) Dorsal view, (F) Central view; [ST, scissorial teeth; SN, scissorial notch; SCR, scrobis; ML, molar lobe; PTA, post-artis; VP, ventral process; S3, third scissorial teeth; STA, stridulatory area; VMS, ventral molar setae; BR, brustia; CA, calx].

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Labio-maxillary structure (Pl. 5 A, B): In ventral view (Pl. 5 A) galea fused with lacinia, and mala formed. Mala cylindrical and provided with 4-6 sclerotized unci at peak. Numerous strong and long setae raised. Maxillary palpus comprises four segments, basal segment being shortest and as long as wide, second segment is two times longer than basal segment, third segment similar to the second segment but slightly wider with a few thin and long setae. Apical segment longer than the previous segments, and its apex somewhat rounded with providing a sensory con. Mentum subdivided into three sclerites: pre-mentum 1 and 2, with post-mentum. Labial palps comprised of two segments, an apical segment nearly the same dimension with a basal segment, barrel-shaped, and gradually tapered towards the terminal end. Labio-Maxillary structure (Pl. 5 B): Stridulatory area with a row of several stridulatory teeth, teeth increase gradually. Glossae prominent and provided with a number of short and long setae. Hypo-pharyngeal sclerome; toothed structure, and irregularly sclerotized, developed from the dorsal surface of the right side, top of left side raised and protruded. Lateral lobes are well defined and provided with long setae.

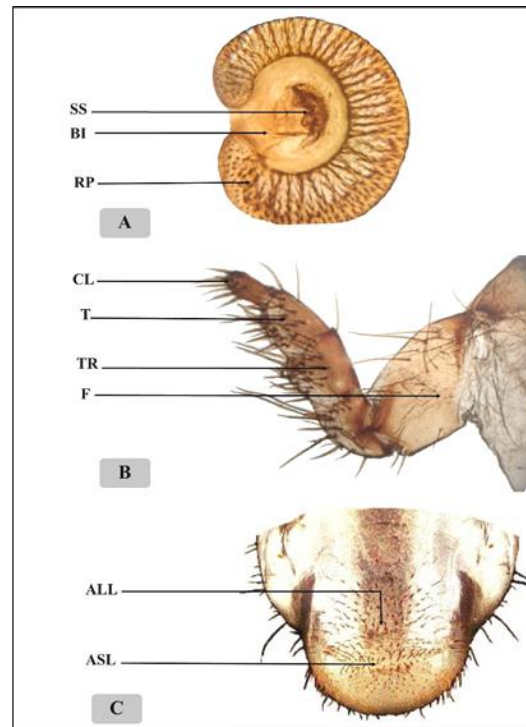


**Plate (5):** Labio-maxillary complex of the third instar grub of *P. algerinus*; (A) Ventral view, (B) Dorsal view. [MP, maxillary palpus; UN-uncus; LP, labial palpus; MA, mala; PRM1, 1<sup>st</sup> prementum sclerite; PRM2, 2<sup>nd</sup> prementum sclerite; PM, post-mentum; CAR, cardo; HSC, hypopharyngeal sclerome; GL, glossa; LAC, lacinia; LL, lateral lobe; SD, stridulatory teeth].

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Thorax with clear region (Pl. 2) and well developed, all three segments distinct. Prothoracic spiracle (Pl. 6 A) about 0.8-1.2 mm long, 0.6-0.8 mm wide; respiratory plate bright tanned, oval and curled body, spiracular-bulla round, marginally raised, respiratory disc with a number of fleapits through its width centre. Notum and pre-scutum of second and third segments slightly convex with a transverse row of long, slender setae. Legs (Pl. 6 B) completely developed, slightly sclerotized. Tarsal claws semicircular; hind tarsal hooks tinier than pro-tarsal and middle-tarsal claws. Coxa, trochanter, femur, and tibio-tarsus of legs characterized by being slightly sclerotized, pale brown to yellowish cream in colour with abundant long and stout setae.

Abdomen cylindrical and thick (Pl. 2), curved inward. Abdominal tergites 1-7 provided with a row of numerous microspinules. Abdominal fold well-defined, and clearly visible on the pleurae. Abdominal segments 1-8 carry a pair of spiracles, which are sub-equal in size with each other, except for the spiracle of the 8th segment that smaller in size. Rapid swelling occurs in the seventh to ninth abdominal segments; abdominal cuticle more expanded so that the interior can be seen, and folds rather decreased. Raster; palidia monostichous with numerous setae and closed anteriorly. Septula elliptical, longer than wide by five times. Tegilla consists of many squarely scattered short, thick setae and fewer long, and slender setae. Anal pore about Y-shaped; dorsal anal lobe provided with sickle-shaped setae, associated with right, tiny setae. Ventral anal lobe with straight, slender setae. Campus with two moderately long setae. Chaetotaxy of ventral anal lip similar to that of tegilla, but with more numerous long setae. Dorsal anal lip with dense, reddish, mid-size setae and some long setae. Anal slit transverse, bordered by dense rows of stout, mid-size setae.

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**Plate (6):** Third instar grub of *P. algerinus*; (A) Mesothoracic spiracle [ SS, spiracular slit; BI, bulla; RP, respiratory plate], (B) Hind leg [CL, claws; T, Tibiotarsus; TR, Trochanter; F, femur], (C) Last abdominal segment [ALL, anal lower lip; ASL, Anal slit].

## DISCUSSION

In the current study, the adult of *P. algerinus* (Fuessly, 1778) was reported in the NCBI GenBank for the first time in Erbil Province, Kurdistan Region, Iraq. Immature identification is limited to the Scarabaedidae species; therefore, a molecular approach based on the mitochondrial gene *COI* was employed to address this issue, which is a modern and precise method for species-level identification. The data analysis of the molecular study magnificently corresponds to *P. algerinus* with 100% query cover. Regarding the morphological description, a lighting microscope with LCD Digital microscope (Huma scope premium) and an attached Viewing Screen (Germany) was used to illustrate fine morphological features. The grub of *P. algerinus* was identified based on the following characteristics: (body shape including thorax, body folds, anal segment; mouthparts (i.e. Labrum, epipharynx with its micro parts, and their arrangement), mandibles and labio-maxillary structure, galea, maxillary palpus, and hypopharyngeal sclerome, spiracles, the claws of legs that are apically blunt, bear several setae. By examining these characteristics accurately, the grub of *P. algerinus* can be recognized from the previously reported Dynastinae species (Ritcher, 1966; Morón, 1977; Morelli, 1991; Morelli and Alzugaray, 1994; Löble and Löble, 2016). However, the characteristics of immatures make it difficult to

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determine the species due to the incompatibility between the taxonomy and the morphology of immature stages. Previous studies are insufficient or not included; therefore, the present study is necessary to fill this gap, providing precise illustrations and detailed descriptions with an addendum of genomic documentation and its registration in NCBI GenBank. We trust that whole descriptions of the Dynastinae immature stages will provide further diagnostic characteristics, which will permit wider and more comprehensive taxonomic reviews and develop the characterizations and identification of species in all life forms.

#### CONCLUSIONS

In the present study, the last instar grub of scarabaeid species *P. algerinus* was precisely described depending on light microscopy. Besides, it was originally registered in NCBI GenBank for the Iraq-Kurdistan Region, Erbil Province. The Mitochondrial *COI* gene sequence was used in molecular techniques which enabled precise identification of the species and its evolutionary relationships. Both followed methods have a prevailing element in identifying this species and its taxonomy.

#### CONFLICT OF INTEREST STATEMENT

"The authors declare that they have no conflict of interest".

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**دراسة جزيئية للنوع (*Pentodon algerinus* (Füessly,1778) مع وصف يرقات الطور الأخير (Coleoptera, Scarabaeidae)**

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**الخلاصة**

في هذه الدراسة، تم تسجيل نوع (*Pentodon algerinus* (Füessly,1778) (رتبة غمدية الالجنحة Coleoptera، العائلة الجعالية Scarabaeidae) وراثياً في بنك الجينات في NCBI باستخدام الجين الجزئي للميتوكوندريا (*COI*) بتقنية جزيئية. ثم تم وصف يرقة الطور الثالث لهذا النوع مظهرياً بناءً على المجهر الضوئي. خلال فترة الدراسة، جمعت 24 يرقة من مناطق مختلفة في محافظة أربيل. وقد تم توضيح معظم الصفات المظهرية ليرقات الطور الأخير، بناءً على اليرقات التي تمت تربيتها إلى الحشرات الكاملة تحت الظروف الحقلية لتأكد التشخيص. على الرغم من إجراء العديد من المحاولات البحثية على دور الحشرة الكاملة، إلا أنه لم يتم إجراء أي عمل على الأدوار غير الناضجة التي تتغذى على جذور الثيل في حقول محافظة أربيل.