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RE-DESCRIPTION OF THE LAST INSTAR LARVAE OF *CAPNODIS TENEBRIONIS* (LINNAEUS, 1760) (COLEOPTERA, BUPRESTIDAE) DEPENDING ON SCANNING ELECTRON MICROSCOPE

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ABSTRACT

The flat-headed borer, *Capnodis tenebrionis* (Linnaeus, 1760) , dispersed extensively in various geographical regions; it is extremely harmful and a dire threat to most stone fruit cultivars, and once the larva enters under the host tree crown, the infested host tree will gradually dry and eventually die. In this study, specimens were collected from several sites in Erbil province, Kurdistan Region- Iraq; then the fully grown larvae were selected for morphological study. In addition, clarification of morphological consequential and implication for most diagnostic characters were studied in all dissected parts. Scanning electron microscope (SEM) was used to further analyze the hidden features of the selected characteristics; those were not shown in the primary mounting. The results impressively presented new conclusive evidence for the descriptive illustration of *C. tenebrionis*, and it confirmed a precise identification of this species in the larval stage.

Keywords: Buprestidae, *Capnodis*, Erbil, Larva, SEM

INTRODUCTION

The genus *Capnodis* Eschscholtz, 1829 (Coleoptera, Buprestidae) is a widely distributed genus that consists of 21 species (Löbl and Löbl, 2016), of which around 10 species recorded in Iraq (Ali, 2007). It is highly important to recognize this species in the larval stage and differentiate it from other borers in the region of this study- Iraq, as it destroys the roots of both saplings and mature trees of cultivated stone-fruits. Perhaps, this due to the damage which this larva cause starts from neonate instar and it is the capability of boring the host plant root systems. Another point which is worth mentioning is the life-span of the larval stage length, and the large numbers of larval instars (nine instars), as well as all these instars, live within the root cortex of the host plant. Noticeably, the economic impact of this species is particularly severe in the Mediterranean countries (Mfarrej and Sharaf, 2010). As a result of larval feeding on the stone-fruit trees, roots are often endangered leading to the devastation of the orchards as a whole (Talhouk, 1976). What is intriguing is that *Capnodis tenebrionis* has

dominated wide geographical regions, including North Africa, Central, and Southern Europe, the Near East and around the Black and the Caspian Sea (Marannino *et al*., 2003).

In addition, the widespread of this species in the Mediterranean countries is due to the suitability of climate conditions in those regions (Bonsignore *et al*., 2008; Sharon *et al*., 2010). Despite the in-depth knowledge available about the damage which this larva causes, the region of this study yet to find suitable solutions to limit or manage the damage by this pest. To further support the latter point, the norm practice to face this kind of epidemic in Kurdistan is by applying a huge amount of chemicals against root colonization, this approach has proven to be unsuccessful (Sakuma *et al*., 2016).

This study found out that there are many important morphological characteristics in the larval body. Besides, it has been promoted by the importance of morphology and identifying larvae. The aim of this study is to clarify and illustrate the most morphological characters. Thus, support accurate identification of the larval stage of *Capnodis tenebrionis* (Linnaeus, 1760) in their taxonomic position within the family Buprestidae.

MATERIALS AND METHODS

Larval collecting and identification

The larvae were collected from the trunk and roots of the apricot, *Prunus armeniaca* L., (1753), and peach, *Prunus persica* (L.) Batsch(1801), started from mid-spring to the end of summer 2018. Then mature larvae (last instar larvae) were chosen for describing. Meanwhile, on the same host plant, a number of larvae were conserved until they reach their adult stage; this was done to obtain an accurate identification of this species. For this stage of the study, we compared it with references of Obenberger (1926); Thery (1936); Knopf (1971); Varandi *et al*. (2009), and Ghahari*et al*., (2015). In addition, the larval stage of *C. tenebrionis* was confirmed to the genus level by Dr. Svatopluk Bily (Czech University of Life Sciences, Prague, Czech Republic) and Dr. Mark Volkovitsh (Zoology, Russian Academy of Science, St. Petersburg, Russia). Finally, the morphological terminology used in present study align to that used in the papers by Bilý (1999); Bílý *et al*. (2011); Bílý and Volkovitsh (2005, 2007); Volkovitsh and Bílý (2015).

Morphological studies

The morphological features in this paper structurally depend on the study of Bily and Volkovitsh (2001, 2007), Levey (2016), Da Silva and Zarbin (2016), and; in which the larval body parts boiled in 10% KOH aqueous solution until the tissues softened and dissolved fat bodies. Then the specimens were rinsed three times in distilled water and diagnosed with the aid of a dissecting microscope (OPTIKA, Italy). After that, the mouthparts separated from the head capsule along the posterior margin of the epistome. Finally, the body cut along a pleural line from the thorax to the last abdominal segment to separate pronotal and prosternal grooves with their sculptures, mesothoracic and abdominal spiracles, and proventriculus.

Slides preparation and taking photos

In the process of mounting specimens for the preparation of slides, the protocol described by Alexeev (1960) was followed, DPX media (a mixture of polystyrene, tricresyl phosphate, and xylene) which acts as a clearing agent. To achieve the latter stage, first, the dissected parts were dehydrated, in four various ethanol concentrations (25%, 50%, 75%, and 99 %) and then they rinsed in Xylol. On the following stage, we placed one drop of DPX on the center of a clean slide, then the prepared parts, which intended to describe, placed into the drop using 0.5 mm microneedle and covered with coverslips. To avoid bubbles creation, the

slides placed horizontally on a hot plate (approximately 36˚C) for 24 - 48 hours. A compound microscope with the camera (Huma scope premium with LCD camera- Germany) is used to display the specimens; only then, the required images were taken. A stage micrometer (Shinuya, Japan) used for measuring and scaling of the analyzed parts. Regarding graphical hand sketching, they are based on microscopic shapes then illustrated using drawing pens (STAEDTLER, pigment liner, Fineliner) in diameters (0.1 - 0.5) mm and tress papers. After scanning, all illustrations are edited then using computer software (Adobe Photoshop7.0 and Paint).

Scanning electron microscope (SEM)

SEM section of the study was carried out in (Electron Microscopy Unit, Research Center of Soran University-Iraqi Kurdistan Region. For this purpose, we followed Kirti and Shipali (2014), and Kownacki *et al*. (2015) protocol. The selected parts of the larva were dehydrated in graded series of ethanol and passed through critical drypoint; then the dehydrated parts were mounted on SEM specimen stubs using only a small strip of double-sided adhesive tape. The specimens were fixed on holders, then sputter-coated with gold and scanned under FEI - Quanta 450 scanning; filament type: tungsten.

RESULTS AND DISCUSSION

Diagnostic characters of the larva: the body of full-grown larva is flat and relatively long, and it has off-brown color, which is a typical feature of buprestid larval shape (morpho-ecological larval type 2); it has reasonably large prothorax and perfectly sclerotized pronotal and prosternal grooves. Body length about 5 - 6 cm; width of prothorax 0.5 - 0.7 cm (Fig.1 a).

Head: head wider than long, and it appeared as a small flexible flat segment inside the prothorax. Sclerotized epistome, hypostome, pleurostome are attached to the head, and they surround the mouthparts (Figs. 1a, b).

Epistome: the epistome has a convex rectangular shape toward the outer side, and full sclerotized, its width is greater about six folds than its length; posterior margin is bisinuate with sharp and acute corners; anterior margins slightly concave between mandibular condyles which are semi-globular (Fig. 1c). The anterior of the middle part of epistome has two groups of sensillae which are very close to each other, and virtually fibrous, every single group is composed of two long sensillae, with one campaniform sensillum. Two long and noticeable setae arise from prestome, near the posterior margin of the epistome (Pl. 1a). Hypostome elongated, sclerotized and narrowed in the middle, divided widely by vertical suture; it bears a campaniform sensillum and trichoid as well as pleurostome without ocelli.

Clypeus: strongly transverse, membranous and smooth (Fig. 1d); the length is about half of its width and the posterior margin is connected to the epistome, but anterior margins slightly arcuate to the posterior margin (Pl. 1b).

Labrum: slightly transverse and resembles a crowned shape with round edges; width considerably greater than its length. Lateral lobes regularly developed between rounded anterolateral corners, which they appear to be round and out words. Apical seta is a pair of short and sharp trichoid, and not extended to the anterior margin of the labrum. Lateral sides closely round outwards and , carry microspinules (Fig. 1d). Palatine sclerites completely developed, and the lateral branches of palatine sclerite are curved and sclerotized more strongly in comparison to the median ones, but the medial branches are not fully developed. Also, four campaniform sensillae, and two long setae -median sensilla- are situated on the top

part of the medial branch of the palatine sclerite. In addition, labrum's anterolateral sensillae (t-trichoid, c-campaniform) include internal –1t+2t+3t and external –1t-2c-3t-4t, a campaniform sensillum situated close to the lateral branches of palatine sclerite. The upper surface of the labrum recognized by a thin transverse space of long micro-setae which creates a bundle of setae on the anterior margin, remaining posterior surface glabrous (Pl. 1b).

Mandibles: (Fig. 1 e) mandible is triangle-shaped; their length is more than the width, widened at the base, sclerotized strongly, and have two obtuse dark brown apical teeth. On the dorsal side near the mandibular condyle, two short and acute setae are located as well as a common round basis (Pl. 1 c). At the same surface on the dorsal side, a tiny area of micro sculpture can be observed.

Labium: labium oblique, with length about 1.5 times greater than width; anterior margin convex moderately, and expanded posteriorly; anterolateral corners widely rounded; transverse prementum about twice as wide as long; external surface of labium decorated by the presence of short, and fine microspinulae, where the conical bundled formed, and distributed in three distinct areas; nearby the anterior margin and two round dense areas between corner sclerites, as a result, a heart shape can be seen (Fig. 1f); The corner sclerite of prementum concave, and curved inward. Every sclerite bears five campaniform sensillae and one long sharp seta. Post mental seta relatively long, but not reach to the anterior microspinuled area (Pl. 1 d).

Maxillae: cardo somewhat round, membranous and smooth; stipes enlarged with arcuated inward, internal sclerite with fully sclerotized, short and sparse of microspinulae found on the outer margin. Maxillary palpus consists of two sclerotized segments, the basal segment "basal palpomere" relatively large, and longer approximately by 1.5 times than width, long sharp seta rise from its anterolateral margin in which extends far above the apical segment, with a presence of few short microsetae in the same area. A campaniform sensillum located on the anterior part of the segment (Fig. 1 g); apical segment "terminal palpomere" with conical, and fully sclerotized, and length twice as width, which carries fine, curved sensillum and not extended to reach the level of the palpomere's apex, the latter holds four extremely fine sensory appendices scattered on its surface. Mala sub-cylindrical and full-sclerotized with a completely developed internal sclerite, it's longer about 1.5 times than the width, mala characterized by the growth of six long and thick trichosensillae on the apical surface, with fine dense setae situated alongside the anterior margin and extended downwards to level with maxillary sclerite; and on the basal part of mala, a campaniform sensillum is located (Pl. 1 e).

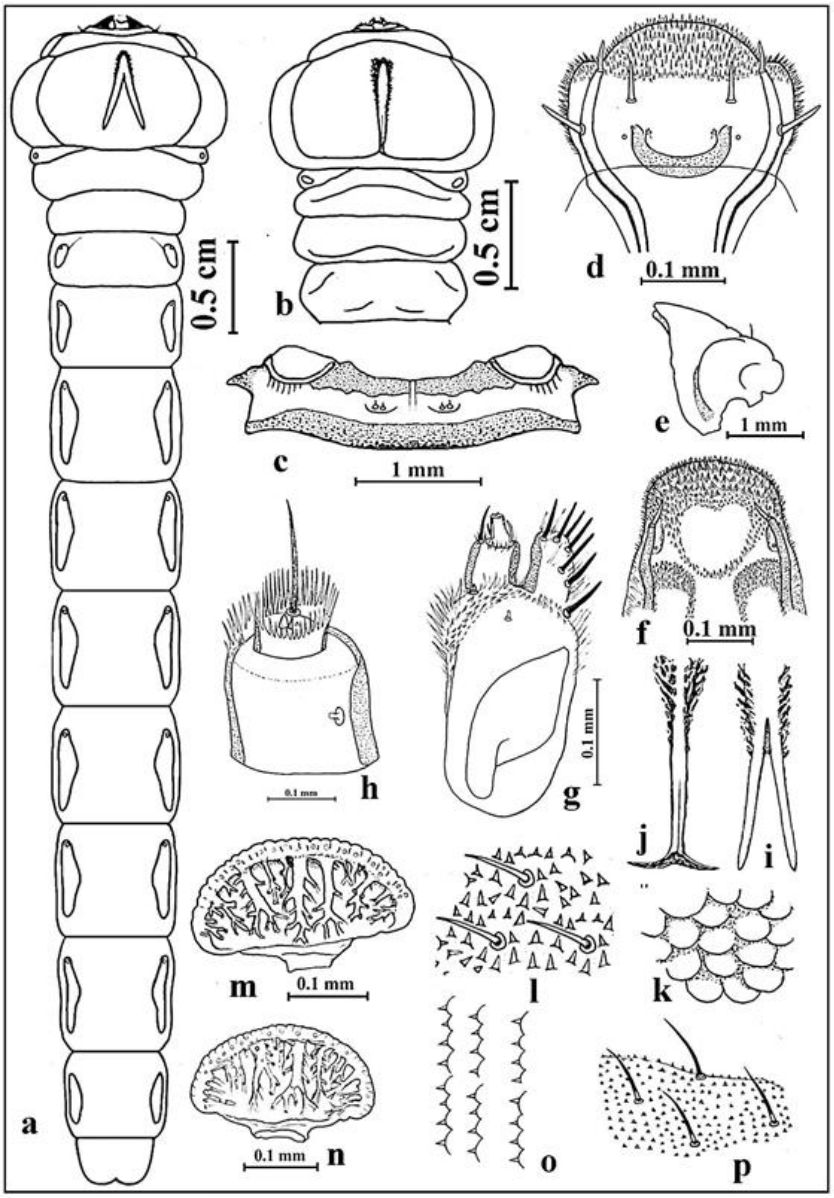

Figure (1): Larva of *C. tenebrionis* (L., 1760); (a) dorsal view of larval body, (b) ventral view of larval body, (c) Epistome, (d) Labrum, (e) Mandible, (f) Labium, (g) Maxilla, (h) Antenna, (i) Pronotalgroove, (j) Prosternal groove, (k) Pronotal sculpture, (l) Pronotal surface, (m) Meso- thoracic spiracle, (n) First abdominal spiracle, (o) Inner sculpture of proventriculus, (p) Integument surface.

Re-Description of the Last Instar Larvae

Antennae: antenna composed of two segments, the basal segment "basal antennomere" sub-cylindrical shaped, with length more noticeable than its width, and it is about 4 times as long as the apical segment. The basal segment originated in the antennal incisions it is located in the posterolateral space of the epistome (Fig.1 h). The articular membrane is rough due to having sharp and short microspinulae. The apical segment "terminal antennomere" equal in length and width and slightly bent towards the apex. The anterior margins of both segments carried the apical crown of microspinulae, but the terminal antennomere was characterized by the growth of a long, thin trichosensilla within this crown that can be seen from the frontal view. The apical cavity of the terminal antennomere obvious and full-grown. In addition, two small palmate sensillae and one large conical sensillum located at the bottom of sensory appendage, and their bases are very close to each other (Pl. 1 f).

Thorax: (Figs. 1 a, b) prothorax rather broad, and has a flat round shape; Pronotal groove moderately sclerotized, and it can be seen clearly; it appears like a shape of an inverted letter V;the groove is darker than the plate, the outer borders of the anterior part are marked by rusty colored small asperities (Fig. 1 i; Pl. 2 a). Prosternal groove takes a straight sclerotized line and slightly bifurcate posteriorly. Small asperities in dark brown colored perplexing at the anterior part and surrounding outer borders (Fig. 1 j; Pl. 2 b). The micro sculptured asperities of pronotal and prosternal plates seem as fish scale marks (Fig. 1 k; Pl. 2 c), these plates are diametrically sclerotized, and they also include dense short microspinulae with few thin setae (Fig. 1 L; Pl. 2 d). Mesothorax transversely, and their width about 3 times more than its length, and it is much wider than the metathorax and abdominal segments. Metathorax slightly smaller and narrower than the mesothorax. Furthermore, the presence of short and dense microspinulae on the cuticle, long sharp flexible bristles emerged, in which condensed on caudal and lateral boundaries.

Spiracles: mesothoracic spiracles are considerably noticeable, resembles the mushroom shape, with dense trabeculae (Fig. 1 m); abdominal spiracles with nearly to elliptic shaped, and similar to prothoracic spiracle, but smaller and fewer number of trabeculae (Fig. 1 n).

Proventriculus: it's like an elongated sac and located under prothorax internally, it has different elements with complicate inner armament, these armaments moderately developed , and arranged on vertical stripes that nearly to globularly shaped. The vertical stripes carried dense, robust, and uni-micro teeth protrusion, which located on the tubercles (Fig. 1o).

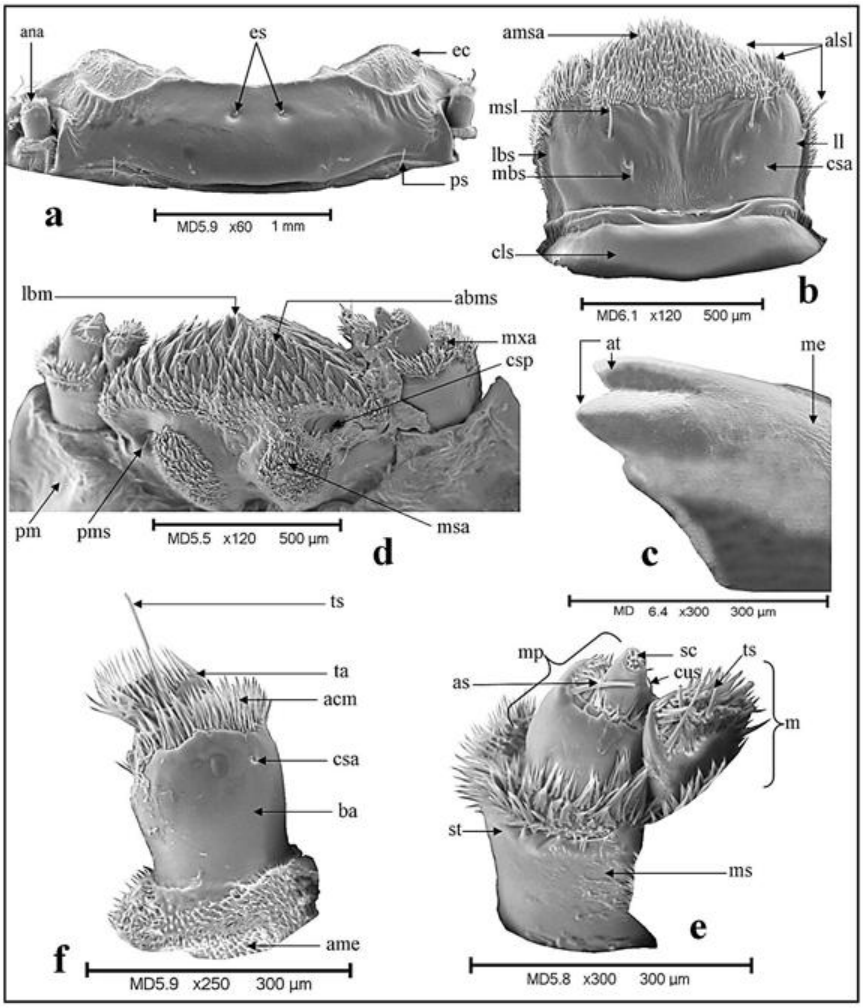

Plate (1): Mouthpart and antenna with their chaetotaxy of larva of *C.tenebrionis* (L., 1760) with abbreviations; (a) Epistome (ana- antenna, ec- epistomal condyle, es-epistomalsensillae, ps-prestomalsensilla), (b) Labrum (alsl- antero-lateral sensillae of labrum, amsa- anterior margin withmicrospinuledarea, cls- clypeus, csa-campaniformsensillae, lbs- lateral branch of palatine sclerite, ll- lateral lobe, mbs-medial branch of palatine sclerite, msl- medial sensillae of labrum), (c) Mandible (at- apical teeth, me- mandible), (d) Labiomaxillary complex (abms- anterior bundle ofmicrospinulae, csp- corner sclerite of prementum, lbm- labium, msa-microspinulae area, mxa-maxilla, pm- pre mentum, pms- postmental seta), (e) Maxilla (as-apical seta, cus-curved sensilla, (m) mala, mp- maxillary palps, ms-maxillary sclerite, sc-sensory cone, st- stipes, ts- trichosensilla), (f) antenna (acm-apical crown of microspinulae, ame- articular membrane, ba- basalantennomere, ta-terminal antennomere, ts-trichosensillum).

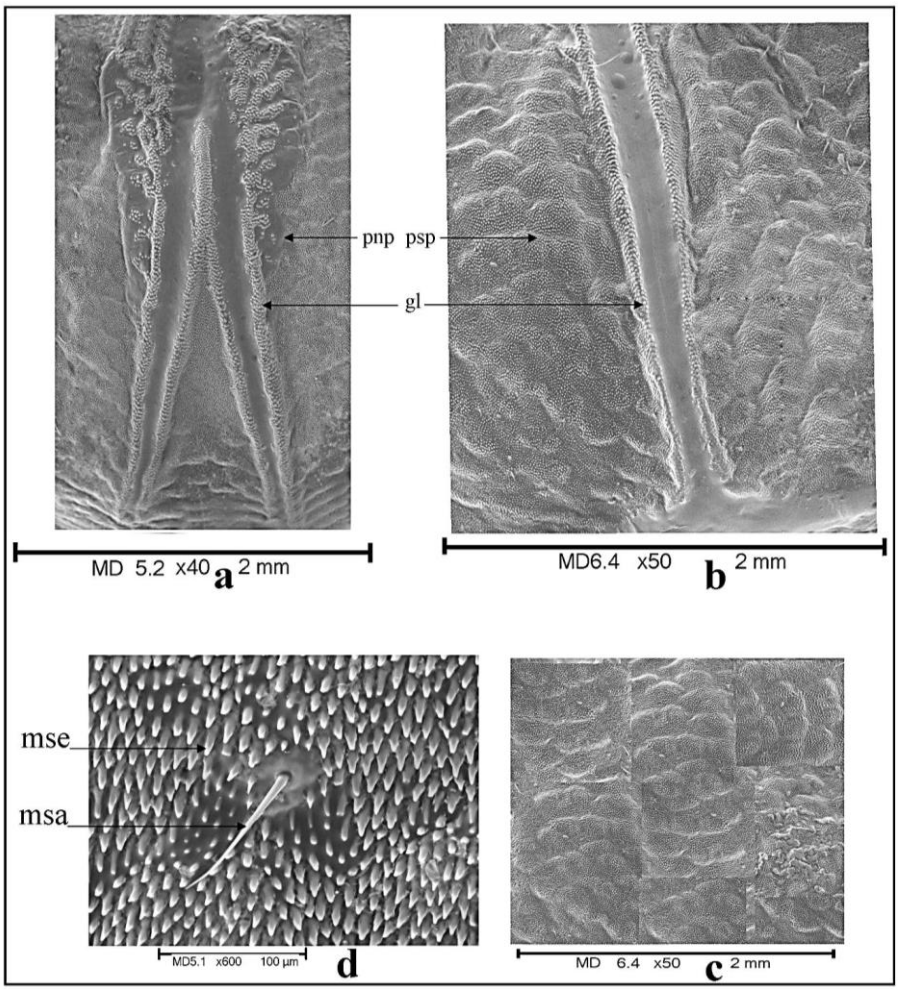

Plate (2): Pronotal, and prosternal plate with their chaetotaxy of the larva of *C.tenebrionis* (L. 1760), with abbreviations; (a) pronotal grove, (b) prosternal groove (gl- groove line, pnp- pronotal plate, psp- prosternal plate), c-prosternal armament, (d) prosternalsculpture (msa- micro seta, mse-microspinulae)

Abdomen: flat, and long; and consists of 10 segments, the dorsal folds made the segments tighten medially inward, so it appears as two folds. The segments 3- 8 have approximately the same length and width, but the first abdominal segment is smaller than the others; while the segments 9th and 10th are different in size and shape (Fig. 1 a). The integument surface has short and dense microspinulae. Furthermore, long thin bristles can be seen on the dorsal and ventral side of the abdominal surface (Fig. 1 p). Abdominal folds take the closed longitudinal figure in which they bent towards the body axis, there are no morphological differences between dorsal and ventral folds. The 8th abdominal segment with conical and it is half the

size of the previous segments. The last abdominal segment smallest, deficiently sclerotized and divided by a vertical anal rim.

CONCLUSIONS

This research investigated the morphological characteristics of the last larval instar of *Capnodis tenebrionis* (L. 1758). Several morphological characteristics of the larval stage were selected for describing and identifying the larvae. After analyzing the details obtained in this study, we can conclude that the description of most taxonomic characteristics for the larval stage of this species in detail by comparing different techniques, which they complement each other. Interestingly, drawing sketches, mounting and scanning electron microscope were the three factors contributed to diagnoses and identification of the larvae species correctly in their taxonomic position. Finally, we were concluding that the species could be determined in immature stages, provided comprehensively and accurately it has been described.

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إعادة وصف الطور اليرقي الأخير لحشرة (Coleoptera, Buprestidae) *Capnodis tenebrionis* Linnaeus, 1760 بالأعتماد على المجهر الالكتروني الماسح

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تأريخ الاستلام: ٢٠١٩/٠٧/٢٩ ، تأريخ القبول: ٢٠١٩/١٠/٠٩ ، تأريخ النشر: ٢٠١٩/١٢/٢٦

الخلاصة

ينتشر حفار ذو الرأس المسطح *Capnodis tenebrionis* Linnaeus, 1760 في العديد من المناطق الجغرافية المختلفة؛ اذ تهدد يرقاته انواع الأشجار المختلفة ذات النواة الحجرية، وبمجرد دخول اليرقة تحت تاج شجرة العائل، يؤدي ذلك تدريجياً إلى جفافها وبالتالي موتها. في هذه الدراسة جمعت نماذج اليرقات من مواقع مختلفة ضمن محافظة أربيل، كوردستان العراق، إذ أختيرت يرقات الطور الأخير كاملة النمو لدراسة المظهر الخارجي، فضلاً عن توضيح أهم الصفات التشخيصية لكل الأجزاء المدروسة. اذ استخدم المجهر الإلكتروني الماسح لإظهار الصفات الدقيقة للأجزاء المختارة والتي يصعب رؤيتها من خلال الطرق التقليدية. حيث إن النائج أظهرت أدلة وصفية جديدة واضحة التي أكدت التشخيص الصحيح للنوع *C. tenebrionis* في الطور اليرقي .