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ORIGINAL ARTICLE

MORPHOLOGICAL CHARACTERIZATION OF BLOWFLY MAGGOTS (DIPTERA, CALLIPHORIDAE) FROM THE MIDDLE OF IRAQ

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ABSTRACT

This study aimed to conduct a comparative analysis of seven calliphorid species of third-instar larvae that were collected from central Iraq. The purpose was to morphologically characterize these species and develop an identification key to differentiate them from closely related species. Scanning Electron Microscope (SEM) graphical images and high-resolution traditional optical microscopes were used to analyze the morphological characteristics of the species *Calliphora vomitoria* (Linnaeus, 1758), *C. vicina* Robineau-Desvoidy, 1830, *Chrysomya albiceps* (Widemann, 1819), *Ch. rufifacies* (Macquart, 1844), *Ch. megacephala* (Fabricius, 1794), *Lucilia cuprina* (Wiedemann, 1830), and *L. sericata* (Meigen, 1826). An identification key was constructed based on the observed morphological differences to enable accurate species separation. The constructed identification key provided a reliable method for species separation based on their morphological features.

This study contributes to the field by providing a comprehensive morphological characterization of the larvae of these blowfly species which are distributed in central Iraq. The developed identification key offers a valuable tool for future research and forensic investigations involving these species, enabling accurate species identification and differentiation.

Keywords: Calliphoridae, Cephalopharyngeal, Identification key, Larvae, SEM.

INTRODUCTION

Blowfly larvae, commonly known as maggots, hold significant ecological and forensic importance. These larvae are highly efficient decomposers, feeding on decomposing organic material, including animal carcasses. They play a crucial role in the decomposition Process, breaking down organic matter and facilitating the recycling of nutrients back into the environment. Additionally, blow fly larvae are essential in the field of forensic entomology, where they are used to estimate the postmortem interval in criminal investigations (Higley and Haskell, 2001).

The scanning electron microscope (SEM) is a powerful tool widely used in entomology to study insects. Unlike traditional light microscopy, SEM provides detailed information and allows for species-level identification. It offers higher resolution and Captures fine details that may be difficult to observe with light microscopes (Jalil, and Ali, 2019; Martín-Viveros and Ollá, 2020; Li *et al*., 2021; Al-Sudani and Al-Amery, 2024).

The third instar larvae or maggots of calliphorid species exhibit variations in their structure and appearance. Before transforming into pre-pupae, these larvae grow to a size between 15 mm and 20 mm. The body is vermiform, tapered at the anterior end and blunt at the posterior end. The anterior spiracles, located laterally at the distal end of the prothorax, display a varying number of papillae among species. The cephalopharyngeal skeleton is heavily pigmented, and an accessory sclerite is present beneath the hook part of the larva, which is absent in the second instar (Ahmad *et al*., 2010).

Sukontason *et al*. (2004) examined four Calliphoridae species, highlighting features such as anterior spiracles, dorsal spines, and posterior spiracles, confirming that these comparisons could aid forensic practitioners in distinguishing third instars. While, Shaheen *et al*. (2005) identified key characteristics of Lucilia sericata species using a scanning electron microscope including, detailed spine distribution, papillae without hairs in abdominal segments, papillae on caudal segment surfaces, and tubercle between respiratory discs.

In Iraq, several studies have explored the abundance of species within this family and their distribution (Mawlood and Abdul-Rassoul, 2011; Augul *et al.*, 2013, 2022; Augul and Al-Saffar, 2015; Al-Saffar and Augul, 2023).

This study aims to analyze morphological characteristics of caliphorid insects using SEM images, focusing on third instar larvae, and to develop a key for their identification based on shape characteristics. The study's implications are important for forensic entomology and related fields, as it includes new and accurate morphological characteristics in the taxonomic key.

MATERIALS AND METHODS

Specimens' collection: This study aimed to investigate Challiphoridae specimens collected from different locations in Iraq. The specimens were collected From Kadhimiya, Baghdad; Haii Al Bisatin, Karbala; and Samarra, Saladin Province. A total of 200 specimens were collected from Chicken, Dog, and Cat carcasses as well as from the liver and lungs of sheep between October 2022 and September 2023. After killing, the maggots were thoroughly washed (4-5 times) before being preserved in 70% alcohol, as described by Knipling (1939).

Identification of maggots: The maggots were identified by using keys from many references (Zumpt, 1965; Erzinçlioğlu, 1987a; Liu and Greenberg, 1989; Queiroz *et al.*, 1997; Wells *et al.*, 1999; Sukontason *et al.*, 2008).

Sampling procedure and specimen's preparation: The sampling procedure involved using sheep liver as bait, following the method described by Kavitha *et al*. (2013). Collection sites were selected based on their geographical distribution and availability of calliphorid populations. The collected specimens were stored in ethanol tubes at room temperature until further processing. The total number of specimens collected from each location ranged from (200-220). However, only 25 specimens were randomly selected and examined from each location for this study. The specimens used in the study were from the third larval instar. This was chosen to ensure that all Samples were at a comparable stage of development.

Terminology: The terminology used in this study was based on Zumpt (1965). This reference was used to ensure consistency and accuracy in the identification and description of the third-instar calliphorid maggots.

Specimen preparation for scanning electron microscopy (SEM): To prepare the specimens for scanning electron microscopy (SEM), the larvae were killed by immersing them in hot water for ten minutes. Afterward, they were washed 2-5 times in alcohol to remove any external debris or contaminants. Dehydration of the specimens was achieved by exposing them to open air for five minutes. This step designed to remove any remaining moisture from the specimens before SEM analysis. To facilitate SEM analysis, the specimens were mounted on a metal plate for support. They were then coated with a 20-30 nm layer of gold. The gold coating improved conductivity and enhanced the quality of the images obtained during SEM analysis (Murtey and Ramasamy, 2016).

Scanning electron microscopy (SEM) Analysis: After an overnight wash in distilled water, the specimens were dehydrated in a graded series of ethanol. The dehydrated specimens were critically point- dried and mounted on aluminum stubs using colloidal silver paste. The mounted specimens were observed and photographed using an AXIA-CHEMISEM, Axia Chmi SEM Scanning Electron Microscope in Baghdad. Resolution and magnification capabilities were applied according to Colwell and O'Connor (2000). The specimens were examined under the SEM, and images were captured. The images were then transferred to a computer for further analysis and documentation.

RESULTS AND DISCUSSION

In this study, a comparative analysis was conducted on seven species of third- instar Calliphorid maggots found in central of Iraq. The species included in the study were *Calliphora vomitoria* (Linnaeus, 1758), *C. vicina* Robineau-Desvoidy, 1830, *Chrysomya albiceps* (Widemann, 1819), *Ch. rufifacies* (Macquart, 1844), *Ch. megacephala* (Fabricius, 1794), *Lucilia cuprina* (Wiedemann, 1830), and *L. sericata* (Meigen, 1826). The objective of the study was to morphologically characterize these species and construct an identification key to differentiate between them based on their morphological characteristics.

This study presents a key for the identification of third- instar larvae which is the first to cover the forensically most important species of blowflies in Iraq. The list of taxa included seven species of confirmed forensic relevance, the species included in the study were

Calliphora vomitoria (Linnaeus, 1758), *C. vicina* Robineau-Desvoidy, 1830, *Chrysomya albiceps* (Widemann, 1819), *Ch. rufifacies* (Macquart, 1844), *Ch. megacephala* (Fabricius, 1794), *Lucilia cuprina* (Wiedemann, 1830), and *L. sericata* (Meigen, 1826). Most of them are widespread in many adjacent countries and were frequently reported on large animal carrion and human corpses (Akbarzadeh *et al.*, 2015). Taxonomically significant characters were in the form of images produced using digital cameras mounted on a compound microscope, stereomicroscope and scanning electron_microscope (SEM). Many Taxonomic keys depend on morphological characteristics studied through the compound microscope (Zumpt, 1965; Erzinçlioğlu, 1987b; Holloway, 1991; Fan *et al.*, 1997; Wells *et al.*, 1999; Szpila, 2009; Prado *et al.*, 2023; Szpila *et al.*, 2024); these features include cephalopharyngeal skeleton, spinnulation, abdominal segments, anterior spiral lobe, and size due to peritreme. Additional traits, such as periodic external whorls, veins in anal segmental plates, and the presence of other distinguishing features were also considered. However, this study enhanced the taxonomic key special characteristics through scanning electron microscope such as: apical spines of dorsomedial processes in both species *Chrysomya albiceps*, *Ch. rufifacies*, the shape and distribution of spinose bands on segments, such as in species *Calliphora vomitoria*, *C. vicina*, *Lucilia cuprina* and *L. sericata* and the presence or absence of inner projection in the peritremal ring of posterior spiracles as observed in both species *Lucilia cuprina* and *L. sericata*.

Based on the analysis of the SEM images and morphological characteristics, the following results were obtained:

1- *Chrysomya albiceps*: This species exhibited a posterior spiral shape similar to *C. vomitoria* and C. vicina, with a unique surface texture, spinnulation on abdominal segments (Pl. 1A), and unique peritreme size.

2- *Ch. rufifacies*: The larvae of this species had a unique posterior spiral shape, rough surface, spinnulation (Pl. 1B), cephalopharyngeal skeleton (Pl. 2E), and a distinctive size due to peritreme compared to other species.

3- *Ch. megacephala*: This species exhibited a posterior spiral shape similar to *Ch. rufifacies*, but with a smoother surface, no spinnulation on abdominal segments, and a distinctive cephalopharyngeal skeleton (Pl. 2F).

4- *Calliphora vicina*: The larvae of this species resembled *C. vomitoria* in posterior spiral shape and absence of spinnulation (Pl. 1C), but showed slight differences in cephalopharyngeal skeleton at their bases (Pl. 2A), direction of pocesses, and peritreme size.

5- *C. vomitoria*: This species exhibited a unique posterior spiral shape, smooth surface, and no spinnulation on abdominal segments (Pl. 1D). Cephalopharyngeal skeleton has a distinctive direction and size (Pl. 2B).

6- ***Lucilia cuprina***: The larvae of this species resembled *Ch. megacephala* in posterior spiral shape, surface texture, and absence of spinnulation (Pl. 1E), but had slight differences in cephalopharyngeal skeleton (Pl. 2D), direction, and size due to peritreme.

7- ***L. sericata***: This species had a posterior spiral shape similar to *L. cuprina*, smoother surface, no spinnulation on abdominal segments (Pl. 1F), a characteristic cephalopharyngeal skeleton, and a unique size due to peritreme.

Overall, the comparative analysis of the seven species of third instar-larvae of calliphorid species using SEM and traditional optical microscopes, revealed distinct morphological characteristics that allowed for their differentiation. The constructed identification key based on these characteristics will be valuable for future studies in forensic entomology and related fields. Further research is warranted to explore the potential applications of these findings in practical forensic investigations.

Additional information on the morphology of important European blowfly species is scattered across various studies (Knipling, 1939; Zumpt 1965; Erzinçlioğlu 1985, 1987a, b, 1988, 1990; Liu and Greenberg 1989; Carvalho Queiroz *et al.*, 1997; Wells *et al.*, 1999; Wallman, 2001; Sukontasan *et al.*, 2003, 2008). Although several taxonomic keys have been combined so far, none of them yet cover the full list of species (Szpila, 2009). Among these, Ishijima key (1967) is considered the most comprehensive, with the exception of *C. albiceps* and *C. mortuorum.* Schumann (1971) provided other important keys; with a notable contribution to the understanding the larval character of the genus *Lucilia* Robineau-Desvoidii in Europe. European, judges are included in the third condition of the third site of both Christina and Cris Mann, an outstanding series of books with keys, published by Draber - Mońko (2004) through mid-and central European-life. About the science of statistics, they looked at the data. Erzinslioglu (1985) proposed a critical study of the morphology of the third stage of Calliphoridae.

Through this work, several morphological characters were identified that could be exploited for taxonomic reasons, such as cephalopharyngeal skeleton and direction of process on posterodorsal corner of their bases, spinnulation on abdominal segments, lobe of anterior spiral, and the size due to peritreme (Chrysomyinae vs others blowflies), along with the presence of many other characteristics. Currently the main criterion for identifying judgmentally important species of tertiary larvae is the identity of nearly all-important SEM images.

Key to the identity of third instar larvae for morphological evaluation:

1- Large, elongate, fleshy tubercles gift on each segment; tip of tubercles bearing several small spines (Pl. 1A, B)…………………… …..………………………………………… 2

- Abdominal segments lacking large, elongate tubercles (Pl.1C, D, E, F) …….……….....… 3

2- Outermost ventral process less cylindrical, greater triangular, and topped with smaller spines; apical spines of dorsomedial processes closely aggregated (Pl. 3A), anterior spiracles with 9-10 lobes (Pl. 4A)………………………………….. *Chrysomya albiceps*

- Outermost ventral process more cylindrical, less triangular, and crowned with larger spines; apical spines of dorsomedial processes more widely spaced (Pl. 3B), anterior spiracles with 10-11lobes (Pl. 4B) …………………………………...…..………. *Chrysomya. rufifacies*

3-Posterior spiracular peritreme incomplete, and more remote from each other (Pl. 5C)………… …..……………………………………….....……..*Chrysomya megacephala*

- Posterior spiracular peritreme complete (Pl..5D, E, F)…… ……………….......................4

4-Cephalopharyngeal skeleton with an accessory oral sclerite. (Pl. 2A, B)…*Calliphora* ..…5

- Cephalopharyngeal skeleton usually without an accessory oral sclerite (Pl. 2C, D) ………………………………………………………………………….... *Lucilia*….. 6

5- Spinose bands on segments with broad, three-or four-or rarely two-pointed spines (Pl. 6B); anterior spiracle with 8 lobes……………………………………..…. *Calliphora vomitoria*

- Spinose bands on segments with less broad, two- or rarely three-pointed spines (Pl. 6A); anterior spiracle with 9 lobes (Pl. 4C) ………………………………….. *Calliphora vicina*

6-Anterior spiracle with 9–10 lobes (Pl. 4F); Inner tubercles of upper margin of anal segment separated by a distance approximately equal to the distance between inner and median tubercles, peritremal ring of posterior spiracles with inner projection, spinose bands on segments with more than three-pointed spines (Pl. 5F) ……………..…….. *Lucilia sericata*

- Anterior spiracle with 7–8 lobes (Pl. 4E); Inner tubercles of upper margin of anal segment separated by a distance approximately equal to the distance between the inner and outer tubercles, peritremal ring of posterior spiracles without inner projection, spinose bands on segments with two- and three-pointed spines. (Pl. 5E) …………………..…. *Lucilia cuprina*

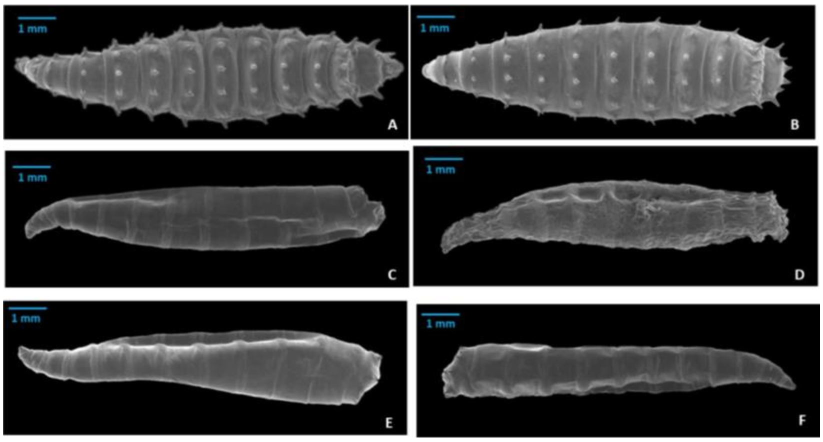

Plate (1): Scanning electron micrographs of a third instar larva of (A) *Chrysomya. albiceps* (B)*Ch. rufifacies* (C) *Calliphora vicina* (D) *Calliphora vomitoria* (E) *Lucilia cuprina* (F) *L. sericata*.

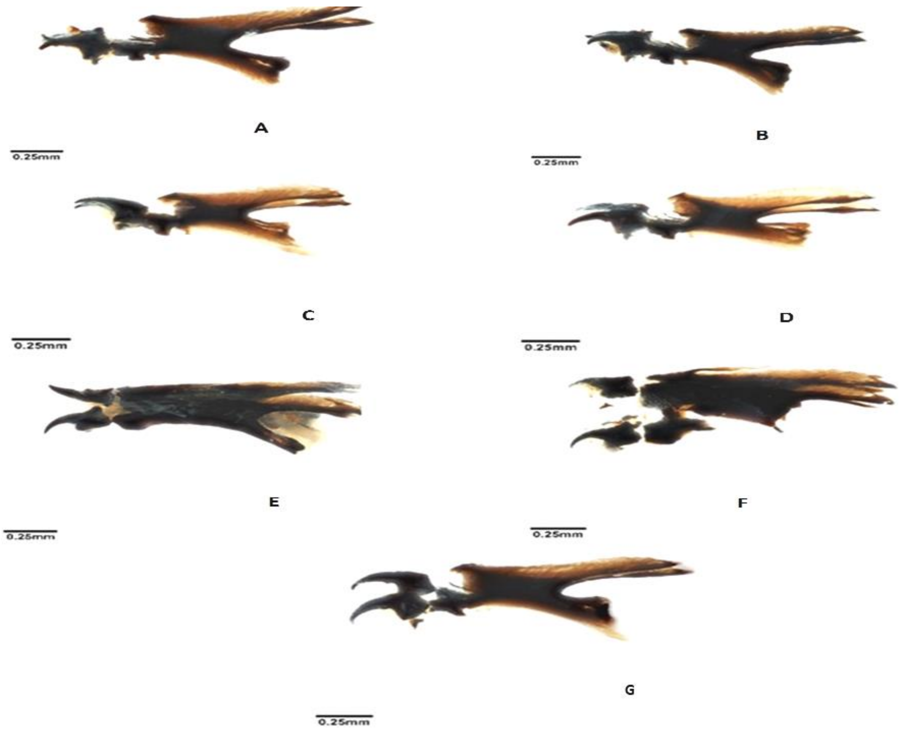

Plate (2): Light microscopic micrographs of cephalopharyngeal skeleton; (A) *Calliphora vicina*, (B) *Calliphora vomitoria*, (C) *Lucilia sericata*, (D) *Lucilia cuprina*, (E) *Chrysomya rufifacies*, (F) *Ch. megacephala* , (G) *Ch. albiceps*.

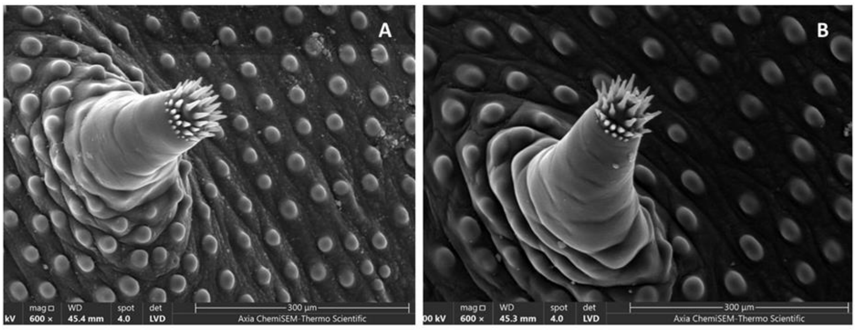

Plate (3) : Scanning electron of apical spines of dorsomedial processes; (A) *Chrysomya albiceps* (B) *Ch. rufifacies*.

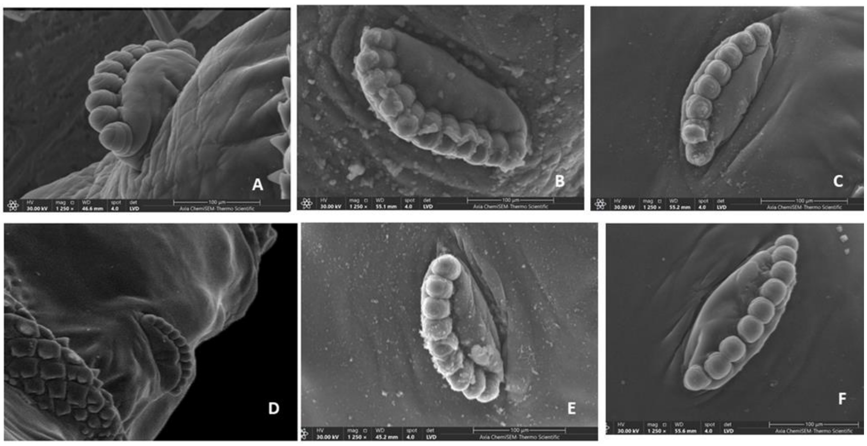

Plate (4): Scanning electron micrographs of anterior spiracle of a third instar larva; (A) *Ch. albiceps*, (B) *Ch. rufifacies*, (C) *Calliphora vicina*, (D) *Ch. megacephala*, (E) *Lucilia cuprina*, (F) *L. sericata*.

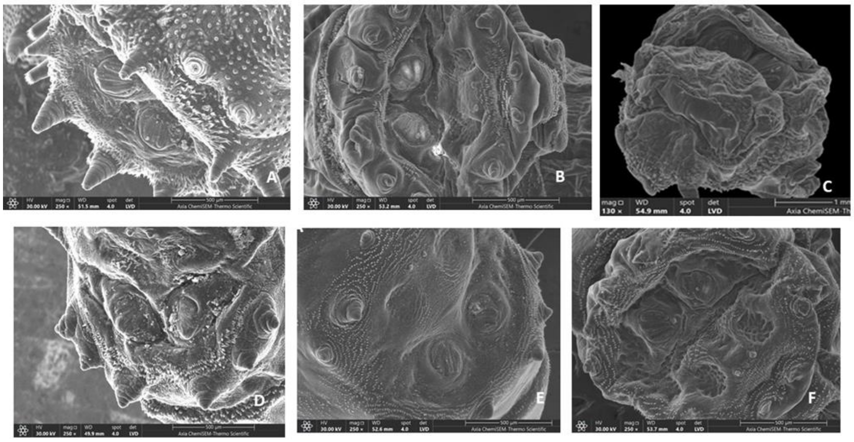

Plate (5): Scanning electron micrographs of posterior spiracle of a third instar larva; (A) *Ch. albiceps*, (B) *Calliphora vicina*, (C) *Ch. megacephala*, (D) *Calliphora vomitoria*, (E) *Lucilia cuprina*, (F) *L. sericata*.

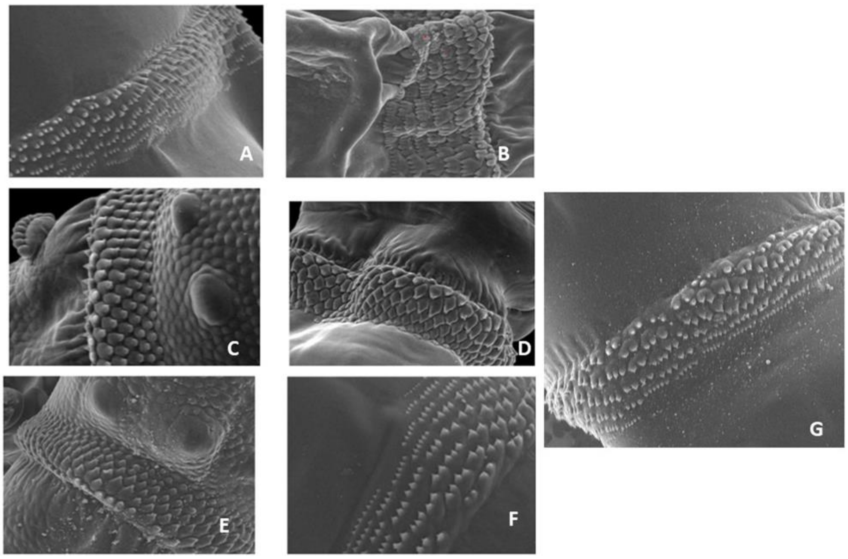

Plate (6): Scanning electron micrographs of spinose bands of third instar larva; (A) *Calliphora vicina* (B) *Caliiphora vomitoria* (C) *Chrysomya albiceps* (D) *Ch. megacephala* (E) *Ch. rufifacia* (F) *Lucilia sericata* (G) *L. cuprina*.

CONCLUTIONS

In conclusion, this study successfully morphologically characterized seven species of third instar Calliphorid maggots found in the middle of Iraq. Through the use of SEM images and traditional optical microscopes, the distinct morphological characteristics of each species were identified and described. An identification key was constructed to differentiate between these species based on their morphological features. The findings of this study have important implications for forensic entomology, forensic biology, and ecological studies. Future research could incorporate genetic analysis and phylogenetic studies to further enhance our understanding of these species.

CONFLICT OF INTEREST STATEMENT

The results of this study are part of the requirements of the Ph. D. in Zoology, Department of Biology, College of Science, University of Baghdad for the first author.

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التوصيف المظهري ليرقات الذبابة المعدني (Diptera, Calliphoridae) من وسط العراق

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الخلاصة

هدفت هذه الدراسة إلى إجراء تحليل مقارن لسبعة أنواع من يرقات الطور الثالث للذباب المعدني الموجودة في وسط العراق. كان الغرض هو توصيف هذه الأنواع شكليًا وتطوير مفتاح تعريف لتمييزها عن بعضها البعض. تم استخدام الصور الرسومية للمجهر الإلكتروني الماسح (SEM) والمجاهر الضوئية التقليدية عالية الدقة لتحليل الخصائص المظهرية للأنواع:

Calliphora vomitoria (Linnaeus, 1758), *C. vicina* Robineau-Desvoidy, 1830, *Chrysomya albiceps* (Widemann, 1819), *Ch. rufifacies* (Macquart, 1844), *Ch. megacephala* (Fabricius, 1794), *Lucilia cuprina* (Wiedemann, 1830), and *L. sericata* (Meigen, 1826).

تم تصميم مفتاح تشخيصي بناءً على الاختلافات المظهرية لتمكين الفصل الدقيق بين الأنواع. نجحت الدراسة في وصف الطور الثالث للأنواع السبعة. يوفر المفتاح طريقة موثوقة لعزل الأنواع بناءً على صفاتها المظهرية. تساهم هذه الدراسة في المجال الميداني من خلال تقديم توصيف مظهري شامل لسبعة أنواع من يرقات الذباب المعدني الموجودة في وسط العراق. يوفر مفتاح التعريف المطور أداة قيمة للبحث المستقبلي وتحقيقات الطب الشرعي التي تشمل هذه الأنواع، مما يتيح التعرف الدقيق على الأنواع والتمايز بينها.