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Ultrastructural Analysis of Eggs of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae): Morphological Traits and Taxonomic Implications

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Abstract: This study aimed to investigate the chorionic ultrastructure of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) eggs from six different geographical populations in Iraq (Babylon, Karbala, Najaf, Wasit, Maysan, and Basrah). Specimens were collected between March 18, 2023, and April 11, 2023, and reared under controlled laboratory conditions. The chorion morphology of the eggs was examined using a Scanning Electron Microscope (SEM) to identify the morphological variations among different regions. The SEM analysis revealed distinct differences in the surface patterns of the egg chorion among the populations. The Samples of egg population from Babylon exhibited a rough and irregular surface texture. In contrast, eggs from Karbala, Najaf, and Wasit displayed varied geometric patterns, while the Basrah population was characterized by a distinct octagonal pattern .The study concludes that Specimens from Najaf showed a significantly different polygonal cell area compared to other regions. In contrast, no significant differences were detected among specimens collected from Wasit, Karbala, Maysan, and Basrah provinces.

Keywords: *Galleria mellonella*‹ TESCN UEGA3 Sem Gzech ‹Chorion ‹ Polygenel cells ‹ Micropyle.

Introduction

The greater wax moth *Galleria mellonella* (Linnaeus, 1758) Lepidoptera: Pyralidae) is a destructive pest of significant economic importance to honeybee colonies, with a wide global distribution (Roversi *et al.,* 2008; Quansah *et al*., 2022). The taxonomic complexity of the Pyralidae family and the influence of phenotypic plasticity make species identification challenging based on morphology alone. To overcome this, our study utilizes the stable patterns of the egg chorion as a reliable tool to differentiate G. mellonella populations and investigate their intraspecific variation. (Solis, 2007; Candan & Bayrakdar, 2008). Females lay eggs in large quantities, a critical trait for ensuring the species' survival. Consequently, these insects exhibit high fertility to enhance their ecological success (Mari *et al*., 2018; Gooch *et al*., 2021).

Generally, the eggs of Lepidoptera are covered by a hard, protective outer layer called the chorion, beneath which lies a thin wax layer that prevents desiccation. The egg also features small funnel-shaped openings (micropyles) at its anterior end, facilitating sperm entry for fertilization (Dolinskaya, 2019).

The chorion exhibits either simple or ornate patterns. It may appear as a network of polygonal cells or consist of transverse or longitudinal ridges forming polygonal structures termed the micropylar rosette. This rosette serves as a taxonomic fingerprint for studying interspecific variation (Hosamani *et al*., 2017; Kwadha *et al*., 2017; Desai *et al*., 2019).

The chorion additionally contains minute pores called aeropyles, visible only via SEM, which facilitate gas exchange. A specialized opening, the micropyle, is also present on the chorion, serving as the entry point for sperm during fertilization. These ultrastructural features cannot be resolved using conventional light microscopy, necessitating SEM for detailed chorion analysis and interspecific differentiation (Chapman, 1998).

The study of egg morphology serves as a powerful tool for insect species identification, particularly in distinguishing between closely related species with similar external characteristics, thereby enhancing taxonomic accuracy and reliability. This study aimed to investigate and scientifically describe fine-scale morphological features of eggs to assess the degree of morphological variation among examined specimens. Such characterization may contribute to improved diagnostic precision for *Galleria mellonella*, complementing other morphological identification criteria. Due to the absence of specialized studies employing scanning electron microscopy (SEM) for egg characterization, and with local research restricted to pest control SEM enables high-resolution morphological characterization of the chorion, revealing taxonomically informative variations among species. Such studies provide critical diagnostic tools for examining chorion microstructure across host populations, genera, and species within Lepidoptera (Kong *et al*., 2019 & Liu *et al*., 2023; Xu & Wang, 2013).

methods, this study utilized SEM technology to enhance morphological taxonomy. This approach improves diagnostic precision and represents the first application of its type in Iraq.

Materials & Methods

Collection and rearing of *G. mellonella*

Adult *G. mellonella* moths were collected from infested honeybee colonies (apiaries) across central and southern Iraq, including the following provinces and GPS coordinates (Table 1).

Sampling was conducted between March 18 and April 11, 2023. The insects were placed on infested wax combs containing various developmental stages of the pest and reared in the Insect Laboratory, Department of Plant Protection, College of Agriculture, University of Basrah. Wax combs containing *Galleria mellonella* were placed in rearing cages (30 × 19 × 19 cm) equipped with old wax frames. The insects were maintained under laboratory conditions, with temperatures ranging from 25–32°C and relative humidity from 10–60%, as monitored using a thermo-hygrometer (MAX-MIN THERMO HYGRO-CLOCK). The mean environmental conditions were 28 ± 2°C and 70 ± 10% relative humidity. Each cage was labeled with the collection site, apiary name, collection date, and corresponding GPS coordinates. After adult emergence, five mated females were isolated and placed individually in 9 cm Petri dishes for oviposition (Firacative *et al*, 2020).

Sample preparation for scanning electron microscope (SEM)

Collected eggs were gently washed with distilled water to separate individual eggs and remove surface debris. The specimens were then divided into two groups for analysis. Eggs from the first group were used to measure egg length and width under a light microscope at 40× magnification. Images were captured using a Nikon digital camera and analyzed with ImageJ software to obtain precise morphometric measurements. Eggs from the second group were carefully transferred using a fine brush onto a piece of black cloth placed inside a Petri dish. They were then dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%) to ensure proper fixation. The eggs were incubated in a graded ethanol series for dehydration as follows: 10 minutes each in 30% and 50% ethanol, 15 minutes each in 70% and 90% ethanol, and 20 minutes in absolute (100%) ethanol (Tattini *et al*., 2007).

Table (1). Geographic coordinates of *G. mellonella* collection sites in central and southern Iraq.

provinces	Region	Latitude (N)	Longitude (E)	Coordinates (Decimal Degrees)
Babylon	**Sadt-Al-hindia**	32°41'28.8"	44°25'01.6"	32.6913, 44.4171
Karbala	**Al-hur**	32°38'54.0"	43°58'28.9"	32.6483, 43.9747
Najaf	**City center**	32°00'40.6"	44°21'12.3"	32.0113, 44.3534
Wasit	**Al-ahrar**	32°33'39.0"	45°48'20.9"	32.5608, 45.8058
Maysan	**Al-emara**	31°51'02.2"	47°06'58.9"	31.8506, 47.1164
Basrah	**Abo-al-khaseeb**	30°25'58.6"	47°52'58.5"	30.4329, 47.8829

Fixation: After dehydration, eggs were stored in 10% formalin within tightly sealed vials for preservation. Samples were sent to the Ministry of Science and Technology's SEM Laboratory (Baghdad). A TESCAN VEGA3 SEM was used for high-resolution imaging. Accelerating Voltage: 5 Kv, Statistical analysis of egg morphological traits using GenStat 2012, Comparative Analysis, and One-way ANOVA to compare: polygonal cell area between specimens collected from different provinces, and micropyle structural variations among populations.

Magnifications: 200x, 500x, 1000x, 5000x, and 10,000x.

Morphological features studied: Chorion surface structure, Micropyle morphology, and polygonal cells (shape, arrangement, and edges). Postgraduate Entomology Laboratory / Department of Plant Protection .

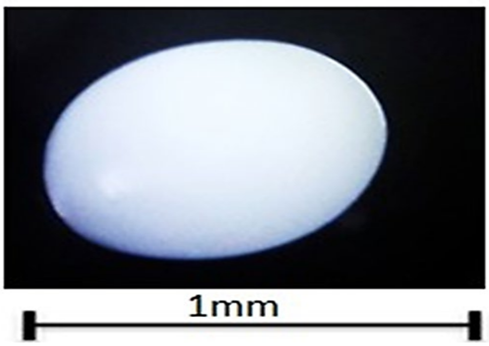

Fig. (1): Size and shape of *G. mellonella* eggs.

Statistical analysis of egg morphological traits using GenStat 2012, Comparative Analysis, and One-way ANOVA to compare: polygonal cell area between specimens collected from different provinces, and micropyle structural variations among populations.

Results & Discussion

Description of eggs using a light microscope

The eggs of *G. mellonella* are exceptionally small (0.4–0.7 mm in length and 0.3–0.6 mm in width) and exhibit an ovoid shape. Initially, they appear glossy white with a pinkish tint, gradually turning yellow during embryonic development (Fig. 1). These findings align with previous descriptions by Ménard *et al.* (2021) and Lange *et al.* (2018), confirming the species' consistent egg morphology across studies.

Significant variations in egg surface area (mm^2) among different geographical populations of *G. mellonella* (Table 2). Najaf population showed the largest egg surface area (0.4687 mm^2), differing significantly ($p<0.05$) from all other regions. Specimens of Basrah (0.4047 mm^2) and Maysan (0.3773 mm^2) populations formed an intermediate group; Specimens of Karbala (0.3387 mm^2) and Wasit (0.1937 mm^2) had the smallest recorded egg sizes. Specimens collected from Najaf showed the largest egg surface area with significant differences from all regions. Results suggest potential geomorphological variation linked to environmental conditions or host plants. This was corroborated by Reavey (1992). Egg surface area is influenced by multiple factors, including diet composition and female age. Environmental factors also play a significant role, as eggs laid during winter exhibit larger dimensions compared to those produced in moderate seasons

Table (2): Egg surface area of *G.mellonella* in the studied regions (mm^2).

province	(Mean ± SD)	Statistical Grouping*
Najaf	0.4687 ± 0.012	a
Basrah	0.4047 ± 0.015	b
Maysan	0.3773 ± 0.009	bc
Karbala	0.3387 ± 0.011	c
Wasit	0.1937 ± 0.008	d
Babylon	0.4510 ± 0.013	a

Different lowercase letters indicate significant differences ($p < 0.05$).

Description chorion scanning electron microscope (SEM)

The results of SEM examination at 1000× magnification (Figure 2, Table 2) revealed a significant geographical variation in chorion architecture among populations of *G. mellonella*. In Karbala & Najaf, the integument exhibited dominance of 4-5 sided polygons with non-angular, rhomboid/elliptical structures (Fig. 2B, 2C). In Wasit, the surface was characterized by fused polygons forming elongated chains without clear boundaries (Fig. 2D). Maysan samples showed 7-sided polygons coexisting with semi-circular ornaments (Fig. 2E), aligning with Kirti & Kaur (2014). In Basrah, the highest geometric diversity (4-8 sides) was recorded, lacking irregular forms (Fig. 2F). while specimens were unique in showing an absence of polygonal cells, with only tubercle-like structures observed (Fig. 2A). These results are similar to what was shown by Cônsoli *et al.* ,(1999) regarding differences in the shapes of the polygons on the chorion of the rice bollworm *Corcyra cephalonica* (Pyralidae). Xu &Wang (2013) showed that there are rounded, oval, and semi-circular shapes on the exochorion. Babylon's tuberculated chorion suggests adaptive divergence, possibly due to local host plant interactions, and Genetic isolation. Basrah's geometric regularity may indicate stable environmental conditions.

Polygonal cell area

The results of the statistical analysis in Table 3 showed that the polygonal cell area was calculated. Najaf population exhibited significantly larger polygonal cell area (0.000224 mm²) compared to other regions ($p<0.001$). No significant differences (NS) among Wasit, Karbala, Maysan, and Basrah specimens ($p>0.05$), Babylon specimens were excluded from analysis due to the complete absence of defined polygonal cells. The 55.4% larger cell area in Najaf specimens suggests potential: local adaptation to environmental factors, genetic divergence in chorion development. The larger polygonal cell area observed in Najaf specimens indicates enhanced cellular activity or may result from genetic regulation of chorion protein expression, as proposed by Wang *et al*., (2010).

Table (3): Polygonal cell shape of *G. mellonella* eggs in the studied regions.

province	Polygonal Cell Shapes	Unique Features
Karbala	Quadrilaterals, pentagons	Irregular rhomboid/elliptical ornaments
Najaf	Quadrilaterals, pentagons	Irregular rhomboid/elliptical ornaments
Wasit	Quadrilaterals, pentagons, hexagons	Elongated fused polygons
Maysan	Pentagons, hexagons, heptagons	Oval/semi-circular irregular edges
Basrah	Quadrilaterals to octagons	No irregular shapes
Babylon	No defined polygonal cells	Tuberculated rough surface

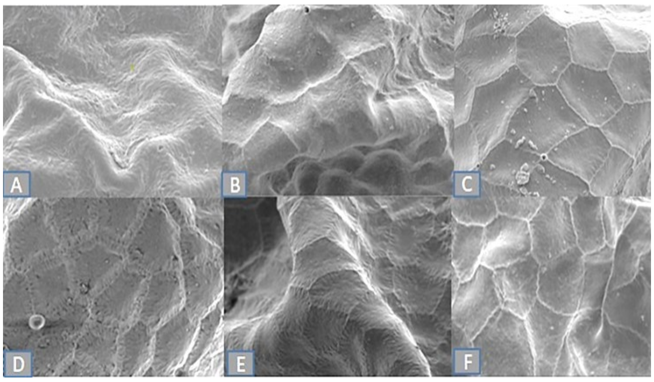

Fig. (2): Description of the polygonal cell shapes of *G. mellonella* eggs in the study regions; (A) Babylon, (B) Karbala, (D) Najaf, (C) Wasit, (E) Maysan , (F) Basrah. [X 1000].

Table (3): Polygonal cell area of *G. mellonella* eggs in the studied regions (mµ).

Province	Mean Area ± SD (µm)	Statistical Grouping*	Significance
Najaf	0.000224 ± 0.000015	a	p<0.001
Wasit	0.000144 ± 0.000009	b	NS
Karbala	0.000129 ± 0.000008	b	NS
Maysan	0.000123 ± 0.000007	b	NS
Basrah	0.0001227 ± 0.000006	b	NS

Polygonal cell edges

Ultrastructural analysis of polygonal cell edges in *G. mellonella* Eggs (Figure 3) (10000x) reveals significant geographical variation in polygonal cell edge morphology across populations: Babylon (Fig.3A) absent defined edges, entire surface covered by irregular tubercles forming rough texture. Specimens from Karbala (Fig.3B) and Najaf (Fig.3C) fused tubercular edges, ovoid/round tubercles forming rough sutures. Specimens of Wasit (Fig.3D) with Keratinized ridges, spherical/ovoid tubercles forming thick, zigzag margins, whereas the specimens of Maysan (Fig.3E) have irregular keratinization, uneven elevations with coarse edges. Specimens of Basrah (Fig.3F) have micro-tubercle fusion, miniature ovoid tubercles with partial edge disappearance. These results are similar to what Arbogast *et al*., (1980) proposed that these irregular keratinized edges reflect a protective egg architecture against environmental stressors.

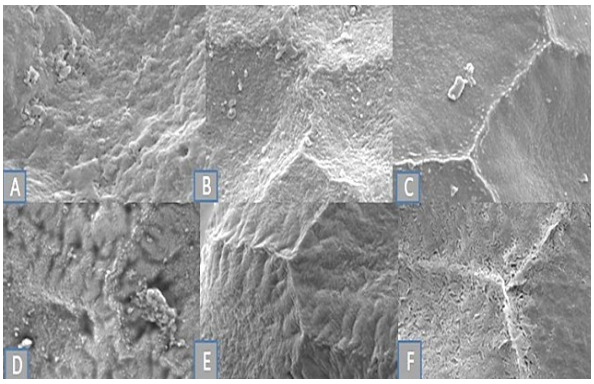

Fig. (3): Polygonal cell edges of *G. mellonella* eggs in the study regions; (A) Babylon, (B) Karbala, (D) Najaf, (C) Wasit, (E) Maysan, (F) Basrah.[10000X].

Micropyle

The results of Figure 4 showed a variation in the micropyle of the studied specimens, as the micropyle in the specimens was a Babylon (4A) micropyle shape , ovoid, with irregular rough edges, and embedded under a tuberculated operculum. Specimens of Maysan (4E) with ovoid shape, defined edges, and distinct marginal articulation, Karbala (4B) and Najaf specimens (4C) with circular, prominent, raised edges, Consistent Pyralidae type morphology. Wasit (4D) circular, recessed, irregular edges, highest cellular connectivity. Basrah (4F) circular, smooth, raised edges, most geometrically perfect form. These results align with the findings reported by Jagielski *et al.* ,(2020).

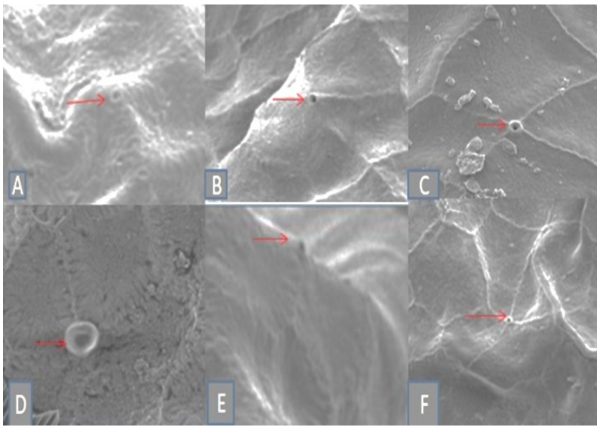

Fig. (4): Micropyle edges of *G. mellonella* eggs in the study regions; (A) Babylon, (B) Karbala, (D) Najaf, (C) Wasit, (E) Maysan, (F) Basrah.[1000X].

Conclusions

This study provides the first ultrastructural characterization of *Galleria mellonella* eggs from Iraq, revealing geographic variation in

chorionic morphology. Specimens from Babylon were unique in lacking polygonal cells and exhibited exclusively tuberculate surface structures. In contrast, populations from Basrah displayed the highest geometric diversity, with polygons ranging from four to eight sides. Specimens from Najaf possessed larger polygonal areas, suggesting that these chorionic features may hold potential taxonomic and diagnostic significance.

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Contributions of authors

Both authors (R.M. Khlaf and M.A. Al-etby) collaborated on field collection, laboratory rearing, and SEM ultrastructural analysis. They jointly performed the data interpretation, statistical analysis of chorion patterns, and finalized the manuscript for publication.

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Conflict of Interest

The authors declare no conflicts of interest associated with its publication.

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التحليل الدقيق لبيض حشرة Galleria *mellonella* L. (Lepidoptera: Pyralidae) : الصفات المظهرية والتداعيات التصنيفية في العراق

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المستخلص: هدفت هذه الدراسة إلى معرفة التركيب الدقيق لقشرة البيض *Galleria mellonella* (Linnaeus, 1758) من ست مناطق جغرافية مختلفة في العراق (بابل، كربلاء، النجف، واسط، ميسان، والبصرة). جُمعت العينات في الفترة ما بين 18 مارس 2023 و11 أبريل 2023، ورُبيت في ظروف مختبرية. تم فُحص قشرة البيض مظهريا باستخدام المجهر الماسح إلالكتروني (SEM) لتحديد الاختلافات المظهرية المحتملة. اوضحت النتائج تباين كبير في انماط شكل القشرة الخارجي بين المناطق المجموعات المدروسة اذ أظهرت مجموعة بابل نسيجًا سطحيًا خشنًا وغير منتظم. في المقابل، أظهرت بيض كربلاء والنجف وواسط أنماطًا هندسية متنوعة، بينما تميزت مجموعة البصرة بنمط ثماني الأضلاع مميز. علاوة على ذلك، كشف التحليل المورفومتري للخلايا المضلعة على سطح البيض عن اختلافات كبيرة. أظهرت مجموعة النجف مساحات خلايا متعددة الأضلاع أكبر بشكل ملحوظ إحصائيًا مقارنةً بجميع المناطق الأخرى المدروسة ($P < 0.05$). ولم تُلاحظ أي فروق جوهرية في هذه السمة بين مجموعات واسط وكربلاء وميسان والبصرة. تشير هذه النتائج إلى أن الموقع الجغرافي قد يؤثر على خصائص القشرة في بيوض *G. mellonella*، مما يُبرز إمكانية وجود تمايز على مستوى المجموعة. الكلمات المفتاحية: *Galleria mellonella*، TESCN UEGA3 Sem Gzech، سطح القشرة، خلايا متعدد الاضلاع، فتحة النقير.

الكلمات المفتاحية: *Galleria mellonella* ، الماسح الالكتروني TESCN UEGA3 Sem Gzech ، قشرة البيض، خلايا متعدد الاضلاع، النقير.