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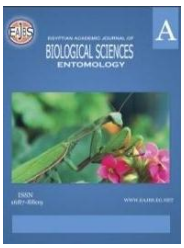

Morphological Characterization and Molecular Barcoding of Angoumois Grain Moth, *Sitotroga cerealella* in Qus, Qena Governorate, Egypt

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ABSTRACT

The Angoumois grain moth, *Sitotroga cerealella*, is a significant global stored grain pest and a vital factitious host for mass-rearing egg parasitoids and certain entomophagous insects in biological control laboratories. Despite its dual importance, the comprehensive characterization of *S. cerealella* populations is often lacking. So, the present study aimed to provide a detailed morphological and molecular identification of *S. cerealella* adults from Qus, Qena Governorate, Egypt. The morphological identification was performed using light microscopy (LM) and scanning electron microscopy (SEM), documenting features like body length, antennae, wings, and male genitalia. Males (avg. 4.27 mm) were significantly shorter than females (avg. 5.354 mm). Molecular identification involved amplifying and sequencing the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene. The resulting 636 bp *COI* sequence under accession number (MW452547) in GenBank. BLAST searches and phylogenetic trees analyses (maximum parsimony, minimum evolution, and neighbour joining) consistently confirmed the specimens' identity as *Sitotroga cerealella*, exhibiting 100% genetic identity with existing GenBank sequences. This integrated morphological and molecular profile of *S. cerealella* from Egypt is crucial for accurate pest identification and optimizing *Trichogramma* mass rearing, which is conducted on a large scale in Egypt and worldwide, thus promoting sustainable biological control strategies to achieve sustainable development goals (SDGs).

INTRODUCTION

The Angoumois grain moth, *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae), is recognized as one of the most important and destructive pests of stored grains worldwide. It is a global pest, infesting numerous crops such as corn, sorghum, soybean, rice, paddy, and their processed derivatives (Trematerra, 2015; Abde-Hameid, 2018; Sowmya *et al*., 2023). This moth alone can cause up to 40% loss, and when combined with other grain pests, economic losses can escalate to 50% (Ashamo, 2010; Trematerra, 2015; Hasan *et al*., 2025). Furthermore, direct consumption of *S. cerealella* infestations

significantly reduces grain quality via contamination (frass, webbing, dead insects), rendering produce unsuitable and necessitating costly cleaning or disposal (Chowdhury *et al*., 2023). The reliance on repeated chemical fumigation for control further elevates expenses and raises environmental and health concerns. These escalating costs and growing pesticide resistance highlight an urgent need for sustainable, economically viable alternatives. Beyond its direct impact as a pest, *S. cerealella* holds significant importance in biological control. The multiplicity of its hosts, coupled with the ease and low cost of its rearing, makes it a pivotal factitious host for the mass production of *Trichogramma* spp., egg parasitoids in biological control laboratories (Muhammad *et al*., 2012; Abdel-Hameid, 2018). Globally, approximately 18 different species of the genus *Trichogramma* are applied to control pests in various crops, including cotton, other field crops, and vegetables (Zang *et al*., 2021; Dodiya *et al*., 2023). For instance, in Egypt, Abdel-Galil *et al*. (2018) reported the use of *S. cerealella* for rearing *T. turkestanica*. Despite its dual significance as both a major pest and a crucial rearing host, few studies provide a comprehensive morphological and/or molecular identification of *S. cerealella*. Most existing research tends to focus on specific organs, such as antennae or ovipositors (Ma *et al*., 2017). However, Li *et al*. (2025) used transcriptome analysis and molecular docking to investigate the olfactory mechanisms of *S. cerealella*, providing insights into how the moth detects odors. Given its critical role as a primary host for *Trichogramma* rearing, it is imperative to fully identify and acquire a complete understanding of its morphological and molecular characteristics. The mitochondrial cytochrome c oxidase subunit 1 (*COI*) protein-coding gene has emerged as a widely adopted, practical, and standardized species-level barcode across most of the animal kingdom (Kachhawa, 2023; Ahmed *et al*., 2024). Therefore, the present study sought to thoroughly characterize the morphological features of adult *S. cerealella* moths and to confirm the strain's identity, both morphologically and molecularly, utilizing *COI* sequencing techniques.

MATERIALS AND METHODS

1-Insect Collecting and Rearing:

Angoumois grain moths were sourced from the *Trichogramma* mass rearing lab in Qus, Qena Governorate, Egypt, to generate enough. These moths were then reared for five generations (23 ± 2 °C, 75 ± 5 % RH, L 16: D 8) at the Biological Control Lab, Plant Protection Department, Assiut University, Assiut, Northern Upper Egypt.

2-Morphological Identification:

2.1. Light Microscope (LM):

For species identification, the wings of adult Angoumois grain moths were spread using pins, and other body morphological characters were also examined. All measurements were conducted with an HDMI MULTI-OUTPUT HD camera (Toup Cam_120) according to Abdel-Galil *et al.* (2023).

2.2. Scanning Electron Microscopy (SEM):

Scanning electron microscopy was performed at the Electron Microscopy Unit, Central Lab, Faculty of Science, South Valley University, Qena, Egypt. Imaging utilized a JEOL JSM-5500 LV Scanning Electron Microscope (JEOL, Japan). Before imaging, adult moths were spread on a holder, fixed in FAE (formaldehyde, acetic acid, and ethanol), and stored in 70% ethanol, following Polilov (2017).

2.3-Morphological Characters Measurements:

Identification of specimens relied on available taxonomical knowledge. Species were first identified morphologically, according to Ma *et al*. (2017) and Shah *et al.* (2020). For morphometric measurements, including scales and all morphological measures on

photomicrographs, Image J 1.48V (Schneider *et al*., 2012) was employed, with all data recorded in millimeters (mm).

3-Molecular Genetic Identification:

3.1-DNA Extraction:

Genomic DNA was extracted from Angoumois grain moth legs following the manufacturer's guidelines using the QIAamp DNA Mini kit (Qiagen, Hiden, Germany).

3.2-PCR Conditions:

The partial mitochondrial *COI* gene was then amplified using the LCO1490 forward and HCO2198 reverse primers (Folmer *et al*., 1994). PCR assays were conducted in 50 µl reactions (25 µL PCR master mix, 1 µL each primer, 1 µL genomic DNA), followed by cycling conditions: initial denaturation at 94 ºC for 240 sec.; 30 cycles of denaturation (94 ºC, 60 sec.), annealing (49 ºC, 60 sec.), and extension (72 °C, 60 sec.); and a final extension at 72 ºC for 10 min. Amplified products were separated on a 1.5% agarose gel containing ethidium bromide and sized using a 100bp DNA Ladder RTU (GeneDireX).

3.3-PCR Product Purification and Sequence Determination:

DNA sequencing was exclusively performed by Macrogen (Seoul, South Korea). The resulting sequences were submitted to the National Center for Biotechnology Information (GenBank/NCBI) to obtain accession numbers.

3.4-Alignment and Phylogenetic Analysis:

Sequence alignment utilized MUSCLE (Edgar, 2004) with its default settings. Phylogenetic tree analyses were conducted using MEGA version 7.018 (Kumar *et al*., 2016), employing Maximum Parsimony (MP), Neighbor-Joining (NJ), and Minimum Evolution (ME) methods, each with 1000 bootstrap iterations (Felsenstein, 1985). Sequence divergences were calculated using Kimura two-parameter distances (Kimura, 1980). To determine sequence similarity against existing data, BLAST searches of the GenBank NCBI database were performed.

3.5-GenBank Accession Number:

The *COI* gene rDNA sequence for Sitotroga, which is discussed in this paper, has been partially deposited and can be found in the DDBJ (www.ddbj.nig.ac.jp/), EMBL (www.embl.de/), and GenBank nucleotide sequence databases ([http://www.ncbi.nlm.nih.gov] (http://www.ncbi.nlm.nih.gov)) under the accession number MW452547.1.

4-Data Processing:

Morphometric measurement data were generated using Image J 1.48V (Schneider *et al*., 2012). The calculation of means ± standard deviation (SD) and t-value were performed in Microsoft Excel 2016.

RESULTS

1- **Morphological Identification:**

As observed under light microscopy, the adult moth exhibits a moderately glossy, grey to brown coloration, with its body densely covered by scales and hairs (Fig. 1). Pronounced sexual dimorphism is evident in the abdomen upon ventral examination: females (Fig. 1& 2A) display a broader, longer, and typically unpigmented abdomen, whereas males (Fig. 1& 2B) present a narrower, pointed, and often blackish abdomen.

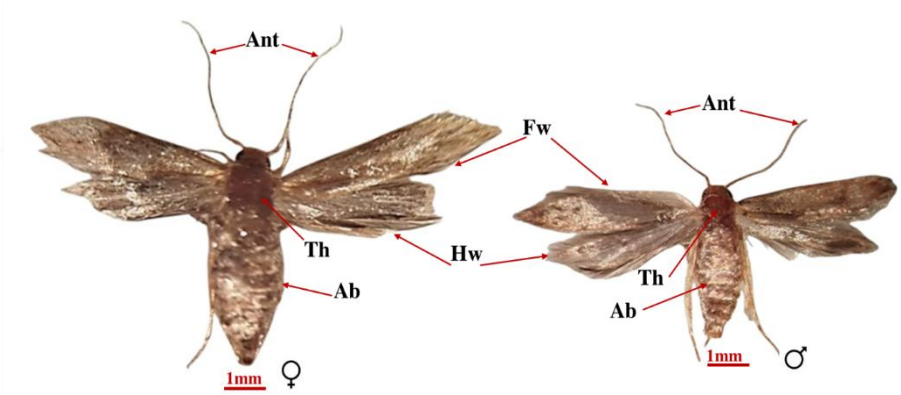

Fig.1: Light photomicrographs illustrate the dorsal view of sexual dimorphism in adult *Sitotroga cerealella*, with **A.** Representing the female, and **B.** The male. Ant: antennae, Fw: Forewings, Hw: Hindwings, Th: Thorax, Ab: Abdomen.

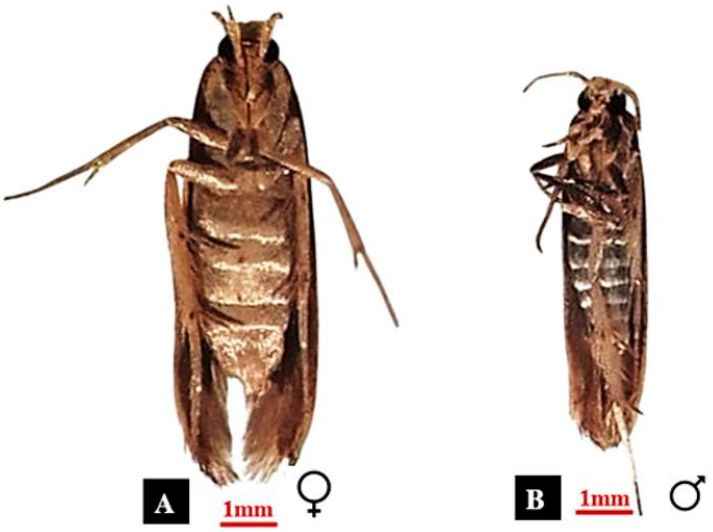

Fig.2: Light photomicrographs illustrate the ventral view of sexual dimorphism in adult *Sitotroga cerealella*, with **A.** Representing the female, and **B.** The male

1.1-The body Length:

Mature female Angoumois grain moth measured 5.354±0.498 mm in length (range: 4.94–6.61 mm), while males averaged 4.27±0.46 mm (range: 4.01–5.46 mm). This significant sexual dimorphism in body length ($t=3.32$, $P<0.0036$, $n=10$ per sex) indicates males are generally smaller than females.

1.2. The Head:

In the adult Angoumois grain moth, the head is relatively small, pale brown to buff, and covered in fine setae, blending with the body. This structure is morphologically significant, containing key sensory organs such as prominent compound eyes and filiform antennae, alongside mouthparts specifically adapted for a non-feeding adult lifestyle (Fig. 3A).

1.2.a. Compound Eyes:

The large compound eyes, occupying a significant portion of the head, are encircled by fine hairs **(Fig. 3B).** High magnification reveals the ocelli within each compound eye to be clean, clear, and arranged in a regular hexagonal pattern (Fig. 3C).

1.2.b. Mouthparts:

Scanning Electron Microscopy (SEM) photomicrographs of Angoumois grain moth adults reveal mouthparts modified into a well-developed proboscis for fluid feeding, along with maxillary and labial palps (Fig. 3A).

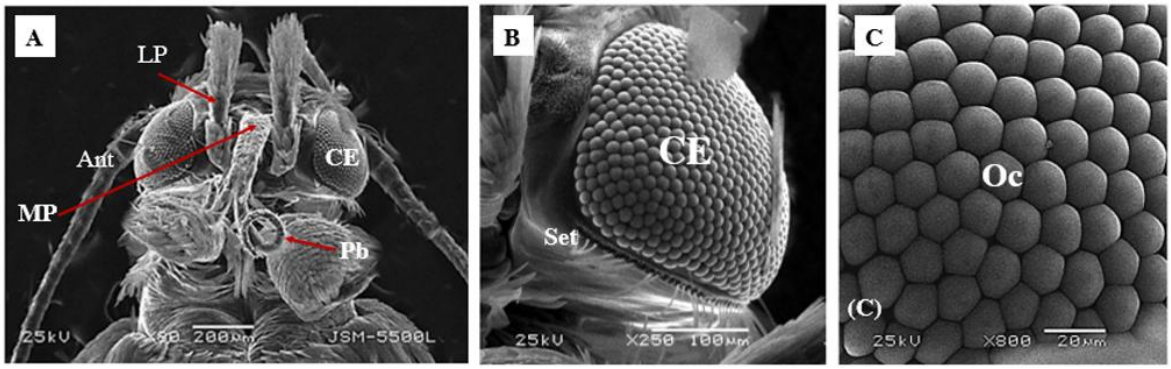

Fig.3: SEM photomicrographs of Adult *Sitotroga cerealella,* head ventral view. **A.** Full ventral view of the head compound eyes, antennae, Labial palp, Maxillary palp, and proboscis. **B.** Magnified view (x 250) of a compound eye illustrating the hexagonal Ocelli and Fine setae surrounding the edge of the compound eye. **C.** Hexagonal pattern shape of the ocelli under high magnification x800.
CE: Compound Eye, Ant: antennae, LP: Labial Palp, MP: Maxillary Palp, PB: Proboscis, Set: Setae, and Oc: Ocelli.

1.2.c. Antennae:

Each slender, thread-like antenna of Angoumois grain moth (Fig. 4) is segmented into a basal scape, a pedicel, and a long, multi-segmented flagellum. These structures are vital chemosensory organs, enabling the detection of pheromones critical for mating and other environmental cues. While female antennae (average: 3.71±0.27 mm, range: 3.11–3.98 mm) and male antennae (average: 3.59±0.21 mm, range: 3.23–3.89 mm), statistical analysis revealed no significant difference in length (t=1.124, P=0.2912).

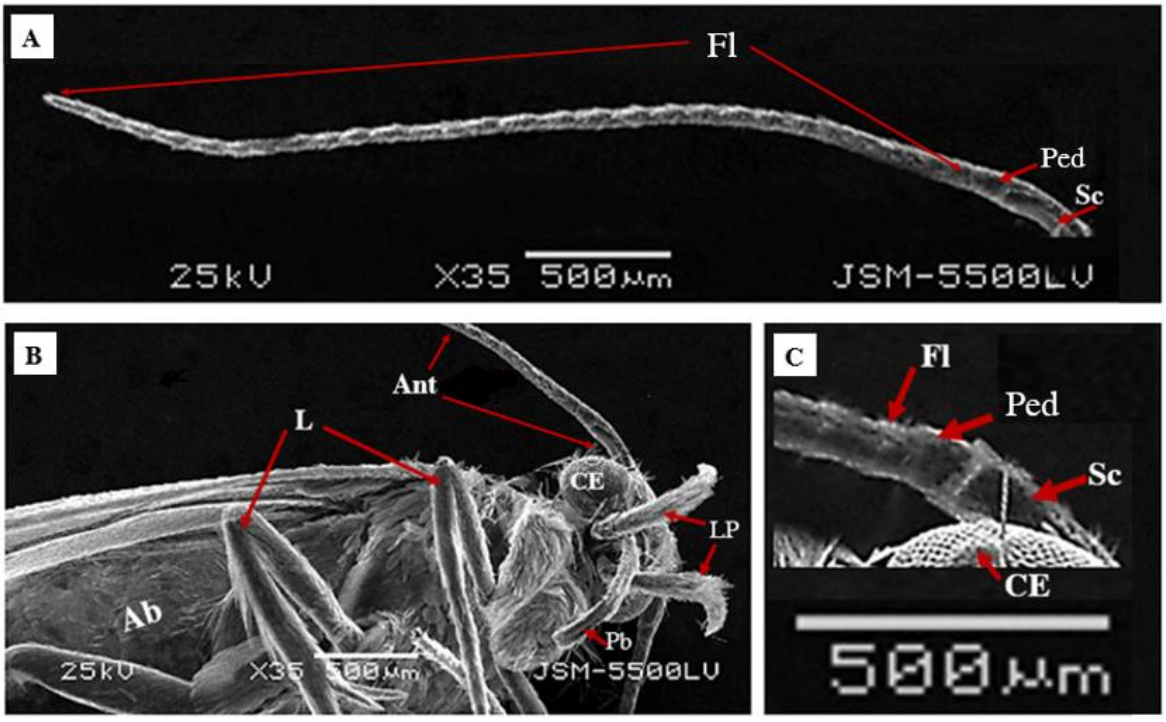

Fig.4: SEM photomicrographs. **A.** The complete adult antenna starts with a basal scape, followed by a pedicel, and terminating in a long, multi-segmented flagellum. **B.** Ventral view of Adult *Sitotroga cerealella* shows the antenna's position. **C.** High-magnification view illustrating specific antennal segments. Sc: Scape, Ped: Pedicel, Fl: Flagellum, Ant: Antennae, CE: Compound Eye, LP: Labial Palp, Pb: Proboscis, L: Legs, and Ab: Abdomen.

1.3-The Thorax:

The thorax of Angoumois grain moth is a pale brown, median body section situated between the head and abdomen. It is anatomically divided into three distinct segments: the prothorax, mesothorax, and metathorax. Each of these segments articulates with a pair of legs, and the thorax collectively provides support for the insect's wings.

1.3.a. Wings:

The forewings of *S. cerealella* are elongated, wide, and covered in small hairs, with longer hairs forming a dense fringe along the perimeter, exhibiting a yellowish-silvery sheen with brown markings. Conversely, the hindwings are diamond to trapezoidal, greyish, and possess an apical extension heavily fringed with hair setae along the posterior margin (Fig. 1). The hindwing of *S. cerealella* is narrower than its forewing (Fig. 5). The forewing venation includes subcosta (Sc), radius (R1-R5), media (M1-M3), cubitus (Cu1-Cu2), and anal veins (A1+2) (Fig. 5A). For the hindwing, the venation consists of subcosta (Sc), radius (R5), media (M1-M3), cubitus (Cu1-Cu2-CuP), and anal veins (A1+2) (Fig. 5B). While commonly referred to as Sc and Rs, the first hindwing vein is typically a fused Sc+R1, and the base of the second vein may be R.

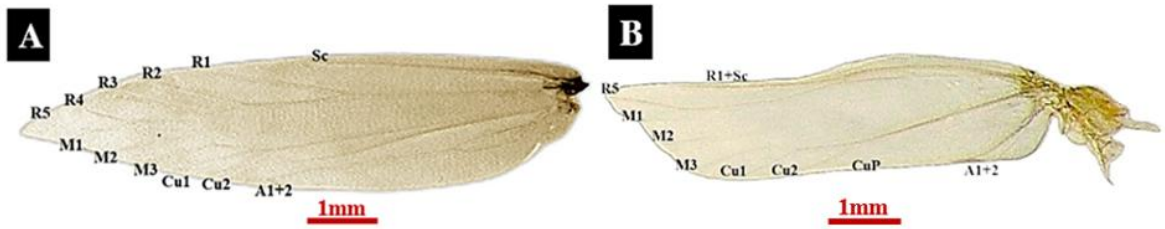

Fig.5: Light Photomicrographs present the wings structures of adult *Sitotroga cerealella* without their typical covering of scales.
A. Forewing venation includes subcosta (Sc), radius (R1-R5), media (M1-M3), cubitus (Cu1-Cu2), and anal veins (A1+2) **B.** Hindwing venation consists of the first hindwing vein is that is typically a fused Sc+R1, radius (R5), media (M1-M3), cubitus (Cu1-Cu2-CuP), and anal veins (A1+2).

1.3.b. Legs:

The legs of the Angoumois grain moth are characteristic insect appendages, each structured with a coxa, trochanter, stout femur, slender tibia, and a multi-segmented (typically 5-segmented) tarsus terminating in claws. These relatively long and slender limbs are adorned with fine hairs, which enhance their capacity for gripping surfaces and environmental sensing. Notably, the tibia's apical region features a well-developed, needle-like pretarsus (Fig. 6C). Collectively, these morphological attributes enable the walking and crawling behaviors crucial for the moth's navigation within stored grain environments during oviposition and subsequent larval development (Fig. 6).

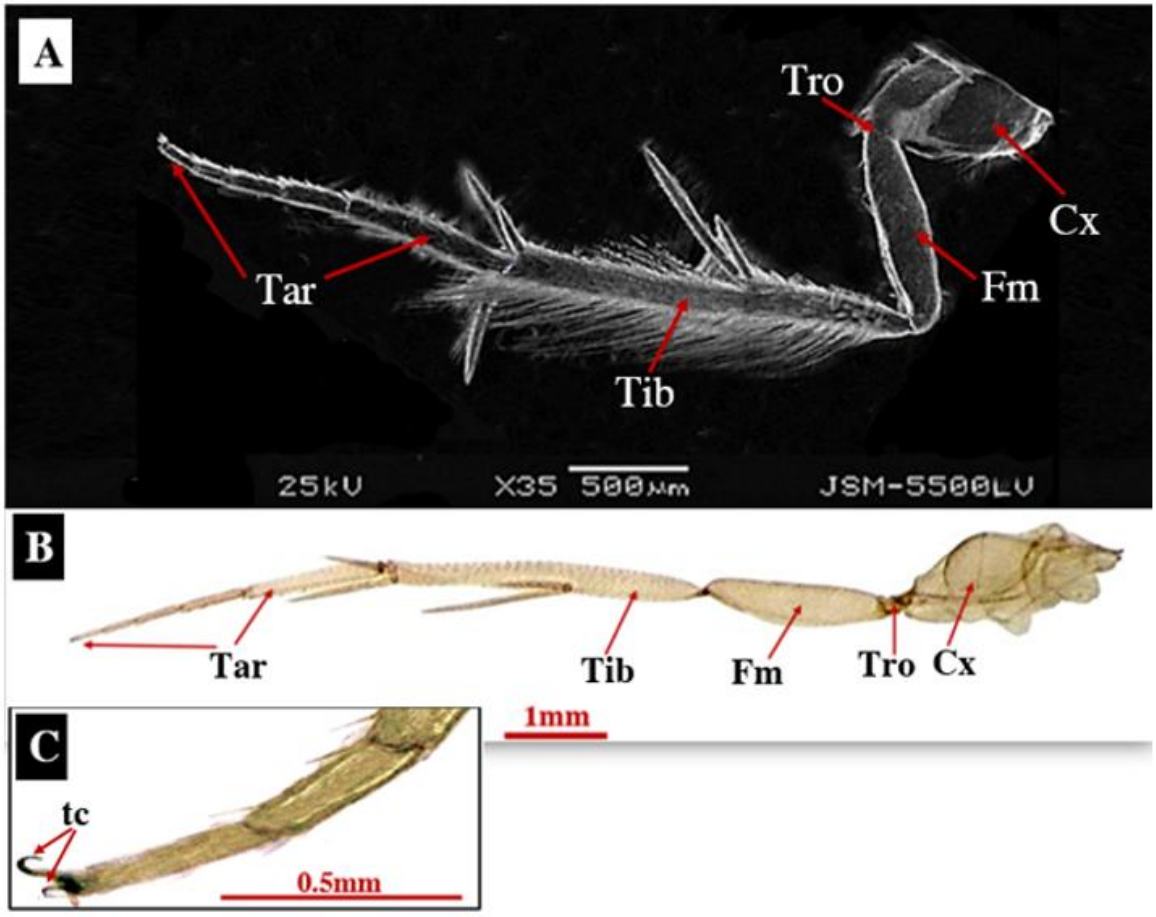

Fig.6: Photomicrographs present the leg structure of adult *Sitotroga cerealella* coxa, trochanter, femur, tibia, and a multi-segmented tarsus ending in claws. **A.** SEM photomicrograph (LM) of structure of Leg. **B.** Light photomicrograph (LM) of structure of Leg. **C.** Light photomicrograph of structure Tarsus show a well-developed claw. Cx: Coxa, Tro: Trochanter, Fm: Femur, Tib: Tibia, Tar: Tarsus, and tc: tarsus claw.

1.4-The Abdomen:

The abdomen of *S. cerealella* is segmented and covered with hairs (Fig. 7 B), displaying marked sexual dimorphism in morphology and associated genitalia.

1.4.a Female Abdomen and Genitalia: The female abdomen is typically bulky, elongated, and often colorless. It comprises eight well-developed segments. The mean female abdominal length is 0.247086±0.010682 mm (range: 0.23159–0.25855 mm), with an average abdominal area of 0.042923±5.381776 mm^2 (range: 0.035365–0.050411 mm^2). The female genitalia are adapted for oviposition and sperm reception. The primary ovipositor is formed by elongated papillae anales (Fig. 7) bearing sensilla, supported by internal apophyses. Sperm reception occurs via the ostium bursae, leading to the corpus bursae (sperm storage sac) through the ductus bursae. Additionally, a dorsal sex pheromone gland is present between the 8th and 9th abdominal segments.

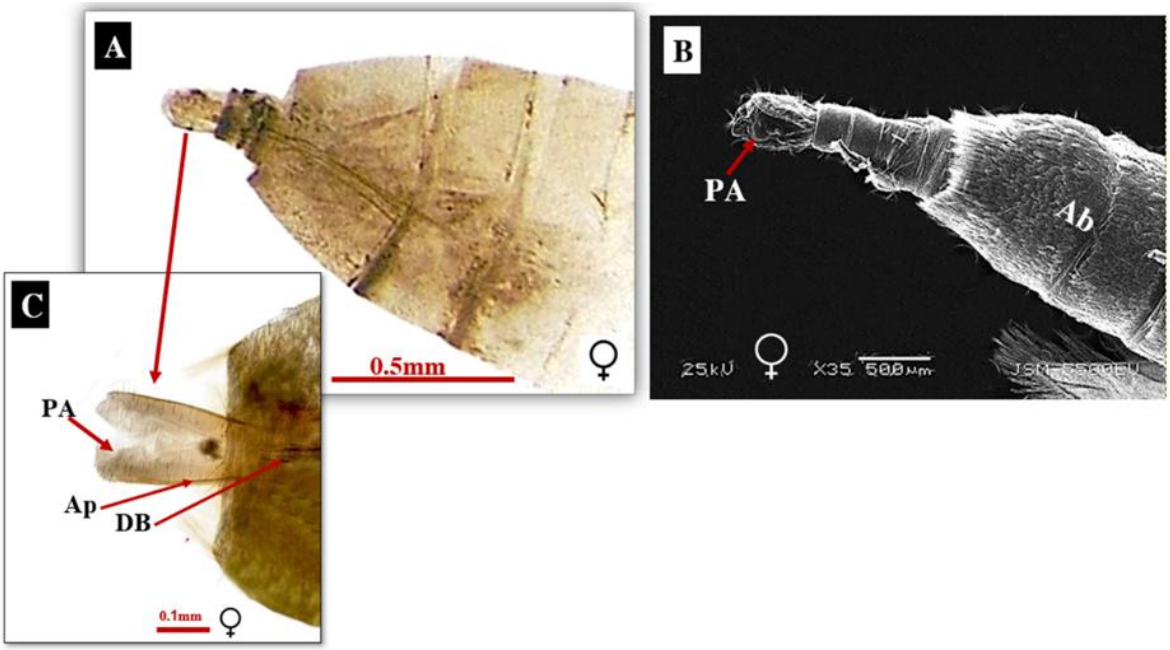

Fig. 7: Photomicrographs. A. Light Photomicrographs of female Adult *Sitotroga cerealella* ovipositor Highlighting Papillae Anales. **B.** SEM Photomicrographs of female ovipositor formed by elongated Papillae Anales. **C.** High-magnification view illustrating Highlighting Papillae Anales, Apophyses, and Ductus Bursae. PA: Papillae Anales, Ap: Apophyses, and DB: Ductus Bursae.

1.4.b Male Abdomen and Genitalia: In contrast, the male abdomen is characteristically blackish, thin, and pointed. The male exhibits complex, sclerotized genitalia crucial for copulation and species identification. Key features include an elongated aedeagus with a long caecum, paired valvae (clasping structures that may have a semi-separated sacculus), a dorsal uncus, a ventral semicircular gnathos that articulates with the uncus, and an elongated, subtriangular saccus extending anteriorly (Fig.8).

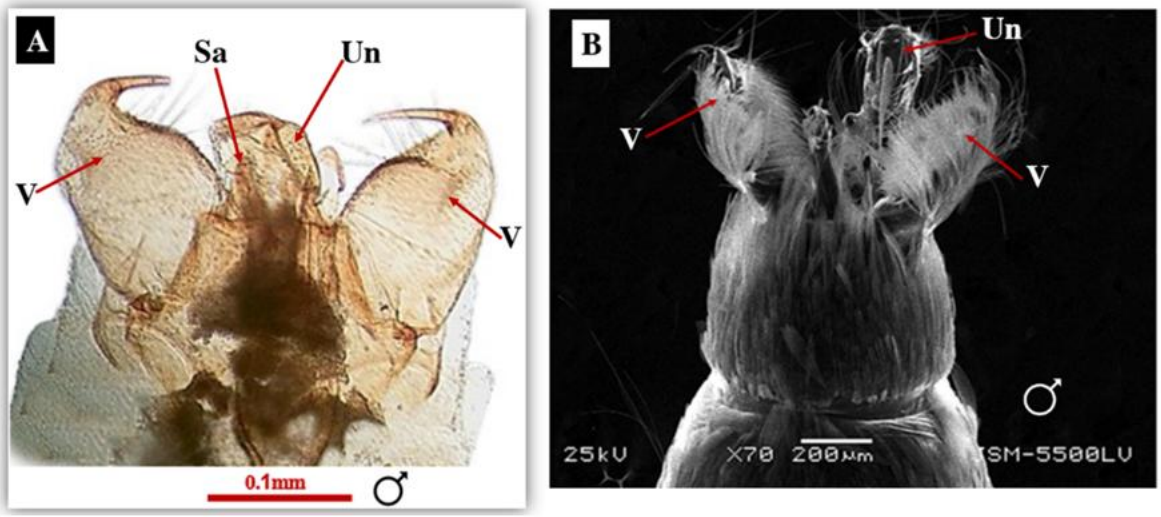

Fig.8: Photomicrographs. **A.** Light Photomicrographs of male Adult *Sitotroga cerealella* genitalia illustrate paired valvae, uncus, and an elongated subtriangular saccus extends anteriorly. **B.** SEM Photomicrographs of male genitalia show paired Valvae and uncus. V: Valvae, Un: Uncus, and Sa: Saccus.

2-Molecular Identification:

The *COI* gene sequence obtained for the present study, spanning 636 bp, has been deposited in GenBank under accession number MW452547. Nucleotide composition analysis showed an average of 31.4% adenine, 15.6% cytosine, 15.3% guanine, and 37.7% thymine. Consequently, the A+T content (69.2%) was found to be notably higher than the C+G content. BLAST/N searches of this *COI* sequence against the NCBI database consistently matched with 13 entries of *S. cerealella* within the subfamily Apatetrinae (Table 1).

Table 1: Comparative Analysis of *COI* Gene Sequences for *Sitotroga cerealella*, Related Species, and Out-group Taxa from GenBank/NCBI.

No.	Species	Accession number
1	*Sitotroga cerealella* isolate SQus	MW452547.1
2	*Sitotroga cerealella* vouche	KY492361.1
3	*Pexicopia dascia* voucher	KF388101.1
4	*Pexicopia* sp._ANIC	JN264909.1
5	*Anisoplaca_achyrota*	JX984205.1
6	*Pexicopia desmanthes*	KF394890.1
7	*Pectinophora endema*	KF389728.1
8	*Anisoplaca bathropis*	KF391162.1
9	*Pexicopia desmanthe*	JN264912.1
10	*Pexicopia catharia* voucher	KF390985.1
11	*Pexicopia proselia* voucher	JN264916.1
12	*Pexicopia pheletes* voucher	JN264921.1
13	*Pexicopia mimetic* voucher	KF391161.1
14	*Platyedra subcinerea* voucher	MN804565.1
15	*Autosticha pachysticta*	MN852878.1
16	*Autosticha* sp.	MZ234710.1
17	*Autosticha modicella* voucher	KF523749.1

Phylogenetic reconstruction using *COI* sequences demonstrated that the understudied sample formed a sister clade with *S. cerealella* (KY492361) from GenBank/NCBI (Fig. 9). This relationship was consistently recovered across Maximum Parsimony (MP) as shown in Fig.9A, Minimum Evolution (ME) as shown in Fig. 9B, and Neighbor-Joining (NJ) analyses as shown in Fig. 9C, with only slight variations in support values. The analysis included 13 related species and 3 out-group species for comprehensive phylogenetic placement.

Pairwise genetic distances for the understudied *S. cerealella* sample and 13 Apatetrinae species exhibited a range of 0.000 to 0.014. The most genetically similar organism was *S. cerealella* (KY492361.1) from GenBank, showing a 0.000 genetic distance. The average genetic distance across all comparisons was 0.089% (Table 2).

Fig. 9 A: Maximum Parsimony Phylogenetic Tree of *Sitotroga cerealella* and Related Species Based on *COI* Sequences from GenBank/NCBI.

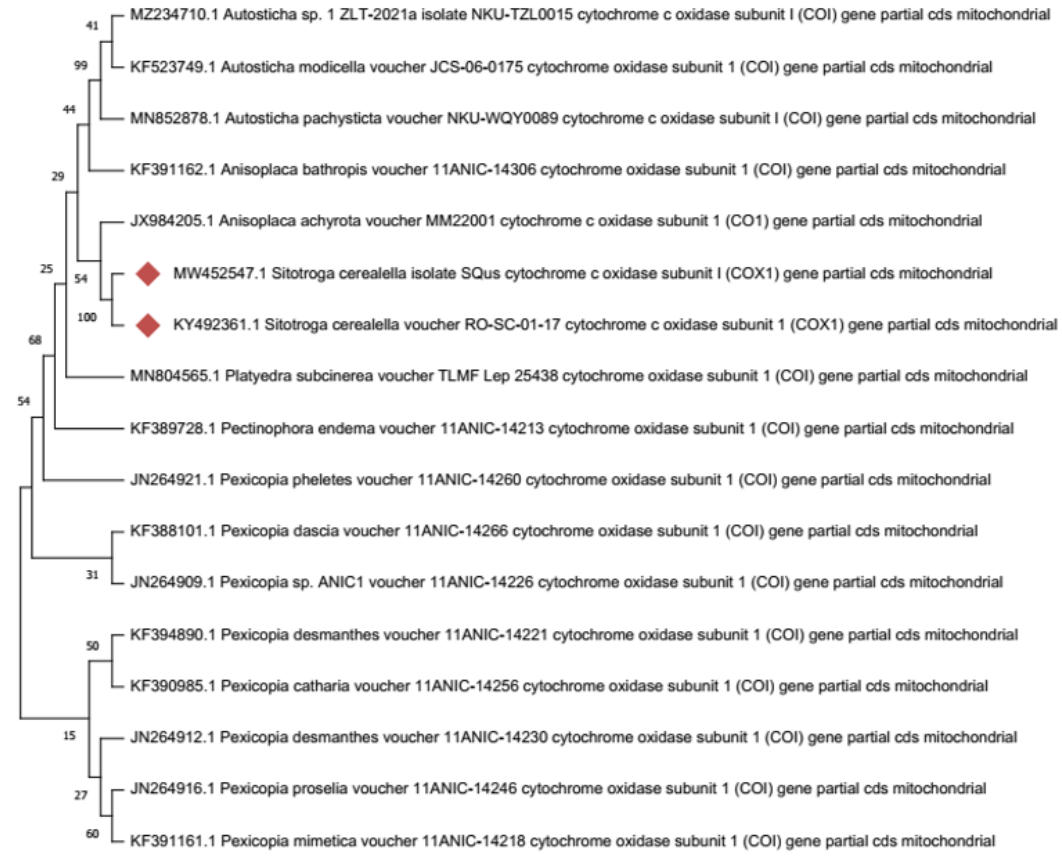

Fig. 9 B: Minimum Evolution Phylogenetic Tree of *Sitotroga cerealella* and Related Species Based on *COI* Sequences from GenBank/NCBI.

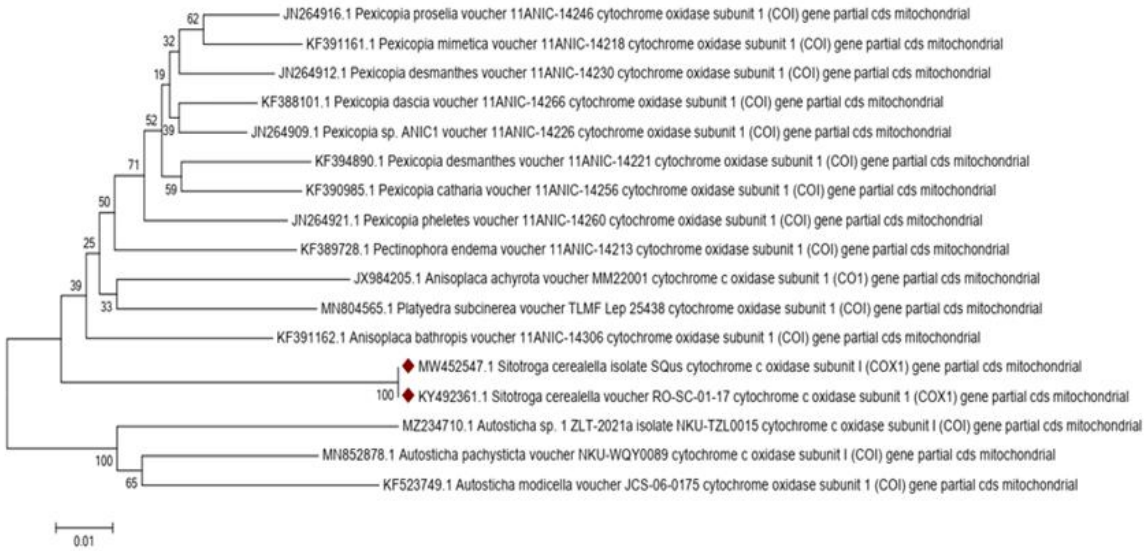

Fig. 9 C: Neighbor-Joining Phylogenetic Tree of *Sitotroga cerealella* and Related Species Based on *COI* Sequences from GenBank/NCBI.

Table.2: Pairwise Genetic Distances of *Sitotroga cerealella* and Related Species Based on GenBank/NCBI *COI* Sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
MW452547.1_Sitotroga_cerealella_isolate_SQus		0.000	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.013	0.015	0.015	0.016
KY492361.1_Sitotroga_cerealella_vouche	0.000		0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.013	0.015	0.015	0.016
KF388101.1_Pexicopia_dascia_voucher	0.090	0.090		0.006	0.011	0.008	0.010	0.010	0.007	0.008	0.008	0.008	0.007	0.010	0.012	0.013	0.013
JN264909.1_Pexicopia_sp._ANIC	0.092	0.092	0.026		0.010	0.007	0.009	0.010	0.007	0.008	0.008	0.008	0.008	0.010	0.013	0.014	0.013
JX984205.1_Anisoplaca_achyrota	0.098	0.098	0.076	0.068		0.011	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.011	0.015	0.015	0.015
KF394890.1_Pexicopia_desmanthes	0.098	0.098	0.046	0.039	0.080		0.010	0.011	0.008	0.008	0.008	0.009	0.008	0.011	0.013	0.014	0.014
KF389728.1_Pectinophora_endema	0.099	0.099	0.061	0.057	0.080	0.068		0.011	0.009	0.009	0.010	0.010	0.010	0.010	0.012	0.014	0.014
KF391162.1_Anisoplaca_bathropis	0.099	0.099	0.059	0.061	0.080	0.078	0.071		0.010	0.011	0.010	0.011	0.011	0.011	0.013	0.014	0.013
JN264912.1_Pexicopia_desmanthe	0.101	0.101	0.032	0.031	0.074	0.047	0.059	0.062		0.008	0.008	0.009	0.007	0.010	0.013	0.014	0.013
KF390985.1_Pexicopia_catharia_voucher	0.101	0.101	0.041	0.041	0.082	0.044	0.059	0.071	0.047		0.008	0.009	0.009	0.011	0.013	0.014	0.014
JN264916.1_Pexicopia_prosella_voucher	0.103	0.103	0.039	0.039	0.073	0.046	0.061	0.062	0.036	0.041		0.008	0.007	0.010	0.013	0.014	0.013
JN264921.1_Pexicopia_pheletes_voucher	0.103	0.103	0.044	0.042	0.080	0.053	0.061	0.074	0.051	0.056	0.042		0.009	0.010	0.013	0.015	0.014
KF391161.1_Pexicopia_mimetica_voucher	0.107	0.107	0.034	0.037	0.080	0.051	0.068	0.069	0.037	0.046	0.031	0.058		0.011	0.013	0.014	0.014
MN804565.1_Platyedra_subcinerea_voucher	0.107	0.107	0.066	0.061	0.076	0.080	0.071	0.074	0.069	0.078	0.064	0.066	0.080		0.013	0.015	0.014
MN852878.1_Autosticha_pachysticta	0.127	0.127	0.090	0.099	0.125	0.106	0.101	0.101	0.106	0.108	0.099	0.108	0.099	0.108		0.012	0.010
MZ234710.1_Autosticha_sp.	0.134	0.134	0.105	0.114	0.134	0.116	0.123	0.116	0.114	0.123	0.117	0.123	0.117	0.129	0.087		0.013
KF523749.1_Autosticha_modicella_voucher	0.138	0.138	0.108	0.106	0.134	0.125	0.121	0.103	0.112	0.129	0.106	0.116	0.110	0.114	0.073	0.094	

DISCUSSION

Sitotroga cerealella is a significant global grain pest causing up to 40% loss, but it's also vital for mass-rearing *Trichogramma* in biological control, like *T. turkestanica* in Egypt (Trematerra, 2015; Ashamo, 2010; Abdel-Galil *et al*., 2018). Accurate identification of insect pests is paramount for effective pest management strategies, particularly in stored grain pests like the Angoumois grain moth, *S. cerealella*. This study successfully provides a comprehensive morphological and molecular characterisation of *S. cerealella* adults from Qus, Qena Governorate, Egypt, addressing the previous lack of detailed characterization for this species despite its dual importance as a major pest and a factitious host for *Trichogramma* parasitoids.

Our morphological observations, utilizing both light microscopy (LM) and scanning

electron microscopy (SEM), corroborate and expand upon existing knowledge regarding *S. cerealella*. The documented average body lengths for males (4.27 mm) and females (5.354 mm) clearly illustrate the significant sexual dimorphism, with females being notably larger. This finding aligns with general lepidopteran characteristics where females often require greater resources for egg production (Wang *et al*., 2023). The detailed imaging of various morphological structures, including compound eyes, filiform antennae, and modified proboscis, provides valuable insights into the sensory and feeding adaptations of this moth. The meticulous description of wing venation and leg segmentation further contributes to a robust morphological baseline for identifying *S. cerealella*. While some studies focus on specific organs like antennae or ovipositors (Ma *et al*., 2017), our research offers a holistic morphological profile, which is essential for accurate identification and differentiation from closely related species.

Beyond morphology, molecular barcoding using the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene proved to be a powerful tool for confirming the identity of the *S. cerealella* specimens. The amplified 636 bp *COI* sequence (GenBank: MW452547.1) exhibited a high A+T content (69.2%), a common characteristic in insect mitochondrial DNA, as noted by various studies in entomological molecular genetics (Ahmed *et al*., 2024). The BLAST searches and phylogenetic analyses (Maximum Parsimony, Minimum Evolution, and Neighbor-Joining) consistently placed the Qus samples within the *Sitotroga cerealella* clade with 100% genetic identity to existing GenBank sequences. This high genetic congruence, evidenced by a pairwise genetic distance of 0.000 with a reference *S. cerealella* sequence (KY492361.1), strongly supports our species identification. This further reinforces the reliability of *COI* as a universal barcode for species-level identification in the animal kingdom, as highlighted by Hebert *et al*. (2003) and Kachhawa (2023). The consistency across multiple phylogenetic methods further strengthens the robustness of our molecular findings.

The integrated approach of combining morphological and molecular techniques is crucial, especially in cases where morphological characters can be subtle or require specialized taxonomic expertise for interpretation (Jalali, 2013). This comprehensive characterization lays a strong foundation for future research aimed at understanding the genetic diversity of *S. cerealella* populations and optimizing their utilization in biological control efforts. Accurate characterization is crucial for both effective pest management and optimized parasitoids production, leading to a sustainable agricultural future.

Conclusion

The present study successfully provided a comprehensive morphological and molecular characterization of *Sitotroga cerealella* adults from Qus, Qena Governorate, Egypt. Morphological analyses, employing light and scanning electron microscopy, detailed features such as body length, antennae, wings, and male genitalia, revealing significant sexual dimorphism with females being larger. Molecular barcoding using the *COI* gene yielded a 636 bp sequence (GenBank: MW452547.1) with a high A+T content. BLAST searches and phylogenetic analyses consistently confirmed the species' identity with 100% genetic similarity to existing GenBank sequences. This integrated approach is crucial for accurate identification, especially where morphological distinctions are subtle. The findings are vital for effective pest management strategies and optimizing Trichogramma mass rearing, thereby promoting sustainable biological control methods in Egypt and globally.

List of Abbreviations

Abbreviation	Full name
Ab	Abdomen
Ant	Antennae
Ap	Apophyses
CE	Compound Eye
Cx	Coxa
Cu	Cubitus
COI	Cytochrome C Oxidase Subunit 1
DNA	Deoxyribo Nucleic Acid
DB	Ductus Bursae0
Fm	Femur
Fl	Flagellum
Fw	Forewings
Hw	Hindwings
LP	Labial Palp
L	Legs
LM	Light Microscopy
MP	Maxillary Palp
MP	Maximum Parsimony
M	Media
ME	Minimum Evolution
NCBI	National Center for Biotechnology Information
NJ	Neighbour Joining
Oc	Ocelli
PA	Papillae Anales
Ped	Pedicel
PCR	Polymerase Chain Reaction
Pb	Proboscis
R	Radius
Sa	Saccus
SEM	Scanning Electron Microscopy
Sc	Scape
Set	Setae
Sc	Subcosta
SDGs	Sustainable Development Goals
Tar	Tarsus
Tc	Tarsus Claw
Th	Thorax
Tib	Tibia
Tro	Trochanter
Un	Uncus
V	Valve

Declarations:

Ethics Approval and Consent to Participate: This study has been granted by the Research Ethics Committee of Faculty of Agriculture at Assiut University in accordance with Egyptian laws and university guidelines for the care of animals (approval no. 03-2025-0034).

Authors Contributions: MABM, GNA, and SEM did the conceptualization. MABM, GNA, and SEM contributed to the formal analysis. MABM, GNA, and SEM took part in the investigation. MABM wrote the original draft. GNA and SEM did the writing–review and approved the final manuscript. All authors read and approved the final manuscript.
Competing Interests: The authors have no competing interests to declare that are relevant to the content of this article.
Availability of Data and Materials: The datasets analysed during the current study are available in the [GenBank/NCBI] repository, web link [https://www.ncbi.nlm.nih. gov/ nuccore/MW452547], under accession number [MW452547]**.**
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ARABIC SUMMARY

التوصيف المورفولوجي والتشفير الجزيئي لفراشة حبوب، *Sitotroga cerealella* في قوص، محافظة قنا، مصر

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تُعد فراشة الحبوب *Sitotroga cerealella*، آفة عالمية مهمة للحبوب المخزونة وعائلًا حيويًا رئيسيًا للتربية الكمية لطفيلات البيض وبعض الحشرات آكلات الحشرات (الطفيلات والمفترسات) في معامل المكافحة البيولوجية. على الرغم من أهميتها المزدوجة، غالبًا ما يفتقر التوصيف الشامل لفراشة الحبوب. لذا، هدفت الدراسة الحالية إلى التعريف المورفولوجي والجزيئي مفصل للحشرات الكاملة من فراشة الحبوب والقاطنة بقوص، محافظة قنا، مصر. تم إجراء التعريف المورفولوجي باستخدام المجهر الضوئي (LM) والمجهر الإلكتروني الماسح(SEM) ، مع توثيق السمات المختلفة والتي شملت طول الجسم، وقرون الاستشعار، والأجنحة، والأعضاء التناسلية الذكرية.

أظهرت النتائج أن الذكور كانت أقصر بدرجة فائقة المعنوية (بمتوسط 4.27 مم) عن الإناث (بمتوسط 5.354 مم)، مما يشير إلى تباين جنسي واضح. تضمن التحديد الجزيئي تضخيم وتسلسل جين (*COI*). أظهر تسلسل *COI* الناتج بطول 636bp (MW452547) ببنك الجينات. أثبتت عمليات البحث باستخدام BLAST وتحليل الأشجار التطورية (الاقتصاد الأقصى، والتطور الأدنى، والانضمام إلى الجوار) تأكيد هوية العينات على أنها *Sitotroga cerealella*، وأظهرت هوية وراثية بنسبة 100٪ مع تسلسلات بنك الجينات الموجودة.

تعد هذه الدراسة المورفولوجية والجزيئية المتكاملة لحشرة فراشة الحبوب *S. cerealella* أمرًا بالغ الأهمية لتحديد الآفات بدقة وتحسين التربية الكمية للترايكوجراما والتي تتم علي نطاق واسع بمصر و العالم، وبالتالي تعزيز اهداف التنمية المستدامة (SDGs).

الكلمات المفتاحية: جيلاشيدى، مورفوجيني ، جين*COI* ، ترايكوجراما، مكافحة بيولوجية.