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# Decoding Chorionic Diversity: Eggshell Microarchitecture Reveals Taxonomically Informative Exo-chorionic Variations in Five Polyphagous Noctuid Pest Species from Northwestern Himalaya

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## Abstract

The present investigation is an attempt to study the fine scale ultrastructural architecture of egg chorions of five economically important Noctuid moth pests from Himachal Pradesh, India i.e., *Agrotis ipsilon* (Hufnagel), *Agrotis segetum* (Denis & Schiffermüller), *Helicoverpa armigera* (Hübner), *Peridroma saucia* (Hübner) and *Trisula variegata* Moore, using scanning electron microscopy (SEM). The egg stage has been relatively understudied in comparison to other life stages; despite its taxonomic and ecological importance this study addresses a major gap in lepidopteran biology. The detailed examination revealed marked interspecific variations in exo-chorionic sculpturing, polygonal reticulations, polygonal cell boundary architecture, organization of micropylar rosette, transition zone, micropyles and aeropylar distribution. These highly specialised chorionic characters show that moth eggs are evolutionarily refined structures, shaped by ecological adaptations and reproductive strategies, rather than passive developmental stages. The observed ultrastructural diversity offers useful diagnostic characters for egg-based morpho-taxonomy and improves systematic interpretations.


## Introduction

Lepidopteran eggs exhibit extensive structural variability morphologically across groups and genera. The egg's shape can be round, half-round, elliptical, conical, cylindrical or flat. It can also be more specialized, like somewhat cuboid, asymmetrical, or lenticular. These differences are generally specific to each species and are affected by the substrate where the eggs are laid, the behaviour of the animals that lay the eggs and the environment. The egg chorion may have simple and basic structure or crinkled surface, consisting of divisions, with transverse or longitudinal ridges. The micropylar region is located on the upper anterior portion of eggs which are globular, conical or cylindrical in shape and on the outer perimeter or rim in flattened or lenticular shaped eggs (Holland, 1898, 1903). This region is guarded with distinct micropylar rosette. The number of micropyles vary from 1–20 (Hinton, 1981).

The micromorphological characteristics of the chorion frequently full-fill physiological roles, such fortifying the eggshell, promoting gas exchange via aeropyles or assisting in water retention. Moreover, surface ornamentation may aid in species recognition and taxonomic classification, offering critical indicators in systematic and evolutionary research of Lepidoptera. In conclusion, the coloration and form of lepidopteran eggs are not only aesthetic characteristics but signify essential biological adaptations. They perform several ecological and physiological roles, such as camouflage, protection, structural integrity and developmental regulation. The variability in these criteria highlights the evolutionary adaptability of the order Lepidoptera and illustrates the complex interplay between morphology, functionality and environmental adaption.

In the realm of biological sciences, particularly in the examination of the embryonic stages of insects, including eggs, larvae and pupae, scanning electron microscopy (SEM) is of paramount importance. Conventional photo-microscopy techniques frequently do not disclose the intricate sculpturing, micropores, setal formations and chorionic ornamentations essential for species identification and comprehension of evolutionary adaptations. On the other hand, SEM makes it possible to accurately record these diagnostic traits, which is a big help to morphological taxonomy and systematics. The resulting images, while monochromatic, are exceptionally clear and informative, enabling researchers to identify even the most subtle morphological distinctions among closely related taxa. Such comprehensive morphological datasets have enhanced the utilization of external and microstructural characteristics in elucidating higher and lower taxonomic hierarchies, hence integrating molecular and morphological methodologies in taxonomy.

Peterson's seminal studies (1960, 1962, 1963a, 1963b, 1964) established the groundwork for contemporary research on lepidopteran egg chorion morphology. His thorough analysis offered the initial extensive descriptions of chorionic sculpturing, micropylar configurations and aeropylar arrangements among many moth and butterfly taxa, highlighting their significance in systematics and interspecific differentiation. Recent studies by Moreira *et al.* (2019), Guedes et al. (2020), Pinto and Fernandes (2020), Gonzaga-Segura et al. (2020), Venancio et al. (2020), and Daquila et al. (2021) had consistently reinforced the significance of SEM-based egg morphology in lepidopteran taxonomy. These investigations utilize contemporary imaging and morphometric analysis, uncovering slight interspecific differences and enhancing the diagnostic reliability. These modern contributions underscore the ongoing and developing importance of lepidopteran egg and juvenile stage morphology as essential instruments in systematic, phylogenetic and evolutionary research.

The present research work involves a thorough examination of the exterior egg architecture in five economically important polyphagous moth pest species across family Noctuidae, utilizing sophisticated scanning electron microscopy (SEM) as the main analytical method. The application of SEM enables a thorough investigation of the microstructural and ultrastructural characteristics of the eggshell surface, therefore providing exceptional accuracy in recording morphological variation among taxa. This technique offers high-resolution visualization to create a comprehensive morphological framework of the eggshell layers, emphasizing surface characteristics including chorionic sculpturing, aeropylar configurations, micropylar patterns and the variety of polygonal cell arrangements. These characteristics demonstrate both conservative (traditional) and variable (mutable) traits, reflecting evolutionary lineage stability while also indicating species-specific adaptations to ecological and reproductive contexts. The morphological data produced from this study will enhance lepidopteran taxonomy for these species by offering further diagnostic characteristics based on egg chorion morphology. Thus, the current research enhances the morphological representation of the examined taxa and creates a methodological framework for future investigations into the structural complexity and evolutionary importance of lepidopteran egg morphology.

## Materials and Methods

- **Survey tours and collection of eggs.** The collection tours were conducted across different localities of Kangra, Kullu and Mandi districts of Himachal Pradesh. The adult moths were collected by using white-sheet-light trap to collect both male and female adult moths and to facilitate natural mating and subsequent procurement of freshly laid eggs. The altitude of the surveyed locations varied from 800 meters above mean sea level to 2591 meters above mean sea level.

In instances where freshly laid eggs were unavailable, some gravid females were also dissected to extract mature eggs directly from the ovaries, following the methodological framework outlined by Dolinskaya (2019).

- **Preservation of adult moths.** The adult moths were euthanized by using standard entomological procedures to ensure minimal damage to morphological features. Each specimen was carefully stretched, pinned and systematically arranged, labelled and preserved for further identification and studies in fumigated, air-tight wooden entomological boxes to prevent fungal contamination and pest infestation.
- **Preservation of eggs.** The freshly laid eggs obtained from the female moths were carefully hand-picked by using fine, soft-tipped brushes and precision forceps to avoid mechanical damage. The collected eggs were immediately transferred to clean glass vials containing a mixture of 70% ethyl alcohol and glycerol in the ratio of 8:2. This preservation medium maintained the integrity of external sculpturing and microstructural features of the chorion.

- **Photography.** The adult moths collected during field surveys were photographed in colour by using a digital camera to capture detailed morphological features and colour patterns essential for subsequent identification.
- **Identification.** The identification of the collected adult moth specimens was carried out by using standard and authoritative taxonomic references, primarily those of Hampson (1892–1894), along with other relevant monographs and diagnostic keys.
- **Preparation of samples and procedure for SEM studies.** The scanning electron microscopic studies were conducted to examine the fine surface architecture and ultrastructural details of the eggshells. The entire sample preparation procedure was carried out by following standard electron microscopy protocols as stated by Dolinskaya (2019) with slight modifications to suit the delicate nature of lepidopteran eggs.

1. *Fixation.* The collected egg samples were initially fixed in 2.5% glutaraldehyde prepared in phosphate buffer solution (PBS; pH 7.4) for a minimum duration of one hour at room temperature.
2. *Dehydration.* After fixation, the eggs were gradually dehydrated through a series of graded ethanol concentrations (50%, 70% and 90%), each for 15 minutes, followed by final dehydration in absolute alcohol to eliminate all traces of moisture.
3. *Mounting and Sputter Coating.* Following dehydration, the dried egg samples were mounted on aluminium stubs by using double-sided conductive carbon adhesive tape. The mounted specimens were then sputter-coated with a fine layer of gold and platinum alloy.
4. *Scanning and Imaging.* The prepared specimens were examined under a JEOL JSM-6510LV Scanning Electron Microscope available at the Sophisticated Instrumentation Centre, Punjabi University, Patiala. The high-resolution micrographs were captured at varying magnifications to examine the chorionic surface pattern, micropylar region, rosette arrangement, aeropyles, ridges and other external ultrastructural features.

- **Nomenclature for naming different parts/ structures.** The terminology and descriptive framework for eggshell structures were followed as per the standards and nomenclature proposed by Döring (1955), Peterson (1964), Zolotuhin and Kurshakov (2009) and Dolinskaya (2019). This ensured uniformity in the interpretation and description of the observed microstructural features.

# Results

**GENUS AGROTIS OCHSENHEIMER**

*Agrotis* Ochsenheimer, 1816, *Schmett. Eur*., **4**: 66; Holloway, 1979, *Series Entom*., **15**: 399; Yoshimoto, 1995, *Tinea*, 14 (suppl. 2): 49; Hacker, 2001, *Esperiana*, **8**: 276; Ronkay *et al*., 2013, *Fibigeriana Suppl*., **1**: 18; San Blas, 2014, *Zootaxa*, **3771**(1): 10.

*Type Species* — *Noctua segetum* Denis & Schiffermüller

*Distribution* — Worldwide

**Key to the studied species of genus Agrotis Ochsenheimer**

1. The bases of polygonal cells are smooth and texture free; micropylar rosette is composed of 10 primary petaloid cells and 14 secondary petaloid cells; micropylar pit has four micropyles; aeropyles are restricted to few rows of polygonal cells……..**Agrotis ipsilon (Hufnagel)**

……. The bases of polygonal cells are textured with wrinkled pattern; micropylar rosette is composed of 16 primary petaloid cells and 25 secondary petaloid cells; micropylar pit has five to six micropyles; aeropyles are present all over the general exo-chorion……**Agrotis segetum (Denis & Schiffermüller)**

**Agrotis ipsilon (Hufnagel)**

## (Common Name: Black Cutworm)

**(Fig. 1 — 6)**

*Phalaena ipsilon* Hufnagel, 1766, *Berlin. Magazin.*, **3**(4): 416.

*Agrotis ipsilon* Hufnagel: Holloway, 1976, *Moths of Borneo*, **1976**: 6; Holloway, 1989, *Malayan Nature J.*, **42**(2–3): 57–225, 65; Poole, 1989, *Noctuidae Lepid. Cat. (N.S.).*, 1989: 118; Yoshimoto, 1994, *Tinea*, **14**(Suppl. 1): 97; Yoshimoto, 1995, *Tinea*, **14**(Suppl. 2): 50; Hacker, 2001, *Esperiana*, **8**: 281; Angulo & Olivares, 2005, *SHILAP Revta. Lepid.*, **33**(130): 138; Olivares *et al*., 2009, *SHILAP Revta. Lepid.*, **37**(148): 394; Ronkay *et al*., 2013, *The Atlas of the Noctuidae Fauna of Taiwan (Lepidoptera, Noctuoidea). Fibigeriana Supplement*, **1**: 9-122; San Blas, 2014, *Zootaxa*, **3771**(1): 47; Lafontaine & Schmidt, 2010, *ZooKeys*, **40**: 1-239; Leley, 2016, *Cat. Ins. Russian Far East*., **2**: 1-812.

*Type Locality* — Vienna region

*Distribution* — Worldwide

*Egg Shape and size* — The egg of this noctuid moth is spherical in shape and its diameter is 573µm.

*Egg Chorion* — The egg chorion of this species is highly sculptured with well-defined horizontal ribs and vertical ridges all over the exo-chorionic surface which is a characteristic feature of family Noctuidae. The general exo-chorion is composed of square, rectangular and pentagonal shaped polygonal cells. These polygonal cells have well-defined raised walls and clear and smooth bases providing distinct shape and boundaries to the polygonal cells. The aeropylar openings are observed at the junction of ridges and ribs forming thick polygonal corners. These aeropyles are restricted upto a very limited area after transition zone.

*Micropylar Region* — In this species, the micropylar region is present at the anterior pole of the egg and centrally placed. The micropylar region is incomplete and composed of primary and secondary petaloid cells. The tertiary petaloid cells are absent. The secondary petaloid cells are further encircled by a distinct transition zone having intermediate shaped polygonal cells separating the general exo-chorionic surface area from the micropylar region.

*Micropylar rosette* — The micropylar rosette is observed centrally at the anterior pole of the egg as a prominent and well-defined structure. This micropylar rosette is formed of 10 primary petaloid cells, present radially and forming a flower shaped symmetric rosette around the micropylar pit. These primary petaloid cells have separate engraved thick raised walls and textured raised basal surface. The primary petaloid cells are further surrounded by 14 small, tear-drop shaped secondary petaloid cells, which are not uniform in their shape and size and have thick uniform raised walls against the smooth texture-free basal surface. The tertiary petaloid cells are absent in this rosette. The micropylar region is completely devoid of aeropylar openings. A distinct transition zone is observed after the micropylar rosette having intermediate shaped polygonal cells separating the micropylar region from general exo-chorionic polygonal field.

*Micropyles* — The micropyles are observed in between the primary petaloid cells in a well-defined squarish micropylar pit. This micropylar pit has four micropylar openings one at each corner. The micropyles are small and round in their formation.

*Aeropyles* — The aeropyles are present upto limited rows of the polygonal cells at the junction of the ribs and ridges. The micropylar rosette is completely devoid of these air pores. The aeropyles are large, cylindrical in shape having thick walls and embedded in the corners of the polygonal cells. Each polygonal cell holds four to six aeropyles as per the shape of the polygonal cell.

**Agrotis segetum (Denis & Schiffermüller)**

## (Common Name: The Turnip Moth)

**(Fig. 7 — 12)**

*Noctua segetum* Denis & Schiffermüller, 1775, *Ank. syst. Schmett. Wienergegend*, 81.

*Agrotis segetum* Denis & Schiffermüller: Stephens, 1829, *Illusts. Brit. Ento.*, **2**:115; Staudinger, 1881; *Stettin Ent. Ztg.*, **42**(10–12): 423; Cockayne, 1952, *Ent. Rec. J. Var.*, **64**(7–8): 189; Lucas, 1959, *Ent. Ber. Amst.*, **19**: 204; Yoshimoto, 1994, *Tinea*, **14**(Suppl. 1): 98, pl. 14, f. 5; Yoshimoto, 1995, *Tinea*, **14**(Suppl. 2): 49; Hacker, 2001, *Esperiana*, **8**: 277.

*Type Locality* — Austria (Vienna)

*Distribution* — Worldwide

*Egg Shape and size* — The egg is spherical in its shape with a diameter of 388.88µm.

*Egg Chorion* — In this species, the egg chorion is highly sculptured with horizontal ribs and vertical ridges all over the egg chorion as a family specific character of these moths. The general exo-chorion is composed of square, rectangular and pentagonal shaped polygonal cells. These polygonal cells are well-defined by raised thin boundaries with wrinkle textured basal areas. These polygonal cells are with distinct aeropylar openings at their thick corners.

*Micropylar Region* — In this egg, the micropylar region is present at the anterior pole of the egg and centrally located. The micropylar region is incomplete and formed by primary and secondary petaloid cells only. The tertiary petaloid cells are absent. The secondary petaloid cells further gradually merge in the transition zone. The transition zone is with intermediate shaped polygonal cells, separating the general exo-chorion from the micropylar region of this egg.

*Micropylar Rosette* — The micropylar rosette is observed at the anterior pole of the egg. This micropylar rosette is incomplete in its formation and composed of primary and secondary petaloid cells. The tertiary petaloid cells are absent. The primary petaloid cells are 16 in number and prominently arranged in symmetrical intricate flower shape. These petaloid cells are with separate thick and raised boundaries. A complete thick and raised outer boundary surrounds the primary petaloid cells and holds the rosette compactly. These primary petaloid cells are further encircled by 25 secondary petaloid cells which are elongated and dissimilar in overall formation, possessing clear thin raised boundaries and texture free bases. The micropylar rosette further merges in the transitional zone, which marks the end of the micropylar rosette by smooth transition area having intermediate shaped polygonal cells.

*Micropyles* — The micropylar openings are present in between the primary petaloid cells, in an irregular oval micropylar pit. This micropylar pit has five to six micropyles along with its circumference. The micropyles are small

and round in shape.

*Aeropyles* — The aeropyles are observed all over the general exo-chorion at the junction of the ribs and ridges. The micropylar rosette is completely devoid of these air pores. The aeropyles are large, cylindrical in shape possessing thick walls and embedded in the corners of the polygonal cells, each polygonal cell holds four to six aeropyles as per the shape of the polygonal cell.

**GENUS HELICOVERPA HARDWICK**

*Helicoverpa* Hardwick, 1965, *Ent. Soc. Canada*, **40**: 1-247; Holloway, 1979, *Series Entom*., **15**: 401; Yoshimoto, 1995, *Tinea*, **14**(Suppl. 2): 49; Hacker, 2001, *Esperiana*, **8**: 312.

*Type Species* — *Noctua armigera* Hübner

*Distribution* — Africa; Asia, India; Australia; Central America; Europe; South America.

**Helicoverpa armigera (Hübner)**

**(Common Name: Cotton Bollworm)**

# (Fig. 13–18)

*Noctua armigera* Hübner, 1805, *Samml. Eur. Schmett*., **4**: pl. 79, f. 370.

*Helicoverpa armigera* Hübner: Holloway, 1989, *Malayan Nature J.*, **42**(2–3): 75, pl. 1, 8, f. 67, 70, 71; Yoshimoto, 1994, *Tinea*, **14**(Suppl. 1): 97, pl. 14, f. 21; Yoshimoto, 1995, *Tinea*, **14**(Suppl. 2): 49; Hacker, 2001, *Esperiana*, **8**: 312; Gyulai & Ronkay, 2001, *Esperiana*, **8**: 665.

*Type Locality* — Europe.

*Distribution* — India: Throughout India; Africa; Asia; Australia; Central America; Europe; South America.

*Egg Shape and Size* — In this noctuid moth, the egg is spherical in shape, having ribs and ridges all over the egg chorion a family specific characteristic. Its diameter is 360.6µm.

*Egg Chorion* — The egg chorion is highly sculptured with thick ridges running longitudinally all around the egg and thin ribs forming the polygonal cells. The general egg chorion is composed of urn-shaped, square and rectangular polygonal cells. These polygonal cells are formed of raised thick ridges and thin ribs and wrinkle textured basal surfaces. The polygonal cells have aeropyles at their corners.

*Micropylar Region* — The micropylar region of the egg is observed centrally placed at the anterior pole of the egg. In this species, the micropylar region is incomplete and formed of primary petaloid cells only. The secondary and tertiary petaloid cells are absent. The primary petaloid cells are further surrounded by a distinct wide plain texture free area, as the transition zone, distinctly separating the micropylar region from the general exo-chorionic surface area. This transition zone along with the micropylar region is devoid of aeropyles.

*Micropylar Rosette* — The micropylar rosette of this egg is observed centrally placed at the anterior pole of the egg. The micropylar rosette is formed of 12 primary petaloid cells only and surround the micropylar pit as an asymmetrically arranged rosette. These primary petaloid cells are observed with thick engraved raised boundaries and raised textured cell basal surfaces. The secondary and tertiary petaloid cells are absent in this micropylar

rosette. The primary petaloid cells are further surrounded by distinct plain texture free transition zone, separating the rosette from polygonal field.

*Micropyles* — The micropylar openings are observed in between the primary petaloid cells, located in a squarish micropylar pit. This micropylar pit has four micropyles, one at each corner of pit. The micropyles are deep and round in shape.

*Aeropyles* — The aeropylar openings are restricted limited upto three to four rows of the polygonal cells on the general exo-chorion. The aeropyles are small and round with thick visible boundaries. In this species, the aeropylar openings are present randomly around the polygonal cells with no clear pattern or number of arrangements as observed in other noctuid species. The micropylar rosette and the transition zone are completely devoid of these aeropyles.

**GENUS PERIDROMA HÜBNER**

*Peridroma* Hübner, 1821, *Verz. Bek. Schmett*., **15**: 227; Lafontaine, 2004, *Am. N of Mexico*, **27.1**: 1-385 19, 32; Ronkay *et al*., 2013, *Fibigeriana* Suppl. **1**: 16; Hacker, 2001, *Esperiana*, **8**: 306.

*Type Species* — *Noctua saucia* Hübner

*Distribution* — Africa; Asia; Europe; India; North America; South America.

**Peridroma saucia (Hübner)**

# (Common Name: Variegated Cutworm)

**(Fig. 19 — 24)**

*Noctua saucia* Hübner, 1808, *Samml. Eur. Schmett*., **4**: pl. 81, f. 378.

*Peridroma saucia* Hübner: Angulo & Jana-Sáenz, 1984, *Gayana Zool*., **48**(3–4): 66; Yoshimoto, 1994, *Tinea*, **14**(Suppl. 1): 99, pl. 14, f. 3; Hacker, 2001, *Esperiana*., **8**: 306; Angulo & Olivares, 2005, *SHILAP Revta. Lepid.*, **33**(130): 155; Olivares *et al*., 2009, *SHILAP Revta. Lepid.*, **37**(148): 396; Ronkay *et al*., 2013, *Fibigeriana* Suppl., **1**: 16; Navarrete *et al*., 2015, *SHILAP Revta. Lepid.*, **43**(170): 289.

*Agrotis saucia* Hübner: Godman & Salvin, 1889, *Biol. Centr.-Amer., Lep. Heterocera*., **1**: 281; Staudinger, 1899, *Naturhist. Mus. Hamburg*., **2**(6): 63.

*Type Locality* — Europe

*Distribution* — India: Himachal Pradesh, Uttarakhand; Canada; China; Japan; New Zealand; Northern Europe; South America.

*Egg Shape* — The egg is spherical in shape having ribs and ridges all over its surface. The egg is 388.88µm in its diameter.

*Egg Chorion* — In this species, the egg chorion is highly textured with thin raised horizontal ribs and thick vertical ridges all over the egg chorion. The general exo-chorion is composed of square, rectangular and pentagonal shaped polygonal cells. These polygonal cells are observed with thick ridges and thin ribs and wrinkle textured cell bases. The aeropylar openings are present at the thick corners of the polygonal cells.

*Micropylar Region* — The micropylar region is located at anterior top of the egg and centrally placed. The micropylar region of the egg is incomplete and composed of primary and secondary petaloid cells. The tertiary petaloid cells and the transition zone are absent in this micropylar region. The polygonal cells of the general exo-chorionic surface area dominate after secondary petaloid cells.

*Micropylar Rosette* — The micropylar rosette is centrally located at the anterior pole of the egg. The micropylar rosette is symmetrically formed of 13 primary petaloid cells having prominent thin raised boundaries with raised and irregularly textured inner basal polygonal surface. A complete thick and raised outer boundary surrounds the primary petaloid cells and holds the rosette compactly. These primary petaloids are further encircled by 12 secondary petaloid cells. The secondary petaloid cells are with thin raised boundaries and plain texture free cell bases. The transition zone is not observed in this species.

*Micropyles* — The micropyles are observed in the centre of the micropylar region. The micropyles are deep and round in shape and present in a plus-shaped micropylar pit. This micropylar pit has four micropyles, one at each corner of the pit.

*Aeropyles* — The aeropyles are observed all over the general exo-chorion surface along the thick polygonal cell corners. The aeropyles are round in shape and with prominent thick circular boundaries. The aeropyles four to six in number as per the shape of the polygonal cells. The aeropyles are absent in the micropylar region of the egg.

**GENUS TRISULA MOORE**

*Trisula* Moore, 1858, in Horsfield & Moore, *Cat. Lep. Ins. Mus. Nat. East India House*, **2**: 420; Roepke, 1941, *Zoologische Mededelingen*, **23**(2), 13–30; Hampson, 1894, *Fauna Moths, India*, **2**: 468; Singh & Shipali, 2014, *Cibtech Jour. Zool.*, **3**(3): 26–27.

*Type Species* — *Trisula variegata* Moore

*Distribution* — India; Indonesia; Nepal; Philippines; South China; Sri Lanka; Thailand.

**Trisula variegata Moore**

## (Common Name: Not Known)

**(Fig. 25 – 30)**

*Trisula variegata* Moore, 1858, *in Horsfield & Moore, Cat. Lep. Ins. Mus. Nat. East India House*, **2**: 420; Roepke, 1941, *Zoologische Mededelingen*, **23**(2), 13–30; Hampson, 1894, *Fauna Moths, India*, **2**: 468; Singh & Shipali, 2014, *Cibtech Jour. Zool.*, **3**(3): 26–27.

*Type Locality* — India (Madras)

*Distribution* — India: Throughout India; Indonesia; Nepal; Philippines; South China; Sri Lanka; Thailand.

*Egg Shape and Size* — The egg is hemispherical and disc-shaped with a flat base for attachment. Its diameter is 1.109mm.

*Egg Chorion* — The egg chorion surface is highly sculptured with raised thick vertical ridges and thin horizontal ribs forming polygonal cells. The polygonal cells are rectangular in shape and some with irregular shapes. These

polygonal cells are observed with sharply defined elevated ribs and ridges, presenting a lace-like textural appearance. The wrinkled texture basal surfaces form deep craters and further enhance three-dimensional relief of the egg chorion. The aeropylar openings are observed in the general exo-chorion, encircling the polygonal cells on thick raised ridges of the egg chorion.

*Micropylar Region* — The micropylar region is located centrally at the anterior pole of the egg. The micropylar region is incomplete and formed of primary and secondary petaloid cells only. The tertiary petaloid cells are absent. The secondary petaloid cells are further surrounded by a clear transition zone with intermediate polygonal cells, creating smooth transition from the micropylar region to the general exo-chorion through gradual reduction in intermediate polygonal cell sizes.

*Micropylar Rosette* — The micropylar rosette is observed at the anterior pole of the egg and centrally located. In this species, the micropylar rosette is formed by 14 tightly arranged and symmetric primary petaloid cells, converging radially towards the central micropyle. These primary petaloid cells are completely distinct in their morphology and formation from the surrounding secondary petaloid cells. These primary petaloid cell are raised, solid, elongated and tapering structures that radiate outwardly. These primary petaloid cells are further encircled by 16 secondary petaloid cells which are elongated, hollow and formed of fine elevated boundaries with wrinkle textured basal surfaces. The tertiary petaloid cells are absent. A distinct transition zone is present with intermediate polygonal cells separating the general exo-chorion from the micropylar apparatus.

*Micropyles* — At the core of the micropylar rosette, a single micropyle is observed. This micropyle is round and deep in its formation and encircled by radiating ridges of the primary petaloid cells. The micropylar pit is absent.

*Aeropyles* — The aeropyles are present on the thick raised vertical ridges at every junction, where the vertical ridges meet the horizontal ribs to form polygonal cell. The polygonal cells are observed with four aeropyles, one at each corner. The aeropyles are smooth, circular, minute with raised thick rims, forming the appearance of hollow cylinders when viewed from sides of the vertical ridges.

## Discussions

The examined species referable to family Noctuidae exhibited a moderate degree of interspecific variation. A recurring family specific trend is observed across all the studied species having ridges and ribs covering entire exo-chorion which prominently resembles to the morphology of butterfly eggs described in Papilionoidea which reflects a conserved structural theme attributable to their common evolutionary lineage. The presence of convergent costal ridging within noctuid further supports the notion of repeated structural evolution of vertical costae in family Noctuidae (Dolinskaya, 2014; Sosa-Gómez et al., 2024). The eggs are uniformly spherical in majority of the studied species and hemi-spherical in *Trisula variegata* Moore. The egg chorion is observed highly sculptured in all examined species with polygonal cells typically ranging in shape from squarish, rectangular and pentagonal. However, the polygonal cells are urn-shaped with square and rectangular cells in *Helicoverpa armigera* (Hübner). The polygonal cells are bounded by raised, thick ridges and thin ribs, forming a distinctive reticulate-ribbed exo-chorionic architecture frequently cited as diagnostic characteristics for noctuid eggs (Dolinskaya, 2016). This reticulate architecture also resembles to the ridged chorion arrays as observed in other noctuid taxa, including *Autographa nigrisigna* (Walker) where vertical chorionic ribs are observed (Xue & Hua, 2018).

The micropylar rosette formation is incomplete in all the studied species and usually comprises of primary petaloid cells and secondary petaloid cells ranging from 10–16 and 12–25 in number respectively. However, in *Helicoverpa armigera* (Hübner), the micropylar rosette is further reduced to only 12 primary petaloid cells. The transition zone

which separates the micropylar region from general exo-chorionic area is observed in all the studied species except *Peridroma saucia* (Hübner). The shape of the micropylar pit is squarish in *Agrotis ipsilon* (Hufnagel) and *Helicoverpa armigera* (Hübner); oval in *Agrotis segetum* (Denis & Schiffermuller); plus shaped in *Peridroma saucia* (Hübner). No distinct pit is observed in *Trisula variegata* Moore. The number of micropylar openings is relatively stable and ranges from 4–6 in majority of the examined species. Only single micropyle is observed in *Trisula variegata* Moore. It has been observed that the aeropylar distribution is restricted to specific rows or ridges in *Agrotis ipsilon* (Hufnagel) and *Helicoverpa armigera* (Hübner) whereas, their number varies from 4–6 as per the shape of the polygonal cells in *Agrotis segetum* (Denis & Schiffermuller), *Peridroma saucia* (Hübner) and *Trisula variegata* Moore. Fehrenbach et al. (1987) attempted to count the number of aeropyles present on the egg chorion in two Noctuid species namely *Heliothis virescens* (Fabricius) and *Spodoptera littoralis* (Boisduval). They recorded 50 aeropyles per egg in *Heliothis virescens* (Fabricius) and 400 aeropyles per egg in *Spodoptera littoralis* (Boisduval). Dolinskaya (2016) affirmed that the micropylar and aeropylar characters can be diagnostic across noctuid subfamilies and supported their systematic utility. The family Noctuidae exhibits high chorionic conservatism in the studied species and confirms the taxonomic and diagnostic usage of egg characters. While species-specific deviations underscore the evolutionary plasticity of chorionic sculpturing as influenced by microhabitat, oviposition strategy and gas-exchange demands supporting the diagnostic reliability of egg characters within family Noctuidae.

## Conclusion

This study employs scanning electron microscopy to examine the fine-scale ultrastructural architecture of the chorion, rectifying a notable gap in lepidopteran biology, where egg-stage morphology has been historically neglected relative to adult and larval traits. A comparative examination of the examined species reveals conserved structural elements amid notable species-specific distinctions. Conversely, various chorionic characteristics, including the shape and size of the eggs, exo-chorion's texture, polygonal boundaries with basal surface granulations, shape of the micropylar pit, number of micropyles, and the count of primary, secondary and tertiary petaloid cells, exhibit considerable variation among species, genera and families. Such data collectively highlight a two-fold pattern of evolutionary conservation and adaptive divergence in egg chorion morphology. The egg chorion serves as a multipurpose interface across all studied taxa, satisfying three essential criteria of early development i.e., safeguarding the embryo, facilitating respiration and regulating fertilisation via micropylar apparatus. This study reveals clear differences in the morphology of the eggs particularly in surface sculpturing of the chorion, distribution of the aeropyles and complexity of the micropylar region. These differences are not arbitrary or just for appearance, but are adaptations that are unique to certain lineages. These characteristics are significant and play important role in egg laying, making conditions favourable in their microhabitats, metabolic needs of their embryos and their life-history strategies. While fundamental architectural components remain uniform among families, but variations in sculptural complexity and structural reinforcement likely reflect ecological adaptations related to oviposition substrate, microclimatic exposure and foetal protection. Incorporating these ultrastructural features in systematic analyses makes it easier to distinguish species apart and shows how SEM-based ultrastructural egg morphology can be a beneficial tool in lepidopteran taxonomy.

# Future scope

The evident diversity in chorionic patterns and adaptations indicates that considerable investigation is still required in this largely overlooked domain. The subsequent investigations may expand taxonomic scope to include other moth families, rare or ecologically specialised species and taxa from under-represented biogeographic locations. Such investigations would enhance our understanding about evolutionary adaptations of the eggs in Lepidoptera to

various settings. The ultrastructural exo-chorionic data possesses practical applications in applied entomology. The detailed morphological data can help to identify species and also be very useful for pest control, monitoring biodiversity and evaluating ecosystems. Understanding the relationship between chorionic structure and environmental resilience, including desiccation resistance, temperature tolerance and oxygen diffusion, could improve methods for predicting population survival in changing climate conditions or habitat destruction.

# Declarations

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**Author contributions** Sainika Jallundhara (collected, processed, photographed the specimen, developed methodology, original description and identified the specimen). Dr. Amritpal Singh Kaleka (corroborated the identification and reviewed description). Both authors prepared and reviewed the manuscript.

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**Competing interests** The authors declare no competing interests.

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# Table

**Table No. 1: Comparative account of ultrastructural egg characters in studied species of Family Noctuidae**

| Egg Characters | ***Agrotis ipsilon* (Hufnagel)** | ***Agrotis segetum* (Denis & Schiffermüller)** | ***Helicoverpa armigera* (Hübner)** | ***Peridroma saucia* (Hübner)** | ***Trisula variegata* Moore** |
|---|---|---|---|---|---|
| Egg shape | Spherical | Spherical | Spherical | Spherical | Hemi-spherical |
| Egg size (diameter) | 573µm | 388.88µm | 360.6µm | 388.88µm | 1.109mm |
| Chorion texture | Highly sculptured | Highly sculptured | Highly sculptured | Highly sculptured | Highly sculptured |
| Polygonal cell shapes | Square, Rectangular, Pentagonal | Square, Rectangular, Pentagonal | Urn, Square, Rectangular | Square, Rectangular, Pentagonal | Rectangular, Irregular |
| Polygonal cell boundaries | Raised thick ridges and thin ribs | Raised thick ridges and thin ribs | Raised thick ridges and thin ribs | Raised thick ridges and thin ribs | Raised thick ridges and thin ribs |
| Polygonal cell base texture | Smooth texture-free | Wrinkled texture | Wrinkled texture | Wrinkled texture | Wrinkled texture |
| Micropylar rosette composition | PPC+SPC | PPC+SPC | PPC | PPC+SPC | PPC+SPC |
| Primary petaloid cells (PPC) | 10 | 16 | 12 | 13 | 14 |
| Secondary petaloid cells (SPC) | 14 | 25 | Absent | 12 | 16 |
| Tertiary petaloid cells (TPC) | Absent | Absent | Absent | Absent | Absent |
| Transition zone | Present | Present | Present | Absent | Present |
| Micropylar pit shape | Square | Oval | Square | Plus | Absent |
| Micropyles | 4 | 5-6 | 4 | 4 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| Aeropyles | Restricted to some rows,<br><br>4-6 per polygon | 4-6 per polygon | Restricted to some rows,<br><br>present randomly on ridges | 4-6 per polygon | 4 per polygon |

## Plates

Plates I-VI are available in the Supplementary Files section.

## Figures

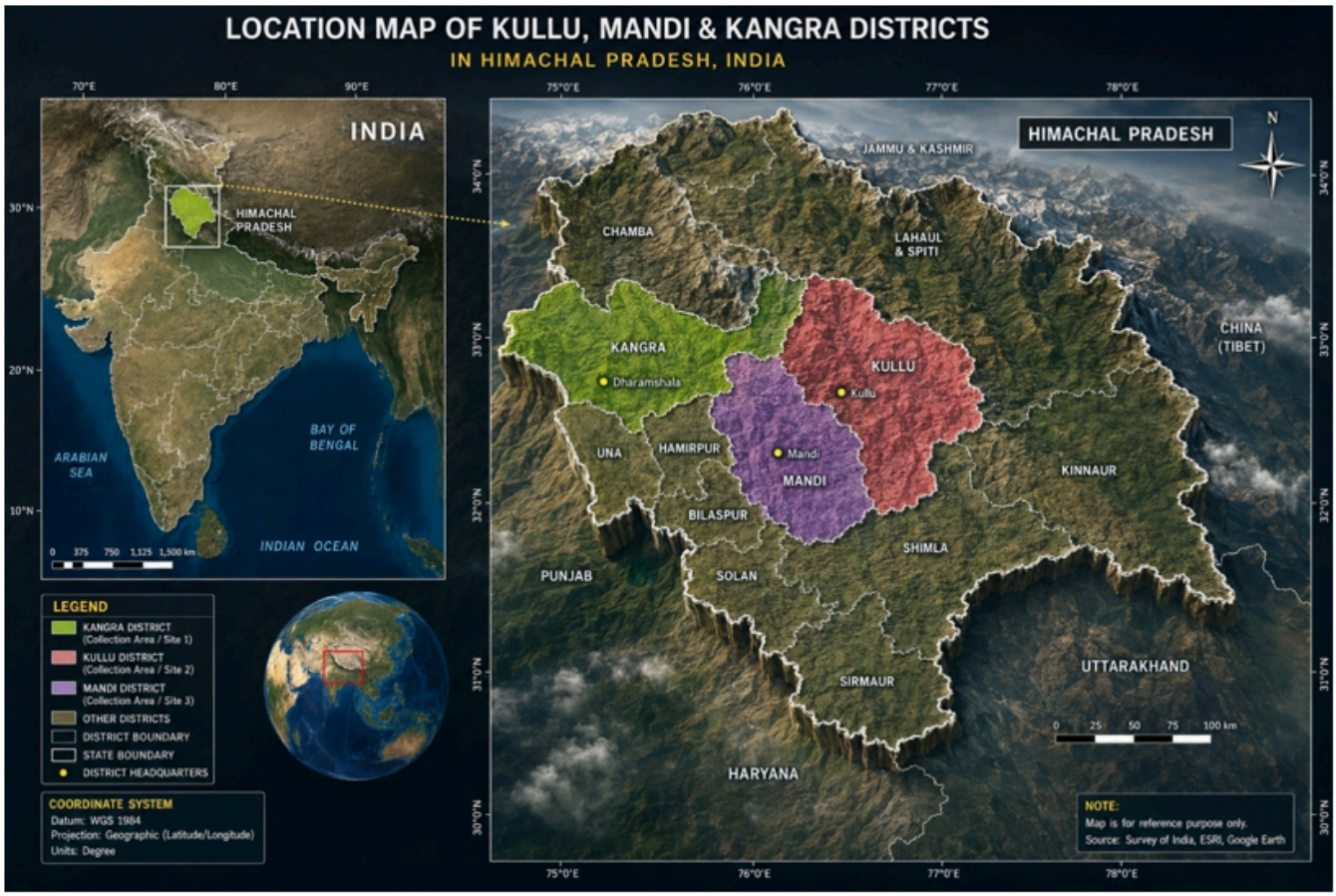



**Figure 1**

Unnumbered image in the Materials & Methods section.

## Supplementary Files

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