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ULTRASTRUCTURE OF EGG CHORION AND ITS SCULPTURE IN TWO ARCTIIDS (EREBIDAE: ARCTIINAE)

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ABSTRACT

The identification of moths has been always dependent on characters like wing maculation, wing venation, male and female genitalic features, but the ultrastructural characters like egg chorion patterns have been completely ignored. These are equally important for identification. In the present study, scanning electron microscope was used to inspect, characterize, and depict eggs of two tiger moth species i.e., *Andala unifascia* Walker, 1855 and *Creatonotos transiens* (Walker, 1855) referable to family Erebidae. On the eggshells of both these species, morphological features have been observed and these are of great taxonomic value at species as well as at genus levels. These characters will enable identifying moths at much earlier stages of their life histories i.e., eggs.

Key words: Aeropyles, *Andala unifascia*, Arctiidae, *Creatonotos transiens*, micropylar region, micropylar rosette, micropyles, polygonal cells, scanning electron microscope, taxonomy, ultrastructure

The eggs are well established mode of reproduction among invertebrates including insects, spiders, mollusks, and crustaceans. Like most other insects, the lepidopterans are oviparous or "egg-bearers" (Gullan and Cranston, 2004). Many species lay their eggs singly in widely spread manner, in tiny clumps or in masses, while others lay the egg masses which are further coated by a hardened fluid from the female's abdomen glands (Holland, 1898; 1903). The taxonomic and phylogenetic significance of egg shell architecture has been demonstrated in several lepidopteran families- Noctuidae (Salkeld, 1984), Lycaenidae (Munguira et al., 2015), Danaidae (Kitching, 1985), Mnesarchaeidae (Kobayashi and Gibb 1995), Rougerie and Estradel 2008), Pieridae (Llorente-Bousquets and Castro-Gerardino 2007; Llorente-Bousquets et al., 2018; Hernández–Mejía et al., 2013), Nymphalidae (García-Barros and Martín, 1995; Freitas and Brown, 2004; Nieves-Uribe et al., 2015) and others.

In Lepidoptera, the eggs are usually elliptical, spherical, hemispherical, flattened, slightly cuboid and asymmetrical in shape. The chorion may have simple and basic structure or highly patterned surface, consisting of divisions with transverse or longitudinal ridges (Noctuidae). The egg shell generally possesses microscopic minute grooves or holes (airspaces, air-pores or aeropyles) which can only be seen in high magnification and resolution power of an electron microscope. These air-pores permit respiratory interchange of gases i.e., oxygen (O_2) and carbon dioxide (CO_2) between the environment and developing larvae with fairly very small amount of water loss. The upper side of the egg is somewhat depressed and creates a small medial chamber with minute aperture known as micropyle (Evans. 1932). It is an exceptional aperture near anterior top of the egg which provides a portal for introduction of male gamete during fertilization in these insects. This micropylar region is located on the upper anterior portion of eggs which are globular, conical, or cylindrical in shapes 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 aim of this paper is to show the use of egg characters for the classification of species. This article also provides keys to the species, based on egg characters, for these two tiger moths in India. In the present study, scanning electron microscope was used to inspect, characterize, and depict eggs of two tiger moth species i.e., *Andala unifascia* Walker, 1855 and *Creatonotos transiens* (Walker, 1855) referable to family Erebidae in order to facilitate the identification tasks of moths at much earlier stages of their life histories i.e., eggs.

MATERIALS AND METHODS

Collection tour was conducted in locality Sainj (Kullu), District of Himachal Pradesh (31.77029° N, 77.30515° E), to collect the eggs of moths during April, 2022. The light trap method was used for collection and mating of adult moths in order to obtain freshly laid eggs (Dolinskaya, 2019); The freshly laid eggs were then hand-picked with fine forceps and brushes and preserved in 70% alcohol and glycerol in the ratio of 8:2; The collected moths, after obtaining eggs were stretched and preserved in duly fumigated and air-tight wooden boxes for further identifications.

The sample eggs were fixed in 2.5% glutaraldehyde for a minimum duration of one hour. Then, the material was shifted into phosphate buffer solution (PBS) having 7.4 pH and rinsed repeatedly for a minimum period of 15 minutes. The eggs were dehydrated by manoeuvring a succession of graded ethyl alcohol (in 50% alcohol for 15 min, followed by 70% and 90% alcohol for 15 min each and thrice changes in 100% alcohol for 10 min each). After dehydration, the sample material was mounted on aluminium stubs with double-sided adhesive carbon tape and sputter coated with a mixture of gold and platinum. The sputtered samples were observed and studied under scanning electron microscope (JEOL JSM-6510LV) available in the sophisticated Instrumentation Centre of Punjabi University, Patiala. The sample material was photographed to explore the egg chorion, micropylar region, arrangement of micropylar rosette, aeropyles and other external ultra-structural characters present. The terminology used Zolotuhin and Kurshakov (2009) and Dolinskaya (2019).

RESULTS AND DISCUSSION

***Andala unifascia* Walker, 1855** (Fig. 1a-1f)

Andala unifascia Walker, 1855; List Spec. Lepid. Insects Colln. Br. Mus. 3: 774; Singh et al., 2014, Rec. Zool. Survey India. Occ. Pap 367: 4.

Alphaea varia Walker, (1865), List Spec. Lepid. Insects Colln. Br. Mus. 31: 297.

Diacrisia unifascia Walker: Hampson, 1901, Cat. Lepid. Phalaenae Br. Mus. 3: 273, pl. 44, f. 7; Rothschild, 1910, Novit. Zool. 17(2): 125.

In present species, the egg is spherical and 0.639 mm in its diameter (appears deflated due to their very delicate egg chorion that easily loses its original shape). The general egg surface area is smooth and lightly textured as observed under scanning electron microscope. The chorionic surface wall is lightly sculptured and uniformly patterned with hexagonal and heptagonal cells. The polygonal cells are observed with slightly marked thin boundaries or walls and these boundaries make them distinct from one another. The small aeropylar openings or respiratory pores are present on these thin boundaries of these polygonal cells. These air-openings are without any special or unique structure. The polygonal cells are clear and smooth in its surface structure without any craters or depressions at the center.

The micropylar region is present at the anterior top of the egg forming a micropylar depression. This micropylar depression has a micropylar pit and primary petaloid-cells forming a rosette followed by a series of secondary petaloid cells. In total 9 petal shaped primary cells are found around the micropylar pit and present in a flower-like fashion to form a distinct rosette. Half the portion of these cells is seen fused with each other with curved and slightly raised edges to demarcate their boundaries. The inner surface area of these cells is seen crimpled and wrinkled or somewhat textured. The primary petal-shaped cells are surrounded by secondary petaloid cells to further decorate the micropylar rosette. Micropyles openings are present in the center

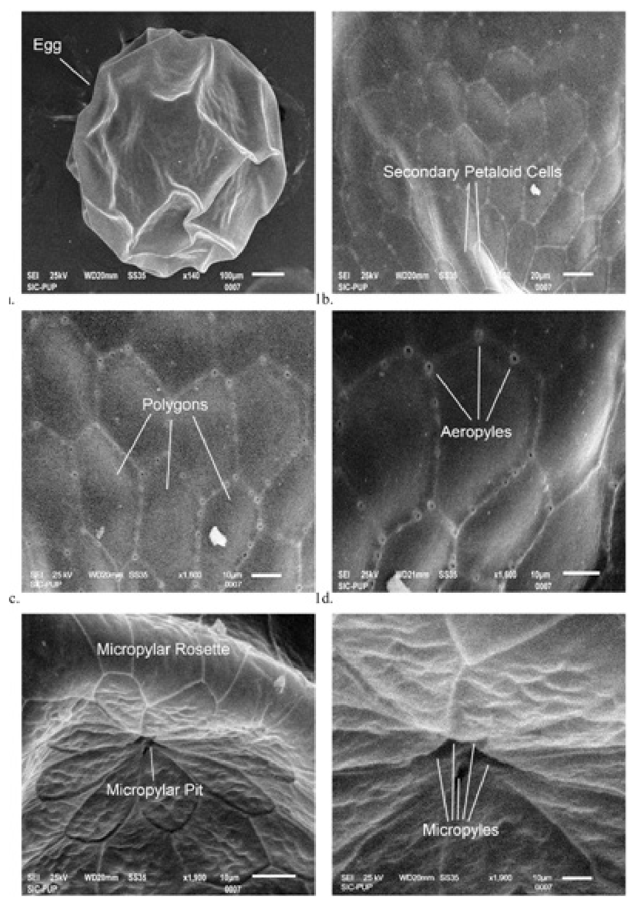

Fig. 1a-1f. Egg of *A. unifascia*
a: dorsal view; b-c: egg chorion, d: aeropyles;
e-f: micropylar region

of micropylar region at anterior end of the egg. A star shaped depressed hollow structure is present in center of the primary cells. In total 5 micropylar openings are present in this star-shaped pit, each at every corner of the star-shaped micropylar pit. The aeropyles are found in an irregular fashion on each side of the polygonal cells present all over the chorionic surface of the egg. The number of aeropylar openings varies from 10-13 per cell according to the shape of the polygonal cell. The structure of the air-cavities is found to be somewhat raised and thickened bordering the tiny holes. The air openings present on the corners of the polygonal cells are bigger and more prominent in appearance than the aeropyles present on the sides of these polygonal cells.

Specimens examined: Himachal Pradesh: Kullu: Sainj: 15. vi. 2022, 3 eggs.

Distribution: North-Western India and Pakistan.

Comments: Walker (1855) described the present genus as a monotypic genus with *unifascia* as its type species and still it is represented by single species.

***Creatonotos transiens* (Walker, 1855) (**Fig. 2a-2f)

Phissama transiens Walker, 1855, Cat. Lep. Het., 3: 675; Moore, 1882, Lep. E. Ind. Co., 1882: 16; Kirby, 1892, Cat. Het., 1: 243; Hampson, 1894, Moths Ind., 2: 29; Seitz, 1910, Macrolep. World, 2: 90; Daniel, 1943, Mitt. Munch. Ent. Ges., 33(3): 743.

Creatonotos transiens Walker: Holloway, 1976, Moths of Borneo, 1976: 4; Barlow, 1982, Moths South-East Asia, 1982: 74; Dubatolov and Holloway, 2007, Bonn. Zool. Beitr., 55(2): 115; Cerny, 2011, Entomofauna, 32(3): 40.

Spilosoma transiens Walker, 1855, List Specimens Lepid. Insects Colln. Br. Mus., 3: 675.

Creatonotus transiens Walker: Hampson, 1901, Cat. Lepid. Phalaenae Br. Mus., 3: 334; Rothschild, 1910, Novit. Zool., 17 (2): 160; Hampson, 1920, Cat. Lep. Phalaenae Br. Mus. (Suppl.), 2: 428; Kirti and Singh, 1994, Geobios New Reports, 13(1): 22.

The egg is almost spherical in shape and 0.763mm in its diameter. The general egg surface is smooth and is observed with a slightly depressed cavity (Micropylar region) present at anterior top of the egg. The chorion surface, under magnification is found highly sculptured with polygonal cells such as tetragonal, pentagonal as well as hexagonal cells all over the egg surface. These cells are completely flat and smooth without any ridges or ribs. Each polygonal cell has tiny aeropylar openings on its boundary and per cell about 20-35 such openings are there. The micropylar region is present in slightly depressed area at the top anterior region of the egg. The micropylar region of the egg consists of micropylar openings and series of petal-shaped primary and secondary cells forming micropylar rosette starting from the center towards periphery. The micropylar rosette is formed of a total of 9 petal-shaped primary cells arising from the central part of micropylar pit. These primary cells are further surrounded by secondary petaloid cells which merges into transition zone (where the micropylar rosette ends and mergers with polygonal cells). No visible ribs and ridges are found around the rosette cells. The micropylar openings are present in the center of micropylar region. These openings are 4 in number and present in each of the corner of the squarish micropylar pit. The aeropyles or air-openings are present on the boundaries or walls of the polygonal cells demarcating their shapes. These air-openings are approximately 20-35 in number and the number varies

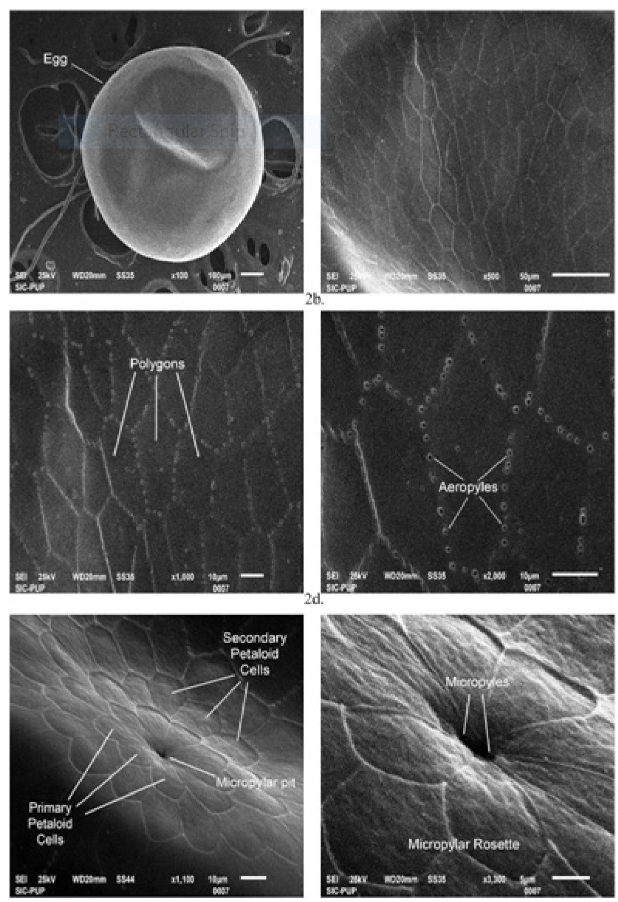

Fig. 2a-2f. Egg of *C. transiens*
a: dorsal view; b-c: egg chorion;
d: aeropyles; e-f: micropylar region

according to the shape of each cell. Each aeropyle is somewhat flat in shape and with a very slightly raised boundary encircling the openings.

Specimens examined: Himachal Pradesh: Kullu: Sainj: 23.vi.2022, 2 eggs.

Distribution: Throughout India, China, Java, Myanmar, and Sri Lanka.

Comments: This genus is represented by three species i.e., *gangis* Linnaeus, 1763; *transiens* Walker, 1855 and *ananthakrishanani* Kirti & Kaleka, 1999 from India. The species of this genus are known for their coremata i.e., specialized scent glands which they possess internally in pockets of 7th and 8th abdominal sternite in males. De la Torre-Buneo (1950) defined these unique glands and these glands emit some volatile chemicals responsible for sex attraction. Hariprasad and Kanaujia (2000) and Kaleka and Bali (2020) also discussed about these glands. These glands are usually twice the length of moth's body.

Key to species (Based on egg)

Egg chorion uniformly patterned with hexagonal and heptagonal cells; 10-13 aeropylar openings present on the boundaries of each polygonal cell. Micropylar rosette textured; micropylar pit star-shaped……...……… *Andala unifascia* Walker, 1855

Egg chorion uniformly patterned with tetragonal, pentagonal, and hexagonal cells; 20-35 aeropylar openings present on the boundaries of each polygonal cell. Micropylar rosette smooth; micropylar pit squarish in shape…………*Creatonotos transiens* (Walker, 1855)

Results reveal that the external morphology or ultrastructure of egg's chorion is distinct in both the studied species referable to two distinct genera with respect to their chorion sculpturing, number of primary cells forming the micropylar region, number, and shape of micropylar and aeropylar openings. The general pattern of egg chorion has been found similar as in other Erebidae investigated by Doring (1955) and Peterson (1963) by means of light microscopy. About aeropylar openings on the egg chorion in lepidopteran eggs quite limited studies are there. Fehrenbach et al. (1987) attempted to count the number of aeropyles present on egg chorion in two noctuid species namely *Heliothis virescens* Fabricius, 1777 and *Spodoptera littoralis* (Boisduval, 1833). They recorded 50 aeropyles/ egg in *H. virescens* and 400 aeropyles/ egg in *S. littoralis*. In the present study, an attempt has been made to examine the number as well as shape of aeropylar openings. The number of aeropyles per polygonal cell has been observed to be 20-35/ polygonal cell in the eggs of *C. transiens* which remains limited to 10-13/ cell in case of *A. unifascia* (Table 1). Based on present work, it can be easily concluded that the ultrastructural features are also remarkable and these can authenticate and strengthen the morphotaxonomy. Further inspection of such features present on the eggs of more species of various moths needs to be conducted.

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AUTHOR CONTRIBUTION STATEMENT

K.S.A: Compiled and analyzed data; J.S: Insect

Table 1. Important ultrastructural egg chorion characters

Studied species	Egg features								
	Egg shape	Egg size (diameter)	Chorion texture	Shape of polygons	Number of aeropyles (per polygonal cell)	Number of micropyles	Primary petaloid cells	Secondary petaloid cells	Shape of micropylar Pit
Andala unifascia Walker	Spherical	0.639 mm	Lightly sculptured	Hexagonal & heptagonal	10-13	5	9 Petaloid	Present	Star shaped
Creatonotos transiens (Walker)	Spherical	0.763 mm	Highly sculptured	Tetragonal, pentagonal & hexagonal	20-35	4	9 Petaloid	Present	Square shaped

collection, photography, scanning and compilation; K.Y: Insect collection and compilation.

CONFLICT OF INTEREST

No conflict of interest.

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