Nota lepid. 23 (1): 50-63; 01.IV.2000

## Eggshell fine structure of some species of Lithosiinae (Arctiidae) of Far East Russia

Igor G. Pljushch & Irina V. Dolinskaya

Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, UA-01601 Kiev MSP, Ukraine

**Summary.** Eggs of 7 species belonging to 6 genera of the Lithosiinae (Arctiidae) from the Primorskiy Krai (Far East Russia) were examined with the use of scanning electron microscopy. Descriptions and a comparative morphological analysis are provided for all of these species. The diagnostic characters for genera and species are chosen.

Zusammenfassung. Eier von 7 Arten aus 6 verschiedenen Gattungen der Lithosiinae (Arctiidae) aus dem Primorskiy Krai in Fernen Osten Rußlands wurden rasterelektronenmikroskopisch untersucht. Fur jede Art werden die Eier beschrieben und eine vergleichende morphologische Analyse gegeben. Diagnostische Merkmale für die Gattungen und Arten werden begrundet.

**Résumé.** Des œufs de 7 espèces, appartenant à 6 genres de Lithosiinae (Arctiidae), en provenance du Primorskiy Krai (Extrême Orient russe), ont été étudiés par moyen d'un microscope électronique à balayage. La description, ainsi que l'analyse comparative de la morphologie des œufs etudiés est présentée pour chacune de ces espèces. Des caractères diagnostiques des œufs pour les genres autant que les espèces sont établis.

Key words: Lithosiinae, Arctiidae, egg, exochorion sculpture, morphology, Far East Russia.

## Introduction

The knowledge of the egg chorion of Arctiidae is still insufficient and fragmentary. The eggs of some members of the subfamily Arctiinae have been studied in some detail (Döring, 1955; Sarlet, 1967; Hinton, 1981; Orlando & Evoneo, 1984), whereas those of the subfamily Lithosiinae remain poorly studied (Döring, 1955; McFarland, 1972; Lafontaine *et al.*, 1982; García-Barros, 1985, 1986). Thus, the aim of our study was, even if partly, to fill this gap.

## **Material and Methods**

The work is based on the original material collected by the authors in the Primorskiy Krai (Far East Russia). The eggs were

obtained from females captured in the field. The moths were placed into pasteboard boxes of a size of  $100 \times 90 \times 50$  mm, overlaid with lichens. Females also laid the eggs at random in the box, without choosing the special substrate. The eggs were examined with the use of scanning electron microscopy (SEM). Both dry egg chorions that were collected after hatching, and fresh specimens fixed with glutaraldehyde-osmium tetroxide or with alcohol were examined. Fixed eggs were first dehydrated in increasing acetone concentrations, and then critical point dried.

The Lithosiinae species were identified with the aid of Daniel (1939, 1951, 1952, 1954) and Inoue (1982). The systematic arrangement follows Dubatolov *et al.* (1993).

## Description

#### Stigmatophora laecrita (Swinhoe, 1894)

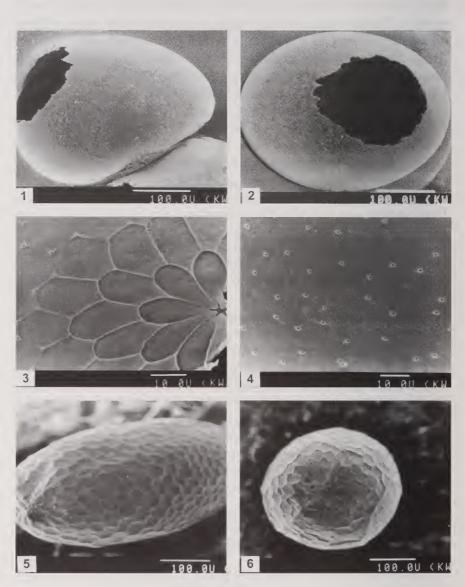
Material. 29, Russian Federation, Primorskiy Krai, Ussuriisky rayon, Gorno-Tayozhnaya Stantsia (Mountain-Taiga Station), 22.VII.1995 (eggs laid the same day; 10 eggs examined).

Egg spherical (Figs 1, 2), height 0.5 mm, diameter 0.5–0.55 mm. Chorion white, hyaline. Exochorion sculpture cellular in the micropylar area and with clearly marked aeropyles on the remaining egg surface. Micropylar area more clearly expressed, represented by 2–3 rows of polygonal cells (Figs 2, 3). Micropylar rosette (50.8– 58.9 µm in diameter) with 10–11 petalled cells, 3 times longer than wide, joined along  $^{2}/_{3}-^{3}/_{4}$  of their length. Central portion of the rosette like a small star-shaped depression (7.6–9.3 µm in diameter), 6.3–6.7 times smaller than rosette diameter, with 5 micropylar openings. The remaining egg surface smooth, with hardly noticeable cells but clearly expressed aeropyles, bordered by roller-like margins (9.8–12.0 µm in diameter) (Fig. 4).

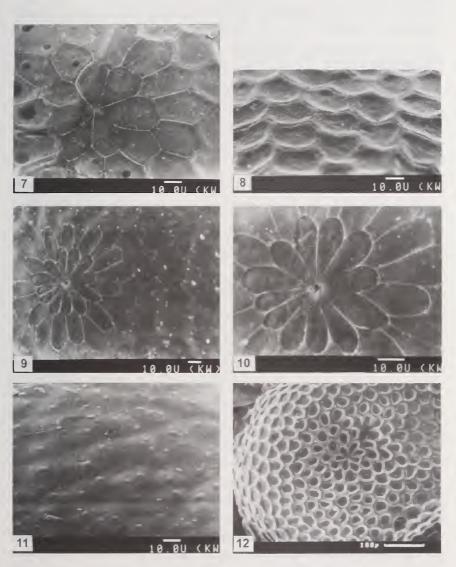
The larva nibbles out a large rounded opening at the apical area of the egg (Figs 1, 2). Caged females laid the eggs in single-layered clusters.

#### Miltochrista miniata rosaria Butler, 1877

Material. 49, Russian Federation, Primorskiy Krai, Ussuriisky rayon, Gorno-Tayozhnaya Stantsia (Mountain-Taiga Station), 6.VII., 8.VII.1995 (eggs laid the same days; 10 eggs examined).



Figs 1–6: 1 – *S. laecrita* (× 240), 2 – *S. laecrita* (× 240), 3 – *S. laecrita* (× 1500), 4 – *S. laecrita* (× 1200), 5 – *M. miniata rosaria* (× 220), 6 – *M. miniata rosaria* (× 200).



Figs 7–12: 7 – *M. miniata rosaria* (× 780), 8 – *M. miniata rosaria* (× 780), 9 – *P. staudingeri* (× 540), 10 – *P. staudingeri* (× 1100), 11 – *P. staudingeri* (× 720), 12 – *L. quadra* (× 190).

Egg high, oblong-ovoid, narrowing towards the base (Fig. 5). Height 0.7–0.85 mm, the largest width 0.3–0.45 mm. Egg pale orange. Chorion pale brown, hyaline. Exochorion sculpture clearly expressed, cellular (Fig. 8). Micropylar area represented by 2–3 rows of polygonal cells with flat cell margins (Figs 6, 7). Micropylar rosette (51.9–68.6 µm in diameter) with 7–8 wide cells, 1.5–2 times longer than wide, joining along 1/2-2/3 of their length. Central portion of the rosette like a small star-shaped depression (4.6–7.1 µm in diameter), 9.7–11.2 times less than rosette diameter with 3–5 micropylar openings (Fig. 8). The cells across the remaining egg surface are concave and show sharply uplifted cell margins reaching 1.3–1.6 µm (Fig. 8).

The larva nibbles out a rounded opening at the apical area of the egg. In captivity, the eggs were laid in single-layered, loose clusters.

#### Paraona staudingeri Alpheraky, 1897

**Material.** 3, Russian Federation, Primorskiy Krai, Ussuriisky rayon, Gorno-Tayozhnaya Stantsia (Mountain-Taiga Station), 4, 9, 11.VII.1995 (eggs laid the same days; 10 eggs examined).

Egg spherical, height 0.5 mm, diameter 0.65 mm. Egg pale green, immediately before eclosion the egg is green. Chorion white, hyaline. Exochorion sculpture cellular in the micropylar area, remaining egg surface faintly folded and with moderately expressed aeropyles (Fig. 9). Micropylar area more clearly marked, represented with 2–3 rows of polygonal cells. Micropylar rosette (72.6–91.5  $\mu$ m in diameter) with 11–13 cells, 2–3 times longer than wide, with margins fusing along  $^{2}/_{3}-^{3}/_{4}$  of their length. Central portion of the rosette looking like a small star-shaped depression (5.0–6.3  $\mu$ m in diameter), 14.5 times smaller than the rosette diameter, with 5 micropyles (Fig. 10). The remaining egg surface is slightly folded, and presents hardly noticeable cells with slender cell margins (0.2–0.3  $\mu$ m) and more clearly identifiable aeropyles (1.95–2.9  $\mu$ m in diameter) bordered by roller-like margins (Fig. 11).

The larva nibbles out a rounded opening at the apical area of the egg. Captive females laid the eggs in single-layered clusters.

## Lithosia quadra (Linnaeus, 1758)

**Material.** 29, Russian Federation, Primorskiy Krai, Ussuriisky rayon, Gorno-Tayozhnaya Stantsia (Mountain-Taiga Station), 7.VII.1994 (eggs laid the same day; 10 eggs examined).

Egg spherical, height 0.5 mm, diameter 0.5–0.6 mm. Egg palegreen. Chorion white, hyaline. Exochorion sculpture clearly expressed, pitted-cellular (Fig. 12). Micropylar area represented by 2 rows of pitted cells. The micropylar area sculpture differs from that on the remaining egg surface by the lack of aeropyles (Fig. 13). Micropylar rosette (67.0–85.0  $\mu$ m in diameter) with 8–9 wide cells, 1.5–2 times longer than wide, with margins fused along  $^{2}/_{3}$ – $^{3}/_{4}$  of their length. Central portion of the rosette looking like a small starshaped depression (7.4–8.5  $\mu$ m in diameter), 9–10 times smaller than the rosette diameter, with 5 micropyles (Fig. 14). Remaining egg surface with wide cell margins (10.2–10.8  $\mu$ m) and minute aeropyles (2.8–3.1  $\mu$ m in diameter) at the margin junctions (Fig. 15).

We consider this type of sculpture to be transitional between the pitted and the cellular sculpture. Its cells are not polygonal (as in the micropylar area or in the Notodontidae) (Dolinskaya, 1987a, b), but are rather rounded or oval with unclear margins.

The larva nibbles out a rounded opening at the apical area of the egg. Captive females laid the eggs in single-layered clusters.

#### Agylla gigantea (Oberthür, 1879), A. collitoides (Butler, 1885)

**Material**. A. gigantea 29, A. collitoides 29, Russian Federation, Primorskiy Krai, Ussuriisky rayon, Gorno-Tayozhnaya Stantsia (Mountain-Taiga Station), 4, 7.VII.1995 (eggs laid the same days; 10 eggs of each species examined).

Egg spherical, height 0.45–0.55 mm, diameter 0.55–0.7 mm. Egg of *A. collitoides* pale-green with faint bluish hue. Chorion white, hyaline. Exochorion sculpture cellular in the micropylar area and remaining egg surface folded with sharply expressed aeropyles united in the cells (Figs 16, 17, 20). Micropylar area represented by 2–3 rows of oblong polygonal cells (Fig. 18). Micropylar rosette (71.7–89.0  $\mu$ m in diameter) with 11–13 petalled cells, 2–3 times longer than wide, joined along  $^{2}/_{3}$ – $^{3}/_{4}$  of their length. Central portion of rosette looking like a small star-shaped depression (8.2– 11.7  $\mu$ m in diameter), 7.6–8.7 times smaller than rosette diameter, with 4–5 micropylar openings (Fig. 20). Remaining egg surface folded, looking sharply expressed, united in the cell aeropyles, bordered by large roller-like margins (4.0–5.7  $\mu$ m in diameter) and with hardly noticeable cell margins (Figs 20, 21).

The larva nibbles out a rounded opening at the apical area of the egg. Captive females laid the eggs in single-layered clusters.

*Note*. No significant differences have been found between both species.

#### Eilema griseolum (Hübner, [1803])

**Material.** 3, Russian Federation, Primorskiy Krai, Ussuriisky rayon, Gorno-Tayozhnaya Stantsia (Mountain-Taiga Station), 16, 18, 23.VII.1995 (eggs laid the same days; 10 eggs examined).

Egg spherical, height 0.55–0.65 mm, diameter ca. 0.7 mm. Egg yellowish white. Chorion white, hyaline. Exochorion sculpture weakly expressed, consisting of hardly noticeable cells (Fig. 22). (Clear border between micropylar area and remaining egg surface lacking). There is a progressive transition in egg surface appearance from the micropylar area towards the egg equator. Sculpture more clearly defined in the first two rows (1–2 rows) of the micropylar area, then gradually disappearing (Fig. 23). Micropylar rosette (66.8–116.0  $\mu$ m in diameter) with 14–17 petalled cells, 2–3 times longer than wide, joining along <sup>3</sup>/<sub>4</sub> of their length. Central portion of rosette looking like a small depression (6.7–8.7  $\mu$ m in diameter), 10.0–13.3 times smaller than rosette diameter, with 4–8 micropyles (Fig. 24).

The larva nibbles out a rounded opening at the apical area of the egg. Eggs laid in single-layered clusters.

## Discussion

Based on the foregoing data, we can reach the following conclusions.

Eggs of the studied lichen moths are radial symmetric (upright type) with the micropylar area located at the pole opposite to the surface. Most of the studied Lithosiinae genera have spherical eggs (*Stigmatophora* Stgr., *Eilema* Hb., *Agylla* Wlk.), while oblong-ovoid-shaped eggs occur in *Miltochrista* Hb.. The size ratio of the

eggs varies: spherical — height 0.45–0.65 mm, diameter 0.5–0.7  $\mu$ m; oblong-ovoid — height 0.7–0.85  $\mu$ m, largest width 0.3–0.45  $\mu$ m.

The coloration of the eggs depends of their contents, and is mostly of a greenish tint (*Paraona* Moore, *Lithosia quadra*, *A. collitoides*). Some species have yellowish-white (*E. griseolum*) or pale orange (*Miltochrista*) eggs. The chorion is hyaline, mostly white (otherwise pale brown: *Miltochrista*).

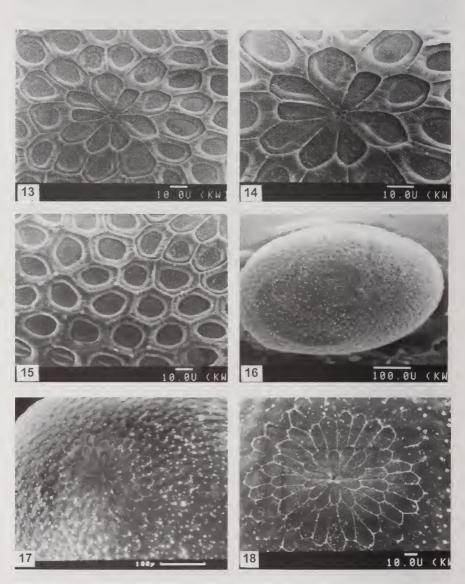
The exochorionic sculpture consists of a reticulate pattern, formed by the cell margins. It may be cellular over the whole egg surface, with sharply pronounced cell margins (*Miltochrista*) or cells developing only in the micropylar area, and the remaining egg surface with clearly expressed aeropyles united in the cells (*Stigmatophora, Paraona Agylla*). Sometimes cells are hardly noticeable (*Eilema*). The sculpture may also be peculiar, pitted-cellular (*L. quadra*). A similar type of sculpture is present in some species of the family Lasiocampidae (*Euthrix potatoria askoldensis* Oberth., *Paralebeda plagiera* Wlk. — authors' original data, unpublished yet).

The cell margins of some Lithosiinae eggs are very narrow  $(0.2-0.3 \ \mu\text{m})$ , weakly marked, or lacking. However, in some species they are sharply pronounced, reaching 1.3–1.6 mm (*Miltochrista*) or 10.2–10.8  $\mu\text{m}$  (*L. quadra*).

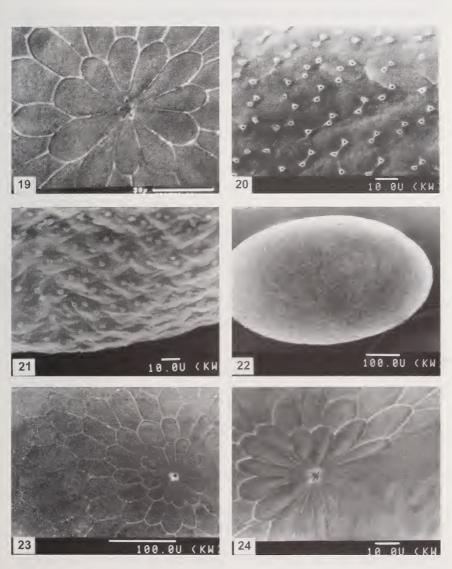
The aeropyles can be either sharply expressed, bordered with large roller-like margins (up to  $4.0-5.7 \ \mu m$  in *Agylla* spp., or  $9.8-12.0 \ \mu m$  in *Stigmatophora*), small ( $2.8-3.1 \ \mu m$  in *L. quadra*), or even not identifiable under SEM (as in *Eilema, Miltochrista*).

The micropylar area is represented by the rosette and 2–3 rows of polygonal cells with clearly expressed cell margins. The size of the micropylar rosette varies from 50.8–68.6  $\mu$ m (*Stigmatophora*, *Miltochrista*) to 71.7–91.5  $\mu$ m (*Agylla*, *Paraona*), and the number of rosette cells amounts to between 7–9 (*L. quadra*, *M. miniata rosaria*) and 14–17 (*Eilema*). The central portion of the rosette looks like a small star-shaped depression, 6.3–14.5 times smaller than the rosette diameter. The number of micropylar openings varies from 3 to 5.

Studied species of Lithosiinae are similar to Arctiinae (Pljushch & Dolinskaya, in press) in having the same oviposition behaviour in captivity. The eggs are laid in single-layer tight clusters, where



Figs 13–18: 13 – *L. quadra* (× 780), 14 – *L. quadra* (× 1200), 15 – *L. quadra* (× 780), 16 – *A. gigantea* (× 160), 17 – *A. gigantea* (× 210), 18 – *A. gigantea* (× 540).



Figs 19–24: 19 – A. gigantea (× 950), 20 – A. collitoides (× 1000), 21 – A. collitoides (× 780), 22 – E. griseolum (× 150), 23 – E. griseolum (× 280), 24 – E. griseolum (× 1000).

they are pressed to one another (*Stigmatophora*, *Paraona*, *Eilema*, *Lithosia*, *Agylla*) or not (*Miltochrista*).

The larva nibbles out a uniformly large, rounded opening at the apical (micropylar) area of the eggs, more rarely in the apical-lateral part.

Comparative table of the character states in studied species of Lithosiinae.

Species >	1	2	3	4	5	6
Shape (A – spherical, B – oblong- ovoid).	A	В	А	А	А	А
Egg colour (A - different green tints, B – pale orange, C – yellowish white)	-	В	А	А	A*	С
Chorion colour (A - white, B – pale brown)	A	В	А	А	А	А
Exochorion sculpture (A – cellular only in the micropylar area, the remaining surface looks like aeropyles, B – cellular over the whole egg sur- face, C – hardly noticeable cells, D - pitted-cellular	A	В	A	D	A	С
Egg margins (A - hardly noticeable, B – clearly noticeable)	A	В	А	В	А	А
Aeropyles (A – moderately or clearly noticeable, B – not visible, C – sharply noticeable)	A	В	A	А	С	В
Oviposition (A – the eggs pressed to one another, B – not pressed).	А	В	А	А	А	А

1 - S. laecrita, 2 - M. miniata, 3 - P. staudingeri, 4 - L. quadra, 5 - A. gigantea & A. collitoides, 6 - E. griseolum. \* Available for A. collitoides only.

The comparative analysis pursued herein allows the evaluation of the following characters (Table).

1. The egg shape can be used to identify some genera (*Miltochrista*) or for grouping of the genera (see below).

2. Such characteristics as the way the larva nibbles out the eclosion opening and the oviposition behaviour in captivity are not as reliable as in tiger-moths.

3. The egg coloration can be used to identify species or genera.

4. The chorion coloration can be used to identify some genera (*Miltochrista*).

5. The exochorion sculpture allows to identify species and genera. It is useful for grouping of the genera (see below).

6. The characters of the micropylar area, such as the number of micropylar area rows, the number of rosette cells, and the number of micropylar openings, are the only quantitative characters representing a continuous clinal variation and are subject to intraspecific variation. Therefore only the extremes of a cline could appear useful for diagnostic purposes.

As a result, all examined genera of Lithosiinae may be placed in two groups according to the egg characters (with three subgroups in one of these):

(1) *Miltochrista* – egg oblong-ovoid, chorion pale-brown.

- (2) *Stigmatophora*, *Paraona*, *Lithosia*, *Agylla* and *Eilema* egg spherical, chorion white.
  - (a) Lithosia exochorion sculpture pitted-cellular.
  - (b) Stigmatophora, Paraona, Agylla exochorion sculpture looking like clearly expressed aeropyles united in the cells (Stigmatophora exochorion sculpture smooth with clearly expressed aeropyles; Paraona exochorion sculpture faintly folded with moderately expressed aeropyles; Agylla exochorion sculpture clearly folded with sharply expressed aeropyles).
  - (c) *Eilema* exochorion sculpture weakly expressed, looking like hardly noticeable cells, aeropyles not visible.

All the examined genera can be placed also into four groups according to the exochorion sculpture:

- (1) Exochorion sculpture cellular only in the micropylar area, the remaining surface looking like clearly expressed aeropyles united in the cells *Stigmatophora*, *Paraona*, *Agylla*.
- (2) Exochorion sculpture cellular on the whole egg surface *Miltochrista*.
- (3) Exochorion sculpture pitted-cellular Lithosia.
- (4) Exochorion sculpture weakly expressed, looking like hardly noticeable cells *Eilema*.

## Acknowledgements

We wish to express our thanks to Dr M. Ponomarenko and Dr E. Belayev (Institute of Biology and Pedology, Vladivostok,

Russia) and to Dr M. Omel'ko (Gorno-Tayozhnaya Stantsia [Mountain-Taiga Station], Primorskiy Krai, Russia) for their assistance during our field work in Primorskiy Krai and granting us their stationary facilities. We are obliged to Dr A. Lvovsky (Zoological Institute, Russian Academy of Sciences, St. Petersburg) and to Mr I. Kostjuk (Zoological Museum of the National Shevchenko University in Kiev, Ukraine), who kindly assisted with the literature. Mr I. Kostjuk provided access to the collection material under his care.

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Zeitschrift/Journal: Nota lepidopterologica

Jahr/Year: 2000

Band/Volume: 23

Autor(en)/Author(s): Pljushch Igor G., Dolinskaya Irina V.

Artikel/Article: Eggshell fine structure of some species of Lithosiinae (Arctiidae) of Far East Russia 50-63