Recent Advances in Insect Embryology in Japan
Edited by H. Ando and K. Miya.
ISEBU Co. Ltd., Tsukuba 1985

Blastodermic Cuticles of the Jumping Bristletail, *Pedetontus unimaculatus* (Microcoryphia, Machilidae)

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Synopsis

The formation and structure of blastodermic cuticles of the machilid, *Pedetontus unimaculatus* are described and illustrated in detail.

The formation of blastodermic cuticle commences in the stage of germ rudiment formation, and completes generally by about the time of the elongation of germ disc. The blastodermic cuticle is composed of three layers. The outermost layer (2-4μm-thick) is dark brown in color. Its surface assumes a polygonal pattern of which polygons are ca. 10μm in diameter, and at the center of each polygon a pointed process (*ca.* 2-3μm-high) stands. The outermost layer also has various-shaped and sized nodal projections on its surface. The middle layer (4-8μm-thick) is hyaline. The innermost layer (5-10μm-thick) is also hyaline and bears radial striation.

The basic plan of blastodermic cuticle in *Pedetontus unimaculatus* is in good agreement with those in machilids hitherto studied. Among the features of machilid blastodermic cuticles the formation in the earlier stages of development is the most noticeable within the ectognathous insects.

Introduction

In not a few insects, the secondary egg membranes, blastodermic or serosal cuticles, are formed during embryogenesis. They play an important role of the protection of egg. The blastodermic cuticles are also known in machilid eggs. Larink (1969, 1972, 1979) reported the formation and structure of blastodermic cuticles of some machilids (four genera, five species). He (1979, 1983) pointed out that the blastodermic cuticles of machilids have common features in the formation and basic structures: i) formation in the early stage of embyogenesis, ii) three-layered construction composed of the outermost pigmented and two hyaline ones, and iii) radial-striated structure in the innermost layer. He also suggested that the surface structures of machilid blastodermic cuticles are clearly specific in species.

We have been studying the embryonic development of a machilidan *Pedetontus unimaculatus* (Machida, 1981; Machida and Ando, 1981). In a series of our work, here we describe and illustrate the formation and structure of the blastodermic cuticle of the insect in detail.

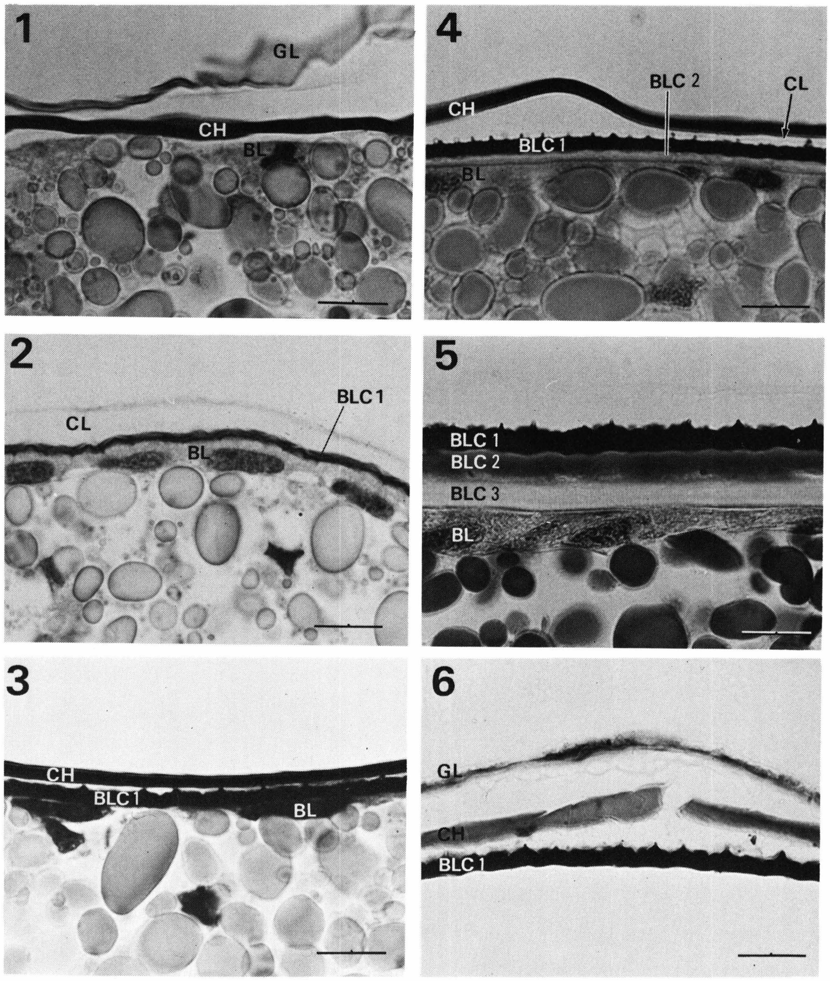
Materials and Methods

Imaginal insects were collected from Shimoda, Shizuoka Pref., Japan. The methods for maintaining them, collecting eggs and fixing the materials were the same as described previously (Machida, 1981). Sections were prepared with the normal paraffin and the JB-4 embedding methods. For scanning electron microscopy, the gelatinous layer and chorion were removed with rolling the egg on a double-coated tape ("Scotch") struck to the slide glass. Some of eggs were ultrasonicated in a detergent solution. After fixation and the penetration of egg membranes with needle, eggs were dehydrated and dried according to the critical point method. Specimens mounted with nail enamel on stubs were coated with gold and examined with a JEOL JSM-T200.

Results

The general character of the egg of *Pedetontus unimaculatus* was described previously (Machida, 1981). The egg is covered with a outermost gelatinous layer (GL) and a chorion (CH) *ca.* 1-2μm and 3μm in thickness respectively (Fig. 1). In the egg just after oviposion, the chorion is elastic, but soon it hardens and turns into light brown from hyaline. Eggs adhere to each other in a clump or to the substratum with the gelatinous layer. In *Pedetontus unimaculatus,* the blastodermic cuticle forms in early stages of the embryonic development as in the other machilids. The stages in the description follow the previous work (Machida, 1981).

The blastoderm forms and the development enters into the germ disc formation. The blastodermic cuticle begins to be secreted during the stage of germ rudiment formation (early in Stage 1). First, externally to the blastoderm a light brown cuticle appears (Fig. 2). It reaches 2-4 μm in thickness, and darkens (Fig. 3). This cuticular layer is named *blastodermic cuticle 1* (BLC 1). A hyaline rough-structured layer (coating layer, CL, in Figs. 2, 4, 11, 12) is observed at the surface of BLC 1. The coating layer of *Pedetontus unimaculatus* corresponds to the *Oberflächemembran* reported by Larink (1969, 1972, 1979) for the other machilids. Wide variations are observed in the thickness of coating layer (CL) regionally in each egg or individual eggs (*cf.* Figs. 2, 4). In Fig. 12, two sublayers of the coating layer are distinguished. Nothing is known on the relation of the coating layer and vitelline membrane. The former may be only a desiccated fluid material existed between the chorion and blastoderm. The surface structure of BLC 1 is illustrated in Figs. 7-12. It has a polygonal pattern of which polygons are *ca.* 10μm in diameter, and a pointed process stands, 2-3μm or lower in height, at the center of each. BLC 1 also has various-shaped and sized nodal projections on its surface. The chorion and the egg contents are now separated from each other with the processes and nodal projections. In a few days after oviposition, the egg color changes from orange into dark brown with the formation of BLC 1.



Figs. 1-6. Photographs showing the successional stages of blastodermic cuticle formation in *Pedetontus unimaculatus*. Scales: 20 μm.

1. Egg with newly formed blastoderm. Egg is surrounded with a gelatinous layer and a chorion. 2. Egg in germ rudiment formation. Blastodermic cuticle 1 (BLC 1) is under formation. A hyaline layer (CL) is observed over BLC 1. 3. BLC 1 thickens and becomes darker. It bears many processes on its surface. 4. An additional hyaline layer, blastodermic cuticle 2 (BLC 2) is under formation. A hyaline layer (CL) is faintly seen over BLC 1. 5. Third layer, blastodermic cuticle 3 (BLC 3), is added under BLC 2. BLC 3 is also hyaline and bears radial striations. BLC 2 is heavily stained with chromotrope 2R in this section. The blastodermic cuticle completes generally by about the time of elongation of germ disc. The egg of this section is one in the earliest stage of germ band (Stage 2). 6. Near hatching, the blastodermic cuticles 2 and 3 are digested. This is a photograph of exuvia. Only the BLC 1 remains intact.

BL blastoderm, BLC 1, 2, 3 blastodermic cuticle 1, 2, 3, CH chorion, CL layer coating blastodermic cuticle, GL gelatinous layer.

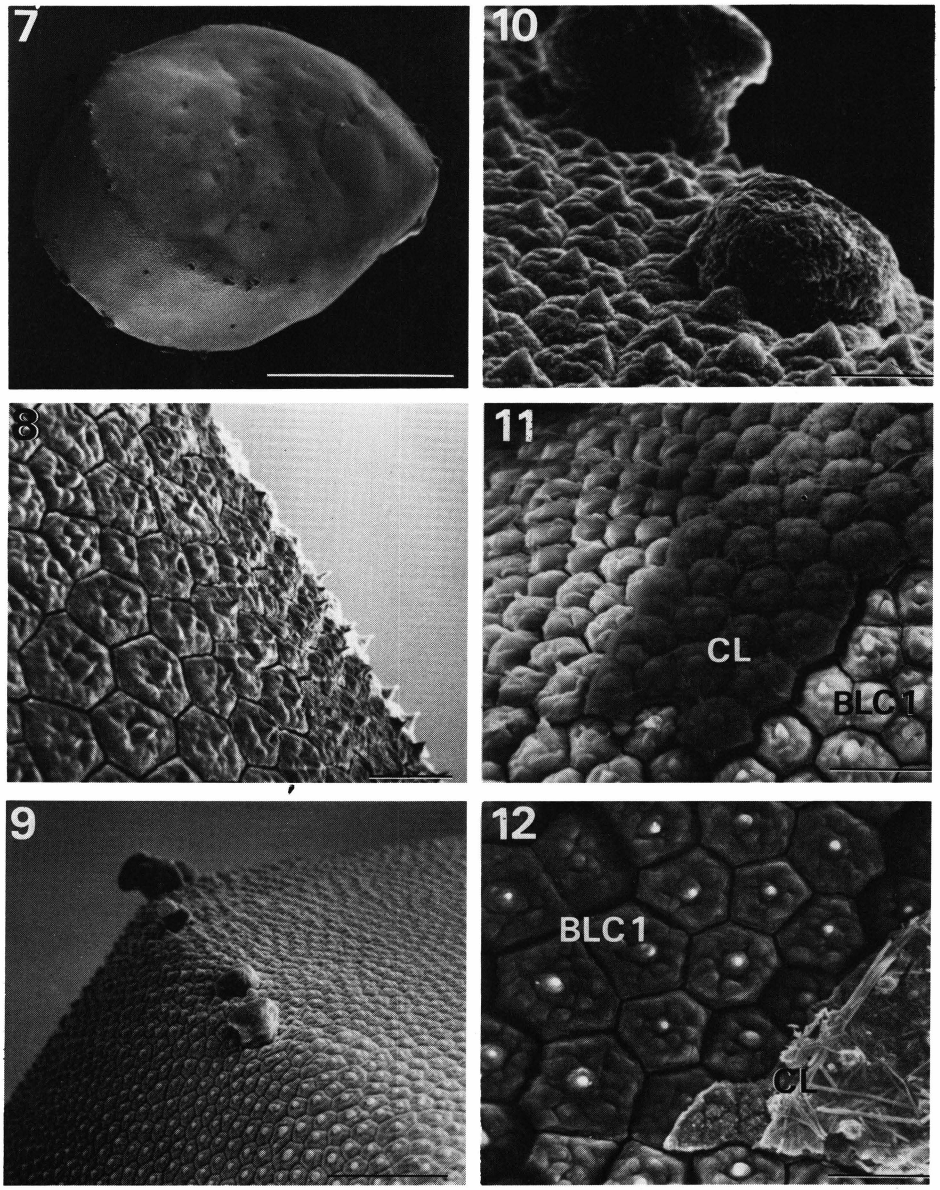
In no time, a hyaline cuticular layer, blastodermic cuticle 2 (BLC 2), is added innerly to the BLC 1 (Fig. 4). It is 4-8μm in thickness, and tends to be heavily stained with dyes such as eosin and chromotrope 2R. The surface facing the BLC 1 assumes a polygonal pattern corresponding to that of BLC 1. Soon, an additional cuticular layer, blastodermic cuticle 3, is formed just under the BLC 2 (Fig. 5). It is 5-10μm in thickness, and it bears radial striation, as in *Trigoniophthalmus alternatus* and *Lepismachilis sp.* (Larink, 1979). The striated structures in *Pedetontus unimaculatus* may correspond to the microcanals as Larink (1979) observed for *Trigoniophthalmus alternatus* and *Lepismachilis sp.*

In this way the blastodermic cuticles are formed, and the formation completes generally by about the time of elongation of germ disc, at most delayed by the earliest germ band stage (Stage 2). The definitive general construction of the egg membrane system in *Pedetontus unimaculatus* is as illustrated in Fig. 13. No changes is observed in the blastodermic cuticles during the following embryonic stages until near the hatching. The thickness of cuticular layers varies from 12-20μm or more in individual eggs. These differences in thickness depend on those of the blastodermic cuticles 2 and 3. In each egg, the blastodermic cuticles have almost a uniform thickness, only under the germ project innerly the cuticular layers into a protuberant process *ca.* 25μm in height, as in *Petrobius brevistylis* (Larink, 1969). It is related to fold-like structure of the embryonic membrane formed under the germ disc. After the completion of blastodermic cuticles, the chorion becomes fragile, and is later cracked often. Now the cuticular layers perform a principal part of egg protection instead of the chorion. We often found that the mycocelia invade the space between the chorion and blastodermic cuticle, or that the space is filled with water when the egg is incubated in a wetty condition. The cuticular layers of *Pedetontus unimaculatus* have a high ability for the protection of egg, as the case in the other machilids (Wygodzinsky, 1941; Larink, 1969, 1972).

Just before hatching (Stage 14), blastodermic cuticles 2 and 3 disappears (Fig. 6), and the larva hatches. Probably some kind of enzyme may be responsible for the disappearance of blastodermic cuticles. But we cannot provide any supportive evidences for it at the present. The digestion of egg membranes is not followed well in the apterygote insects. In a thysanuran *Thermobia domestica,* an evidence is presented for the serosal cuticle is digested by a substance secreted from pleuropodia (Woodland. 1957).

Discussion

The formation and structure of the machilidan blastodermic cuticle were reported for *Petrobius brevistylis, Petrobius maritimus, Trigoniophthalmus alternatus, Dilta hybernica* and *Lepismachilis sp.* (Larink, 1969, 1972, 1979). The blastodermic cuticles in *Pedetontus unimaculatus* are in good agreement with those in the above four genera and five species machilids in basic plans: i) formation in the very early stage of embryogenesis, ii) three-layered construction, iii) outermost pigmented layer (BLC 1) with a complex surface structure, iv) hyaline middle and innermost layers (BLC 2 and 3), and v) radial-striated structures in the innermost layer (BLC 3). It is supportive for Larink's (1979, 1983) view on the features of the machilid blastodermic cuticles. He also pointed out that the surface structures of the outermost layer are clearly specific in species, and we have no objection also to his pointing-out. The surface structure of the outermost layer in *Pedetontus uni-*



Figs. 7-12. Photographs showing surface structures of the blastodermic cuticle (BLC 1) of *Pedetontus unimaculatus.* The specimens in these photographs are in the middle stages of embryogenesis (Stages 8-10). See the text.

7. Dechorionated egg. Scale: 500μm. 8. General surface structure of BLC 1. Scale: 10μm. 9. Nodal projections of BLC 1. The BLC 1 is thinly coated with a layer, the coating layer. Scale: 50μm. 10. Ibid. Scale:10μm. 11. BLC 1 and coating layer (CL). Scale:10μm. 12. Ibid. Scale:10μm.

maculatus is relatively simple among the machilid hitherto studied.

The blastodermic cuticle in the Microcoryphia and the serosal cuticle formed in most of pterygote insects and the Thysanura (Heymons, 1897; Sharov, 1953; Woodland, 1957) are homologized with each other. It deserves specieal emphasis, within the ectognathous insects, that in machilids the cuticles are formed in the very early stages of development: *i. e.*, generally from the germ rudiment formation- to germ disc-stages. It may be related to the fact that the chorion in machilids is not so protective, or that the structure to be corresponding to the amnioserosal fold formed in the Thysanura-Pterygota is not well developed or, in strict sense, lacking in *Pedetontus unimaculatus*, probably the machilids (unpublished). The conditions in machilids resemble those in collembolans (*cf.* Jura, 1972). Within the Antennata possessing only the serosa or blastoderm as the embryonic membrane, the blastodermic cuticles are formed in the Collembola (Jura, 1972), Diplura (Uzel, 1898; Tiegs, 1942), Symphyla (Tiegs, 1940), Pauropoda (Tiegs, 1947) and Diplopoda (Dohle, 1964). The formation occurs generally in the early stages of development in these aminals, although it might be not easy to compare the stages in different animals. Only among these the formation is relatively delayed in the Diplura.

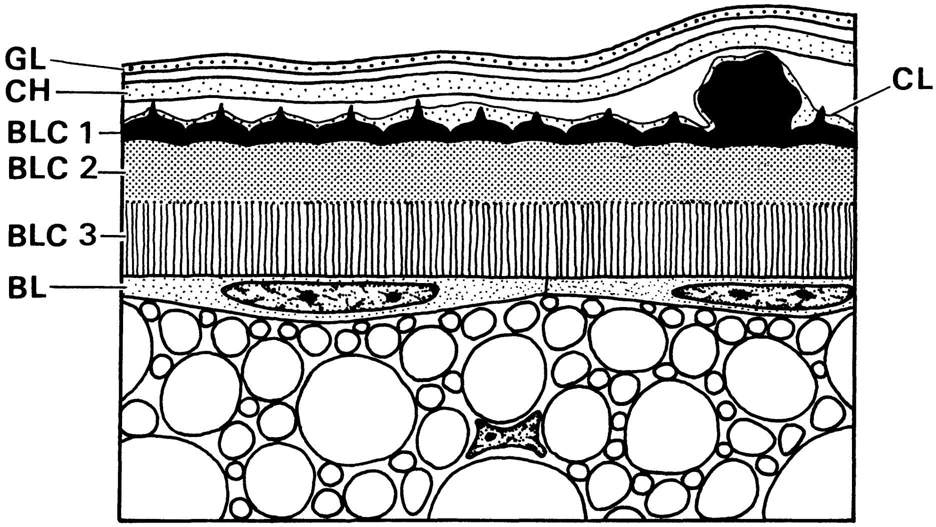


Fig. 13.. Diagram illustrating the structure of egg membrane in *Pedetontus unimaculatus*.
See the text. BL blastoderm, BLC 1, 2, 3 blastodermic cuticle 1, 2, 3, CH chorion, CL layer coating blastodermic cuticle, GL gelatinous layer.

Acknowledgements

We thank to Profs. K. Miya and M. Kurihara of Iwate University for their valuable suggestions on the preparation of JB-4 sections. The work was carried out while the senior author, R. Machida, was in receipt of a grant from the Japan Society for the Promotion of Science which is gratefully acknowledged.

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