

# Development of the Pleuropodia in the Embryo of the Glowworm *Rhagophthalmus ohbai* (Rhagophthalmidae, Coleoptera, Insecta), with Comments on Their Probable Function

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## Abstract

The development and degeneration of the pleuropodia in the embryo of the glowworm *Rhagophthalmus ohbai* were described, and their probable function was discussed in connection with the change of the serosal cuticle. The pleuropodia of this insect fully developed into cup-shaped ones at the stage just before revolution. The three-layered, tough serosal cuticle, on the other hand, was completed by several days before revolution and covered whole egg surface. The granular substances appeared just after revolution. They gradually increased in number and were scattered throughout the space between the embryo and the serosal cuticle until several days before hatching. It was assumed that the substances were secreted from the pleuropodia, because the shape of the pleuropodial cells changed rapidly after revolution and the pleuropodia as a whole degenerated by the time of hatching. At several days before hatching, the middle and innermost layers of the serosal cuticle decayed into numerous granules. On the basis of the timing of these structural changes in both the pleuropodia and serosal cuticle, it was strongly suggested that the granular substances cause the serosal cuticle to decay and consequently facilitate the process of hatching.

## Introduction

Pleuropodia are a pair of appendage-like projections that arise on the first abdominal segment of many insect embryos, and are thought to be homologous to the thoracic legs or a part of them. The pleuropodia, however, show a typical glandular structure in certain embryonic stages and degenerate toward the end of the egg period. In her experimental study, Slifer (1937) demonstrated that a secretory substance from the pleuropodia of the grasshopper *Melanoplus differentialis* dissolves the inner layer of the serosal cuticle, one of the egg coverings, hence acts as a hatching enzyme. A similar function of the pleuropodia has been suggested in the apterygotan *Zygentoma* (Woodland, 1957) and the hemimetabolous Phasmida (Louvet, 1973). However, in holometabolous insects, there is no reliable work showing the function of pleuropodia.

*Rhagophthalmus ohbai* is one of the glowworm whose females are completely larviform and luminescent. The structure of the newly laid eggs and the outline of its embryogenesis have been described in our previous papers (Kobayashi *et al.*, 2001, 2002), but a fuller description of the formation of the pleuropodia has not been given there. In the present article, we describe the formation and degeneration of the pleuropodia of this insect and also pay our attention to the condition of the serosal cuticle in order to discuss their probable function.

## Materials and Methods

Mated females of *Rhagophthalmus ohbai* were collected at Iriomote-jima and Ishigaki-jima, Okinawa Prefecture, Japan, in December of 1997 to 1999, with the permission of the Environmental Agency of Japan, the Agency for Cultural Affairs, and the Educational Committee of Okinawa Prefecture to N. Ohba. The methods for maintaining the

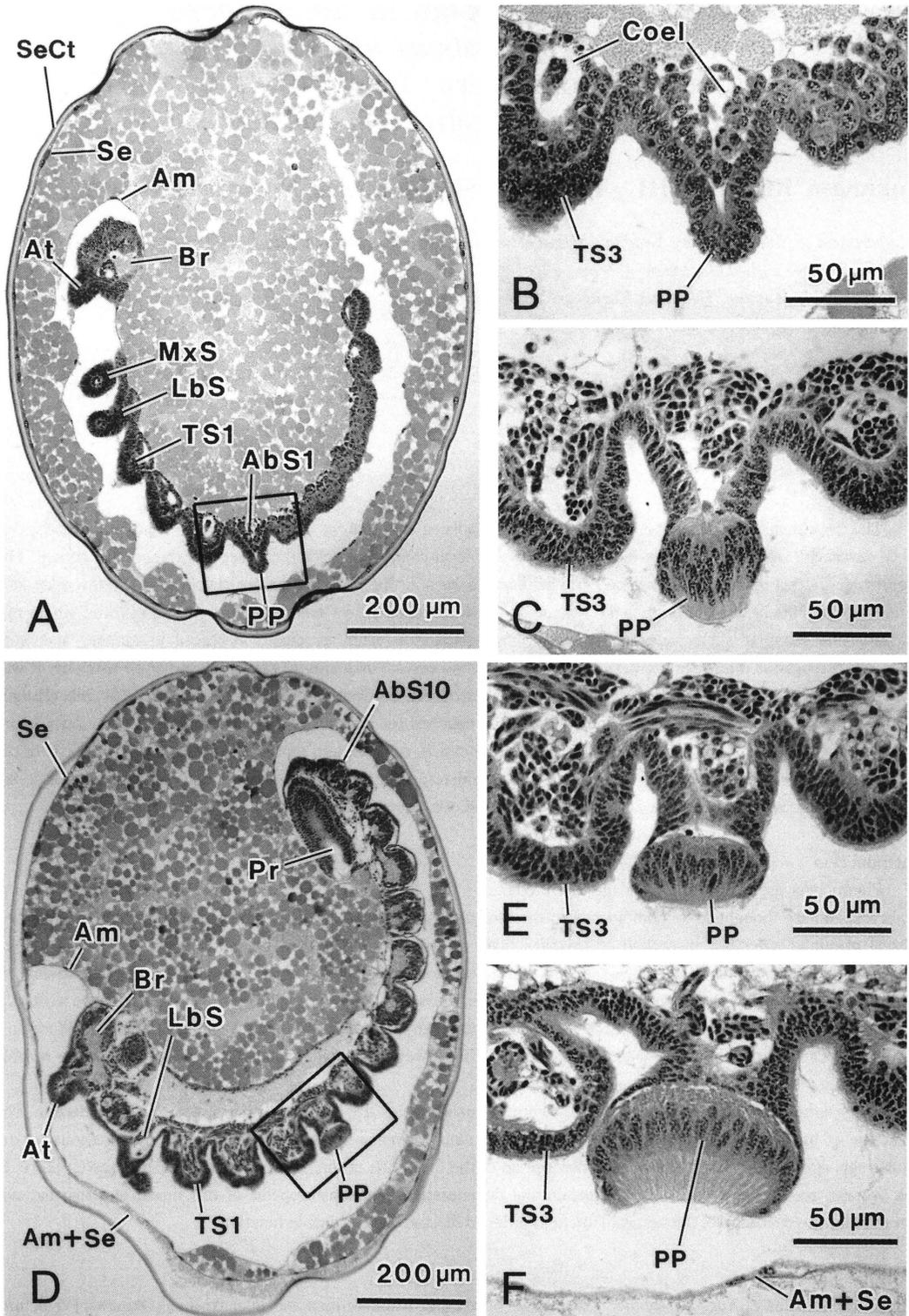


Figure 1

insects, collecting, fixing, and sectioning the eggs were the same as described by Kobayashi *et al.* (2002). Sections of 5 or 6- $\mu\text{m}$  thickness were stained with Delafield's haematoxylin and eosin.

## Results

The timetable of embryonic development of *Rhagophthalmus ohbai*, with emphasis on the formation of the pleuropodia and serosal cuticle, is shown in Table 1.

### *Development and degeneration of pleuropodia*

At about 9 days after oviposition (a. o.), the fully elongated embryo is completely immersed in the yolk and its segmentation is almost finished; that is, the embryo is composed of the protocephalon, four gnathal, three thoracic, and 10 abdominal segments excluding the nonsegmental abdominal end or telson. A pair of rudimentary appendages then appears on each gnathal and thoracic segment except for the gnathal intercalary one. At about 10 days a. o. when the thoracic appendages become elongated, the pleuropodia appear as a pair of minute, appendage-like projections of about 35- $\mu\text{m}$  long in the first abdominal segment (Fig. 1A, B). At this stage the cells of the pleuropodia can not be distinguished from the surrounding ectodermal cells of the segment in shape and stainability, and the inner surface of the pleuropodial cells are not lined with mesodermal cells. By about 13 days a. o., however, the pleuropodia become

Table 1 Timetable of embryonic development of *Rhagophthalmus ohbai*, incubated at about 22°C, with emphasis on the formation of the pleuropodia and serosal cuticle.

Hours/days after oviposition	State of development
0-6 hours	Maturation division
6-9 hours	Fertilization
9-33 hours	Cleavage
33-48 hours	Formation of blastema
48-60 hours	Formation of blastoderm
60-72 hours	Formation of germ disk
72-96 hours	Formation of germ rudiment
4.5-6 days	Formation of germ band or embryo; formation of inner layer; beginning of serosal cuticle formation
6-8 days	Elongation and segmentation of embryo
8-10 days	Appearance of gnathal and thoracic appendages; formation of coelomic sacs, proctodaeum, and stomodaeum
10-12 days	Appearance of ganglion; appearance of pleuropodia; completion of three-layered serosal cuticle
12-14 days	Fusion of cephalo-gnathal region; differentiation of secretory cells in pleuropodia
14-17 days	Embryo widens and becomes superficial in position; pleuropodia develop into cup-shaped ones
17-18 days	Revolution; secretion of granular substances from pleuropodia
18-21 days	Completion of dorsal closure
21-26 days	Embryonic moulting; degenerating pleuropodia are retracted into body wall
26-30 days	Formation of setae; pigmentation of mandibles; disappearance of pleuropodia
30-34 days	Hatching; inner layer of serosal cuticle becomes spongy and decays

Fig. 1 Longitudinal sections of eggs of *Rhagophthalmus ohbai*, showing successive changes of developing pleuropodia. A. About 10-day after oviposition (a. o.) egg. B. Higher magnification of rectangle in A. C. About 13-day a. o. egg. D. About 14-day a. o. egg. E. Higher magnification of rectangle in D. F. About 17-day a. o. egg. AbS1, 10: 1st and 10th abdominal segments, Am: amnion, At: antenna, Br: brain, Coel: coelomic sac, LbS: labial segment, MxS: maxillary segment, PP: pleuropodium, Pr: proctodaeum, Se: serosa, SeCt: serosal cuticle, TS1, 3: prothoracic and metathoracic segments.

globose and composed of long spindle-shaped cells whose nuclei are located in their middle part (Fig. 1C). The lateral faces of the pleuropodia are covered with flat cells extended from the surrounding ectodermal cells; hence the pleuropodial cells are easily distinguished from ectodermal ones. At about 14 days a. o., the pleuropodial cell mass as a whole becomes disk-like one whose surface is flat (Fig. 1D, E). At about 17 days a. o., the diameter of the pleuropodia increases to about 100  $\mu\text{m}$  owing to the enlargement of the constituent cells, and their surface becomes concave (Fig. 1F). The nuclei of the pleuropodial cells become shifted to their proximal part and the distal half of the pleuropodia is composed of many minute rods set side by side extending from the pleuropodial cells. During this period (13–17 days a. o.), the embryo becomes superficial in position from its anterior part; that is, the protocephalon and gnathal segments assume a superficial position first and finally the whole embryo becomes observable from the outside. Embryonic revolution then occurs at about 18 days a. o., and the embryonic membranes, the amnion and serosa, which have covered the ventral side of the embryo, rupture. They are incorporated into the dorsal side of the yolk mass, and the serosa forms the secondary dorsal organ (Fig. 2A). Just after revolution, numerous granules become observed in the wide space between the embryo and serosal cuticle. These granular substances are supposed to be secreted from the pleuropodia, because the pleuropodial cells change in shape rapidly at this stage, that is, their distal half become long cone-shaped and the nuclei further shift to the proximal part (Fig. 2B). At 1 to 3 days after revolution, the pleuropodia are retracted into the body wall of the embryo and their round external orifices become covered with a thin, probably cuticular, membrane, or the embryonic cuticle (Fig. 2C). The pleuropodia then degenerate (Fig. 2D) and completely disappear by about 28 days a. o., or 2 to 6 days before hatching. The granular substances, however, are still scattered throughout the space between the embryo and serosal cuticle after the degeneration of pleuropodia, and some of them adhere to the inner surface of the serosal cuticle.

#### *Formation and decay of serosal cuticle*

The newly laid eggs are covered with a very thin and delicate eggshell (below 1  $\mu\text{m}$  in thickness) composed of the outer chorion and the inner vitelline envelope. At about 4 days a. o., the circular germ disk invaginates into the yolk to form a spherical germ rudiment, and the serosa covers the whole egg surface below the vitelline envelope. At about 5 days a. o., the serosal cuticle begins to be secreted on the entire surface of the serosa (Fig. 3A). It increases in thickness gradually (Fig. 3B) and the three-layered serosal cuticle of about 9  $\mu\text{m}$  in thickness is completed by 10 days a. o. (Fig. 3C). Almost no variations are observed in the thickness throughout the egg surface. The outermost layer (serosal cuticle 1) is about 2.5  $\mu\text{m}$  in thickness and heavily stained with eosin. The middle layer (serosal cuticle 2) is 2.5–4  $\mu\text{m}$  and lightly stained with haematoxylin, whereas the innermost one (serosal cuticle 3), whose thickness is also 2.5–4  $\mu\text{m}$ , is stained neither with eosin nor haematoxylin. The difference of these three layers is retained until about 27 days a. o. or about 9 days after revolution, but after that time the boundary between the serosal cuticles 2 and 3 becomes obscure (Fig. 3D). At about 31 days a. o. or 1 to 3 days before hatching, the merged serosal cuticles 2 and 3 become spongy and decay into numerous granules (Fig. 3E, F). The full-grown embryo or the first instar larva probably swallows these granules by the time of hatching. The remaining thin egg coverings, the chorion and serosal cuticle 1, are torn by the mandibles of the full-grown embryo, and hatching occurs at about 34 days a. o.

#### **Discussion**

The formation of pleuropodia has been reported in the embryos of many ectognathous insect orders including the apterygotan Archaeognatha and Thysanura, although pleuropodia are lacking sporadically in several orders, *e. g.*, Ephemeroptera, Dermaptera, Mecoptera, and Diptera. The structures, however, have not been found in entognathous orders, *i. e.*, Collembola, Protura, and Diplura. The formation of pleuropodia, therefore, can be considered as an autapomorphic character in the embryogenesis of Ectognatha. In spite of the ubiquity of the structures, however, Slifer's (1937) work is the only experimental study demonstrating that secretory substances from the pleuropodia of the locust *Melanoplus differentialis* dissolve the inner layer of the serosal cuticle, formerly white cuticle, and they act as hatching enzymes. In a later ultrastructural study on the pleuropodia of the stick insect *Carausius morosus*, though not experimental one, Louvet (1973) also speculated that the granules secreted from the pleuropodia act as hatching enzymes. In other insects, however, several authors questioned the function of the pleuropodia as a producer of hatching enzymes (Ando, 1953; Bullière, 1970; Novak and Zambre, 1974; Stay, 1977). In Odonata, for example, the pleuropodia degenerate soon after revolution and the serosal cuticle is not reduced in thickness during the post-

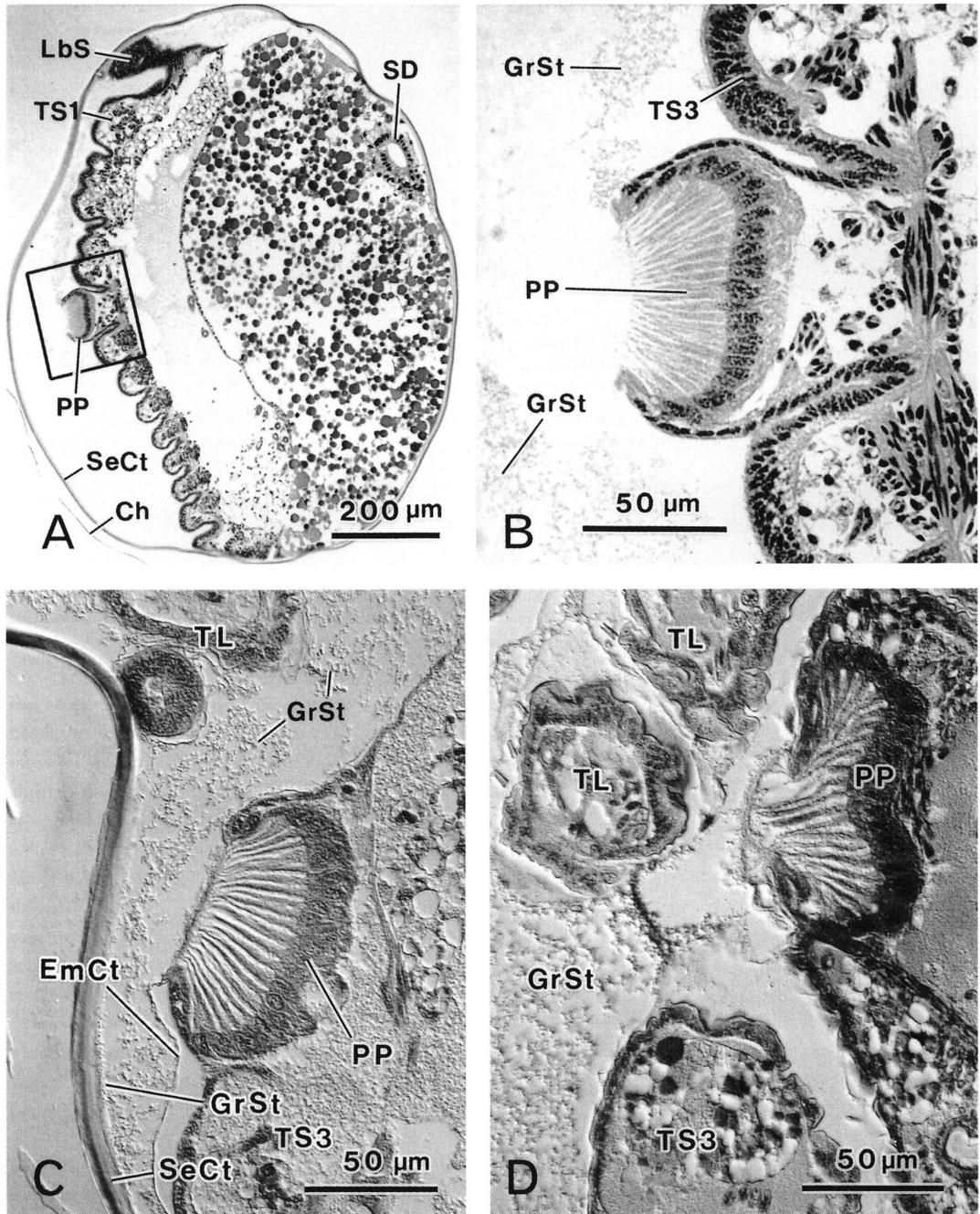


Fig. 2 Longitudinal sections of eggs of *Rhagophthalmus ohbai*, showing successive changes of degenerating pleuropodia and granular substances probably discharged from them. A. About 18-day after oviposition (a. o.) egg. B. Higher magnification of rectangle in A. C. About 19-day a. o. egg (differential interference microscopy). D. About 27-day a. o. egg (differential interference microscopy). Ch: chorion, EmCt: embryonic cuticle, GrSt: granular substance, LbS: labial segment, PP: pleuropodium, SD: secondary dorsal organ, SeCt: serosal cuticle, TL: thoracic leg, TSL, 3: prothoracic and metathoracic segments.

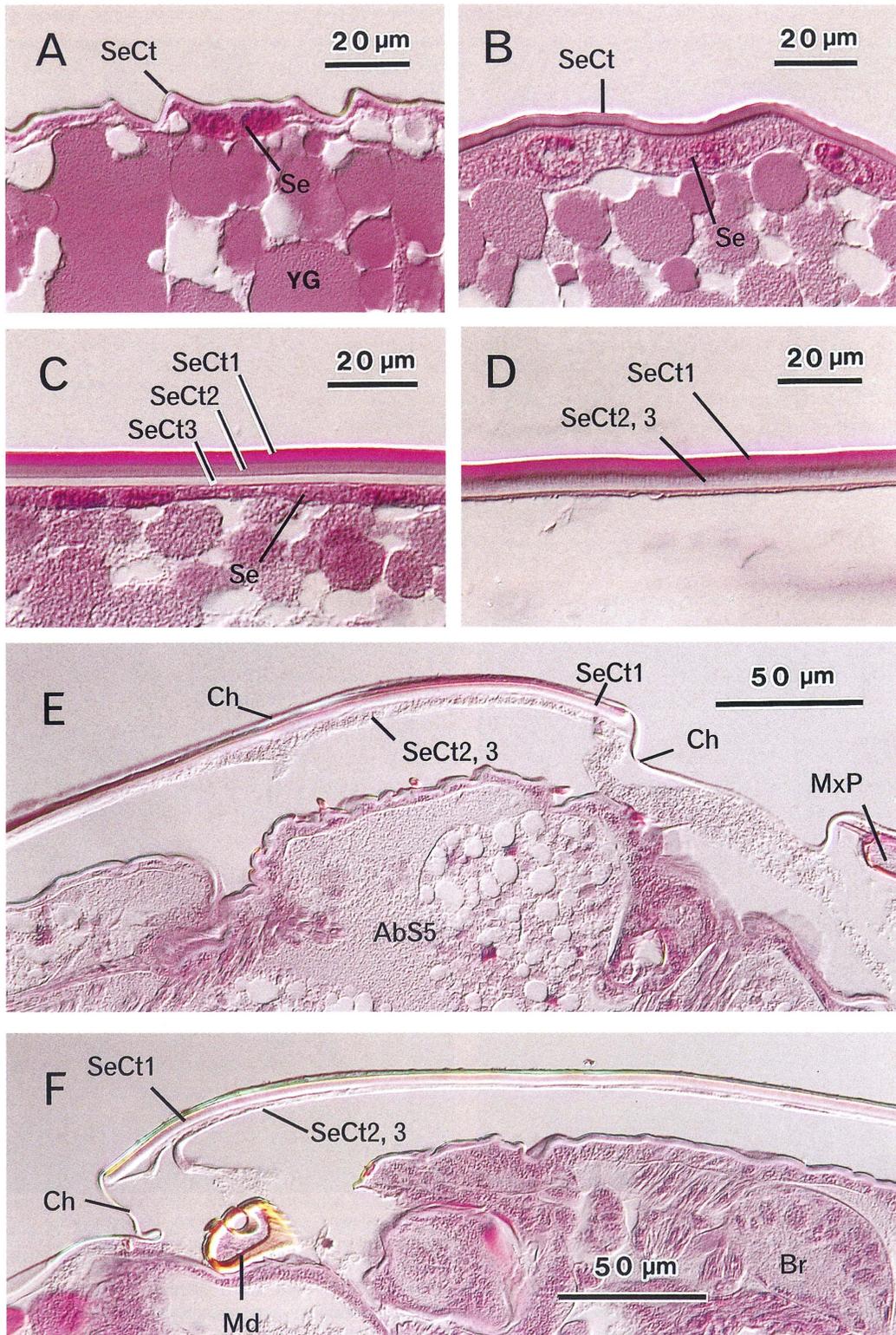


Figure 3

revolutionary stages; thus, the enzymatic function of the secretion is doubtful (Ando, 1953, 1962).

In Coleoptera, the pleuropodia have been found in not a few families, *e. g.*, the Hydrophilidae, Dytiscidae, Scarabaeidae, Meloidae, Tenebrionidae, and Lampyridae (Heider, 1889; Wheeler, 1890; Graber, 1891; Korschelt, 1912; Williams, 1916; Hussey, 1926; Ullmann, 1967; Stanley and Grundmann, 1970; Church and Rempel, 1971; Ando and Kobayashi, 1975; Kobayashi, 1987). The formation of the pleuropodia, therefore, must be a common event in coleopteran embryogenesis, although they are clearly lacking in the Cantharidae and some Chrysomelidae (Miya, 1965; Fujiwara and Kobayashi, 1987). Although there is a variety of their forms in Coleoptera, the pleuropodia of *Rhagophthalmus ohbai* closely resemble those of the lampyrid *Photuris pennsylvanica* (Williams, 1916) and dytiscid *Dytiscus marginalis* (Korschelt, 1912) in their developmental mode and the shape of the full-grown pleuropodia (mushroom-shaped). Their secretory activities have been observed in some of the coleopteran insects, and the production of a hatching enzyme is eventually suggested in *Tribolium confusum* (Stanley and Grundmann, 1970). These observations and assumptions may afford circumstantial evidence that the function of the pleuropodia is the production of hatching enzymes as in the case of *Melanoplus*. However, not only in Coleoptera but also in Holometabola, there is no report concerning the successional changes of the serosal cuticle in connection with the development of pleuropodia.

The pleuropodia of *R. ohbai* become fully developed by the time of revolution, but the granular substances, which are probably discharged from the pleuropodia, do not appear until the rupture of embryonic membranes after revolution. After this time, the three-layered, tough serosal cuticle is separated from the serosal cells and comes in touch with the granular substances directly. The boundary of the middle (serosal cuticle 2) and innermost (serosal cuticle 3) layers becomes obscure at about 27 days a. o. (about 9 days after revolution) and the two layers become spongy to decay into numerous granules around 31 days a. o. or 1 to 3 days before hatching. Although the decay of the two layers begins about 9 days after the discharge of the granular substances, it is reasonable to assume that the granular substances are involved in the decay of the serosal cuticle, because the substances still persist at this stage and some of them adhere to the inner surface of the serosal cuticle. The decay of serosal cuticle, therefore, may proceed very slowly.

The three-layered organization of the serosal cuticle as observed in *R. ohbai* is also found in the archaeognathan *Pedetontus unimaculatus*, but is here reported for the first time in the pterygotan insects. In *P. unimaculatus*, the middle and innermost layers of the serosal cuticle also disappear just before hatching, and the presence of hatching enzymes is also assumed, because the large pleuropodia are formed in this insect (Machida and Ando, 1985; Machida *et al.*, 1994). The three-layered organization thus might be ubiquitous in the hexapod serosal cuticle and might be also comparable to the tripartite organization of the cuticles of larval or adult insects, *i. e.*, the epicuticle, exocuticle, and endocuticle. In fact, in *Melanoplus*, Slifer and Sekhon (1963) regard the inner layer of the serosal cuticle, which is dissolved by hatching enzymes as cited before, as the endocuticle and outer layer as the epicuticle (they do not assume the presence of the exocuticle). In *R. ohbai*, it might be possible to assume that the serosal cuticle 1 corresponds to the exocuticle and both the cuticles 2 and 3 to the endocuticle, because the sclerotized exocuticle of larval or adult insects is often stained deeply with eosin as in the case of the serosal cuticle 1. We, however, need further ultrastructural study to understand the nature of these layers in *R. ohbai*.

*Acknowledgments:* We are grateful to Messrs. Y. Goto (Yokohama City), Y. Sato (National Research Institute of Vegetables, Ornamental Plants and Tea), I. Kawashima (Yokohama City), and N. Sakaguchi (Environmental Agency of Japan) for their help in collecting the materials.

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Fig. 3 Longitudinal sections of eggs of *Rhagophthalmus ohbai*, showing successive changes of serosal cuticle (differential interference microscopy, chorion removed in A-D). A. About 5-day after oviposition (a. o.) egg. B. About 7-day a. o. egg. C. About 11-day a. o. egg. D. About 27-day a. o. egg. E, F. About 31-day a. o. egg. AbS5: 5th abdominal segment, Br: brain, Ch: chorion, Md: mandible, MxP: maxillary palp, Se: serosa, SeCt: serosal cuticle, SeCt1–3: serosa cuticles 1 to 3, YG: yolk granule.

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