Description of Early and Middle Developmental stages in Embryos of the Firefly, *Luciola cruciata* MOTSCHULSKY (Coleoptera : Lampyridae)*

by

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INTRODUCTION

Since the order Coleoptera is one of the insect orders best studied embryologically, there have been numerous reports published on the coleopteran embryology up to the present.

Most of these reports are connected with the following families, Chrysomelidae, Curculionidae, Scarabeidae, Hydrophilidae, Ditiscidae, Tenebrionidae and others. Therefore the embryogenesis of these beetles are well known. However, many other families of this order have been only fragmentally studied and almost all are unknown embryologically.

The embryology of fireflies was briefly studied by WILLIAMS (1916), HESS (1922) and BUGNION (1922). The first author studied the photogenic organs and embryology of American lampyrids, *Photuris pennsylvanica* and *Photinus consanguineus*, and he published a short report on the embryonic development of these fireflies. The second author investigated the origin and development of the same organs of *P. pennsylvanica* and touched on the development of this organ during the embryonic life. The last author studied the anatomy and embryology of the light organs of the Europian firefly, *Lampyris bellieri* and commented to some extent upon the embryos. The main aim of the study of the aforesaid works to confirm that this organ is derived from either the ectoderm or mesoderm or both ectoderm and mesoderm, so that the observations of the embryonic development were rather neglected. As a result the embryogenesis of Lampyridae has remained studied but little up to now.

The family Lampyridae, together with families Drilidae, Cantharidae and Lycidae, belongs to the superfamily Cantharoidea which is thought to be a diverged group in the suborder Polyphaga (CROWSON, 1960) and it may be of interest in studying the coleopteran phylogeny.

This paper deals with the description of the early and middle developmental stages of the embryos of the Japanes firefly, *Luciola cruciata* MOTSCHULSKY, mainly concentrating on the external forms of the embryos. A full description of the embryogenesis of the insect will be given in future papers.

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MATERIAL AND METHOD

The eggs of *Luciola cruciata* MOTSCHULSKY are used as the material in this study. The full-grown larvae are obtained at Matsuokyo valley of the Tenryu River, Nagano Prefecture, Central Japan and kept in cages containing with soil for pupation. The duration of pupation is about three weeks. The newly emerged male and female beetles are put in a cage with a mass of moistened moss. Copulation takes place in this cage and females lay their eggs on the moss.

The eggs were fixed with alcoholic BOUIN's fluid warmed to 70°C for 20 minutes. They were cut into 7 micra thickness and stained with DELAFIELD's haematoxylin and eosin. For the observation of the fixed embryos, GRENACHER's borax carmine staining was used. All drawings were made with ABBE's camera lucida.

DESCRIPTION ON STAGES

The newly laid eggs of *Luciola cruciata* MOTSCHULSKY are nearly spherical in shape and whitish yellow in color. They are about 0.58 by 0.6 milimeters in diameter in fixed eggs. According to WILLIAMS (1916), the egg of *Photuris pennsylvanica* DE GEER is subspherical, pale lemon yellow, and measures about 784 by 677 micra in diameter. The chorion of *L. cruciata* egg is smooth and no distinctive sculupturing is evident. A single micropyle is located at one pole of the egg as in *P. pennsylvanica* (WILLIAMS, 1916). The polarity of the egg is indistinguishable by external observation because the egg is almost perfectly spherical in shape.

In *L. cruciata* total time duration from oviposition to hatching of the eggs, incubated at 25°C, was about three weeks, i. e. circa 21 days. In the laboratory, the eggs of *P. pennsylvanica* and *Photinus consanguineus* hatch in less three weeks (WILLIAMS, 1916), and HESS (1922) mentioned that the eggs of the former species require an average of about 26 days to complete their embryonic development.

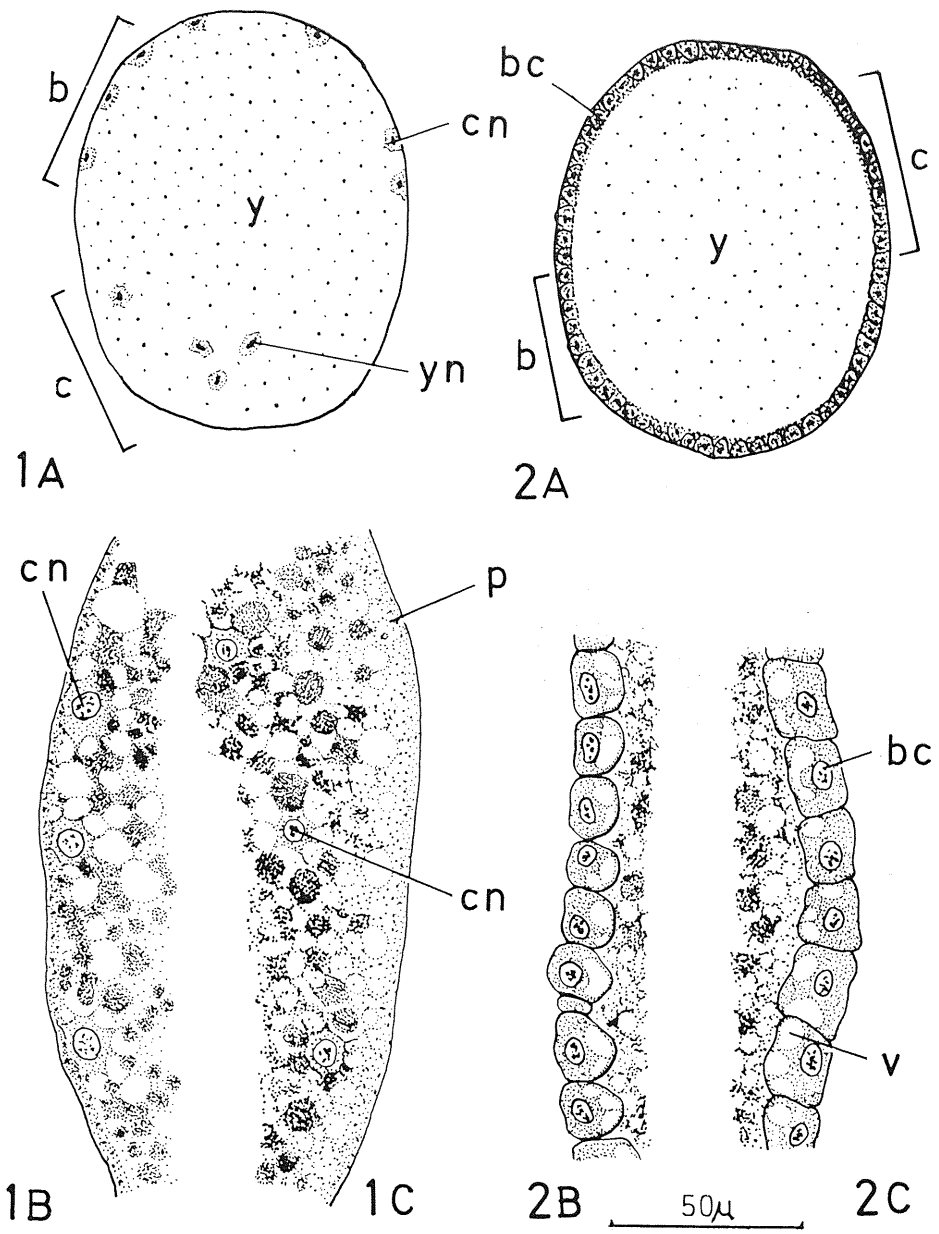
The successive processes of the embryonic development of the firefly can be divided into a number of stages based on the external morphology of the embryos.

The structure of the newly laid eggs, maturation divisions, fertilization, and cleavage of eggs of *L. cruciata* will be described in another paper.

1. Developmental stages of embryos of *L. cruciata*

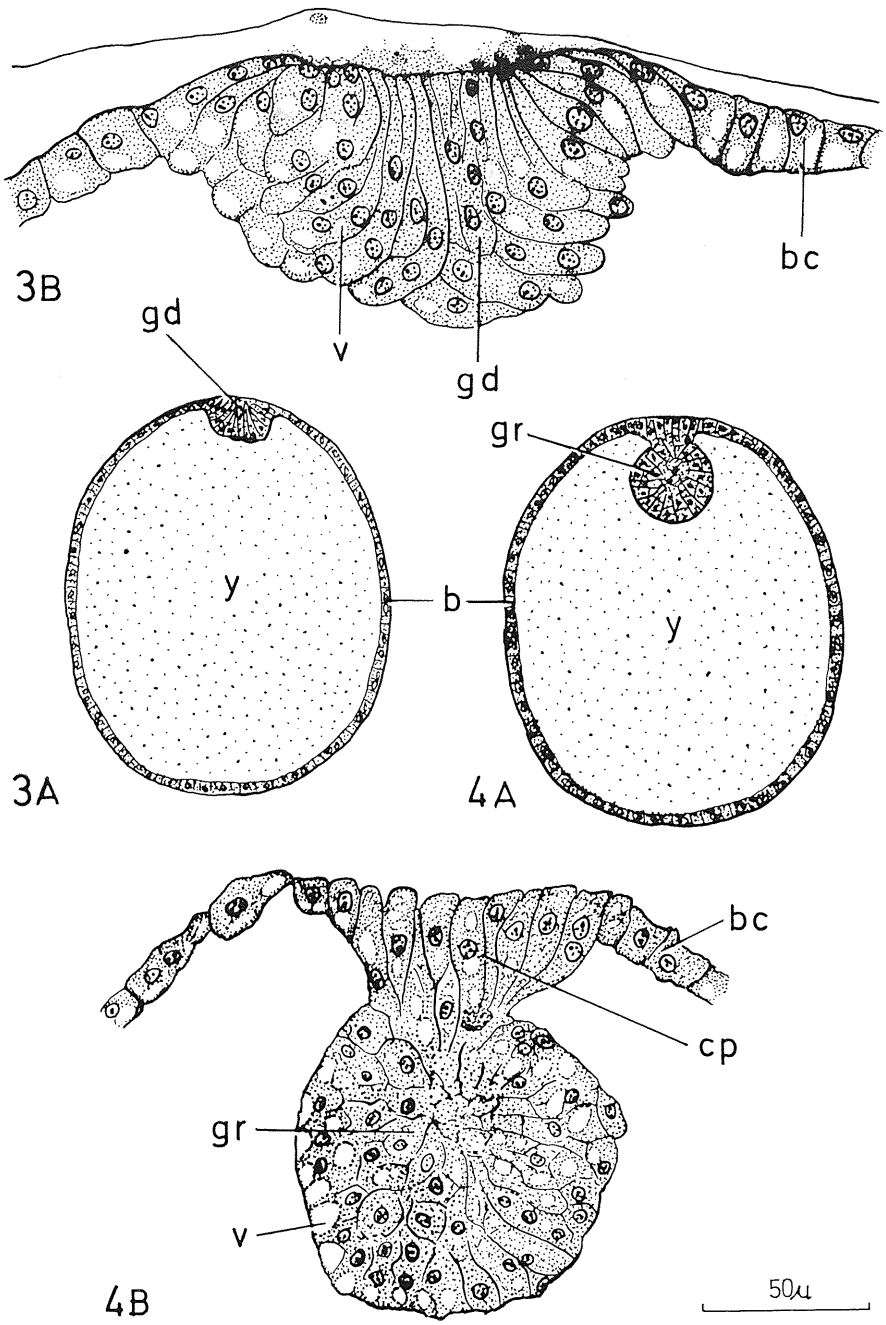
Stage 1. Formation of blastoderm (about 10-13 hours after oviposition) Figs. 1, 2

At about ten hours after oviposition, the cleavage nuclei reach the periplasm on nearly half of the egg surface, but in the remaining half the peripheral migration of nuclei is delayed and they have not attained the periplasm. The cellularization or formation of the blastema begins at the area penetrated by cleavage nuclei. The blastema stages in



Figs. 1, 2. Blastoderm formation of *L. cruciata*

1A. Longitudinal section of egg (10h : 20m after oviposition, shematical), 1B. Cleavage nuclei at egg surface in zone b, 1C. Cleavage nuclei in peripheral migration in zone c, 2A. Long. sec. of egg (13h : 20m a. ovipo., shematical), 2B. Blastoderm cells in zone b, 2C. Blastoderm cells in zone c. bc : blastoderm cells, cn : cleavage nucleus, p : periplasm, v : vacuole, y : yolk, yn : primary yolk nucleus



Figs. 3, 4. Formation of germ disk and germ rudiment of *L. cruciata*
3A. Longitudinal section of egg (16h : 20m after oviposition, shematical), 3B. Long. sec. of germ disk, 4A. Long. sec. of egg (19h : 20m a. ovipo., shematical), 4B. Long. sec. of germ rudiment. b, blastoderm, cp : connecting part, gd : germ disk, gr : germ rudiment, v : vacuole, y : yolk

P. pennsylvanica egg (WILLIAMS, 1916) closely resembles that of *L. cruciata*.

At about thirteen hours after oviposition, the uniform blastoderm cells cover the entire egg surface. Not very many nuclei remain in the yolk and they differentiate into primary yolk nuclei or cells. Thus the blastoderm is completed prior to the formation of the germ disk, and the differentiation of the embryonic and extra-embryonic areas in the blastoderm follows. Figs. 2B and 2C show that the blastoderm cells in the same egg differentiate into two morphological types, that is both the round and cuboidal blastoderm cell groups are found at this time.

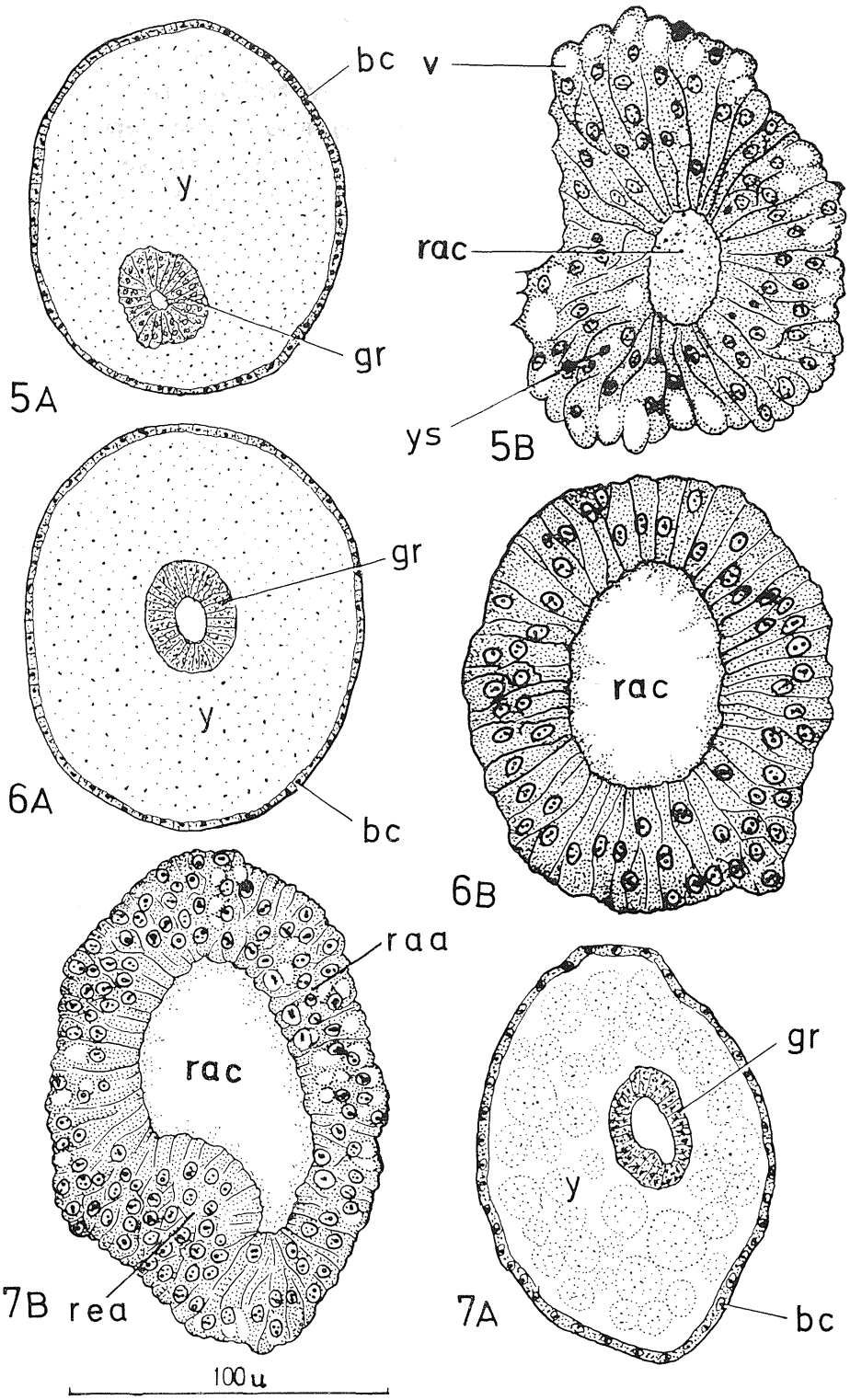
Stage 2. Formation of germ disk and germ rudiment (about 16-19 hours after oviposition) Figs. 3, 4

At about 16 hours after oviposition, a small germ disk is formed in the blastoderm. In longitudinal section of the egg there is seen the germ disk, about 77 by 128 micra in size, invaginated into the yolk. It is semicircular in shape and composed of elongated cells arranged roughly radially. At about 19 hours after oviposition, the germ disk develops and becomes a round germ rudimet, about 95 by 92 micra in diameter. It projects into the yolk and is connected with the blastoderm or developing serosa by a short thick pedicel-like part. In this stage the germ rudiment has no central lumen or cavity, that is it is a solid celluar body. The cells of the germ rudiment and the connecting part contain considerable vacuoles.

According to the description and figures given by WILLIAMS (1916), the early embryo stage in *P. pennsylvanica* may correspond with the earliest germ rudiment stage in *L. cruciata*. He showed the very early embryo in cross section in Fig. 33 of his paper, and, judging from the figure, the invaginated round embryo of *P. pennsylvanica* already has a large central cavity continuing to the exterior and no pedicel-like part. There is a difference between these morphological points in the germ rudiments of *L. cruciata* and in the early embryo of *P. pennsylvanica*.

Stage 3. Appearance of central cavity and differentiation of embryonic area in germ rudiment (about 25-46 hours after oviposition) Figs. 5-7

At about one day after oviposition, the oval germ rudiment, about 130 by 100 micra in size, leaves the developing serosa and is immersed into the yolk. The cells of the connecting part are fragmented and degenerating. Now, the small lumen, about 35 by 22 micra in size, appears in the center of the rudiment. With lapse of time, the germ rudiment, about 137 by 115 micra in size, sinks into the central region of the egg and the central lumen enlarges, about 70 by 58 micra in size. In about 37 hours after oviposition, the cells composing of the germ rudiment are uniform, but at the end of this stage (about two days after oviposition, about 155 by 105 micra in size) the differentiation of the embryonic and amniotic areas occurs. Hitherto the wall of the rudiment has been of uniform thickness, but now the embryonic area becomes thicker than the amniotic. In the embryonic area, about 57 micra in thickness, there are observed cells densely distributed, while on the other hand, in the amniotic area, bout 24 micra in thickness,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cells are rather sparsely distributed. Mitoses, yolk spherules and vacuoles are also found in both areas.

Stage 4. Beginning of germ band segmentation (about three days after oviposition) Fig. 6

At 24 hours after stage 3, the round germ rudiment develops into the small germ band completely immersed in the yolk. The germ band in this stage begins to bend dorsally and in the lateral view it is U-shaped. Its ventral side is covered by the amnion and the dorsal side is filled up by the yolk. The germ band is segmented into six regions, that is, the protocephalic lobes, rudiments of three gnathal, one thoracic segments and one large posterior segment-forming zone. The embryo of *P. pennsylvanica* showed by WILLIAMS (1916, Fig. 37) resembles the germ band of the present species.

Stage 5. Completion of germ band segmentation (about four days after oviposition) Fig. 7

In this stage, the germ band has been so elongated that its anterior and posterior ends come close together and it is a nearly circular in shape in the lateral view. The germ band has been completely metamerized and consists of the protocephalic lobes with rudimental antenna, the mandibular, maxillary, labial, three thoracic and ten abdominal segments. The germ band is entirely immersed in the yolk similar to the former stages.

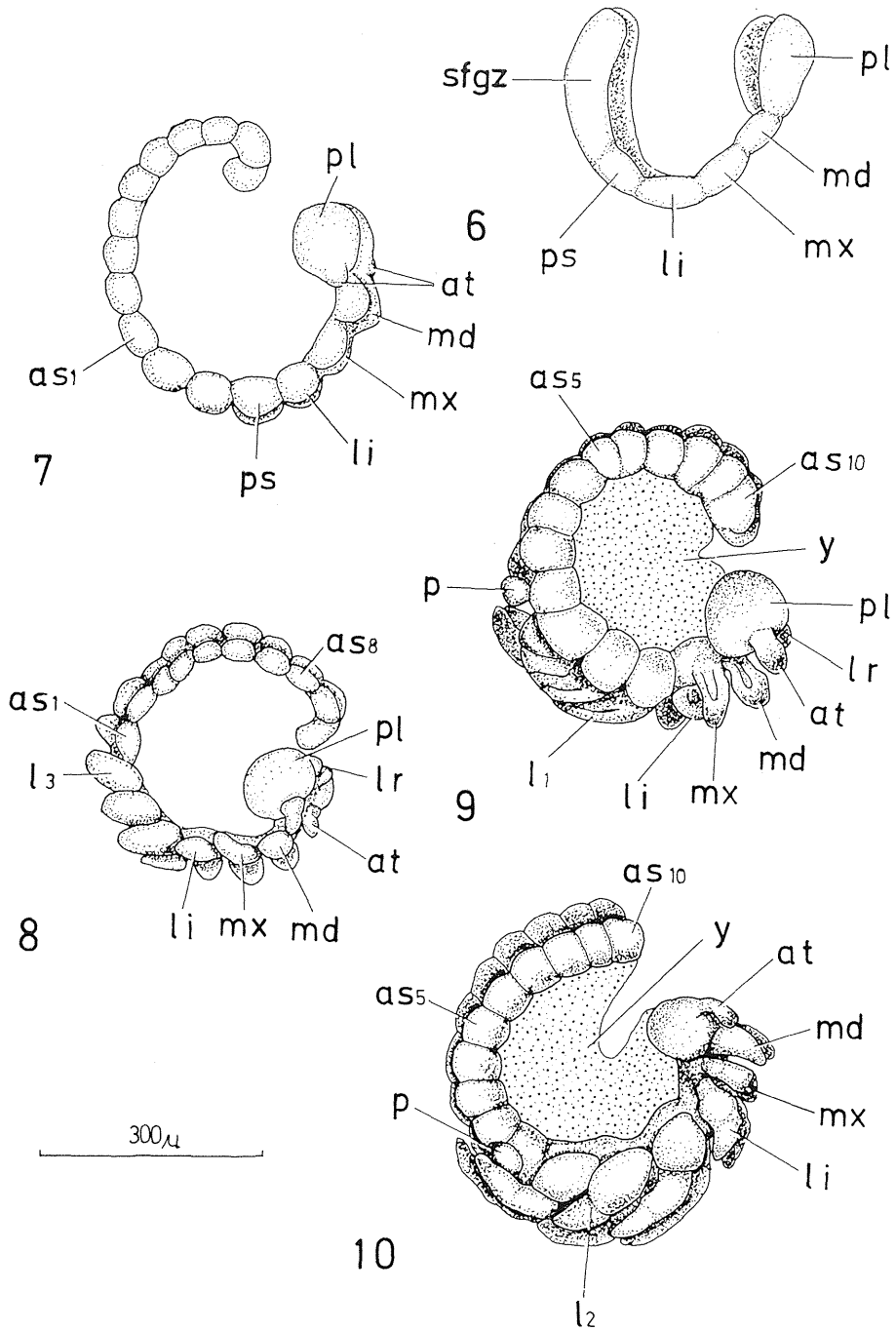
Stage 6. Early embryo with appendages (about five days after oviposition) Fig. 8

Both ends of the slender embryo touch each other, therefore the embryonic form is completely circular in the lateral view. The protocephalon has two pairs of rudimental labra and antennae, and the gnathal region has three pairs of rudimental appendages. The thoracic region of the embryo has three pairs of non-metamerized leg rudiments. In this stage, the anlagen of the pleuropodia are not found by external observation. The present embryo of *L. cruciata* corresponds with the embryo of *P. pennsylvanica* showed in Fig. 40 by WILLIAMS (1916).

Stage 7. Embryo with developing pleuropodia (about seven to nine days after oviposition) Figs. 9, 10

The six days and twenty-two hour old embryo is more advanced than those of the former stage. Also in the present stage the embryo curves dorsally as in the early stages of development. Now the length of the embryo decreases, but the embryo considerably increases in thickness and width, and as a result the embryo assumes a usual definite form in the middle stage of the embryonic development in Coleoptera. The gnathal region moves forward by the progression of cephalization. All appendages of the embryo develop remarkably and at this stage a pair of bell-shaped pleuropodia form in the first

Figs. 5-7. Germ rudiments of *L. cruciata*
5A. Longitudinal section of egg (25h : 20m after oviposition, shematical), 5B. Long. sec. of germ rudiment, 6A. Long. sec. of egg (37h : 20m a. ovipo., shematical), 6B. Long. sec. of germ rudiment. 7A. Long. sec. of egg (46h : 20m a. ovipo., shematical), 7B. Long. sec. of germ rudiment. bc : blastoderm cells or developing serosa, gr : germ rudiment, raa : rudimental amniotic area, rac : rudimental amniotic cavity, rea : rudimental embryonic area, v : vacuole, y : yolk, ys : yolk spherule



Figs. 6-10. Several stages of germ band and embryos of *L. cruciata*
6. Germ band (2d : 22h after oviposition), 7. Embryo (3d : 22h a. onipo.), 8. Embryo(4d : 22h a. ovipo.), 9. Embryo (6d : 22h a. ovipo.), 10. Embryo (8d : 22h a. ovipo.). as 1, 5, 8, 10 : 1st, 5th, 8th, 10th abdominal segments, at : antenna, l1, 2,3 : fore, middle, hind legs, li : labium, lr : labrum, md : mandible, mx : maxilla, p. : pleuropodium, pl : protocerebral lobe, ps : prothoracic segment, sfgz : segment forming growth zone, y : yolk

abdominal segment.

At the end of stage 7, eight days and twenty-two hours after oviposition, the embryo attains the early pre-revolution stage and its protocephalon becomes smaller in size than those in early embryos of the present stage. The appendages of the embryo are more elongated and enlarged, and in the thoracic legs three segments are formed. The embryo of the species is quite similar to that of *P. pennsylvanica* showed in Fig. 41 by WILLIAMS (1916).

The germ bands and embryos in the abovementioned stages of *L. cruciata* appear a usual feature of coleopteran embryos excepting for the fact that they are completely sunken in the yolk like those of the lepidopteran embryos such as *Pieris rapae* (EASTHAM, 1930) and *Chilo suppressalis* (OKADA, 1960).

A description of the further developmental stages from stage 7 will be given in the next paper.

2. Ball-shaped germ rudiments in *L. cruciata* with special reference to the germ band types in Coleoptera

In insect eggs in general, the types of germ bands are divided into three, that is, the superficial, invaginated and immersed ones. Accordingly, the coleopteran germ bands are usually formed on the ventral surface of the eggs and they are classified into the superficial type of germ band, for instance, *Hydrophilus piceus* (Hydrophilidae, HEIDER, 1889), *Lytta viridana* (Meloidae, REMPEL & CHURCH, 1968), *Epilachna vigintioctomaculata* (Coccinellidae, MIYA & ABE, 1966), *Phyllophaga forvida* and *Phy. hirticula* (Scarabeidae, LUGINBILL, 1953) and others. However, according to ZAKHVATKIN (1968) in the family Chrysomelidae two types of germ bands, i. e. superficial and invaginated, appear, *Timarcha coriaria* having a superficial type and *Galerucella tenella* and *Chalcoides aurata* having an invaginated, and a similar tendency also may be found in the family Curculionidae (KRZYSZTOFOWICZ, 1960).

If the abovementioned classification of the germ bands is accepted, the type of the germ bands of *L. cruciata* and *P. pennsylvanica* (WILLIAMS, 1916) is classified as the immersed type.

In the present study, it is not known whether all lampyrids have ball-shaped germ rudiments or not. Since HESS (1922) and BUGNION (1922) studied the light organs of the grown embryos, it is only natural that they did not observe the early embryos of the fireflies, and did not describe them. However at least one of the most important characteristics of the lampyrid embryogenesis is the presence of ball-shaped germ rudiments. According to CROWSON (1955), lampyrid larvae seem among the most primitive and Lycids and Cantharids the most advanced from the comparative morphology of larvae in Cantharoidea, and these facts may suggest that the family Lampyridae has an unique or primitive position in the superfamily Cantharoidea.

Judged from WILLIAMS' description and figures in *P. pennsylvanica*, the ball-shaped germ rudiments are formed by the invagination of the blastoderm cells, but in *L. cruciata*

the grm disk is formed by the way of the proliferation of the blastoderm cells restricted to a small area. If the invagination and proliferation of the blastoderm cells in the process of the germ rudiment formation are thought to belong to the same category, they may join each other in this important period of development.

Recently MIYAGAWA (1973) studied the embryology of the caddisfly, *Stenopsyche griseipennis* and found that the round germ rudiments as that of *Luciola* are formed in the species, and the senior author and TANAKA (unpublished studies) observe that the round germ rudiments are also formed in *Endoclyta signifer*, one of the most primitive moths belonging to the family Hepialidae (Monotrysia). These similarity in the form of germ rudiments may show a close affinity between these two orders, that is, Trichoptera and Lepidoptera. It may be important in the insect phylogeny that the abovementioned species of Coleoptera, Trichoptera and Lepidoptera have round germ rudiments respectively.

SUMMARY

1. The early and middle stages of the embryonic development in the firefly, *Luciola cruciata* MOTSCHULSKY are described in the present paper.
2. The germ disk of *L. cruciata* is formed by the proliferation of the blastoderm cells restricted to a small area. It is invaginated into the yolk, and develops a solid round germ rudiment completly immersed in the yolk.
3. The later germ rudiment has the central cavity or rudimental amniotic cavity and becomes ball-shaped.
4. The presence of ball-shaped germ rudiments is an important characteristics of the lampyrid embryogenesis.

LITERATURE CITED

BUGNION, E. 1922. Études relatives a l'anatomié et a l'embryologie des vers luisants ou Lampyrides. Bull. biol. d. France d. Belg., **56** : 1-53.

CROWSON, R. A. 1955. The natural classification of Coleoptera. Nathaniel Lloyd, London.

Crowson, R. A. 1960. The phylogeny of Coleoptera. Ann. Rev. Entomol., 5 : 111-134.

EASTHAM, L. S. 1927. A contribution to the embryology of *Pieris rapae*. Quart. Jour. Micr. Sci., **71** : 353-394.

HEIDER, K. 1889. Die Embryonalentwicklung von Hydrophilus piceus L. Gustav Fischer, Jena.

HESS, N. W. 1922. Origin and development of the light-organs of Photurus pennsylvanica De Geer. Jour. Morph., **36** : 245-277.

KRZYSZTOFOWICZ, A. 1960. Comparative investigations on the embryonic development of the weevils (*Coleoptera, Curculionidae*), and an attempt to apply them to the systematics of this group. Zoologica Poloniae, **10** : 3-27, 1959-1960.

LUGINBILL, P., Jr., 1953. A contribution to the embryology of the May beetle. Ann.

Entomol. Soc. Amer., **46** : 505-528.

MIYA, K. & T. ABE 1966. The early embryology of *Epilachna vigintioctomaculata* Motschulsky (Coccinellidae, Coleoptera), including some observations on the later development. Jour. Fac. Agr., Iwate Univ., **7**, 277-289.

MIYAGAWA, K. 1973. The embryology of the caddisfly Stenopsyche griseipennis MacLachlan (Trichoptera : Stenopsychidae.) I. Early stages and changes in external form of embryo. Kontyû, **41**, 413-425.

OKADA, M. 1960. Embryonic development of the rice stem-borer, *Chilo suppressalis*. Sci. Rep. Tokyo Kyoiku Daigaku, Sec, B, **9** : 243-296.

REMPEL, J. G. & N. S. CHURCH 1969. The embryology of *Lytta viridana* Le Conte (Coleoptera : Meloidae). V. The blastoderm, germ layers, and body segments. Canad. Jour. Zool., **47** : 1157-1171.

WILLIAMS, F. X. 1916. Photogenic organs and embryology of Lampyrids. Jour. Morph., **28** : 145-207.

ZAKHVATKIN, Y. A. 1968. Comparative embryology of Chrysomelidae. Zool. Jour. Acad. Sci. S. S. S. R., XLVII, 1333-1342 (in Russian).

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