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External Morphogenesis of the Embryo of *Ascalaphus ramburi* (Neuroptera, Ascalaphidae)

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Synopsis

External form of the developing embryos of *Ascalaphus ramburi* MacLachlan is observed and the normal plate of the embryonic development is given. Micropyles are observed at each pole of the egg and a girdling or hatching line presents at the anterior pole of the egg. The large, broad ventral plate differentiates in the blastoderm and the embryo remains superficial during its entire development. The segmentation of the germ band has occurred before the inner layer formation. A large secondary dorsal organ is formed.

Introduction

The Ascalaphidae are actively flying neuropteran insects and widely distribute in the world. In Japan four species belonging to four genera are known, and the Japanese Ascalaphidae were studied taxonomically by Okamoto (1909) and autoecologically by Sonan (1938), but the embryonic development of Ascalaphidae was not studied up to this time.

Concerning the Neuroptera and Megaloptera works dealing with the embryonic development are on *Chrysopa perla* (Bock, 1939, 41), *Sialis lutaria* (Strindberg, 1915; DuBois, 1936, 38), *S. mitsuhashii* (Suzuki *et al*, 1981) and *Protohermes grandis* (Miyakawa, 1979, 80).

In the present paper the outline of the embryonic development of *Ascalaphus ramburi* is described, and the detailed studies on embryogenesis of this species will be published in the near future.

Material and Methods

The egg of *Ascalaphus ramburi* MacLachlan were collected during late May and mid June in Kaida and Ueda in Nagano Prefecture and Shirotori in Gifu Prefecture of Japan, and they were reared in plastic cases at room temperature.

Almost all egg-masses were oviposited on dead stems of various grasses or dead twigs of trees in grass-fields surrounded by trees.

Observations of the developing embryos were done under living or fixed conditions. Especially at early stages of the embryonic development, living eggs were mostly used for observation. The eggs were fixed with F. A. A., Carnoy's solution or Bouin's solution. F. A. A. and Bouin's solution were heated at 60°C to 70°C before use. When the eggs are observed, the outer layer of the chorion was dissolved by 4% antiformin.

Results

1. External Structure of the Egg

The eggs of *Ascalaphus ramburi* are creamly-white in color and oval in shape, about 2 mm in length and 1.3 mm in width. Eggs are deposited in rows upon grass stems or twigs, the ventral side of the egg being laid over these substrata. Though in the American Ascalaphidae modified eggs or repagula encircling the twig beneath the egg-masses were reported (Henry, 1972, 78), no repagula were found in egg-masses of *A. ramburi.* The chorion is covered with a reticulate pattern, and there is observed a micropylar area at each pole of the egg (Figs. 1, 20). The chorion consists of two layers, that is, colored outer layer and transparent inner, respectively. The inner layer is more resistive to sodium hypochlorite (antiformin) than the outer layer.

There is a whitish girdling line near the anterior pole of the egg (Figs. 1, 2), and the first instar larva hatches out from this part.

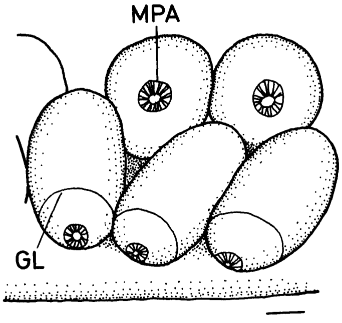
2. Changes in the External Form of Developing Embryos

The egg period of *A. ramburi* is about 18 days at 19°C-28°C. The embryonic development of *A. ramburi* is divided into the following 16 stages.

Stage 1 (about 1 day after oviposition, Fig. 2)

It is very likely that the maturation division and fertilization undergo early this stage, but these processes could not observed in the present study.

The blastoderm formation follows as the cleavage nuclei *i. e.,* the energids arrived at the egg surface late at this stage. The nuclei at the egg surface undergo the mitotic divisions repeatedly and the nuclear density increases, and then the cellular blastoderm is formed.



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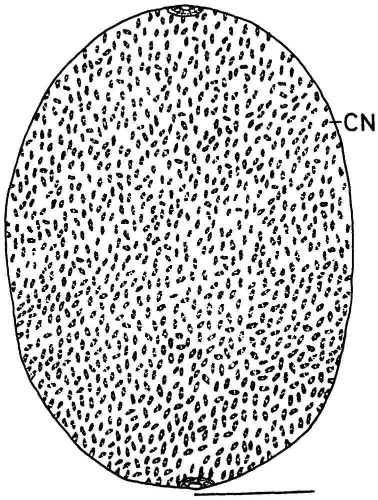

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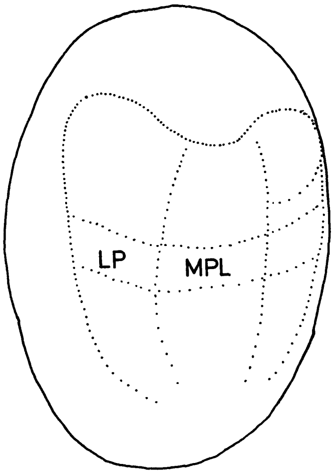

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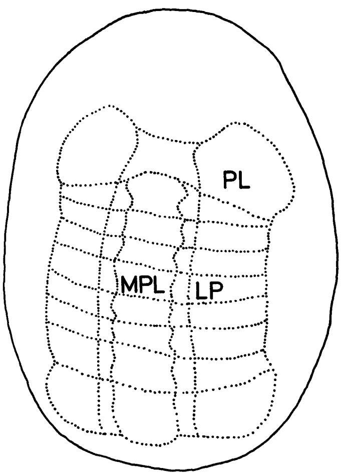

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Fig. 1. Eggs of *Ascalaphus ramburi*.
Fig. 2 Egg of Stage 1.
Fig. 3 Egg of Stage 2, ventral view.
Fig. 4 Egg of Stage 3, ventral view.

CN cleavage nucleus, GL girdling line, LP lateral plate, MPA micropylar area, MPL middle plate, PL protocephalon. Scales: 0.5 mm.

Stage 2 (about 2 and 1/8 days after oviposition, Fig. 3)

The large heart-shaped ventral plate differentiates in the blastoderm. In the meanwhile the rudimental middle and lateral plates begin to differentiates in the ventral plate. The segmentation of the ventral plate also begins at this stage.

Stage 3 (about 2 and 1/4 days after oviposition, Fig. 4)

The germ band or ventral plate of this stage decreases in width, and its segmentation proceeds. As the result of this, the protocephalon, intercalary, three gnathal, three thoracic segments and an unsegmented abdominal lobe are formed. The middle and lateral plates have differentiated but the inner layer has not yet formed.

Stage 4 (about 2 and 3/8 days after oviposition, Fig. 5)

The germ band further decreases in width and the primitive groove along the middle line of ventral plate begins to form. The protocephalon, intercalary, mandibular, maxillary, labial segments and the first to the third thoracic segments with a large abdominal lobe become more apparent.

Stage 5 (about 2 and 3/4 days after oviposition, Fig. 6)

The germ band becomes more elongated and width of the germ band becomes narrower. The primitive groove also becomes clear, and the segmentation of the inner layer begins at this time. The amniotic folds develop at the anterior and posterior ends of the germ band.

Stage 6 (about 3 days after oviposition, Fig. 7)

The width of the germ band is much narrower than that in the former stage. The segmentation proceeds to the fourth abdominal segment whereas a large posterior part of the abdomen remains unsegmented.

Stage 7 (about 3 and 1/2 days after oviposition, Fig. 8)

The embryo or germ band continues to elongate further and its caudal end reaches near the posterior egg pole. A pair of the labrum and antennal rudiments appear on the protocephalic lobes, and the rudiments of paired appendages are formed in each gnathal and thoracic segments except the intercalary. There are ten abdominal segments at this stage. Therefore now the embryo consists of the protocephalic lobes, intercalary, three gnathal, three thoracic and ten abdominal segments. The stomodaeal pit is seen just behind the labral rudiments.

Stage 8 (about 4 days after oviposition, Fig. 9)

The intercalary segment is gradually reduced, and a pair of appendages of the first abdominal segment becomes distinguishable at this stage. The appendages observed in the first ab-

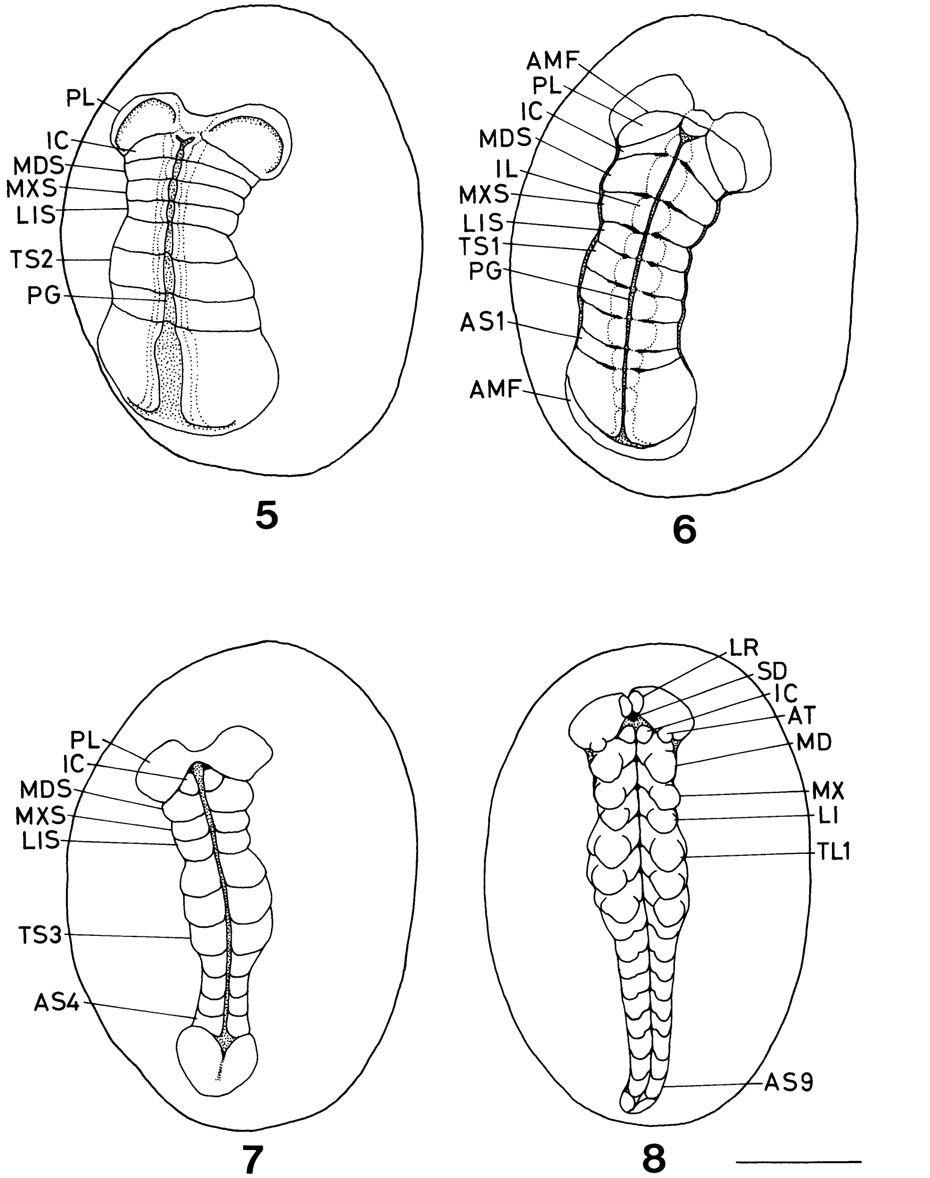


Fig. 5. Egg of Stage 4, ventral view.
Fig. 6 Egg of Stage 5, ventral view.
Fig. 7 Egg of Stage 6, ventral view.
Fig. 8 Egg of Stage 7, ventral view.

AMF · amniotic fold, AS1, 4, 9, 1st, 4th, 9th abdominal segments, AT antenna, IC intercalary segment, IL inner layer, LI labium, LIS labial segment, LR labrum, MD mandible, MDS mandibular segment, MX maxilla, MXS maxillary segment, PG primitive groove, PL protocephalon, SD stomodaeum, TLI 1st thoracic leg, TSI-3 1st-3rd thoracic segments. Scale 0.5 mm.

dominal segment are the pleuropodia, which are similar to the thoracic appendages in shape.

Stage 9 (about 4 and 1/2 days after oviposition, Fig. 10)

The width of anterior part of the embryo increases, which may be seen especially from the protocephalic lobes to the thoracic region. A pair of the rudimental eyes appear on both lateral sides of the protocephalic lobes, and the antennal rudiments elongate to the mid-ventral line of the embryo. The stomodaeal pit is covered gradually by the developing labrum. The rudiments of the mandible, maxilla and labium also develop, and the maxillary palpal rudiments are formed on each maxilla.

Stage 10 (about 5 and 1/2 days after oviposition, Fig. 11)

The embryo becomes much wider than that in Stage 9. The stomodaeal opening is covered by the labrum, and the rudiments of eyes increase in size. The developing mandible and maxilla elongate further, and the labium moves forwards and becomes to be located posteriorly to the maxilla. The rudimental appendages of the thorax and the pleuropodia develop further. A pair of developing spiracles appear in the second and third thoracic segments and the first to the eighth abdominal segments. The caudal end of the embryo bends to the ventral side and has the paired anal lobes.

Stage 11 (about 6 and 1/2 days after oviposition, Fig 12)

The rudiments of lateral ocelli in the eye area are clearly observed in this stage. The labium is hidden under the other gnathal appendages. It seems that the pleuropodia begin to secrete. Brownish pigments appear over the surface of the head and both lateral sides of the thorax and abdomen of the embryo. The dorsal closure of the embryo begins, consequently the width of the embryo increases further.

Stage 12 (about 7 and 1/2 days after oviposition, Fig. 13)

The embryo develops so as to occupy the whole ventral side of the egg, and the dorsal closure advances further. The basal parts of the mandible and maxilla begin to fuse, and a pair of labial palpi appear at this stage. The scoli begin to develop on the second and third thoracic segments. The pleuropodia are larger than those at the previous stage, and each spiracle slightly rises from its original position. The tenth abdominal segment on which the anus is located reaches the six abdominal segment, and the ninth and tenth abdominal segments fuse with each other.

Stage 13 (about 8 and 1/2 days after oviposition, Fig. 14)

The maxillae are located under the mandibles and the tips of these appendages attain to the first abdominal segment. The developing eye composed of seven rudiments of lateral ocelli changes into a thick and short process projecting from the head surface.

The thoracic legs elongate greatly, and the tips of metathoracic legs reach the seventh abdominal segment. The basic pattern of the leg-segments is definitely formed. The tenth

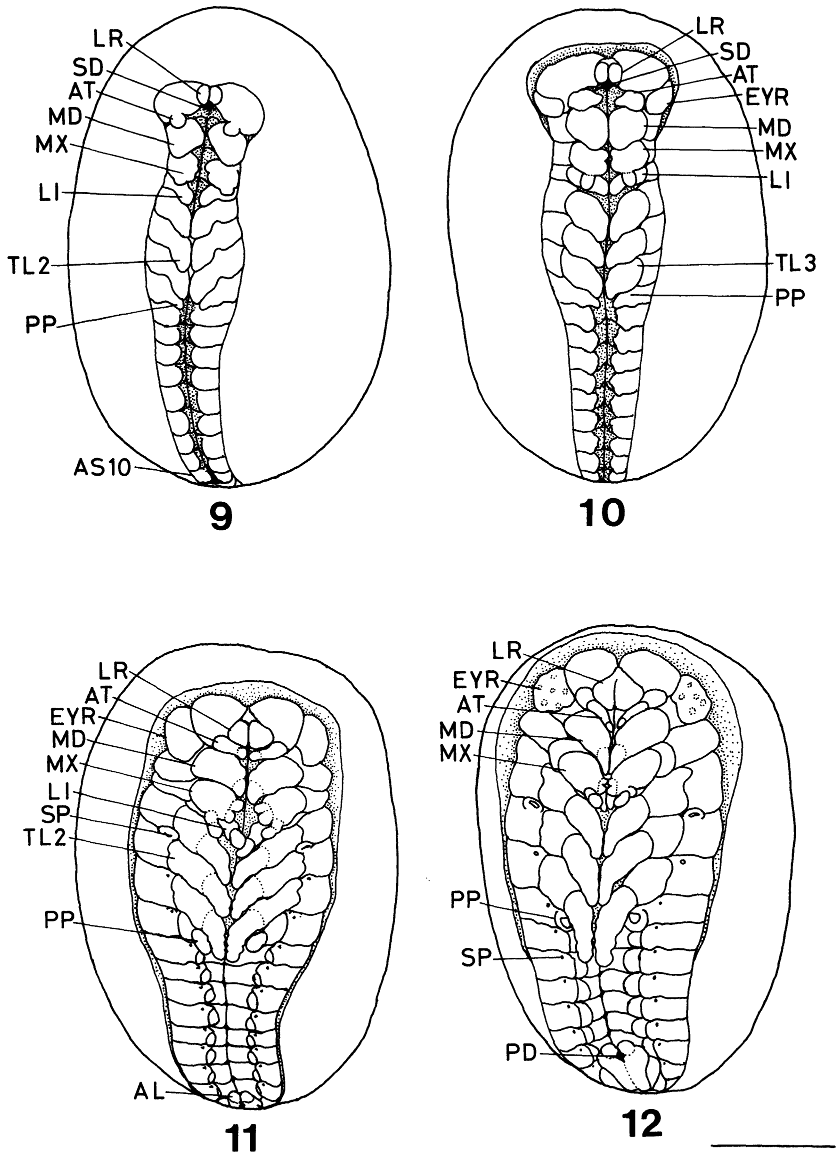


Fig. 9 Egg of Stage 8, ventral view.
Fig. 10. Egg of Stage 9, ventral view.
Fig. 11 Egg of Stage 10, ventral view.
Fig. 12. Egg of Stage 11, ventral view.

AL anal lobe, AS10 10th abdominal segment, AT antenna, EYR eye rudiment, LI labium, LR labrum, MD mandible, MX maxilla, PD proctodaeum, PP pleuropodium, SD stomodaeum, SP spiracle, TL 1, 2, 3 1st, 2nd, 3rd thoracic legs. Scale: 0.5 mm.

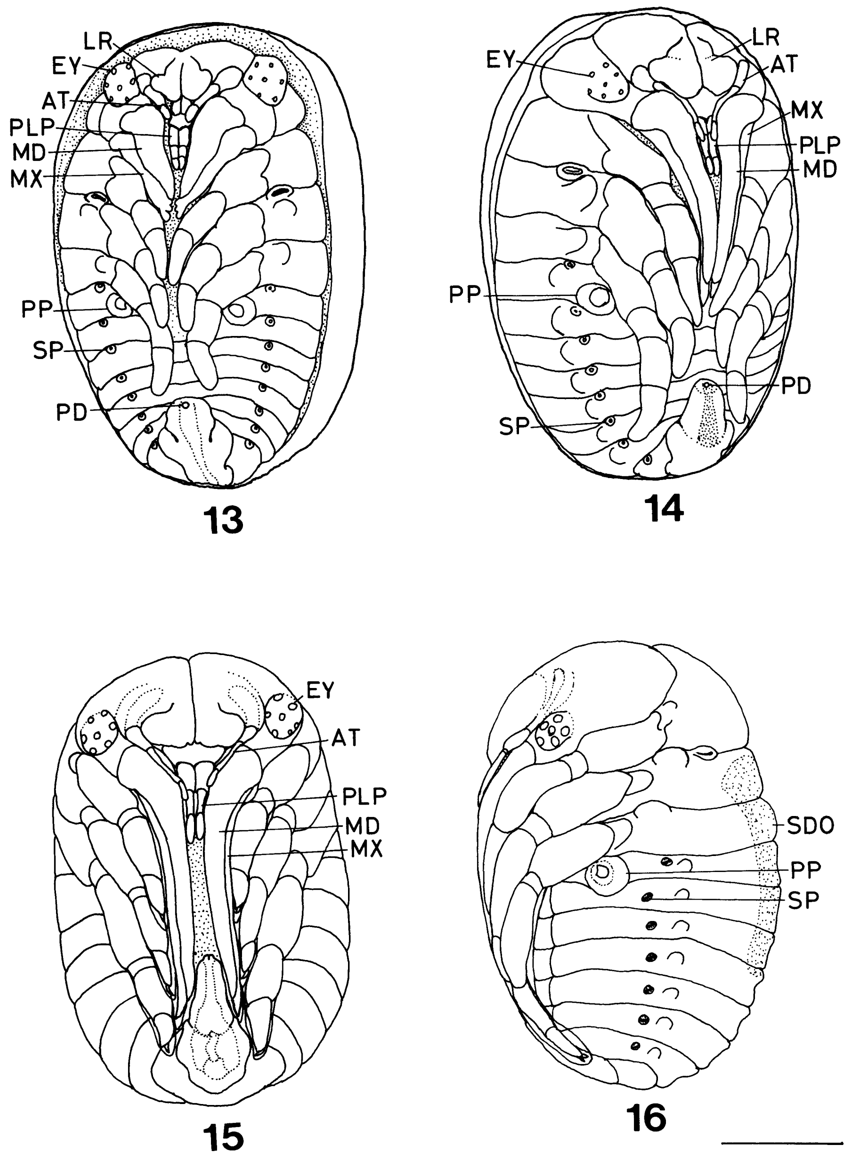


Fig. 13. Egg of Stage 12, ventral view.
Fig. 14. Egg of Stage 13, ventral view.
Fig. 15. Embryo of Stage 13, ventral view.
Fig. 16. Embryo of Stage 14, lateral view.

AT antenna, EY eye, LR labrum, MD mandible, MX maxilla, PD proctodaeum, PLP labial palp, PP pleuropodium, SDO secondary dorsal organ, SP spiracle. Scale: 0.5 mm.

abdominal segment is pointed-cone shaped.

Stage 14 (about 10 days after oviposition, Figs. 15, 16)

The dorsal closure has already finished by this time and the large secondary dorsal organ is formed on the dorsal side of the first thoracic to the third abdominal segments. The external form of the first instar larva has been completed except the scoli, tail and lack of the hairs.

Stage 15 (about 14 days after oviposition, Figs. 17, 18)

The eggs at this stage become dark in color and to be remarkably hollowed. The fully grown embryo is almost similar to the first instar larva. The lateral ocelli turn dark brown, the body is pigmented brownish, and the all bristles come out remarkably.

Stage 16 (about 19 days after oviposition, Fig. 19)

The newly hatched larvae are brown, but they then change dark brown within a short time after hatching. They are a typical ascalaphid larva in many respects and about 5.2 mm in length including jaws. They stay on the egg-masses subsequent several days and then fall on the earth.

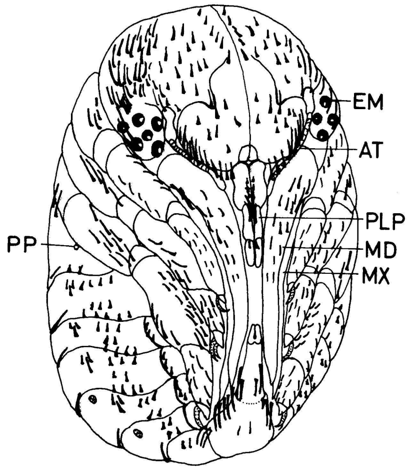
Discussion

In general the form and size of the eggs of *Ascalaphus ramburi* resemble those described for other ascalaphids such as *Hybris subjacens* (Sonan, 1938), *Ululodes mexicana, Ascaloptynx furciger* (Henry, 1972), and *Byas albistigma* (Henry, 1978). The structure and location of the micropyles observed in *A. ramburi* are basically the same as those in other species of Ascalaphidae. *A. ramburi* also shares with other owlflies a girdling line at the anterior pole of eggs for the hatching of larva, and the repagula are not formed.

In the egg of *A. ramburi* the large, broad ventral plate differentiates and this feature of the ventral plate resembles those of *Chrysopa perla* (Bock, 1939) and *Sialis mitsuhashii* (Suzuki *et* al., 1981), and moreover it bears also a resemblance to those of the scorpionfly, *Panorpa.*

The segmentation of ectodermal part of the ventral plate in *A. ramburi* has occurred before the formation of the inner layer as reported in *C. perla* (Bock, 1939). Therefore the differentiation of middle and lateral plates and the segmentation in early developmental stages seem to be the common features in the neuropteran embryogenesis.

The embryo of *A. ramburi* develops on the egg surface as observed in *S. lutaria* (Strindberg, 1951; Du Bois, 1938), *C. perla* (Bock, 1939) and *Protohermes grandis* (Miyakawa, 1979). The caudal end of the embryos in *S. lutaria, C. perla* and *P. grandis* reaches the dorsal side of the egg, and the katatrepsis of the embryo is remarkable, but the embryonic caudal end of *A. ramburi* attains to only the posterior pole of the egg because of the shortness of embryo, and conspicuous katatrepsis does not occur. Seven lateral ocelli develop in *A. ramburi,* whereas six in *S. lutaria, C. perla* and *P. gandis.*



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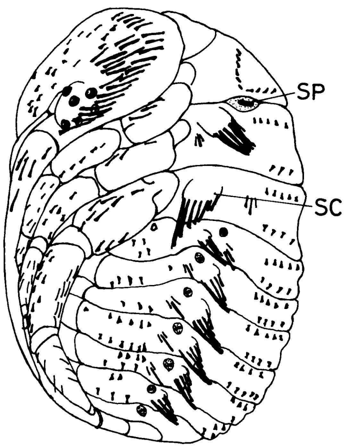

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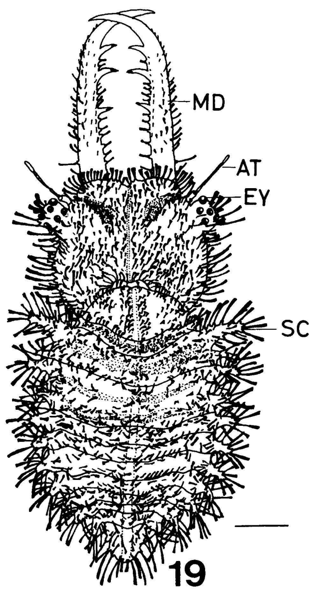

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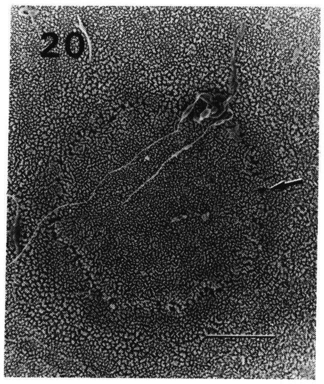

Fig. 17. Embryo of Stage 15, ventral view.
Fig. 18. Embryo of Stage 15, lateral view.
Fig. 19. 1st instar larvae (Stage 16), dorsal view.
Fig. 20. Micropylar area at anterior pole of egg (arrow, micropyle) (X360)

AT antenna, EY eye, MD mandible, MP micropyle, MX maxilla, PLP labial palp, PP remnant of pleuropodium, SC scolus, SP spiracle. Scales: 50 μm in Fig. 20, 0.5 mm in others.

General features of external morphogenetic process observed in *A. ramburi* may allow us to consider that they belong to those commonly found in the primitive holometabolous insects.

Acknowledgement

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