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Embryogenesis of the Leather Winged Beetle, *Athemus suturellus* Motschulsky (Coleoptera, Cantharidae)*

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and

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Synopsis

External morphological events during embryogenesis in *Athemus suturellus* Motschulsky are studied briefly. The embryo of *A. suturellus* develops on yolk surface without immersion into yolk, and belongs to the superficial type of germ band. The relationship between *A. suturellus* and fireflies is considered from the embryological standpoint.

Introduction

Although many studies on the embryogenesis of coleopteran insects have been done, the embryonic development of the leather winged beetles, family Cantharidae of Cantharoidea, which contains *Athemus suturellus* has not been studied up to now. Other families in the superfamily, Lampylidae and Drilidae (Omethidae) were studied embryologically (William, 1916; Bugnion, 1922; Hess, 1922; Ando and Kobayashi, 1975; Kobayashi and Ando, 1985). Cantharoidea is supposed to be primitive group in polyphagous coleopterans, but the systematic situation of this group in Coleoptera has not yet been determined. In the present paper the authors described briefly the embryonic development of *A. suturellus*, by external observation and compared the obtained results with those of fireflies.

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Material and Methods

Adults of *Athemus suturellus* Motschulsky were collected at Sugadaira, Nagano Prefecture of Japan, from June to early August. The captured insects were reared by one pair in each plastic case with plaster bottom. They were fed with aphids. Females deposited eggs on plaster of the case.

Eggs were fixed with alcoholic Bouin's fluid or F. A. A. at room temperature for 24 hr. Then they washed and preserved in 70% alcohol. The chorion of eggs was removed with the aid of fine needles, and embryos were stained Mayer's haemalum for the study of the external morphology. The eggs were stuck with needle, and they were embedded in paraplast and were cut at 7 μm-thick. The sections were stained with Delafield's haematoxylin and eosin. First instar larvae were stained with eosin for two days and were mounted on hollow slide glasses. The figures were drawn with the Abbe's apparatus.

Results

Total time duration from oviposition to hatching in *Athemus suturellus* is about 240 hours at room temperature, and the development process is divided into nine stages as shown in Table 1.

Table 1. Time table of embryonic development in *Athemus suturellus*.

Hours after Oviposition	Stage of Development
0 - 48 *hr*	Cleavage
48 - 68 *hr*	Formation and Completion of Blastoderm
68 - 80 *hr*	Formation of Germ Band
80 - 130 *hr*	Initiation and Completion of Segmentation
130 - 150 *hr*	Formation of Appendages
150 - 180 *hr*	Formation of Mouthparts
180 - 200 *hr*	Rotation of Embryo
200 - 230 *hr*	Dorsal Closure of Embryo
230 - 240 *hr*	Hatching

1. *Newly laid egg*

The eggs of *A. suturellus* are laid as a mass of 200 to 350 in number, cohered with sticky substance. The newly laid eggs are ellipsoidal and 600 × 450 μm in size on an average, and they are soft, lustrous, smooth, and milky in color.

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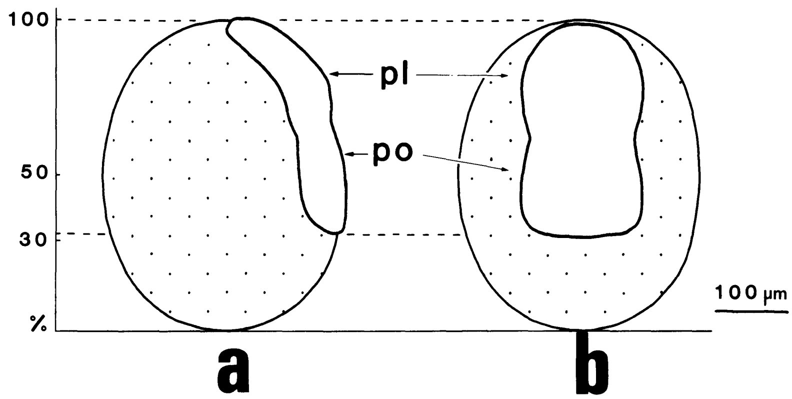

Fig. 1. Germ band of *Athemus suturellus*, about 80 hr after oviposition. a: lateral view, b: ventral view. pl, protocephalon; po, protocorm.

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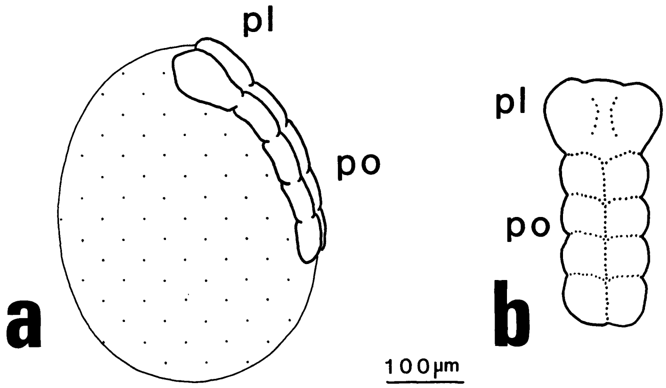

Fig. 2. Young embryo of *Athemus suturellus*, about 90 hr. a: lateral view, b: ventral view. pl, protocephalon; po, protocorm.

2. *Cleavage*

Although the process of maturation, fertilization and early cleavage could not be observed in detail, it was noted that one nucleus with the cytoplasmic coat existed near the anterior pole of the egg at a few hours after laying. The nucleus, which to be a synkaryon, repeatedly divides mitotically during the subsequent two days.

3. *Blastoderm formation*

At about 48 hours after oviposition, energids begin to migrate gradually toward the periplasm but some ones remain in the yolk as future primary vitellophages. After the penetration of energids, about 25 μm in diameter, into the periplasm, the periplasm thickens and exhibits knobby protuberances inside. The nuclei (7−9 μm in diameter) in the periplasm continue to divide and distribute uniformly over there, to form a "syncytial blastoderm" . Then cell membranes appear between each nucleus, in result of this a "cellular blastoderm" completes, of which cells are spindle-shaped, 2 × 13 μm in size.

4. *Formation of ventral plate and germ band*

At about 72 hours after oviposition, an elliptic ventral plate is formed on the ventral region of the eggs, covering two thirds of egg length from the anterior pole, which is about 200 μm in width and 300 μm in length.

At 80 hours after oviposition, the ventral plate begins to elongate and differentiates into the protocephalon anteriorly and the protocorm posteriorly, which is 250 μm in width and 350 μm in length (Fig. 1), and increases in thickness.

5. *Segmentation of germ band*

The protocephalon becomes concave at middle part, and the protocorm elongates gradually and its segmentation begins (Fig. 2). At about 95 hours after oviposition, the protocorm is composed of the mandibular, maxillary, and labial segments, and a growth zone which is longer than other segments, and a primitive groove appears in the protocorm. The germ band increases its length, along ventral side of egg, as the segments are added one by one.

At about 5 hours later, in the germ band three gnathal, three thoracic and three or four abdominal segments are visible, but the intercalary segment is not clear. At the same time the labral rudiment appears as a pair of small lobes in the anterior region of protocephalon (Fig. 3). At about 110 hours, the segmentation of the germ band continues further, and the caudal end of the germ band or embryo attains to the egg dorsal side over the posterior pole of the egg. The neural groove occurs along the midventral line of the embryo as a plain groove.

At about 120 hours, the segmentation of the embryo completes (Fig. 4), and thor-

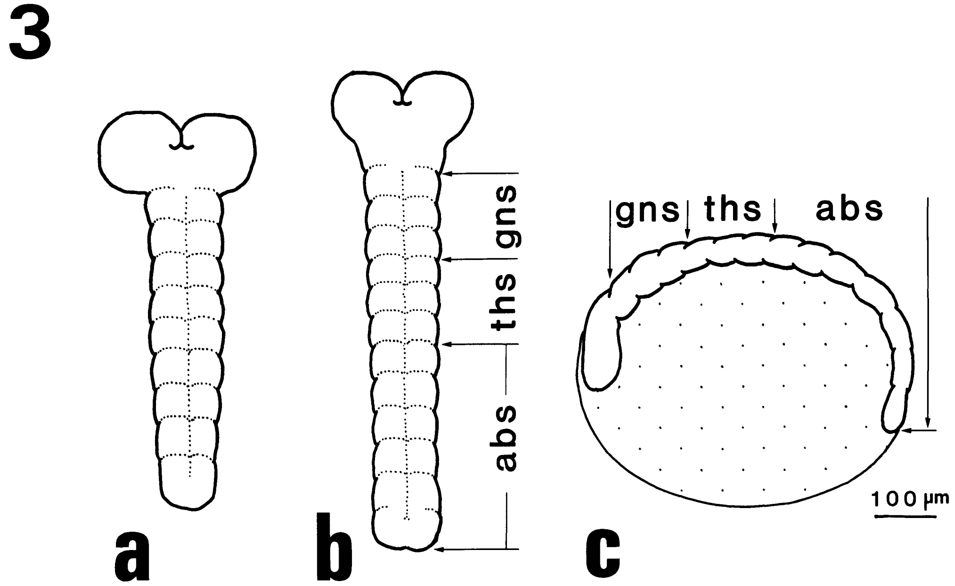

Fig. 3. Segmentation of embryo of *Athemus suturellus*. a: about 100 hr, ventral view, b: about 110 hr, ventral view. c: about 110 hr, lateral view. abs, abdominal segment; gns, gnathal segment; ths, thoracic segment.

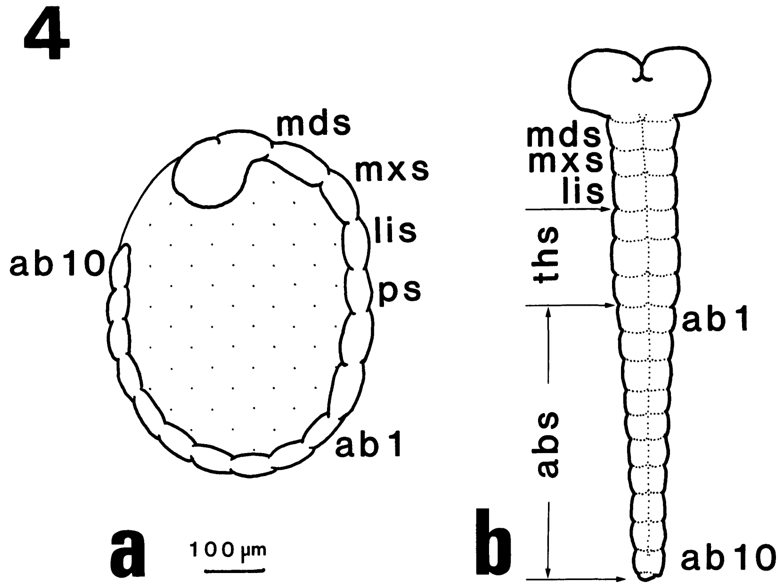

Fig. 4. Completion of segmentation in embryo of *Athemus suturellus*, about 120 hr. a: lateral view, b: ventral view. abs, abdominal segment; ab1, 10, first and tenth abdominal segments; lis, labial segment; mds, mandibular segment; mxs, maxillary segment; ps, prothoracic segment; ths, thoracic segment.

acic segments are broadest of all, and the fourth to tenth abdominal segments appear being thinnest in the width. The stomodaeal invagination appears behind the paired labral rudiments and the neural groove becomes clearer. At this stage the embryo consists of head lobes, intercalary, other three gnathal, three thoracic and ten abdominal segments with the telson. At about 130 hours, the embryo attains to the longest stage and encircles the yolk mass.

6. *Formation of appendages*

With the beginning of appendage formation, the embryo becomes dwindled by degrees. At about 135 hours after oviposition, the rudiments of appendages are formed as small protrusions on the gnathal and thoracic segments (Fig. 5 A), and the neural ridges become clear in the abdominal segments by development of the ganglia. Invagination of the stomodaeum deepens further behind the level of mandibular rudiments, and three pairs of swellings appear in the head lobes which may correspond to the future protocerebral lobes.

The segmentaion of rudiments of the antennae, mandibles, maxillae, labium, and fore, mid and hind legs takes place at about 150 hours. The legs are directed medio-posteriorly. The mandibular rudiments are prominent in the gnathal appendages, and the maxillary ones are long and slender. Now the tips of antennal ones are over the intercalary segment (Fig. 5 B). In *Luciola cruciata* a pair of prominent pleuropodia is formed at the first abdominal segment (Ando and Kobayashi, 1975), but in *A. suturellus* they are not found.

7. *Formation of mouth parts*

The remarkable morphogenesis in the embryo is the cephalization including transformation and translocation of the gnathal segments and appendages. At about 160 hours after oviposition, each segment of the thorax and abdomen becomes distinct, in consequence of shrinkage of the embryonic length (Fig. 6 A) . The head lobes and gnathal segments begin to gather to form the future head at about 170 hours (Fig. 6 B). The labral rudiments unite to form a single developing labrum in front of the stomodaeum, and the antennal ones move latero-dorsally. The rudiments of mandible, maxilla, and labium are assembled around the stomodaeum to form the future mouth parts together with the developing labrum. Figure 10 represents diagrammatically this process in sequence. At this time the thoracic appendages are also segmented into the coxa, trochanter, femur, tibia and pretarsus.

8. *Revolution and dorsal closure of embryo*

Until pre-cephalization stage the embryo has existed on the convex ventral surface of egg, but it begins to shorten again with the extension of both lateral extremities of the embryo. At about 180 hours the caudal end of the embryo reaches back near the

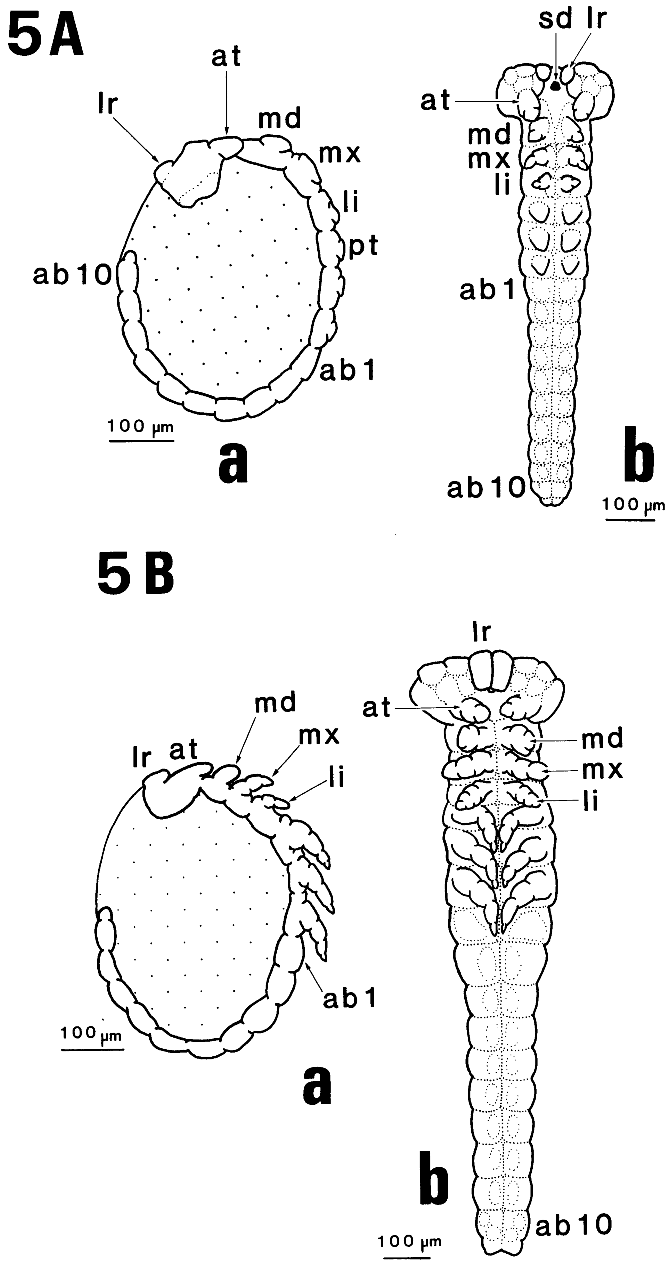

Fig. 5. Appendage formation of embryo of *Athemus suturellus*. A: about 135 hr, B: about 150 hr. A-a: lateral view, A-b: ventral view. B-a: lateral view, B-b: ventral view. ab1, 10, first and tenth abdominal segments; at, antenna; li, labium; lr, labrum; md, mandible; mx, maxilla; sd, stomodaeum; pt, prothorax.

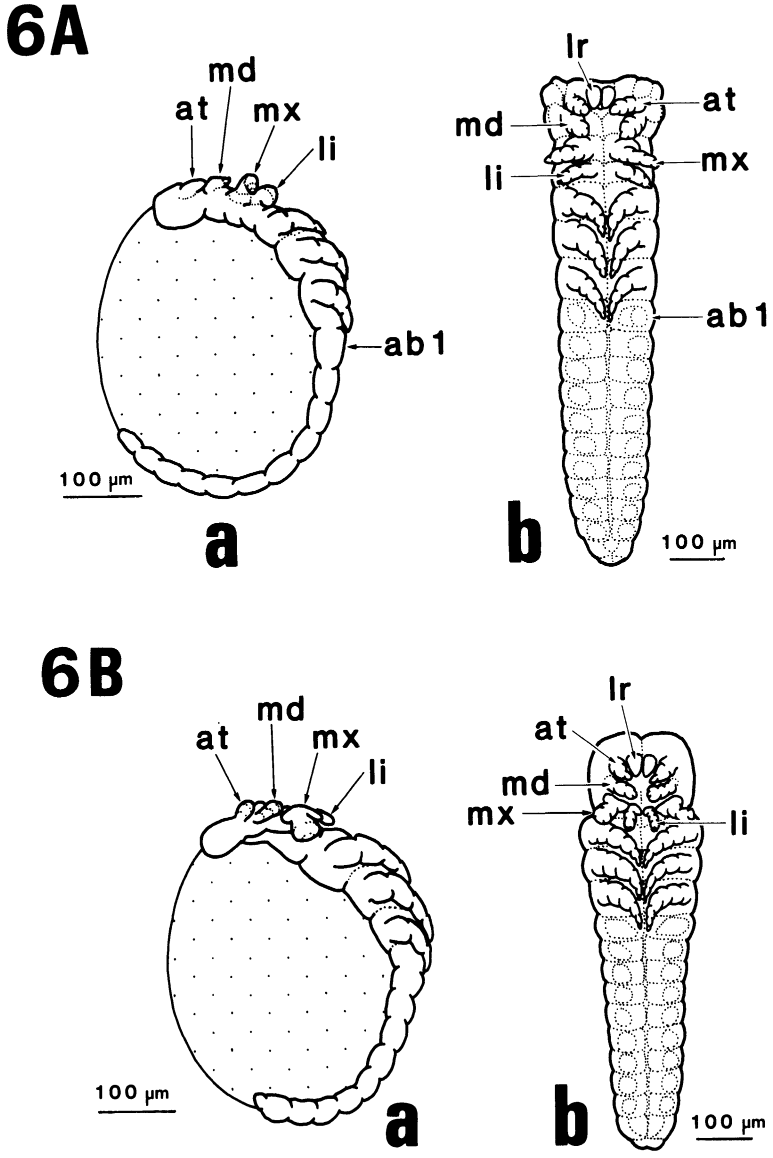

Fig. 6. tion of mouth parts of *Athemus suturellus*. A: about 160 hr, B: about 170 hr. A-a: lateral view, A-b: ventarl view. B-a: lateral view, B-b: ventral view. ab1, first abdominal segment; at, antenna; li, labium; lr, labrum; md, mandible; mx, maxilla.

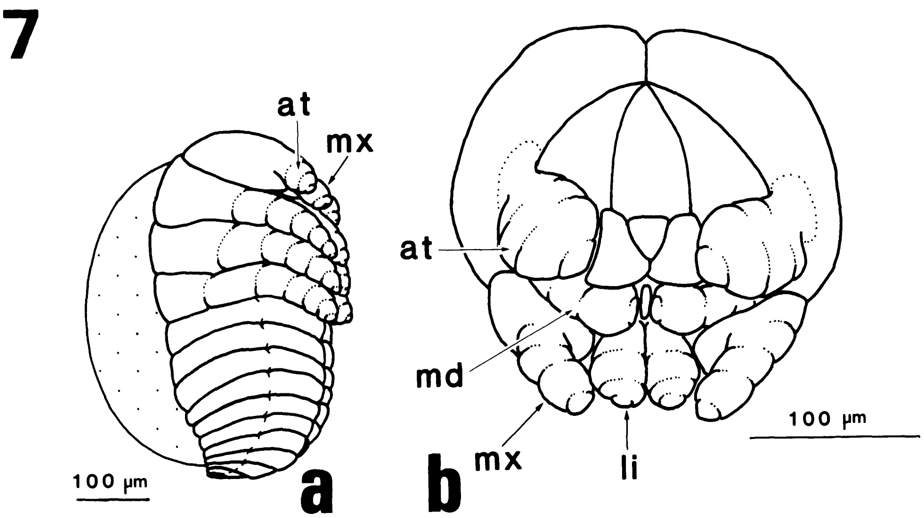

Fig. 7. Pre-dorsal closure in embryo of *Athemus suturellus*, about 180 hr. a: lateral view, b: head. at, antenna; md, mandible; mx, maxilla; li, labium.

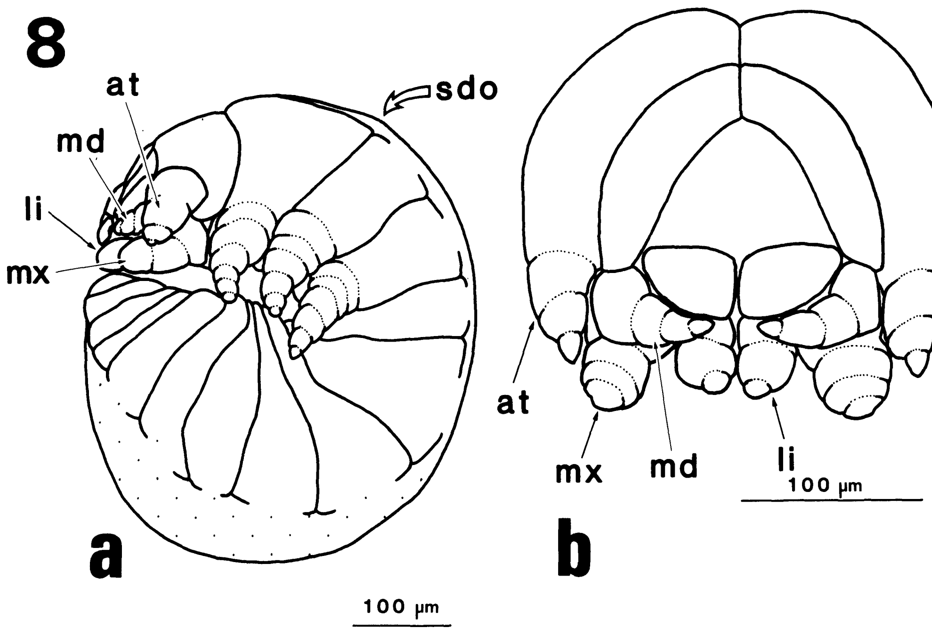

Fig. 8. Near completion of dorsal closure in embryo of *Athemus suturellus*, about 200 hr. a: lateral view, b: head. at, antenna; md, mandible; mx, maxilla; li, labium; sdo, secondary dorsal organ.

posterior pole of the egg, and the revolution of the embryo finished. Several hours after the revolution the dorsal closure of embryo progresses (Fig. 7). During this time, a spindle-shaped secondary dorsal organ is formed in the dorsad of the embryo. The coronal and frontal sutures appear, and the clypeus and gena are distinguished.

In about 190 to 195 hours, the embryo begins to lengthen again, and its caudal end pushes forward along the ventral surface of the egg, and as a result of this growth, the curvature of the embryo is reversed. The secondary dorsal organ becomes a discoidal form.

9. *Embryo after dorsal closure*

The coronal and frontal sutures become distinct, and the frons, clypeus and gena acquire their definitive forms. The tip of mandible becomes pointed, the maxilla turns to the outside, the labrum retracts inside the mandibles, and the antennae move more laterally. Consequently, the mouth parts of the first instar larva are completed (Fig. 8).

The labrum, clypeus and frons meet one another more compactly, and the labrum becomes to be not visible from the outside.

10. *Hatching*

After the accomplishment of the dorsal closure, the young larva rends the chorion in the site between the head and the abdominal end, and hatches out at about 10 days after oviposition (Fig. 9). The first instar larva, which is milky in color, is about 1 mm or more in length. It has some microtrichiae on the thorax and the abdomen, except the head, and a row of pili presents in the proximal part of each segment.

Discussion

The germ bands of insects are generally divided into three categories, that is, the superficial, invaginated and immersed. In Coleoptera the germ bands are generally formed on the ventral surface of the egg, and there the further development also progresses. Therefore they belong to the superficial type, for instance, as in *Hydrophilus piceus* (Heider, 1889), *Lytta viridana* (Rempel and Church, 1969), *Epilachna vigintioctomaculata* (Miya and Abe, 1966) and so on.

The germ band of *Athemus suturellus* also belongs to this type, and the similar condition is found in other members of Cantharidae, *Themus cyanipennis*, *T. midas* and *Prothemus ciusianus* (Fujiwara, unpublished).

In Lampyridae (Ando and Kobayashi, 1975; Kobayashi and Ando, 1985), however, one of the families of Cantharoidea, a ball-shaped germ rudiment is formed and then sinks deeply into the yolk, and the following embryonic development proceeds within the yolk. Consequently, it belongs to the so-called "immersed type" of germ band which is the rare case in Coleoptera. As to the phylogeny of cantharoidean families, Crowson (1955) said that larvae of Lampyridae are the most primitive from the

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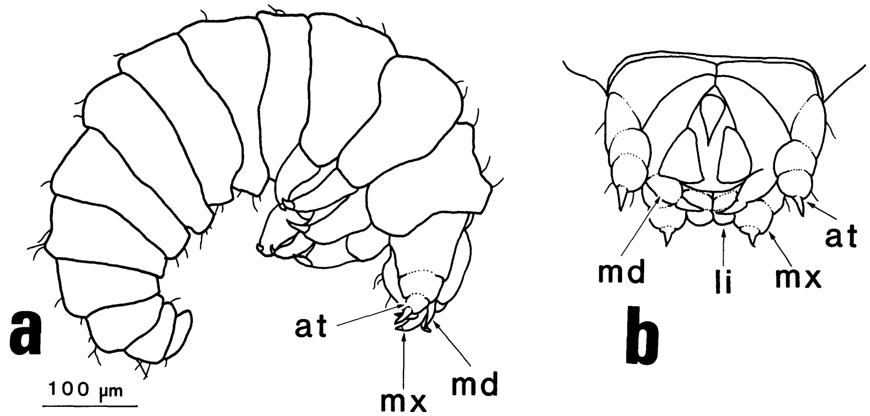

Fig. 9. First instar larva of *Athemus suturellus*. a: lateral view, b: head. at, antenna; md, mandible; mx, maxilla; li, labium.

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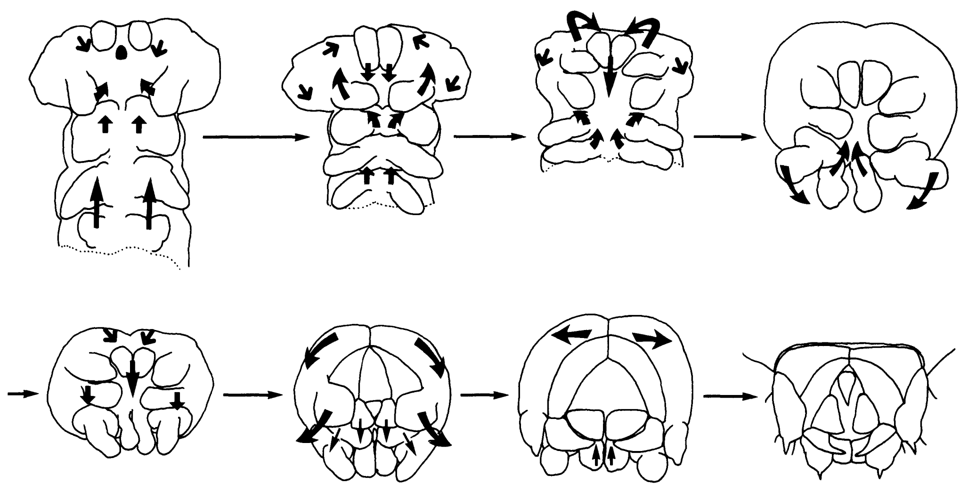

Fig. 10. Diagrams of head formation of embryo of *Athemus suturellus*.

comparative morphology, on the contrary, Lycidae and Cantharidae are the most advanced. According to him, Lampyridae has the most primitive larvae in Cantharoidea, but it has very specialized germ bands. In contrast, *A. suturellus* has the most specialized larvae in the same superfamily, but it has a general type of germ band, namely superficial one thought of more fundamental type in Coleoptera and other holometabolan orders.

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