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External Features of *Sialis mitsuhashii* Embryo through Development (Megaloptera, Sialidae)

Hiroshi ANDO
Kozo MIYAKAWA
and
Satoru SHIMIZU

Synopsis

Changes in external features of *Sialis mitsuhashii* embryo during the development observed with light and scanning electron microscopes were described. The successive formation of mouth-parts is shown in photographs and the external features of developing embryos in *S. mitsuhashii* and *Protohermes grandis* (Corydalidae) were compared and the primitive features of them were discussed.

Introduction

Megaloptera has been considered as the most primitive order of holometabolous insects because the fossil records of them were known as early as at the Permian period. It is likely that the common ancestor of Megaloptera which diverged at the Upper Carboniferous period gave rise Mecoptera, Rhaphidioptera and Neuroptera. The modern Megaloptera (or super-family Sialoidea) consists of two families, Sialidae and Corydalidae. Observation on the life-history of the Megaloptera exhibits that this insect group maintains the most primitive features of endopterygote insects: they are; 1. the larvae are aquatic; 2. the imagoes are incapable of dispersal far from the larval habitat; 3. the eggs are deposited on leaves or rocks above the water as a batch consisted of many hundred eggs; 4. the pupae are exarate and decticous, and have a good resemblance to adults.

The embryological features of the Megaloptera were hitherto studied for three species re-ferable to two families; Sialidae, *Sialis lutaria* L. – general embryology with special re-ference to histological feature (Strindberg, 1915), – experimental embryology with statements of normal embryogenesis (DuBois, 1938), *S. mitsuhashii* Okamoto –early embryogenesis (Suzuki, Shimizu and Ando, 1981), –oogenesis (Matsuzaki and Ando, 1977); Corydalidae, *Protohermes grandis* Thunberg –changes of external features of the embryos during development (Miyakawa, 1979), –homology of abdominal appendages (Miyakawa, 1980). Nevertheless, the amount of embryological informations for this order is poor as compared to other insect orders.

This paper aims to provide the changes of external features in the embryos of *Sialis mitsuhashii* Okamoto during the development, as observed with both the light and scanning electron microscopes, and to give a comparison with those in the embryos of *Protohermes grandis* Thunberg.

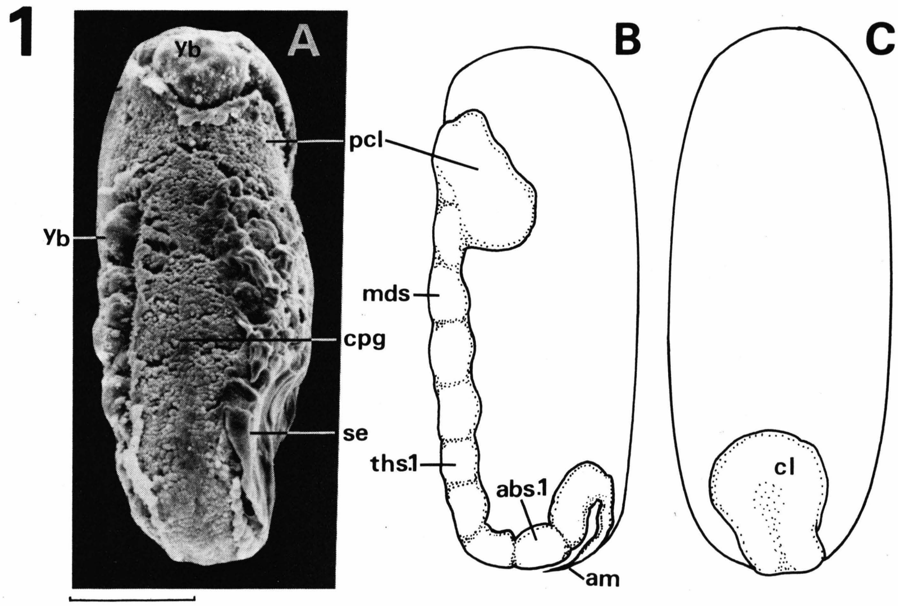
Material and Methods

Adults and eggs of *Sialis mitsuhashii* Okamoto were collected at the Goshiki Lake on the foot of Mt. Bandai, Fukushima Prefecture of Japan in mid-June, 1982. The external forms of early embryos were described in previous paper (Suzuki et al., 1981). For scanning electron microscopy, after removal of chorion and embryonic envelopes, the fixed embryos were dehydrated with an ethylalcohol isoamyl acetate series, dried by critical point drying method, and coated with gold. Observation was done under the scanning electron microscope, JSM T-200 of JEOL.

Observations

Two-day old. (Fig. 1) The protocephalon and protocorm differentiate in the embryo covered ventrally with the amnion. The primitive groove becomes more conspicuous and then it is closed. The protocorm increases in length and decreases in width, exhibiting a sign of the metamerization, *viz.* four gnathal, three thoracic, and two abdominal segments with a large caudal lobe, and the caudal end attains to the egg dorsal side over the posterior pole of the egg. The yolk cleavage is clearly observed around the germ band.

Three-day old. (Fig. 2) The embryo elongates further due to the longitudinal growth of the abdomen. The abdomen turns dorsally at the posterior pole of the egg and its tip reaches the level at about one-third of the egg length from the anterior pole. Metamerization of the embryo becomes distinct except at the posterior portion of the abdomen. In the protocephalon there are the primordia of the labrum, antennae and the stomodaeal pit. The rudiment of the labrum is at first one-lobed. In the gnathal region rudiments of mandible, maxillae and labia are also formed as paired swellings. No clear boundary is detected between the narrow intercalary segment and mandibular one. Thoracic segments become somewhat broader than the gnathal ones and bear medioposteriorly pointed small leg buds. The abdominal part is narrow and long which consists of eleven segments each of which having nearly the same length, but the width is as much the half as that of the thoracic ones. No abdominal swellings are observed at this stage. The size of the cells composed of the embryo becomes different in this stage.



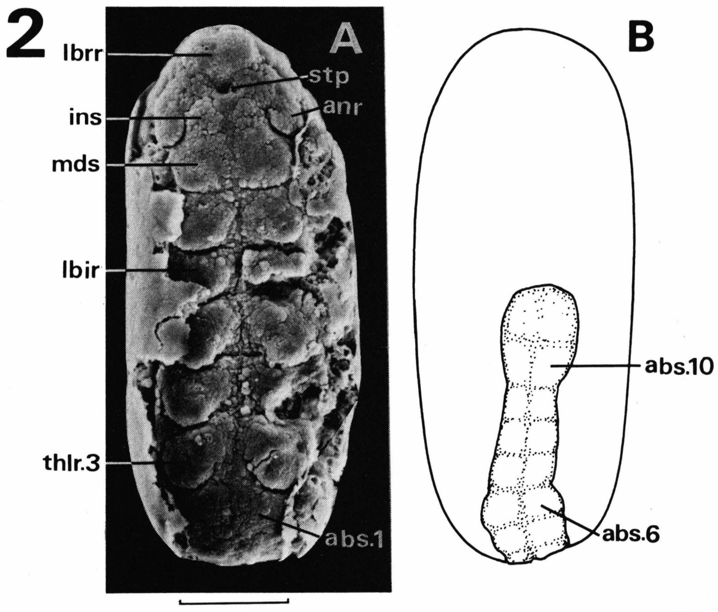
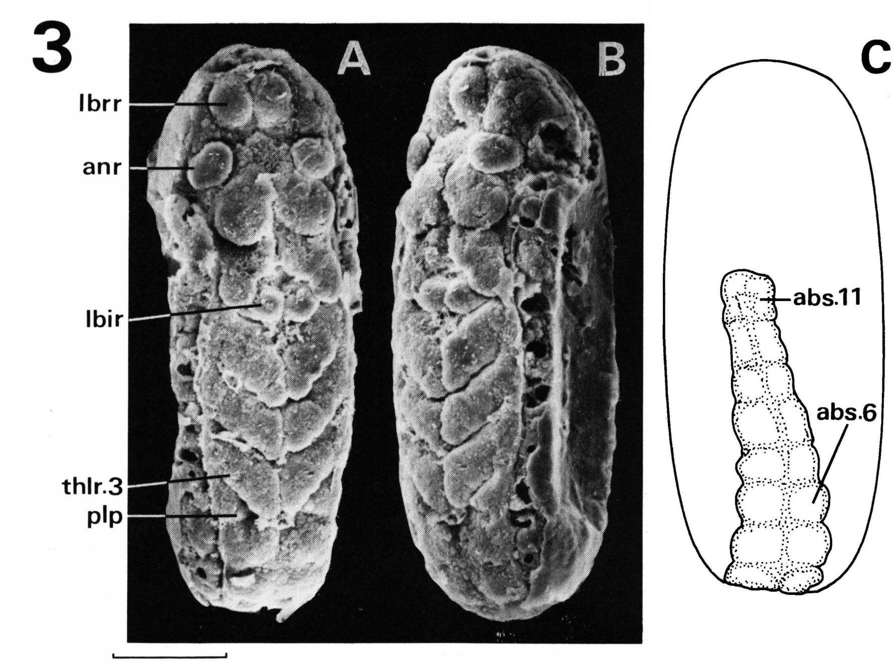


Fig. 1. Embryo (2 days old) A. Ventral view, B. Lateral view, C. Dorsal view. Scale: 100μm

Fig. 2. Embryo (3 days old) A. Ventral view, B. Dorsal view. Scale: 100μm

abs. 1, 6, 10 1st, 6th, 10th abdominal segments, am amnion, anr antennal rudiment, cl caudal lobe, cpg closed primitive groove, ins intercalary segment, lbir labial rudiment, lbrr labral rudiment, mds mandibular segment, pcl protocephalic lobe, se serosa, stp stomodaeal pit, thlr.3 rudiment of 3rd thoracic leg, ths.1 1st thoracic segment, yb yolk block.



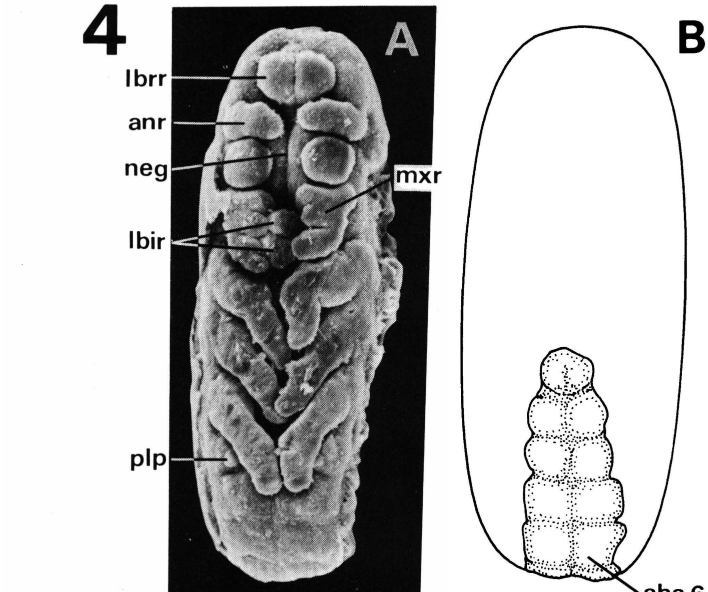


Fig. 3. Embryo (4 days old) A. Ventral view, B. Nearly lateral view, C. Dorsal view. Scale: 100μm

Fig. 4. Embryo (5 days old) A. Ventral view, B. Dorsal view. Scale: 100μm

abs. 6, 11 6th, 11th abdominal segments, anr antennal rudiment, lbir labial rudiment, lbrr labral rudiment, mxr maxillary rudiment, neg neural groove, plp pleuropodium, thlr.3 rudiment of 3rd thoracic segment.

Four-day old. (Fig. 3) The entire length of the embryo reaches at its maximum in the pre-revolution period, occupying about seven-eighths along the long perimeter of the egg.

The rudiments of appendages are developing. The labral rudiment now changes into bilobed. The antennal and mandibular ones become prominent. The maxillary ones have two developing points, the distal and medio-basal, respectively. The paired labial ones are observed between the distal points of maxillae. The rudiments of thoracic legs are three-segmented and grow medioposteriorly. In the first abdominal segment, the pleuropodia appear as a pair of tiny lobes. Segmentation of the abdomen becomes complete and ten segments are counted. The neural ridge is not so clear. The invagination of the stomodaeum becomes pronounced. The cells composed of the surface of rudimental appendages become smaller in size and smooth in appearance.

Five-day old. (Fig. 4) The embryo begins to increase in width and decreases in length. Forward movement of the labial segment begins.

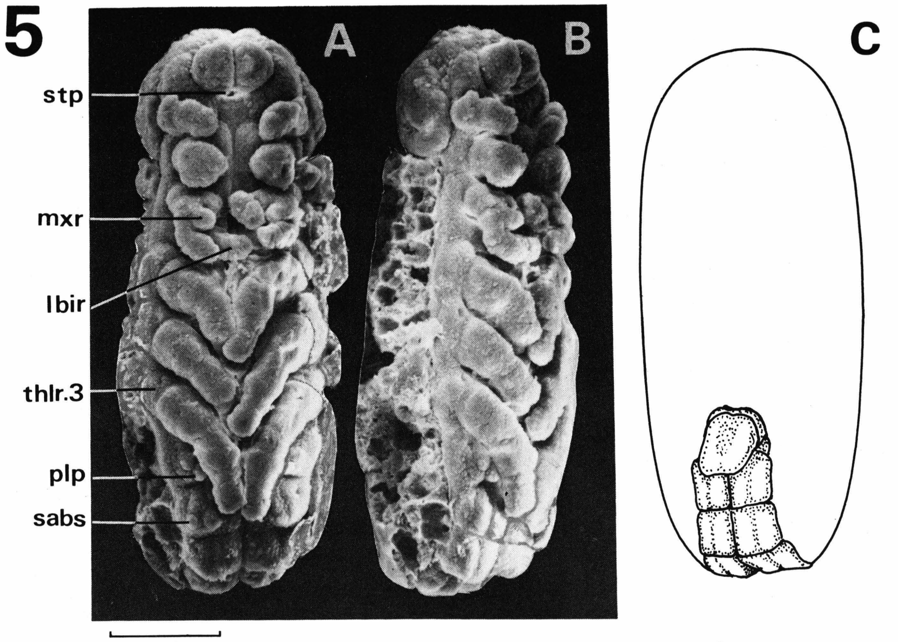
The antennal rudiments become three-segmented. The maxillary ones which had two points in the previous stage now have three developing points. These are rudiments of, from the base, the lacinia, galea and the maxillary palpus. Apices on the paired labial rudiments become to be located anteroposteriorly on the midline between the maxillary rudiments, due to narrowness of the space surrounded by the latter. The thoracic leg rudiments become three-segmented and their distal ends reach the following segment. The neural ridge becomes deep except in the posterior portion of the abdomen.

Six-day old. (Fig. 5) The embryo widens especially in the thoracic and first seven abdominal segments laid on the ventral side of the egg, and the eighth abdominal one is located at the posterior pole, and the ninth and tenth segments remain on the dorsal side of the egg. Morphogenetic movement proceeds in the gnathal region.

The bases of the labial rudiments move forward along the midline and reach the level of maxillary ones. Along with this morphogenetic movement the labial segment loses its territory from the body sides.

Spiracular pits appear in the meso- and metathoracic segments, and three pairs of swellings are observed outside the ganglionic area of each abdominal segment. The one is located medially to the spiracle, whereas the other two just posterior to the former and on the posterior border of a segment. In the first abdominal segment, the median one of the posterior swellings is the pleuropodium and the anterior and lateral ones appear later. In the second abdominal segment these three swellings appear simultaneously. In the following abdominal segments, a pair of a swelling appears at the site just medially to the tracheal pit, which later develops into the abdominal filament or gill. This swelling is serially homologous with two lateral (anterior and posterior) swellings of the first two abdominal segments, and quite resembles that found in corydalid *Protohermes grandis* embryo (Miyakawa, 1979).

Seven to eight-day old. (Fig. 6a, b) Early revolution stage. The embryo increases in width to cover more than half of the ventral side of the egg, and decreases in length with a ventralward turn of the abdominal end. The cephalic lobes grow and their distal ends extend posteriorly to the posterior end of the gnathal region, thus forming the basic shape of the head as a union of the cephalo-gnathal regions. The last two (ninth and tenth) abdominal segments at first turn to the ventral side of the egg with the completion of dorsal close-



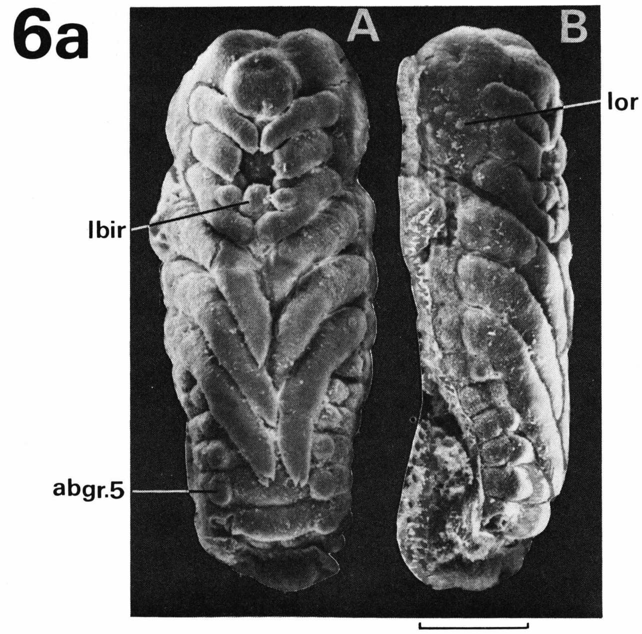
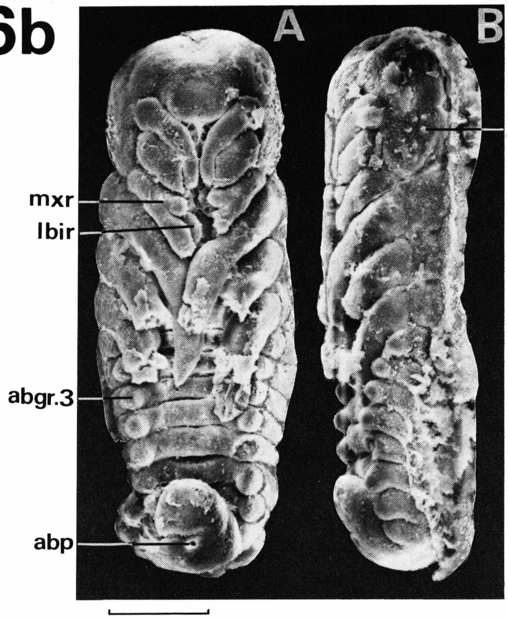


Fig. 5. Embryo (6 days old) A. Ventral view, B. Nearly lateral view, C. Dorsal view. Scale: 100μm

Fig. 6a. Embryo (7 days old) A. Dorsal view, B. Lateral view. Scale: 100μm

abgr.5 5th abdominal gill rudiment, lbir labial rudiment, lor rudiment of lateral ocelli, mxr maxillary rudiment, plp pleuropodium, sabs swelling of abdominal segment, stp stomodaeal pit, thlr.3 rudiment of 3rd thoracic leg.

6b



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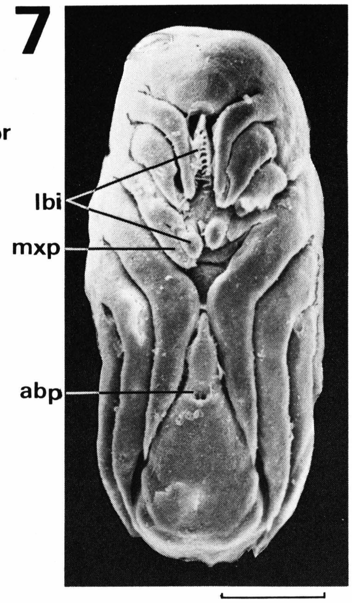

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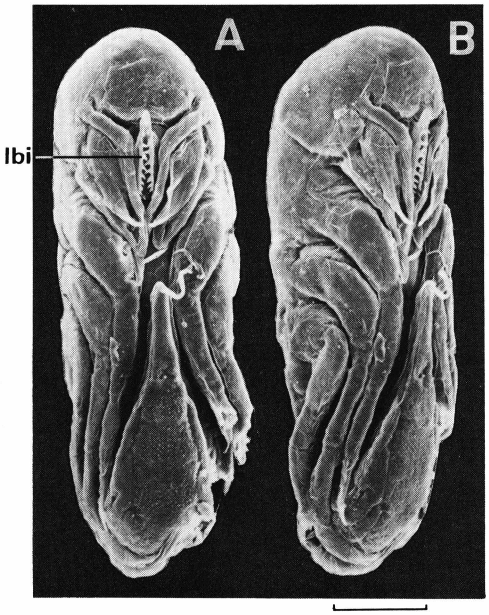

Fig. 6b. Embryo (8 days old) A. Ventral view, B. Lateral view. Scale: 100μm

Fig. 7. Embryo (9 days old) Ventral view. Scale: 100μm

Fig. 8. Embryo (10 days old) A. Ventral view, B. Nearly lateral view. Scale: 100μm

abdr.3 3rd abdominal gill rudiment, abp pore of abdominal end, lbi labium, lbir labial rudiment, lor rudiment of lateral ocellus, mxr maxillary rudiment, mxp maxillarypalp.

ure in this portion. As development proceeds, the eighth abdominal segment also turns ventrally at the posterior end of the egg. In this area the yolk mass still remains and the dorsal closure is postponed at this period.

The labral rudiment remains of paired condition. All rudiments of appendages in seven days embryos increases in size; *e. g.* the maxillary ones extend to the prothoracic segment, the rudiments of prothoracic legs to the first abdominal segment, those of mesothoracic legs to the third abdominal, and those of metathoracic legs to the fifth abdominal segment. Among three abdominal swellings appeared in the previous day, the posterolateral ones (just posteromedial to the tracheal pit) are seen in the first seven abdominal segments as conspicuous lobes; They are the rudiments of abdominal filaments. The pleuropodia and corresponding swellings observed from the second to seventh abdominal segments at the position just lateral to the ganglionic region do not develop further, and the anterior swellings disappear or have been incorporated into the growing posterolateral lobes.

The rudiments of lateral ocelli appear as seven tiny protrusions on the distal portion of the cephalic lobes at the level of mandibular and maxillary segments. These are the first sign of seven ocelli distributed in two rows, three in the dorsal side and four in the ventral (to the longitudinal body axis). The pit of abdominal end becomes observable.

Nine-day old. (Fig. 7) Late revolution stage. The embryo expands laterally to envelop the yolk on its dorsum. This development is accompanied with a rapid consumption of considerable amount of yolk and the longitudinal expansion of the abdominal part. The abdomen increases five-thirds times in length during this development, whereas increase in length of the head and thorax is not so significant. The abdomen turns ventrally as its seventh segment localizing at the posterior pole of the egg, the tenth segment arriving at the level of the first abdominal one, and the tip of the caudal projection reaches the mesothoracic one. Thus, the revolution of the embryo is completed. The antennal rudiments become three-segmented, and the mandibular rudiments become bifurcated. The developing thoracic legs become four-segmented, which consist of the coxa, femur, tibia and tarsus.

On the midline of the gnathal region a forceps-shaped structure (in ventral view) which lies longitudinally and bilaterally just posterior to the labral rudiment and between the antennal ones. The joint of this forceps is located anteriorly and ventral edges of the structure bear nine to ten dentations. This structure is originated from the anterior part of the labial rudiments.

Ten-day old. (Fig. 8) Completion of the dorsal closure. The posture of the embryo is approximately the same as in the previous stage. The whole embryo undergoes further extension and development of the appendages. The mouth parts unite more compactly and their apical portions become sharply pointed and sclerotization proceeds. Each thoracic leg elongates further and bears setae at the coxa and the femur. The tarsal claws are sclerotized.

Eleven-day old. (Fig. 9) The embryo becomes full grown and hatches out from the ventral surface of the egg-shell.

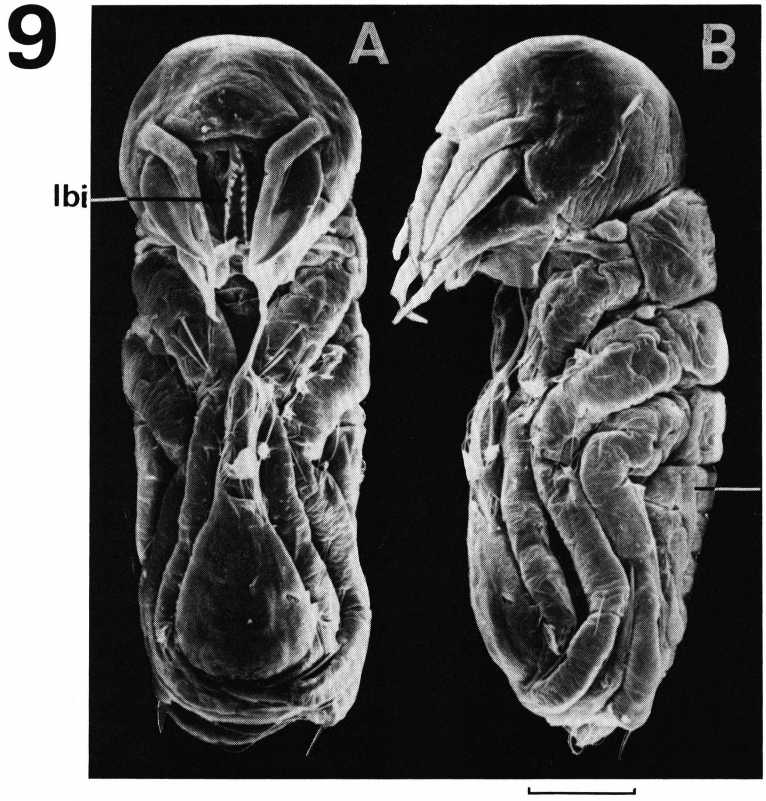

Fig. 9. Embryo (11 days old) A. Ventral view, B. Lateral view. Scale: 100μm
abg.1 1st abdominal gill, lbi labium.

Discussion

In many holometabolous orders, the germ disc is formed by aggregation of the blastoderm cells along the ventral midline of the egg, and the embryonic rudiment remains over the yolk surface without sinking into the yolk. The same is true for the megalopteran embryos except that they have an exceedingly broad germ disc or ventral plate (Suzuki et al., 1981).

One of the conspicuous features of the megalopteran embryos is seen in the stage at about beginning of the tracheal invagination in *Sialis mitsuhashii* (Fig. 5) and *Protohermes grandis* (Miyakawa, 1979, Fig. 5). Both embryos, at this stage, exhibit two or three swellings at either side of abdominal segments, the most lateral one of which gives rise to the tracheal gill or abdominal filament, whereas other swellings are vestigial and degenerate later. Similar swellings were found in the abdominal segments of the embryos at the corresponding stages of neuropteran *Ascalaphus ramburi* (Kamiya and Ando, 1984) and mecopteran *Panorpa* and *Bittacus* (Suzuki, 1982; Ando and Miyakawa, unpublished) among the holometabolous orders. On the other hand, similar lobes were recently found in embryos at the corresponding stage of lower insect orders: Thysanura (*Pedetontus,* Machida, 1981), Odonata (*Euphaea,* Norling, 1982), Dermaptera (*Anisolabis,* Fuse and Ando, 1983). These embryonic abdominal swellings or lobes are found widely in the primitive forms of the lower insect orders. From these evidences, authors believe that the megalopteran embryos exhibit a primitive feature among those of holometabolous orders.

Turning to comparison of the embryos between Sialidae and Corydalidae within the order Megaloptera, some differences are observed. At the stage when the protocephalon and protocorm are differentiated, the embryonic area relative to the total egg surface and that of protocephalon relative to the total embryo are larger in Sialidae than in Corydalidae. In *S. mitsuhashii* and *S. lutaria* (DuBois, 1938) embryos, the cephalic lobes extend posterolaterally and reach the level of the mandibular segment, whereas in *P. grandis* embryo, the cephalic lobes extend anterolaterally and do not reach the mandibular segment. This difference of initial state of the cephalic lobes is maintained also in the later developmental stages. The other differences are found in the labium and anal appendages of both species. These structure become definitive at the late embryogenesis when the revolution and the dorsal closure of the embryo take place. The outer part of labium becomes conspicuous in *S. mitsuhashii* as a pair of comb-like structures (or dentate ligulae) which covers on the midline of mouth-parts throughout, whereas it does not develop in *P. grandis.* The anal lobes develop in sialid embryos as a non-paired extension, *i. e.,* the terminal filament, whereas those in corydalid embryos develop into a pair of pygopods with sclerotized distal claws. These differences of the labium and the anal appendages between Sialidae and Corydalidae, however, are of postembryonic characters adaptive to the environment in their larval lives.

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Authors' address: Prof. H. Ando & Mr. S. Shimizu,
Sugadaira Montane Research Center,
University of Tsukuba, Sanada, Nagano
386-22, Japan

Dr. K. Miyakawa,
1024, Imafuku, Kawagoe 356, Japan