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A Preliminary Note on the Embryonic Development of a Diving Beetle, *Hydaticus pacificus* Aubé (Insecta: Coleoptera, Dytiscidae)*

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The Coleoptera, the largest order in Insecta, is composed of four suborders, Archostemata, Myxophaga, Adephaga, and Polyphaga. In spite of many phylogenetical studies, the intersubordinal relationships are still controversial (Beutel, 2005). A comparative embryological approach is one of the useful methods for phylogenetic discussion, but most of the embryological studies of Coleoptera have been concentrated on the largest suborder Polyphaga occupying about 90% species of this order. However, as for the other three suborders, embryological information is old and fragmental. In the aquatic adephagan family Dytiscidae, the embryogenesis of *Dytiscus marginalis* was studied about 100 years ago (Korschelt, 1912; Blunck, 1914). The full grown embryo of another dytiscid species *Agabus bipustulatus* and its hatching process were then described by Jackson (1957, 1958). In the terrestrial adephagan family Carabidae, the external morphology of the *Carabus nemoralis* embryos was fragmentally described by Kemner (1918). In the light of such insufficient embryological knowledge of Adephaga, we have started an embryological study of this suborder focusing on *Carabus insulicola* (Carabidae), *Dineutus mellyi* (Gyrinidae), and *Hydaticus pacificus* (Dytiscidae). In this paper, we report the outline of the embryonic development of *H. pacificus* with emphasis on middle and late stages by external observation.

Eggs of *H. pacificus* were deposited on water grasses by breeding females in a laboratory. Progenitors of the females were captured at several places in Fukushima Prefecture, Japan. The egg period was about 50 hours under the condition of 28℃. The eggs were fixed with alcoholic Bouin's fixative in every 1 to 3 hours. To observe the external form of developing embryos, egg shells (chorion and serosal cuticle) and embryonic membranes (serosa and amnion) were removed from the egg by fine needles. The observation of embryos was made using a stereoscopic microscope.

The newly laid egg is nearly cylindrical with a slight convexity at the ventral side. It is about 2.20 mm long and 0.68 mm wide. As development proceeds, the egg becomes 2.70 mm long and 0.85 mm wide in maximum. The chorion is smooth and thin, and almost transparent throughout the whole egg surface.

For the convenience of description, the developmental process of *H. pacificus* was divided into 10 stages; stages 1 to 3 (early stages), stages 4 to 7 (middle stages), and stages 8 to 10 (late stages). Maturation division, fertilization, cleavage, and the formation of the blastoderm are assumed to occur in early stages (1–3 hours after oviposition, a.o.), but the histological description of these stages will be given in a future paper. External features of embryos in stages 4 to 10 are as follows.

In stage 4 (4–6 hr, a.o.), a long germ band, occupying about four fifths of the egg length, is formed on the ventral side of the egg. In this stage, the amnion and serosa are formed by fusion of amnio-serosal folds at the ventral side of the germ band. In the late of this stage, the germ band differentiates into a bi-lobed protocephalon and a slender protocorm.

In stage 5 (6–12 hr, a.o.), the posterior end of the germ band elongates towards the dorsal side of the egg, thus the germ band assumes nearly J-shaped in a lateral view. At the posterior end of the protocephalon, antennary rudiments appear as a pair of minute projections. At the anterior half of the protocorm, segmentation of future gnathal and thoracic regions occurs; that is, mandibular, maxillary, and labial segments appear in the former region, and three thoracic segments in the latter. Late in this stage, segmentation also begins

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in the future abdominal region, and a pair of minute projections, or pleuropodia, appears in the first abdominal segment. Near the anterior end of the protocephalon, a stomodaeum appears as a shallow invagination in the latter half of this stage. Although the formation of a primitive groove is assumed to occur at the previous stage, the groove is observed along the ventral midline of the protocorm at the beginning of this stage, but it disappears by the end of this stage.

In stage 6 (12–19 hr, a.o), the germ band widens, but its length somewhat contracts, so that the abdominal end becomes situated just at the posterior pole of the egg. Abdominal segmentation completes and this region becomes 10-segmented. The labral rudiment appears as a large bi-lobed projection just anterior to the stomodeaum. In the gnathal segments, mandibular appendages appear as a pair of globose lobes (coxopodites), but maxillary and labial ones are formed as a pair of two-segmented projections composed of coxopodites and telopodites (palpi). In thoracic segments, their appendages are formed as thick projections developing postero-medially. In the first abdominal segment, pleuropodia first develop into globose projections, but later they become flat and cup-shaped. In the mesothoracic and metathoracic segments and the first eight abdominal segments, spiracular invaginations appear on both sides of each segment. A neural groove appears along the ventral midline of the germ band. A small proctodaeal invagination is observed at the abdominal end.

In stage 7 (19–24 hr, a.o.), the integration of the cephalognathal region advances. That is, the labral rudiment becomes flat and extends posteriorly, and the labial lobes on both sides shift medially and fuse each other to form a labium. The mandibular lobes slightly extend postero-medially, and the maxillary and labial palpi further elongate. The thoracic appendages also further elongate and their tips attain to the ventral midline. By the end of this stage, in the mesothorax, metathorax, and the first seven abdominal segments, the openings of the spiracles close and disappear, but the openings in the eighth abdominal segment remain as spiracles. The neural groove also closes and disappears. At the abdominal end the ninth and 10th abdominal segments fuse, and thus the abdominal region consists of nine segments.

In stage 8 (24–33 hr, a.o.), it is assumed that the revolution of the germ band occurs in the middle of this stage (27 hr, a.o.), but the detailed observation of this process has been missed in this study. After revolution, the abdominal end flexes ventrally and extends anteriorwards. At the abdominal end, urogomphi appear as a pair of slender projections. The integration of the cephalognathal region further proceeds, and thus the basic form of the larval head is completed. That is, the antennae, maxillary and labial palpi elongate posteriorly, and the mandibles also elongate and become sickle-shaped. The fused labium shifts anteriorly between the maxillae and almost becomes in contact with the labrum. The thoracic appendages also extremely elongate posteriorly and the tips of the meso and metathoracic appendages almost attain to the posterior end of the egg. By the end of this stage, lateral walls of the germ band extend dorsally and fuse each other; thus dorsal closure completes.

In stage 9 (33–45 hr, a.o.), the segmentation of appendages excluding mandibles completes; that is, the antennae, maxillary and labial palpi are three-segmented, and the thoracic appendages become five-segmented. The labrum becomes flat and wide.

In stage 10 (45–50 hr, a.o.), the first instar larva completes and hatching occurs at 50 hr, a.o.. The medial part of the mandibular distal half assumes dark brown. In lateral view, a thick lateral tracheal trunk running from the prothoracic to the eighth abdominal segments is observed as a dark brown band through the body wall. The pleuropodia gradually degenerate and disappear by the time of hatching. At the top of the head capsule, a pair of brown sclerotized spines, or egg teeth, appears. The spines remain at the head of the hatched larva.

The germ band of *H. pacificus* belongs to the superficial type as observed in several polyphagan Coleoptera, for example, *Hydrophilus piceus* (Heider, 1889) and *Lytta viridana* (Rempel and Church, 1969). The change of the external shape of the developing embryos in *H. pacificus* is fundamentally the same as that observed in *Dytiscus marginalis* (Korschelt, 1912; Blunck, 1914).

The formation of the secondary dorsal organ, which is confirmed in *Dytiscus marginalis* (Korschelt, 1912), and the developmental origin of urogomphi have not been observed in detail in the present study. These processes together with those of early development are the subjects to be observed in the future.

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