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Embryonic Development of a Whirligig Beetle, *Dineutus mellyi* Régimbart: External Features of the Egg and Developing Embryos (Insecta: Coleoptera, Gyrinidae)*

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The order Coleoptera is composed of four suborders, Archostemata, Myxophaga, Adephaga, and Polyphaga. In spite of many phylogenetical analyses, there are several competing hypotheses regarding inter or intra-subordinal relationships (Beutel, 2005). A comparative embryological approach is one of the useful methods for discussing the phylogeny of higher taxon levels such as suborders or superfamilies. The embryological studies of Coleoptera, however, have been concentrated on the largest suborder Polyphaga occupying about 90% of this order, and nothing is known about the embryogenesis of the suborders Archostemata and Myxophaga. In the suborder Adephaga, to which the Gyrinidae belongs, while there are old embryological studies on a diving beetle, *Dytiscus marginalis* (Korschelt, 1912; Blunck, 1914), little is known about the embryogenesis in other adephagan taxa. Then in order to understand the embryological features of Adephaga from the perspective of comparative embryology and phylogeny, we have begun to study embryogenesis of three adephagan species which belong to different families; *Carabus insulicola* (Carabidae), *Dineutus mellyi* (Gyrinidae), and *Hydaticus pacificus* (Dytiscidae). Among these species, the larvae of a whirligig beetle, *Dineutus mellyi*, are unique in having eight pairs of gill projections, or tracheal gills, on the first eight abdominal segments, and two pairs of them on the ninth segment. In this paper, we report the outline of external features of the egg and developing embryos of *Dineutus mellyi*, focusing on the origin of these unique projections.

About 20 females of *Dineutus mellyi* Régimbart were collected at Ogimi, Okinawa Prefecture, in February of 2008. The female kept in an aquarium laid 10 to 20 eggs side by side at a time on the surface of waterweeds in water. A total of about 500 eggs were obtained from about 20 females. The egg period was 288–312 h (12–13 days) under the condition of about 22℃.

The newly laid eggs are white, long ellipsoid, and 2.3×0.65 mm in size. As development proceeds, the egg size increases to 2.4×0.9 mm just before hatching. The eggs loosely adhere on their dorsal surface to the substrate by mucous substances.

The chorion is about 20 μm in thickness, and with numerous minute hill-shaped projections all over the egg surface. After 6 to 7 days after oviposition, however, the chorion is widely cleaved along the split line which runs at the ventral midline of the egg; thus the serosal cuticle, which has been formed beneath the chorion by this time, becomes exposed. The split line before cleavage can be detected in the newly laid eggs as a faint line in which the arrangement of hill-shaped projections is irregular and discontinuous: the interior of each projection is hollow, so that the surface of the projections is often depressed in the hollow space.

At the anterior end of the egg, the surface is slightly sunken and the hill-shaped projections of the chorion are smaller in size. At the center of the dent, there is a cylindrical process of about 40 μm in height and 10 μm in diameter at its proximal part. This process is distally enlarged like a lotus pip, and many minute pores are scattered on the enlarged surface. These pores are probably micropyles, so we call this process the micropylar projection. The micropylar projection and the split line on the chorion have not been observed in other Adephaga and Polyphaga, and may be characteristic of the eggs of the Gyrinidae. It is noteworthy that, in spite of some differences in structural details, the micropylar projection of the Gyrinidae looks similar to the micropylar projections found in Neuropterida (Megaloptera, Rhaphidioptera, and some Neuroptera), which is a proposed sister group of Coleoptera.

The egg period was divided into 11 stages, on the basis of the changes in external embryonic features. As mentioned before, the larvae of the Gyrinidae largely differ from other adephagan larvae in possessing a pair of gill projections on each of the first eight abdominal

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segments, two pairs of gill projections on the ninth abdominal segment, and four terminal hooks at the 10th abdominal segment. Gill projections on the first abdominal segment are formed from a pair of swellings appeared on the lateral sides of pleuropodia. Gill projections on the second to eighth abdominal segments are also formed from paired swellings situated on the positions homologous to those of the first abdominal segment. Of two pairs of gill projections on the ninth abdominal segment, the lateral ones are formed from the swellings homologous to those of other abdominal segments, but another pair of projections (dorsal gill projections) originates from swellings appeared on the dorsal side of lateral projections. Therefore, regarding the pleuropodium on the first abdominal segment as a homologue of the thoracic appendages (Machida, 1981), the gill projections except dorsal gill projections on the ninth abdominal segment are interpreted as projections born on the position homologous to the basal part, or coxopodial part of thoracic appendages. The developmental process of gill projections and the positions where they arise on the abdominal segments are very similar to those observed in the embryogenesis of a megalopteran, *Protohermes grandis* (Miyakawa, 1979).

Tracheal invaginations are formed on the mesothoracic, metathoracic, and the first eight abdominal segments in the early embryos, but their openings disappear completely at middle embryonic stages. The last abdominal segment is formed by fusion of the 10th and 11th abdominal segments. Four terminal hooks on the last abdominal segment originate in four swellings appeared on the posterior end of the 11th abdominal segment, but it is uncertain whether they can be homologized with the gill projections.

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