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Formation of a spherical germ rudiment in the glow-worm, *Rhagophthalmus ohbai* Wittmer (Coleoptera: Rhagophthalmidae), and its phylogenetic implications

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Introduction

Rhagophthalmus ohbai is one of the glow-worms, inhabiting only Iriomote-jima, Ishigaki-jima, and Kohama-jima of the Ryukyu Islands. The females are larviform and luminescent, and breed their egg mass until or just after hatching. Owing to their peculiar shape and habits, Olivier (1910) established the family Rhagophthalmidae for the genus *Rhagophthalmus* and other related genera. Wittmer and Ohba (1994) described this species, according to Olivier's view. McDermott (1964), on the other hand, included these genera among the Lampyridae as a subfamily Rhagophthalminae, but more recent authors treated this subfamily as that of the Phengodidae distributed in both the Old World and New World (Crowson, 1972; Lawrence *et al.*, 2000). A recent molecular phylogenetic study has revealed that *R. ohbai* forms a cluster with *Stenocladius* within the family Lampyridae (Suzuki, 1997). Thus the relationship of this queer insect has yet to be clarified and the systematics of lampyrids in higher level has been confused.

Embryological study on the luminescent beetles has hitherto been restricted to the Lampyridae, such as *Photuris* (Williams, 1916; Hess, 1922), *Photinus* (Williams, 1916), *Lampyris* (Bugnion, 1922), *Luciola* (Ando and Kobayashi, 1975; Kobayashi and Ando, 1985; Kobayashi, 1987), and *Hotaria* (Kobayashi and Ando, 1985; Kobayashi, 1987). We have, however, no information on the embryogenesis of luminescent beetles other than Lampyridae. In the present paper, we describe the embryogenesis of the glow-worm, *R. ohbai*, with special reference to the formation of germ rudiment, in order to evaluate its phylogenetic relationships from the embryological standpoint.

Materials and Methods

Mated females of *Rhagophthalmus ohbai* were collected at Iriomote-jima and Ishigaki-jima, Okinawa Prefecture in December of 1997 to 1999, with the permission of the Environmental Agency of Japan, the Agency for Culture Affairs and the Educational Committee of Okinawa Prefecture to N. Ohba. They were reared in plastic petri dishes (7 by 2 cm) loosely packed with moistened soft paper (Kimwipe, Kimberly-Clark) in order to allow them to oviposit. The laid eggs were transferred to other petri dishes paved with moistened filter paper, and incubated at about 23℃. The egg period was 30 to 34 days under this condition. They were fixed with hot alcoholic Bouin's fluid (about 55℃ for 30 min) every 3 h for the first 36 h after oviposition (a. o.). From 36 h a. o. to 96 h (4 days) a. o. they were fixed every 12 h. From 4 days a. o. onward they were fixed every day or every 2 days.

For histological observation, eggs were dehydrated in a series of ethanol, embedded in paraffin, sectioned at 5 μm, and stained with Delafield's haematoxylin and eosin. For observation of whole embryos by light microscopy, fixed eggs were stained with alcoholic borax carmine after piercing the chorion with fine needles.

Results

The newly laid eggs of *Rhagophthalmus ohbai* are yellowish white, short ellipsoid and 1,085 by 775 μm in size. The egg size increases twice in stages, to 1,125 by 900 μm at 8 days after oviposition, and to 1,150 by 950 μm at 17 days. The eggs are soft and fragile just after oviposition because of their thin chorion, but they become firmer at about 8 to 9 days owing to the formation of serosal cuticle. At the one end of the longitudinal axis of the egg, 11 to 19 micropyles are arranged in a small circle. This area is supposed to correspond to the anterior end of the egg.

Egg cleavage is of typical superficial type, and cleavage nuclei or energids migrate toward the egg periphery. The energids reach the periphery after the ninth division at about 36 h, and they further divide twice there, and thereby a thin blastoderm is formed at about 48 h. The newly formed blastoderm soon differentiates into two areas; the embryonic area composed of small flat cells having one nucleus (Fig. 1A) and the extra-embryonic one composed of large cells having two or four nuclei (Fig. 1B). The cells of the former area then aggregate into the anterior side of the egg, to form a thick circular germ disk at about 72 h (Fig. 1C). All marginal regions of the germ disk soon flex, being accompanied with the extra-embryonic area, to form an amnioserosal fold, whereas the central part of the germ disk begins to sink into the yolk (Fig. 2A). The amnioserosal fold extended from all margins of the germ disk eventually meets and fuses, and a sac-like germ rudiment is formed at the anterior end of the egg at about 3.5 days (Fig. 2B). The extra-embryonic area also meets and fuses, and the serosa covers the whole egg surface. The germ rudiment itself further sinks into the yolk, and becomes spherical, having a large lumen or amniotic cavity at its center (Fig. 2C). At about 5.5 days, the spherical germ rudiment begins to elongate (Fig. 2D), and its inner side becomes thick to develop into a long germ band or embryo at 7 to 8 days, whereas the outer side facing the egg periphery becomes thin to differentiate into an amnion.

The embryo then continues to develop in the submerged condition until about 12 days, and after that time it acquires a completely superficial position until hatching. Details of the development of the embryo including organogenesis will be described in a future paper.

Discussion

Formation of the germ rudiment of fireflies has been reported on *Photuris pennsylvanica* (Williams, 1916), *Luciola cruciata* (Ando and Kobayashi, 1975), *L. lateralis* (Kobayashi and Ando, 1985), and *Hotaria parvula* (Kobayashi and Ando, 1985). In these lampyrid species, a spherical germ rudiment is formed by the invagination of the circular germ disk into the yolk just under the micropylar region (probably the anterior end of egg), and we may accept this type of germ rudiment formation as being representative of the Lampyridae. In these lampyrids, the germ rudiment then becomes a long germ band or embryo in the yolk, and the submerged condition of the embryo persists until just before embryonic revolution. On the other hand, in the leather winged beetle, *Athemus suturellus*, belonging to the Cantharidae which is put in the superfamily Cantharoidea as the Lampyridae is, the germ rudiment forms at the antero-lateral side of the egg surface, and develops into a long germ band *in situ* (Fujiwara and Kobayashi, 1987). In most Coleoptera as in many other holometabolous insects, the germ rudiment or early germ band is formed at the egg surface from the beginning, although in a chrysomelid beetle, *Atrachya menetriesi*, a spherical germ rudiment is formed by the invagination of the germ disk into the yolk near the posterior end (Miya, 1965). Therefore the formation of the submerged spherical germ rudiment of Lampyridae near the anterior end is absolutely unique in coleopteran embryogenesis, and should be regarded as an autapomorphy of this family.

The present results reveal that also in *Rhagophthalmus ohbai* the spherical germ rudiment is formed near the anterior end of the egg after the closure of the amnioserosal fold, and the germ rudiment then develops into a long germ band in the submerged condition. This is the very feature now designated as an autapomorphy of Lampyridae and strongly suggests that *R. ohbai* should be referred to the Lampyridae. A recent molecular datum (Suzuki, 1997) supports this conclusion.

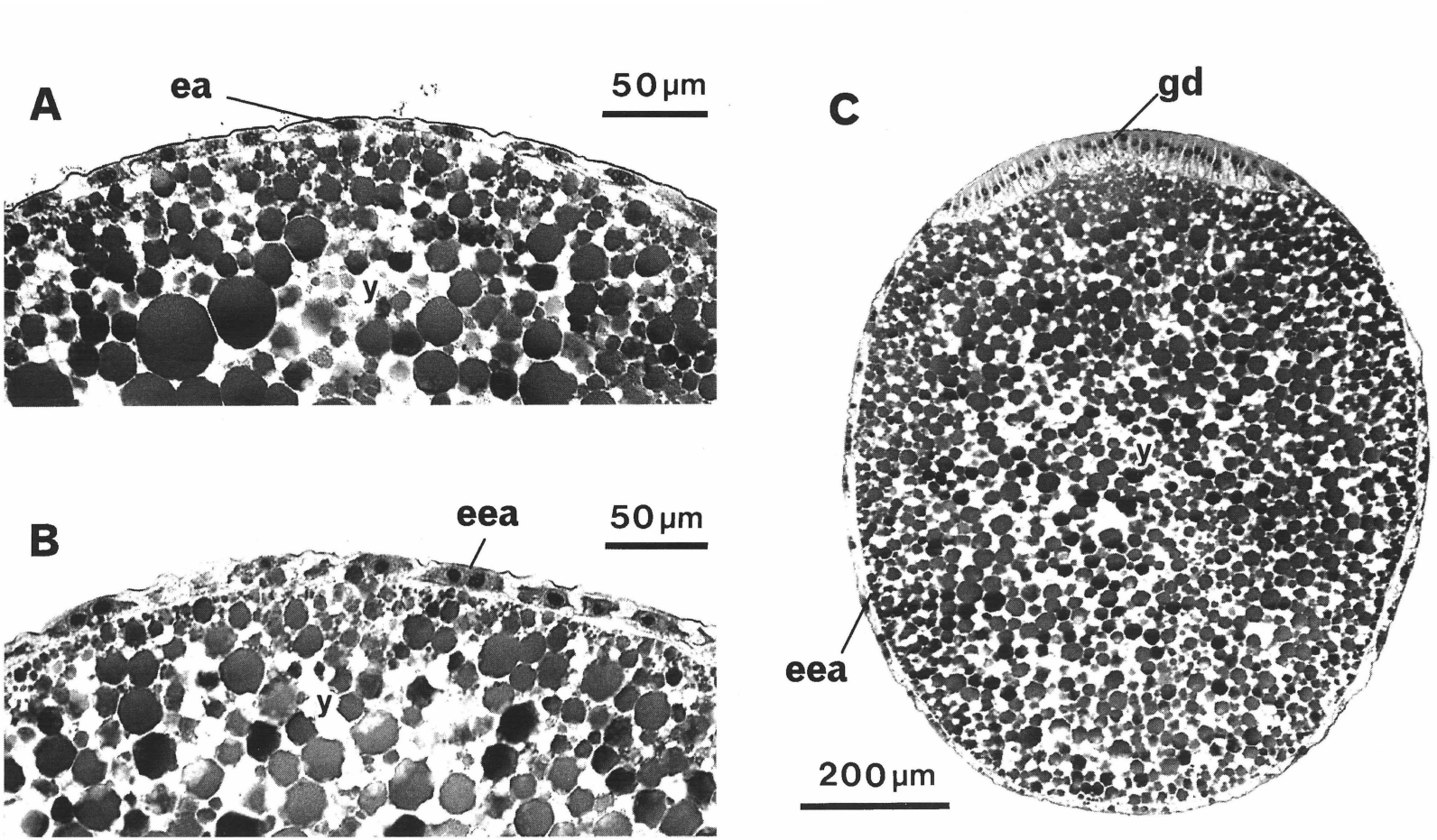

Fig. 1 Longitudinal section of the egg of *Rhagophthalmus ohbai* (chorion removed). A, B. Sections of about 54-h-egg, showing blastoderm cells in the future embryonic (A) and extra-embryonic (B) areas. C. A section of about 72-h-egg, showing the differentiation of the germ disk. ea: embryonic area, eea: extra-embryonic area, gd: germ disk, y: yolk.

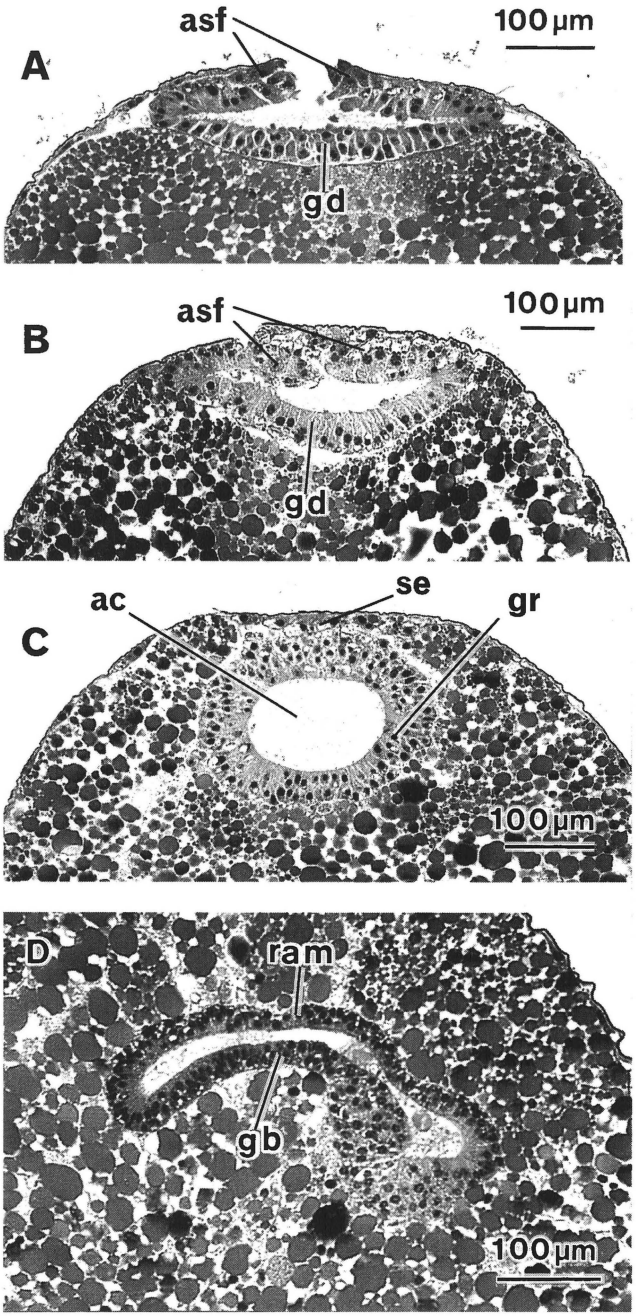

Fig. 2 Longitudinal sections of the egg of *Rhagophthalmus ohbai* (chorion removed), showing the formation of germ rudiment. A. About 3.5-day-egg. B. About 3.5-day-egg. C. About 4-day-egg. D. About 5.5-day-egg. ac: amniotic cavity, asf: amnioserosal fold, gb: germ band, gd: germ disk, gr: germ rudiment, ram: rudimentary amnion, se: serosa.

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