DIVERSITY AND CONSERVATION OF FIREFLIES

PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON DIVERSITY AND CONSERVATION OF FIREFLIES

EDITED BY

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ROYAL INITIATIVE PROJECT ON THE STUDY OF FIREFLIES IN THAILAND
QUEEN SIRIKIT BOTANIC GARDEN (QSBG)

Proceedings of the International Symposium on
Diversity and Conservation of Fireflies
in Commemoration of
HM Queen Sirikit's 76th Birthday Anniversary
held at Queen Sirikit Botanic Garden (QSBG)
Chiang Mai, Thailand
26-30 August 2008

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Organized by
The Royal Initiative Committee on the Study of Fireflies in Thailand
Queen Sirikit Botanic Garden (QSBG)
The Botanical Garden Organization (BGO)

Sponsored by
Petroleum Authority of Thailand (PTT)
The Sanga Sabhasri Research Foundation
TNT Express Worldwide (Thailand)

2009

Published by: Queen Sirikit Botanic Garden, Botanical Garden Organization

Citation: Napompeth, B. (ed.). 2009. Diversity and Conservation of Fireflies. Proceedings of the International Symposium on Diversity and Conservation of Fireflies, held at Queen Sirikit Botanic Garden, Chiang Mai, Thailand, 26-30 August 2008.

ISBN: 978-974-286-745-4

Cover photo: *Vesta* sp. by Somkhuan Suk-ieam, QSBG

Printed by: Nantakarn Graphic 120 Soi 7 (Pongsuwan) Changpuak Rd., T.Sriphum A.Muang Chiangmai 50200

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EMBRYO GENESIS OF FIREFLY: PHYLOGENETIC IMPLICATION OF THE FAMILY RHAGOPHTHALMIDAE

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Introduction

Rhagophthalmus ohbai Wittmer is a firefly (glowworm) discovered in Iriomote-jima Island, Ryukyu Islands, southwest of Japan, and was described by Wittmer in Wittmer and Ohba (1994). According to Wittmer and Ohba (1994) and Ohba et al. (1996), the morphology and ecology of this species is very unique. The adult male has no light organ, and the posterior part of eyes is indented in dorsal. Furthermore, the adult female is completely apterous and laviform. She has a large lantern in the last abdominal segment and three small lanterns in each segment from the mesothorax to the ninth abdominal segment. After sun set, she lifts up her abdomen and glows from the lantern of the abdominal end to attract males. Just within 20 min after sun set, the female and the male copulate, and the female gets into the soil. Then, she spawns eggs and broods on an eggs mass until hatching. At this time, she changes her glow style from the lantern of the abdominal end to that of other segments.

Such peculiar habits have not been reported in this genus until the description by Wittmer and Ohba (1994), and the taxonomic and phylogenetic position of this genus has not been clear yet. Namely, the genus *Rhagophthalmus* was treated in the member of Lampyridae, Rhagophthalmidae or Phengodidae by some taxonomists and its phylogenetic position is also contradictory by molecular analyses. To clarify the taxonomic and phylogenetic problem of *Rhagophthalmus*, we focused on the early embryonic development of *Rhagophthalmus ohbai* at the view point of embryogenesis.

Taxonomy and Phylogeny of Rhagophthalmidae

The genus *Rhagophthalmus* was established by Motschulsky (1853) based on *Rhagophthalmus scutellatus*. Olivier (1910) erected the family Rhagophthalmidae for three genera, *Dioptoma*, *Ochotyra* and *Rhagophthalmus*. One year later, however, he replaced *Rhagophthalmus* to Lampyridae (Olivier 1911). Crowson (1955) gave the subfamily Rhagophthalminae in the Lampyridae, and this treatment was followed by McDermott (1964). In 1972, however, Crowson placed the Rhagophthalminae in the family Phengodidae. Later Wittmer and Ohba (1994) replaced *Rhagophthalmus* to the Rhagophthalmidae with the description of five new species and of luminosity and behavior of *R. ohbai*.

Crowson (1972) constructed a phylogenetic tree based on male morphology. In his tree, *Rhagophthalmus* was treated in the member of Phengodidae. Branham and Wenzel (2001) also constructed a phylogenetic tree based on male morphology by cladistic analysis, but members of the two families, *Dioptoma*, *Diplocladon* and *Rhagophthalmus* (Rhagophthalmidae) and *Cenophengus*, *Phrixothrix*, *Pseudophengodes* and *Zarhipis* (Phengodidae), were included in two different clades for each. Namely, Rhagophthalmidae and Phengodidae were recognized as distinct families.

By molecular studies, on the other hand, Suzuki (1997) constructed a molecular phylogenetic tree of 26 lampyrid species distributed in four subfamilies (Luciolinae, Cyphonocerinae, Lampyrinae and Ototretinae) and including one rhagophthalmid by mitochondrial 16S ribosomal RNA gene. Lycidae was used as an outgroup taxon. In his result, *R. ohbai* was included in Lampyridae and was closely related to *Stenocladius*. On the other hand by luciferase gene analysis, Ohmiya et al. (2000) compared the amino acid sequences of luciferase among the four families, Elateridae, Lampyridae, Phengodidae and Rhagophthalmidae, and suggested that the Rhagophthalmidae is more closely related to the Phengodidae than the Lampyridae. Bocakova et al. (2007) constructed a phylogenetic tree of Elateriformia including 28 families by mitochondrial and nuclear ribosomal RNA genes, and their result was the same as Ohmiya et al. (2000). Sagegami-Oba et al. (2007) also showed the same result by nuclear 18S ribosomal RNA gene. Furthermore, the result obtained by Stanger-Hall et al. (2007) was the same as Suzuki (1997) that *R. ohbai* is included in the Lampyridae but is more closely related to *Pterotus* than *Stenocladius*. Thus, the phylogenetic position of Rhagophthammidae (*Rhagophthalmus*) is still problematical both in morphological and molecular analyses.

Embryogenesis of Firefly

Generally the cleavage type of early embryonic development is categorized as superficial cleavage in insects. Namely, fertilized nucleus divides several times without cell division, and nuclei increase in number in the yolk. Most of the nuclei migrate toward the surface of egg, and cytoplasmic divisions occur at the egg surface. Then, a layer of cells are formed, and it is called a blastoderm. After blastoderm formation, a part of blastoderm cells proliferates actively and forms a germ disk (Fig. 1A), and the germ disk develops into a superficial germ band (Fig. 1E). Then the embryo develops at the superficial position of the egg. This is the normal development of insect embryos. On the other hand in Lampyridae, the germ disk is invaginated in the yolk and it becomes a spherical cell mass. It is called a germ rudiment (Fig. 1B, 1C). Then, the germ rudiment migrates toward the center of the egg (Fig. 1D), and the embryo develops in the immersed condition of the yolk.

It was the first that Williams (1916) described the embryogenesis of fireflies and discovered spherical germ rudiment formation in *Photuris pennsylvanicus* of the subfamily Photurinae. Later, the spherical germ rudiment formation was confirmed in three lucioline species, *Luciola cruciata* (Ando and Kobayashi 1975), *L. lateralis* and *Hotaria parvula* (Kobayashi and Ando 1985) and one lampyrine species, *Pyrocoelia rufa* (Kobayashi et al. 2006).

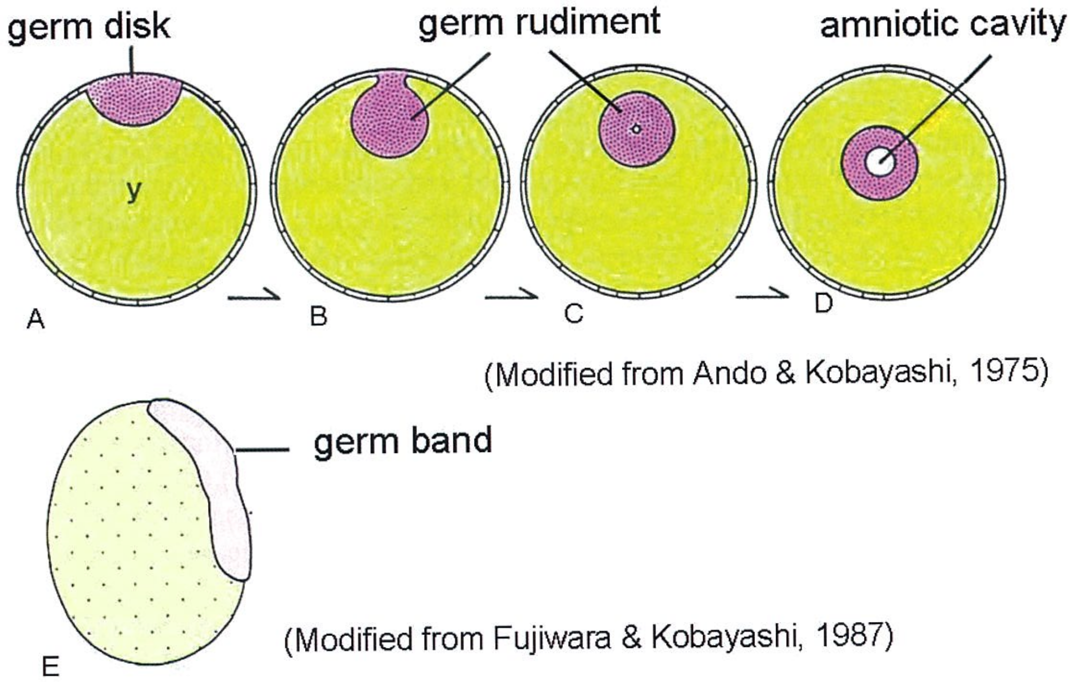

Fig. 1. Diagram of embryogenesis of Lampyridae (modified from Ando and Kobayashi (1975) (A to D) and Cantharidae (modified from Fujiwara and Kobayashi (1987) (E). A. Germ disk formation; B. & C. Germ rudiment Formation; D. Migration of germ rudiment into the yolk; and E. Germ band formation.

The spherical germ rudiment formation is a unique character in insects. The character is found in five species of the three subfamilies of Lampyridae and two primitive moth species, *Endoclita signifer* (Hepialidae) and *Neomicropteryx nipponensis* (Micropterygidae) (Ando and Tanaka 1976; Kobayashi and Ando 1988), although early embryonic development has been observed widely in insects. The germ rudiment stays at the superficial position of the egg and continues embryonic development in the two moth species. However in Lampyride, the germ rudiment migrates toward the center of the yolk, and the embryo develops in the immersed condition of the yolk. This character is specific only in Lampyridae.

Taxonomic Position of Rhagophthalmidae

Early embryonic development of *Rhagophthalmus ohbai* was observed by the histological method with light microscopy (Kobayashi et al. 2001, 2002). After blastderm formation, the germ disk is differentiaed from a part of blastderm cells (Fig. 2A). The germ disk is invaginated in the yolk (Fig. 2B) and becomes the spherical germ rudiment (Fig. 2C). Then, the germinal rudiment migrates into the center of the yolk, and the embryo develops in the immersed condition of the yolk (Fig. 2D). Thus, the spherical germ rudiment formation of *R. ohbai* is very similar to that of

Lampyridae, and the result supports close relationship between Lampyridae and Rhagophthalmidae.

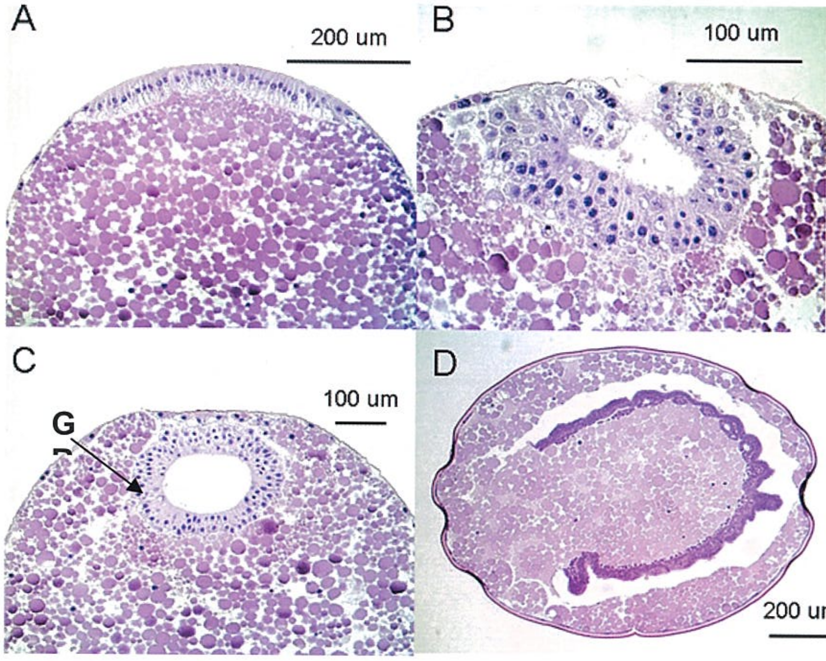

Fig. 2. Longitudinal section of egg of *Rhagophthalmus ohbai*, showing the spherical germ rudiment (GR) formation. A. 72-h egg; B. 3.5-day egg; C. 4-day egg; and D. 10-day egg.

The spherical germ rudiment is deeply immersed in the yolk. This is the specific character for the lampyrid and rhagophthalmid species. On the other hand, in cantharid species, a family member of the superfamily Cantharoidea, the germ disk develops into a superficial germ band, and the embryo develops at the superficial position of the egg (Fig. 1E; Fujiwara and Kobayashi 1987). This character is common in Coleoptera. Then, the embryonic characters are mapped on the phylogenetic tree of Stanger-Hall et al. (2007) (Fig. 3). Based on the tree, the character, spherical germ rudiment formation, of lampyrid and rhagophthalmid is considered as apomorphic, while the character, superficial germ band formation, of cantharid is considered as plesiomorphic in Cantharoidea phylogeny. Furthermore, Fig. 3 indicates that the embryonic characters of Phengodidae and Lycidae would help to evaluate the phylogenetic relationships among Cantharoidea families.

In conclusion, the early embryonic development of *Rhagophthalmus ohbai* is fundamentally the same as that of Lampyridae. This result is supporting the ingroup hypothesis by McDermott (1964), Suzuki (1997), and Stanger-Hall et al. (2007). But other molecular phylogenetic studies suggest close relationship between Rhagophthalmidae and Phengodidae. To clarify the early embryonic development of Phengodidae and Lycidae, that would be a key for phylogenetic consideration among family members of Cantharoidea.

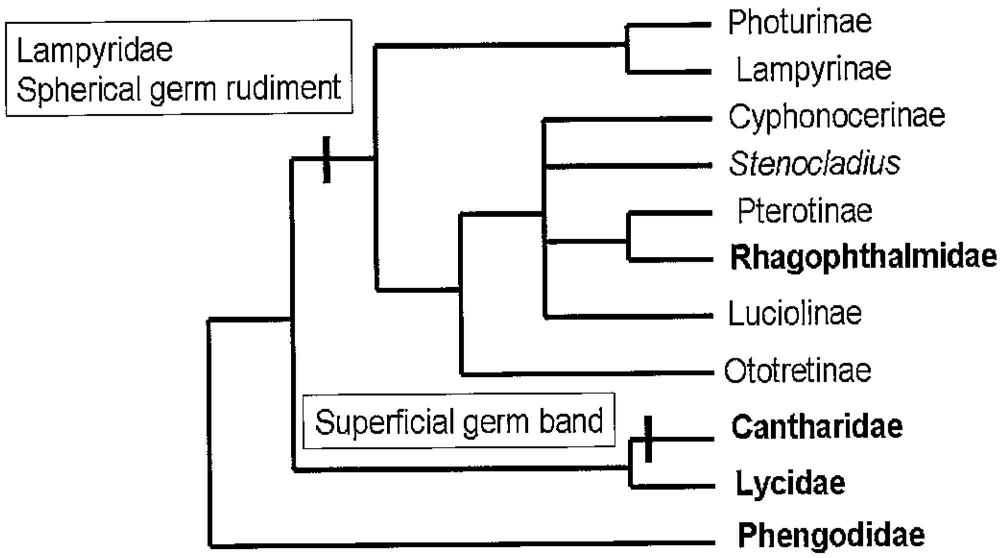

Fig. 3. Mapping two embryonic characters, spherical germ rudiment and superficial germ band, on the tree proposed by Stanger-Hall et al. (2007).

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