

Observations on the luminescence configurations of eight firefly genera and their immature stages

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ABSTRACT

Long exposure photography was used for recording luminescence configuration of eight firefly genera in Hong Kong, namely: *Rhagophthalmus*, *Stenocladus*, *Lamprigera*, *Pyrocoelia*, *Diaphanes*, *Aquatica*, *Luciola* and *Pteroptyx*, all sampled in Hong Kong. It was found that luminescence configuration does not necessarily coincide with light organ configuration of the same firefly. Additionally, a large portion of the fireflies in this study are capable of displaying bipartite light spots from their obround or rectangular light organs. *Stenocladus* can display three different types of luminescence configurations, including diffused light from the whole body. This study also shows that male and female adult of *Lamprigera* retain their larval light organs while other species develop additional light organs with their larval light organs remaining intact. Possible implications of the luminescence configurations are proposed and discussed.

Keywords: Fireflies, bioluminescence, Lampyridae, Rhagophthalmidae, aposematic, light organs, Hong Kong.

INTRODUCTION

Fireflies are the best known animals displaying bioluminescence. The light emitting part of a firefly body is called lantern, luminescent organ, photic organ or light organ. Light organs of fireflies often appear as conspicuous white patches on preserved specimens. However, for some species, the light organs are not conspicuous or even not visible externally. In some cases, the light produced is very dim or only lasts for a short period of time during the development from larva to adult. As a result, they could sometimes be overlooked. In this study, long exposure photography was used to record the luminescence configuration of fireflies.

MATERIALS AND METHODS

As the light emitted by fireflies is very dim, special camera settings were required to clearly show the light. To increase exposure of the firefly luminescence to the camera sensor, long exposure time (from 1/8 sec. to 120 sec., depending on species) was used, with a tripod. In many cases, sensor sensitivity would be adjusted to a higher value (ISO 400 to ISO 3200), and/or large lens aperture would be selected. Although the glowing light organs are shown, the other parts of the body are not exposed to light when the firefly is in the dark. Consequently, in order to show the whole body and therefore the relative positions of the glowing light organs, two methods were used in this study: 1. As

soon as the long exposure photo was taken, another photo with flash light was produced, so that the relative position of the light organs could be seen by comparing the two photos; 2. using fill-in light. This was done by either pointing a torch towards the objects nearby, allowing the reflected light to shine onto the firefly, or simply using fill-in flash light from the camera system. The intensity of fill-in light was controlled such that it would not overshadow the light emitted by the light organs. All photos were taken with either an Olympus E3 digital camera connected to a Zuiko Digital ED 50 mm f/2.0 Macro lens, or an Olympus E620 digital camera connected to the same lens, or a Canon 550D digital camera connected to a EFS 60 mm f/2.8 Macro USM lens or to a MP-E65 mm f/2.8 1-5X macro lens.

The study involved 10 species of fireflies belonging to eight genera, collected from 2006 to 2010 in Hong Kong, China, namely:

1. *Rhagophthalmus motschulskyi* and *Rhagophthalmus* sp. (Rhagophthalmidae). Two females of *Rhagophthalmus motschulskyi* were seen glowing in the wild and in captivity respectively, and long exposure photos were taken at the moment when they glowed. The identity of the female was inferred from another female with the same morphological features found in the same locality, which mated with an identified male of *R. motschulskyi*. A pair of unknown *Rhagophthalmus* sp. were found mating in the wild and were collected. The female was observed and a series of long exposure photos was taken during oviposition.

2. *Stenocladus* spp. (Lampyridae: Ototretadrilina-Ototretinae complex). More than 50 females were observed in about 20 night trips in three different well vegetated localities in Hong Kong, from November, 2009 to January, 2010, and from November 2010 to December, 2010. Long exposure photos were taken to show the luminescence configurations of eight different female individuals in the wild. Two females were collected for intensive observation and for taking long exposure photos in an indoor environment. A light emitting male of a *Stenocladus* sp. found on a spider web was collected and long exposure photos were taken during the short moment when it emitted light. A *Stenocladus* sp. larva was collected in the wild and reared; long exposure photos were taken when it glowed. Because of the very different markings of two types of larvae found (another one without long exposure photo taken), it could be inferred that there are two different species of *Stenocladus* in Hong Kong. Therefore, it is possible that the females and males mentioned in this study belong to different species. From their distinct external morphology, there is no doubt that they belong to this genus. Two events of attempted mating

were observed in the wild, which gave support to the association between the male and the female.

3. *Lamprigera* sp. (Lampyridae: Lampyrinae)

A female larva of an unknown *Lamprigera* was collected in the wild and reared; it pupated but died before emergence of the adult. Long exposure photos were taken when light was emitted from the larva and from the pupa. Another female and a male of the same unknown *Lamprigera* were seen glowing in the wild and long exposure photos were taken. All the individuals mentioned above were found in a very restricted locality on Tai Mo Shan.

4. *Pyrocoelia* sp. (Lampyridae: Lampyrinae)

A female larva of an unknown *Pyrocoelia* sp. was collected in the wild and reared. It was observed intensively and a series of long exposure photos were taken in the course of its development from pre-pupa to adult. Another female and several males of the same *Pyrocoelia* sp. were seen glowing in the wild and long exposure photos were taken.

5. *Diaphanes citrinus* (Lampyridae: Lampyrinae)

A male adult was collected and series of long exposure photos were taken in an indoor environment. Another male adult was seen glowing in the wild and a series of long exposure photos were taken.

6. *Aquatica* cf. *ficta* (Lampyridae: Luciolinae)

A female pupa was obtained in February, 2011, by rearing larvae. A series of long exposure photos were taken in the course of its development into an adult.

7. *Luciola terminalis* (Lampyridae: Luciolinae)

A male adult was collected in the wild and kept in a transparent plastic vial. An AVI format (30 frames per second) video (duration: 1min. 24 sec.) was taken when it emitted light. The video was taken with an Olympus EPL1 digital camera connected to a Zuiko Digital ED 50 mm f/2.0 Macro lens. The video was played frame by frame to see the changes in the luminescence pattern.

8. *Pteroptyx* sp. (Lampyridae: Luciolinae)

A female pre-pupa of *Pteroptyx* sp. was obtained in October, 2010, by rearing larvae. A series of long exposure photos were taken when it glowed during larval development. A male adult was collected in the wild and kept in a transparent plastic vial. An AVI format (30 frames per second) video (duration: 45 sec.) was taken when it emitted light. The video was taken with the same camera and lens mentioned above. The video was played frame by frame to see the changes in the luminescence pattern.

The numbering of abdominal segments and ventrites refers to the true segments.

In some cases, light is emitted from the light organs located just beneath the ventrites, but in other cases, especially in immature stages, light is emitted from somewhere inside

the abdominal segment or from a whole abdominal segment. They are termed differently in the results section.

Abbreviation: V= Abdominal ventrite

RESULTS

1. *Rhagophthalmus motschulskyi* and *Rhagophthalmus* sp. Two different luminescence configurations were observed on adult female specimens. The first one was a large light spot that appeared on V8 (Fig. 1.1, 1.2). The second one was during oviposition, a bright light spot appeared near the right posterolateral margin of mesothorax, metathorax, and 1st-9th abdominal segment (presumably there are corresponding light spots at the opposite side, but they are not shown in the photo records). An additional light spot appeared near the posterodorsal margin of each of the 11 segments mentioned above. The lateral light spots on each of the abdominal segments were glowing during observation, but the lateral light spots on the two thoracic segments as well as the dorsal light spots were sometimes not emitting light (Fig. 1.3-1.6). No well-defined light organs were seen in the preserved specimens.

2. *Stenocladus* spp.

The larva displays one pair of light spots was evident on the 7th and 8th abdominal segments (Fig. 2.1). Three different patterns of luminescence were observed on adult female specimens. The whole body, except the anterior part of pronotum and the head, glowed evenly (Fig. 2.2) and this was the most common pattern observed. Less frequently, the whole body, except the anterior part of the pronotum and the head, glowed evenly, and at the same time a pair of large, brighter light spots appeared on the 8th abdominal segment (Fig. 2.4). Finally, a pair of large light spots appeared on the 8th abdominal segment and at the same time the whole body glowed very weakly, there was also a pair of tiny light spots close to the left posterolateral margin of abdominal segment 2-4 (presumably, the same pairs could be seen on the right side of the body, but is not shown in the photos). It appeared that tiny light spots also appeared in the metathorax, but they were not definitely shown in the photos (Fig. 2.5). Adult males seldom emit light, and only two instances were observed amongst about 20 individuals in the wild; both were caught in spider webs. A pair of weak light spots appeared on V6 (Fig. 2.6). No well defined light organs are seen in the preserved specimens.

3. *Lamprigera* sp.

The larva displayed one pair of "C"-shaped light spots on V8 (Fig. 3.1), which were also shown by the female pupa and adult. The male adult showed one pair of light spots on V8 (Fig. 3.4).

4. *Pyrocoelia* sp.

The larva displayed one pair of large light spots on the eighth abdominal segment (Fig. 4.1) which was the same

for the female prepupa and pupa (Fig. 4.2, 4.3). On the last day before emergence, an additional pair of small light spots appeared on V6 (Fig. 4.4). In the adult female, an additional pair of larger spots appeared on V7 on the second day after emergence. All three spots glowed simultaneously (Fig. 4.5). The light spots at the eighth abdominal segment gradually dimmed and finally disappeared on the fourth day after emergence (Fig. 4.6). In the adult male there was an obround light spot on V6 and V7 (Fig. 4.7). When the intensity of light given out was low, it appeared as 2 spots at both end of the obround light organs (Fig. 4.8).

5. *Diaphanes citrinus*

The male adult emitted light on V6 and V7, sometimes in an obround shape (Fig. 5.1), but when the intensity of light emitted was low, it appeared as 2 spots at both end of the obround light organs (Fig. 5.2).

6. *Aquatica* cf. *ficta*

Glowing larvae under water were occasionally seen, but no long exposure photo was successfully taken to show this. Clearly defined light organs could be seen as a pair of spots on the eighth abdominal segment (Fig. 6.1) On a five days old pupa a pair of light spots appeared on the eighth abdominal segment and a pair on V6 (Fig. 6.2). When the same pupa was 8-day old (two days before emergence), the light spots on V6 became obround shaped (Fig. 6.3). When the intensity of light emitted was low, the two extremes of the stripe were brighter (Fig. 6.4). The light spots on the eighth abdominal segment diminished as the pupa became more mature. On the first day after emergence of an adult female, the same obround light spots appeared on V6. When the intensity of light emitted was low, it appeared as two-spotted form (Fig. 6.5). On the third day after emergence, the light spots on the eighth abdominal segment became very small (Fig. 6.6) and disappeared on the fifth day after emergence (Fig. 6.7). The light source on V6 appeared as two spots when it was not glowing fully (Fig. 6.8). No flashing (only intermittent glow) was observed until the twelfth day after emergence.

7. *Luciola terminalis*

The male adult emitted light from V7. It was continuous and steady throughout the recording period and was more or less even across the whole surface of V7 except on the terminal portion. The light emitted from V6 was flashing and bipartite throughout the recording period (Fig. 7.1). The light organs on V6 and V7 are well defined white patches covering nearly the whole surface of V6 and V7 (Fig. 7.2).

8. *Pteroptyx* sp.

Larvae were occasionally seen glowing either in captivity or in the wild, but no long exposure photo was successfully taken to show the glowing pattern. Two light organs could be seen on the eighth abdominal segment, although they were not very well defined (Fig. 8.1). The prepupa displayed

two light spots on the eighth abdominal segment, which coincided with the light organs of the larva (Fig. 8.2). A two days old female pupa displayed a pair of light spots on V8 (Fig. 8.3). The last day before emergence (10-day old pupa), an obround light organ was clearly visible on V6 (Fig. 8.4). As soon as the pupa turned into an adult, light was emitted as two spots on V8 and evenly from the light organ on V6 (Fig. 8.5). No flashing was observed. In the male adult, a flashing light appeared either on V6 as a pair of spots with none on V7 (Fig. 8.6), or from both V6 and V7 as a bipartite structure (Fig. 8.7). Only when the light organs were glowing fully, did they appear as rectangular structures (Fig. 8.8). The light organs beneath V6 and V7 were well defined white patches covering nearly the whole surface of these ventrites (Fig. 8.9), leaving only the median posterior projection of V7.

DISCUSSION

From the results, it is shown that long exposure photography with a single macro lens reflex camera can be a useful tool to record the luminescence configuration of fireflies, which does not always coincide with the configuration of light organs of the same firefly. In this study, light organs of *Rhagophthalmus* and *Stenocladus* are even not visible in both dry specimens and specimens preserved in alcohol.

All members of the Lampyridae observed in this study and in both sexes, are capable of displaying bipartite light spots from their obround or rectangular light organs (*Pyrocoelia*, *Diaphanes*, *Pteroptyx*); in the genus *Lamprigera*, the light organ is already bipartite. Therefore, the obround or rectangular light organ may be regarded as a combination of two enlarged spot light organs in an abdominal segment, rather than being two distinct structures. All larvae and larviform females involved in this study had two spotted light. This two spotted configuration could be explained by their proximity to the two spiracles of each abdominal segment, which supply oxygen necessary for the bioluminescence's chemical reaction; or the result of uneven innervation. In males the enlargement of the two spotted light organs merging into an obround light organ may well be the result of competition for females by displaying larger light patch(s). It is interesting to note that in *Lamprigera*, both the adult male and female retain the two spotted pattern on V8 of their larval stage, but *Pyrocoelia* and *Diaphanes*, while members of the same family, change their light organs to V6 and V7. Close examination of the specimens showed that in order to make room for the genitalia, there was no fat deposited in segment eight of *Pyrocoelia* and *Diaphanes* (both sexes). Although segment eight of *Lamprigera* is not substantially larger, a considerable amount of fat is deposited in this segment and the preceeding one which showed no light. In *Pyrocoelia*, *Aquatica* and *Pteroptyx*, adult females developed their light organs when their larval light organs on V8 were still intact and functioning. Therefore the light

organs of the adults of these three species are not directly derived from their larval stage.

Luminescence configuration of *Stenocladus* is exceptional in that it is not localised as spots. Buck (1948) suggested that photogenic cells were typically grouped together in one or more compact localised masses with specific tracheal and nervous supplies; and regarded the light emitted in *Phengodes*, which is produced by loose independent giant cells, apparently without tracheae, as exceptional. The light produced by *Stenocladus* is more evenly distributed and the light producing mechanism may possibly be even more loosely organised than that of *Phengodes*. Chen (2003) reported that the prepupa (sex not mentioned) and the male pupa of *Stenocladus bicoloripes* in Taiwan produced diffused light and the larvae of *Stenocladus* do not have such diffused luminescence. Adult females of *Stenocladus* gain the ability of producing spot light as larvae, as is in many other fireflies, therefore what would be the reason for having additional diffused luminescence? Males have well developed antennae used in sexual interaction, therefore communication between the two sexes may not solely rely on light signals and chemical signaling may also play an important role. In view of their soft body surface and low mobility, aposematic display may be an explanation to added luminescence. Many fireflies were found to be able to produce unpalatable or toxic substances (Blum and Sannasi, 1974; Eisner *et al.*, 1978; Fu *et al.*, 2009) and it has been proposed and tested that luminescence is an aposematic display in firefly larvae (Buschman, 1988; De Cock, 2000; Fu *et al.*, 2009, Sivinski, 1981). For the wingless and less mobile adult females of *Pyrocoelia*, *Diaphanes* and *Lamprigera*, their comparatively very bright light organs may also play an important role of aposematic display.

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FIGURES



Fig. 1.1. *Rhagophthalmus motschulskyi* adult female— Large light spot on V8.



Fig. 1.2. *Rhagophthalmus motschulskyi* adult female— Large light spot.

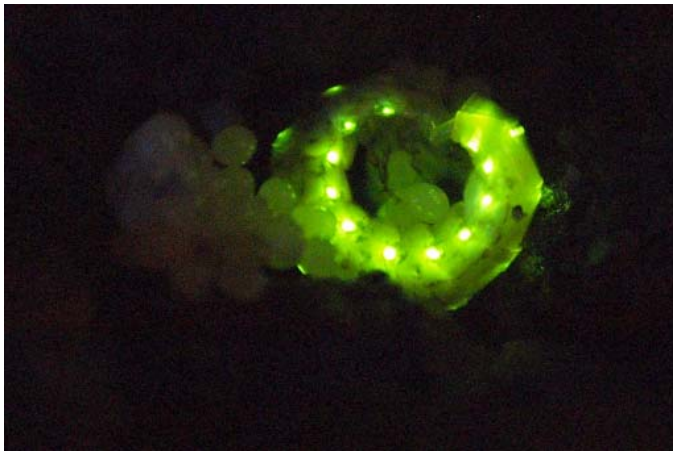


Fig. 1.3. *Rhagophthalmus* sp. adult female— luminescence during oviposition.



Fig. 2.1. *Stenocladus* sp. larva — light spots on seventh and eighth abdominal segments.

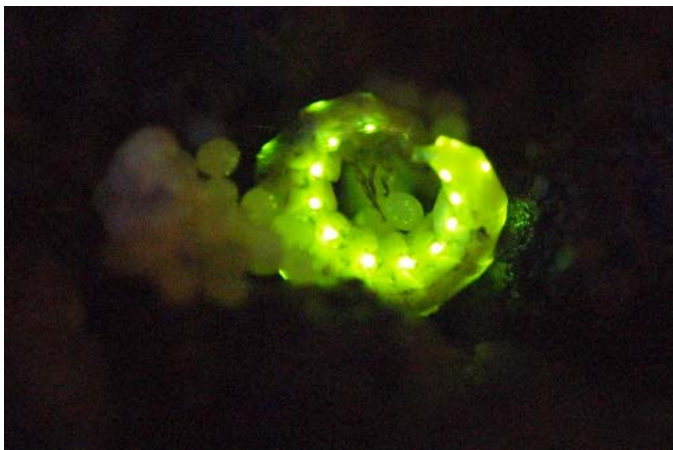


Fig. 1.4. *Rhagophthalmus* sp. adult female— luminescence during oviposition

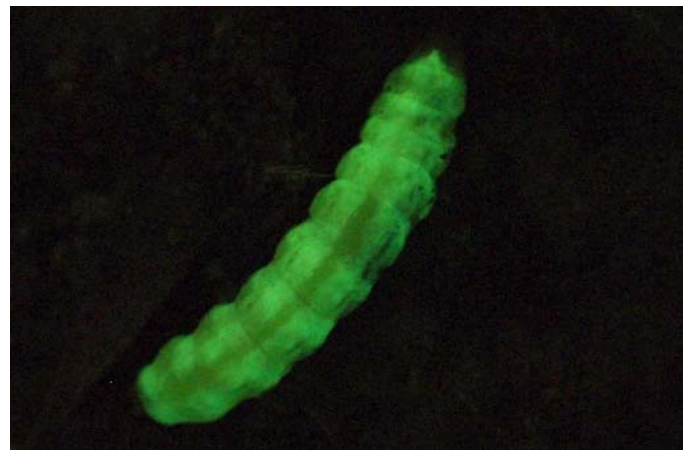


Fig. 2.2. *Stenocladus* sp. adult female — diffused glow from the whole body.



Fig. 1.5. *Rhagophthalmus* sp. adult female — luminescence during oviposition



Fig. 2.3. *Stenocladus* sp. adult female — whole body under flash light.



Fig. 2.4. *Stenocladius* sp. adult female — diffused glow from the whole body with two spotted light on the eighth abdominal segment.



Fig. 3.1. *Lamprigera* sp. larva — one pair of “C”-shaped light spots on V8.

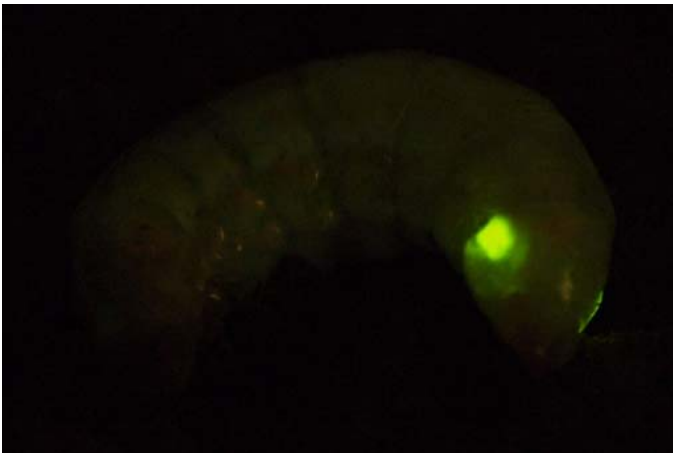


Fig. 2.5. *Stenocladius* sp. adult female — light spots on the eighth abdominal segments, whole body glows weakly with tiny light spots on the second to fourth abdominal segments.



Fig. 3.2. *Lamprigera* sp. female pupa — one pair of “C”-shaped light spots on V8.



Fig. 2.6. *Stenocladius* sp. adult male — light spots on V6.



Fig. 3.3. *Lamprigera* sp. adult female — 1 pair of “C”-shaped light spots on V8.



Fig. 3.4. *Lamprigera* sp. adult male — 1 pair of light spots on V8.



Fig. 4.3. *Pyrocoelia* sp. Female pupa — one pair of large light spots on the 8th abdominal segment (dorsal view).



Fig. 4.1. *Pyrocoelia* sp. larva — one pair of large light spots on the 8th abdominal segment.



Fig. 4.4. *Pyrocoelia* sp. female pupa (last day before emergence) — one pair of light spots on the 6th & 8th abdominal segments.



Fig. 4.2. *Pyrocoelia* sp. prepupa — one pair of large light spots on the 8th abdominal segment.



Fig. 4.5. *Pyrocoelia* sp. adult female (2-day old) — one pair of light spots on the 6th, 7th & 8th abdominal segments.



Fig. 4.6. *Pyrocoelia* sp. adult female (4-day old) the light spots on the 8th abdominal segment have disappeared.



Fig. 5.1. *Diaphanes citrinus* adult male — obround light organ in full glow on V6 & V7.



Fig. 4.7. *Pyrocoelia* sp. adult male — obround light organ in full glow on V6 & V7.



Fig. 5.2. *Diaphanes citrinus* adult male — 2-spotted light from the obround light organ on V6 & V7.



Fig. 4.8. *Pyrocoelia* sp. adult male — 2-spotted light from the obround light organ on V6 & V7.



Fig. 6.1. *Aquatica* cf. *ficta* larva — light organ on the 8th abdominal segment.



Fig. 6.2. *Aquatica cf. ficta* pupa (5-day old) — a pair of light spots on V6 and V8.



Fig. 6.5. *Aquatica cf. ficta* adult female (1-day old) — a pair of light spots on V6 and V8.



Fig. 6.3. *Aquatica cf. ficta* pupa (8-day old) — a pair of light spots at V8, obround shaped light on V6.



Fig. 6.6. *Aquatica cf. ficta* adult female (3-day old) — light spots on V8 diminished.



Fig. 6.4. *Aquatica cf. ficta* pupa (8-day old) — a pair of light spots on V8 and V6.



Fig. 6.7. *Aquatica cf. ficta* adult female (5-day old) — light spots on V8 have disappeared.



Fig. 6.8. *Aquatica cf. ficta* adult female (3-day old) — 2-spotted light on V6.



Fig. 8.1. *Pteroptyx* sp. larva — a pair of light spots on the 8th abdominal segment.

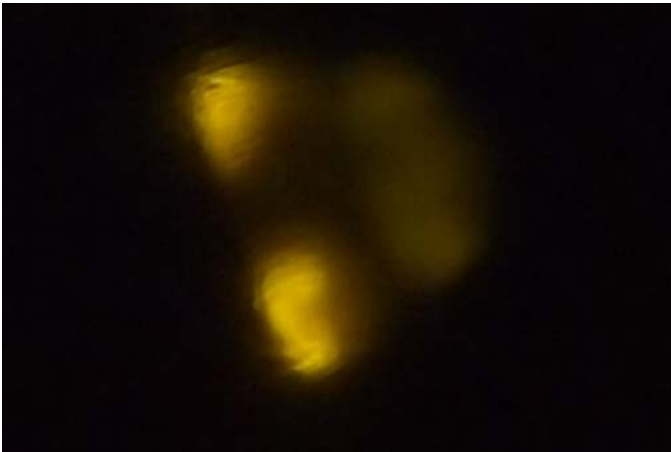


Fig. 7.1. *Luciola terminalis* adult male — partial light from V7 and 2-spotted light from V6.



Fig. 8.2. *Pteroptyx* sp. prepupa — a pair of light spots on the 8th abdominal segment.



Fig. 7.2. *Luciola terminalis* adult male — light organs on V6 and V7 occupying the whole ventrite surface.



Fig. 8.3. *Pteroptyx* sp. Pupa (2-day old) — a pair of light spots on the 8th abdominal segment.



Fig. 8.4. *Pteroptyx* sp. Pupa (10-day old, last day before emergence) — a pair of light spots on the 8th abdominal segment & obround light organ on V6.

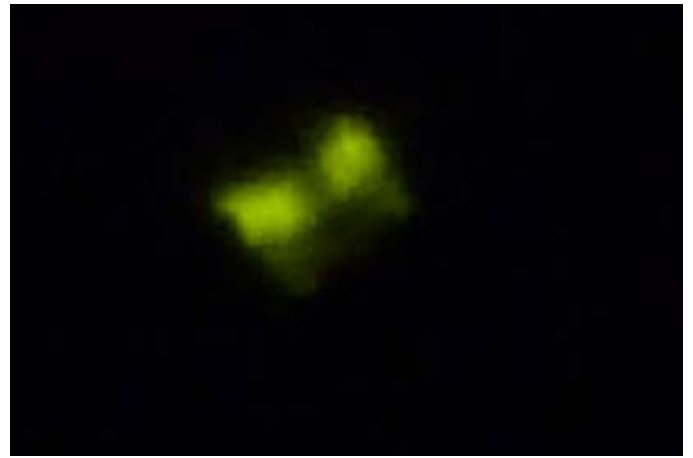


Fig. 8.7. *Pteroptyx* sp. adult male — 2-spotted light from V6 and 2-spotted light from V7.



Fig. 8.5. *Pteroptyx* sp. adult female (1-day old) trapped in pupal case — 2-spotted light from V8 and obround shaped light from V6.

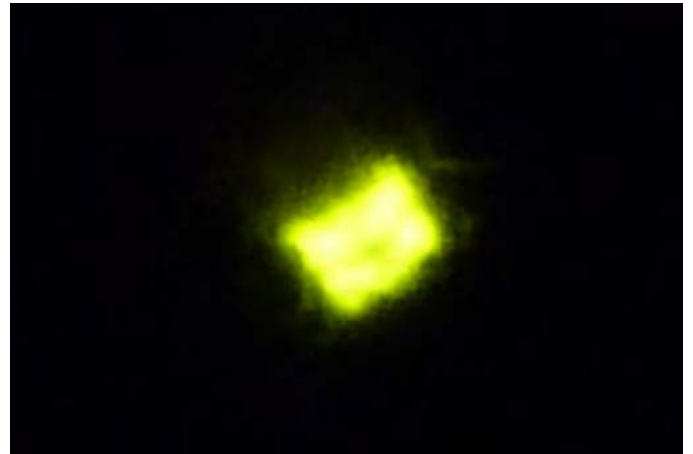


Fig. 8.8. *Pteroptyx* sp. adult male — Light from V6 and V7 in full glow.



Fig. 8.6. *Pteroptyx* sp. adult male — 2-spotted light from V6.



Fig. 8.9. *Pteroptyx* sp. adult male — Light organs on V6 and V7.