THE BIOLOGY AND REARING OF FIREFLY *Pteroptyx malaccae* Gorham.

ชีววิทยาและการเพาะเลี้ยงหิ่งห้อยชนิด *Pteroptyx malaccae* Gorham.

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Pteroptyx malaccae Gorham เป็นหิ่งห้อยชนิดที่พบได้ตามพื้นที่น้ำกร่อยและป่าชายเลน ปัจจุบันหิ่งห้อยชนิดนี้มีจำนวนลดลงมากเนื่องจากการเปลี่ยนแปลงและถูกทำลายของแหล่งอาศัยโดยเฉพาะอย่างยิ่งพื้นที่ป่าชายเลน งานนี้จึง มีเป้าหมายที่จะศึกษาวิธีการเพาะเลี้ยงและชีววิทยาของหิ่งห้อย เพื่อ เป็นการสร้างความรู้พื้นฐานในการอนุรักษ์สายพันธุ์หิ่งห้อย โดยเฉพาะ การอนุรักษ์หิ่งห้อยชนิด *P. malaccae* ซึ่งเป็นชนิดที่ได้รับความสนใจในด้านการท่องเที่ยวเชิงอนุรักษ์ นอกจากนั้นยังสามารถใช้เป็นแนวทางในการเพิ่มปริมาณเพื่ออนุรักษ์หิ่งห้อยในธรรมชาติได้อยู่อย่างยั่งยืน ทั้งนี้ โดยการศึกษาชีววิทยาและวงจรชีวิต ซึ่งประกอบด้วยภาชนะและวัสดุที่เป็นแหล่งอาศัยของหิ่งห้อย ได้แก่ ดิน กิ่งและใบลำพู น้ำ และอาหาร โดย ใช้แอปเปิ้ลเป็นอาหารในระยะตัวเต็มวัย และหอยชนิด *Assiminea* sp. เป็นอาหารในระยะตัวหนอน วงจรชีวิตของหิ่งห้อยในห้องปฏิบัติการประกอบด้วย 4 ระยะ ได้แก่ ไข่ ตัวหนอน ดักแด้ และตัวเต็มวัย ใช้เวลาตลอดทั้งวงจรเฉลี่ย 122.90 วัน แต่ละระยะใช้เวลา 12.15, 97.83, 9.83 และ 12.33 วัน ตามลำดับ และเมื่อเป็นตัวเต็มวัยจะมีสัดส่วนเพศผู้ต่อเพศเมียเท่ากับ 4 : 1

คำสำคัญ : หิ่งห้อย /*Pteroptyx malaccae* / วงจรชีวิต

Pteroptyx malaccae Gorham (Coleoptera: Lampyridae) is a semiaquatic firefly found in a brackish and mangrove ecosystem. They are currently decreasing due to the destruction their habitats, especially mangrove forest. Therefore, rearing techniques, and the biology were necessary investigated in order to provide basic knowledge necessary for firefly conservation, especially *P. malaccae*. that becoming a prioriity as eco-tourism attractions in Thailand, In addition, it is also maintaining sustainability of natural firefly populations . This work focus on biology, life cycle, morphology and rearing methods. With proper conservation practices, these firefly populations can persist and increase in natural habitats. The successful rearing allowed the study of its life cycle and biology. Insect rearing methods and conditions are described, consisting of a rearing container with suitable substrates (food, soil, plants, moisture) for shelter and oviposition site. Adults and larvae were fed with fresh apple and snail, *Assiminea* sp. The life cycle of this firefly was 3-4 months under laboratory conditions, consisting 4 stage of egg, larva (4 instar), pupa, and adult. The average life cycle was 122.90 days, consisting 4 stage as egg, larva, pupa, and adult. Adult lives an average of 12.33 days. Egg, larva, and pupa lasted an average of 12.15, 97.83 and 9.83 days, respectively. Eventually, adults emerged with a sex ratio of male : female as 4 : 1

Key words: Firefly / *Pteroptyx malaccae* / Life cycle

1. INTRODUCTION

Fireflies (Coleoptera : Lampyridae) are found in most regions of the temperate and tropical environments around the world. Worldwide, almost 2,000 species were listed by McDermott (1966). Literature survey on fireflies reveals that the majority of research has been focused on the bioluminescent behavior and flash communication of some European, American, and

Asian species (Ohba 1983; Vencl *et al.* 1994; Branham 1996; Vencl and Carlson 1998; Xinhua *et al.* 2005). However, several authors have also investigated the life history and flash communication of fireflies in Asia (Nobuyoshi, 1988, 1996; Chen and Chen 1997).

The habitat of larvae is generally found in damp environment. The terrestrial species; *Photuris* and *Photinus,* are found under stone in ground depression, under leaf litter. Many species in Asia such as *Luciola cruciata* (Suzuki, 1997, Yuma and Hori, 1981), *L. owadai* (Suzuki, 1997), *L. japonica* (Lloyd et al., 1989) are aquatic lives that occur in freshwater marshes or in wet, wooded areas where their larvae have more abundant sources of food. However, *Pristolycus lucifera* is semiaquatic species which larvae have ability to survive under water.

The interesting characteristics of fireflies their bioluminescent behavior showing synchronous flashing while they aggregate and perch on trees and luminous after sunset (Lloyd 1971; Suzuki *et al.* 1996; Copeland & Moiseff 1997). The flashing of certain Southeast Asian firefly species is congregational, long running and precise especially on mangrove tree species, such as *Sonneratia caseolalis* Engler, *Avicennia officinalis* L., *Nypa frutican* Wurmb (Buck, 1988), while North American fireflies are described as non congregational, often local, and/or sporadic (Moiseff and Copeland 2000; Buck, 1988).

One species of fireflies, *Pteroptyx malaccae* Gorham having the interesting characteristics, the synchronous flashing while they aggregate and perch on trees and luminous after sunset. It applicates to maintaining firefly populations as eco-tourism attractions, which are of considerable interest in Thailand. However, the information concerning biology, and rearing has not been available in the literature. Thus, the study provides basic knowledge, which focus on life cycle, morphology and rearing methods of *P. malacca*e, the most species, which is of onsiderable interest in Thailand. With proper conservation practices and the sustainable eco-tourisms, these firefly populations can persist in natural habitats and increase in distribution to include already disturbed or threatened areas.

2. MATERIALS AND METHODS

2.1 Insect Rearing

Male and female adults were collected from Ban-Lam estuary in Samaae chai, Bang-krok , Sub-district, Ban-Lam District located at 5 kilometers from the mouth of Phetchaburi river, Phetchaburi Province, Thailand. The collecting site is a brackish water area in the estuarine habitat with various kinds of mangrove trees where congregations of fireflies are found. Flying or flashing adults on trees were catched by sweepnet at night.

Insect breeding experiments were conducted in a dark room maintained at ambient temperature. Adults (in the ratio of female : male = 1 : 3) were placed in a mating plastic

or glass container (17 x 20 x 9 cm), lined with a suitable substrate for oviposition as soil, fresh leaves, moist filter paper and provided with an apple slice which was refreshed every two days (Moiseff and Copeland, 2000). Dead leaves and small pieces of bark were added as shelter (De Cock and Mathysen, 2005).

When females were placed in the container with more than one male, there was competition among males for mating (Demacy, 2005). Copulation generally lasts for 1-2 hours and females mate only once a night (Lewis and Wang, 1991). Then the female will deposit her eggs on or in the soil.

After egg deposition took place, eggs were removed to an aluminium tray (size 30 x 30 x 6 cm.), with 1/3 soil. The newly hatched larvae and later instars feed on a brackish snail, *Assiminea* sp. until the end of larvae stage. About 4 weeks after hatching, the larvae were more visible and could be transferred to a new tray with about 3 cm depth of soil. Larvae were transferred to a new tray every week after the observation of number and stage until pupation.

Pupae were placed in a small plastic container, with soil. As soon as the adults emerged, they were fed with fresh apple and water until they died.
In this study, we monitored the growth rate after each moult, the duration and the number of larval instars.

2.2 Biology of *Pteroptyx malaccae* Gorham

In this study, life cycle were monitored the growth rate after each moult, the duration and the number of larval instars. For a morphology study, each instar were measured the body length and recorded the morphological characteristics.

3. RESULTS AND DISCUSSION

3.1 Life cycle of *Pteroptyx Malaccae* Gorham

Results of the studies in Table 1 indicated that *P. malaccae* could be reared successfully in dark room at ambient temperature. The average life cycle was 122.90 days. The adults of *P. Malaccae* lived an average of 12.33 days. They mated within 3 - 10 days after emergence and began laying eggs right after. The mated female used their ovipositor to search for oviposition sites. Eggs were laid singly or in a group of 2 - 10 eggs into the soil. Each female deposited an average of 18.30 eggs during their life span and lived another 1 - 2 days before they died.

Three hundred and forty three *P. malaccae* eggs were initially in the life history study. The egg stage lasted an average of 12.15 days. Of 243 eggs developed to first instars, 76 developed to second instars, 61 developed to third instars, and 55 developed to fourth

instars. The larval stage lasted an average of 97.83 days. Only 45 pupae were obtained and the pupal stages lasted an average of 9.83 days. Eventually, 36 adults emerged with 29 females (80.55%) and 7 males (19.44%) or a sex ratio of male : female as 4 :1

The mortality rate during egg stage, first instars, second instars, third instars, fourth instars, and pupal stage were 29.15%, 68.72%, 19.73%, 9.84, 18.18% and 20.00% respectively (Table 1).

Table I. Developmental biology of *Pteroptyx malaccae* Gorham reared at ambient temperature

Stage of development	Number of specimens	Developmental period (day)	Percent mortality
Egg	20	12.15 ± 1.39	29.15
Larval			
First instar	20	25.87 ± 1.81	68.72
Second instar	20	23.10 ± 1.81	19.73
Third instar	20	26.15 ± 1.18	9.84
Fourth instar	20	13.00 ± 1.12	18.18
Total larval stage		97.83 ± 3.56	
Pupal	12	9.83 ± 0.72	20.00
Adult	30	12.33 ± 2.76	-
Male (days)	10	13.70 ± 2.11	
Female (days)	10	10.40 ± 1.35	
Total life cycle		122.90 ± 5.28	-

Generally, when females and males *P. malaccae* were placed in a glass container, males become so aroused by the female that they even try to copulate with other males. While trying to copulate with the female, male probes its posterior genitalia and seeking for a union even after a female has already been copulated with another male. While copulating, female pulls male and faces upward on leaves, stems or clings to a slick glass surface. Sometime the female will drag the male around to find a new perch or walking up stems or leaves where they may escape the attention of other males. Copulation generally lasts for 1 - 2 hours. Then a female will deposit eggs in the soil by probing her ovipositor on or into the crack crevices.

The habitat of *P. malaccae* larvae in nature is in mangrove or brackish water area, composing with high density of mangrove trees affected by tidal movement, where they lived in a damp soil with some substrates such as barks and other organisms. The larvae are principal member of the invertebrate fauna which inhabit the area. They fed on snails, earthworms, larvae of other insects, copepods and probably other soft - bodied animals on and in soil (Mass and Dorn, 2005).

In rearing experiment, newly hatched larvae were fed primary with brackish snails, *Assimenae* sp. Fresh snails were replaced every 2 days. Larvae attacked by climbing into snail, and injecting toxin from their falcate and began feeding when the snail was

immobilized (Buschman, 1984; Labella and Lloyd, 1991). An average of developmental period from first to fourth instars was 91 - 103 days.

During the first instar, one snail was fed on by 3 - 8 larvae (1 - 14 days old) or 2 - 3 larvae (14-30 days old) per day. The first instar larvae are very sensitive due to their small size. Mortality could be high if rearing conditions are not favorable.

During second to fourth instar, larvae were stronger and had higher survival rate. They were voracious and one snail was fed to each individual larva within 1 - 2 days. Larvae can also emit light during the second instars (34 - 40 days after emergence). They always glow and move during night until fourth instars. All instars have a similar shape and color but their width and length of body are different.

The mature larvae became immobile and curled their bodies. When disturbed, the larvae glowed for a short time and occasionally left the pupal cell to build a new one.

P. malaccae larvae were carnivorous, feeding on snails, and slugs. They could crawl on the bottom of the tray and cling to soil which assist the larvae in capturing snails while searching for their snail prey. Similarly, they could live and had ability to survive under water by absorption of oxygen through the membranous cuticle or the caudal grasping organ.

During pupal stage, no food is required. When pupal cells were completed, they pupated underground in the excavated cell. The pupae would glow and wiggle their abdomen when disturbed. Eyes, antennae, legs and wings including light organs were developed and became obvious.

The adult stage, when a pupa shed off its exuviae, the preimago remained in the pupal cell for 2 - 4 hours (Xinhua *et al*, 2005). The elytra of newly emerged adults were pale and very soft. Adults of *P. malaccae* emerged from the pupal cell several hours later. All researches revealed that adults did not feed. (Lloyd, 1997). Females incorporate spermatophore derived protein, “nuptial gift” into developing oocytes during mating (Rooney and Lewis, 1999, van der Reijiden *et al*, 1997; Cratley *et al,* 2003; Lewis *et al*, 2004; Demacy, 2005). The adult of *P. malaccae* died within 12 - 16 days, while *Lampyris noctiluca* died within a week (Mass and Dorn, 2005).

3.2 Morphological Characteristics of *Pteroptyx malaccae* Gorham

The duration of developmental stages which obtained from this study were composed of egg, larvae, pupae, and adults stages. Their morphological appearance and developmental period were recorded for each stage as the followings:

3.2.1 Eggs

The egg of *P. malaccae* was spherical in shape. Newly laid eggs are smooth, pale yellow color and do not change during incubation. An average egg sizes was measured as 0.5 - 0.75 mm. in diameter (Fig. 1A) Eggs were usually deposited singly or in a group of 2 - 10 eggs on or 0.3 - 0.5 mm deep in the soil. Incubation periods of eggs averaged 12.15 days with the ranged of 10 to 15 days.

3.2.2 Larvae

The larval stage of *P. malaccae* was composed of 4 instars, with a similar shape of elongate, slender, sub - parallel - side (tapering a little in front and behind) and slightly flattened. (Fig. 1B).

The first instar larvae were red brown color with a long and narrow body and measured on average of 1.5 mm. They have red brown body segments. Newly hatched larvae were noticeable when they move and began to feed about 2 days after hatching. The second, third, and fourth instar larvae became more obvious. Their lengthes were 4.0 - 5.0, 8.0 - 10.0, and 12.0 - 13.0 mm, respectively. (Table II)

Larvae have three thoracic and nine abdominal segments. The dorsal surface of body segments was black or dark brown with irregular small pale depressed areas on all terga and a narrowly pale median line runs from the anterior margin of prothorax to the posterior margin of body segment. The color of ventral surface was pale cream.

Larvae can emit light since the second instar (34 - 40 days). They have a bilateral pair of light organ on the ventral side which became obvious when glowing.

Before moulting, the larvae have thick and fat body. They curled and changed their bodies to C shaped of pink color within 3 - 4 days in an excavated cell. The larval body was pale pink after moulting and changed to black color within 3 - 5 hours.

Table II. Body length of different larval instar.

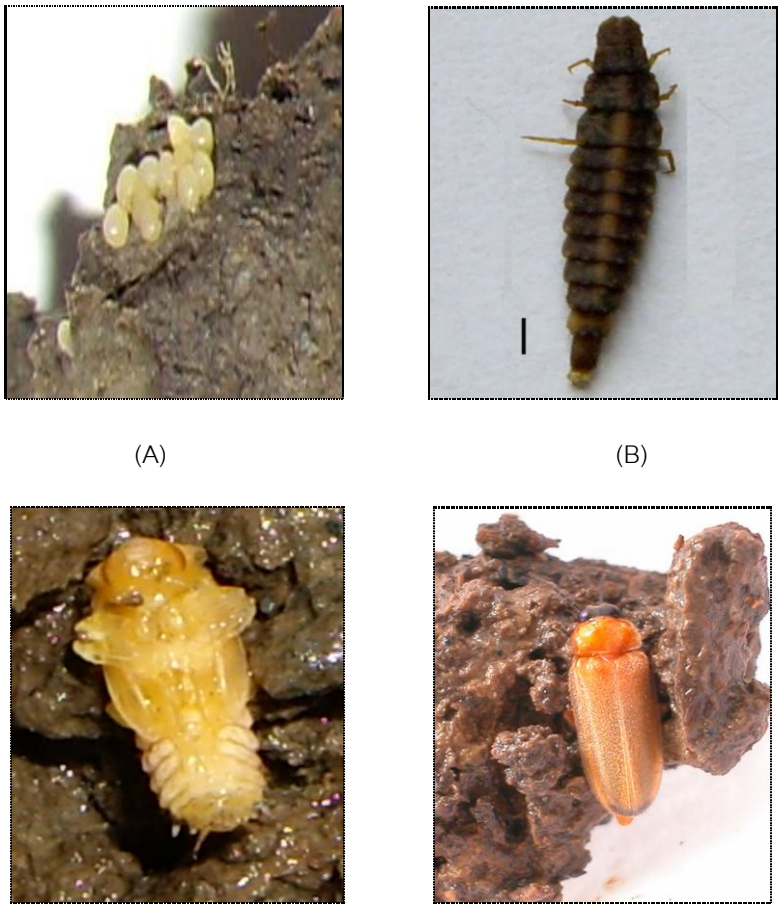
instar	Larval body length (mm)
instar:	
First instar	1.0 - 2.0
Second instar	3.0 - 5.0
Third instar	8.0 - 10.0
Fourth instar	11.0 - 13.0

3.2.3 Pupae

Pupae were exarate type with light yellow color at the time of pupation (Fig. 1C) and measured 6.0 - 8.0 mm in length. The mature larvae climbed into soil and constructed pupal cells to pupate underground in the excavated cell.

Three days after pupation, the compound eyes became brown. The light organs are situated on the abdominal sternum in the form of two round, white spots. At 5 days, the eyes and antennae were black, legs and wings were dark brown and became obvious. The light organ and shape of the last abdominal could be used for sex determination during in this stage; male had 2 light organs and the tip of ventral abdominal segments was U shaped, while female had only one light organ and the tip of ventral abdominal segments was V shaped. The developing adult lanterns became white and their margins distinctly recognizable.

3.2.4 Adults



(A) (B)

(C) (D)

Fig. 1. *Pteroptyx malaccae* in various stages
(A) eggs; (B) larva; (C) pupa; (D) adult

The color of *P. malaccae* was orange brown with elongated and soft body. The body length is 6.5 - 8.0 mm as shown in Fig. 1D. Male is smaller than female. The head segment was dark brown, the compound eyes and the filiform antennae segments were apparently black in color. Adults fireflies have yellow abdominal sternites with a stark white area of light organ at posterior margin of ventral abdominal segments. The females are distinguished from the males by their pointed posterior abdominal segment and ovipositor. Males are observed that they have two light organs on the fifth and sixth abdominal segments, while females have only one rectangular light organ, located on the fifth abdominal segment.

4. CONCLUSIONS

The studied biology of firefly, *Pteroptyx malaccae* provides basic knowledge. In addition to understanding the important aspects of how to minimize know the life cycle and appropriate habitat. With the developed rearing technique, the life cycle could be maintained from generation to generation. The rearing technique allows mass culture under laboratory condition. As well, it probably is useful for eco-tourism attractions, which are considerable interest in Thailand. Although the conservation of firefly species is difficult, because of the specific habitat and many limiting factor in the environment. The effective conservation of *P. malaccae* is feasible by incorporating the basic knowledge in biology and rearing technique.

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