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Captive rearing of *Papilio polymnestor* and *Chilasa clytia* butterflies in the campus of Jahangirnagar University, Bangladesh

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Abstract
Captive rearing of *Papilio polymnestor* (Blue mormon) and *Chilasa clytia* (Common mime) were carried out in the campus of Jahangirnagar University, Bangladesh during March 2016 to October 2016. The *Citrus limon* and *Citrus maxima* were used as larval host plants for *P. polymnestor*, while the *Litsea glutinosa* was used as larval host plant for *C. clytia*. The egg was laid singly by *P. polymnestor* on the underside of leaves of the host plant, whereas *C. clytia* was preferred to lay egg singly on the bottom of the tender leaves. The duration of different developmental stages (egg, larva and pupa) of these two butterfly species were recorded. *P. polymnestor* and *C. clytia* took 36.4±0.22 and 31.6±0.22 days respectively to develop from egg to adult. The average temperature and relative humidity during the rearing period were 33°C±1.04 and 81±1.21%. Captive rearing may be a part of the revival program for many threatened species; therefore, this study may be helpful in the process of conservation of endangered butterflies and in developing ecotourism industry in Bangladesh.

Keywords: Captive rearing, butterfly, *Papilio polymnestor*, *Chilasa clytia*, JU campus

Introduction
Butterflies are fascinating creature due to their vivid coloration and important roles in the ecosystem. Adult butterflies accomplish pollination in the environment of many economically important plants [1]. They are also used in the food chain, as prey for birds, bats, lizards and various other animals [2-4]. Besides, it is a good biological indicator because they are very much related to their host plants. Butterfly species diversity intimately related to rich in plant diversity in a given area, hence they are indicators to study the ecological balance in an ecosystem as well [5].

There are approximately 20,000 species of butterflies throughout the world [6, 7]. While there are only about 400 species of butterflies exist in Bangladesh [8, 9]. Among them, 305 species have been well documented and their threat status was determined by the IUCN-Bangladesh [8-13].

Adult butterflies consume all sorts of nectar as their prime food, but their caterpillars need plant leaves which are called larval host plants. It is believed that the conservation of butterflies is related to the conservation of larval host plant [14]. During oviposition, the female carefully examines the leaves and parts of the plant so that the caterpillar does not have to search for appropriate host plant [15]. Forests are the habitat of butterflies and these forests contain the larval food plant for these creatures and due to their habitat destruction along with changes in climate and weather patterns, the abundance of butterfly is declining day by day [13]. The rate of survival of butterflies in nature is very low, which is about 5-10% [16]. On the other hand, captive rearing of *Danaus* chrysippus in the campus of Jahangirnagar University, the survival rate was achieved 35.04% [17]. Moreover, around 200 butterflies were released into nature following this captive rearing scheme [17]. Consequently, this effort has the potential to play a significant role in the recovery of endangered butterflies. Considering the above facts, for certain invertebrates, rearing in captive condition are increasingly being suggested as a very helpful and cost-effective conservation tool. [18]. Therefore, the present study was undertaken to standardize the rearing protocols of *Papilio polymnestor* and *Chilasa clytia.*

Materials and Methods

Study area

This study was carried out in the Butterfly park and research centre (BPRC) (23°52'33 N 90°16'6 E) of Jahangirnagar University campus (JU campus), Bangladesh. Adult butterflies of *P. polymnestor* and *C. clytia* were collected from different habitats of JU campus by insect sweeping net. The identification of butterflies was conducted by using the keys of Bingham (1905, 1907), Wynter-Blyth (1957) and Talbot (1939, 1947) [19-23].

Larval host plants

The plant *Citrus limon* and *Citrus maxima* were used as larval host plants for *P. polymnestor* while the *Litsea glutinosa* was used as larval host plant for *C. clytia* (Fig. 1A, B, C). The "Pictorial guide to nursery plants" [24] and "Encyclopedia of flora and fauna of Bangladesh" [25] were used for the identification of those plants.

Rearing time and method

The study was conducted during March 2016 to October 2016. The average temperature and relative humidity during the rearing period were 33°C±1.04 and 81±1.21%. The plant *C. limon*, *C. maxima* and *L. glutinosa* were planted in the earthen pots. A net cage (height 6ft× width 4ft× width front side 5.5ft) was prepared for the rearing of the butterflies which was covered by a mosquito net. When the larval plants attained a height of about 2-2.5ft then two host plants along with the earthen pots were placed inside the net cage. One pair of wild male and female were captured from the nature and then left in the cage provided with their nectar plants. The net cage was placed in such place where the direct sunlight was reached. Butterflies were provided with sugar solution, placing into a plastic plate soaked in cotton along with their nectar plants. One pair of male and female mate in the net cage and female lay egg singly on the young leaves of the larval food plant. The eggs were measured by a microscope equipped with measuring scale. 10 plastic boxes were prepared for the larval rearing which covered with mosquito net. After hatching out from egg to larva, the larva was transferred on the plastic boxes and provided with available fresh green leaves of the respective larval food plants. Brush was used to transfer very small larva and blunt forceps to transfer larger one. Overcrowding was avoided in a box with keeping a limited number of larvae. The excreta of larvae cleared properly twice in a day. Sometime larvae were affected by bacterial diseases and died, the affected and died larvae were immediately removed to avoid spread of the diseases. Caterpillars went to pupation on the plastic boxes. Pupal changes were determined every day in the box. The lengths of the egg, each instar of the larva and pupa were measured by the scale and duration was counted. The study of developmental period of each species was replicated 5 times.

Fig 1: Larval host plants of *P. polymnestor* [A (*C. limon*), B (*C. maxima*)] and *C. clytia* [C (*L. glutinosa*)

Results and Discussion

Captive rearing of *Papilio polymnestor* and *Chilasa clytia* were carried out in the campus of Jahangirnagar University, Bangladesh. The average temperature and relative humidity during the rearing period were 33°C±1.04 and 81±1.21%. The results on captive rearing of these two butterflies are given below:

Captive rearing of *P. polymnestor*

P. polymnestor has four stages in the life cycle viz. egg, larva, pupa and adult. Among them, larva had five instars. *C. limon* and *C. maxima* were used as larval host plants for *P. polymnestor* (Fig 1 A, B).

Egg: The eggs were laid singly on the underside of young leaves. The spherical egg was pale creamy yellow with a diameter of about 1.5-1.8mm (Fig. 2a). The incubation period was with an *average* of 3.4±0.22 days (Table 1) and the newly hatched larva was a body length of about 3-3.5mm.

Larva

Instar I: The newly hatched larva was transparent, grayish white on the dorsal side and laterally, it was dark brown with faded whitish markings. Afterward, the whitish dorsal markings changed to greenish brown with distinct white markings on the prothorax and subsequent abdominal segments. After an average of 3±0 days (Table 1), the larva molted to the next instar (Fig. 2b). The larva measured up to 5-6mm in length.

Instar II: The larva measured 15-16.5mm in length and the larval duration lasted an *average* of 3.6±0.22 days (Table 1). The color of the body was distinct greenish yellow with prominent white markings on the anterior, middle and posterior body segments (Fig. 2c).

Instar III: The larva attained to a maximum of 20-22mm in length. There was no drastic change in physical appearance except that the larva became greener in color (Fig. 2d). This instar lasted an *average* of 4±0 days (Table 1).

Instar IV: The next molt was transferred the caterpillar to its 4th instar with a drastic change in appearance. The body color was becoming bright green with cryptic markings mixed with white bands throughout the body (Fig. 2e). The body length attained about 32-34mm. This instar lasted an *average* of 3.6±0.22 days (Table 1).

Instar V: The larva attained a maximum of 43-45mm in length (Fig. 2f) and lasted an *average* of 3.4±0.22 days (Table 1). The body color was bright green with two eye spots on the third thoracic segment and, on the abdominal segments 1 and 2, a transverse band was present. Moreover, oblique bars were present on the mid-abdominal segments. Eventually, on the surface of a leaf, the caterpillar came to rest in an upright position (Fig. 2f).

Pupa: Pupa was greenish with large yellowish marking. It has cephalic horns and humped in the thoracic area (Fig. 2g). The pupa measured up to 37-38mm in length and fully developed adult normally emerged out after an *average* of 15.4±0.22 days (Table 1).

Adult*:* At the time of emerging from pupa to fully grown adult, pupal cage split at the head, warning legs were thrust out and the insect hauls it out of the case (Fig. 2h). At that time, the wings were shrunken, cramped and wet as well as the abdomen distended. After that wing span was expanded to the normal size rapidly. It took an *average* of 36.4±0.22 days from egg to adult (Table 1).

Revathy and Mathew (2013) [26] reported an average of 43±1.80 days from egg to adult, while this value was 36.4±0.22 days in the present study (Table 1). Ecological factors, especially temperature, humidity and availability of larval host plants might be the possible determinant to influence the different development period of the butterflies. In the present study, an average temperature and relative humidity were 33°C±1.04 and 81±1.21%, while Revathy and Mathew [26] didn't mention any information regarding these two parameters. In the present study, *C. limon* and *C. maxima* were recorded as the larval host plants of *P. polymnestor*, whereas over 10 larval host plants of the Rutaceae family were reported by Revathy and Mathew [26]. In any adverse situation, this wide host plant range provides opportunities for a better adaptation of a butterfly [27].

Table 1: Duration (days) of different developmental stages of *P. polymnestor* and *C. clytia*

Developmental stages		*P. polymnestor* Mean Duration (Days) (±)SD	*C. clytia* Mean Duration (Days) (±)SD
Egg		3.4±0.22	2.8±0.18
Larval stages	Instar I	3±0	3±0
	Instar II	3.6±0.22	3.2±0.18
	Instar III	4±0	3.6±0.22
	Instar IV	3.6±0.22	3±0
	Instar V	3.4±0.22	4±0
Pupa		15.4±0.22	12±0
Total development period		36.4±0.22	31.6±0.22

*Each data represent the mean of five replications

Fig 2: Different developmental stages of life cycle of *P.polymnestor* (a-h)

Captive rearing of *C. clytia*

C. clytia has four stages in the life cycle viz. egg, larva, pupa and adult. Among them, larva had five instars. *Litsea glutinosa* was used as larval host plants for *C. clytia* (Fig. 1 C).

Egg: The eggs were laid singly on the young leaves of the host plant. It was spherical and creamy white. The surface of the egg coated with a rough layer of orange-yellow granulated materials (Fig. 3a). Diameter of the egg was 1.5-1.8mm. The egg took with an *average* of 2.8±0.18 days to hatch into 1st instar larva (Table 1).

Larva

Instar I: The 1st instar larva attained to a length of about 6-7mm. After an *average* of 3±0 days, the 1st instar larva was molted to the 2nd instar and the dark lateral markings were decolorized and disappeared (Table 1). There was a white saddle and white patch on the 3rd-4th abdominal segments and on the posterior abdominal segments respectively (Fig. 3b).

Instar II: This instar lasted an *average* of 3.2±0.18 days (Table 1). The body length reached about 11-12mm before the next molt. The 2nd instar larva was a similar appearance to the 1st instar larva. The body processes became long and stout with the bright orange color on dorsal patches. Moreover, white color on the saddle and posterior abdominal segments were very much distinctive (Fig. 3c).

Instar III: The 3rd instar larva has taken an *average* of 3.6±0.22 days to transfer to the next instar and reached the body length about 20-20.5mm (Fig. 3d) (Table 1).

Instar IV: The 4th instar larva contained distinct white markings on its body. These white markings had extended gradually to the whole of the abdominal segments. On the other hand, white lateral patches appeared on the thoracic segments of the body (Fig. 3e). This instar lasted with an *average* of 3±0 days with the body length reached about 30-32mm (Table 1).

Instar V: The 5th instar larva lasted an *average* of 4±0 days and the body length reached up to 50-52mm (Fig. 3f) (Table 1).

Pupa: The pupa was resembled a broken twig and about 40-42mm in length (Fig. 3g). It looked brownish with streaked and blotched. Average incubation period of pupa was 12±0 days (Table 1).

Adult: After completed the pupation period, a full-grown adult emerged out of the pupal case (Fig. 3h). It took an *average* of 31.6 ± 0.22 days from egg to adult (Table 1).

In the present study, the development period is consistent with Revathy and Mathew's (2014) [28] finding for *C. clytia* (Table 1). On the other hand, Revathy and Mathew [28] *reported Litsea coriacea (in addition to L. chinensis and L. deccanensis), as a larval host plant for C. clytia*, whereas in the present study it was *L. glutinosa*. These diverse host plants facilitate the butterflies for their better survival.

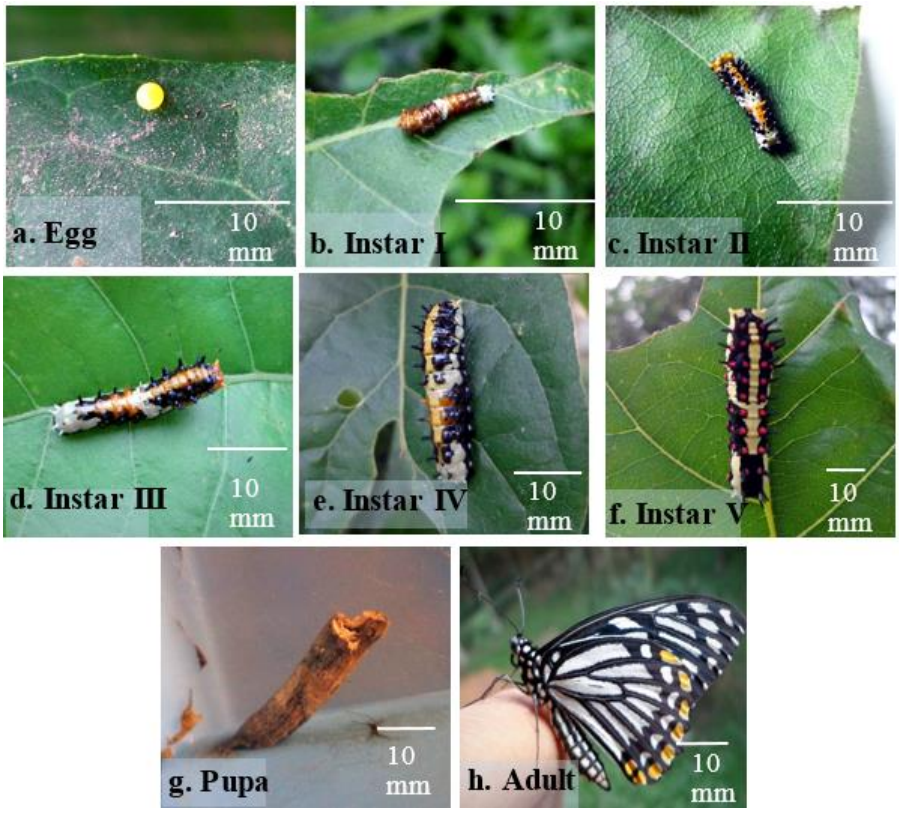

Fig 3: Different development stages of life cycle of *C. clytia* (a-h)

Works on captive rearing of butterflies is scanty in Bangladesh [14, 17, 29, 30]. In Bangladesh, declining the population of butterflies and their destruction of natural habitat occurs at an alarming rate [13]. On the other hand, captive rearing has the potential to boost declining populations [16]. Besides the ecological importance there are other aspects of captive rearing, which is butterfly farming or/and ecotourism industry. Butterfly farming/ecotourism can

be beneficial in providing local employment and reduces pressure on the beneficial insects [31]. Ecotourism by butterfly has become common in many countries including Singapore, Philippines, Thailand, Malaysia, Taiwan, Tanzania, Kenya, Papua New Guinea, Costa Rica, Guyana and Mexico [32-38]. Thus, this piece of work will be an essential step to conserve the endangered butterfly and in developing ecotourism industry in Bangladesh.

Conclusion

The present study was carried out on *P. polymnestor* and *C. clytia* to standardize their rearing protocols. These results of captive rearing may be helpful for the conservation of endangered butterflies and in developing butterfly ecotourism in Bangladesh.

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