Univ. j. zool. Rajshahi. Univ. Vol. 36, 2017 pp. 49-56

http://www.ru.ac.bd/zoology/university-journal-of-zoology-rajshahi-university/

ISSN 1023-6104

Biology and morphometrics of the common mormon butterfly, *Papilio polytes* Linnaeus (Lepidoptera: Papilionidae) rearing in laboratory condition

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Abstract: Biology and morphometrics of common mormon butterfly (*Papilio polytes*) was studied on Citrus leaves under laboratory condition. The incubation period was 3.54±0.45 days followed by five larval instars covering a total period of 15.5±0.89 days (3.17 ± 0.59 days for first, 2.47±0.61 days for second, 2.47±0.49 days for third, 2.77±0.73 days for fourth and 4.62±1.16 days for fifth instar). Pre pupal and pupal period lasted 1.03±0.12 days and 10.58±0.47 days, respectively. The development of *P. polytes* from egg to adult took on an average of 30.65±2.91 days. The entire life cycle completed on an average of 39.29±3.16 days and 42.57±3.83 days and have no statistical significant difference on life cycle durations (P>0.05) of male and female, respectively. Mortality rate for larval stages (1st to 5th instar) were 7.61%, 4.71%, 7.41%, 9.33% and 4.17% and mortality at pupal stage was recorded as 7.69%. The female butterfly lived longer (11.92±8.24 days) than male (8.64±7.33 days) and longevity of male and female were not statistically significant (P>0.05). The morphometric variations of different life stages of the *P. polytes* butterfly were recorded.

Key words: Biology, morphometrics, rearing, butterfly, *Papilio polytes*.

Introduction

Papilio polytes is a common species of swallowtail butterfly which both sexes have tail (lobe like structure) at the end of the hind wing and belongs to the family Papilionidae. It is a tropical or subtropical butterfly distributed from Southeast Asia to Southwestern Island of Japan (Corbet & Pendlebury, 1992; Nakayama *et al*., 2003 & Nakayama & Honda, 2004). The male of this species is monomorphic while the female is polymorphic (Corbet & Pendlebury, 1992; Otsuka, 2001). The larval/caterpillar stages of *P. polytes* most serious destructive, which cause the considerable economic damage to various citrus plants as well as various crops of agriculture and forestry.The caterpillar has wide varieties of feeding adaptation and this feeding adaptation has made the *P. polytes* a serious pest in citrus orchard and young plantations (Revathy & Mathew, 2014). The caterpillar is a foliage feeder prefers blossoms and young nurseries of host plants (Gaikwad *et al*., 2011). In Bangladesh, *P. polytes* is identified as minor pest of citrus plants, *e.g*. lime, lemon, shaddock, mandarin, shatkora, adajamir and rough lemon but it has the potential to become a major pest because it shows rapid Population growth under favorable environment. It is the serious and regular pest of curry leaf, *Citrus* spp. of tropical region (Corbet & Pendlebury, 1992) and other plants of rutaceae (Haribal, 1992; Gunathigalraj, 1998). Information on the biology of *P. polytes* under laboratory condition in Bangladesh and their morphometrics are poor. So, the present study was undertaken to know the biology and morphometrics of *P. polytes*, which would strengthen the information in relation to butterfly biology and morphometrics in Bangladesh. The result will also be useful to control and conserve this species in this country.

Materials and Methods

The study was carried out in the laboratory in Radiation Entomology and Acarology Division at the Institute of Food and Radiation Biology (IFRB) in Atomic Energy Research Establishment (AERE); Dhaka, Bangladesh. Adult male and female butterflies were collected from AERE campus. After collection male and female butterflies kept in rearing cage (44cm×38cm×38cm) covered with mosquito net. The butterflies were fed with 10% sugar solution daily to ensure

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reproductive success. For oviposition young branches of host plant **(***Citrus* sp.**)** placed inside the cage. Two 100 watt incandescent bulbs were inflamed from outside of the cage during the oviposition time only. The branch with egg was kept in plastic container (26 cm×17 cm×7 cm) for hatching. Newly hatched larvae were separated and placed in 10 plastic containers (21.5 cm×13.5 cm×10.5 cm) and each container 10 larvae with citrus leaves. To keep the leaves fresh, a moist piece of cotton was placed around the cut tip and wrapped with aluminum foil. The leaves were changed at interval of 24 hours for the first, second and third instars but in case of fourth instar leaves were replaced interval of 12 hours and for the fifth instar leaves replaced interval of 8 hours during the rearing time. Morphometric study of different life stages was carried out by taking 10 replication of each stage, *viz*. egg, first instar, second instar, third instar, fourth instar and fifth instar larvae as well as pre-pupa, pupae and adults for linear measurements. In addition to the above parameters color, shape, size, molting, feeding behavior and duration of eggs, larvae, pupa and adults were also recorded. The entire experiment was maintained at mean temperature of 29.57°C (±1.59) with mean relative humidity 72.5 % (±4.95) and the study was conducted from August to October 2018. Statistical analysis was performed using Student's *t*-distribution under the null hypothesis.

Result and Discussion

The results of duration of different stage, mortality and morphometrics data of *P. polytes* on citrus leaves are presented in Table 1, 2 and 3.

Egg: Female *P. polytes* deposited egg singly on young stem, ventral and dorsal side of young leaves of the host plant. For the egg laying, female butterfly hold the leaves with the help of legs, wing arranges in vertically and bending the abdomen placed a single egg by the ovipositor. The egg was small and spherical with a diameter 0.94-1.3 mm (1.12±0.13mm). Gaikwad & Bhawane (2013) found that the egg diameter was 0.98mm to 1.12mm and Tan (2011) reported egg nearly spherical with a diameter of about 1.2mm. The newly laid eggs seem smooth in appearance but finally appearance turn finely roughened surface. The freshly laid eggs greenish-yellow in color (Fig. 1A) but after one day it turns into pale yellow to creamy in color with black taint on the surface of the egg (Fig.1B). The color of eggs become brownish black in the third day (Fig.1C). The incubation period was 3.0- 4.0 days on an average of 3.54±0.45 days. The more or less similar incubation period of *P. polytes* was found as 3-5 days (Revathy & Mathew, 2014; Halloran & Wason 2013), 4.0 days (Gaikwad & Bhawane 2013) and 3.0 days (Tan, 2011).

Larvae/Caterpillar: *P. polytes* have complete metamorphosis and passes through distinct egg, larval, pupal, and adult stages. In the present study, five instars of larvae were observed. Instar wise description and duration of these larval stages are given below.

First Instar: The first instar larva eats its egg shell before commencing to feed on the leaves of the host plant. The newly hatched larvae was yellowish dark brown dorsally and dark brown in laterally. Two off-white bands encircle the first and last abdominal segments. The larvae with small and large setae present in the head and lateral sides of the body. A row of tubercles present lateral sides of last two abdominal segments. Head with prominent shallow grooves sutures, two conspicuous horns (primary setae) and hypognathous mandibles. The last abdominal segment bears whitish-brown caudal horns (Fig. 2A). The length of the first instar larvae was 3.0-5.0mm (4.09±0.65 mm). The duration of the first instar larvae was found 3.0-5.5 days on an average of 3.17 ± 0.59 days that was longer than ranges reported in the literatures, specifically 2.60±0.54 days reared on curry leaves (*Murraya koenigii*) at 38- 45 °C by Ellarao *et al*., (2016), 2.71±0.13 days on bael (*Aegle marmelos*) leaves and 2.51±0.11 days on citrus leaves (*Citrus medica*) at 27±1°C by Shobana *et al*., (2010).

Second Instar: The newly molted second instar larvae look like as the late of first instar larvae excluding more extricate white markings on the middle and posterior body parts. In connection with age of larvae the white patches become more widened dorsally and laterally of the body. Just before the second molting moment the body color turned into darker and anterior part of body heighten and widened than posterior part of the body. A series of quadrilateral structure found from 1st thorax segment to last abdominal segment. The quadrilateral structure of abdominal parts more conspicuous than that the quadrilateral structure of thoracic parts (Fig. 2B). It measured with 5-10 mm (7.33±1.41 mm) and the second instar continued for 2.0-4.0 days on an average 2.47±0.61days. Salmah*et al*., (2007) showed that duration of second instar as 2.20±0.07, 2.07±0.05 and 2.17±0.07 days on leaves of different host plants, *Citrus*

aurantifolia, C. hystrix, and *C. reticulate,* respectively which were more or less similar in our study.

Third Instar: After the second molting, the larvae called third instar**.** This instar was darker with distinguish white furrow lateral sides of the middle and posterior body segments and the more prominent whitish-brown saddle marks on the dorsal side. Dorsally three pairs of white-bluish spots present on the second to fourth abdominal segments. Caudal horns were thicker, conspicuous and color is snow-white looking. One pair of white spiky projection visible dorsally on the beginning of the fifth abdominal segment (Fig. 2C). Toward the end of this stage the body color changed into grayish-green. The third instar spent 2.0-3.0 days (2.47±0.49 days) and the body length reaching up to 12.0- 17.0 mm (14.03±1.33mm). Similar duration of third instar larvae (2.50±0.10, 2.07±0.05 and 2.33±0.09 days) on leaves of different host plants, *Citrus aurantifolia, C. hystrix,* and *C. reticulate* were recorded by Salmah *et al*., (2007).

Fourth Instar: This instar larvae look like to the late third instar larvae ordinarily but has greasy appearance with greenish coloration and lateral white streak enclosed the first and second abdominal segments entirely. The third thoracic segment bears four conspicuous white-bluish spots. As growth continues the enigmatic markings of light to dark green amalgamated with white streaks becomes increasing molted. In this instar, caudal and cephalic horns molted and molted horns were looked as jelly like spiky projection (Fig. 2D). Fourth instar expended 2.0-5.0 days (2.77±0.73 days) and body length was 18.0-28.0 mm (22.76±2.94mm). Salmah *et al*., (2007) reported that the duration of fourth instar larvae were 3.17±0.07, 2.93±0.11 and 2.83±0.07 days on leaves of different host plants, *viz*. *Citrus aurantifolia, C. hystrix,* and *C. reticulata* respectively and also found 3.07±0.11days on curry leaves (*Murraya koenigii*).

Fifth Instar: The first to fourth instar larvae were more or less similar appearance except size and color but the fifth or final instar larvae bears drastic change in physical appearance from previous instar. The newly exuded larvae become light green but after one day this color gradually changed into pure green. Two transverse sinus marking bands present dorsally; one is connected two eye spots of third thoracic segment and another one occurs between third and the first abdominal segment. A pastel purplish bluish slits existing in the sinus designs. Kohl like stripe arises just behind the last thoracic segments. In the earlier phase of the fifth instar larvae, 3 pairs bluish spots present; one pair on third thoracic segments, rest of two pairs on third and fourth abdominal segments, respectively but later stage bluish spots disappear from the body. Six oblique black bars present in the either sides of the abdominal segments; first bars both each side run from the base of the abdominal segments three to the top of segment four**,** second bars is present each sides of six abdominal segments and third bars is much shorter and occurred at the two sides of the last abdominal segments (Fig. 2E). Although the larvae bears legs all instar but in the 5th instar, thoracic (true legs) and abdominal legs (pseudo legs) more conspicuous than from the previous instars. Thorax has three segmentations and abdomen have eleven segmentations but nine to eleven segments fused together. Each thoracic segment bear one pair of legs but in case of abdominal segmentations only 3, 4, 5, 6 and fused segments (9-11) bears prolegs. Thoracic legs whitish and spongy at the base and bears hooks like structure. All the prologs are stumpy, whitish and spongy. Observation made on the larval duration of the final instar 2.5-8.0 days on an average 4.62±1.16 days. In the early stage body length was 28.0-35.0mm (30.94±2.05mm) and the length of final stage this instar was 39.0-50.0 mm on an average of 45.8±3.74mm. Before pupation the larvae stops feeding and passes into pre-pupal stage. Shobana *et al*., (2010) recorded that the duration of fifth instar larvae of *P. polytes* varied from 4.23±0.16 to 4.61±0.08 days on *Murraya koenigii, Citrus medica, Toddalia asiatica, Glycosmis pentaphylla* and *Aegle marmelos* leaves at 27±1 °C. Among the five instars the fifth instar lasted longer period and these findings similar with larval duration of above mentioned literatures. The voraciously feeding by larvae started from the fourth instar and continued till fifth instar before pupating. The fifth instar larvae consumed the highest amount of lemon leaves and similar result also reported by Karim *et al*., (2007).

Total larval period: The duration of total larval period (first to fifth instar) was on an average of 15.5±0.89 days and ranged from 14.0 to 25.5 days, similar to that reported by Ellarao *et al*., (2016) and Halloran & Wason (2013). But the some literatures showed wide range of larval periods, specifically 18.44±0.80 days by Shobana *et al*., (2010), 20.0-23.0 days by Revathy & Mathew (2014), and 20.94 days by Gaikwad & Bhawane (2013). The

longer periods may be due to the cool-weather larval development or in the different temperatures ranges.

Pre-pupa: Toward the end of the fifth instar larvae stopped the feeding and take shelter far from the feeding site. The large amount of excreta and water spilled from the body. The body gradually shortens in length and attaching in substrate with frontal and posterior parts of the body by bending upwards mid-dorsally. This hanging stage looks like as looping structure of hydra locomotion (Fig. 3A). Pre-pupal period lasted 1.0 to 1.5 days (1.03±0.12 days) but most of the cases the pre-pupal period was 24 hours. Gaikwad & Bhawane (2013) & Shobana *et al*., (2010) recorded 1.0 & 0.99±0.02 days and Salmah *et al*., (2007) found that the1.0 days for pre-pupal duration of *P. polytes*.

Pupa: Following the pre-pupa the pupation takes place 24 hours later. Pre-pupa and pupal stages differ significantly from their morphological characterisitics. The pupa of *P. polytes* is called chrysalis which dangles itself with a black silk sash from the stem. The silk sash ascends from mid ventral abdominal parts and posterior side of the caudal horn. Two pupal colors, *viz*. green and brown were found. The green color pupae predominantly green with two yellowish diamond patches on the dorsum of the abdominal segments (Fig. 3B). In the brown pupa was mainly grayish to darker shades of brown (Fig. 3C). Each pupal characteristic with a dorsum thoracic hum, a pair of cephalic horns and bloated of abdominal side view. The pupa turned into black in color before eclosion. First and third day old pupal weight were 1.18-1.35gm (1.28±0.057) and 0.93-1.18gm (1.04±0.084), respectively. Pupal length was 25.0-32.0 mm (29±2.26mm). We found the length of pupal stage 10.0-11.0 days with an average of 10.58±0.47 days which was similar to that reported in the literature specifically, 10.30 days in Gaikwad & Bhawane (2013), 10.80±1.30 days in Ellarao *et al*. (2016), 10.02±0.56 days in Shobana *et al*. (2010) and 9.6-10.3 days in Salmah *et al*. (2007). However, it differs slightly from the observation of Revathy & Mathew (2014) who reported 12.0-15.0 days. The pupal duration can be varied more or less depending on the temperature and relative humidity of the study area.

Adults: Both sexes of adult *P. polytes* were tailed. The male was black. Forewing has terminal series of white spots, decreasing in size towards the apex and hind wing with complete discal band of elongated white, which were well separated from each other (Fig. 4A). The female, fore wing with pale streaks between the veins. Hind wing with 4 elongated white spots (2 spots in middle of 4 were more elongated) adjacent to the cell and a paler series of narrow red spots on termen (Fig. 4B). The female butterfly was larger in size than male. Male wingspan varied from 77.0mm to 96.00 mm (87.4 ± 6.15mm). Whereas, the wingspan of female varied from 87.0 mm to 105.0 mm on an average of 95.2 ± 6.19 mm. The longevity of adult butterfly was counted from the emergence of the adult till its death. The male lived from 4.0 to 21.0 days (8.64±7.33 days) while the longevity of female ranged from 4.0 to 26.0 days (11.92±8.24 days). Shobana *et al*. (2010) found that the longevity for male and female were 4.38±0.17 and 6.81± 0.26 days respectively, which is shorter than the present findings. The development of *P. polytes* from egg to adult took on an average of 30.65±2.91 days. These observations are more or less in agreement with those reported by Halloran and Wason (2013), Tan (2011), Shobana *et al*. (2010) and Salmah *et al*. (2007). The present study revealed that the *P. polytes* complete its life cycle (egg to the death of adult) separately for male 39.29±3.16 days and female 42.57±3.83 days. Gaikwad & Bhawane (2013) recorded the total duration of life cycle of *P. polytes* as 40.38 days, Halloran and Wason (2013) reported this as 30.0 to 43.0 days and Shobana *et al*. (2010) reported 35.0 and 36.50 days for male and female respectively. Out of four life stages of *P. polytes*, larval period was longest and only it's larval stage damaging the host plant.

Mortality rate: In the present study mortality rates of immature stages were 8.0% (eggs), 7.61% (first instar), 4.71% (second instar), 7.41% (third instar), 9.33% (fourth instar), 4.17% (fifth or final instar) and 2.86% (pre-pupal stage). The mortality rate of pupal stage was 7.69%. Halloran and Wason (2013) found highest mortality during the egg stage, Suwarno (2010) reported highest mortality at the fifth instar but this rate may differ depending on predators, parasitoids, weather and also human error.

Conclusion

The present findings on the biology of common mormon are important to develop proper management strategies as well as conservation of butterflies in Bangladesh. Bangladesh environmental conditions would be favorable for the growth and development of common mormon.

Table 1. Durations of different developmental stages of *P*. *polytes*.

Developmental stages	Duration (Days)		
	Minimum	Maximum	Mean±Sd
Incubation period	3.0	4.0	3.54±0.[45]
Larval period			
First instar	3.0	5.5	3.17±0.59
Second instar	2.0	4.0	2.47±0.61
Third instar	2.0	3.0	2.47±0.49
Fourth instar	2.0	5.0	2.77±0.73
Fifth instar	2.5	8.0	4.62±1.16
Total larval period	14.0	25.5	15.5±0.89
Pre-pupa	1.0	1.5	1.03±0.12
Pupa	10.0	11.0	10.58±0.47
Total (Egg to adult emergence)	28.0	38.0	30.65±2.91
Longevity			
Male	4.0	21.0	8.64±7.33[a]
Female	4.0	26.0	11.92±8.24[a]
Life cycle (egg to the death of adult)			
Male	32.0	59.0	39.29±3.16[a]
Female	32.0	64.0	42.57±3.834[a]

Means followed by the same letter within a Column are not significantly different (Student *t*-test; P≥0.05).

Table 2. Mortality (%) of different life stages of *P*. *polytes*.

Life stages	Total Mortality	Mortality (%)
Egg	8	8.0
First instar	7	7.61
Second instar	4	4.71
Third instar	6	7.41
Fourth instar	7	9.33
Fifth instar	3	4.17
Pre pupa	2	2.86
Pupa	5	7.69

Table 3. Morphometrics analysis of different life stages of *P*. *polytes*.

Developmental stages	Minimum	Maximum	Mean±Sd
Egg (mm)	0.94	1.13	1.12±0.13
Larvae length (mm)			
First instar	3.0	5.0	4.09±0.65
Second instar	5.0	10.0	7.33±1.41
Third instar	12.0	17.0	14.03±1.33
Fourth instar	18.0	28.0	22.76±2.94
Fifth instar	28.0	35.0	30.94±2.05
Fifth instar (late stage)	39.0	50.0	45.8±3.74
Pupal length (mm)	25.0	32.0	29±2.26
Pupal weight (1st day) gm	1.18	1.35	1.28±0.057
Pupal weight (2nd day) gm	0.93	1.18	1.04±0.084
Male Wingspan (mm)	77.0	96.0	87.4±6.15
Female Wingspan (mm)	87.0	105.0	95.2±6.19

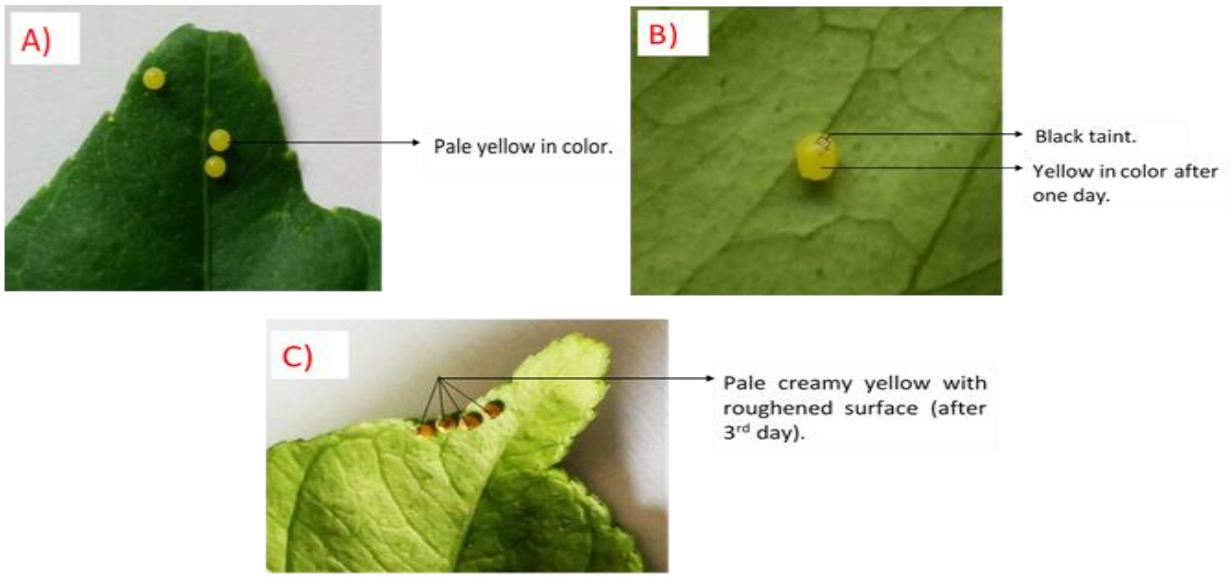

Fig. 1. Egg stages; A) Fresh egg (just after laying), B) 2nd day old egg and C) 3rd day old egg.

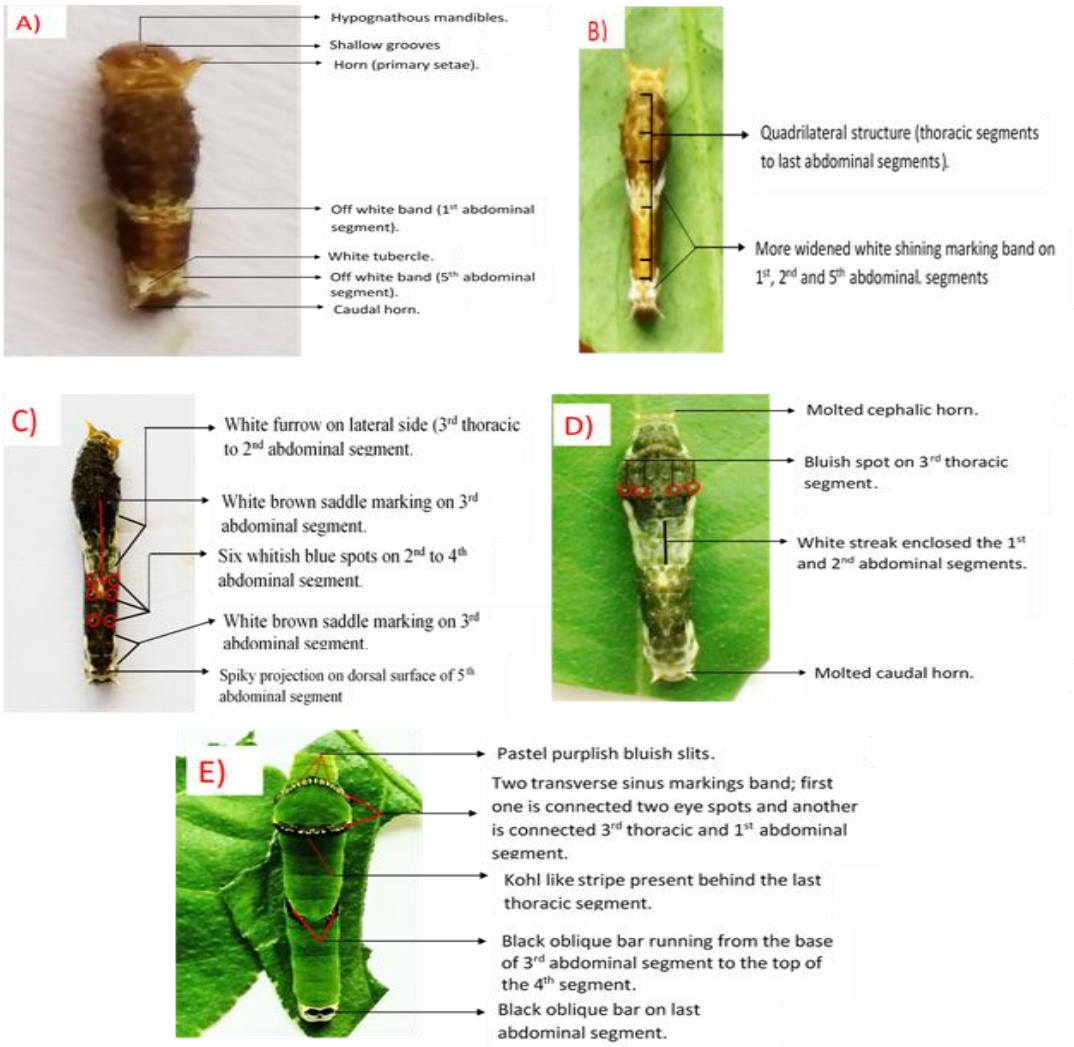

Fig.2. Caterpillar; A) 1st instar, B) 2nd instar, C) 3rd instar, D) 4th instar and E) 5th instar.

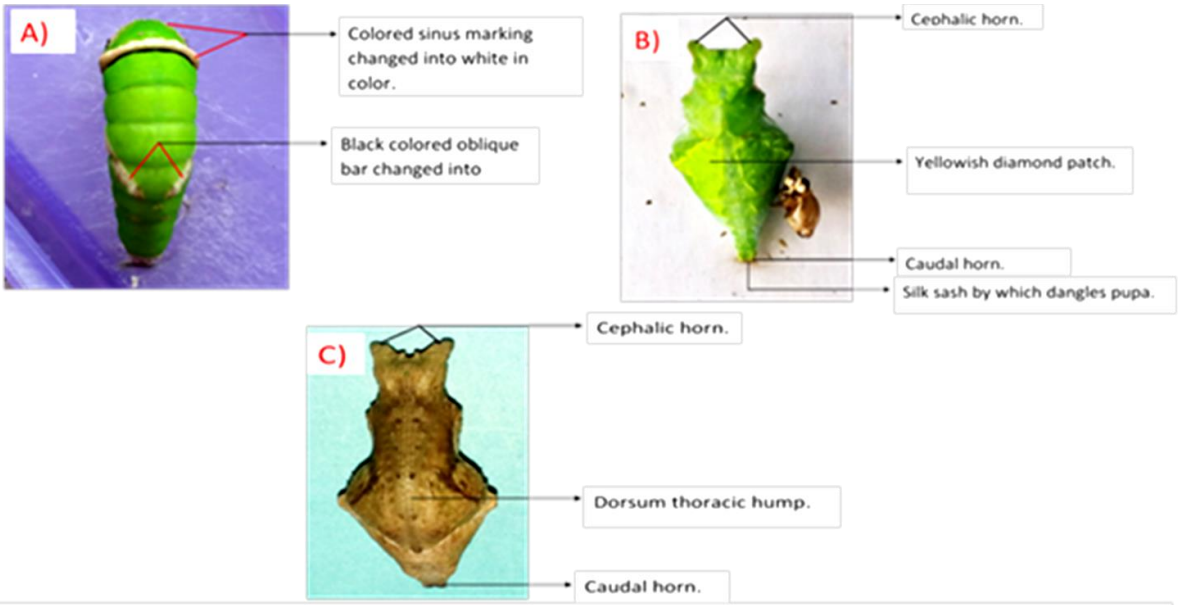

Fig. 3. Pupa; A) Pre-pupa, B) Green pupa (ventral view) and C). Brown pupa (dorsal view).

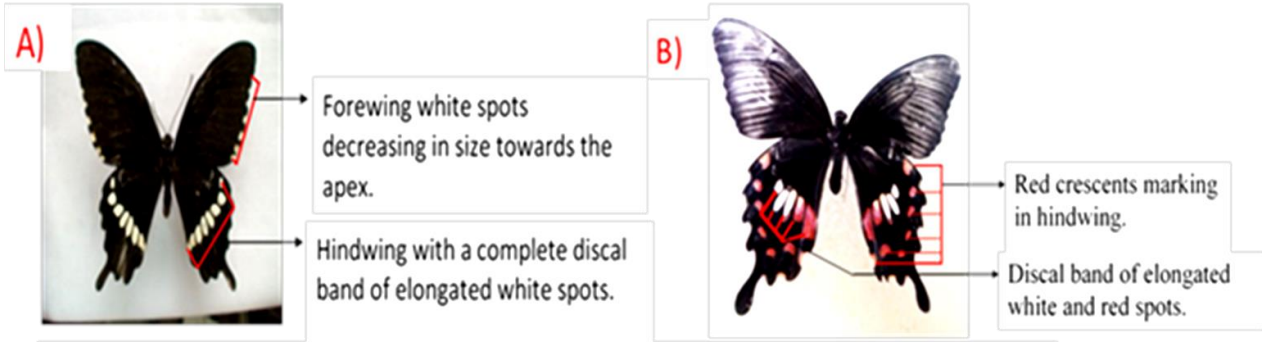

Fig. 4. Adults; A) *P. polytes* (male) and B) *P. polytes* (female).

Acknowledgements

This work was conducted under the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Bangladesh Atomic Energy Commission (BAEC) and Faculty of Science, Yamaguchi University, Japan agreement for research cooperation (2015-2020).

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