

Life history of *Hypolimnias bolina philippensis* (Butler) (Lepidoptera: Nymphalidae) on *Ipomoea batatas* (L.) Lam.

Dianne Joy D. Aguilon^{1*}, Celia dR. Medina², Luis Rey I. Velasco²

¹Department of Forest Biological Sciences, College of Forestry and Natural Resources, University of the Philippines Los Baños, College, Laguna, Philippines;

*Email: ddaguilon@up.edu.ph

²Institute of Weed Science, Entomology and Plant Pathology, College of Agriculture and Food Science, University of the Philippines, Los Baños, College, Laguna, Philippines

ABSTRACT. *Hypolimnias bolina philippensis* (Butler) is one of the three subspecies records of *Hypolimnias bolina* (L.) or the Great Eggfly in the Philippines. Despite its wide distribution range, knowledge of the life history patterns of *H. bolina philippensis* on its host is lacking. This study aimed to elucidate some aspects of the life history of *H. bolina philippensis* (Butler) on *Ipomoea batatas* (L.) Lam. The description of the duration, habits, behavior, and morphological characteristics of the various stages of development of *H. bolina philippensis* on *I. batatas* was studied under laboratory conditions using first- and second-generation individuals. Based on the results, *H. bolina philippensis* completed its life cycle on *I. batatas* in 51–81 days with an egg incubation period of 4–5 days, larval period of 25–39 days having six instars, pupal period of 8–11 days and the female longevity of 10–17 days while 8–14 days for males. Each stage of development possessed distinct characteristics and habits. The eggs were laid in clusters on *I. batatas* leaves that were dome-shaped, pale to yellow green, and ribbed. Larvae have orange heads and brown bodies covered with urticating setae. Young larvae were gregarious but become solitary as they mature and feed actively at night. The obtect pupa was brown blotched with black, which turned into dark brown before emergence. Male butterflies have dark indigo-blue wings, while females generally have dark-olivaceous brown wings. Mating occurred two to three days after emergence. Female butterflies lived longer than males. The adult sex ratio during the study was variable. The description of the life history traits of *H. bolina philippensis* in this study can be a baseline to compare and study the other subspecies. In addition, local butterfly farms can further utilize the study results to improve the mass production of *H. bolina philippensis*. However, more investigations on the biology and ecology of other subspecies should be done to compare better and understand how the different subspecies adapt to fluctuating global climate conditions, especially in the Philippine setting.

Keywords: life cycle, mating behavior, morphology

INTRODUCTION

Hypolimnias bolina (L.), or the Great Eggfly, is a common nymphalid butterfly distributed throughout Southeast Asia and Pacific and Indian oceanic regions (Clarke & Sheppard 1975). The high migratory tendency of *H. bolina* was observed in various countries such as the Philippines, Japan, southern China, and the Indochinese Peninsula (Mitsuhashi *et al.* 2011 as reviewed in Shirouzu 1960;

Kawazoe & Wakabayashi 1976; Fukuda *et al.* 1983; Fukuda & Nicho 1988).

H. bolina is highly polyphagous, utilizing at least 28 different host plants (Vane-Wright *et al.* 1977). Its wide host range includes plant families such as Moraceae, Portulacaceae, Malvaceae, Leguminosae, Convolvulaceae, Euphorbiaceae,

Acanthaceae, Amaranthaceae, Polygonaceae, Urticaceae, Asteraceae or Compositae, and Rubiaceae. *H. bolina* was reported (female's actual laying/ovipositing) in *I. batatas* (Clarke & Sheppard 1975) and as larval food plants (Holloway & Peters 1976; Marsh *et al.* 1977).

The genus *Hypolimnias* Hubner belongs to the family of Nymphalidae or brush-foot butterflies. In the Oriental region, there are three *Hypolimnias* forms recorded, *H. antilope*, *H. bolina*, and *H. missippus* (Corbet & Pendlebury 1945). Among the different forms, *H. missippus* and *H. bolina* remain the best-studied in terms of their distribution, polymorphism, genetics, ecology, mimicry, behavior, and biochemistry (Smith 1976; Clarke & Sheppard 1975; Morishita 1976; Fukuda *et al.* 1976).

The female *H. bolina* is considered one of the most variable butterflies in the world (Corbet & Pendlebury 1945). Despite its adaptability to various host plants and environmental conditions and its migratory ability, it is surprising that many subspecies of *H. bolina* have been found in almost every location of its geographic range. As such, several subspecies have been identified in almost every geographic location where it is found. In the Oriental region alone, the Malayan Peninsula has an insular subspecies, *H. bolina bolina* (Linnaeus), and a continental species of *H. bolina jacintha* (Drury) (Saji & Lovalekar 2020; Hoi-Sen 1983). The Philippines has three different subspecies records, namely *H. bolina philippensis* (Butler), *H. bolina joloana* Fruhstorfer and *H. bolina kezia* (Butler). Of these three subspecies, the subspecies *philippensis* is widely distributed in almost all parts of the Philippine archipelago, except Bogao, Jolo; Sanga-Sanga, Sibutu; and Tawi-Tawi, areas in which subspecies *joloana* is distributed. The lesser-known subspecies *kezia* can only be found in Batanes (Baltazar 1991; Treadaway 1995; Treadaway *et al.* 2012). *H. bolina philippensis* was also recorded in Borneo and Japan (Baltazar 1991; Mitsuhashi *et al.* 2011).

Among these subspecies, *H. bolina philippensis* is the most common and the local favorite, which is mass-produced in many butterfly farms using sweet potato, *Ipomoea batatas*. Despite its highly polyphagous and voracious nature, there were no reports of serious damage to sweet potatoes under natural conditions. It is apparent from these observations that there is much more to know about the biology and ecology of subspecies *philippensis*. Regionally, detailed and specific knowledge of the life history of *H. bolina philippensis* on *I. batatas* is limited. Hence, a description of the life history traits provided in this study would be basal in any subsequent ecological studies of this highly variable species. This study aimed to elucidate some aspects of the life history of *H. bolina philippensis* on *I. batatas* under laboratory conditions. Specifically, it sought

to: 1) determine the duration of the developmental stages; 2) describe their life-history traits, habits, and behavior of the larval and adult stages; and 3) describe the morphological features of the various stages of development.

METHODOLOGY

The life history of *H. bolina philippensis* on host plant *I. batatas* was carried out in the laboratory at the University of the Philippines Los Baños, Laguna. Insect rearing was done at room temperature or ambient laboratory conditions, where the temperature was controlled inside using an air conditioner as necessary.

Stock culture of *H. bolina philippensis* came from a butterfly farm in Marinduque, an island province in the Southern Tagalog region in the Philippines. A total of 100 pupae were reared to adulthood. The pupae were prepared by gluing the silk thread of the pupal cremaster in bamboo sticks placed inside the nylon mesh screen cages (60 x 50 x 30 cm) to keep the pupa suspended on its cremaster for proper adult emergence. Newly emerged adults were provided with potted sweet potato plants or cuttings soaked in water as oviposition sites. Cut flowers of *Ixora* spp. as nectar sources were likewise given and replaced regularly. Also, sugar solution and water were given to supplement adult food.

The first- and second-generation offspring were used to observe the life-history traits. There were three cohorts used in the life history study. Adult females which emerged from the pupal stock culture were observed for oviposition. The leaves with freshly laid eggs were gathered carefully in the host plants. The freshly laid eggs in the leaves were transferred to clean Petri dishes (10 cm diameter and 1.5 cm depth) lined with moist absorbent paper and maintained in moist conditions until hatching.

The eggs were examined at a six-hour interval daily to record the hatching time. Larvae emerging from eggs were removed and placed in groups of 15–20 for the early instars while in a group of four for the later instars, sealed separately in rectangular plastic containers (approximately 160 × 110 mm) lined with moist absorbent paper. The properly rinsed excised fresh sweet potato leaves pre-soaked in 1% bleach solution for 10 mins served as larval food. Throughout the study, proper sanitation was done to prevent disease by cleaning the containers daily (wash with 1% hypochlorite then dried) and regularly changing the absorbent paper as needed.

Larvae and pupae were monitored once daily for pupation and emergence. Larval and pupal dimensions were measured using a ruler. Each instar's egg and larval head capsule were

measured using a calibrated eyepiece micrometer with a 40x magnification. After emergence, adults were sexed, and the wingspan was measured using a ruler.

The duration of all developmental stages from egg to adult eclosion was observed and recorded daily. Description of the different stages from egg to adult was adapted from De Niceville (1890) on *Hypolimanas bolina*. Adult life span, sex ratio, and mating behavior were observed from the three cohorts of the laboratory population. Larval behaviors such as feeding, aggregation, and cannibalism were also noted. All data were analyzed statistically for range, mean, mode, and standard error.

RESULTS AND DISCUSSION

H. bolina philippensis completed its life cycle on *I. batatas* within 51–81 days, passing through the egg, six larval instars, pupal, and adult stages. The duration of the different stages of development is indicated in **Table 1**. The summary of measurements of all the developmental stages and width of the head capsule of the larval instars are shown in **Tables 2** and **3**, respectively. Pictorial illustrations or color photographs of all the developmental stages are depicted in **Figure 1**.

Table 1. Duration of the developmental stages of *H. bolina philippensis* reared on *I. batatas* under laboratory conditions.

Stage	Population (N)	Range (days)	Mode (days)	Mean $\pm$ standard deviation (days)
<i>Developmental stage</i>				
Egg	48	4–5	4	4.41 $\pm$ 0.49
Larva				
First instar	48	3–4	3	3.37 $\pm$ 0.48
Second instar	48	3–4	3	3.22 $\pm$ 0.42
Third instar	48	3–4	3	3.35 $\pm$ 0.48
Fourth instar	48	3–5	4	3.19 $\pm$ 0.53
Fifth instar	48	3–7	4	4.47 $\pm$ 0.68
Sixth instar	47	6–10	7	7.31 $\pm$ 0.78
Pupa	60	8–11	10	9.68 $\pm$ 0.65
<i>Post developmental stage</i>				
Adult				
Male	34	8–14	9	10.55 $\pm$ 1.59
Female	26	10–17	16	15.46 $\pm$ 1.79
Total		51–81		

Table 2. Body length measurements of the developmental stages of *H. bolina philippensis* reared on *I. batatas* under laboratory conditions.

Stage	Population (N)	Range (mm)	Mean $\pm$ standard deviation (mm)
<i>Developmental stage</i>			
Egg	25	0.40–0.56	0.47 $\pm$ 0.04
Larva			
First instar	25	1.80–3.50	2.70 $\pm$ 0.49
Second instar	25	2.80–7.80	6.10 $\pm$ 1.54
Third instar	25	6.20–12.00	8.89 $\pm$ 1.51
Fourth instar	25	13.50–21.90	17.46 $\pm$ 2.55
Fifth instar	25	19.30–36.20	26.54 $\pm$ 4.47
Sixth instar	25	30.70–56.00	39.40 $\pm$ 8.30
Pupa	25	19.00–25.00	22.77 $\pm$ 1.91
<i>Post developmental stage</i>			
Adult wingspan			
Male	15	72.50–93.40	79.80 $\pm$ 7.20
Female	15	81.50–96.80	88.20 $\pm$ 5.60

Table 3. Head capsule measurement of all the larval instars of *H. bolina philippensis* reared on *I. batatas* under laboratory conditions.

Stage	Population (N)	Range (mm)	Mean $\pm$ standard deviation (mm)
First instar	25	0.32–0.40	0.35 $\pm$ 0.01
Second instar	25	0.44–0.46	0.50 $\pm$ 0.02
Third instar	25	0.68–0.80	0.74 $\pm$ 0.05
Fourth instar	25	1.00–1.32	1.12 $\pm$ 0.08
Fifth instar	25	1.60–1.88	1.70 $\pm$ 0.08
Sixth instar	25	2.12–2.68	2.41 $\pm$ 0.15

Duration of developmental stages and some notes on behavior

Egg. The incubation period ranged from 4 to 5 days. The eggs were regularly laid in clusters on the sweet potato leaves' petioles, branches, and sides. Most of the eggs were found on the underside of the leaves. Newly laid eggs appeared moist and were pale or light green. On the fourth and fifth days, they turned white and transparent. Then the embryo head slowly became distinct on the top portion of the egg. The majority of the eggs hatched on the fourth day after oviposition. The egg was the most critical stage of

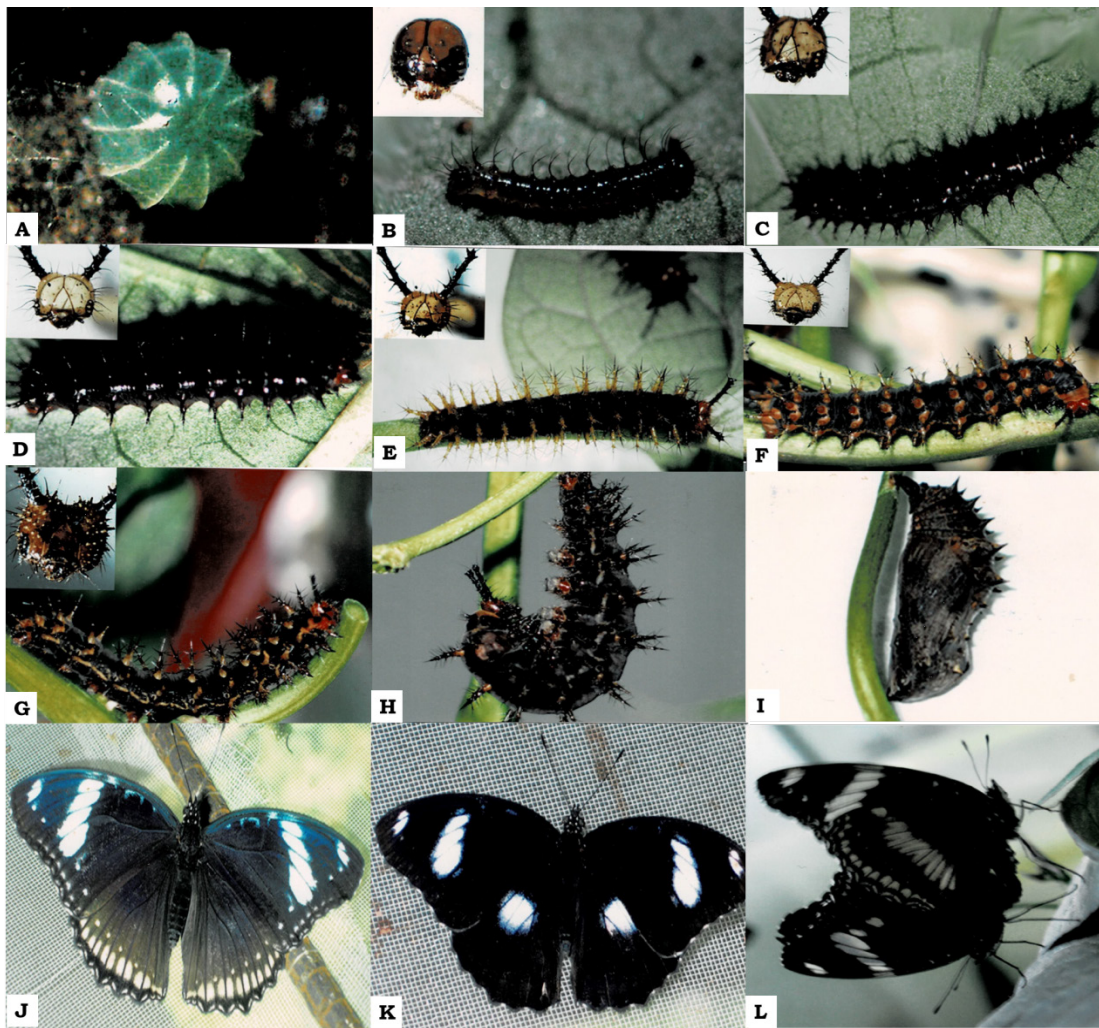


Figure 1. Developmental stages of *H. bolina philippensis* reared on *I. batatas* under laboratory conditions. **A:** Egg, **B:** First instar larva, **C:** Second instar larva, **D:** Third instar larva, **E:** Fourth instar larva, **F:** Fifth instar larva, **G:** Sixth instar larva, **H:** Pre-pupa, **I:** Pupa, **J:** Adult, female **K:** Adult, male **L:** Mating female (top) and male (bottom).

development because it is prone to predators like ants. After six days, eggs that failed to hatch shriveled, dried, and turned dark green.

Larva. There were six larval instars. The whole larval period ranged from 25–39 days. The different larval durations were as follows: first instar (3–4 days), second instar (3–4 days), third instar (3–4 days), fourth instar (3–5 days), fifth instar (3–7 days), and sixth instar (6–10 days). Upon hatching, the first instar chewed the top portion of the egg with the head coming out first, and then the whole body gradually appeared. It then crawled under the leaf and produced silk for additional adherence. Larval feeding in the leaves started immediately after emergence. The first and second instar larvae were gregarious, mostly aggregating under the leaves of the food plants. These early instars preferred to feed on

the soft leaves, producing characteristic holes. Third instar larvae started to become solitary as they grew older. They consumed the whole leaf, starting from the margin to the inner portion of the leaf. Fourth, fifth, and sixth instars were highly voracious, consuming even the midribs of the leaves. They fed actively at night while hiding under the leaves in the daytime. The first to fourth instar frass was compact and dry, unlike the fifth and last instar, which was soft and moist.

Molting was initiated when the scoli on the head of the emerging instar appeared as two black spots at the posterior part of the head. In the first to fifth instar, the larva freed itself from the old cuticle through a longitudinal slit originating from the prothorax to the abdomen. The old head capsule was pushed forward with the contractions of the larva, which was detached entirely during the early instar molting. Newly

molted larva had yellow scoli in the head and body, which changed later into its normal coloration. The sixth instar larva molted through the ecdysial line of the head capsule.

Pupa. Pupal duration ranged from 8–11 days. Prepupal stage consisted of the last one to two days of the sixth instar, where the transformation from larva to pupa occurred. The fully grown sixth instar larva ceased to eat and stayed in certain spots in the rearing cage, such as petiole, leaves, and even corners. It then became posteriorly compressed before fastening itself to these spots by producing silk threads from the abdomen's posterior part. After the pupating larva suspended itself, it curled up and remained motionless until its anterior dorsal portion expanded. The old skin was washed off through peristaltic movements, finally showing off the cremaster. Young chrysalis was moist, soft, and dark brown. After several hours, the pupa hardened without producing silk. Pupae kept in a head-down position during rearing produced normal adults. Observed abnormalities in the pupal stage mostly consisted of an underdeveloped puparium with gaps between segments and incomplete shedding of the larval cuticle. These pupae were not viable.

Adult. Females lived longer than males. The longevity of females was 10–17 days while 8–14 days for males. Adults usually emerge early in the morning. It emerged from the pupa through an ecdysial line that runs longitudinally along its antero-dorsal surface. Newly emerging adults have crumpled and soft wings, which later hardened and expanded after constant fanning while their legs were still held in the puparium. Male butterflies in each cohort emerged earlier than the female. A variable sex ratio was observed in the different cohorts. The cohorts reared from July to September and September to November (female=49, male=41) were female-biased (female=28, male=24), while the ones reared in January to March were male-biased (female=24, male=31). Based on the three cohorts used in the study, there was a slight variability in the sex ratio of *H. bolina philippensis*. This result attributable to the possible presence or absence of male-killing lines found in the species is in concordance with the previous studies of Mitsuhashi *et al.* (2011).

An adult usually mated 2 to 3 days after emergence. In the courtship flight, the male fluttered around the cage, and approached the female head-to-head, and vibrated its antenna with the female antenna. It moved away for some time, then went back and approached the same female from behind with its head touching the tip of the female's abdomen. The male then bent its abdomen and tried to insert its aedeagus into the female. This mating ritual was repeated with different females until the male found his successful mate. Paired individuals mated in a tail-to-tail position. Mating lasted for about 30 minutes to 4 hours. Mating

usually occurs late in the morning or early in the afternoon. *H. bolina philippensis* males showed an aggressive territorial mate-locating behavior which was also documented in other studies on *H. bolina* (Rutowski 1992; Kemp 2001).

The pre-oviposition period was three to five days after mating. The gravid female has a very large and distended abdomen. Most were not active and rested under the plant's leaves. They alighted under a leaf during oviposition and bent their abdomen to deposit the eggs. The eggs were laid on either side of the leaves, petiole, and sweet potato stems. Interestingly, females preferred to lay eggs on plant parts where other females previously laid them. Fecundity was not recorded systematically as it seemed to decrease with the succeeding generation of the laboratory parent population. This could be attributed to the decreasing heterogeneity of the gene pool of the laboratory population due to interbreeding.

Description of the developmental stages

Egg. The egg was dome-shaped, pale to yellow, and measured 0.40–0.56 mm in diameter. The chorion was ornamented with 11–12 longitudinal ribbings that meet at a hollow point in the cephalic pole where the microphile is found (**Figure 1A**).

Larva. The larva was cylindrical, slightly thickened in the middle, brown to black, and covered with urticating setae. Its hypognathous head was sclerotized, larger than the prothorax, and surrounded by numerous setae. Its hypognathous head was sclerotized, larger than the prothorax, and surrounded by numerous setae in the dorsal and lateral areas. A pair of antenna-like scoli was found in all instars except the first instar larva. The stemmata were arranged in a semicircle on a pigmented area anterior-laterally. Four stemmata, which lie equidistant in a traverse line, were distinctly large and lay anterior to the two small stemmata. A seta with black to orange chalazae was found at the center of these stemmata and in between the posterior lying stemmata. The thorax was equally divided into three segments with a pair of legs. Unlike the meso- and metathoraces, the prothorax has a shield and a large spiracle. Various hairs and setae surrounded all legs. The setae in the prothorax were variable, having only subspiracular scoli from the first to the third instar and developing a supraspiracular scoli starting on the fourth instar. The meso- and metathoraces have a verruca above the legs, two lateral scoli, and one subdorsal scoli.

The abdomen has ten segments. Abdominal segments III–IV and Abd X have a pair of prolegs on each. The prolegs have subventral setae, black plantae, and uniserial triordinal crochet. Abd I–VIII have spiracles, which were black and elliptical with one opening.

In all larval instars, the number and position of scoli in the abdominal segments were arranged as follows: Abd I have 3 subspiracular, 1 supraspiracular, 2 subdorsal, and 1 mediodorsal scoli. Abd II has ventral, 1 verruca, 2 subspiracular, 1 supraspiracular, 2 subdorsal, and 1 mediodorsal scoli. Abd III-VI have a verruca above the proleg, 1 subspiracular, 1 supraspiracular, 2 subdorsal, and 1 mediodorsal scoli. Abd VII has 2 ventral scoli, 1 subspiracular, 1 spiracular, 1 supraspiracular, 2 subdorsal scoli, and 1 mediodorsal scoli. Abd VIII has 2 ventral, 2 spiracular, 2 subdorsal, and 2 mediodorsal scoli. Abd IX-X has 2 subdorsal scoli and numerous setae.

First instar. The first instar larva was cream to dark brown with a transparent cuticle through which the midgut was visible. It measures 1.80–3.50 mm long. The head capsule was dark orange with hairs and few setae in the cranial area, measuring 0.32–0.40 mm in width. The body segments have numerous setae as described above. The seta has a black chalaza with long slender hair (Figure 1B).

Second instar. The second instar larva was dark brown and shiny, measuring 2.80–7.80 mm long. The head capsule was light orange, with more prominent hairs and setae in the cranial area, and measures 0.44–0.46 mm in width. Compared with the first instar, the head has a pair of antenna-like black scoli, and the stemmata were more distinct. The setae covering the body have developed into scoli and verrucae. The most prominent scoli were the subspiracular setae in the prothorax, the lateral and subdorsal setae in the meso- and metathoraces, and the supraspiracular the mediodorsal setae of the abdominal segments. The prothoracic shield has become apparent and bears three anterior pairs and one posterior pair of setae (Figure 1C).

Third instar. The third instar larva closely resembled the second instar larva except for the size and the scoli color. It has grown to a length of 6.20–12.00 mm with a head capsule of 0.68–0.80 mm in width. The pair of scoli in the head has doubled in length. The spiracle increased in size as well. The scoli in the body were now dark orange chalazae with black branches (Figure 1D).

Fourth instar. The fourth instar was generally black with a light orange head. It measures 13.50–21.90 mm in length when fully grown. The head capsule was 1.00–1.32 mm in width, and some of its setae have light yellow chalazae. The scoli in the head and the body increased in size significantly. As described in the second instar, the prominent scoli in the body was more visible due to golden yellow chalazae. The prothorax now has the supraspiracular scoli aside from the subspiracular scoli. The prothoracic shield has changed its color from black to orange (Figure 1E).

Fifth instar. The fifth instar larva was brown to black with an orange head, 19.30–36.20 mm body length, and a head capsule width of 1.60–1.88 mm. The head capsule of this instar could be distinguished from the other instars by its granular texture, presence of two distinct pairs of setae on the front, and yellow branches of antenna-like scoli. The black pigmentation on the adfrontal and epicranial suture, a distinguishing characteristic of the sixth instar, started to develop in some individuals. The larva's body has a yellow-orange longitudinal line below the spiracles. All thoracic legs were densely covered with setae (Figure 1F).

Sixth instar. The sixth instar was powdery black with a dark orange head and 30.70–56.00 mm body length. Among the larval instars, it has the largest head capsule with a width of 2.12–2.68 mm. All structures in the head were bigger and well developed. The processes of the setae were dark orange and prominent, making the head's surface very granular. As described in the previous instar, the dark pigmentation along the epicranial and adfrontal suture was more distinct. All the body scoli have dark orange chalazae with black setae and apex (Figure 1G).

Pupa. The obtect pupa was brown blotched with black, which turned into dark brown before emergence. The pupa was 19.00–25.00 mm. The Head is dorso-mesal and dorso-lateral tubercles. Dorso-mesal tubercles were always present on Abd III-VIII and usually on Abd II. Cremaster is longer than broad, with lateral projections on each base side (Figure 1I).

Adult. The adult's head was black with white dorsal bands and typically scaled. The eyes were naked. The palpi were pointed and produced above the head. The antennae were well developed with a finely pointed club. The thorax was dorsally convex and covered with long black scales with white speckles on the ventral side. The abdomen was black with two rows of white angular spots approximately lying on the ventral side of Abd II, Abd II and III, and Abd IV. The females were usually larger than the males (Figure 1J-L).

The male generally has dark indigo-blue wings. The upper surface of the forewing was black, with a big central white spot tinged with iridescent purple and three apical white spots. The lower surface of the forewing was brown with an apical white spot, two bluish spots in the distal area, and two rows of marginal white spots. The upper surface of the hindwing was black, with a circular central white spot tinged with iridescent purple. The undersurface of the hindwing was brown with a central white spot that extends from the costa to the dorsum, white discal spots, and two rows of marginal white spots. Male has wingspan of about 72.50–93.40 mm.

The female was generally dark olivaceous brown. The upper surface of the forewing was black with a similar apical band to the male. Below these apical bands were three small white spots in the lower central white patch and two rows of marginally white spots. The lower surface of the forewing was brown with the same marking as the upper surface of the forewing with additional three small white spots and two bluish spots. The upper surface of the hindwing was black with a central white area, bluish marking arranged linearly in tornus or rows of narrow marginal white spots. The lower surface of the hindwing was brown with a large whitish band (but in some individuals, this characteristic is lacking), many rows of discal small white spots, and two rows of marginal white spots, which were larger and more distinct compared to the ones present in males. The female has a wingspan of about 81.50–96.80 mm.

CONCLUSION

This study described the life history of *H. bolina philippensis* on *I. batatas* under laboratory conditions. The life cycle was completed in 51–81 days. The egg, larva, pupa, and adults were described morphologically. Each stage of development possessed distinct characteristics. Body dimensions and head capsules of the six larval instars were measured. The information on the life history of *H. bolina philippensis* on *I. batatas* can be used as a baseline for further study on the other two subspecies of *H. bolina* in the Philippines. Also, results from the study can help improve the mass production of local butterfly farms. However, more investigations on the other subspecies and aspects of their biology and ecology are still needed to further understand the adaptation of these subspecies to changing climatic conditions, particularly at the regional level or in the Philippine setting.

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