The First Report of Life Cycle

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The First Report of Life Cycle for *Ornithoptera croesus* (Wallace, 1859) Endemic Butterfly Found Bacan Islands

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ABSTRACT

This study reports for the first time the survival and length of time the perfect metamorphosis of *Ornithoptera croesus* endemic butterflies from Bacan Island. The purpose of this study is to describe the metamorphosis phase of *O. croesus* for the length of time needed for metamorphosis and *O. croesus* survival ability in Mussaenda (*Mussaenda pubescens*) and Asoka (*Saraca asoca*) flowers as a database of endemic butterfly conservation policy in Bacan Island. This research was conducted from July to October 2020. The eggs were collected from mussaenda and asoka plants in the Mount Sibela nature reserve on Bacan Island, and then reared in captivity with a cage size of 50x50x50 cm. The results showed that the survival percentage of *O. croesus* was in the low category with a value of 55%. Furthermore, it is found out that the time required for the metamorphosis of *O. croesus* is 93-100 days, including the longer category compared to other *Papilio*. The results of this study recommend that *O. croesus* needs attention in the conservation of local resources, considering the low life span and long metamorphosis are feared to be extinct.

Key words: Life cycle, butterfly, hostplant, *Ornithoptera croesus*.

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INTRODUCTION

Ornithoptera croesus butterflies in their development experience a life cycle better known as metamorphosis. Metamorphosis is a series of changes in shape and size from eggs to imago. Butterflies undergo a perfect metamorphosis which includes egg stages, larvae, cocoons and imago (Snodgrass, 1961; Jumar, 2000; Helmiyetti et al, 2013). *O. croesus* butterflies choose specific food plants such as the mussaenda and asoka plants to lay their eggs (Mas'ud et al, 2019). The larvae of the Papilionidae butterfly are plant eaters, while the adult butterfly feed is flower nectar (Jordano & Gomariz, 1994; Borror, Triplehorn, & Johnson, 1996; Zulnawati, Dahelmi, & Rahayu, 2018; Mas' ud, Corebima, Haerullah, Hasan, & Alisi, 2019). The relationship of *O. croesus* with its host plants shows a pattern of relationship, especially in the larval phase. In the larval phase the *O. croesus* butterfly requires feed from a specific host plant.

There are six host plant families of Papilionidae species, namely Aristolochiaceae, Rutaceae, Lauraceae, Annonaceae, Magnoliaceae, and Piperaceae (Soekardi, 2012). It is stated that the life cycle of same species butterflies maintained in different host plants would have differences in larval phase length and imago size (Suwarno et al, 2007; Lytan & Firake, 2012). Furthermore, Suwarno et al, (2007) reported that *Papilio polytes* butterflies reared on the *Citrus reticulata* host plants had shorter larval phases. Whereas *Papilio polytes* that are maintained on the *Citrus hystrix* host plant have a larger imago size.

The research findings of Suwarno et al, (2007) can be explained that the availability of host plants influences the life cycle length of the butterfly. Some possibilities that can arise in butterflies due to the various qualities of the host plant include, a species can experience a long life cycle and become a normal-sized adult individual or experience a short larval stage and then become a small-sized or disabled adult. For growth, larvae need water and nitrogen obtained from the host plants that they eat (Lincoln, Couvet, & Sionit, 1986; Fajer, 1989; Baylis & Pierce, 1991; Helmiyetti et al, 2010).

Mussaenda and asoka plants are food plants of the *O. croesus* butterfly located in the Sibela mountain nature reserve of Bacan Island (Mas'ud, 2018). At the height of 20 meters above sea level, there is a butterfly breeding area owned by a researcher and conservationist of the Bacan island butterfly, which has been breeding several taxa of endemic local butterflies, Bacan island, including *O. croesus, Papilio ulysses telegonus, Papilio deiphobus, Papilio lorquinianus gelia, Papilio fuscus lapathus, Troides hypolitus, Troides criton, Graphium milon*, and *Hebomoia glaucippe sulphurea*. Information about the life cycle from eggs to imago of *O. croesus* butterflies that live on the mussaenda and asoka host plants is still limited and has not been carried out, so research needs to be carried out with the aim to collect more complete data on the life cycle of butterflies. The *O. croesus* butterfly is kept in captivity by providing mussaenda and asoka host plants. It is hoped that the results of this study can be used as a reference in endemic butterfly conservation policies on Bacan Island.

MATERIAL AND METHODS

Provision of eggs

This research has been carried out from July to October 2020 using the ex post facto method, to find the causes that allow for changes in the butterfly development phase starting from the egg phase to the imago phase. The tools used in this study include (1) measuring devices in the form of calipers, (2) psychrometers, (3) label paper, (4) petri dishes, (5) room thermometers, (6) stretching boards, (7) ovens, (8) digital scales and (9) digital camera. The research procedure is as follows.

O. croesus butterfly eggs collected from mussaenda and asoka plants in the Sibela mountain nature reserve area of Bacan island, and kept in the Bacan island butterfly breeding with a maintenance cage size of 50x50x50 cm are eggs that have just been laid by the female butterfly of *O. croesus*. The eggs are observed. Each collected egg is placed on a petri dish and labelled with the date and egg identification number. Then the eggs are kept in a maintenance cage so that their life cycle can be observed until they reach the adult stage. (egg stage, larvae, pre pupae, pupa to become imago).

Life cycle of *O. croesus*

The life cycle of a butterfly is calculated from egg to adult. During the observation, measurements were taken of the duration of the egg, larvae, pre-pupa and pupa stages. Measurements were made on the shape, color, diameter and height of the egg, body length and diameter of the head shell on the larvae, the length of the antennae the span of the wing and the length and width of the front and hind wings. The *O. croesus* butterfly cycle data and morphological characteristics of each *O. croesus* individual stage obtained were analyzed descriptively. Furthermore, the egg stage is observed until the eggs hatch into larvae. Instar larvae are counted since larvae hatch from eggs. The second instar larvae are counted after the larvae have changed their first skin until their second skin changes, and so on until the last instar larvae (fourth instar) are finished. During maintenance, feeding of larvae in the form of mussaenda and asoka leaves is carried out. Larvae are transferred from petri dishes to feed leaves placed in a glass from the larvae entering the second instar to the fourth instar larvae, each labelled with information about each larva. At the time of the study also carried out measurements of temperature using a thermometer in the maintenance room and the relative humidity of the air using a psychrometer.

RESULTS

The results of this study obtained information that 4 *O. croesus* butterflies were found in the host plants of mussaenda and asoka as many as 4 individual *O. croesus* butterflies with a life cycle as shown in Table 1. In this study, 8 eggs were successfully collected, but only 5 eggs out of 8 successfully metamorphosed into larvae.

Table 1.Developmental phases of 4 *O. croesus* individuals

Developmental phase	time
1) egg preparation period	7 days
2) egg development period	28 ays
3) The period of development of larvae / caterpillars	28- 30 days
4) Development of cocoons	28 ays
5) Egression time from pupa as a young *O. croesus*	1 minute, 34 seconds
6) Life time as an adult	30-35 days

Three eggs did not succeed in hatching eggs because parasites were exposed. 5 larvae that live, which managed to grow into imago only 4 larvae, 1 larvae died during instar 3. The description of the length of time required for each phase of *O. croesus* metamorphosis is: the mating phase (copulation) of the *O. croesus* butterfly for 9 hours. Furthermore, the egg preparation phase on the host plant lasts for 7 days. The time for egg development is 28 days and the larval development period is 28-30 days. Furthermore, the pupa development period was 28 days and the time of aggression from pupa to young *O. croesus* was 1 minute 34 seconds. The time required for the development of imago (young butterflies) into adults is 30-35 days. The total time required for the developmental phase of the life cycle of the *O. croesus* butterfly started from the egg preparation period, the egg development period, the larval development period, the pupal development period, the expulsion period into young *O. croesus*, and the butterfly development period. Imago (young butterflies) become adults for 93-100 days. The environmental conditions consist of a temperature of around 29.5^{0}C, relative humidity of about 79%, light intensity of 112.3, and rainfall of around 212.3.

Furthermore, the results of the study also obtained information about the description of the phases of the metamorphosis process in the egg preparation phase which begins with copulation in *O. croesus* butterflies between males and females lasting for 9 hours (Fig. 1).

Fig 1. Copulation (marriage) of *O. croesus* butterflies on mussaenda plants.

Eggs are the first stage in the life cycle of *O. croesus*. Eggs are full of nutritious liquid that caterpillars use for their growth. The egg has a special hard shell to protect the caterpillars that grow inside. Eggs are usually placed under the leaves so that predatory animals do not find it and it is difficult to eat it.

Fig 2. *O. croesus* eggs.

Egg stadia of *O. croesus* on mussaenda and ashoka host plants

After mating, *O. croesus* lays his eggs on the lower of the leaves of the mussaenda and asoka plants. The leaves in the vines (mussaenda and asoka) chosen for oviposition are usually light green leaves and not stiff for them to lay eggs in the tip area (30 cm from the tip) to full. Female butterflies generally do not lay eggs on the terminal part of the leaf, and usually one egg is placed on only one leaf (Fig. 2).

Stadia larva/caterpillar of *O. croesus* on mussaenda and asoka host plants

Instar larvae will hatch from eggs in summer by making holes. The initial hatching of the egg becomes a caterpillar with a caterpillar length of approximately 2 millimeters. The caterpillar is dark brown in color and has spines that vary from 60 to 66 spines. During the instar growth process, the caterpillar molts 4 times. Then after 7 days the caterpillar molts again and then eats the skin on 14 days later the caterpillar releases its skin and cocoon.

The first and second instars are black and soft and there are tapered spines in all segments but the 6th segment has a bright yellow tapered thorn with a black tip, and a black head. The third and fourth instars are also black or purplish brown with yellow spines except in the 6th segment. The third and fourth instars vary according to body color; starting from black, chocolate or cream with thorns in the 6th segment brown orange and black tipped. The back color of the larvae is very uniform and ends in blackish brown except on the 6th segment with pale yellow to orange spines. The head of the larvae is brown with bright yellow, the first part of the thoracic segment is dark in color with yellow osmeterium (Fig. 3). When an adult larva can reach 7 cm long.

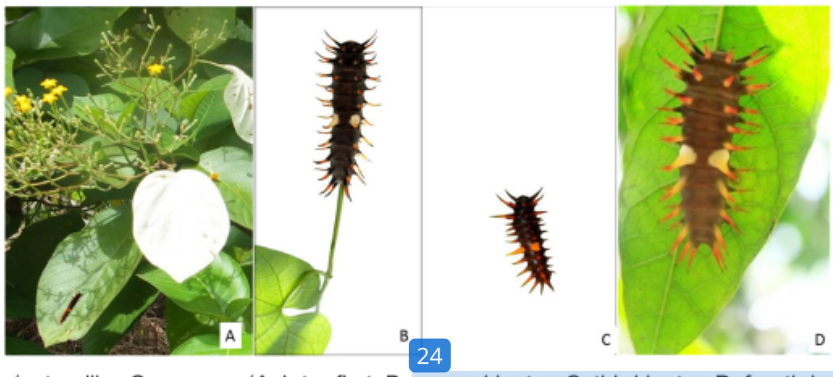

Fig 3. Larvae/caterpillar *O. croesus* (A. intar first, B. second instar, C. third instar, D. fourth instar).

At rest, the larvae under the leaves also do a complete ecdysis and they will always consume the skin removed. The body of the larvae of *Ornithoptera* sp. undergoes a change into a solid mass (concentrated mixture) of cells that will turn into butterflies within 14 days. Furthermore, the number of thorns and the pairs legs can be seen in Fig. 4.

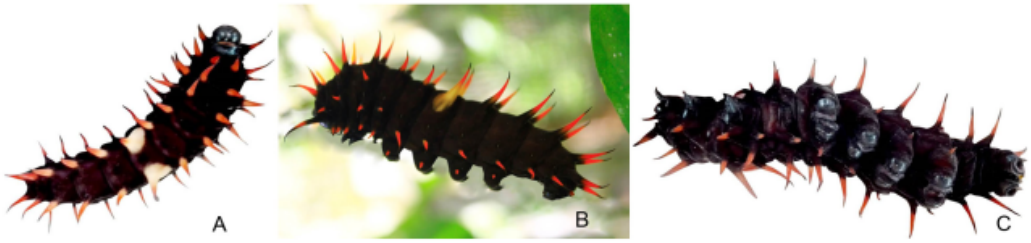

Fig 4. Larvae/caterpillar *O. croesus* (A = number of thorns; B = pairs legs; C = five pairs of legs in small meaty structure).

Based on Fig. 4 it can be explained as follows: (1) the number of upper spines is 24, (2) the number of right and left spines is 18 and 21, respectively. (a; true legs ie there are three pairs of true feet candidates, (b; mid abdominal prolegs that is there are four pairs of small structured meat found in the mid-abdomen to walk and hold and (c; anal proleg that is a pair of small structured flesh found in the the back end (the anus) functions to walk and hold, then on the last day, the larvae ascend to a safe place to spin and hang their heads down in a "J" shape for 12 hours. (Fig. 5)

The first, second and third instars experience nitrogen concentrations increase more quickly in the summer when the nutrient concentration in the leaves is also high, so that the size of the instar becomes larger compared to the size of the instar when eating less nutritious leaves.

Fig 5.*O. croesus* larvae spinning and hanging their heads down in the shape of the letter "J".

Fig 6. *O. croesus* cocoon (*O. croesus* cocoon is usually seen attached to the foodplant).

Stadia pupa of *O. croesus* on mussaenda and asoka host plants

O. croesus butterflies that have been obtained as many as 4 individual *O. croesus*, 1 male butterfly and 3 female butterflies have developed into adulthood by undergoing a change in metamorphosis from larvae to cocoons for 14 days or two weeks. When the instar prepares itself to become a cocoon, the adult larva instar leaves the leaf of the host plant (Fig. 6), the adult larvae move to the bush and look for a new place for approximately 1-2 days, the adult larvae release a black liquid (meconium), then spin and attach the tail segment and proleg terminal section towards the bottom of the leaf, forming a silk thread that will support the weight of the pupa. Before removing

the skin, the larvae of the butterfly extend, grow up and remain silent for 2 days.

The final ecdysis process takes place quickly because the larvae skin is rolled back and finally ejected again by a flick of the terminal segment, before being reinserted into the cremaster attached to a silk base on the leaf. Overall in the process of metamorphosis from larvae to cocoons takes about 3-4 days.

Butterflies young of *O. croesus* on mussaenda and ashoka host plants

Imago *O. croesus* has a long process of separating the skin from its shell into a young *O. croesus* butterfly with a time of 1 minute 34 seconds. While during the development of young *O. croesus* becomes an adult that is for 30-35 days. *O. croesus* young butterfly who broke away from his shell by crawling out. Next they look for food and drink in the nectar of the mussaenda flower, using special organs such as the mouth called "proboscis". Furthermore, the process of separating the imago skin from its shell into young *O. croesus* as shown in Fig. 7 is: a) After about 2 weeks in the form of pupae, the cocoon (chrysalis) begins to appear transparent. It is cocoon open when *O. croesus* is ready to come out, usually early in the morning, b); This stomach is filled with hemolymph, fluid like blood, and will be flowed into its wings in order to develop fully, c); *O. croesus's* stomach continues to flow hemolymph into wings, the proboscis begin to be removed and extended from its mouth. d); Wingtip is the last part to be filled with fluid. In addition to flowing hemolymph into its wings, *O. croesus* also releases orange-colored liquid which is a waste product /impurities resulting from the process of metamorphosis. e); in this phase the *O. croesus* wing has fully developed, it takes about 1 hour for the drying process before *O. croesus* is ready to fly, and f); *O. croesus* is ready to fly.

At the time of its initial growth, the body of *Ornithoptera* spp. was generally wet, wrinkled, its wings softer than silk and its body filled with a fat-like liquid. *Ornithoptera* spp. butterflies remove themselves from the cocoon by pushing the panel legs. The phases of the perfect metamorphosis process of *O. croesus* can be seen in Fig. 8.

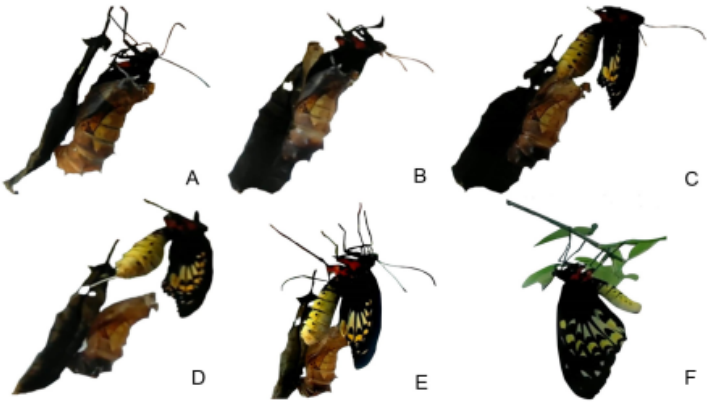

Fig 7.Imago *O. croesus* has a long process of separation of the skin of imago from its shell into a young *O. croesus*.

DISCUSSION

The results of this study indicate that the *O croesus* butterfly has a survival rate of 50%-62,5% on collection data. Based on these results, it can be said that O croesus has a lower survival rate than the *Papilio demoleus* butterfly 80% (Helmiyetti et al, 2010), the *Papilio polytes* butterfly 66.66% (Astuti, 2005). Butterflies have different survival abilities depending on environmental conditions and adaptability. In the butterflies *Graphium agamemnon*, *Papilio demoleus* and *Papilio polytes* have different life cycles at temperatures around 27º-29ºC in the host plants *Michellia champaka* (Magnoliaceae) and *Annona muricata* (Annonacea). In general, insects on host plants *Michellia champaka* (Magnoliaceae) and *Annona muricata* (Annonacea) have a minimum temperature range of 15ºC, an optimum temperature of 25ºC and a maximum temperature of 45º (Jumar, 2000).

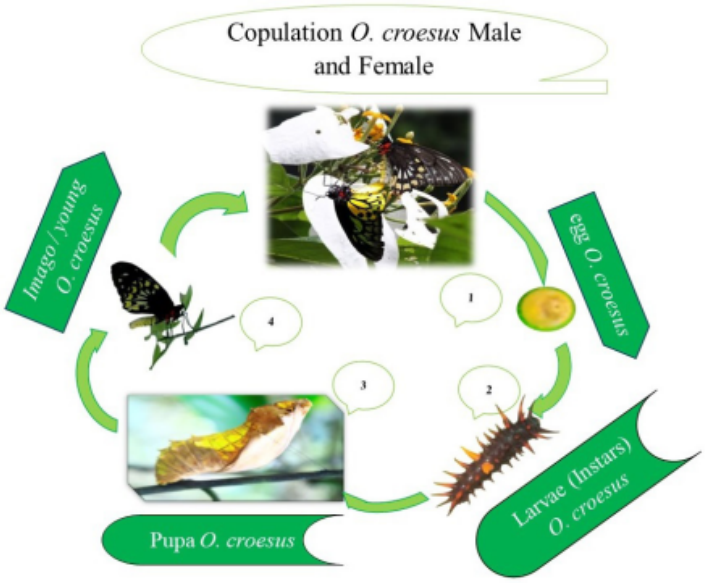

Fig 8.Development phase of the perfect metamorphosis process of *O. croesus*. (1. egg; 2. larvae; 3. pupa; 4. imago).

The results of research by Dahelmi, Salmah, & Yulnetti (2013) found that the life cycle of *Graphium agamemnon* lasts for 33-43 days, but in the host plant *Citrus aurantifolia* the life cycle of *Papilio demoleus* and *Papilio polytes* lasts for 31-42 days (Astuti, 2005). In this study, it is known that the life cycle of the butterfly *O. croesus* is 93 to 100 days. The day long description for metamorphosis starts from the egg preparation period for 9 hours, the egg development period for 7 days, the larval development period for 28-30 days, the development period for pupae for 28 days, the long expiration time being *young O. croesus* 1 minute 34 seconds and the development period imago (young butterfly) becomes an adult of 30-35 days. The life cycle of the butterflies *Papilio demoleus* and *Papilio polytes* which on the host *Citrus calamansi* is shorter because it has a higher temperature at the time of the study (Helmiyetti et al, (2013) and Astuti (2005). As also stated by Jumar (2000) generally

if the temperature rises, the metabolic process will also be faster and the time to complete the insect development cycle is also faster. Faster metabolic rates cause a reduction in substances needed by insects. This makes the age of the insect will be shorter. In addition to the temperature factor, the type of host plant is also thought to influence the length of the butterfly's life cycle.

In line with research Helmiyetti et al (2010) reported that the life cycle of *Papilio demoleus* for 22-24 days and *Papilio polytes* for 22-26 days on the host plant *Citrus maxima*. The life cycle of *Papilio demoleus* became longer, namely 27-31 and the life cycle of *Papilio polytes* for 27-28 days on different host plants, namely *Citrus cala*nsi. The results of this study indicate the duration of 93-100 days in *O. Croesus*. Based on the results of the study note that the water content of *Citrus calamansi* leaves is 66.58 ± 0.02%; in gr fruit (*Citrus maxima*) contains a water content of 71.56% (Helmiyetti et al, 2010). Water and protein content is very important for larval growth, especially in the early days. The quality of the host plant will affect the length of time needed for larvae to carry out further development and affect the size of the l ae (Jordano & Gomariz, 1994; Ojeda-Avila, Woods, & Raguso, 2003; Suwarno et al, 2007; Zulnawati et al, 2018). In this study the Asoka and Musaenda plants have a relatively little water content compared to citrus plants.

At the egg stage of *O. croesus*, lays its eggs under the leaves of Mussaenda and Asoka. In line with Sands & New (2013) states that female butterflies begin to collect their eggs under the leaves of plants that become food (foodplant). The egg stadia of *Papilio demoleus* and *Papilio polytes* on the hos lant of *Citrus calamansi* was 3 days. This statement is in line with the research of Suwarno, Salmah, M.R., Hassan, A.A., & Norani (2007) who reported that the egg stadia in *Papilio polytes* on four types of host plants lasted for 3 days. However, it is relatively shorter when compared with Helmiyetti et al (2010) 2-4 days and Astuti (2005) which lasts 2-5 days. In *O. croesus* the egg stadia is 7 days old. Selvey (2008) measured the eggs of *Ornithoptera* spp in general as long as 2.3-2.5 mm in diameter and were initially greenish yellow, becoming pale yellow in a few days and brown yellow one or two days before hatching. Furthermore, Selvey (2008) states that after the eggs incubate, the larvae move to the host plant (foodplant) and look for soft leaves to eat small hairs (trichomes) at the bottom of the leaf as well as a ace to stick.

In general, the time required for the larval stage of the *O. croesus* butterfly is 28-30 days. Furthermore, the duration of the 1st instar larvae to 4th instar larvae on pilio demoleus lasted for 2-3 days. In instar larvae 5, the time needed is 5-6 days. This is also in line with the results of research Helmiyetti et al (2010, 2013); Dahelmi et al (2013) and Astuti (2005) who reported that the 5th instar larvae in this type of butterfly lasted for 4-5 days.

The concentration of nitrogen in the host plant can affect the number of instars in butterflies and the level of instar development (larvae), size and productivity of adult butterflies (Taylor, 1984). In the rainy season the instars experience a decrease in nitrogen concentration which is influenced by lower nutrient concentrations in the leaves (Taylor & Sands, 1986). Birdwing butterflies usually eat the leaves of vines

(mussaenda), but there are certain species such as *O. richmondia, O. euphorion* and *O. priamus*, usually eating shoots, flowers, seed capsules and stems (Feeny, 1995). Birdwing butterflies generally prefer the leaves of vines because in addition to obtaining food they are also used as a place to lay eggs, breed and can be used as a place to live in limited conditions to protect them from predators and parasitoids (Feeny, 1995; Omura & Honda, 2005; Scott, 2006). Generally, birdwing butterfly species only develop on one or two leaves (Sands, 2008). Common & Waterhouse (1981) and Feeny (1995) stated that *Ornithoptera* spp. larvae generally have tubular organs, these organs are used when there is danger by producing volatile odors that are used to expel predators. It further state that the development phase of *Ornithoptera* spp. larvae generally digest food for 22-46 days until it enters the cocoon stage. The pupa stage of *O. crosesus* was 28 days and the pupa stage of the *Papilio demoleus* butterfly was 9-11 days. This is different from the research of Helmiyetti et al (2010) and Astuti (2005) with a shorter pupa stage time and the results of Suwaro et al (2007) research with a longer pupa stage time than the *O. crossus* pupa stage. The difference in the duration of the pupal period is thought to be due to the different host plants used. When danger is present, all larval instars will bend the body backward and open two yellow osmeterium branches at the anterior end of the prothoracic segment to protect themselves (Common & Waterhouse, 1981; Feeny, 1995; Selvey, 2008; Sands & New, 2013). At the end of the pupa stage the event of the emergence of Ornithoptera spp is called “eclosion” (Common & Waterhouse, 1981; Feeny, 1995; Selvey, 2008; Sands & New, 2013).

In general, the results of this study indicate that the survival of *O. croesus* butterflies is in the low category when compared to other types of Papilio butterflies. Likewise, the length of the life cycle or the length of metamorphosis of *O. croesus* butterflies in the old category compared to other types of butterflies. Based on the results of this study, the *O. croesus* butterfly is a type of butterfly that needs special attention from conservationists considering its ability and low life cycle so that it is feared threatened with extinction.

CONCLUSIONS

The study reported that the survival rate of the *O. croesus* butterfly category was low at 55%. Larvae hatched from only 5 out of 8 eggs collected. 3 eggs did not succeed in hatching eggs because parasites were exposed. 5 larvae that live, which managed to grow into imago only 4 larvae, 1 larvae died during instar 3. Furthermore, it was also reported that the length of the life cycle of *O. croesus* in the long category is 93-100 days for the 9-hour egg preparation phase, the period egg development 7 days, larval development period 28-30 days, pupa development period 28 days, long expulsion to be young *O. croesus* 1 minute 37 seconds, and the development period of imago (young butterfly) becomes adult 30-35 days.

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