

Original Article

Discriminatory of some morphometry and biological aspects of the Cycad blue butterfly, *Chilades pandava* reared on *Cycas* and *Zamia* ornamental palms

Discriminação de alguns aspectos morfométricos e biológicos da borboleta azul Cycad, *Chilades pandava*, criada em palmeiras ornamentais *Cycas* e *Zamia*

G. M. Hassan^{a*} , M. A. Batt^b  and M. R. El-Aassar^a 

^aAgricultural Research Center – ARC, Plant Protection Research Institute, Vegetable, Medicinal, Aromatic and Ornamental Pests Research Department, Giza, Egypt

^bAgricultural Research Center – ARC, Plant Protection Research Institute, Wood Borers and Their Products Research Department, Giza, Egypt

Abstract

The serious blue butterfly, *Chilades pandava* -Horsfield, 1829- (Lepidoptera: Lycaenidae) is consider one of the main destructive insect pests for ornamental palms *Cycas* and *Zamia*. Biological and morphological measurements were carried out of *C. pandava* stages reared on *Cycas revoluta* (Cycadaceae) and *Zamia encephalartoides* (Zamiaceae). In description details, non-significant variations were recorded between the two gender of cycad blue butterfly in the obtained data, but the male adult was more densely blue or violet than female adult. By the aid of SEM, *C. pandava* all stages were distinct by long and thick hairs covered all the body. The morphometric characters namely, length, width and venation of wings, body length, forewing, hindwing could be as a guide for taxonomic discrimination. The data showed that the life cycle duration of *C. pandava* was ranged between 20.64 to 21.7 days. The developmental periods of different *C. pandava* stages are slightly higher on zamia than cycas palms. This investigation detected that a high survival rate was found on *Cycas* palms (86%) than the survived rate recording on *Zamia* palms (82%). In the present study, the described morphometric characters could be used as a guide for taxonomic discrimination of this pest. Consequently, this study added a valuable knowledge about *C. pandava* to have sound decisions for proposal of its management and conservation in Egypt.

Keywords: biology, *Chilades pandava*, *Cycas*, description, Lepidoptera, Lycaenidae, morphology, *Zamia*.

Resumo

A borboleta azul séria, *Chilades pandava* – Horsfield, 1829 (Lepidoptera: Lycaenidae), é considerada uma das principais pragas de insetos destrutivas para as palmeiras ornamentais *Cycas* e *Zamia*. Medidas biológicas e morfológicas foram realizadas em estádios de *C. pandava* criados em *Cycas revoluta* (Cycadaceae) e *Zamia encephalartoides* (Zamiaceae). Nos detalhes da descrição, variações não significativas foram registradas entre os dois gêneros de borboleta azul cicadácea nos dados obtidos, mas o adulto masculino era mais densamente azul ou violeta do que o adulto feminino. Com o auxílio do MEV, *C. pandava*, todos os estágios foram diferenciados por pelos longos e grossos que cobriam todo o corpo. Os caracteres morfométricos a saber, comprimento, largura e venação das asas, comprimento do corpo, asa anterior e posterior, podem servir de guia para a discriminação taxonômica. Os dados mostraram que a duração do ciclo de vida de *C. pandava* variou entre 20,64 e 21,7 dias. Os períodos de desenvolvimento dos diferentes estágios de *C. pandava* são ligeiramente maiores em zamia do que em cicas. Essa investigação detectou que uma alta taxa de sobrevivência foi encontrada em palmeiras *Cycas* (86%) do que a taxa de sobrevivência registrada em palmeiras *Zamia* (82%). No presente estudo, os caracteres morfométricos descritos podem servir de guia para a discriminação taxonômica dessa praga. Consequentemente, este estudo agregou um conhecimento valioso sobre *C. pandava* para ter decisões acertadas para a proposta de seu manejo e conservação no Egito.

Palavras-chave: biologia, *Chilades pandava*, *Cycas*, descrição, Lepidoptera, Lycaenidae, morfologia, *Zamia*.

Introduction

Ornamental palms, *Cycas revoluta* and *Zamia encephalartoides* are very important economic palms in Egypt (Batt et al., 2019). They are widely cultivated in a

garden landscape and tourist villages and resorts and also have been exported to many regions around the world (Al-Sayed et al., 2014). Lycaenidae is defined as "Gossamer-

*e-mail: Gamal.hassan@arc.sci.eg dr; jimy.hassan@gmail.com

Received: March 30, 2022 – Accepted: May 31, 2022



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

winged butterflies” with large diversity in the world (Ackery and Vane-Wright, 1984; Fiedler, 1996). The Lycaenids belong subfamily Polyommatae are dispersal in wide range of geographical areas of world from North America, Europe and Asia (Eliot, 1973) and also in North Africa (Batt et al., 2019). The large butterflies of subfamily Polyommatae were commonly distinguished as well as “Blues”. Lycaenidae family including *Chilades* genus (Order: Lepidoptera) contained large scale of species over than 6,000 species (Pierce et al., 2002). The menace of cycad blue butterfly, *C. pandava* (Horsfield, 1829) was recently a main insect pest of ornamental palms, *Cycas* and *Zamia* (Batt et al., 2019).

Blue cycad butterfly, *C. pandava* was firstly recorded through 2012 in a private farm at Birqash village, Giza, Egypt (Fric et al., 2014). Recently due to world climate change, *C. pandava* caused economic damages and had ability to produce numerous generations every year in *Cycas* palms in Egypt (Abu-shall et al., 2014; Batt et al., 2019). *C. pandava* and *Chilades lajus* were described by morphometric aspects of these butterflies (Mahdi et al., 2018). An important tool of separation and identification of insects either species or groups was a morphometric variation and measurements (Daly, 1985; Digo et al., 2015; Akand et al., 2018). In addition, both shape and size of the insect body and wings was play important role to identify between the closely related taxa within and between insect species (Riva et al., 2001; Baylac et al., 2003; Tuzun, 2009). Moreover, the variation in lengths of body, antenna, wings and legs for two butterfly species *C. pandava* and *C. lajus* in Bangladesh (Mahdi et al., 2018).

The extensively studies including the shape of insect wing were carried out to identify between numerous insects (Miller, 1991; Riva et al., 2001; Villegas et al., 2002; Aytakin et al., 2007). The most evidential characters of any organism were morphological shape which provided a linkage between species type and its environment (Ricklefs and Miles, 1994). The butterflies –environment interaction affected on the development of butterflies (Mutanen et al., 2007).

The ecology and behavior of the *C. pandava* development, survivorship, and reproduction were studied under different temperatures on cycad plants (Ravuiwasa et al., 2011). In addition, the life history of *C. pandava* in Taiwan were studied by Lee (1989). Few previously issues were mainly highlighted on biology, life history and morphology patterns of *C. pandava* (Chang, 1989; Wu et al., 2010).

Due to lack data about *C. pandava* in Egypt, therefore, the present study objective has not only flags on the morphological measurements of the menace of cycad blue butterfly, *C. pandava* stages on *Cycas* palms, but also provides a obviously information case of its biological aspects when reared on *Cycas* and *Zamia* palms in Egypt.

2. Materials and Methods

2.1. Morphological and taxonomic aspects of *C. pandava* on *Cycas* palms

Damaged parts of *Cycas* and *Zamia* palms infested by *C. pandava* were collected from Abou-Ghaleb village, Giza

Governorate, Egypt. These samples were identified at insect identified unit, Survey & Taxonomy Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

During the period from April to July 2021, *C. pandava* stages were collected using a fine hair camel brush from the laboratory culture mounted on stubs with carbon tape, and examined under low vacuum scanning electron microscope model Quanta 250 FEG (Field Emission Gun) attached with EDX unit (Energy Dispersive X-ray Analyses) with accelerating voltage 30 K.V., magnification 14x up to 1000000 and resolution for gun at the central laboratories sector, the Egyptian mineral resources authority, ministry of petroleum, Cairo, Egypt. Pictures of the different stages were also taken using camera (64MP) to describe and determine by using of compu eye, leaf and symptom area program by Bakr (2005). A clear morphological scale measurement was used for the identifying variables according to Akand et al. (2018). All the morphological characteristics (viz. body length and width, head capsule and length of antenna, abdomen, thorax and legs). Wing venation and morphometric analysis for forewing and hindwing were measured and described by Kunte and Tiple (2009); Abu-shall and Tawfeek (2015) and Mahdi et al. (2018).

2.2. Biological development of *C. pandava* stages on *Cycas* and *Zamia* palms

2.2.1. Insect culture

Numerous samples of *C. pandava* stages were initially collected at April to July 2021 from Abou Ghaleb village, Giza, Egypt and then reared on newly shoots, buds and leaves of *Cycas* and *Zamia* nurseries in two wooden cages under laboratory conditions ($26\pm 5^\circ\text{C}$ & $65\pm 5\%$ RH). Each cage (80 cm length x 50cm width x 90 cm height) covered by agrylic clothes (anti *C. pandava* screening) (Figure, 1). Newly fronds of *Cycas* palms were daily provided for *C. pandava* oviposition and feeding. Additionally, the adults were feed on 15% honeybee as nutrient solution (Ravuiwasa et al., 2011) in a saturated cotton wick.

2.2.2. Monitoring of developmental stages and biological aspects

Ten of males and females of *C. pandava* were collected from insect culture and transferred to separately small cage (30 cm length x 20 cm width x 30 cm height) (Figure, 1) to allow females to lay eggs under laboratory conditions. This small cage contained a saturated cotton wick of 15% honeybee solution and newly shoots or young leaves of both two palms. Directly after 24 h, about fifty newly deposited eggs (0-12 h age) on each of *Cycas* and *Zamia* were randomly selected and removed any exceeded eggs. First instar larva was individually kept immediately after egg hatching in a plastic box (20 cm length x 13 cm width x 10 cm height) covered by agrylic cloths and were continuously added and supplied with newly fronds or young leaves of *Cycas* and *Zamia* nurseries. All developmental of larval stages were daily determined and recorded until the adult emergence. Moreover, both of the newly emerged females and males were separated into small cages in Figure 1 (20 cm length x

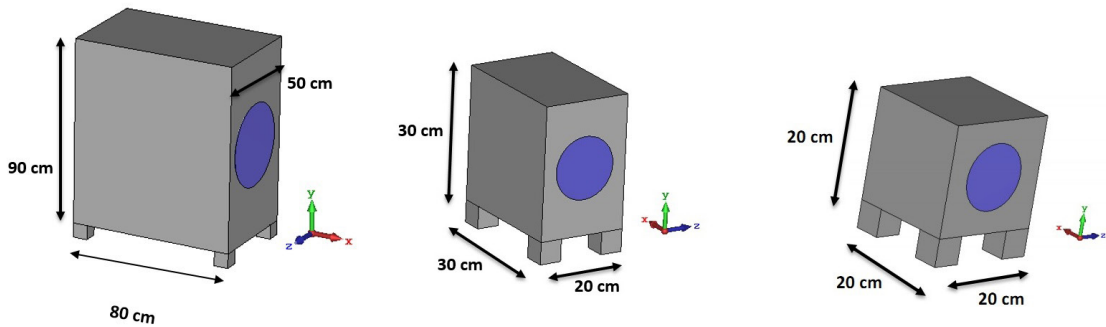


Figure 1. Wood rearing cages.

20 cm width x 20 cm height). This cage was supplied with 15% honeybee as nutrient solution in a saturated cotton wick for the adults, and young leaves of both two palms until the adult death. Additionally, the survival rate, life cycle, longevity and sex ratio were determined.

The obtained data were statistically analyzed using SAS software including t-test (SAS, 2003) at 0.05 levels of probability.

3. Results

3.1. Morphological studies on *Chilades pandava* stages on *Cycas palms*

Cycad blue butterfly, *C. pandava* belong to subfamily Polyommatae and family Lycaenidae. The morphological features of *C. pandava* from egg-adult are studied using both scanning electron microscope and digital camera in order to fully describe this invasive pest.

3.1.1. Egg

Chilades. pandava female laid individually eggs on the undersides of young fronds of the newly leaves. The egg is slightly elevated and likely disc-shaped or oval (Figure 3B). Egg is ~ 0.498 mm in length and ~ 0.446 mm in width (Figure 2). Each egg surface carries numerous irregular-shape sacs in equal parallel rows (Figure 3B). The sacs showed a high refractive index as a whitish in appearance when compared to the dark body background of the egg. The sac is ~0.028 mm long (Figure 2). Through SEM photo of the egg exhibited the sac membranes to appear as like as a wrinkled cloth. These egg sacs appear to connect with them by irregularly shaped of transfer canal as a spider spinning or net. Moreover, the sacs ridged in eggshell are hexagonal-shape, each one had 6 triangles with fine pores which provided the oocyte by ambient air. Between the transfer canal, the small tubercles (ST) were arranged irregularly on a darker area of egg (Figure 3A). After hatching, the exit hole (exiting opening) is oval and is about ~0.27 mm in diameter (Figures 2 and 3B).

3.1.2. Larvae

C. pandava eggs hatch into 1st larval instar. *C. pandava* have four larval instars. larvae were no apparent sexual

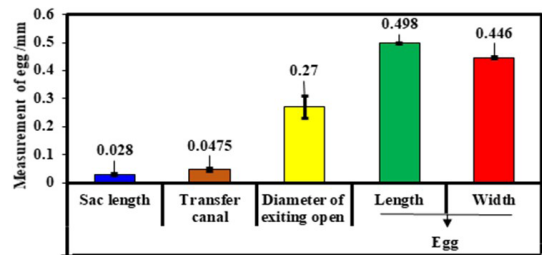


Figure 2. Measures of *Chilades pandava* egg.

dimorphism. All larval instars are elongate and slightly flattened extremely especial in the 4th larva, and also, there had very intensive or thickly hairs on all body surface (Figures 4 and 6). The 1st larva is very small and slightly brown in colour (~ 1.43 ± 0.01 mm in length and ~ 0.33 ± 0.02 mm in width) (Table 1 and Figure 6). The 2nd larval instar was slightly dark brown, about ~ 3.88 ± 0.03 mm in length and ~ 1.12 ± 0.03 mm in width, its' head capsule was ~ 0.44 ± 0.2 mm long and 0.54 ± 0.01 mm wide (Table 1).

Both 3rd and 4th larval instar are deeply dark brown, about ~ 10.8 ± 0.07 and 14.7 ± 0.06 mm in length and 1.97 ± 0.01 mm and 3.97 ± 0.09 mm in width, respectively, its' head capsule was conspicuous (about ~ 0.84 ± 0.01 & 0.96 ± 0.01 mm long and 0.94 ± 0.003 & 1.083 ± 0.01 mm wide, respectively) (Table 1, Figures 4-6). Also, the anal plate in fall grown larva was distinguishing and apparent (Figure 5). In view of Figure 6, the 3rd and 4th larval instars have three dark longitudinal lines extending from the anterior part to the posterior of the body. These lines are surrounded by thin, disconnected and irregular white lines (Figure 6). The abdomen of larval instars has ten segments, and have a pair of prolegs on the abdominal segments, the proleg of larvae is a fleshy conical projection sitting on each side of the segment. However, the abdominal segments are not distinguished in the first instar (Figures 4 and 6).

3.1.3. Pupae

Pupation processing occurred on the leaf in tunnel formed by larva or in a rolled leaf shelter. The prepupa are greenish colour (~ 12.8 ± 0.06 mm long & ~ 6.00 ± 0.08 mm wide), also, it has three longitudinal deep green lines in dorsal surface. Pupa is an obtect or not free which has all

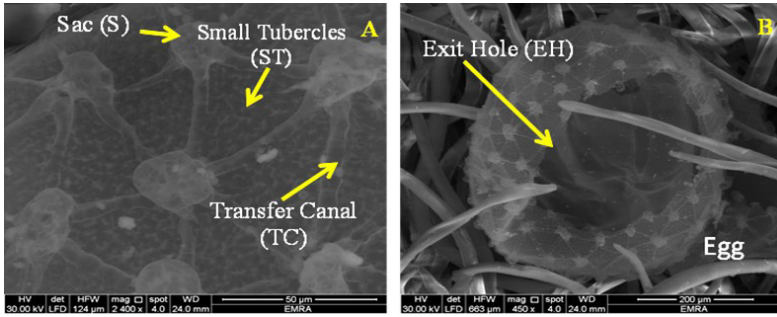


Figure 3. SEM of *Chilades pandava* egg.

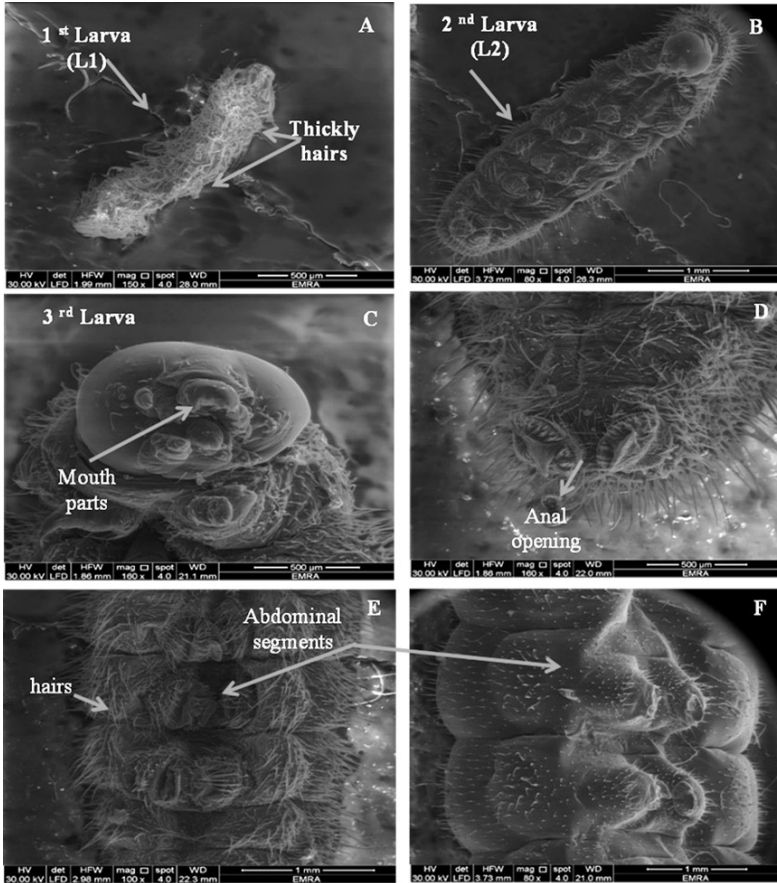


Figure 4. SEM of *C. pandava* larvae: Larvae had thickly hairs in all body parts, A) 1st larva, B) 2nd larva, C) head capsule of 3rd larva, D) anal opening of 3rd larva and E) & F) the abdominal segment of 3rd larva.

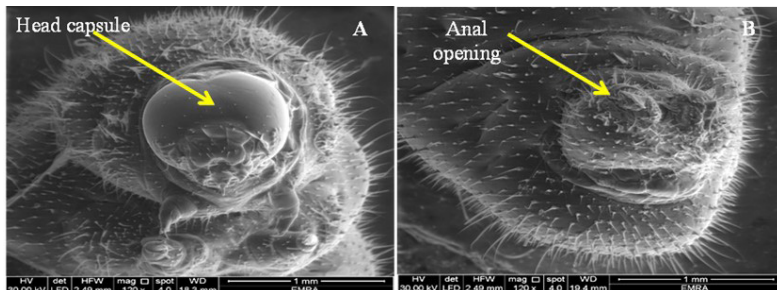


Figure 5. SEM of 4th larva of *C. pandava*: A) head capsule & mouth parts, B) anal opening.

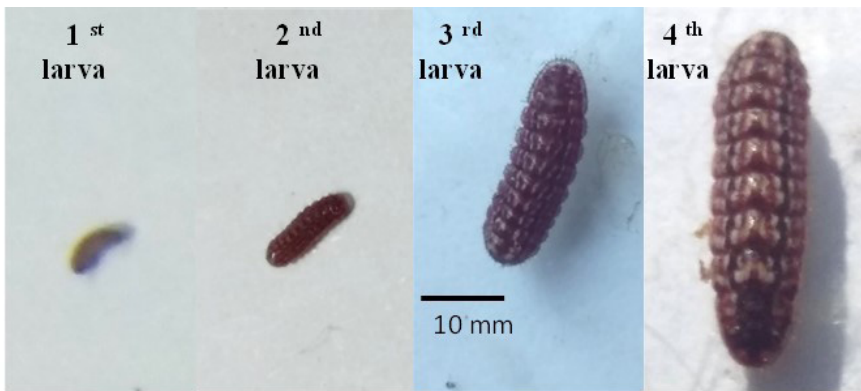


Figure 6. Larvae of *C. pandava* by camera (64MP).

Table 1. Measurements of *Chilades pandava* larvae and pupae on Cycas palms.

Parameters	Measurement (mm) Mean + SE						
	1 st larva	2 nd larva	3 rd larva	4 th larva	Pre-pupa	Pupa	
Length	1.43± 0.01	3.88± 0.03	10.8 ± 0.07	14.7 ± 0.06	12.8 ± 0.06	13.3 ± 0.04	
Width	0.33 ± 0.02	1.12 ± 0.03	1.97± 0.01	3.97± 0.09	5.7 ± 0.08	5.7± 0.04	
Head capsule	length	0.09 ± 0.003	0.44 ± 0.2	0.84 ± 0.01	0.96 ± 0.01	—	—
	width	0.14 ± 0.003	0.54 ± 0.01	0.94± 0.003	1.083 ± 0.01	—	—

appendages adhered to body, and gradually greenish to brown colour with blotchy dark brown areas and about $\sim 13.3 \pm 0.04$ mm long and $\sim 5.7 \pm 0.04$ mm wide. Pupa is smooth depending on full grown larva (Figure 7 and Table 1).

3.1.4. Adult females

Adults of Cycad blue butterfly, *C. pandava* females and males are a medium in size. Both female and male adults are lavender-blue/ or violet-blue in colour but the female is a slight pallid with few bright blue scales which concentrated on the wing base. Generally, the body is appeared to be dorsally blue and ventrally light brown (Figure 8). Results in Table 2 and illustrated in Figure 9 showed that the length of adult female is $\sim 8.19 \pm 0.025$ mm with small head capsule ($\sim 0.96 \pm 0.006$ mm long and $\sim 1.58 \pm 0.009$ mm wide). Head has largely compound eye, with shorted hairs beside and between two eyes, and measured about $\sim 0.58 \pm 0.025$ mm in diameter, the ocelli is absent. The clavate antenna covered with densely scales was observed in both female and male (Figures 9E and 10D), with length $\sim 6.04 \pm 0.013$, and provided with a pair of sense organs (Figure 9A, E). Thorax of female is nearly closed in both length and width being $\sim 3.17 \pm 0.007$ mm and $\sim 3.48 \pm 0.011$, respectively (Table 2 and Figure 9B). Female legs (fore, middle and hind) are homologous in size and shape and have five segments "namely: coxa, trochanter, femur, tibia and tarsus". Tarsus has a pair of forked claws (Figure 9D). Three pairs of legs were $\sim 4.15 \pm 0.006$, $\sim 5.47 \pm 0.010$ and $\sim 4.72 \pm 0.015$ mm for fore, middle and hind legs, respectively (Table 2). Abdomen of *C. pandava* female has

10 segments ($\sim 4.06 \pm 0.012$ mm long & $\sim 1.44 \pm 0.023$ mm wide, Table 2), and the last two segments are extensively modified to genitalia form. Abdomen is covered by densely long hairs (Figure 9C). Adult female as well as in the most of lepidopteran species has two genital openings; the first one is located between 7th and 8th abdominal segments using in mating, and the another one is situated for egg-laying at the end of abdomen (Figure 9C).

3.1.5. Adult males

Sexual dimorphism is mainly characteristically pronounced in *C. pandava* adult. The male measures $\sim 8.42 \pm 0.079$ mm in length with small head capsule ($\sim 0.90 \pm 0.006$ mm long and $\sim 1.54 \pm 0.006$ mm wide). As in female, head has largely compound eye with shortly hairs beside and between two eyes ($\sim 0.56 \pm 0.015$ mm in diameter), and provided with a pair of sense organs (Figure 10A). Male thorax is smaller than female in both length and width being $\sim 2.54 \pm 0.062$ and 1.75 ± 0.015 mm, respectively (Table 2 and Figure 10B). Average length of fore, middle and hind legs of male are $\sim 3.17 \pm 0.017$, 4.43 ± 0.012 and 4.29 ± 0.012 mm, respectively. Abdomen of male is measured 4.98 ± 0.011 mm in length and 0.78 ± 0.009 mm in width (Table 2). Both of 9th and 10th abdominal segments in male are formed the external male genitalia with densely like hairs was observed and covered a genitalia plate of male (Figure 10C).

3.1.6. Wings

In forewing, the dorsal view/ or the upperside of female wing is appeared a slightly shiny blue or violet-blue

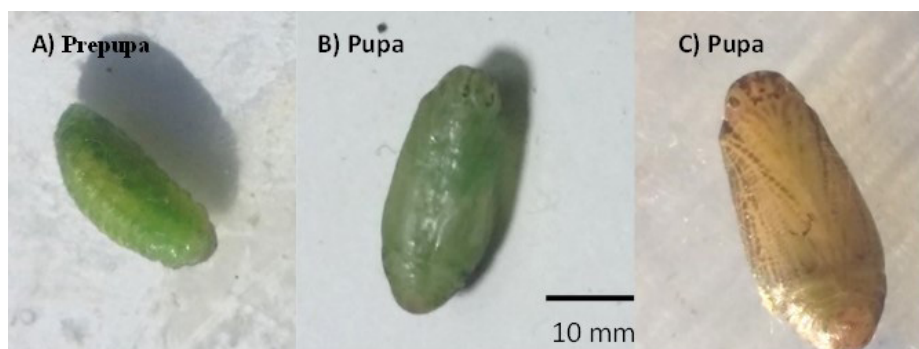


Figure 7. Pupae of *C. pandava* by camera (64MP).



Figure 8. Adults of *C. pandava* by camera (64MP): A & B) Female, C & D) Male.

in colour which concentrated nearly at basal of wing (Figure 11D) with grayish cilia at terminal edge (Figure 11B). However, the ventral view or underside is lightly grayish brown in colour with forewing of female (Figure 11C). The undersides of fore wing for blue butterfly female and male are greater uniform, and lightly grayish brown that have numerous bands of dark brown spots surrounded by dull white edge (Figure 11C, E). In addition, both male and female wings have longitudinal veins that connected with them by transverse lines with are edged by densely dark longitudinal hairs (Figure 11A, B),

The hindwing of female and male (Figure 12) is broader than forewing, bluish in colour but lightly than fore wing. In upperside view, the submarginal edge of female wing contained eight of blackish spots covered by the whitish and gray ring. Six of them are great big and two are small in size. First and second spots that nearly to apical edge of the wing are obsolescent or vanish. While, 6th darkly spot is larger than others and has an orange crown cap in female hindwing (Figure 12B). Contrariwise, in view of upperside of male, all of the eight black spots are nearly obsolescent or vanish in appearance due to the found the

very densely longitudinal hairs except 6th darkly spot is a large and slightly dark in male wing (Figure 12D).

The underside of hindwing in both female and male (Figure 12A, C) are not uniform in appearance. In female, the underside of hindwing has grayish brown bands covered by white lines. The terminal end of hind wing has 9 blackish spots surrounding by a white ring. The 6th, 8th and 9th spots are deeply black in colour, additionally, the orange cap was observed overhead of 6th and 8th spots. In outer edge of hindwing, a dark black spot was found in the medium. Nearly of the base, two separated spots were found in the underside of female hindwing (Figure 12A). Regarding of the underside of male hindwing, all of black spots are nearly obsolescent or vanish in appearance except the 6th darkly spot is appeared a pale dark in colour but it hasn't orange cap (Figure 12C). Cilia or hairlike structures were observed to be longitudinally extended in large numbers on the all surface of hindwing specially in male (Figure 12). The wings of *C. pandava* are covered by intensive or densely scales (Figures 11-12).

The presented results in Table 2 showed that the wing morphometric parameters as well as the length of

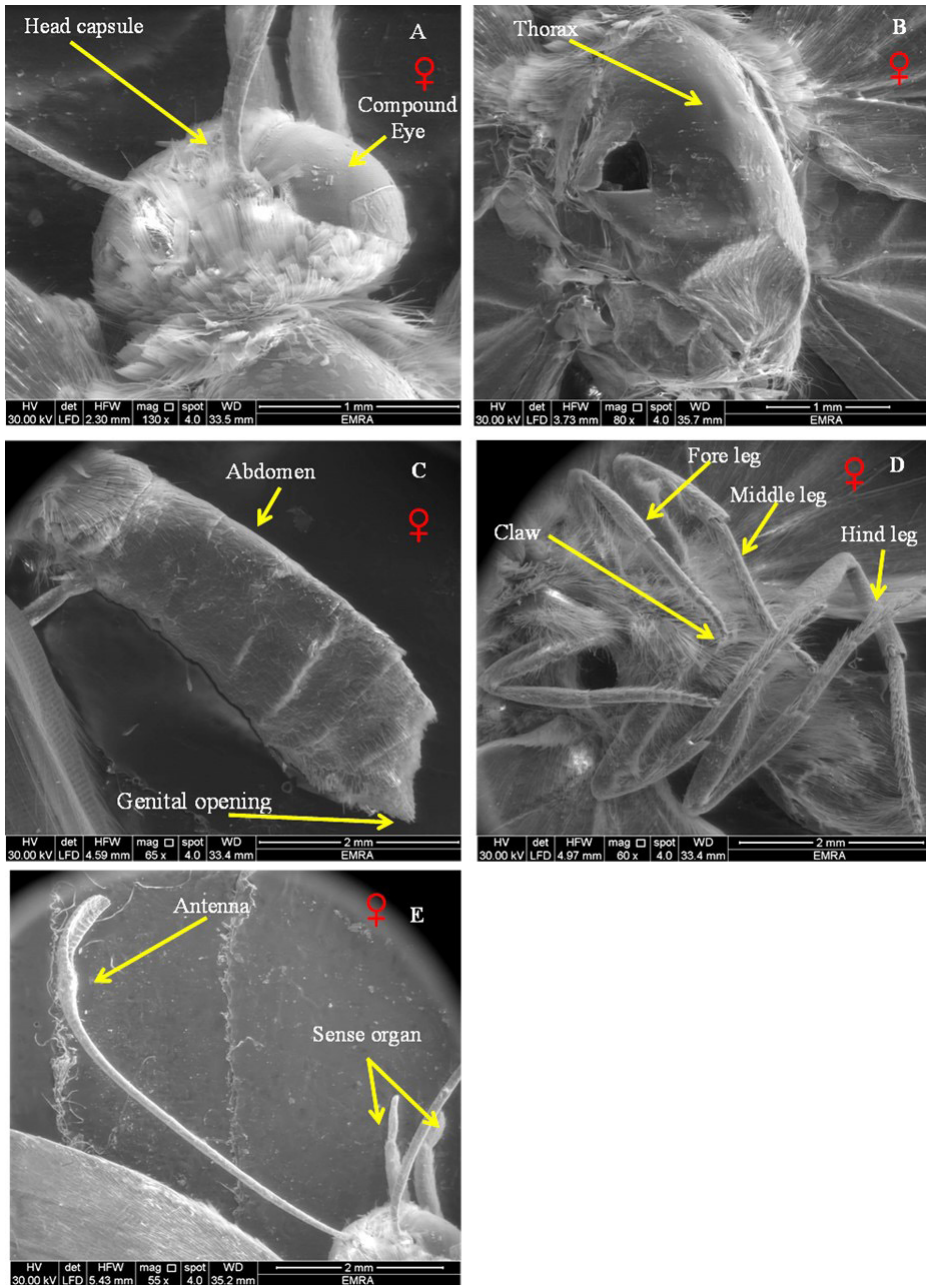


Figure 9. SEM of female adult of *C. pandava*: A) Head capsule & Eye, B) Thorax, C) Abdomen, D) Legs, E) Antenna.

base-tornus, base-apex and apex-tornus for the forewing and hindwing were detected. Also, both of three wing veins namely the Cubitus 2 (C2), Radius 2 (R2) and anal (A) of *C. pandava* forewing were measured (Table 2 and Figure 12B). Regarding forewing, the lengths of base-apex (BA) was $\sim 12.88 \pm 0.05$ mm in female and 11.29 ± 0.12 mm in male of *C. pandava* with statistically significant appearance ($Pr > |T| = 0.04$). Also, a significant difference was stated with base-tornus (BT) ($\sim 10.56 \pm 0.09$ and 9.27 ± 0.08 mm with female and male at $Pr > |T| = 0.02$, respectively). However, a non-significant was recorded

apex-tornus (AT) ($\sim 8.54 \pm 0.05$ mm in female and 8.48 ± 0.05 mm in male at $Pr > |T| = 0.89$, respectively) (Table 2 and Figure 11C). For hindwing, a non-significant difference by statistically analysis was observed with base-apex (BA) ($\sim 10.57 \pm 0.06$ and 9.65 ± 0.05 mm with female and male) and base-tornus (BT) ($\sim 8.62 \pm 0.033$ and 8.07 ± 0.091 mm with female and male at $Pr > |T| = 0.20$). But a considered statistically significant was reported with Apex-Tornus (AT) of hindwing ($\sim 8.27 \pm 0.047$ and 6.76 ± 0.003 with probability a level 0.05 equal 0.03). According to forewing venation, the length of veins viz radius 2 and cubitus

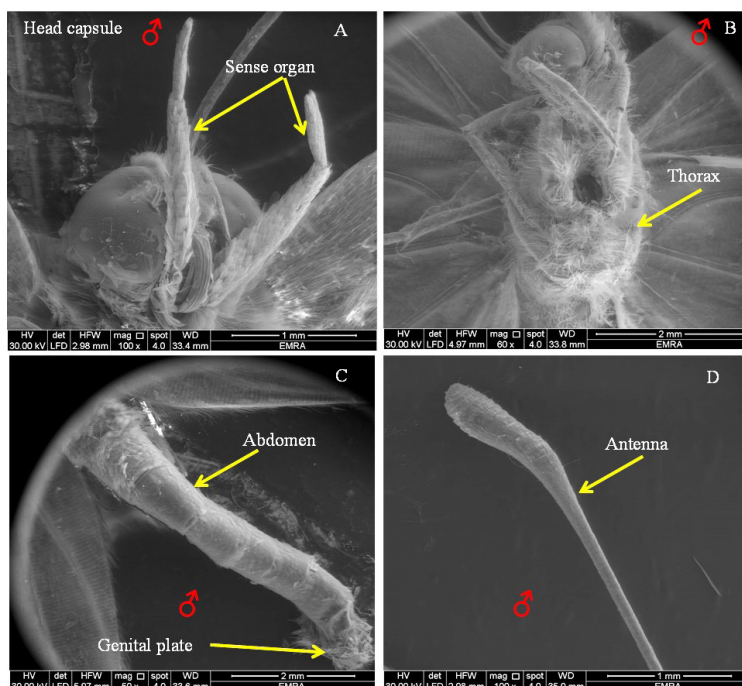


Figure 10. SEM of adult male of *C. pandava*: A) Head capsule & Eye, B) Thorax, C) Abdomen, D) Antenna.

Table 2. Measurements of different parts of adult stage of *Chilades pandava* on Cycas palms.

Parameters	Measurement (mm)		Significant level	
	Female	Male	t-value	Pr > T
Adult length	8.19 ± 0.025	8.42 ± 0.079	- 0.43	0.69
Head capsule	Length	0.96 ± 0.006	0.90 ± 0.006	4.65
	Width	1.58 ± 0.009	1.54 ± 0.006	0.41
Diameter of Eye	0.58 ± 0.025	0.56 ± 0.015	0.35	0.74
Antenna length	6.04 ± 0.013	5.95 ± 0.011	1.33	0.25
thorax	Length	3.17 ± 0.007	2.54 ± 0.062	1.93
	Width	3.48 ± 0.011	1.75 ± 0.015	3.37
			0.03*	
abdomen	Length	4.06 ± 0.012	4.98 ± 0.011	- 1.62
	Width	1.44 ± 0.023	0.78 ± 0.009	2.57
			0.06	
Fore leg	4.15 ± 0.006	3.17 ± 0.017	7.49	0.002*
Middle leg	5.47 ± 0.010	4.43 ± 0.012	2.83	0.05*
Hind leg	4.72 ± 0.015	4.29 ± 0.012	0.96	0.39
Forewing	Base-Apex (BA)	12.88 ± 0.05	11.29 ± 0.12	2.97
	Base-Tornus (BT)	10.56 ± 0.09	9.27 ± 0.08	3.59
	Apex-Tornus (AT)	8.54 ± 0.05	8.48 ± 0.05	0.14
				0.89
Hindwing	Base-Apex (BA)	10.57 ± 0.06	9.65 ± 0.05	1.84
	Base-Tornus (BT)	8.62 ± 0.033	8.07 ± 0.091	1.53
	Apex-Tornus (AT)	8.27 ± 0.047	6.76 ± 0.003	3.24
				0.14
				0.20
				0.03*
Radius 2 (R2) for vein of fore wing	11.89 ± 0.09	10.25 ± 0.11	3.07	0.04*
Cibitus 2 (C2) for vein of fore wing	12.76 ± 0.04	11.01 ± 0.06	3.99	0.02*
Anal (A) for vein of fore wing	10.63 ± 0.17	9.70 ± 0.1	1.71	0.16

Pr = Probability. *Significant.

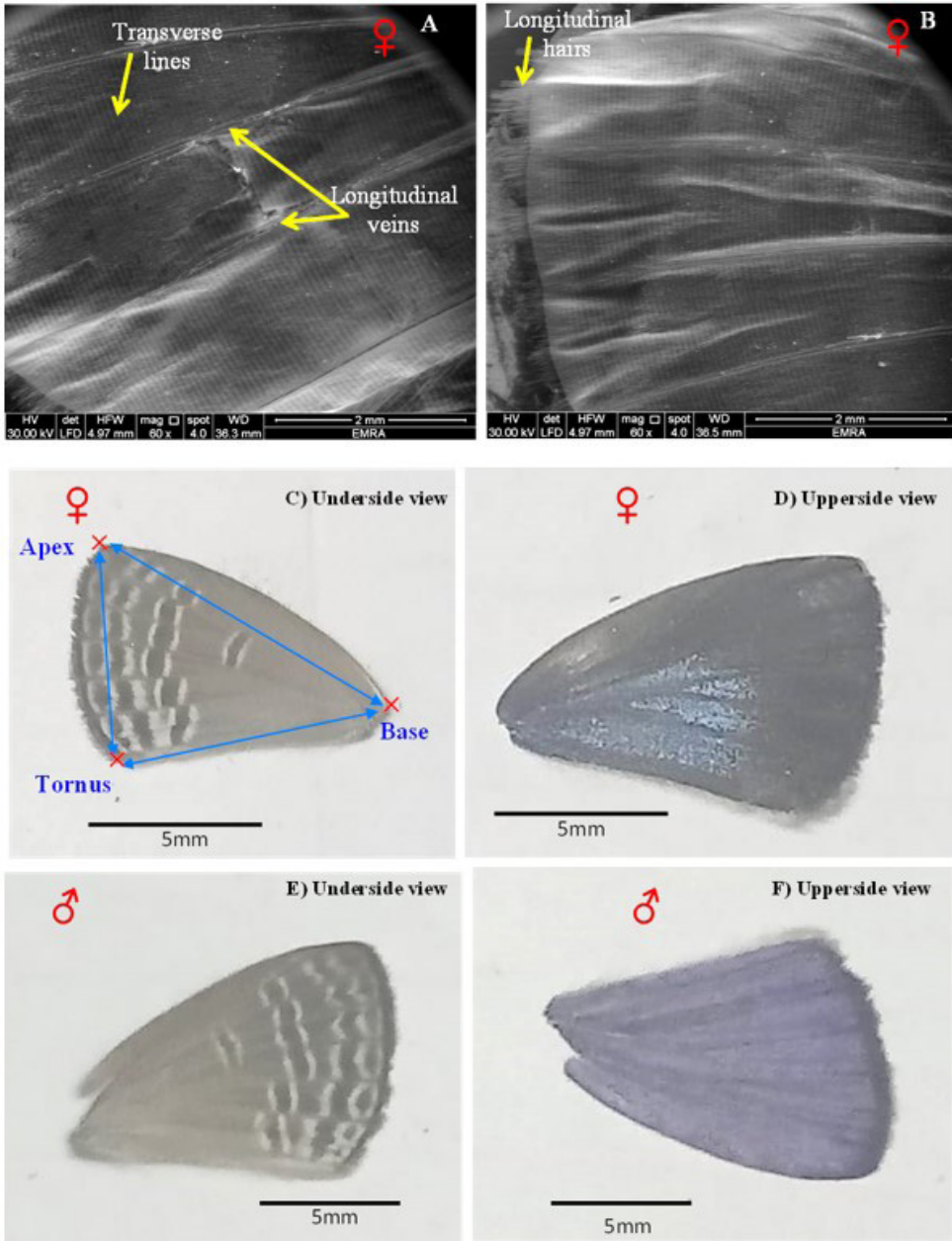


Figure 11. Forewing female and male adults of *C. pandava* by SEM and camera (64MP).

2 was considered significant, but the anal vein length did not indicate any significant differences between female and male (Table 2).

3.2. Biological development of *C. pandava* stages on ornamental palms, cycas and zamia

The influence of two ornamental palms, *Cycas* and *Zamia* on the biological features of *C. pandava* under laboratory conditions was observed. Cycad blue butterfly, *C. pandava* life cycle passed through egg, larval instars, pre-pupa, pupa and adult stages. Data presented in Table 3 indicated

that the developmental period of all immature stages was very nearly closed similar in duration between both tested plants. The incubation period of egg was lower on cycas than zamia plants (1.84 ± 0.07 and 1.88 ± 0.05 days, respectively). However, 1st larval duration was equal value in both two investigated plants (3.7 ± 0.16 days) (Table 3). From 2nd larva to pupa, the cycas palm was recorded a lower duration of *C. pandava* development than other tested palm except pre-pupa stage has small value on zamia palm (2.1 ± 0.15 days). Concerning the two tested ornamental palms, a non-significant difference was observed with all

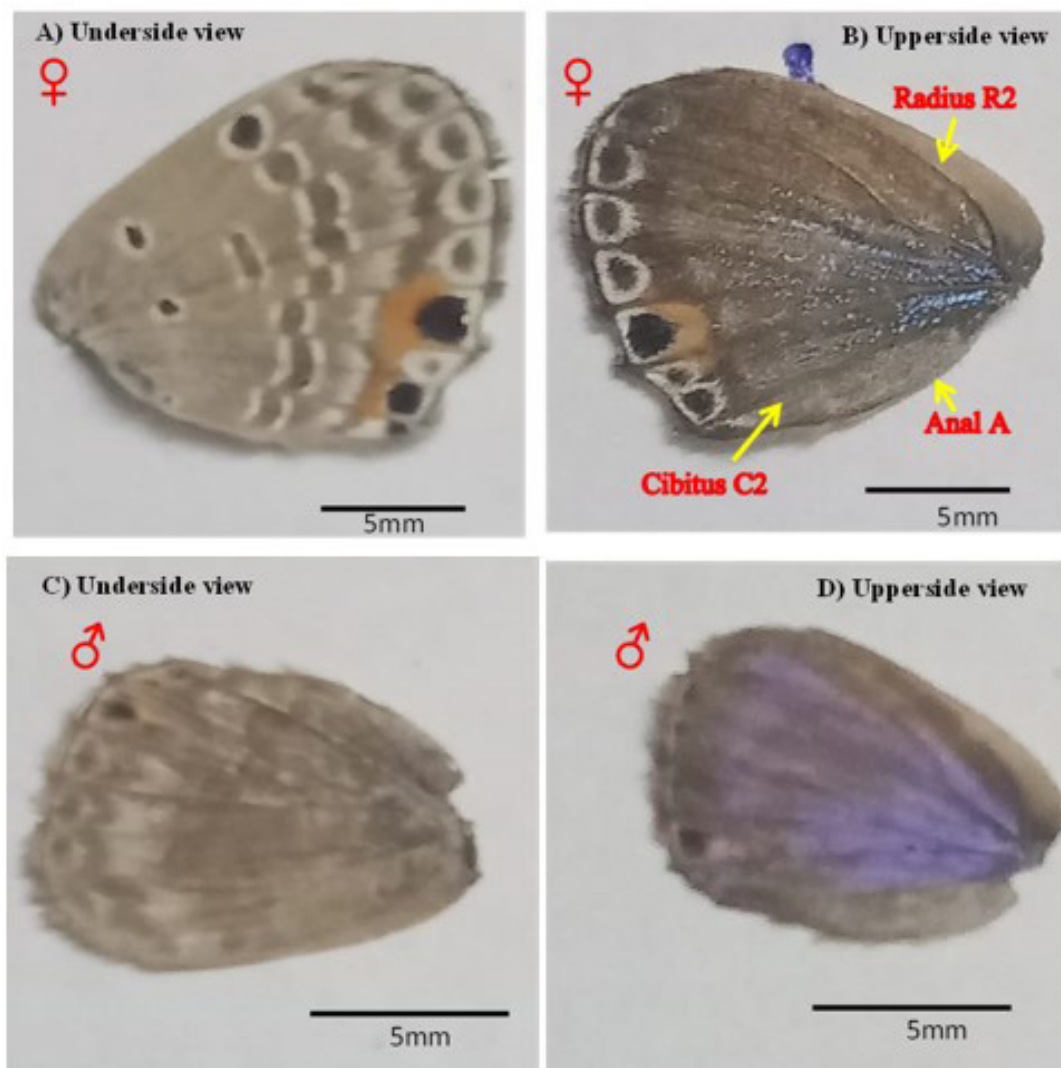


Figure 12. Hindwing female and male adults of *C. pandava* by SEM and camera (64MP).

immatures of *C. pandava*. In general, the larval and pupal durations were recorded longer period on zamia than cycas palms. So that, *C. pandava* life cycle (egg - adult) was slightly higher on zamia than cycas palms (Table 4).

The influence of two ornamental palms on the adult longevity (immediately from pupa moult to adult death) was stated confining by females and males just emergence immediately on two tested host palms, zamia and cycas (Table 4). The adult longevity was prolonged non-significantly when fed on cycas than zamia palms in case of *C. pandava* female and male (Table 4). Moreover, a high survival rate (86%) was detected on cycas palm than zamia (82%). The present data was indicated that a slightly higher sexual percent (Female %) was reported on zamia (54%) than cycas (53%) palms (Table 4) but this difference was not significant. So doubtless, the cycas palm was suitable diet for *Chilades pandava* development under laboratory conditions.

4. Discussion

4.1. Morphological studies for *C. pandava* stages reared on Cycas palms

In the present work, cycad butterfly, *C. pandava* in order Lepidoptera belonging to family Lycaenidae and subfamily Polyommatae. The genus *Chilades* was widely distributed on a varied range of ornamental plants, but *C. pandava* was restricted to be on the two palm species, Cycas and Zamia, in Egypt as that reported by Batt et al. (2019) and Abu-shall and Tawfeek (2015). Also, observed in other countries especially tropical regions including Guinea (Tennent, 2014), Taiwan, India, China, Sri Lanka and Southeast Asia (Igarashi and Fukuda, 2000; Hsu et al., 2002). In view of our results, *C. pandava* female laid individually eggs on the undersides of young leaves or newly fronds. The egg has numerous sac-like shape on its surface which connected by numerous transfer and

Table 3. Developmental periods (Mean \pm SE) of *C. pandava* stages reared on two palm types under laboratory conditions.

Stage	Incubation period of egg (days)	Larval duration (days)				Pupal duration			Larval duration (days)	Pupal duration (days)
		1 st larva	2 nd larva	3 rd larva	4 th larva	Pre-Pupa	Pupa			
Mean \pm SE	1.84 \pm 0.07	3.7 \pm 0.16	2.9 \pm 0.13	3.18 \pm 0.16	3.14 \pm 0.21	2.24 \pm 0.14	3.64 \pm 0.23	12.92 \pm 0.17	5.88 \pm 0.70	
(Min. -Max.)	(1 - 2)	(2 - 5)	(2 - 5)	(3 - 6)	(3 - 9)	(2 - 3)	(2 - 6)	—	—	
Mean \pm SE	1.88 \pm 0.05	3.7 \pm 0.16	3 \pm 0.19	3.4 \pm 0.21	3.7 \pm 0.25	2.1 \pm 0.15	3.92 \pm 0.26	13.8 \pm 0.17	6.02 \pm 0.91	
(Min. -Max.)	(1 - 3)	(3 - 5)	(2 - 7)	(3 - 6)	(3 - 9)	(1 - 3)	(3 - 6)	—	—	
t-value	-0.06	0.00	-0.17	-0.87	-1.36	0.93	-0.43	-1.25	-0.27	
Pr > t	0.96 ^{NS}	1.00 ^{NS}	0.87 ^{NS}	0.43 ^{NS}	0.25 ^{NS}	0.40 ^{NS}	0.69 ^{NS}	0.28 ^{NS}	0.80 ^{NS}	

Pr = Probability; NS = Not significant; SE = Standard error.

Table 4. Biological aspects of *C. pandava* fed on two palms under laboratory conditions.

Biological parameters	Host plants		Significant level		
	Cycas palm	Zamia palm	t-value	Pr > T	
Life cycle (days) (Mean ± SE)	20.64 ± 1.11	21.7 ± 1.26	- 0.90	0.42	
Longevity (days) (Mean ± SE)	Female	11.87 ± 0.49	10.93 ± 0.37	1.28	0.27
	Male	8.60 ± 0.95	7.60 ± 0.73	2.04	0.11
Survival rate %	86%	82%	—	—	
Sex ratio (Females %)	53%	54%	—	—	

Pr = Probability; NS = Not significant.

longitudinal canals. Moreover, there is a non-significant of variation between all stages in the gender of cycad blue butterfly in description details here, but the adult male was more densely blue or violet than adult female. Similarly, it was described by Kehimkar (2008); Batt et al. (2019) and Abu-shall and Tawfeek (2015). In the abdomen of adult, the last two segments are extensively modified to the genitalia form (Braby, 2000). By using of SEM, the obtained results showed that all stages of *C. pandava* were to be distinct by long and thick hairs covered all the body (Abu-shall and Tawfeek, 2015).

Additionally, in the present work, the cycad blue butterfly description based on the photographed camera as that in Feulner et al. (2014) and Abu-shall et al. (2014). In our obtained data, it is stated that the morphometric characters viz. pattern, length, width and venation of wings, body length, forewing, hindwing could be used as a guide for taxonomic discrimination (Azrizal-Wahid et al., 2016; Akand et al., 2018; Mahdi et al., 2018). Finally, a scarce /or rare morphological studies was conducted on this pest throughout the previously years, consequently, this study was to be added a valuable knowledge about *C. pandava* in Egypt.

4.2. Biological development of *C. pandava* stages on ornamental palms, cycas and zamia

C. pandava female laid individually eggs on the undersides of young leaves or newly shoots as in Batt et al. (2016). *C. pandava* life cycle passed through egg, four larval instars, pre-pupa, pupa and adults. This present data is in line with Liu et al. (2003); Ravuiwasa et al. (2011); Abu-shall et al. (2014) and Batt et al. (2016), they found that the life cycle of this species passes through these previously stages. Life cycle duration was ranged between 20.64 to 21.7 days in the present study as a previous study carried out by Lee (1989) who shows that it took 20-30 d to *C. pandava* develop from egg to adult. Also, Batt et al. (2016) conducted that life cycle of this species was extended between 13 to 21 days. In the present study, a new emerging female longevity was slightly longer than male (Hsu, 1987; Lee 1989; Ravuiwasa et al., 2011; Parsons, 1999; Wu et al., 2010).

The obtained data provides an insight on both of these endangered and endemic species, *C. pandava* and its host plant cycas and zamia, in which, the development

of *C. pandava* was slightly higher on zamia than cycas palms. So doubtless, the cycas palm was suitable diet for *C. pandava* development under laboratory conditions. Similarly, this butterfly was infested numerous cycas species up to 85 species (Marler et al., 2012). The extant issues deservedly mentioned the relevant factors for interpreting the data of our work. For cycas and zamia palms–*Chilades* system, Batt et al. (2019) mentioned to the variability of *C. pandava* infestation on both of cycas and zamia palms and its control methods. Moreover, a cycad palms was exclusively attacked by *C. pandava* as a preferred host plant (Marler et al., 2012). Variations in *C. pandava* infestation and occurrence were mentioned on zamia and cycas palms by Batt et al. (2019).

Little studies were focused on the morphology, biology and life history of *C. pandava* in details, but this article added realistic data in details on the description, survival, life cycle, and biology of *C. pandava* in comparison between cycas and zamia palms that have never been illustrated before in Egypt. In this study, the disparate studies between morphological characters and biology of *C. pandava* and caveats that we argued here may explain the relation between this lepidopteran pest and commingled cycas and zamia palms in a garden landscape and arboretum. The real case likely is a conflation of multiple tactics to have sound decisions for proposal of *C. pandava* management and conservation.

5. Conclusion

The endemic and endangered blue butterfly, *Chilades pandava* (Lepidoptera: Lycaenidae) is one of the main pests on the ornamental palms, cycas and zamia in Egypt. The morphometric characters viz. pattern, length, width and venation of wings, body length, forewing, hindwing could be used as a guide for taxonomic discrimination of this pest. The development of *C. pandava* was slightly higher on zamia than cycas palms. So that, the cycas palm was suitable diet for *C. pandava* development under laboratory conditions and in a garden landscape.

References

- ABU-SHALL, A., RAMADAN, H.M. and ABU-GHONEM, M.A., 2014. Immature stages of *Chilades pandava* (Lepidoptera: Lycaenidae), a

- new pest of *Cycas* spp. in Egypt. *Alexandria Journal of Agricultural Research*, vol. 59, no. 3, pp. 197-204.
- ABU-SHALL, A.M.H. and TAWFEEK, M.E., 2015. Description of the Egyptian form of *Chilades pandava* Horsfield (Lepidoptera: Lycaenidae: Polyommatainae) and ultrastructure of antennal sensilla. *Journal of Entomology*, vol. 12, no. 2, pp. 67-76. <http://dx.doi.org/10.3923/je.2015.67.76>.
- ACKERY, P.R. and VANE-WRIGHT, R.I., 1984. *Milkweed butterflies: their cladistic and biology. Being an account of the natural history of the Danainae, a subfamily of the Lepidoptera, Nymphalidae*. London: British Museum, 425 p.
- AKAND, S., BASHAR, M.A., RAHMAN, S. and KHAN, H.R., 2018. Morphometric variation in the species of two subfamilies of Lycaenid butterflies (Lepidoptera: Lycaenidae) of Bangladesh. *Journal of Biodiversity Conservation and Bioresource Management*, vol. 3, no. 1, pp. 9-16. <http://dx.doi.org/10.3329/jbcbm.v3i1.36756>.
- AL-SAYED, A.S., RAHMAN, A.A. and KESBA, H., 2014. Phytonematode community structure and dynamics on ornamental plantations of Egypt. *Egyptian Journal of Agronomy*, vol. 13, no. 1, pp. 26-43. <http://dx.doi.org/10.21608/ejag.2014.63629>.
- AYTEKIN, M.A., TERZO, M., RASMONT, P. and ÇAĞATAY, N., 2007. Landmark based geometric morphometric analysis of wing shape in *Sibirico bombus* Vogt (Hymenoptera: Apidae: *Bombus* Latreille). *Annales de la Société Entomologique de France*, vol. 43, no. 1, pp. 95-102. <http://dx.doi.org/10.1080/00379271.2007.10697499>.
- AZRIZAL-WAHID, N., SOFIAN-AZIRUN, M. and RIZMAN-IDID, M., 2016. The significance of wing and body morphometry in discriminating six species of *Eurema* butterflies (Lepidoptera: Pieridae) of peninsular Malaysia. *Sains Malaysiana*, vol. 45, no. 10, pp. 1413-1422.
- BAKR, E.M., 2005. A new software for measuring leaf area, and area damaged by *Tetranychus urticae* Koch. *C. Journal of Applied Entomology*, vol. 129, no. 3, pp. 173-175. <http://dx.doi.org/10.1111/j.1439-0418.2005.00948.x>.
- BATT, M.A., ABBAS, M.K.A. and BATT, A.M., 2016. *Zamia* palm, a new host of cycad borer, *Chilades pandava* Horsfield (Lepidoptera: Lycaenidae) in Egypt. *Minufiya Journal of Agricultural Research*, vol. 41, pp. 195-201.
- BATT, M.A., HASSAN, G.M. and EL-AASSAR, M.R., 2019. A study on infestation factors of cycas and *Zamia* palms with butterfly, *Chilades pandava* and its control in Egypt. *Pakistan Journal of Biological Sciences*, vol. 22, no. 10, pp. 477-485. <http://dx.doi.org/10.3923/pjbs.2019.477.485>. PMID:31930837.
- BAYLAC, M., VILLEMANT, C. and SIMBOLOTTI, G., 2003. Combing geometric morphometrics with pattern recognition for the investigation of species complex. *Biological Journal of the Linnean Society*, vol. 80, no. 1, pp. 89-98. <http://dx.doi.org/10.1046/j.1095-8312.2003.00221.x>.
- BRABY, M.F., 2000. *Butterflies of Australia: their identification, biology and distribution*. Melbourne: CSIRO Publishing, 101 p. <http://dx.doi.org/10.1071/9780643100770>.
- CHANG, Y.C., 1989. Morphology, life history and damage of cycas blue butterfly *Chilades pandava* as well as pathogenicity of entomogenous fungus to its larva. *Bulletin of Taiwan Forestry Research Institute New Series*, vol. 4, no. 1, pp. 43-50.
- DALY, H.V., 1985. Insect morphometrics. *Annual Review of Zoology*, vol. 30, pp. 415-438.
- DIGO, E.O., ABAD, K.L.M., GUINO-O, I.J.B., SAMILLANO, L.K.C., EDUQUE JUNIOR, R.M., TORRES, M.A.J. and REQUIERON, E.A., 2015. Application of geometric morphometrics in the body shapes of flying fish (*Parexocoetus brachypterus*) in Maitum, Sarangani province. *International Journal of the Bioflux Society*, vol. 8, no. 6, pp. 1027-1030.
- ELIOT, J.N., 1973. The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. *Bulletin of the British Museum (Natural History) Entomology*, vol. 28, no. 6, pp. 371-505. <http://dx.doi.org/10.5962/bhl.part.11171>.
- FEULNER, G.R., ROOBAS, B., CARLISLE, T. and MEYER, H., 2014. First UAE and Arabian record of *Chilades pandava*, the Cycad Cupid butterfly, an introduced Oriental species (Lepidoptera: Lycaenidae) hosted by the ornamental sago plant *Cycas revoluta*. *Tribulus*, vol. 22, pp. 48-56.
- FIEDLER, K., 1996. Host-plant relationships of lycaenid butterflies: large-scale patterns, interactions with plant chemistry, and mutualism with ants. *Entomologia Experimentalis et Applicata*, vol. 80, no. 1, pp. 259-267. <http://dx.doi.org/10.1111/j.1570-7458.1996.tb00931.x>.
- FRIC, Z., DICKINSON, R., FETOUH, G., LARSEN, T.B., SCHON, W. and WIEMERS, M., 2014. First record of the cycad blue, *Chilades pandava*, in Egypt- a new invasive butterfly species in the Mediterranean region and on the African continent (Lepidoptera: lycaenidae). *African Entomology*, vol. 22, no. 2, pp. 315-319. <http://dx.doi.org/10.4001/003.022.0205>.
- HSU, Y.-F., 1987. Notes on *Chilades pandava pandava* Horsfield from Taiwan (Lepidoptera, Lycaenidae). *TYO TO GA*, vol. 38, pp. 9-12.
- HSU, Y.-F., HUANG, C.-L.G. and LIANG, J.-Y., 2002. *Pieridae*. Luku: Forestry Bureau Council of Agriculture. Butterfly Fauna of Taiwan, vol. 2.
- IGARASHI, S. and FUKUDA, H., 2000. *The life histories of Asian butterflies*. Tokyo: Tokai University Press.
- KEHIMKAR, I., 2008. *The book of Indian butterflies*. Mumbai: Bombay Natural History Society, 497 p.
- KUNTE, K. and TIPPLE, A., 2009. The polyommata wing pattern elements and seasonal polyphenism of the Indian *Chilades pandava* butterfly (Lepidoptera: lycaenidae). *News of the Lepidopterists' Society*, vol. 51, no. 3, pp. 86-88.
- LEE, J.Y., 1989. Notes on the life history of *Chilades pandava pandava* Horsfield (Lepidoptera, Lycaenidae) in Taiwan. *Gekkan-Mushi*, vol. 215, pp. 4-5.
- LIU, G.H.A., LU, Y.Y.E., GAN, Y.H.N.G. and ZENG, L., 2003. The biology and population dynamics of the butterfly *Chilades pandava*. *Entomological Knowledge*, vol. 40, no. 5, pp. 426-428.
- MAHDI, S.H.A., FERDOUS, M.E.M. and ARA, N., 2018. Assessment of morphometric characters of the *Chilades pandava* and *Chilades lajus* (Lepidoptera: Lycaenidae) butterflies. *Scholars Academic Journal of Biosciences*, vol. 6, no. 6, pp. 459-464.
- MARLER, T.E., LINDSTROM, A.J. and TERRY, L.I., 2012. *Chilades pandava* damage among 85 cycas species in a common garden setting. *HortScience*, vol. 47, no. 12, pp. 1832-1836. <http://dx.doi.org/10.21273/HORTSCI.47.12.1832>.
- MILLER, W.E., 1991. Body size in North American lepidoptera as related to geography. *Journal of the Lepidopterists Society*, vol. 45, no. 2, pp. 158-168.
- MUTANEN, M., ITAMIES, J. and KAITALA, L., 2007. *Heliozelare splendella* (Stainton, 1851) and *H. hammoniella* Sorhagen, 1885: two valid species distinguishable in the genitalia of both sexes and life histories (Heliozelidae). *Nota Lepidopterologica*, vol. 30, no. 1, pp. 79-92.
- PARSONS, M., 1999. *The butterflies of Papua New Guinea*. London: Academic Press, 736 p.
- PIERCE, N.E., BRABY, M.F., HEATH, A., LOHMAN, D.J., MATHEW, J., RAND, D.B. and TRAVASSOS, M.A., 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera).

- Annual Reviews*, vol. 47, no. 1, pp. 733-771. <http://dx.doi.org/10.1146/annurev.ento.47.091201.145257>. PMID:11729090.
- RAVUIWASA, K.T., TAN, C.W. and HWANG, S.Y., 2011. Temperature-dependent demography of *Chilades pandava peripatria* (Lepidoptera: lycaenidae). *Journal of Economic Entomology*, vol. 104, no. 5, pp. 1525-1533. <http://dx.doi.org/10.1603/EC11034>. PMID:22066181.
- RICKLEFS, R.E. and MILES, D.B., 1994. Ecological and evolutionary inferences from morphology: an ecological perspective. In: P.C. WAINWRIGHT and S.M. REILLY, eds. *Ecological morphology*. Chicago: The University of Chicago Press, pp. 13-41.
- RIVA, J., PONT, F., ALI, V., MATIAS, A., MOLLINEDO, S. and DUJARDIN, J.P., 2001. Wing geometry as a tool for studying the *Lutzumyia longipalpis* (Diptera: Psychodidae) complex. *Memórias do Instituto Oswaldo Cruz*, vol. 96, no. 8, pp. 1089-1094. <http://dx.doi.org/10.1590/S0074-02762001000800011>. PMID:11784928.
- SAS, 2003. *SAS version 9.1*. Cary, NC, USA: SAS Institute Inc.
- TENNENT, W.J., 2014. Two new subspecies of *Mycalesis terminus* (Fabricius, 1775), from the islands of Milne Bay province, Papua New Guinea (Lepidoptera, Satyrinae). *Tropical Lepidoptera Research*, vol. 24, no. 2, pp. 62-66.
- TUZUN, A., 2009. Significance of wing morphometry in distinguishing some of the Hymenoptera species. *African Journal of Biotechnology*, vol. 8, no. 14, pp. 3353-3363.
- VILLEGAS, J., FELICIANGELI, M.D. and DUJARDIN, J.P., 2002. Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *Rhodnius robustus* from Merida (Venezuela). *Infection, Genetics and Evolution*, vol. 2, no. 2, pp. 121-128. [http://dx.doi.org/10.1016/S1567-1348\(02\)00095-3](http://dx.doi.org/10.1016/S1567-1348(02)00095-3). PMID:12797988.
- WU, L.W., YEN, S.H., LEES, D.C. and HSU, Y.F., 2010. Elucidating genetic signatures of native and introduced populations of the cycad blue, *Chilades pandava* to Taiwan: a threat both to Sago Palm and to native *Cycas* populations worldwide. *Biological Invasions*, vol. 12, no. 8, pp. 2649-2669. <http://dx.doi.org/10.1007/s10530-009-9672-4>.