Species

Metamorphosis of silver-striped hawkmoth (*Hippotion celerio L.*) (Lepidoptera: Sphingidae) collected and reared in Bukidnon, Philippines

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ABSTRACT

This research examined at the life cycles of the species that were raised on *Colocasia esculenta* and used DNA barcoding to identify them. Based on National Center for Biotechnology Information (NCBI) and Barcode of Life Data Base (BOLD) with 98.91% and 100% identity respectively, genomic DNA of the reared species revealed its identity as *Hippotion celerio* species with a common name Silver-striped hawkmoth belonging to family the Sphingidae. The complete life cycle of Silver-striped hawkmoth (*H. celerio*) are composed of egg, larvae (first instar, second instar, third instar, and fourth instar), pupa and adult. The morphological description of each life stages was described in detailed. Duration of their life cycle at the emergence of an adult ranged 32 to 34 days.

Keywords: *Hippotion celerio,* instar, life cycle, Genomic DNA, BOLD, NCBI

1. INTRODUCTION

Hippotion celerio (L.) belongs to the Class Insecta, Order Lepidoptera, Family Sphingidae and Subfamily Macroglossinae. It is commonly known as taro hawkmoth, grapevine sphinx or silver-striped hawkmoth. Like butterflies, these hawkmoths are holometabolous insect in which they possess a complete life cycle from egg, larva, pupa and adult (Powell, 2009). The presence of caudal horn during their larval stage is their distinguishing characteristics as well as their narrow forewing and shorter hindwing, bullet-shaped bodies, rapid flyers and long proboscis are the hallmarks of this family (Johnson et al., 2016; Akkuzu et al., 2007; Mohagan et al., 2018; Stöckl & Kelber, 2019). They are nocturnal insects and feed on flowers that contain heavy fragrance (Stöckl & Kelber, 2019). *H. celerio* are known to feed on several plants like *Caladium, Colocasia,* spread hog weed, four o'clock and lettuce tree (Jeenkoed et al., 2016).

Hawkmoths play an important role in the stability of our ecosystem in which they act as herbivores and even responsible for the pollination of tropical flora

(Primo et al., 2013; Powell, 2009). De Camargo et al., (2016) assert that their existence is of significant ecological and economic value. Sometimes, *H. celerio* larvae are pests to some plants. Beetroot *(Beta vulgaris* L.), Sweet potatoes (*Ipomoea batatas* (L.) Lam.) and grapes are occasionally seriously hampered by the *H. celerio* species. The larvae consume the leaf, leaving behind just the veins, severely defoliating the plant. Taro (*Colocasia esculenta* (L.) Schott) leaves can be eaten all the way to the ground, together with the tender young stems and shoots (Jeenkoed et al., 2016). Due to the high mortality from extreme weather conditions and natural adversaries, outbreaks are rare (Primo et al., 2013). *H. celerio* developmental duration may vary depending on their consumed host plant. This study focused on the metamorphosis of *H. celerio* species.

2. MATERIALS AND METHODS

Hippotion celerio (silver-striped hawkmoth) eggs were collected from the taro leaves near the lakes or ponds around the vicinity of Musuan, Bukidnon. Eggs collected were placed inside a plastic disposable container with proper ventilation and was provided with tissue underneath to avoid moisture. This study was carried out at room temperature. Upon hatching of the silver-striped hawkmoth eggs, it was fed with *Colocasia esculenta* until their last instar. Food was replenished every day to assure the development and growth was not limited. Larvae were checked twice a day to see if it undergone molting and record all possible changes in terms of its morphological characteristics. Different stages were also viewed under a stereo microscope. Molecular Identification was carried at Natural Science Research Center (NSRC), Central Mindanao University (CMU). Identification of the species reared were subjected to DNA Barcoding by isolating its genomic DNA from its adult legs and undergone Polymerase Chain Reaction (PCR) followed by electrophoresis to visualize the bands of the PCR product. Primer LepF (ATTCAACCAATCATAAAGATATTGG) and LepR (TAAACTTCTGGATGTCCAAAAAATCA) (Koch, 2010; Wilson, 2012) were utilized in this study. Successful PCR product was sequenced at Macrogen South Korea.

3. RESULTS AND DISCUSSION

Isolated DNA of reared species revealed its identity based on (National Center for Biotechnology Information) NCBI and Barcode of Life Data Base (BOLD) as *Hippotion celerio* with 98.91% and 100% percentage identity respectively. *Hippotion celerio* also known as taro hawkmoth or grapevine sphinx belongs to the family of sphingidae in which they possess a complete life cycle from egg, larva, pupa (chrysalis) and adult (Figure 1). This family are voracious eaters and specific to the food plants they eat during their larval stage (Halder *et al*., 2018).

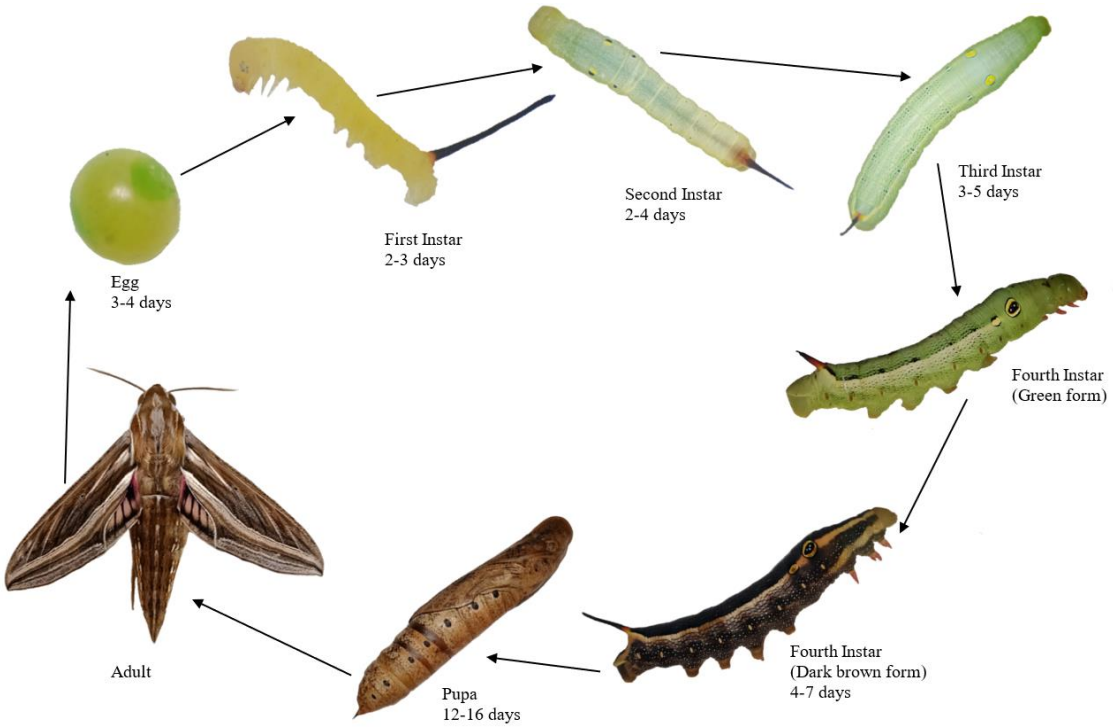

Figure 1 *Hippotion celerio* (Linnaeus, 1758) Complete Metamorphosis

Egg

The eggs of *H. celerio* measured 1.0 mm and were round, clear, smooth surface, bluish green in color, but turns to yellowish color prior to emergence. During the actual searching of eggs on the taro leaf found within the vicinity of Musuan they were laid singly on the lower part of the leaf. According to Goldberg et al., (2015) & Messenger, (1997), eggs laid singly to help them survive when they hatched so that they will not starve due to the insufficient amount of food available. However, cultured hawkmoth species laid their eggs in masses since only one plant were placed inside their cage, they usually oviposit their eggs underneath the leaves of the plant. Additionally, some eggs were discovered on the cage's screen, the stalk and the upper side of a leaf. The incubation period is 3–4 days long (Figure 2).

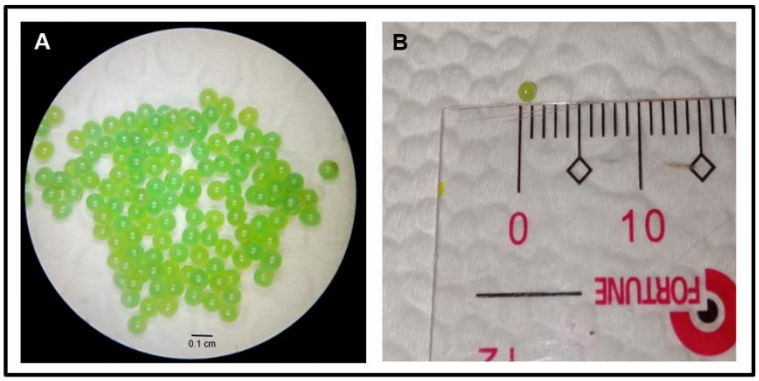

Figure 2 *Hippotion celerio* (Linnaeus, 1758) – (A) Egg showing typical colour (20X), (B) measurement. (3-4 days)

Larva

Larva of family sphingidae can easily recognized by the presence of caudal horn or button and by 6-8 annulets on each body segments (Devi & Ramaju, 2015; Pittaway & Kitching, 2020: Messenger, 1997). Their body consists of tagmata namely head, thorax and abdomen. They are voracious eaters in which their growth depends on the quality and quantity of plants eaten. *H. celerio* undergone four (4) instars with the duration of nine (9) to sixteen (16) days and varies in color from yellow (1st instar), green (2nd to 4th instar) to brown (4th instar).

First Instar

On hatching, the larva starts to consume its egg shell. Its body is slender, cylindrical, smooth and pale-yellow color but after feeding, its thorax and part of its abdomen turns to pale greenish color with long black horn. Head still pale-yellow color without markings. True legs, ventral prolegs and anal plate are similarly colored to its body. When threatened, they started to wiggle their horn. Duration about 2-3 days (Figure 3).

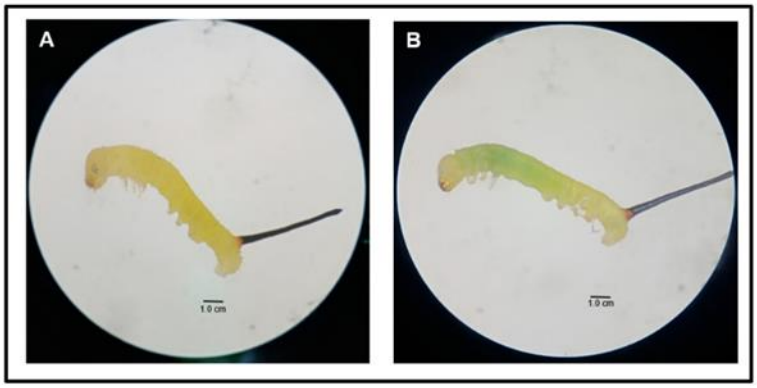

Figure 3 *Hippotion celerio* (Linnaeus, 1758) – (A) 1st instar soon after hatching (20X), (B) 1st instar after feeding (20X) (2-3 days)

Second Instar

The body is long (with ten abdominal segments), slender and cylindrical, light to mid-green coloration with white spots all over its body, yellowish on abdominal segment 8 and beyond. Eye-spots is circle with black and yellow coloration appear on the first abdominal segment and larger than the black eyespots on the second abdominal segment. Head pale yellow without markings.

Caudal horn is long and black with small spikes, slightly orange at the bottom near the anal part. Thorax bears a ventral true leg (T1-foreleg, T2-mid leg and T3- hind leg) while A3, A4, A5 and A6 bears a caudal prolegs usually with crochets (bi ordinal homoideous mesoseries). A7, A8 and A9 does not bear a pro leg. True legs, ventral prolegs, claspers and anal plate are similarly colored to its body but a little paler. Duration about 2-4 days (Figure 4).

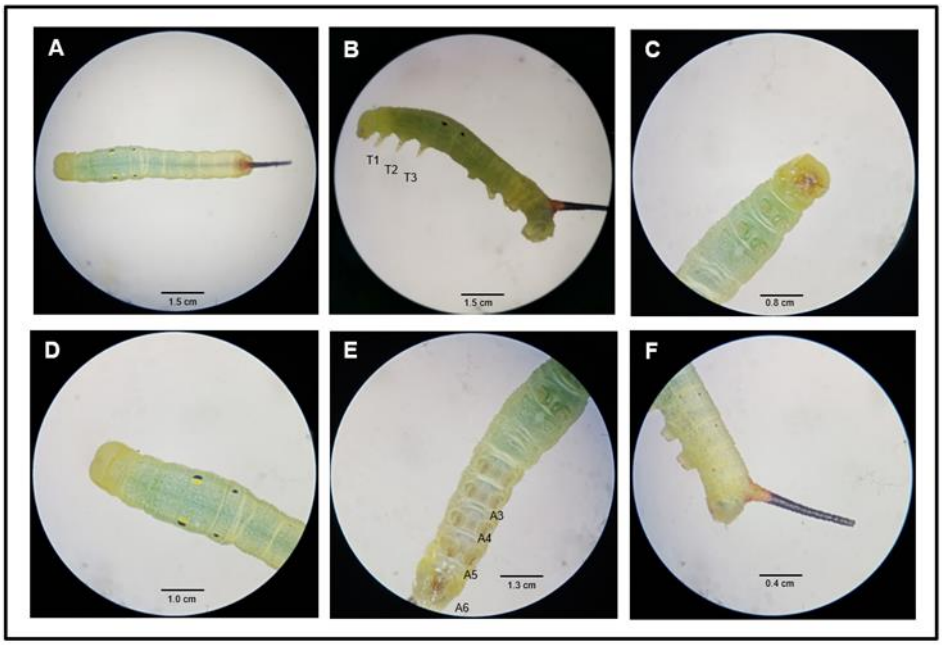

Figure 4 *Hippotion celerio* (Linnaeus, 1758) – 2nd Instar (A) Dorsal view, (B) Lateral view, (C) Head ventral view and ventral true legs, (D) Eyespots on 1st abdominal segment (large) and 2nd abdominal segment (smaller), (E) Ventral prolegs with crochets (bi ordinal homoideous mesoseries), (F) Caudal horn (20X) (2-4 days).

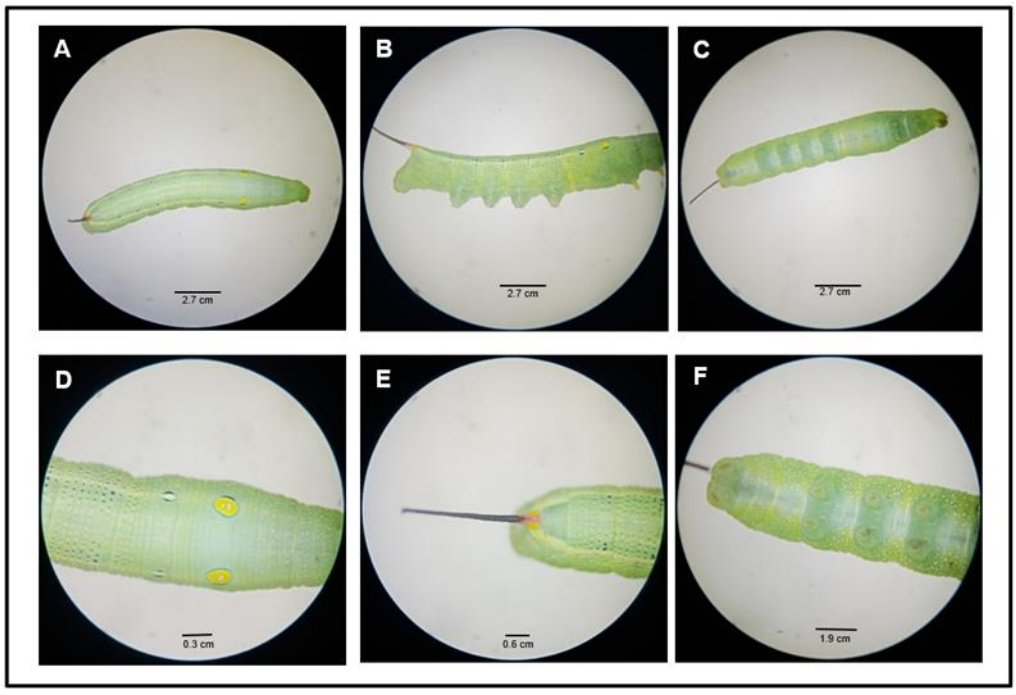

Figure 5 *Hippotion celerio* (Linnaeus, 1758) – 3rd Instar (A) Dorsal view, (B) Lateral view, (C) Ventral view and ventral true legs, (D) Eyespots on 1st abdominal segment (large) and 2nd abdominal segment (smaller), (E) Caudal horn, (F) Prolegs with crochets (bi ordinal homoideous mesoseries) (20X) (3-5 days).

Third Instar

The body is long (with ten abdominal segments) and cylindrical, light to green coloration with white spots present on the ventral part of its body while black spots were observed dorsally along with the dorso-lateral white lines appears from 2nd abdominal segments down to the base of the horn. Eye-spots is larger than the second instar with black circle line, dominantly colored yellow inside and composed with two smaller light green circles on the 1st abdominal segment while eyespots on the 2nd abdominal segment is smaller with black and white coloration. Head is green with no markings. Caudal horn is long and black with small spikes, with pinkish and orange coloration near the base at the anal plate. Thorax bears a ventral true leg (T1-foreleg, T2- mid leg and T3- hind leg), yellow in color, while A3, A4, A5 and A6 bears a caudal prolegs usually with crochets (bi ordinal, homoideous mesoseries). A7, A8 and A9 does not bear a pro leg. Duration about 3-5 days (Figure 5).

Fourth Instar

Last instar varies in color from green to dark brown coloration. According to Eacock et al., (2019), it serves as a cue that it has finished eating (or soon will finish eating) and seek a spot to either diapause or form a chrysalis. Sometimes this color change goes from the background color of their host plant (green) to a more earth tone shade (brown) similar to the likely background color of where they will diapause or pupate (Figure 6 & 7).

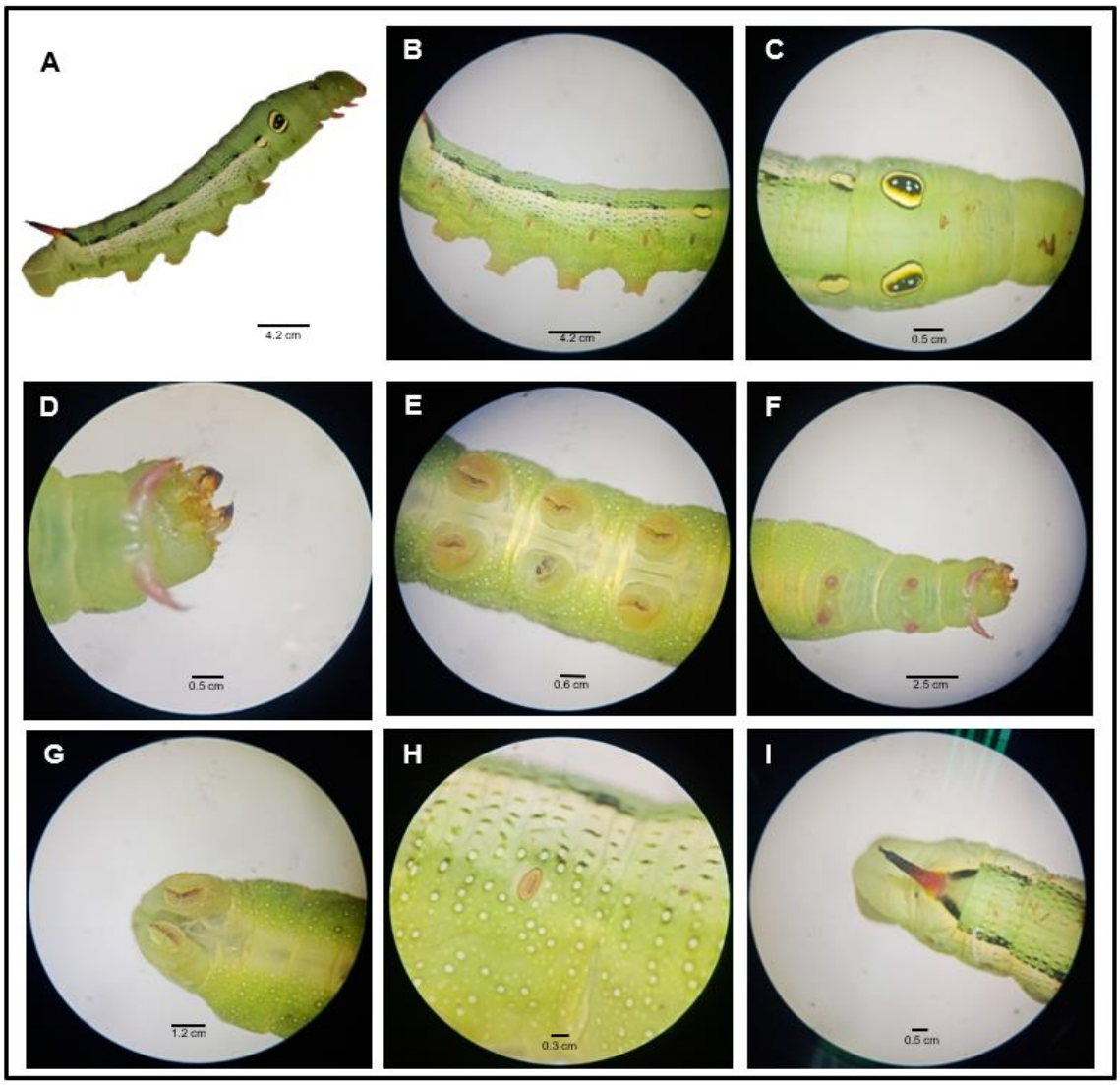

Figure 6 *Hippotion celerio* (Linnaeus, 1758) – 4th Instar (A) Lateral view, (B) Lateral view of its prolegs, (C) Eyespots on 1st abdominal segment (large) and 2nd abdominal segment (smaller), (D) Ventral view of head with setae (chalaza), (E) Prolegs ventral view with crochets (biordinal homoideous mesoseries), (F) Ventral view of true legs, (G) Ventral part of last abdominal segment (A10) bears pro leg with crochets, anal plate, (H) Spiracle, (I) Caudal horn (20X)

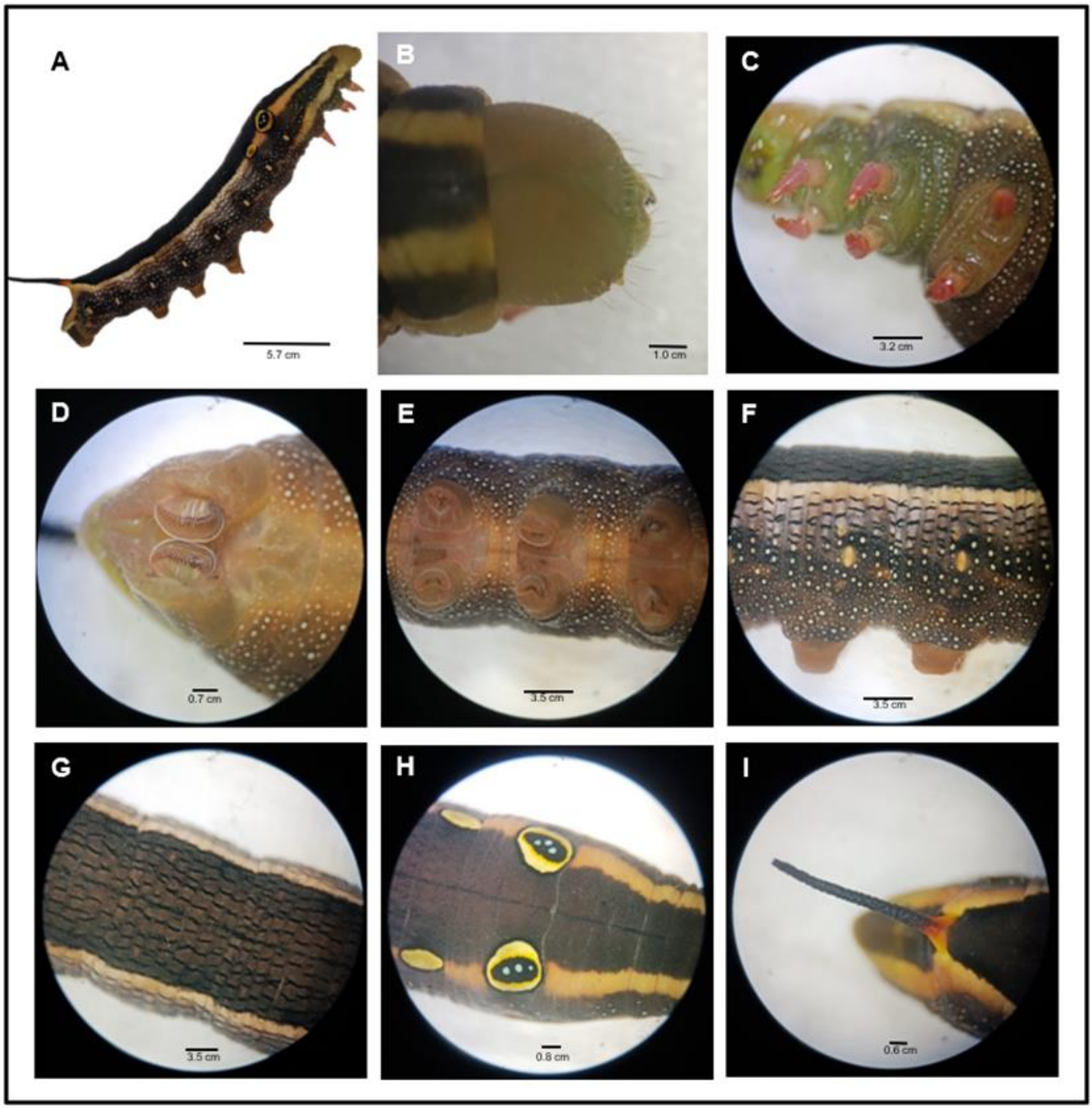

Figure 7 *Hippotion celerio* (Linnaeus, 1758) – 4th Instar (A) Lateral view, (B) Head dorsal view with setae (chalaza), (C) True legs (D) Ventral part of last abdominal segment, anal plate, A10 bears a pair of pro leg with crochets, (E) Prolegs ventral view with crochets (biordinal homoideous mesoseries), (F) Spiracles, (G) Dorsal body part (H) Eyespots, (I) Caudal horn (20X) (4-7 days).

Green form

The body is long (with ten abdominal segments) and cylindrical, green coloration with white spots present on the ventral-lateral part of its body while black spots were observed dorsally along with the thick dorso-lateral white lines and black broken lines that appears from 3rd abdominal segments down to the base of the horn. Eyespots has black circle line coloration, while the inside has white, yellow and black color with four small white dots at the center. Eyespots present on second abdominal segment, dominantly yellow color with pinch of black on one side. Head is green with no markings but setae (chalaza) were present. Caudal horn is long and black with small spikes, with pinkish and orange coloration near on the base at the anal plate. Thorax bears a ventral true leg (T1- foreleg, T2- mid leg and T3- hind leg), pinkish in color, while A3, A4, A5 and A6 bears a caudal prolegs usually with crochets

(biordinal, homoideous mesoseries) as well as the anal prolegs (A10). Its prolegs consists of two parts, the proximal base which bears setae (chalaza) and distal planta which never bears setae (chalaza) but it is where crochets arise. A7, A8 and A9 does not bear a pro leg. Spiracles is oval, brownish color with dark vertical center (Figure 6).

Dark brown form

The body is still long (with ten abdominal segments) and cylindrical, dark brown coloration were observed all throughout its body with white spots present on the ventral-lateral part of its body while thick dorso-lateral white lines appears from 2nd thoracic segments down to the base of the horn. Dorsal part of its abdomen as well as its dorso-lateral part has series of wavy black lines. Eyespots have black circle line coloration, while the inside has white, yellow and black color with three small white dots at the center. The eyespots present on its 2nd abdominal segment is yellow color surrounded with black circle line. Head is dark green with no markings but setae (chalaza) were present. Caudal horn is long and black with small spikes, with pinkish and orange coloration near on the base at the anal plate. Dorsal part of thorax has two yellowish bands from head down to the third thorax. It bears a ventral true leg, pinkish in color and setae (chalaza) were present. Abdominal segment A3, A4, A5 and A6 bears a caudal prolegs usually with crochets (biordinal, homoideous mesoseries) as well as the anal pro leg (A10). Its prolegs consists of two parts, the proximal base which bears setae (chalaza) and distal planta which never bears setae (chalaza) but it is where crochets arise. A7, A8 and A9 does not bear a pro leg. Spiracles oval, still brownish coloration. Its body continued to contract accompanied by the release of fluids from within. In preparation to turn into pupa, it stops eating and make web silken threads onto the leaves to where it covers itself (Figure 8). Duration about 4-7 days (Figure 7).

Figure 8 Prepupal phase, body contracted with the release of fluids

Figure 9 Events that takes place from last instar to pupa with the duration of 3 minutes and 44 seconds)

Pupa

Formation of pupa from last instar takes 3 minutes and 44 seconds to fully shed off its skin (Figure 9). Upon completion of pupation, the head, proboscis, antenna, spiracles, fused wings were still visible and has white coloration ventrally, while patches of brown were seen on the dorsal part of the body along with the dark line on its abdominal segments. After an hour, it turns into its final coloration (Figure 10 A, B, C). Dorsal part is dominantly brown in color, the latter part of its abdominal segments was movable. Spiracles turn black in color. Ventral body part consists of black patches and spots. Cremaster (Figure 10 D, E, F) is pointed, black coloration. Duration about 12-16 days (Figure 10).

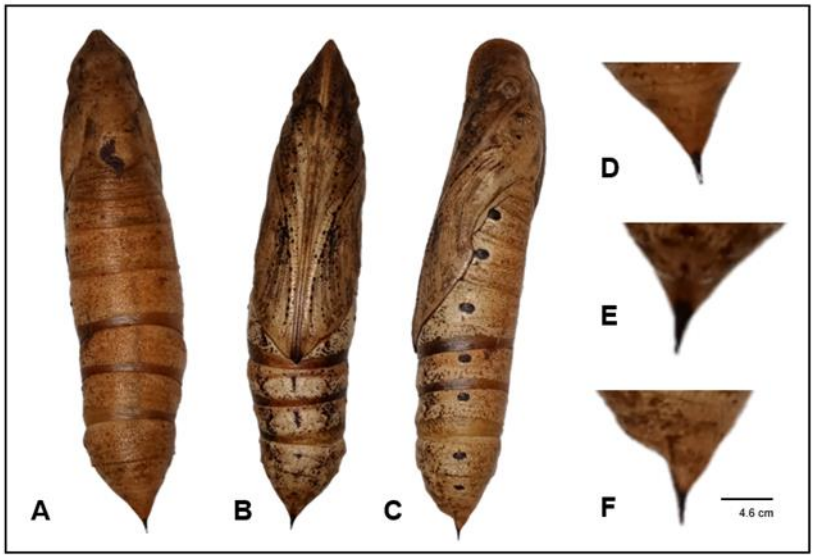

Figure 10 Pupa– (A) Dorsal view, (B) Ventral view, (C) Lateral view; Cremaster of pupa- (D) Dorsal view, (E) Ventral view, (F) Lateral view (12-16 days).

Figure 11 Newly emerged adult producing meconium from its wings

Adult

When the adult emerged, it climbs up to fully expand their wings. The wings are wet and limb. A greenish fluid was observed on the wings of a newly emerged adult hawkmoth. Unpleasant smelling liquid called meconium (Figure 11) was produced upon hatching. It takes an hour to harden their wings before flight. During dusk, they start flying and mating happens in a short period of time. When they are threatened, they flap their wings faster. A day after, females start to lay their egg with the duration of almost

one week (Figure 12). Female can lay up to 189-807 eggs. Male and female can be distinguished by the structure of frenulum (Figure 12 K & L) and structure of its antenna (Figure 12 I & J).

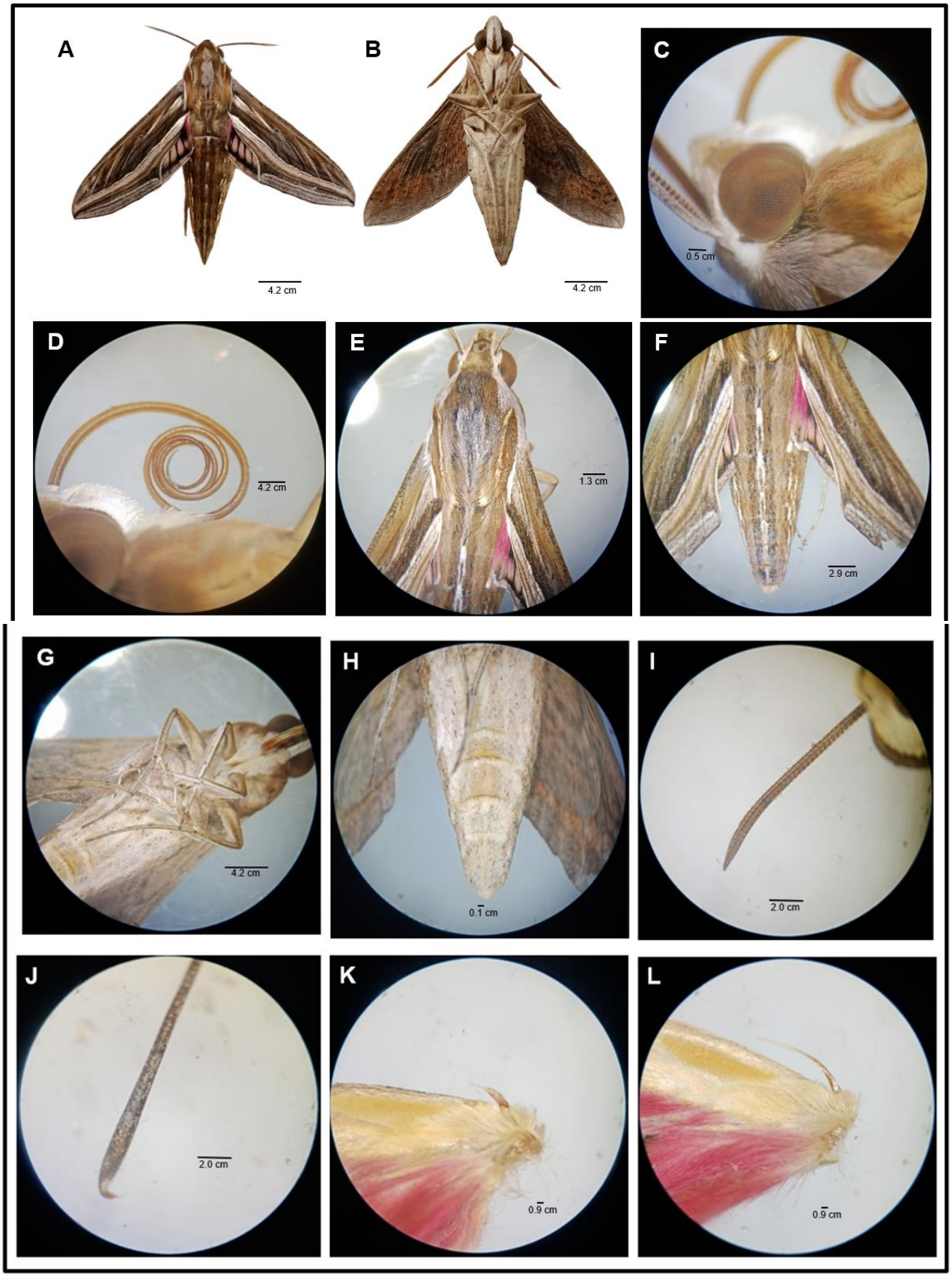

Figure 12 Adult – (A) Dorsal view, (B) Ventral view, (C) Compound eyes, (D) Proboscis, (E) Thorax dorsal view, (F) Abdomen dorsal view, (G) Legs, (H) Abdomen ventral view, (I) Antenna ♂, (J) Antenna ♀, (K) Frenulum ♀, (L) Frenulum ♂ (20X)

The head was dominantly covered with brown and white line anterior its eyes on its dorsal part while ventral part is covered with white. The antennae ranged 0.4 cm -0.5 cm, proboscis (3.6 cm) and compound black eyes (0.4 cm – 0.5 cm). Dorsal part of its thorax is covered with brown color and ash white on its center part while ventral part is dominantly white: Prothorax (0.5 cm- 0.6 cm), mesothorax (0.3 cm) and metathorax (0.3 cm). Abdomen ranged 2.1 cm – 2.3 cm and its dorsal part is brown with golden stripes on both sides and on its center while ventral part is dominantly white with black dots. Forewing ranged to 3.1 cm – 3.3 cm and its dorsal part is dominantly dark coloration with several white lines. Black dot is present near discal cell. Costal margin is straight with pointed apex. Ventral part dominantly colored gray near the base and light orange with dark spots on post median part. Light yellow above inner margin near the base. Hindwing ranged 2.0 cm – 2.1 cm and its dorsal part has discal cell with yellow and pink coloration, black bands on basal and sub-median part. Outer margin consists of white fringe while ventral part is dominantly pale orange black spots.

Duration of the life cycle of *H. celerio* ranged 32 days to 34 days until it turns into an adult. This species is voracious eaters that it can consume all preferred food given to them. It is important to study insect's life cycle to understand how they live, functions and reproduces. This will also help in the management of the said species.

4. CONCLUSION

DNA barcoding is a great tool in identifying an organism. Silver-striped hawkmoth (*Hippotion celerio)* species reared undergone four different life stages namely: Egg, larva, pupa and adult. This species are voracious eaters that it only takes 32-34 days before they could complete their life cycle.

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Authors contributions

All authors contributed equally.

Ethical approval

The life cycles of the species *Colocasia esculenta* was reported in the work. The ethical guidelines are followed in the study for species observation & identification.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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