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FIRST RECORD OF THE EARLY IMMATURE STAGES OF THE WHITE FOUR-RING *YPTHIMA CEYLONICA* (INSECTA: LEPIDOPTERA: NYMPHALIDAE), AND A NOTE ON A NEW HOST PLANT FROM INDIA

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COMMUNICATION

First record of the early immature stages of the White Four-ring *Ypthima ceylonica* (Insecta: Lepidoptera: Nymphalidae), and a note on a new host plant from India

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Abstract: Documentation of the early immature stages (egg, larva, chrysalis) of the White Four-ring (*Ypthima ceylonica* Hewitson, 1865), including larval morphology and behaviour, is described for the first time from India. A new host plant (*Cynodon dactylon* (L.) Pers.) is also reported for this butterfly.

Keywords: *Cynodon dactylon*, juvenile biology, life history, Poaceae, Western Ghats.

வெள்ளை வளையன் (ஓயிட் ஃபோர்-ரிங், *யுப்திமா சிலோனிகா*, ஹூயிட்சன், 1865) பட்டாம்பூச்சியின் வளர்ச்சிப்பருவ முதல் நிலைகளான முட்டை, புழு மற்றும் கூட்டுப்புழு நிலைகள், அந் நிலைகளில் அவற்றின் புறத்தோற்றம் மற்றும் நடத்தை, இந்தியாவில் முதல் முறையாக ஆவணப்படுத்தப்பட்டுள்ளன. இந்த பட்டாம்பூச்சிக்கு ஒரு புதிய உணவுத்தாவரமும் (*சைனோடான் டேக்டைலான்* (எல்) பெர்ஸ்.) கண்டறியப்பட்டுள்ளது.

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Author contribution: HT—involved in field sampling, data analysis and manuscript drafting. HR—involved in field sampling, data collection, data supervision and manuscript editing. AP—involved in data analysis, data validation and manuscript editing.

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INTRODUCTION

The genus *Ypthima* under the family Nymphalidae (Satyrinae) was first described by Hübner in 1818. These butterflies have dull to dark brown wings with a large ocellus on the forewing and a series of submarginal ocelli on the hindwing. Currently, *Ypthima* includes nearly 113 species widespread across the southeastern fringe of the Palearctic Region, Afrotropical Region, and Oriental Region (Shima & Nakanishi 2007). In India, 35 species are known to occur (Varshney & Smetacek 2015).

The White Four-ring *Ypthima ceylonica*, is an uncommon butterfly distributed over the southern Indian states (Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, and Telangana), a West Indian state (Maharashtra), East Indian states (Odisha and West Bengal) and several regions of Sri Lanka. The species was first described from Sri Lanka in 1865 by Hewitson. Moore (1880) recorded the species from Gale and Colombo, Sri Lanka. In India, Marshall & de Nicéville (1882) recorded that the species was distributed across southern India starting from Travancore and Madras to Odisha and a similar observation was made by Moore (1893). Hampson (1888) recorded the species from the Nilgiris Hills. Bingham (1905) extended the distribution to Bengal and also considered *Y. ceylonica* as a race of *Ypthima huebneri* Kirby, 1871, rather than treating them as separate species. However, the male genitalia has been shown to provide important information for the identification of *Y. ceylonica* (Elwes & Edwards, 1893). The recent study by Chandra et al. (2007) has expanded this species' range to the states of Madhya Pradesh and Chhattisgarh.

The first incomplete observation of *Y. ceylonica*'s early stages was made from Sri Lanka in 1910 by Green. This was followed by a detailed description and colour images by van der Poorten & van der Poorten (2012). Though there are two detailed descriptions of the immature stages of *Y. ceylonica* from Sri Lanka, to the best of our knowledge and after extensive literature review, there is no documentation on the early immature stages from India. Thus, we take this opportunity to describe the various instars and report a new larval host plant for *Y. ceylonica*.

MATERIALS AND METHODS

A female *Y. ceylonica* was found ovipositing on two grass species, *Cynodon dactylon* and *Axonopus compressus* (Sw.) P.Beauv. One egg and plant material were collected from the first author's garden (11.030N, 76.902E) located at Coimbatore, Tamil Nadu, India. The collected egg and plant material were placed in a plastic rearing container. The various stages of egg, larva, chrysalis and adult were photographed using a Sony HX60V digital camera. The size of the egg was measured using the Digimizer image analysis software, and the size of the larva and chrysalis were measured using a standard measuring scale. The excreta of the larva was removed and the container was cleaned daily to prevent microbial infection. The larva was supplied with fresh

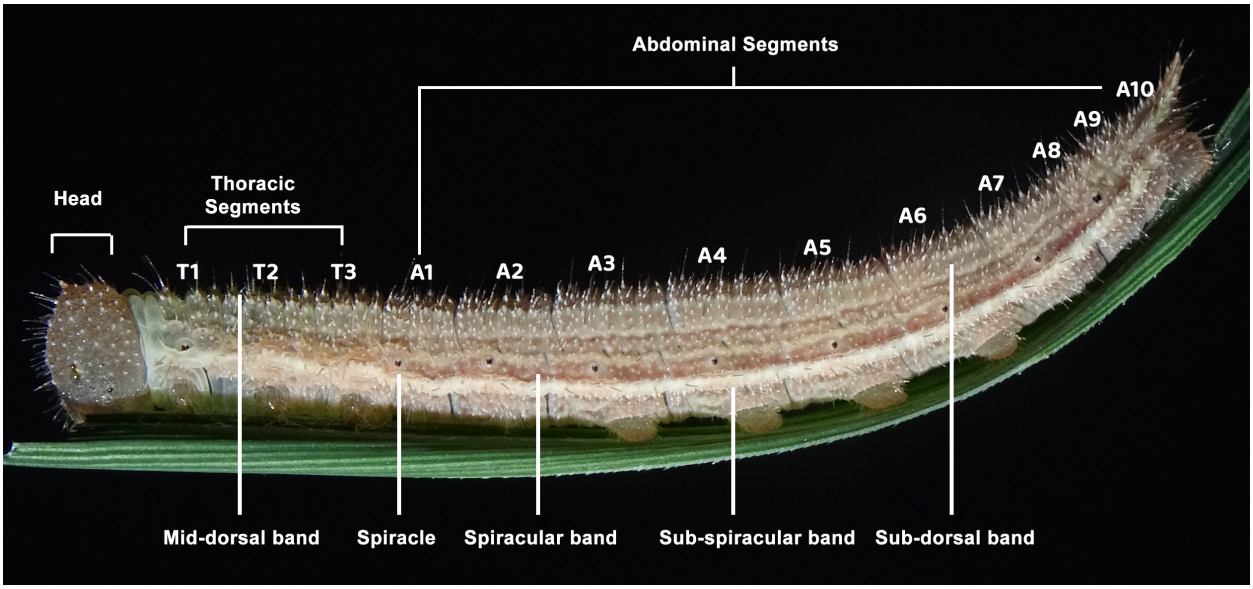

Image 1. Annotated larval segments. © Hari Ramanasaran

leaves of its host plant, *C. dactylon*, whenever required. The described larval segments and morphology are based upon the annotated image shown in Image 1.

RESULTS

Egg

The adult female laid eggs on the underside of both green and dry leaf blades of *C. dactylon* and *A. compressus* very close to the ground. The female was also observed to lay eggs on such nearby objects as a plant's dry leaf, branches, sticks and a stem found in close proximity to the host plant (Image 2). The collected egg measured 0.75mm at its longest diameter. The egg was dull white, almost globular with a nearly flat base and top, and the surface had many small irregular polygonal facets (Image 3a–f). The egg started to develop dark pink striations on day 4 (Image 3d) that continued till the egg matured on day 6 (Image 3f).

Image 2. Ovipositing on a nearby plant's leaf adjacent to the host plant *Axonopus compressus*.

First instar

At the end of day 6 (Image 4a), the neonate larva enclosed by nibbling a portion of the egg. The hatchling completely consumed the eggshell as its first meal. The first-instar larva was cylindrical and measured 2.5mm in length. The head was pale brownish pink and covered with numerous setae. The body was pale pink with a dark

Image 3. Egg of *Ypthima ceylonica* laid on a leaf of *Axonopus compressus*: a—Day 1, 12 June 2017 | b—Day 2, 13 June 2017 | c—Day 3, 14 June 2017 | d—Day 4, 15 June 2017 | e—Day 5, 16 June 2017 | f—Day 6, 17 June 2017. © Hari Theivaprakasham

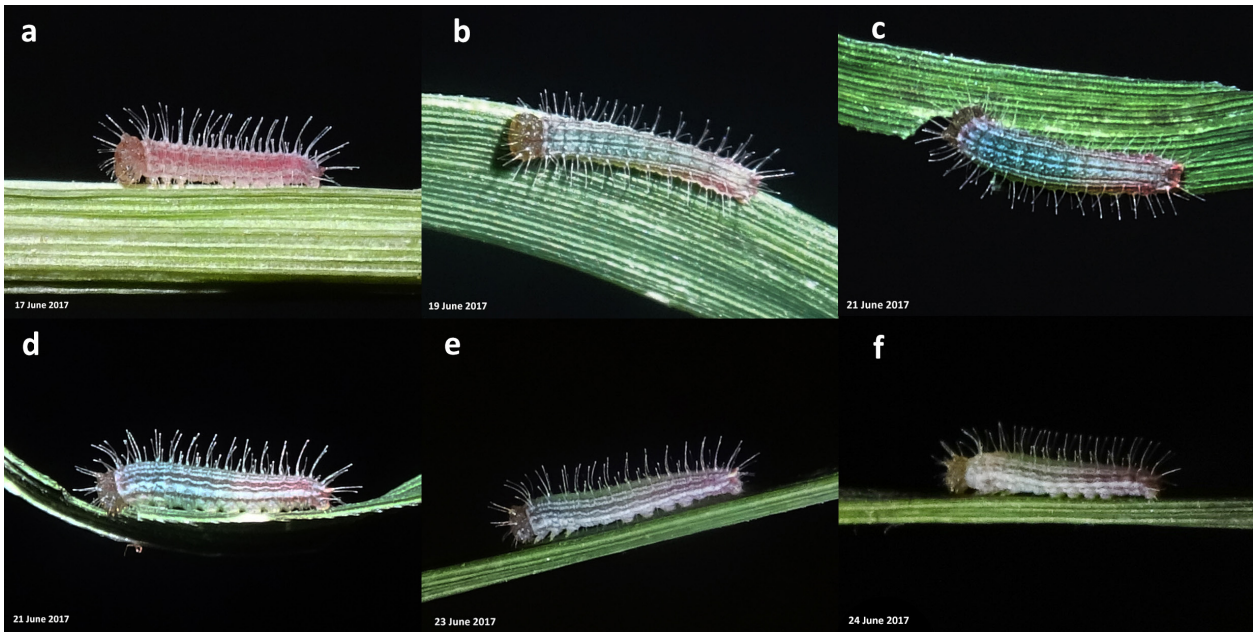

Image 4. First-instar larva of *Ypthima ceylonica*: a—Day 6, 17 June 2017 | b—Day 8, 19 June 2017 | c—Day 10, 21 June 2017 | d—Day 10, 21 June 2017 | e—Day 12, 23 June 2017 | f—Day 13, 24 June 2017. © Hari Ramanasaran

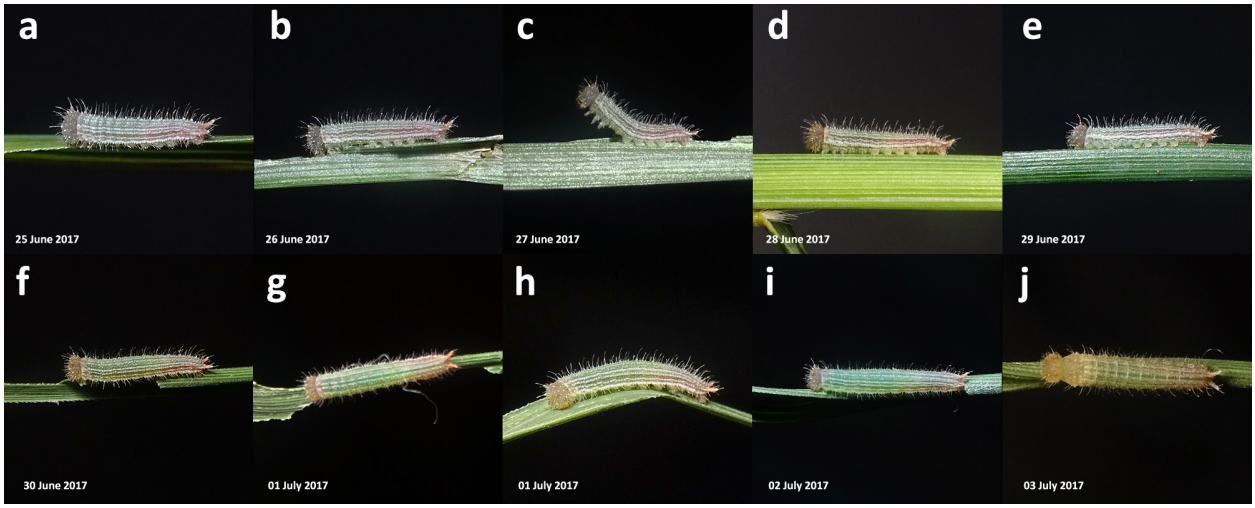

Image 5. Second-instar larva of *Ypthima ceylonica*: a—Day 14, 25 June 2017 | b—Day 15, 26 June 2017 | c—Day 16, 27 June 2017 | d—Day 17, 28 June 2017 | e—Day 18, 29 June 2017 | f—Day 19, 30 June 2017 | g—Day 20, 01 July 2017 | h—Day 20, 01 July 2017 | i—Day 21, 02 July 2017 | j—Day 22, 03 July 2017. © Hari Ramanasaran

pink mid-dorsal band bordered with thin white bands. The sub-dorsal band was thin, dark pink and bordered with thin white bands. The broad spiracular band was pink and bordered with a thin dark white band. The thin sub-spiracular band was pink. All dorsal and ventral bands ran longitudinally from the head to the anal segment. Numerous setae arose from the tubercles on its body. The larva had a pair of short projecting conical horns on the dorsolateral portion of its head and a pair of pointed conical anal processes. On day 8 (Image 4b), the larva fed on young tender grass blades and started to acquire its pale green undertone, which increased day by day (Image 4a–f). The broad pink spiracular band also started to become progressively thinner. After 14 days (Image 4f), the larva moulted and the body length increased to a maximum of 3.2mm.

Image 6. Third-instar larva of *Ypthima ceylonica*: a—Day 23, 04 July 2017 | b—Day 24, 05 July 2017 | c—Day 25, 06 July 2017 | d—Day 26, 07 July 2017 | e—Day 28, 09 July 2017 | f—Day 29, 10 July 2017 | g—Day 30, 11 July 2017 | h—Day 31, 12 July 2017 | i—Day 32, 13 July 2017 | j—Day 33, 14 July 2017 | k—Day 34, 15 July 2017 | l—Day 35, 16 July 2017 | m—Day 36, 17 July 2017 | n—Day 37, 18 July 2017. © Hari Ramanasaran

Second instar

In the second instar (Image 5a–j), the body was dull white initially, with the larva acquiring a green undertone over the next few days (Image 5f–j). The sub-spiracular and spiracular bands became darker, while the mid-dorsal band turned dark green and the sub-spiracular band turned white. The head, which was dark pinkish at the end of the first instar, started to lighten. The length of the body setae gradually decreased in size, while the anal processes became darker, and grew thicker, longer and more pointed. The second-instar larva was more active at night than during the day. Whenever disturbed, the larva, which preferred to eat fresh grass, instantly dropped from the leaf to the bottom of the container. The second instar lasted for eight days. The body length increased from 3.2mm to 5.2mm starting from day 14 (Image 5a) until day 22 (Image 5j).

Third instar

The third-instar larva (Image 6a–n) was light brownish in colour on day 23 (Image 6a), turning pale greenish on day 24 (Image 6b). The setae were drastically reduced in size compared to the second instar. The body was pale green with a dark green mid-dorsal band, and the spiracular band was bordered by a thin white line. The sub-dorsal band was thin, dark green and bordered with thin alternating white and dark green lines that ran longitudinally from the head to the anal segment. The conical anal processes were pale pink, the head turned pale green from its earlier pale brown colour and the sub-spiracular band was white. Starting with day 30 (Image 6g), the upper border of the sub-spiracular line developed thin brown markings. The third instar lasted about 14 days with the body length increasing from 5.2mm to 13mm. The larva moulted on day 38.

Fourth (final) instar

The fourth-instar larva (Image 7a–j) was different compared to the third instar. The body was pale brown and the mid-dorsal band was dark brown with thin white borders. The broad dark brown spiracular band was bordered with thin white lines, and a white line was observed running close above the spiracles. The sub-spiracular band remained white. The spiracles were black and became more prominent compared to earlier instars, while the head and the anal processes were pale brown. The larva fed voraciously on the host plant grass, usually choosing a long blade and feeding from the tip to its base. The larva was observed to reach a leaf's topmost part to feed during the night and return to the bottommost part by morning. It was

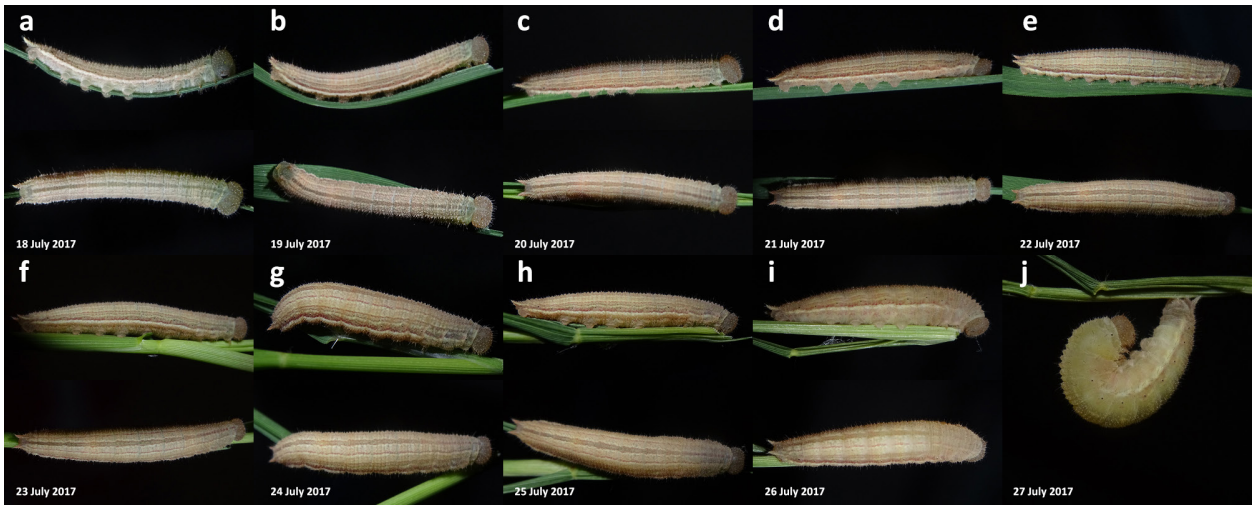

Image 7. Fourth-instar larva of *Ypthima ceylonica*: a—Day 37, 18 July 2017 | b) Day 38, 19 July 2017 | c—Day 39, 20 July 2017 | d—Day 40, 21 July 2017 | e—Day 41, 22 July 2017 | f—Day 42, 23 July 2017 | g—Day 43, 24 July 2017 | h—Day 44, 25 July 2017 | i—Day 45, 26 July 2017 | j—Day 46, 27 July 2017. © Hari Ramanasaran

also observed to rest lengthwise on half-eaten blades. The interesting behaviour of forceful frass ejection was also noted, which is best known in species of shelter-building Hesperiidae, but also witnessed with larvae in the Papilionidae, Pieridae, Nymphalidae and numerous moth families (K. Wolfe, pers. comm.). Besides Indian *Y. ceylonica* and *Ypthima striata* Hampson, 1889 (TH, pers. obs.), this likely predator-distancing strategy is practiced elsewhere by *Ypthima huebneri* (Tan, 2015), *Ypthima pandocus corticaria* Butler, 1879, and *Ypthima baldus newboldi* Distant, 1882 (Tan 2014a,b). The fourth instar lasted for eight days, and on day 45 (Image 7i), the body length increased from 13mm to a maximum of 18mm. After day 44, the larva stopped feeding on the leaves and started searching for a suitable place to pupate. The larva chose a blade of grass on which to pupate and gradually reduced its body length to 8mm. The larva remained stationary and pupated on day 46 (Image 7j), the pensile chrysalis being attached by its cremaster to a silken pad spun by the larva.

Chrysalis

The chrysalis (Image 8) was dull yellow, covered with brown striations and measured 10mm. The general profile was elongated and convex except for a conspicuous bump near the junction of the thorax and abdomen. The ocular caps were pointed and short while the wing cases were bordered with a brown line. The chrysalis turned increasingly darker each day. On day 53, the pupal case became transparent and the subapical ocelli marking of the pharate butterfly became visible. On day 54, an adult female emerged in the early morning and was seen resting upside down, drying its wings and ejecting red meconium fluid. Overall, the chrysalis stage lasted for nine days.

The total growth period from egg to adult spanned 54 days, with the development of egg (six days), first instar (eight days), second instar (eight days), third instar (14 days), fourth instar (nine days) and chrysalis (nine days).

DISCUSSION

Our observations in this study in India had various striking differences in host plant selection, larval and chrysalis stages when compared with the earlier descriptions (Green 1910; van der Poorten & van der Poorten 2012) from Sri Lanka. The following discussion focuses on comparisons with those earlier studies.

Host plant

Various grass host plants for *Ypthima ceylonica* are reported from Sri Lanka and India, all from Poaceae. In Sri Lanka, Green (1910) reported *Phalaris arundinacea* L. and van der Poorten & van der Poorten (2012) reported *Axonopus compressus* and *Cyrtococcum trigonum* (Retz.) A.Camus as host plants. In India, Nitin et al. (2018) reported *Setaria barbata* (Lam.) Kunth and Kalesh & Prakash (2015) reported *A.compressus* as host plants. Our finding in this study, however, showed for the first time that *Cynodon dactylon*, a perennial grass, is also used as a larval food plant by *Y. ceylonica*. Before this addition, *C. dactylon* was known as a host plant for only

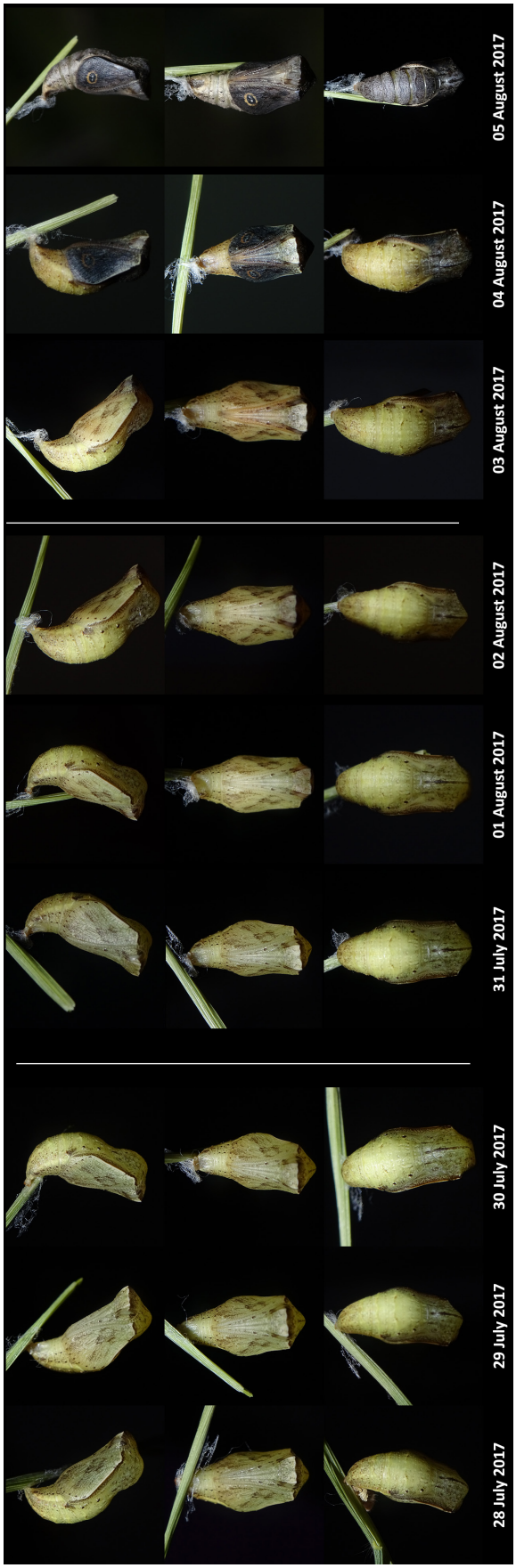

Image 8. Chrysalis of *Ypthima ceylonica* from Day 47 to Day 54. © Hari Ramanasaran

Ampittia dioscorides Fabricius, 1793, *Melanitis leda* L., 1758 (Sawant 2020), and *Ypthima striata* (Agavekar et al. 2020).

Egg

The colour of the egg was white/dull white and not pale blue, but other descriptions of the egg resembled that reported by van der Poorten & van der Poorten (2012).

Larva

We observed only four larval instars compared to five as noted by Green (1910) and Jayasinghe & Rajapakshe (2020). Variation in the number of instars in Lepidoptera is relatively normal and also species dependent (Esperk et al. 2007). The variations in geographical location, environmental conditions and choice of different host plants may affect development (Braby 1994). These assumptions as to *Ypthima ceylonica*, however, need to be further validated by future scientific studies. Four larval instars is not unusual in satyrids. For example, Afrotropical *Ypthima impura* Elwes & Edwards, 1893 (Williams 2020) and Neotropical *Cissia pompilia* C. & R. Felder, 1867, and *Taygetis rufomarginata* Staudinger, 1888 (K. Wolfe, unpub. data) are known to undergo only four instars.

Our observations of the first instar closely resembled the description by Green (1910). In the second instar, the pink base colour was not replaced by whitish green nor were the dorsal, sub-dorsal and sub-spiracular lines replaced with dull green as noted by Green (1910). Instead, our second-instar larva remained nearly the same colour as the first instar with alternate white and pink sub-spiracular and spiracular bands. The transition of Green's (1910) third instar larva resembled the description of our second instar, with the third and fourth instars matching those stages as described by Green (1910). Additionally, the third instar of *Ypthima ceylonica* closely resembled the third and fourth instars of *Ypthima huebneri* (Saji & Das 2020). Our observations of the fourth (final) instar were completely different from the earlier works. The fourth instar's base colour was pale brown with a light brown sub-spiracular band and pale brown head. Whereas the earlier works of Green (1910) and van der Poorten & van der Poorten (2012) reported a green base colour with a green subdorsal band and brownish-green head. The final instar of *Y. ceylonica* closely resembled the final instars of *Ypthima singala* R. Felder, 1868 (van der Poorten & van der Poorten, 2012) and *Ypthima striata* (Agavekar et al., 2020).

Image 9. Freshly eclosed female adult of *Ypthima ceylonica*.

Chrysalis

Green (1910) and van der Poorten & van der Poorten (2012) reported two distinct chrysalis forms from Sri Lanka: grass-green colour and pale grayish-brown. But our chrysalis from India was dull yellow and comparatively different from those described in the earlier studies. Moreover, we noticed that the shape and colour of the chrysalis closely matched that of *Ypthima huebneri* (Saji & Das, 2020).

CONCLUSION

The description of the early immature stages of butterflies are of great value for the identification of juveniles in the field. It also provides supporting data for taxonomic and phylogenetic studies. In this study, the early immature stages of *Ypthima ceylonica* were described in detail for the first time from India, and a new host plant was also reported. Our observations of the early immature stages from India had several variations from the erstwhile descriptions from Sri Lanka. These variations may have occurred due to geographical isolation, subspecies or regional variation, choice of different larval food plants or variations in environmental factors such as temperature, rainfall, relative humidity, and photoperiod. Future morphological and genetic studies on the early immature stages of *Y. ceylonica* from different locations in India need to be performed to better understand the reasons for such variations.

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