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RELATIONSHIP BETWEEN PUPAL COLOUR AND SEX RATIO OF THE COMMON MORMON, *Papilio polytes romulus* CRAMER, 1775 (INSECTA: LEPIDOPTERA), SRI LANKA

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Abstract
A free ranging female common Mormon (*Papilio polytes romulus*) butterfly was observed laying eggs on a *Citrus aurantiifolia* (Family: Rutaceae) plant in a home garden in Battaramulla. 16 eggs were collected and reared in the lab. The duration and measurements of each stage were recorded. After eclosion the sex and wingspan of all adult butterflies were recorded and they were released back to the wild. Pupae were observed in two colours: green and brown. Out of 16 pupae, 12 were green (75%) and four were brown (25%). The four brown pupae gave rise to two males (50%) and two females (50%). The 12 green pupae gave rise to 10 males (83%) and two females (17%). The sex ratios regardless of the pupal colour showed male dominance with 12 males (75%) and four females (25%). All four females were of the *romulus* form. A strong relationship between pupal colour and substrate texture was observed, but no relationship between pupal colour, sex and sex ratios was seen.

***Key words*:** sex ratio, male dominance, Papilionidae, pupal colour, sexual dimorphism

Introduction

In Sri Lanka, the Papilionidae family consists of 15 species of butterflies of which two species are endemic and there are six endemic subspecies (MOE & RE 2014). *Papilio polytes romulus* Cramer, 1775 is one of the most common and most widely distributed swallowtail butterflies found in the country. It is also found in India (Saji *et al*. 2021), Singapore (Tan 2011), Bangladesh (Islam *et al*. 2017, Khan *et al*. 2019), Thailand (Hawkeswood *et al*. 2018), Malaysia, Nepal, Laos, Vietnam, Cambodia and Myanmar (Inayoshi 2014). Females are polymorphic existing in three forms: *romulus*, *cyrus* and *stichius*. The *cyrus* form mimics the male of the same species, the *romulus* form mimics the crimson rose butterfly (*Pachliopta hector*) and the stichius form mimics the common rose butterfly (*Pachliopta aristolochiae ceylonica*) (see Jayasinghe 2015, Jayasinghe *et al*. 2015, van der Poorten & van der Poorten 2016, 2018, De Silva Wijeyerathne 2019).

In Sri Lanka, 14 larval food plants of *P. p. romulus* have been recorded by van der Poorten & van der Poorten (2011, 2016), Jayasinghe *et al*. (2014, 2021), and De Silva Wijeyerathne (2019), and it has been reported that the larval food plant distribution and the distribution of *P. p. romulus* complement each other (van der Poorten & van der Poorten 2011). The immature stages have been described by van der Poorten & van der Poorten (2011), and Jayasinghe *et al*. (2021). The pupal colour dimorphism has been reported by van der Poorten & van der Poorten (2011), Tan (2011), Khan *et al*. (2019), Jayasinghe *et al*. (2021).

The seasonal abundance of *P. p. romulus* has also been studied by Jayasinghe *et al*. (2021). The complete life cycle of *P. p. romulus* is known to be about 32–36 days with the adult stage about 8–10 days in the wild and females living longer than males (Tan 2011, Halloran & Wason 2013). In captivity, adults only live 4–5 days because they refuse to accept any sort of food such as honey or sugar syrup (Gaikwad 2008, Gaikwad & Bhawane 2008). Females are known to lay anywhere between 20–25 eggs in one sitting (Halloran & Wason 2013). The relationship between pupal colour, sex and sex ratios of *P. p. romulus* have not been studied before. In this study we report on this relationship.

Material and methods

On 16 June 2021 at around 1115 h, a free ranging female *P. p. romulus* butterfly was observed laying eggs on a lime plant, *Citrus aurantiifolia* Swingle, 1913, in a home garden in Battaramulla (6.896834ºN, 79.927238ºE). 16 eggs were found on the edge of the upper surface of young leaves and once the eggs hatched the larvae were collected with the leaves and taken to the laboratory where they were reared. The rearing was done at the Research Lab of the Faculty of Science, Horizon Campus, Malabe (8 km from the collection site).

The larvae were placed in a glass tank of dimensions 70 (length) × 18 (width) × 18 (height) cm and a mosquito net was used to cover the open top during the night, held in place by a rubber band, while during the day the mosquito net was removed. Frass and partially eaten leaves were removed from the container on a daily basis and after it was wiped clean with a dry cloth, the larvae were placed back into the tank. Fresh leaves were also provided on a daily basis. Water was sprayed on the leaves every two days. Immature and mature stages were photographed using Nikon D750 with sigma 105mm f/2.8 EX DG OS HSM macro lens.

Measurements were taken using ImageJ, image analysis program (Abràmoff *et al*. 2004) and a digital vernier calliper. The duration of each immature stage was also recorded. Towards the end of the final instar sticks were placed in the container in the vertical position to encourage the larvae to climb on to pupate. After eclosion the sex and wingspan of all adult butterflies were recorded and they were released back to the wild. Immature stages were identified using Tan (2011), van der Poorten & van der Poorten (2011), Jayasinghe *et al*. (2021). Mature stages were identified using Jayasinghe *et al*. (2015, 2021), van der Poorten & van der Poorten (2016, 2018), and the larval food plants were identified using Jayasinghe *et al*. (2014, 2021).

Results

***Hatching*:** Three days after the eggs were laid they matured and became darker. The eggs always hatched in the morning between 0500 and 0600 h. The newly emerged 1st instar fed on its egg shell and consumed it completely before beginning to feed on young leaves.

***Feeding and moulting*:** The 1st and 2nd instar exclusively fed on young leaves. The 3rd, 4th and 5th instars fed on both young and mature leaves. Just before the instar moulted it stopped feeding for about half a day and then crawled out of its skin. About 30 seconds after it moulted it turned back and fed on its moulted skin except for the head cap. The 1st to 4th instars resembled a bird dropping on the leaf presumably to help evade predators such as birds. The final instar was green in colour and blended in with the mature leaf colour to provide camouflage. About half an hour after the final instar had its last feed it excreted a large amount of a semi-solid green coloured material, which appeared to be different to its normal frass before moving to and fro searching for a suitable place to pupate.

***Pupation*:** The final instar larvae each climbed on to a suitable substrate, shrunk in size and formed a 'C' shaped pre-pupa which was held in an upright position anchored to the substrate from the posterior end by cremaster, and made a silk girdle around the body to suspend itself so that the head faced upwards (Fig.1A). This stage lasted only a day.

Plate 17

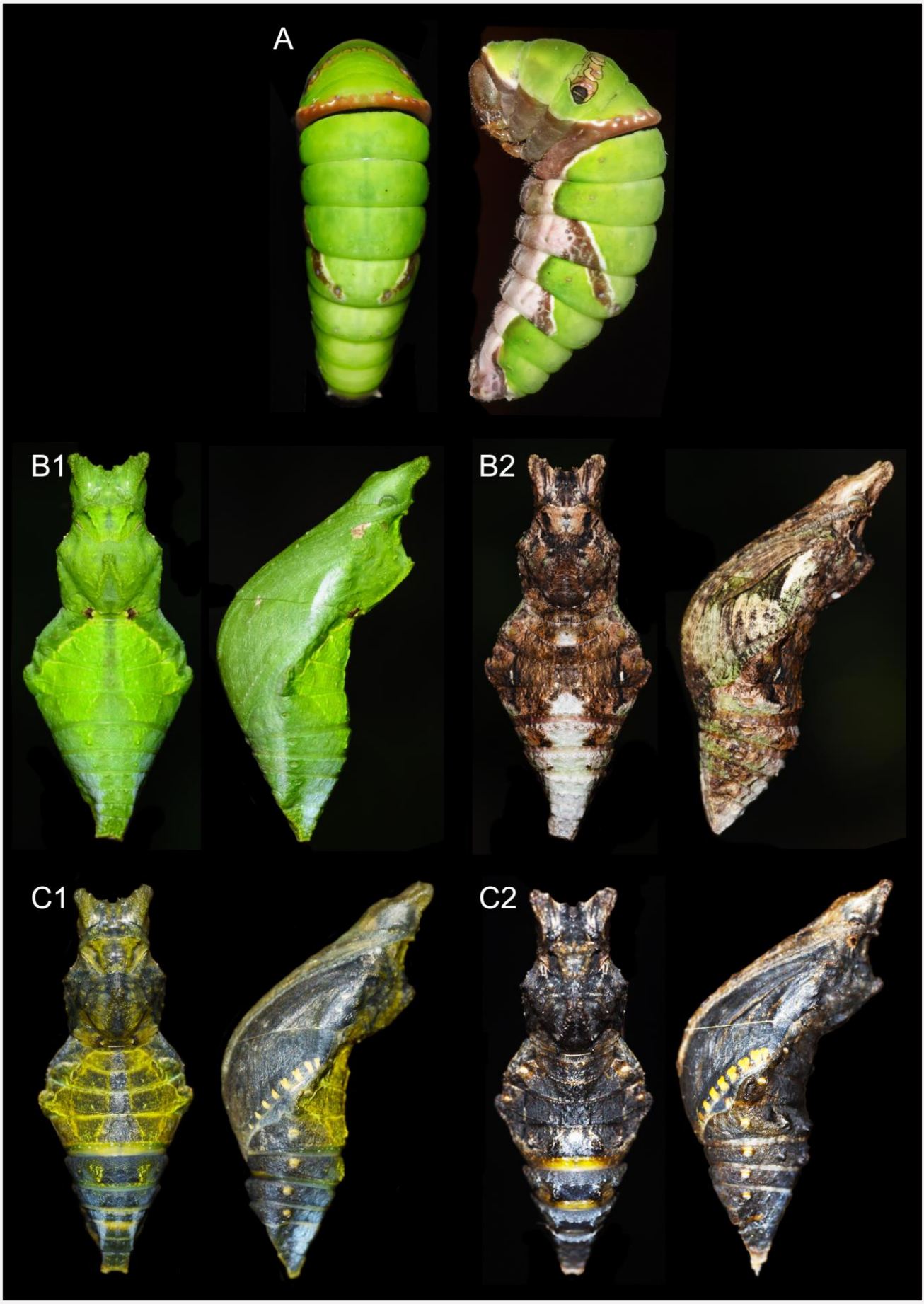

Figure 1. Different stages of *Papilio p. romulus* pupae shows dorsal and lateral views of **(A)** pre-pupa; **(B1)** green and **(B2)** brown pupa; **(C1)** green and **(C2)** brown mature pupa; not to scale

Pupation took place only in the evening between 2000 and 2200 h and took about 30 minutes to complete. About a minute after the colour changed either to green (Fig. 1B1) or brown (Fig. 1B2). The pupae appeared inanimate, however they responded to touch and vibration and if disturbed jerked and even made a short 'hiss' noise to deter predators. After 11 or 12 days the pupa matured, darkened and became black. The green pupa retained a green/yellow tinge (Fig. 1C1) while the brown pupa did not (Fig. 1C2). Even though all eggs were laid at the same time not all the larvae pupated on the same day. When pupation took place, they were at their most vulnerable and if environmental conditions are unfavourable or if predators are present all pupae may be lost. Pupation taking place on different days helps to overcome this problem.

***Eclosion*:** Eclosion took place only in the morning between 0600 and 0800 h and took only a minute to complete. The butterfly pushed out of the pupa head first and jerked downwards to move out of the pupa. The butterfly then hung down and the wings slowly spread open. Blood was pumped through the capillaries in the wings to open them up fully. This took about 45–60 minutes. They flapped their wings several times appearing to test them before finally flying off. Just like pupation, eclosion also happened on different days for the same reason as mentioned above. Females were larger than males in general. Zero mortality was recorded in this study.

***Pupal colour, sex and sex ratios*:** Table 1 shows that in this study green pupae were dominant over brown pupae, however it was evident that there was no relationship between pupal colour and sex as both colour pupae gave rise to both sexes. It also shows that the sex ratio irrespective of pupal colour showed male dominance.

Table 1. Relationship between pupal colour, sex and sex ratios of *Papilio p. romulus*; N, n = number of individuals; M = male, F = female

Pupal colour	Green		Brown	
N	12		4	
Percentage	75%		25%	
Sex	M	F	M	F
n	10	2	2	2
Percentage	83%	17%	50%	50%

In the month of July eggs from a single female on *Citrus aurantiifolia* showed male dominance, but this should be verified with repeated studies over the next couple of years. All four females from this study were of the *romulus* form. In this study the average temperature from the day of laying eggs to eclosion was 26.8 ºC and relative humidity was 86.5%.

Discussion

According to studies by Jayasinghe *et al*. (2021) the lowest seasonal abundance in Sri Lanka was seen in the month of July and August. It is assumed that this was because they lay the least number of eggs (20) (Halloran & Wason 2013). 16 of these eggs were studied in this research accounting for 80% of the total number of eggs a single female may lay in one sitting. Therefore, the results from this study have high statistical significance.

Immature stages of *P. p. romulus* in Sri Lanka have been described by van der Poorten & van der Poorten (2011) and Jayasinghe *et al*. (2021) and some details of duration are mentioned by van der Poorten & van der Poorten (2011). However, detailed information on duration and measurements of immature stages from Sri Lanka were not found in the literature. The duration and measurements of immature stages of *P. p. romulus* was reported by Tan (2011) in Singapore, by Islam *et al*. (2017) and Khan *et al*. (2019) in Bangladesh, and by Gaikwad (2008) and Nagalakshmi *et al*. (2018) in India. Adult butterfly's duration was not recorded in this study as they were released back to the wild. Tan (2011), Gaikwad (2008), Islam *et al*. (2017), Nagalakshmi *et al*. (2018), Khan *et al*. (2019) findings are similar to results with minor variations (see Table 2).

Table 2. Duration and measurements of *Papilio p. romulus*; N, number of individuals; M, male; F, female; — not recorded.

Development stage	N	Duration (days)	Body length (mm)	
			range	mean ±sd
egg	16	3–4	0.8–1.2	1.0±0.2
1st instar	16	3–4	3–5	4.0±1.0
2nd instar	16	2–3	6–10	8.0±2.0
3rd instar	16	2–3	13–16	14.5±1.5
4th instar	16	2–3	20–26	23.0±3.0
final instar	16	5–6	30–46	38.0±8.0
pre-Pupa	16	1	25–30	27.5±2.5
pupa	16	11–12	31–32	31.5±0.5
adult M	12	—	80–100	90.0±10.0
adult F	4	—	105–115	110.0±5.0

Some details of hatching, defence mechanisms of immature stages and adult behaviour were described by van der Poorten & van der Poorten (2016). Detailed observations on hatching, moulting, pupation and eclosion of *P. p. polytes* from India have been reported (Gaikwad & Bhawane 2008, Gaikwad *et al*. 2009, Gaikwad & Bhawane 2013). However, no such detailed records on *P. p. romulus* from Sri Lanka were found in the literature.

Pupal colour, sex and sex ratios were reported by Gaikwad & Bhawane (2008) from a batch of eggs collected from a single plant on a given season with either male dominant or female dominant up to 80%. However, they did not mention specifically which larval food plant or which months were male/female dominant and they also did not mention if the eggs were from a single female or not. Environmental factors influencing pupal colour plasticity have been studied in *P. polytes* in India. Factors such as temperature, relative humidity, pupation on plant vs off plant, substrate texture, substrate diameter and background colour have been identified (Mayeka & Kodandaramaiah 2017). However, the effect of these factors on *P. p. romulus* in Sri Lanka has not been studied previously. Substrate texture was found to be the most dominant factor affecting pupal colour plasticity followed by background colour (Smith 1978, Mayeka & Kodandaramaiah 2017). In this study all four brown pupae were made on sticks while 11 of the 12 green pupae were made on the wall of the glass container (see Table 3).

Table 3. Relationship between pupal colour and substrate texture of *Papilio p. romulus*; N, n = number of individuals; S = smooth, R = rough

Pupal colour	Green		Brown	
N	12		4	
Substrate texture	S	R	S	R
n	11	1	0	4
Percentage	92%	8%	0%	100%

This is consistent with the fact that smooth substrate texture (glass wall) favours green pupa formation while rough substrate texture (sticks) favours brown pupa formation. Other environmental factors such as season, type of larval food plant, captive vs non-captive setting and distribution zones also need to be investigated in order to fully understand this relationship. Studies by Jayasinghe *et al*. (2021) showed that the seasonal abundance of *P. p. romulus* in Sri Lanka was lowest in July and August and highest in November. The effect of season on the pupal colour plasticity might be the subject of a future study. Since 14 larval food plants of *P. p. romulus* have been identified in Sri Lanka (Jayasinghe *et al*. 2014, Jayasinghe *et al*. 2021), the effect of the type of larval food plant on pupal colour ratios and sex ratios is also another potential future study. All results found in the literature and those reported here are from captive rearing studies. However, in non-captive condition these results could be different. This also needs to be investigated.

According to the distribution of butterflies, Sri Lanka can be divided into five major zones: lowland wet zone, Lowland dry zone, intermediate zone, hill country and northern zone (Jayasinghe 2015) with *P. p. romulus* having been reported in all these zones (Jayasinghe *et al*. 2021). Any variation in pupal colour plasticity in different zones also needs to be investigated. The molecular mechanism behind pupal colour dimorphism in *P. polytes* in Japan revealed that the brown colour was determined by melanin synthesis genes, *tyrosine hydroxylase* and *laccase 2* genes while the green colour was determined by expression of both multiple *bilin binding protein*-related genes (blue pigmentation) and multiple *juvenile hormone binding protein*-related genes (yellow pigmentation) together (Yoda *et al*. 2020). These genetic factors have not been studied in *P. p. romulus* in Sri Lanka previously or in this study.

In conclusion, we can report from this study there was no relationship between pupal colour, sex and sex ratios. It can also be reported that in the month of July eggs from a single female on *Citrus aurantiifolia* showed male dominance. Further we can confirm that pupal colour has a strong relationship with substrate texture.

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Literature cited

Abràmoff, M.D., P.J. Magalhães, and S.J. Ram (2004). Image processing with Image J. *Biophotonics International*, 11(7): 36–42.

De Silva Wijeyerathne, G. (2019). *A Naturalist's Guide to the Butterflies & Dragonflies of Sri Lanka*, 2nd ed. John Beaufoy Publishing Ltd., Oxford: 91pp.

Gaikwad, S.M. (2008). Life history of butterfly *Papilio polytes polytes* L. *Proceeding on National Seminar on "Wonderful world of Insects"*. Shivaji University, Kolhapur: 28–29.

Gaikwad, S.M. and G.P. Bhawane (2008). Observations on emergence of butterfly, *Papilio polytes polytes* (Lepidoptera: Papilionidae). *Bionotes*, 10(3): 100.

Gaikwad, S.M. and G.P. Bhawane (2013). Life history including behaviour, parasitism and population density of *Papilio polytes polytes* (L). *Entomology, Ornithology & Herpetology: Current Research*, 2(2): 69.

Gaikwad, S.M., S.R. Aland., D.L. Bharmal *et al*. (2009). Hatching, moulting, pupation and eclosion behaviour in *Papilio polytes polytes* Linnaeus. *Geobios*, 36(1): 91–93.

Halloran, K. and E. Wason (2013). *Papilio polytes*. <https://animaldiversity.org/accounts/Papilio_polytes/> Accessed on 21 July 2021.

Hawkeswood, T.J., K.L. Dunn, and B. Summung (2018). A new larval host plant for *Papilio polytes* L., 1758 (Lepidoptera: Papilionidae) from Ubon Ratchathani Province, Thailand. *Calodema*, 685: 1–5.

Inayoshi (2014). *Papilio polytes romulus*, a checklist of butterflies in Indo-China. <http://yutaka.it-n.jp/pap/10350010.html> Accessed on 21 July 2021.

Islam, S., A.T.M.F. Islam, M.M. Rahman, and A. Yamanaka (2017). Biology and morphometrics of the common mormon butterfly, *Papilio polytes* Linnaeus (Lepidoptera: Papilionidae) rearing in laboratory condition. *Rajshahi University Journal of Zoology*, 36: 49–56.

Jayasinghe, H.D. (2015). *Common Butterflies of Sri Lanka*. Colombo, Sri Lanka: Ceylon Tea Services PLC., Colombo: 40pp.

Jayasinghe, H.D., S.S. Rajapaksha, and C. de Alwis (2014). A compilation and analysis of food plants utilization of Sri Lankan butterfly larvae (Papilionoidea). *Taprobanica*, 6(2): 110–131.

Jayasinghe, H.D., S.S. Rajapaksha, and C. de Alwis (2015). *A Pocket Guide to the Butterflies of Sri Lanka*, 2nd ed. Butterfly Conservation Society of Sri Lanka, Colombo: 27pp.

Jayasinghe, H.D., S.S. Rajapakshe, and C. de Alwis (2021). The story of Sri Lankan butterflies. Butterfly Conservation Society of Sri Lanka. <http://www.slbutterflies.lk/> Accessed on 21 July 2020.

Khan, M.M.H., M.N. Molla, and M.H. Rahman (2019). Development, host preference and leaf consumption of the lime swallow tail butterfly, *Papilio Polytes romulus* Cramer (Papilionidae: Lepidoptera) on citrus. *Journal of the Asiatic Society of Bangladesh* (Science), 45(1): 111–125.

Mayekar H.V. and U. Kodandaramaiah (2017). Pupal color plasticity in a tropical butterfly, *Mycalesis mineus* (Nymphalidae: Satyrinae). *PLoS One*, 12(2): 1–14

MOE & RE (2014). *Butterfly Conservation Action Plan of Sri Lanka*. Biodiversity Secretariat/Ministry of Environment & Renewable Energy, Colombo: 101pp.

Nagalakshmi, P., K. Suryanarayana, and S.P. Venkata Ramana (2018). Life history of common mormon *Papilio polytes* (Lepidoptera: Rhopalocera: Papilionidae) from Sri Lankamalleswara Reserve Forest, Eastern Ghats, Andhra Pradesh. *International Journal of Research & Analytical Reviews*, 5(4): 457–460.

Saji, K., S.N. Prashanth, T. Karmakar, and J.A. Jacob (2021). *Papilio polytes* Linnaeus, 1758. Butterflies of India, Indian Foundation for Butterflies <http://www.ifoundbutterflies.org/sp/603/Papilio-polytes> Accessed on 21 July 2021.

Smith, A.G. (1978). Environmental factors influencing pupal colour determination in Lepidoptera. I. Experiments with *Papilio polytes*, *P. demoleus* and *P. polyxenes*. *Proceedings of the Royal Society of London* (Biological Sciences), 200: 295–329.

Tan, H. (2011). Life history of the common mormon (*Papilio polytes romulus*). Butterflies of Singapore <https://butterflycircle.blogspot.com/2011/10/life-history-of-common-mormon.html> Accessed on 21 July 2021.

van der Poorten, G.M. and N.E. van der Poorten (2011). New and revised descriptions of the immature stages of some butterflies in Sri Lanka and their larval food plants (Lepidoptera: Papilionidae). *Journal of Research on the Lepidoptera*, 44: 123–124.

van der Poorten, G.M. and N.E. van der Poorten (2018). *Field guide to the Butterflies of Sri Lanka*. Lepodon Books, Toronto: 250pp.

van der Poorten, G.M. and N.E van der Poorten (2016). *The Butterfly Fauna of Sri Lanka*. Lepodon Books, Toronto: 418pp.

Yoda, S, E. Otaguro, M. Nobuta, and H. Fujiwara (2020). Molecular mechanisms underlying pupal protective colour switch in *Papilio polytes* butterflies. *Frontiers in Ecology & Evolution*, 8: 51.