Journal of Threatened Taxa | www.threatenedtaxa.org | 26 October 2015 | 7(12): 7945–7949

ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

OPEN ACCESS

Immature stages and the larval food plant of *Nacaduba pactolus ceylonica* Fruhstorfer, 1916 (Lepidoptera: Lycaenidae) in Sri Lanka

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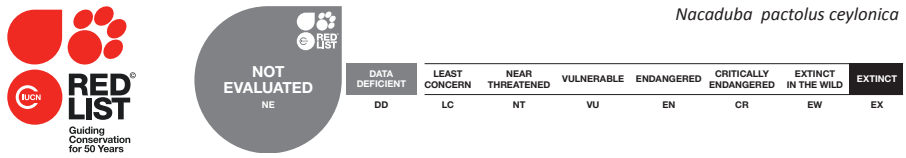
The genus *Nacaduba* Moore, 1881 (Lepidoptera: Lycaenidae: Polyommatinae) is distributed throughout the Indo-Australian region. Its range extends from Sri Lanka to the Fiji Islands (Brower 2008). It represents eight species in Sri Lanka of which two are endemic to the country (MOE 2012a). Although *Nacaduba pactolus* is wide spread in the tropics of its range, it is an uncommon butterfly in Sri Lanka. The subspecies, *Nacaduba pactolus ceylonica,* Fruhstorfer 1916 is endemic to the island (MOE 2012b) and listed as near threatened (MOE 2012a). It is the largest species of the genus in the island and the only member which has a characteristic white-tipped antennae (Woodhouse 1949; d'Abrera 1998). The larval food plants of *N. pactolus* have been recorded from India; *Entada pursaetha* (Fabaceae) (Bean 1964; Bean 1988), Taiwan; *Entada rheedii* (Fabaceae) (Hsu et al. 2004) from Singapore; *Entada spiralis* (Fabaceae) (Tan 2009). So far the larval food plant of this endemic subspecies was unknown and the early stages have not been documented. In this study we report its larval food plant, life history and immature stages.

Material and Methods: Field observations were carried out from February 2013 to May 2015. Close observations were made in two locations, Katepola, Sabaragamuwa Province, Ratnapura District (6.6972^{0}N & 80.2429^{0}E; elevation 144m) and Samanala Nature Reserve, Sabaragamuwa Province, Ratnapura District (6.8332^{0}N & 80.4274^{0}E; elevation 397m). The immature stages (eggs and larvae) were collected with the young shoots and tendrils of their food plant from a home garden in Katepola Village and brought to the laboratory to rear them. The lab-rearing studies were carried out



Nacaduba pactolus ceylonica

DOI: http://dx.doi.org/10.11609/JoTT.o4359.7945-9

Editor: George Mathew, Former Emeritus Scientist, KFRI, Peechi, India. **Date of publication:** 26 October 2015 (online & print)

Manuscript details: Ms # o4359 | Received 17 July 2015 | Final received 02 October 2015 | Finally accepted 10 October 2015

Citation: Priyadarshana, T.S., I.H. Wijewardhane, S. Sarang & N. Wijayathilaka (2015). Immature stages and the larval food plant of *Nacaduba pactolus ceylonica* Fruhstorfer, 1916 (Lepidoptera: Lycaenidae) in Sri Lanka. *Journal of Threatened Taxa* 7(12): 7945–7949; http://dx.doi.org/10.11609/JoTT.o4359.7945-9

Funding: Self funded.

Conflict of Interest: The authors declare no competing interests.

Acknowledgements: We wish to thank Dr. Madhava Meegaskumbura, Lahiru S. Wijedasa, Bushana Kalhara, Sameera Karunarathna, Lahiru Ratnayaka, Rohana Gunawardana, Sadaruwan Hettiarachchi, Mo Mu Bahir, Kanishka Ukuwela, Thasun Amarasinghe, Sandun J. Perera, Sachini Rasadari and Chamara Nandasena for their support and encouragement on this study.

at the Department of Agriculture, Karapincha, Kuruwita (6.7494^{0}N & 80.3460^{0}E; elevation 37m). Each immature stage was placed in transparent plastic containers (13cm in height & 25.5cm in diameter). We opened the rearing containers twice a day to refresh the air inside. Larval excrement as well as the post-feeding remnants of young leaves, stems and tendrils in the rearing containers were cleaned routinely. They were then wiped clean with a dry cloth and the larvae placed back in their respective containers with a fresh supply of food plant when the plant materials were consumed or no longer suitable. All observations were made when the rearing containers were cleaned. Larval stages were identified, measured and photographed. Eggs and early instars were measured using Image J, image analysis program (Abràmoff et al. 2004) and a Carrera Precision digital vernier caliper was used to take measurements of later instars and pupae. A Canon EOS 60D digital SLR camera fitted with a Canon EF-S 18-55 mm lens was used to take pictures. The larval food plant was identified using the Revised Handbook to the Flora of Ceylon volume 1 (Kostermans 1980) and by following the Plant Name database, the Plant List (2013) for the updated Latin names.

Results: Egg-laying of *Nacaduba pactolus ceylonica* was observed in Katepola, on 03 April 2015 around 09:40hr and they were laid singly on young leaves

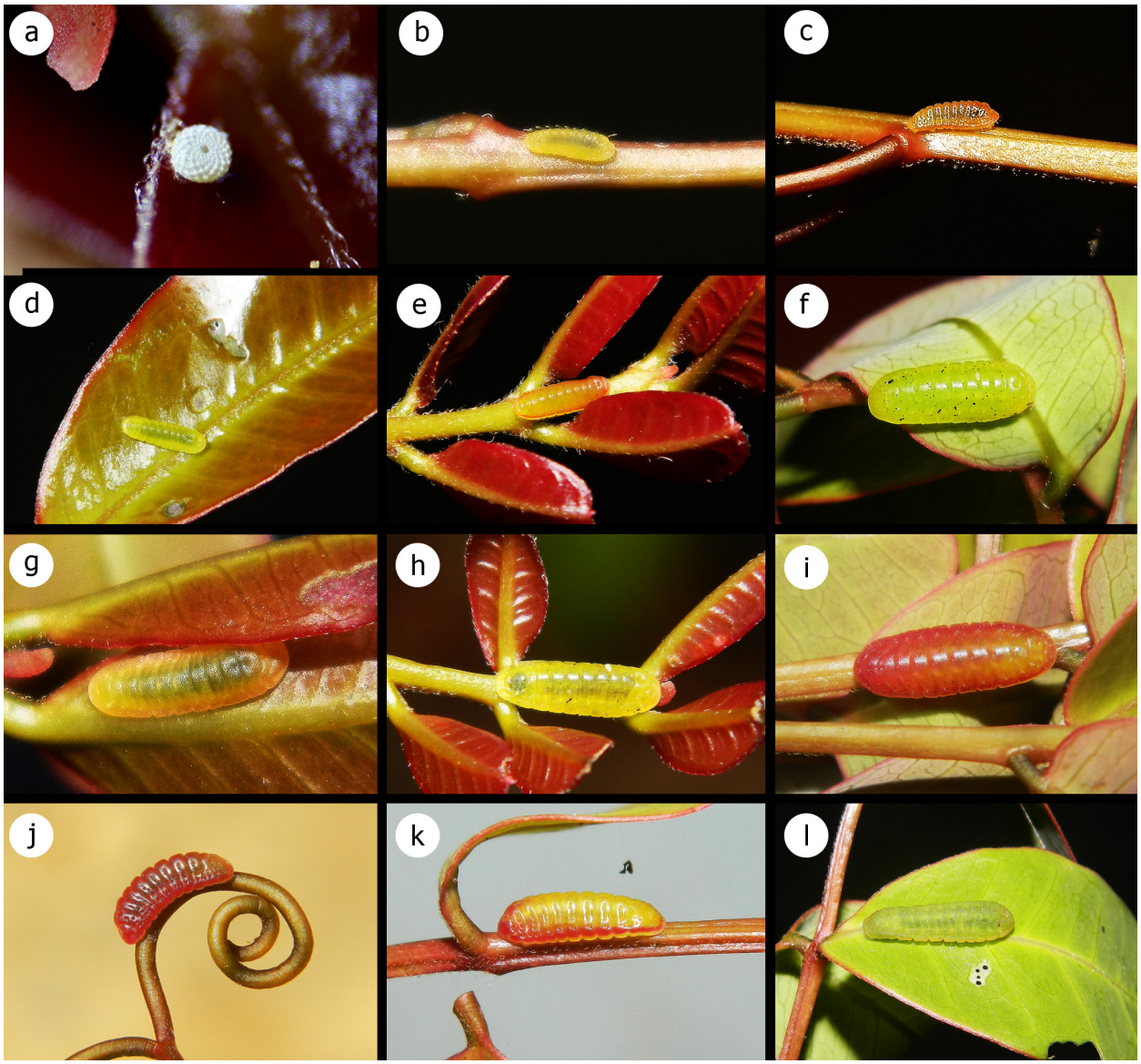


Image 1. Early stages of *N. pactolus ceylonica*. a - Egg; b - First-instar; c - Young second-instar; d - Mature second-instar; e - Yong third-instar; f–k - Mature third-instar; l - Mature fourth-instar. © Authors

and young shoot of a woody climber, *Entada rheedii* (Fabaceae). Also we observed some immature stages (eggs and larvae) near the entrance to the Samanala nature reserve, "Kuruwita-erathna" foot path, in Ratnapura District, Sabaragamuwa Province, on 18 April 2015.

Egg: 0.52 ± 0.02 mm in diameter (n=4), flattened, button-shaped, white to pale yellow coloured, criss-crossed with a network of ridges on the surface (Image 1a). The eggs hatched in three days after they were laid. Upon emergence, larvae consumed the top part of the egg shell.

1st instar: pale yellowish-green with whitish primary setae occurring dorsally and sub-spiracularly. Anal plate and pro-thoracic shield are diamond shaped and light green (Image 1b); length 1.50 ± 0.01 mm (n=3); molt in three days.

2nd instar: setae absent; early second instar was purplish-brown with a yellowish light green tinge (Image 1c) and gradually the colour changed to yellowish light green (Image 1d); dorsum of the thorax slightly depressed; length 3.55 ± 0.06 mm (n=3); molted in two days.

3rd instar: light green (Image 1f) or reddish-pink (Image 1i) while some larvae shows intermediate colours (Image 1e–k); dorsal nectary organ (DNO) and tentacular

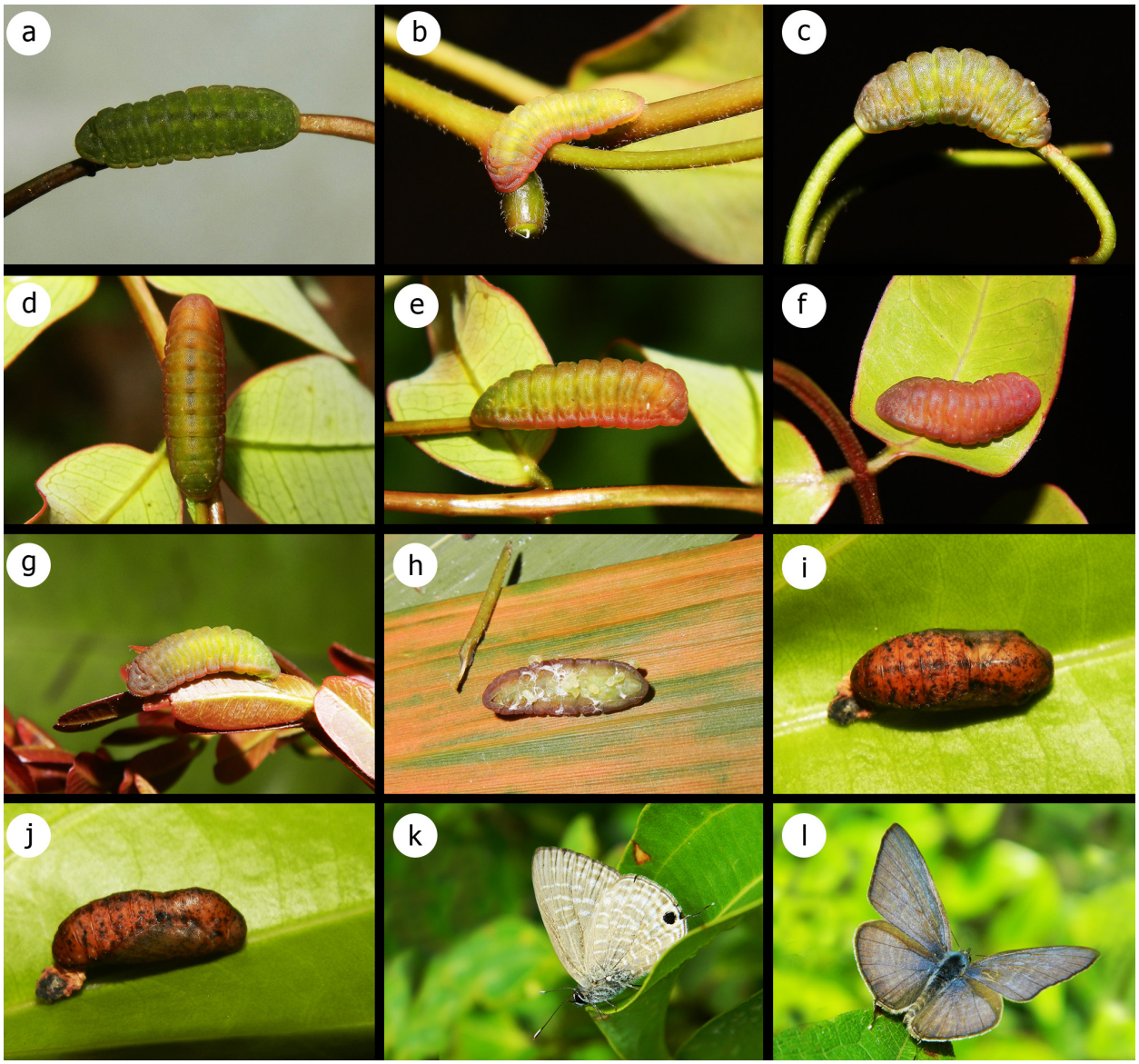


Image 2. Early stages and adult male of *N. pactolus ceylonica*. a–b - Mature fourth-instar; c–e - final-instar; f–g - Mature final-instar; h - Unknown parasitoid larvae on mature final-instar; i - Pupa, dorsal view; j - Pupa, lateral view; k - Male, underside; l - Male, upperside. © Authors

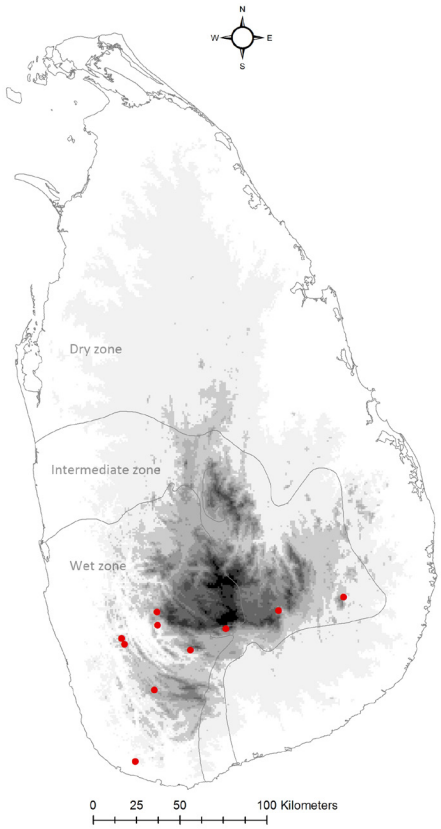


Figure 1. Distribution of *N. pactolus ceylonica* in Sri Lanka.

organs (TO) are barely distinguishable; prothoracic shield white with slight greenish tinge but it is not prominent; length 8.08 ± 0.13 mm (n=6); time taken to molt was three days.

4th instar: ranged from green to reddish-pink (Image 1l, 2a and 2b); DNO and TO well distinguished; white prothoracic shield; length 14.02 mm ± 0.10 mm (n=6); took four days to molt.

Final instar: little or no change in colour (Image 2c –g); length 15.92 ± 0.07 mm (n=5), development time was five days. Parasitoid larvae were observed in a final instar larva (Image 2h) which was collected from the field while the parasitoid larvae were not reared and identified.

Only the 1st and 2nd instar larvae fed on leaf buds while other instars fed on young leaves, stems and tendrils. None of the larval stages consumed the green color leaves and they always fed on pinkish-brown color young leaves. Larvae usually preferred stems, tendrils and rachis for resting. Pupation occurred on top of a mature leaf blade. Final instar larvae secreted a silk-pad on top of leaf blade, and created a silk girdle across the thorax which attached to the silk-pad. Pupae was a typical lycaenid shape (Image 2i–j) and 10.31 mm ± 0.03 mm in length (n=3). In the laboratory, it took 28 days from oviposition to adult emergence.

During the survey we recorded *N. pactolus ceylonica* from 10 locations from wet and intermediate zones of the island (Fig. 1), Samanala-Kuruwita-erathna foot path (6.8332^{0}N & 80.4274^{0}E; elevation 397m), Belihuloya (6.7460^{0}N & 80.7845^{0}E; elevation 978m), Gilimale (6.7649^{0}N & 80.4302^{0}E; elevation 96m) forest reserve, Hiyare forest reserve (6.0592^{0}N & 80.3151^{0}E; elevation 113m), Katepola (6.6972^{0}N & 80.2429^{0}E; elevation 144m), Kukulugala (6.6667^{0}N & 80.2592^{0}E; elevation 345m), Pettigala (6.6365^{0}N & 80.6005^{0}E; elevation 265m) and Sinharaja forest reserve (6.4293^{0}N & 80.4139^{0}E; elevation 482m) in Ratnapura District, Sabaragamuwa Province. Maragala (6.9107^{0}N & 81.3943^{0}E; elevation 184m) in Moneragala District**,** Uva Province and Ella (6.8415^{0}N & 81.0575^{0}E; elevation 666m) in Badulla District, Uva Province. All the larval observations were made in March, April and May.

Discussion: Our observations suggest that March, April and May are the best seasons to observe the early stages of *N. pactolus ceylonica* and it seems to fly year-round in the wet and intermediate zones of the island excluding high elevations. Also they occur in the dry zone along the river banks where the larval food plant, *Entada rheedii* is abundant.

The sea bean, *Entada rheedii* (previously recognized as *Entada pursaetha*) is the only representative member of the genus in Sri Lanka (Kostermans 1980) which is distributed throughout the wet zone of the island including river banks and estuaries. Previous studies have identified *E. rheedii* as the larval food plant of two subspecies of *N. pactolus* in India and Taiwan (Bean 1964, 1988; Hsu et al. 2004). In Singapore the butterfly larvae feed on a different species belonging to the same genus, *Entada spiralis* (Tan 2009). So in its known range of distribution, *N. pactolus* is found to use only one plant genus. So this study confirms the fact that *N. pactolus* is a stenoligophagous species. Exploring *Entada* sp. in its range would help to identify the larval food plant in other countries where the butterfly occurs.

In Sri Lanka six other butterfly species also depend on the same plant as their host for larvae, *Eurema blanda citrine* (Three Spot Grass Yellow), *Charaxes psaphon psaphon* (Tawny Rajah), *Curetis thetis* (Indian sunbeam), *Rathinda amor* (Monkey-puzzle), *Jamides celeno tissama* (Common Cerulean) and *Cheritra freja pseudojafra* (Common Imperial) (Jayasinghe et al. 2014) which may compete for resources. But, as *E. rheedii* is a common wide-spread species, it may not be a limiting factor for this rare subspecies. Not only as a larval host

plant but also as a nectaring plant *Entada rheedi* is an important species for the island butterfly fauna.

The stenoligophagous nature of *N. pactolus ceylonica* across its natural range will make it vulnerable to extinction if the host plant population declines. This highlights the need for proper conservation planning of rare butterflies that takes into consideration the larval stages and larval food plants.

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