Notes on the immature stages and larval host plant of the Nilgiri Tit (*Hypolycaena nilgirica* Moore, [1884]) in the Western Ghats, India

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Abstract: The immature stages of the butterfly Nilgiri Tit *Hypolycaena nilgirica* Moore, [1884] family Lycaenidae, were studied in the foothills of the Nilgiris, Western Ghats, India. The species' life cycle on its newly identified host plant *Eulophia epidendraea* (J.Koenig ex Retz.) C.E.C.Fisch. (Orchidaceae) is reported for the first time in India. The immature stage development, from oviposition to adult eclosion, took approximately 25-32 days. Distribution records of the butterfly species in the region of the type locality are documented and a description of the larval stages is provided. This study provides basic information on the species' life cycle and host plant in India.

Key words: Life cycle; Lycaenidae; Nilgiri Biosphere Reserve; Orchidaceae.

INTRODUCTION

The genus Hypolycaena C. & R. Felder, 1862 (Lepidoptera: Lycaenidae) belongs to the subfamily Theclinae and tribe Hypolycaenini, which has 25 species in the Afrotropical region (Stempffer, 1967) and 11 species in the Indo-Malayan Region, with five in India (Kunte, 2015). In south India, the genus is represented by two species, Nilgiri Tit Hypolycaena nilgirica Moore, [1884], endemic to Western Ghats (Tamil Nadu and Kerala) and Sri Lanka, and Orchid Tit Hypolycaena othona Hewitson, (1865), occurring from southwest India to Malaysia and Indochina (Fiedler, 1992). The species Hypolycaena nilg*irica* was described in 1884 by Moore based on a single male specimen from Coonoor, 1000 feet elevation, Nilgiris, Western Ghats (Moore, 1884-1887; Swinhoe, 1911), and later recorded by several authors from the type locality (Hampson, 1888; Evans, 1932; Wynter-Blyth, 1944, 1945; Larsen, 1987; Jeevith et al., 2017). It has since been recorded from Sri Lanka (Ceylon) first reported by Moore (1884-1887) and later Hampson (1888). In India, until the late 1980s, the species was observed only from the foothills of the Nilgiris at Kallar by Wynter-Blyth (1944) and Larsen (1987), with a few records from Palnis (Larsen, 1987). However, since Larsen's observations in the 1980s, many sightings have been registered from different regions of Western Ghats, including the Geddai slopes of Nilgiris District, Aiyannar Falls of Virudhunagar District, Anamalais of Coimbatore District and Kalakkad Mundanthurai Tiger Reserve of Thirunelveli District in Tamil Nadu, Chinnar Wildlife Sanctuary of Idukki district, and Silent Valley National Park of Palakkad District in Kerala (Sadasivan et al., 2023) (Fig. 1). The species has also been recorded from several locations in Sri Lanka (iNaturalist.org). The species is protected under Schedule II of the Indian Wildlife (Protection) Act, 1972 (New,

1993). Some information has been published on the larval food plants of Nilgiri Tit from Sri Lanka (Poorten *et al.*, 2013), but in Western Ghats, India, the larval host plant was until now unknown, as reported by Bhakare and Ogale (2018), Jeevith *et al.* (2017), and Nitin *et al.* (2018). Indeed, there are no records of the immature stages or definitive larval host plant records from anywhere India. In this paper, the complete life biology of the Nilgiri Tit from egg to adult, with its feeding habits and larval food plant, is reported for the first time from India.

MATERIALS AND METHODS

The study was carried out through biweekly butterfly surveys in the Kallar Horticulture Garden (11°20'32.39"N, 76°50'21.13"E), in the Nilgiri Foothills of southern Western Ghats (Fig. 1), in the type locality region (Moore, 1884). Records from this region and notes on butterfly behavior are provided in Table 1. Observations were made on multiple visits to Kallar Horticulture Garden from January 2020 to December 2021 to document the ovipositing behavior and early instars of the species. The immature stages were observed *in situ*

 Table 1. Observations of Nilgiri Tit in the region of its type locality,

 Nilgiris, Western Ghats, India.

Location	Month	Habitat	Activity (Encounters)
Kallar, Coonoor slopes, Nilgiri Foothills	July, August, October, November, December and January	Moist deciduous forest and rock slopes	Basking and Ovipositing (1) Puddling (1)
Kolikarai, Kotagiri slopes	January	Semi-evergreen and moist deciduous forest	Basking (1)
Kunjappanai, Kotagiri	September	Semi-evergreen and moist deciduous forest	Puddling (1)
Geddai, Manjoor slopes	November	Moist deciduous forest	Puddling (1)

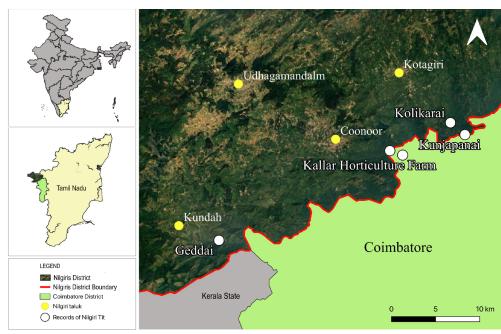


Figure 1. Locations where Niligiri Tit has been recorded in study region.

regularly throughout the life cycle. Identification of instar stage was based on comparison with observations in Poorten et al. (2013). Individuals were not marked or enclosed in containers, but the authors visited the field regularly throughout the study period and observations reported here represent best estimates. In particular, there is some doubt about the identity of fourth and fifth instars, since these are similar in size, color, and feeding pattern, and observations are therefore pooled for these instars. The immature stages were photographed using Nikon 90D, 7200, and 7500 DSLRs with Tamron 90mm and Nikkor 100mm macro lenses. The plant species were identified with the help of pertinent literature (Joseph, 1987; Jalal and Jayanthi 2012) and the nomenclature was updated based on POWO (2023). Field observations of the plant were also made, including habit and habitat, inflorescence, phenology status, and distribution of the species, and photographs were taken in situ.

RESULTS

Nilgiri Tit *Hypolycaena nilgirica* lays eggs on the inflorescence of the larval host plant *Eulophia epidendraea* (J.Koenig ex Retz.) C.E.C.Fisch. (Orchidaceae), a terrestrial orchid species (Fig. 2). This is the first known record of the butterfly species utilizing this plant as a larval host plant in India. In Sri Lanka, Orchidaceae species including *Malaxis versicolor, Peristylus trimenii, Arundina graminifolia, Spathoglottis plicata, Arachnis flos-aeris, Vanda tessellata* and *Vanda testacea* were reported as larval host plants for *H. nilgirica* (Poorten *et al.*, 2013; plant name authorities provided in that paper). Observation of the immature stages of the butterfly in the study area are given in Table 2 and estimates of the duration of each instar are provided in Table 3.

Larval Host Plant

Eulophia epidendraea is a large terrestrial orchid (Fig. 2), with pseudobulbous stems. Leaves grass-like and plaited, and base

sheathing has a pseudobulb. Flowers greenish yellow with a white, purple-streaked lip, 2 cm long; flower-cluster-stalk is round, about 45-48 cm long, erect, bearing 9 membranous sterile bracts. Floral bracts ovate, tapering; dorsal petals lanceolate-oblong, apiculate; lateral sepals, obovate-lanceolate, apiculate; petals broadly lanceolate; sepal lip obovate-oblong; lateral lobes of lip small, erect; mid-lobe broadly oblong, rounded, crenulate, recurved; spur short, cylindrical (Joseph, 1987).

Distribution: The plant species is distributed in India, Sri Lanka and Bangladesh (POWO, 2023).

Phenology: October - March (flowering and fruiting).

Habitat and associated species: This terrestrial orchid was found on rocky slopes in humid areas. The species can also be cultivated *ex situ* with suitable climatic conditions (Joseph, 1987). In the study area, *Eulophia epidendraea* was mainly associated with *Cymbopogon flexuosus* (Steud.)., *Chrysopogon nodulibarbis* (Hochst. ex Steud.) Henrard., *Melinis repens* (Willd.) Zizka., (Poaceae), *Urena lobata* L. (Malvaceae), *Tri*-

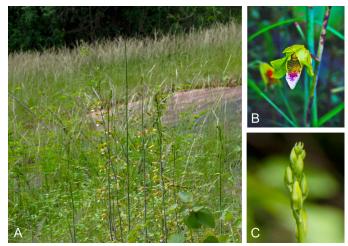


Figure 2. *Eulophia epidendraea* (Orchidaceae) hostplant of Nilgiri Tit *Hypolycaena nilgirica*. A. Habitat, B. Flower, C. Flower bud. © Selvaraj Jeevith.



Figure 3. Nilgiri Tit *Hypolycaena nilgirica* early stages. **A & B.** Female ovipositing on *Eulophia epidendraea*; **C.** Egg; **D.** First instar larva; **E.** Second instar larva; **F.** Third instar larva; **G.** Fourth instar larva - pink morph; **H.** Fourth instar larva, with associated ant *Camponotus* sp.; **I.** Fourth instar larva, with associated ant *Anoplolepis gracilipes.*; **J.** Fifth instar larva - green morph; **K.** Newly formed pupa; **L.** Pupa with white curvature marking; **M.** Pupa lateral view; **N.** Pupa ventral view; **O.** Pupa dark morph; **P.** Pupa fully matured; **Q.** Adult freshly eclosed upper wing; **R.** Underwing. © Manoj Sethumadhavan and Felix Nirmal Dev.

dax procumbens L., (Asteraceae), and the tree *Chloroxylon swietenia* (Roxb.) DC. (Rutaceae).

Notes on Immature Stages

Egg: Females laid eggs generally around noon on hot,

sunny days. Eggs were laid singly on the flower buds (Fig. 3A), stem or other parts of the inflorescence of the host plant. Eggs were initially translucent green in color (Figs 3B, C), which on hardening turned chalky white, within a few seconds after oviposition. The eggs were hemispherical with chorionic

 Table 2. Observations of adults and early stages of Nilgiri Tit in the study area.

Date of Observation	Time (hrs) Butterfly stages	
05, 21, 27 & 28 January 2020	1100 - 1400	Adult
07 June 2020	1000 - 1400	Adult
21, 28 June 2020	1030 - 1400	Adult
15, 17, 27 July 2020	1000 - 1300	Adult
23, 27 September 2020	1100 - 1400	Adult
29 & 30 November 2020	1130 - 1330	Adult (f), eggs, larva (3 & 4
29 & 30 November 2020	1130 - 1330	instar), pupa
01 to 06 December 2020	1000 - 1300	Adult
10 to 12 December 2020	1130 - 1330	Adult
14 & 15 December 2020	1100 - 1300	Adult
25 February 2021	1000 - 1200	Adult
26 August 2021	1230 - 1400	Pupa on host plant
12 October 2021	1530 - 1630	Adult, egg, larva on host
12 October 2021	1550 - 1650	plant
20 October 2021	1230 - 1330	Adult, egg, larva & pupa on
20 October 2021	1230 - 1330	host plant
24 October 2021	1100 - 1330	Adult, egg, larva & pupa on
24 October 2021	1100 - 1550	host plant
30 October 2021	1130 - 1330	Adult, egg, larva & pupa on
50 October 2021	1150 - 1550	host plant

 Table 3. Life biology of Nilgiri Tit Hypolycaena nilgirica.

Butterfly stage	No of Days	Remarks
Egg	4 - 5	
1st Instar larva	2 - 3	
2nd Instar larva	2 - 3	
3rd Instar larva	5 - 6	
4th/ 5th Instar larva	2 - 3	Feeding stopped
		during early 5th
		instar
Pupal stage	10 - 12	The pupa turns
1 0		from green to
		ochreous
Total	25 - 32	

sculpturing of thick, distinct reticulated hexagonal ribs, as is typical for lycaenid ova (Downey & Allyn 1981, 1984). As in other lycaenid species, the eggs were also found on flower buds and the plant stem (Fig. 3B). Empty eggshells and unhatched eggs were observed on the stem and leaf nodes. Most eggs were observed on the terminal and lateral buds. Occasionally, eggs were also seen on the flower petals, but no eggs were observed on the leaves. The eggs took 4 to 5 days to hatch, with no further changes in coloration, and usually hatched during early morning hours.

Larval stages: Two larval morphs were observed, one pink and one green. However, the white morph reported from Sri Lanka (Poorten *et al.*, 2013) was not observed in our study.

First Instar: The egg shells were not eaten by the larvae and 10 individual hatched egg shells were observed on the inflorescence of the plant. The first instar larva was covered fully with small setae. The larva was translucent yellow with pale red disjointed lines running laterally. As soon as the eggs hatch, the larvae bored into the buds and remained there feeding inside the bud throughout the first instar. Frass pellets were observed on the buds, stem, and nodes; however, it was unclear if the larvae exited the bud to excrete or made holes to excrete from inside the buds. This instar lasted approximately 2 to 3 days (Fig. 3D).

Second Instar: After molting from the first instar, the second instar larva emerged out of the buds. The color

became pale green with pale red lines and with fewer hair-like structures. The setae became much more reduced in number, organized linearly, pronounced and distributed along the lateral reddish lines. Once the second instar emerged from the bud, they kept feeding on the outer buds as well as the flower petals. At this stage, the head could be retracted under the sclerotized prothoracic plate and the feeding pattern changed. The larvae started to feed either on the flower buds or plant stem or moved on to feed on fully grown flowers. This instar lasted approximately 2-3 days (Fig. 3E).

Third Instar: At the end of 5-6 days after eclosion, feeding increased considerably. The third instar larva fed on flower petals and on average half the flower was consumed at the end of the first day after molting. By the end of the second day, we estimated that the larva was able to consume approximately 70% of a flower, based on examination of sequential photographs. The length of the larva increased considerably, the hair-like structures were completely replaced with tiny bristles, and the spiracles became visible. This instar lasted for approximately 3-4 days. (Fig. 3F).

Fourth/Fifth Instar: At 9-10 days after eclosion, the larva entered the fourth instar and the feeding became intense, feeding on two complete flowers in two days. The lateral pale red markings disappeared in the mid-section of the larva and remained visible on the dorsal part of the head capsule and posterior sections. The larvae changed color to pale yellow or pale green. The spiracles became more conspicuous. The pale red markings became much duller and the lateral bands clearer both above and below the spiracles. It was difficult to separate the 4th and 5th instars during *in situ* observations and from images, since these instars were similar in size and color, but it is assumed that five instars are estimated to have each lasted approximately two days (Figs. 3G-J).

Pupal Stage: The pupa was pale green throughout. On the anterior part of the head, there was a pale red patch. The pupa was attached to the stem at its posterior tip and fastened by a thin silk girdle across the abdomen. Just above the wing cases, the green turned to ashy white with a distinct blackish patch. After 8-9 days the pupa turned ochreous and the coloration of the wings and body developed rapidly (Figs. 3K-P).

Adult: Four observed adults eclosed early in the morning at 0530 to 0630. The expansion and drying of the wings took approximately one hour (Figs. 3Q,R). Overall, we estimate that the immature life stages took approximately 25 to 32 days from oviposition to eclosion.

Arthropod species associated with *Eulophia* epidendraea: Several other species of insect were observed on *E. epidendraea*. Several species of ants including *Camponotus* sp., *Meranoplus bicolor*, *Anoplolepis gracilipes* and *Crematogaster* sp. were observed on the particular plant on which *H. nilgirica* immatures were feeding, with no predation of larvae of *H. nilgirica* observed. *Anoplolepis gracilipes* were observed feeding on secretions from *H. nilgirica* larvae (Fig. 31). Author FND observed a wasp species attending and preying on *H. nilgirica* during their pupal stage, and also a parasitic wasp emerging from one pupa.

DISCUSSION

This is the first description of the immature stages of Nilgiri tit from India, and although more intensive studies are needed, it provides additional data to compare and contrast with previous observations of the species (Poorten et al., 2013). In our study, the butterfly larva fed on a terrestrial orchid species, but elsewhere in Western Ghats (Kerala), Kalesh (pers. comm.) observed a few epiphytic orchid species as hostplants for H. nilgirica, including Vanda tessellata (Roxb.) Hook. Ex G.Don and Aerides ringens (Lindl.) C.E.C.Fisch. Further study is needed on ant associations, larval forms, and larval host preferences at different elevations. Breeding in captivity for further genetic and morphological study would also be desirable. The larva of Nilgiri Tit has several color morphs (Poorten et al., 2013), with a white form reported in Sri Lanka that was not observed in this study. The presence of such a form in Sri Lanka could be due to larval adaptation to the surroundings, the stem, the inflorescences, or the white flowers of the host plant in that region. Alternatively, it may be that the white form is present in India but was not recorded by us due to the limited sample size. The feeding pattern of H. nilgirica generally followed that of Hypolycaena othona Hewitson, 1895 as documented in West Malaysia, which was also usually seen feeding by way of tunneling into fleshy tissues of the larval host plant (Fiedler, 1992). Similarly, H. nilgirica also avoided feeding on leaves, as in the case of *H. othona*. However, contrary to *H. othona*, *H.* nilgirica was seen feeding on flowers.

Pierce et al. (2002) categorized lycaenid-ant association into three categories as obligate, facultative and non-ant associated. Obligate interactions are necessary during at least some portion of the life cycle wherein the immature butterfly is dependent on ants for its survival. Such interactions can be either mutualistic or parasitic and typically involve a single species or genus of ant. By contrast, in facultative associations, the larvae are found only intermittently associated with ants and do not require attendant ants for survival. The association is also non-specific, with more than one genus or subspecies of ants attending the larvae. These associations are mostly mutualistic, although a few species are also predaceous. In the case of H. nilgirica in India, the species did not appear to require the attending ant for its survival, based on our regular field observations. We thus follow previous authors in regarding this type of interaction as a moderate facultative relationship (Pierce, 2002; Poorten et al., 2013; Sujitha et al., 2023).

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