Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Immature Stages of the Brazilian Crescent Butterfly *Ortilia liriope* (Cramer) (Lepidoptera: Nymphalidae)

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Keywords
Acanthaceae, gregarious caterpillar, *Justicia*, Melitaeini, Phyciodina

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Edited by Marcelo Duarte – MZ/USP

Received 07 May 2010 and accepted 13 October 2010

Abstract

We provide the first information on the morphology of the immature stages (egg, larva, and pupa), oviposition and larval behavior, and host plant, for the Brazilian crescent butterfly *Ortilia liriope* (Cramer), based on material from Santarém Municipality, Pará State, Northern Brazil. Females of *O. liriope* lay eggs in clusters. After hatching, larvae eat the exochorion and remain gregarious in all but the final instar. The host plant recorded in the study site is *Justicia* sp. (Acanthaceae). Despite the scarcity of data on the immature stages of Neotropical Melitaeini, we can already say that some morphological and behavioral traits observed in the immature stages of *O. liriope* are also present in all known genera in this tribe.

Introduction

Melitaeini, one of the six recognized tribes of the Nymphalinae (Nymphalidae), is a relatively small group of ca. 250 butterfly species present only in the Holarctic and Neotropical regions (Wahlberg & Freitas 2007, Wahlberg *et al* 2009). The group is considered monophyletic (Zimmermann *et al* 1999, Freitas & Brown 2004, Wahlberg *et al* 2005), and the few clear synapomorphies consist of characters of male and female genitalia (Higgins 1981, Kons 2000).

The Melitaeini are divided into five distinct clades, including the subtribes Euphydryina, Melitaeina and Phyciodina, the *Chlosyne*-group, and the *Gnathotriche*-group (Wahlberg & Zimmermmann 2000, Wahlberg *et al* 2005). General information about Melitaeini is not evenly distributed among the five clades, being clearly biased towards the temperate Euphydryina and Melitaeina species (Wahlberg & Freitas 2007).

Phyciodina is composed mainly of tropical species, and alone comprises almost half of all species of Melitaeini. In spite of that, data on basic natural history for this subtribe, such as descriptions of early stages and host plants, are known for only a small fraction of the species (Beccaloni *et al* 2008).

The genus *Ortilia* Higgins was erected based on poorly defined characters of male genitalia, and originally included 10 species of typically orange-black melitaeines (Higgins 1981), which later were reorganized to represent nine species by Lamas (2004). Recently, a molecular study by Wahlberg & Freitas (2007) showed that *Ortilia* is in fact a polyphyletic assemblage, with their members distributed among three distinct clades within the Phyciodina. Thus far, immatures have not been described in detail for any species of *Ortilia* (*sensu* Lamas 2004), and information on host plant use remains scarce and uncertain (Beccaloni *et al* 2008, Neild 2008).

Ortilia liriope (Cramer) (Fig 1a), the type species of the genus, together with *Ortilia gentina* Higgins and *Mazia amazonica* (Bates) (Wahlberg & Freitas 2007) form a clearly distinct clade. The species occurs in northern South America, in Amazonia, usually in association with open sunny areas, such as tree gaps, forest edges and secondary forests. The present paper describes the early

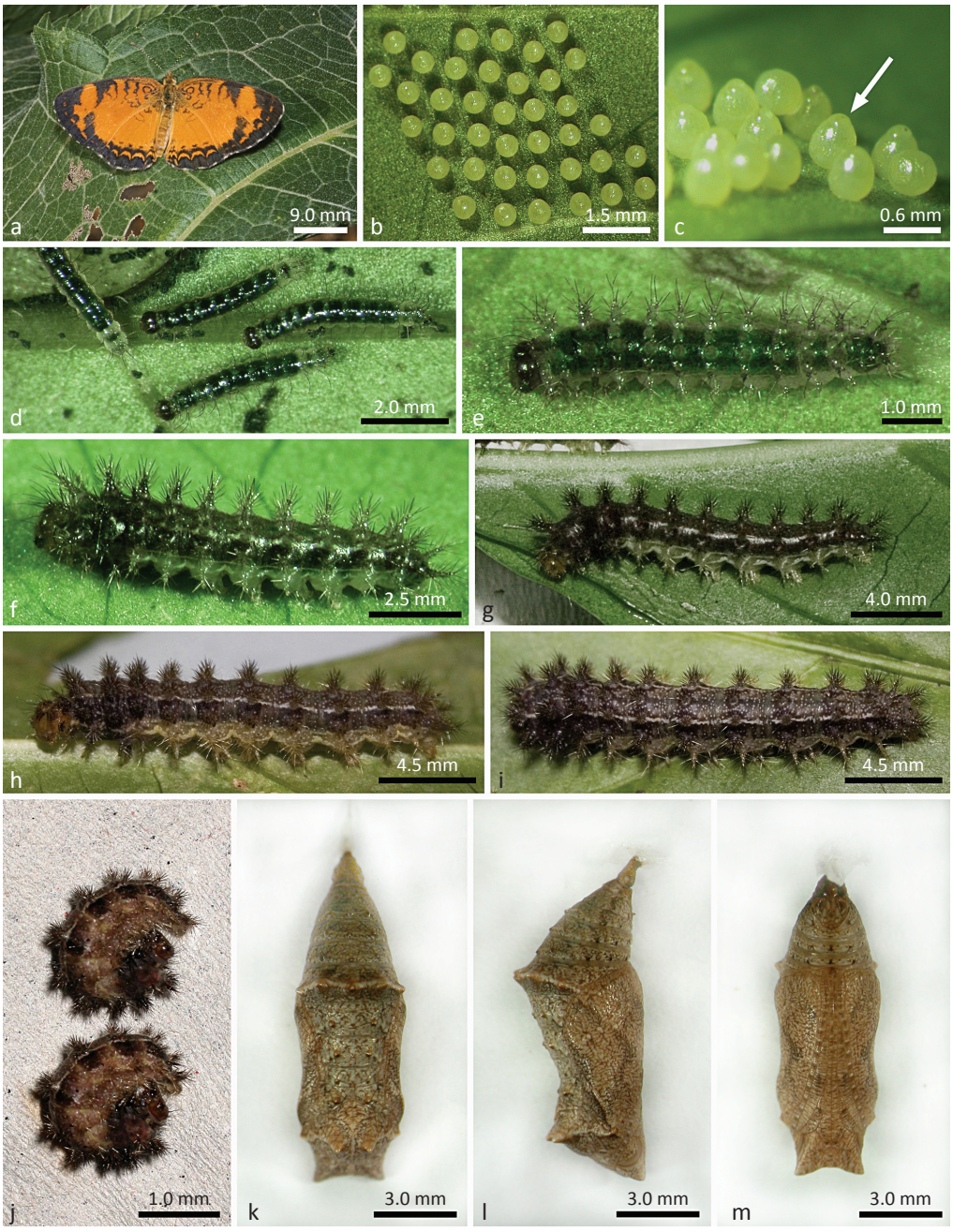

Fig 1 Life stages of *Ortilia liriope*. a) adult female; b) egg cluster (dorsal view); c) egg cluster in lateral view (white arrow indicating pointy apex); d) first instar; e) second instar; f) third instar; g) fourth instar; h) fifth instar (lateral view); i) fifth instar (latero-dorsal view); J) two prepupae; k) pupa (dorsal view); l) pupa (lateral view); m) pupa (ventral view).

stages of *O. liriope*, compares the immatures with those of other known Melitaeini, and briefly discusses host plant use within the Phyciodina.

Material and Methods

Adults and immature of *O. liriope* were collected near the Urumari stream (2°27'S, 54°41'W), in the suburban area of Santarém Municipality, Pará State, Northern Brazil, in 2009-2010. Descriptions of immature stages are based on egg clusters collected in nature on the host plant *Justicia* sp. (Acanthaceae), and reared in laboratory following standard procedures for larval rearing (Freitas 1991) with food *ad libitum* using another species, *Justicia brasiliana*, that was well accepted by the larvae. Data were taken on behavior and development times for all stages. Measurements and general morphology were assessed using a Leica® MZ7.5 stereomicroscope equipped with a micrometric scale. Egg size was measured as height and width. The size of the larval head capsule was measured as the distance between the most external stemma (as in Freitas & Brown 2008). Head and body chaetotaxy were described following Stehr (1987). Immatures were fixed

in Kahle solution (Triplehorn & Johnson 2005); all adults, preserved larvae, head capsules, and pupal skins were deposited at Unicamp (Museu de Zoologia, ZUEC).

Results

Natural history

At the study site, *O. liriope* was observed using *Justicia* sp. as its larval host plant. Females of *O. liriope* laid eggs in clusters of about 50-60 eggs (n = 2). Oviposition occurred with the female sitting on the upper surface of the leaf and curling the abdomen around the leaf edge so that eggs were deposited under the leaf.

After hatching, larvae ate the exochorion and remain aggregated until the final instar. Most larvae passed through five instars, but some have an extra, sixth stadium (18 out of 157 individuals). Final instars dispersed to feed solitarily until pupation. In the first two instars, larvae fed by scraping the under surface of the leaf, but in later instars they fed on the whole leaf, leaving only the largest veins. Prepupae hung from a support by the anal prolegs, curling the body in a ring with the head almost touching the apex of the abdomen (Fig 1j).

Description of immature stages

Egg (Fig 1b-c). Width (diameter) 0.46-0.56 mm, height 0.54-0.64 mm (n = 10). Light yellow, remaining unchanged until hatching. Pear-shaped with a conspicuously pointy apex; with 19-21 poorly defined vertical ridges and numerous inconspicuous horizontal ridges. Duration: five days (n = 15).

First instar (Figs 1d, 2, 3a). Head capsule width 0.30 mm (n = 10). Maximum body length 4.0 mm. Head capsule dark brown, without horns or spines. Body translucent, with dark green gut contents clearly visible; legs, prolegs and anal plate light beige; setae relatively long (ratio between setal length/segment height c. 1.2); setae above spiracles are black, and light below. Frontal head chaetotaxy and lateral body chaetotaxy are shown in Figs 2 and 3a, respectively; in the body the ventral group of setae is present, but is not shown. Duration: 3-5 days (n = 30).

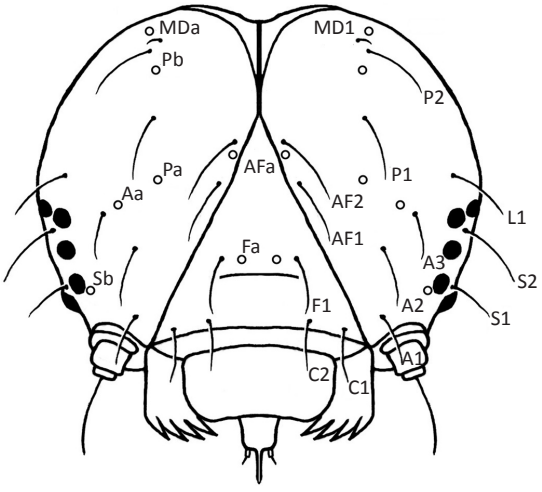

Fig 2 *Ortilia liriope*. Frontal head chaetotaxy of first instar (right side: setae nomenclature; left side: pore nomenclature).

Second instar (Fig 1e). Head capsule width 0.40-0.48 mm (n = 10). Maximum body length 6.0 mm. Head capsule light brown, with conspicuous long brown setae and without horns or spines. Body dark green dorsally, white ventrally; covered with short light brown scoli from T2 through A10; T1 without scoli, but bearing long black setae; legs brown, prolegs translucent, anal plate light brown. Scoli distribution remains the same until the final instar (Fig 3b). Duration: 2-3 days (n = 30).

Third instar (Fig 1f). Head capsule width 0.56-0.68 mm (n = 10). Maximum body length 11 mm. Head capsule orange-brown with black stemmata. Body dark greenish brown dorsally, light cream ventrally on abdominal segments; scoli dark brown, except for the subspiracular abdominal series, which is light cream; legs brown, prolegs translucent, anal plate brown. Duration: 3-4 days (n = 30).

Fourth instar (Fig 1g). Head capsule width 1.00-1.30 mm (n = 10). Maximum body length 16 mm. Head capsule color and shape as in previous instar. Body dark brown dorsally, light cream ventrally in abdominal segments; scoli dark brown, except for the subspiracular abdominal series, which is light cream; legs dark brown, prolegs translucent, anal plate brown. Duration: four days (n = 30).

Fifth (last) instar (Figs 1h-i). Head capsule width 1.90-2.04 mm (n = 6). Maximum body length 19 mm. Head capsule reddish brown with black stemmata; shape as in previous instar. Body dark brown dorsally, light cream ventrally in abdominal segments; scoli dark brown, except for the subspiracular abdominal series, which is light cream; legs dark brown, prolegs translucent, anal plate brown. Duration: six days (n = 30). Some larvae can have six instars; in these larvae, the fifth instar can reach 19 mm in length, but with the head capsule more similar in size to those of the fourth instar (head width 1.20 mm – 1.48 mm). Prepupa without marked changes in coloration (Fig 1j).

Pupa (Figs 1k-m). Length 11-12 mm (n = 14). Light brown with sparse darker marks all over the wing caps and abdomen. General profile elongated; ocular caps short and pointed like little cat ears. A series of short subdorsal pointed knobs from T2 through A7 segments as follows: T2-A1 with one subdorsal pair, those of T2 conspicuously

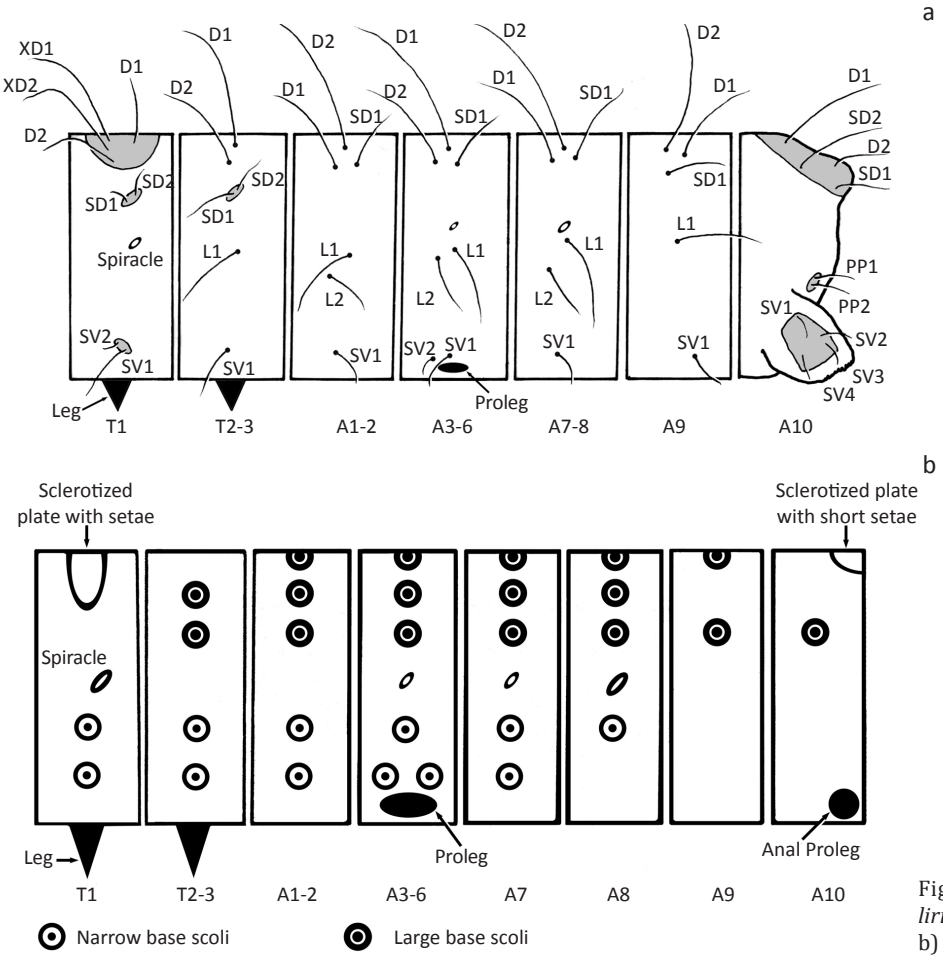

Fig 3 Larval body diagrams of *Ortilia liriope*. a) Lateral first instar chaetotaxy; b) Fifth instar scoli distribution.

enlarged and positioned over a pronounced bump; A2-A3 and A5-A7 with a single dorsal knob in addition to the subdorsal pair; A4 with a conspicuous transverse keel bearing five enlarged knobs, a single dorsal knob and two subdorsal pairs; all remaining segments without knobs (T1, A8-A10). Cremaster broad, dark brown. Abdominal segments beyond wing pad apices mobile. Duration: six days (n = 10).

Discussion

Host plant use

The genus *Justicia* used by *O. liriope* at the study site is used by several other species of Phyciodina (Beccaloni *et al* 2008). Unfortunately, reliable host plant records are available for very few species of Phyciodina, thus no additional discussion on patterns of host plant use in this subtribe is possible.

Although Nymphalinae displays a diversity of host plant use (Beccaloni *et al* 2008, Talsma *et al* 2008, Janzen & Hallwachs 2010), species of the subtribe Phyciodina have been reported on only a few host plants, mainly pertaining to Asteraceae and Acanthaceae, with scattered records on Verbenaceae and Scrophulariaceae (Scott 1986, Feldman & Haber 1998, Beccaloni *et al* 2008). Additional reports of other host plant families used by Phyciodina await confirmation (Beccaloni *et al* 2008).

Morphology of the immature stages

A number of morphological and behavioral traits observed in the immature stages of *O. liriope* are present in all known Melitaeini. These include egg clusters and gregarious larvae, absence of head horns, and the body scoli short and densely branched (Young 1973, DeVries 1987, Freitas 1991).

The pale yellow egg with weakly marked ridges is common to species spread throughout the Melitaeini (Young 1973, Freitas 1991, Wahlberg 2000). The number of vertical ridges appears to be quite variable among Melitaeini (from 18 to 23) (Scott 1973, Wahlberg 2000), and the eggs of *O. liriope* do fit the reported range. Pear-shaped eggs, with a conspicuously pointy apex, have been described for the following taxa of Phyciodina: *Anthanassa frisia hermas* (Hewitson) (D'Almeida 1941), *Eresia ithomioides alsina* Hewitson (Young 1973), *Eresia*

lansdorfi (Godart), *Ortilia ithra* (Kirby) (AVLF unpublished data) and *Tegosa claudina* (Eschscholtz) (Toledo 1979, Freitas 1991). In all other known Melitaeini, the eggs have a flattened apex, including species of the genera *Chlosyne* Butler (Scott 1986, AVLF unpublished results), *Euphydryas* Scudder (Williams *et al* 1984, Wahlberg 2000), *Melitaea* Fabricius (Wahlberg 1997, 2000) and *Phyciodes* Hübner (McDunnough 1920, Genc *et al* 2003).

The setae present in the first instar of *O. liriope* are relatively long (longer than the segment height) compared with many other Nymphalidae groups (Freitas *et al* 1997, Freitas & Brown 2004). Long setae appear to be a widespread feature in Nymphalinae except for the Coeini (Scott 1973, Nakanishi 1988, Freitas 1999), and this character was considered a good synapomorphy of Nymphalinae (Freitas & Brown 2004). The distribution of body scoli in the remaining instars is similar to those of all known Melitaeini (Freitas 1991, Wahlberg 1997).

The pupa of *O. liriope* is very similar in general shape to those of *Tegosa* (Toledo 1979, Freitas 1991) and *Phyciodes* (McDunnough 1920, Scott 1986, Gatrelle 2004), both of which have a conspicuous keel on A4. This keel has not been observed in any of the other Melitaeini genera cited above, and might be of taxonomic importance.

Unfortunately, further comparisons regarding immature morphology and host plant use are not possible at the moment due to the general lack of information, especially for Neotropical species. Based on the sparse published information concerning the immature stages, Phyciodina have usually been considered a homogeneous group. The present results suggest that this picture could change easily once more information has been added to the few data available.

Acknowledgments

We are very grateful to Fosca P P Leite for allowing us to use the stereomicroscope for some larval measurements and to Chieno Suemitsu for the identification of the host plant species. We also thank Harri Lorenzi and Edgar F Souto from the Instituto Plantarum for the donation of host plants for rearing the larvae, and Daniela Rodrigues, Keith Willmott, Niklas Wahlberg, Annette Aiello and Marcelo Duarte for suggestions on the manuscript. AVLF thanks FAPESP (grant 04/05269-9) and the Brazilian CNPq (fellowship 300282/2008-7); LAK thanks CNPq for a fellowship (140183/2006-0); EPB thanks CAPES for a fellowship.

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