

RESEARCH ARTICLE

Immature stages of the *Selaginella*-feeding *Euptychia mollina* (Nymphalidae: Satyrinae) from the Brazilian Amazon

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ABSTRACT. The present paper describes the immature stages of the Neotropical satyrine butterfly *Euptychia mollina* (Hübner, [1813]) from the Brazilian Amazon Forest. Eggs were laid singly on the under surface of the fronds of its host plant, *Selaginella* sp. (Bryophyta: Selaginellaceae). Larvae are solitary in all instars, presenting a color pattern and shape that make them cryptic on its host plant. The pupa is short, smooth and varies from rusty brown to green. Despite the lack of a close phylogenetic relationship, larvae of *Euptychia* are very similar to those of the paleotropical satyrines *Ragadia* and *Acrophthalmia*, suggesting that camouflage is likely to be one of the factors explaining the similarities among them.

KEY WORDS. Camouflage, caterpillar, Euptychiina, Lepidoptera.

INTRODUCTION

In recent years, several aspects of Euptychiina have been extensively studied, including their taxonomy and phylogeny, natural history, biogeography and conservation (Peña et al. 2010, Marín et al. 2011, 2017, Freitas et al. 2012, Zacca et al. 2018 and references therein). As a result, the systematics of the subtribe has been revised from the species to the subtribal level, with significant reorganization in most of the large genera (see references above). Nevertheless, some important points remain under discussion, including the monophyly of the subtribe in relation to its nominal genus *Euptychia* Hübner, 1818 (Peña et al. 2010, Marín et al. 2017, Espeland et al. 2019).

As for other large genera of Euptychiina, *Euptychia* has also been the target of several studies in recent years, and since the checklist of Lamas (2014), the number of recognized species in the genus has increased from 13 to 34 (Freitas et al. 2012, Neild et al. 2014, 2015, Nakahara et al. 2014, 2015a,c,d, 2016, Fratello et al. 2015, Warren et al. 2018, see also Freitas et al. 2013 and Nakahara et al. 2015b), with at least 15 additional species awaiting to be described (S. Nakahara, unpublished data). Species of *Euptychia* are present in most Neotropical forests, although they are more common in lowland and submontane forests, where they can be locally common (AVLF personal observation). Despite their

prevalence in some habitats, very little is known about their ecology, behavior and natural history. For example, *Euptychia* is well-known for being one of the few butterflies to use Lycosida as larval host plants (Singer et al. 1971, Singer and Mallet 1986, DeVries 1987). However, although there are some images of immature stages available for *Euptychia westwoodi* A. Butler, 1867 (Hamm and Fordyce 2016, Janzen and Hallwachs 2018) and partial descriptions for four additional Costa Rican species (Singer et al. 1983, DeVries 1987), a detailed, complete description of the immature stages for any species of *Euptychia* is still lacking.

In the present paper, descriptions of the immature stages for *Euptychia mollina* (Hübner, [1813]) are presented. This is the first paper illustrating in detail all life stages for a species of *Euptychia* and also the first report on immature stages from a species outside of Costa Rica.

MATERIAL AND METHODS

A single egg was obtained through the observation of an oviposition event in the rural region of Belterra municipality, Pará State, Northern Brazil, on August 19, 2018 (2°35'47"S; 54°57'00"W). The oviposited plant was an unidentified *Selaginella* (Lycopsida: Selaginellaceae) that was brought to the laboratory and potted to be used to feed the larva. The egg was brought to

laboratory and, after hatching, the larva was reared in a plastic container cleaned daily and provided with fresh plant material (see above) every two or three days (following Freitas 2007). Data were recorded on morphology and development time for all stages. Dry head capsules and the pupal case were retained in glass vials. Voucher specimens of the immature stages and adults (the ovipositing female and the emerged adult) were deposited in the Museu de Zoologia "Adão José Cardoso" (ZUEC-AVLF), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Additional material was obtained from the Teles Pires River, Alta Floresta municipality, northern Mato Grosso State, Central Brazil, on June 20, 2000 ($9^{\circ}38'12''S$; $55^{\circ}56'24''W$). Several eggs and larvae were also collected in the field on fronds of *Selaginella* sp. along a forest edge following a dirt road running parallel to the river.

Measurements were taken and general aspects of morphology were observed using a Leica® MZ7.5 stereomicroscope equipped with a micrometric scale. Scanning electron microscopy (SEM) was conducted using a JEOL® JSM-5800 microscope, and samples were prepared in accordance with the following protocol: Sample critical point dried using Bal-tec® – CPD030 equipment and attached with double stick tape to aluminum stubs; gold/palladium coated with a Bal-tec® – SCD050 sputter coater. Egg size is presented as height and diameter, and head capsule size is the distance between the most external stigmata (as in Freitas 2007). Terminology for early stages descriptions follows García-Barros and Martín (1995) for eggs and Stehr (1987) for larvae and pupae.

RESULTS

The morphological description and measurements of the immature stages below are based on material from Belterra, Pará. Additional data from Teles Pires river, Alta Floresta, Mato Grosso, are included at the end of this section.

Egg (Figs 1, 2). Green, round with poorly marked irregular cells. Height and diameter 0.60 mm. Duration 7 days (n = 1).

First instar (Figs 3, 4, 17–19). Head capsule width 0.40 mm; head scoli 0.08 mm. Head black, bearing a pair of short scoli on vertex (Figs 18, 19). Third stema larger than other stigmata. Body light green with several small protuberances; caudal filaments short. Legs and prolegs light green. Setae light green; most dorsal and subdorsal setae clubbed at tip (see detail in Figs 17, 19). The chaetotaxy of the first instar is presented in Fig. 20. Maximum length 3 mm. Duration 8 days (n = 1).

Second instar (Figs 5, 6). Head capsule width 0.58 mm; head scoli 0.22 mm. Head black, with two short pointed scoli on vertex. Body green, with light green poorly marked longitudinal stripes and covered with small protuberances giving a rough appearance to the larva; caudal filaments short. Legs and prolegs light green. Maximum length 5 mm. Duration 5 days (n = 1).

Third instar (Figs 7, 8). Head capsule width 0.80 mm; head scoli 0.46 mm. Very similar to second instar in color and general shape. Maximum length 8 mm. Duration 7 days (n = 1).

Fourth instar (Figs 9, 10). Head capsule width 1.20 mm; head scoli 0.72 mm. Head black, with two diverging short pointed scoli on vertex. Body green, with several short dorsal and lateral white chalazae, with oblique dark/light markings converging dorsally; legs and prolegs green; caudal filaments short. Maximum length 13 mm. Duration 7 days (n = 1).

Fifth (last) instar (Figs 11, 12). Head capsule width 1.70 mm; head scoli 0.80 mm. Head green, bordered with light brown markings, with two diverging light brown short scoli on vertex and a frontal rounded bump just below the scoli. Body green, with several short dorsal and lateral white chalazae, with oblique dark/light markings converging dorsally; legs and prolegs green; caudal filaments short, green with a reddish tip. Maximum length 21 mm. Duration 10 days (n = 1).

Pupa (Figs 13–15). Short and smooth; mostly rusty brown, with short ocular caps; cremaster broad, dark in ventral portion; dorsal abdomen with a paired series of short subdorsal light brown protuberances and a conspicuous dorsal shelf on A4. Total length 7.5 mm. Duration 8 days (n = 1).

Additional rearing data. Measurements of the immature stages from Alta Floresta (Mato Grosso, Brazil): Egg: duration 6 days (n = 2), diameter 0.66–0.68 mm, height 0.66 mm (n = 2); first instar: Duration 3 days, maximum length 3.5 mm (n = 2), head width 0.40–0.42 mm (mean = 0.41 mm, SD = 0.009), scoli 0.08–0.10 mm (mean = 0.09 mm, SD = 0.010) (n = 8); second instar: Duration 5 days, maximum length 6 mm (n = 2), head width 0.52–0.58 mm (mean = 0.56 mm, SD = 0.023), scoli 0.22–0.30 mm (mean = 0.26 mm, SD = 0.025) (n = 9); third instar: Duration 9 days, maximum length 9 mm (n = 2), head width 0.78–0.86 mm (mean = 0.81 mm, SD = 0.026), scoli 0.44–0.54 mm (mean = 0.49 mm, SD = 0.033) (n = 8); fourth instar: Duration 14 days, maximum length 15 mm (n = 3), head width 1.10–1.20 mm (mean = 1.16 mm, SD = 0.040), scoli 0.56–0.72 mm (mean = 0.63 mm, SD = 0.062) (n = 7); fifth (last) instar: Duration 17 days, maximum length 19 mm (n = 3), head width 1.50–1.74 mm (mean = 1.63 mm, SD = 0.107), scoli 0.72–0.84 mm (mean = 0.78 mm, SD = 0.050) (n = 4); pupa: general color either mostly green or rusty brown; duration 55–56 days, length 10 mm (n = 2).

Behavior and natural history. *Euptychia mollina* (Fig. 16) is very common all year round in several habitats where its host plant *Selaginella* sp. occurs (Fig. 23), including forest gaps, sand forests with open canopy, secondary vegetation and forest edges (Figs 21–23). In some of these places, *E. mollina* can be locally abundant, such as in Alta Floresta, where dozens of individuals were observed flying in a 200 m transect. The eggs were placed singly on the under surface of the fronds of its host plant (*Selaginella* sp., see above). The larvae are solitary in all instars, and its color pattern and shape make it cryptic on fronds of *Selaginella* sp. Two Braconidae parasitoids (Hymenoptera) were obtained from two field-collected larvae from Mato Grosso in 2000. Adults have a low flight near the ground, rarely flying above 2 m. Males were not observed exhibiting territorial behavior.



Figure 1–16. Life stages of *Euptychia mollina*: (1–2) egg, lateral and dorsal; (3–4) first instar, lateral and dorsal; (5–6) second instar, lateral and dorsal; (7–8) third instar, lateral and dorsal; (9–10) fourth instar, lateral and dorsal; (11–12) fifth (last) instar, lateral and dorsal; (13–15) pupa, lateral, ventral and dorsal; (16) reared adult.

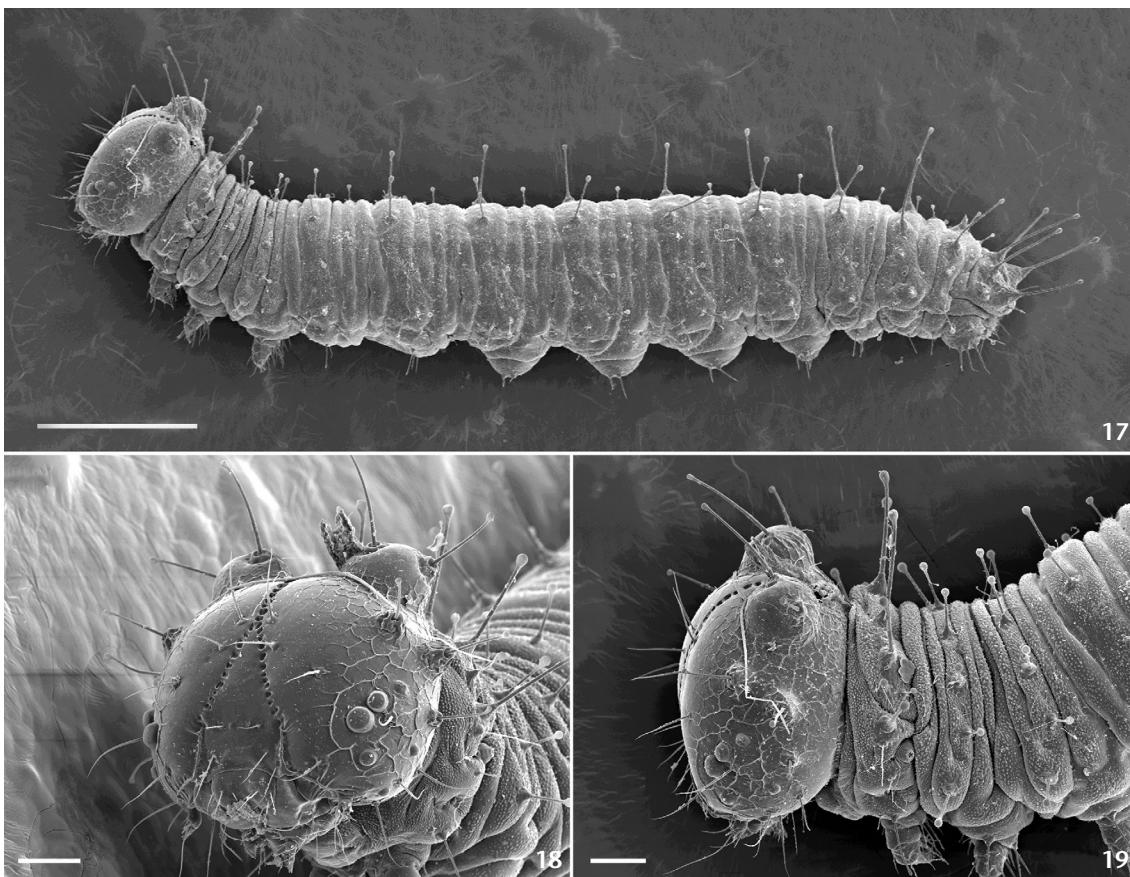


Figure 17–19. Scanning electron microscopy of first instar of *Euptychia mollina*. (17) general view; (18–19) frontal and lateral view of head, respectively. Scale bars: 17 = 0.5 mm, 18–19 = 0.1 mm.

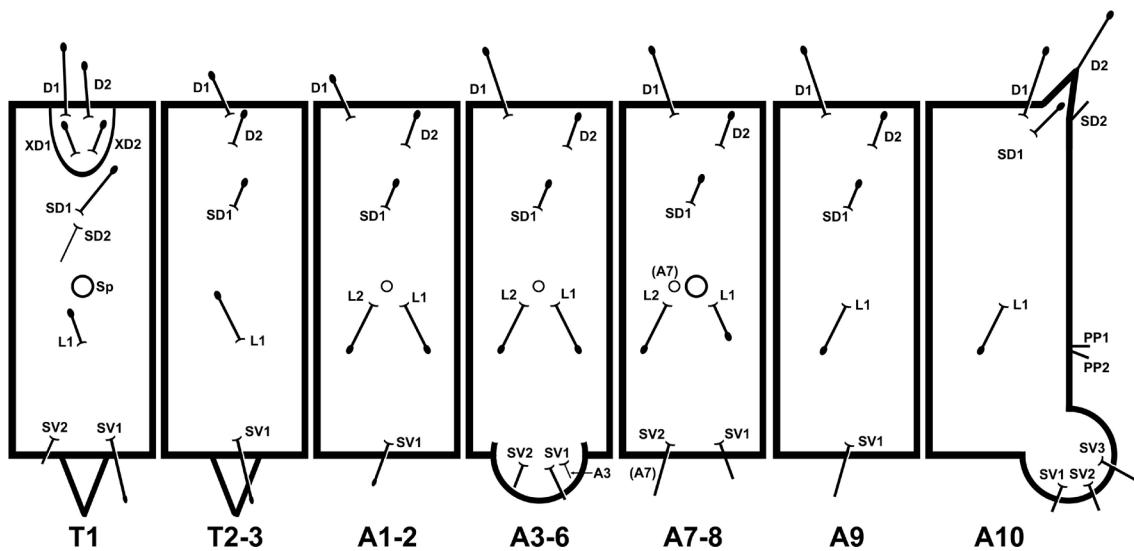
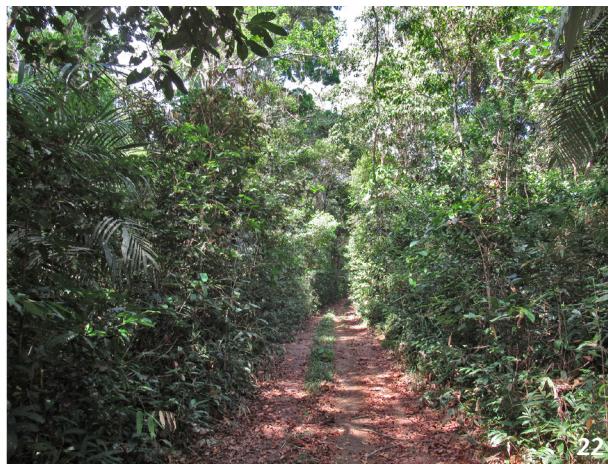


Figure 20. Body diagram showing larval chaetotaxy of first instar of *Euptychia mollina*. For chaetotaxy abbreviations see Stehr (1987) and Murray (2001).



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Figure 21–23. Habitat of *Euptychia mollina* in Belterra, Pará, Brazil. (21) general view of the forest showing tall trees and an open canopy; (22) view of the trail where the ovipositing female was observed; (23) view of the larval host plant, *Selaginella* sp.

DISCUSSION

As previously mentioned, *Euptychia* species are well-known for using non-monocots as larval host plants, mainly *Selaginella*, but also *Neckeropsis* mosses (Bryophyta: Neckeraceae) (Singer et al. 1971, Singer and Mallet 1986, DeVries 1987). The use of Lycopsida as host plants is known in only two other butterfly genera, coincidentally also in the tribe Satyrini, namely *Ragadia* Westwood, [1851] and *Acrophthalmia* C. and R. Felder, 1861 (Fukuda 1983, Igarashi and Fukuda 1996). Curiously, both *Ragadia* and *Acrophthalmia* are Paleotropical genera not related to each other (as previously suggested by Miller 1968 and Fukuda 1983) or to the Neotropical *Euptychia* (Wahlberg et al. 2009, Espeland et al. 2019), suggesting that the use of *Selaginella* as hostplants is a result of independent host shifts, as proposed for insects in general (Mound et al. 1994).

Nevertheless, despite the lack of a close phylogenetic relationship among *Euptychia*, *Ragadia* and *Acrophthalmia*, larvae in these three genera are surprisingly similar in shape and color pattern (Igarashi and Fukuda 1996 and this paper), although diverg-

ing in several other morphological characters. For example, while larvae of all known species of *Euptychia* present a pair of head scoli, larvae of both *Ragadia* and *Acrophthalmia* lack conspicuous head scoli (Igarashi and Fukuda 1996). The pupal shape is also quite distinct, with *Ragadia* and *Acrophthalmia* presenting pupae that are much more rounded and globose than those of *Euptychia* (Igarashi and Fukuda 1996). Given this scenario, independent evolution of camouflage explains the similar appearance of the caterpillars in all these cases, as previously suggested (Singer and Mallet 1986, DeVries 1987, Igarashi and Fukuda 1996), which results in a color pattern convergence among larvae that feed on *Selaginella*. Moreover, larvae of *Orsotriaena* Wallengren, 1858 and *Zipaetis* Hewitson, 1863, two genera phylogenetically close to *Ragadia* (Wahlberg et al. 2009, M. Espeland et al. unpublished data), feed on monocots and are quite distinct from *Ragadia* larvae (Sourakov and Emmel 2001, Kalesh and Valappil 2018, Saji and Kunte 2018).

As additional detailed descriptions of immature stages of Satyrini come to light, coupled with natural history observations,

it becomes clear that camouflage is possibly one of the major strategies of larvae in this group to escape from predators (e.g. DeVries 1987, Freitas 2002, 2017, Freitas et al. 2016).

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