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Volume 70, Number 4 271

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IMMATURE STAGES AND NATURAL HISTORY OF THE NEOTROPICAL SATYRINE PAREUPTYCHIA OCIRRHOE INTERJECTA (NYMPHALIDAE: EUPTYCHINA)

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ABSTRACT. The present paper describes the immature stages of the common Neotropical satyrine butterfly *Pareuptychia ocirrhoe interjecta* (R.F. d'Almeida, 1952). The solitary eggs are white and round, turning black 4 to 6 hours after oviposition. The four solitary larval instars are predominantly green and feed on grasses (Poaceae), including *Setaria* in nature and several other native and introduced species in captivity. The pupa is short and smooth and entirely green. Except for the black eggs, the immature stages are similar to those of other forest species of Euptychiina. The most remarkable and unique characteristic of *P. ocirrhoe interjecta* is the shiny black eggs, a possible synapomorphy for *Pareuptychia* not known in any other Euptychiina.

Additional key words: Atlantic Forest, Cepheuptychia, Godartiana, Taydebis, Zischkaia

The Euptychiina (Nymphalidae: Satyrinae) is one of the largest and most diverse butterfly subtribes, contributing a significant portion of Neotropical butterfly diversity (Peña et al. 2010, Marín et al. 2011). With over 400 recognized species (Lamas, 2004), Euptychiina butterflies occur in virtually all habitats and vegetation types from sea level to over 3500 m (DeVries 1987, Marín et al. 2011). However, despite this large species richness, immature stages have been described for a very few species and genera of Euptychiina (DeVries 1987, Murray 2001, Freitas et al. 2016). For the genus Pareuptychia, for example, immature stages have never been described in detail, and available information includes only textual descriptions in DeVries (1987) and pictures of larvae and pupae in Janzen and Hallwachs (2015).

Species of *Pareuptychia* are associated with forested habitats across the Neotropics (DeVries 1987), and they may be abundant. The genus is very homogeneous, and its eight described species (Nakahara et al. 2016) are very similar in wing pattern and morphology, suggesting that this is a monophyletic group (except maybe for *Pareuptychia lydia* (Cramer, 1777)). However, because of similarity in wing patterns, species limits and identities are not well established and the genus needs to be studied from both morphological and molecular perspectives (Marín et al. in prep.). In addition, knowledge about the immature stages of *Pareuptychia* could be of help in understanding the taxonomy of this group.

In this context, the present paper offers a detailed description of the immature stages of *Pareuptychia ocirrhoe interjecta* (R.F. d'Almeida, 1952) and compares them with those of other neotropical Euptychiina butterflies.

MATERIALS AND METHODS

Study sites. Adults and immatures of *P. ocirrhoe interjecta* were studied in four different localities in São Paulo State, Southeastern Brazil: 1) Reserva Biológica Municipal da Serra do Japi, Jundiaí (900–1100 m; 23°13'S, 46°57'W); 2) ARIE Mata de Santa Genebra, Campinas (600–620 m; 22°49'S, 47°6'W); 3) Fazenda Santa Elisa, IAC, Campinas (630-650 m; 22°51'S, 47°5'W); 4) Parque Estadual Xixová-Japuí, São Vicente (20–200 m; 23°59'S, 46°23'W).

Sampling and rearing of immature stages. Fertile eggs were obtained from wild-captured females confined in plastic bags warmed by a 40W bulb and provided with leaves of several species of native and introduced grasses. Larvae of P. ocirrhoe interjecta and of the additional species mentioned in the discussion section were reared in plastic containers cleaned daily, with fresh plant material provided every two or three days (following Freitas 2007). Data were recorded on behavior and development time for all stages. Dry head capsules and pupal cases were retained in glass vials. Immature stages were fixed in Kahle-Dietrich solution (Triplehorn & Johnson 2005) when the number of specimens was sufficient. Voucher specimens of the immature stages were deposited in the Museu de Zoologia "Adão José Cardoso" (ZUEC-AVLF), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Morphology. 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 (JEOL Ltd., Japan), and samples were critical-point dried in a Bal-tec® – CPD030 (Leica Microsystems, Germany), attached with double-sided

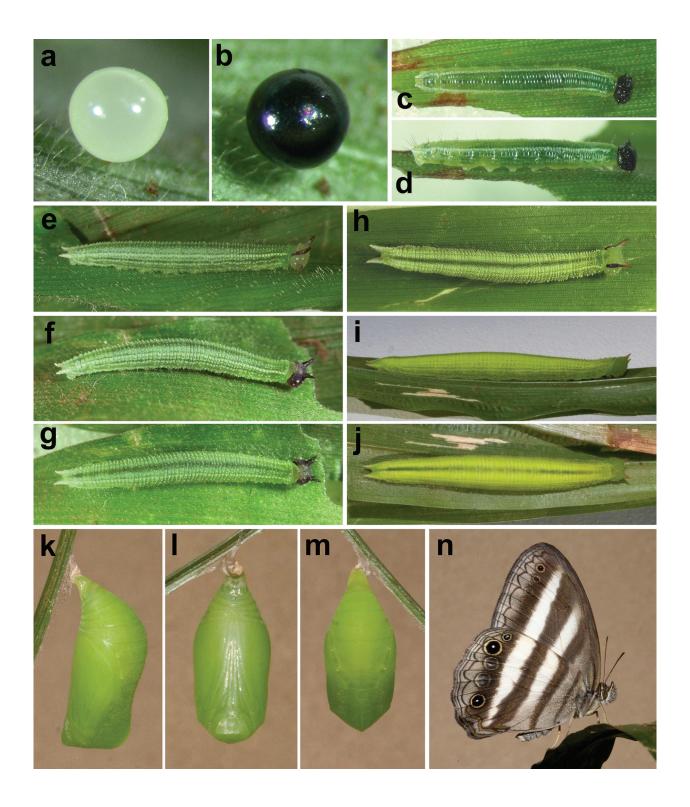


Fig. 1. Immature stages and adult of *Pareuptychia ocirrhoe interjecta*. **a, b.** egg, white and black; **c, d.** first instar, dorsal and lateral; **e.** light head capsule second instar, lateral; **f, g.** dark head capsule second instar, dorsal and lateral; **h.** third instar, dorsal; **i, j.** fourth (last) instar, lateral and dorsal; **k, l, m**. Pupa, lateral, ventral and dorsal; **n.** Adult male.

Volume 70, Number 4 273

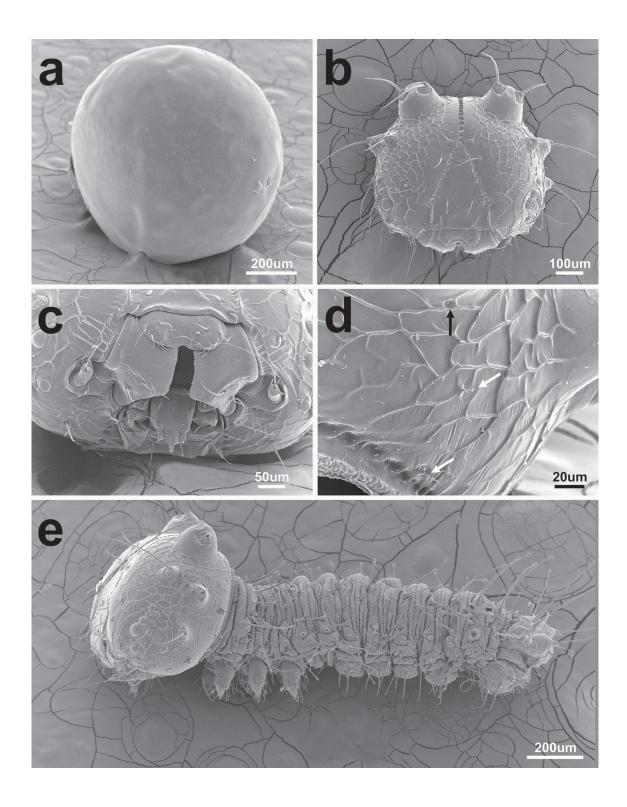


FIG. 2. Egg and first instar of *Pareuptychia ocirrhoe interjecta*. **a.** egg; **b.** Head capsule, general frontal view; **c.** Detail of frontal region; **d**. Detail of posterior region: white setae show microsetae and black seta show a pore; **e.** Early first instar, general lateral view.

tape to aluminum stubs, and coated with gold/palladium with a Bal-tec® – SCD050 sputter coater (Leica Microsystems, Germany). Egg size is presented as height and diameter, and head capsule size is the distance between the most external stemmata (as in Freitas 2007). Terminology for early stages descriptions followed García-Barros & Martín (1995) for eggs and Stehr (1987) for larvae and pupae.

RESULTS

Description of immature stages. The following descriptions and measurements are based on material reared from one female from Serra do Japi, Jundiaí, São Paulo (material reared from three other localities gave similar results).

Egg (Figs. 1a,b-2a). White, round, turning black 4 to 6 hours after oviposition. Height and diameter 0.94-0.96 mm; duration 5-6 days (n=8).

First instar (Figs. 1c,d - 2b-e). Head capsule width 0.64-0.70 mm (mean = 0.66 mm; SD = 0.026 mm; n = 5); head scoli 0.08-0.10 mm (mean = 0.09 mm; SD = 0.008 mm; n = 5). Head black, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow black setae. Third stemma larger than other stemmata. Body light green, with white longitudinal stripes, turning dark green after first meal; caudal filaments very short. Legs and prolegs light green. Setae light green from T1 to A7, black from A8 to A10, all dorsal and subdorsal clubbed at tip (Fig. 2e). Maximum length 5 mm. Duration 6-7 days (n = 8).

Second instar (Figs. 1e,f,g). Head capsule width $0.90-1.00~\mathrm{mm}$ (mean = $0.94~\mathrm{mm}$; SD = $0.052~\mathrm{mm}$; n = 5); head scoli $0.40-0.46~\mathrm{mm}$ (mean = $0.43~\mathrm{mm}$; SD = $0.022~\mathrm{mm}$; n = 5). Head green with two short dark brown scoli on vertex (Fig. 1e,g); in some individuals head is mostly dark brown (Figs. 1f,g). Body green, striped longitudinally with white; caudal filaments short. Legs and prolegs light green. Maximum length 9 mm. Duration $4-8~\mathrm{days}$ (n = 8).

Third instar (Fig. 1h). Head capsule width 1.38-1.46 mm (mean = 1.45 mm; SD = 0.056 mm; n = 5); head scoli 0.60-0.66 mm (mean = 0.62 mm; SD = 0.024 mm; n = 5). Head green, with two diverging very short scoli on vertex; these are reddish with a posterior dark brown line extending to head capsule. Body light green with a conspicuous dark green dorsal stripe and several additional longitudinal markings; caudal filaments short. Legs and prolegs light green. Maximum length 15 mm. Duration 7-9 days (n = 6).

Fourth (last) instar (Figs. 1i,j). Head capsule width 2.18-2.20 mm (mean = 2.20 mm; SD = 0.016 mm; n = 4); head scoli 0.74-0.82 mm (mean = 0.78 mm; SD = 0.034 mm; n = 4). Head green, with two diverging short scoli with reddish apex on vertex. Body light green with a conspicuous dark green dorsal stripe and several fine longitudinal stripes; caudal filaments short and reddish on apex. Legs and prolegs green. Maximum length 25 mm. Duration 7-10 days (n = 6).

Pupa (Figs. 1k,l,m). Short and smooth; entirely green; short rounded ocular caps; cremaster light green; dorsal abdomen smooth without projections. Total length 10.0-10.5 mm (n = 5). Duration 9-14 days (n = 4).

Behavior and natural history. *P. ocirrhoe interjecta* (Fig. 1n) is common in several environments in the Atlantic Forest from sea level to 1000 m altitude. Oviposition behavior was not observed in the field, but two solitary eggs, two larvae and one pupa were collected in the field in an unidentified native species of *Setaria* (Poaceae) in a shaded narrow trail inside the forest. Eggs were laid singly in the laboratory and larvae

easily accepted the same hostplant mentioned above. Larvae were solitary in all instars and did not exhibit cannibalistic behavior (several larvae of different instars were reared together in small pots). Adults were easily observed along forest edges and in clearings flying low in the understory, rarely rising above 2 m above the ground. Flight was erratic and fast and, when disturbed, butterflies performed unpredictable aerial maneuvers with alternating flashes of white and dark, which makes them difficult to capture in flight. Adults feed on fermenting fallen fruits, feces and several other decaying substances, being never observed visiting flowers. However, there are reports of adults feeding on extrafloral nectar (Barbosa 2013; AVLF pers. obs.).

DISCUSSION

The immature stages of P. ocirrhoe interjecta are quite morphologically simple and similar to those of several other species of Neotropical Euptychiina: larvae lack body scoli, present short head horns and caudal filaments and pupae are short and smooth. The entirely green last instar, although not rare in Euptychiina, is apparently uncommon; most known last instars of small forest Euptychiina are predominantly brown or have color tones that make them cryptic on the background of dead leaves and stems (Singer et al. 1983, DeVries 1987, Murray 2001, Kaminski & Freitas 2008, Janzen & Hallwachs 2015). Species with similar green last instars and/or pupae includes other species of Pareuptychia (AVLF unpublished; Janzen & Hallwachs 2015), Cepheuptychia cephus (Fabricius, 1775), Taydebis peculiaris (A. Butler, 1874), Zischkaia pacarus (Godart, [1824]), Godartiana muscosa (A. Butler, 1870) (Figure 3) and Forsterinaria pronophila (Freitas & Peña 2006). Although Cepheuptychia and Taydebis are genera related to Pareuptychia in part of the "Pareuptychia clade" (Peña et al. 2010), the similarity in color pattern is not related to phylogenetic relationships. For example, other forest species belonging to the "Pareuptychia clade" present predominantly non-green last instars, including Chloreuptychia arnaca (Fabricius, 1776), Megeuptychia antonoe (Cramer, 1775) (Janzen & Hallwachs 2015), Splendeuptychia furina (Hewitson, 1862) and Splendeuptychia doxes (Godart, [1824]) (Freitas, unpublished). The greenish last instars of Satyrotaygetis satyrina (H. Bates, 1865) are quite distinct from those of Pareuptychia, especially in the longer, strongly diverging head horns.

The most remarkable and unique characteristic of the immature stages of *P. ocirrhoe interjecta* is the shiny black eggs. This trait, present also in other species of *Pareuptychia* (DeVries 1987, AVLF unpublished), is unique among all known Euptychiina (however, color

Volume 70, Number 4 275

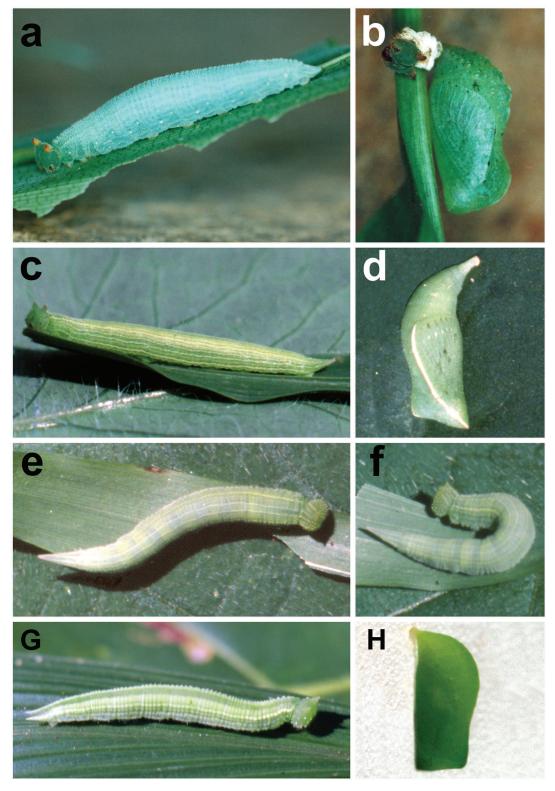


FIG. 3. Immature stages of Euptychiina (all from Brazil). **a, b.** *Cepheuptychia cephus*, fourth (last) instar and pupa (both lateral), Upper Juruá River, Marechal Thaumaturgo, Acre; **c, d.** *Taydebis peculiaris*, fourth (last) instar and pupa (both lateral), Santa Virgínia, São Luis do Paraitinga, São Paulo; **e, f.** *Zischkaia pacarus*, third instar, dorsal and lateral, Morro Grande, Cotia, São Paulo; **g, h.** *Godartiana muscosa*, fourth (last) instar and pupa (both lateral), Serra do Japi, Jundiaí, SP.

change in fertile eggs is not unique in Pareuptychia, and has been reported in Calisto Hübner, [1823] (Sourakov 1996)). Although the possible adaptive significance of this striking egg color is unknown, the similarity of these black eggs to the parasitized eggs of other species of Euptychiina is evident (Freitas et al. pers. obs.). Since it is widely known that visual cues are important for ovipositing butterflies (e.g. Rausher 1979, Williams & Gilbert 1981, Shapiro 1981, Freitas & Oliveira 1996, Sendoya et al. 2009), this opens the possibility that such cues are equally important for ovipositing females of egg parasitoids, which are known to show a low preference by black eggs (Lobdell et al. 2005). In this context, the black eggs of Pareuptychia could mimic parasitized eggs, thus reducing parasitoid oviposition and decreasing egg parasitism. Field and laboratory studies, however, are needed to test this hypothesis.

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