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IMMATURE STAGES OF THE NEOTROPICAL MISTLETOE BUTTERFLY *CUNIZZA HIRLANDA PLANASIA* FRUHSTORFER (PIERIDAE: ANTHOCHARIDINI)

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ABSTRACT. The immature stages of the Neotropical mistletoe butterfly *Cunizza hirlanda planasia* Fruhstorfer (Pieridae: Anthocharidini) are described and illustrated for the first time from cerrado areas of Central Brazil Plateau. Eggs are laid in small loose clusters (2–7 eggs) on new red leaves of the mistletoe *Passovia ovata* (Pohl ex DC.) Kuijt (Loranthaceae). Larvae are semi-gregarious and underwent five instars. Host plant use, morphology, and behavior of immature stages are similar to those of related genera in the *Hesperocharis* group of Anthocharidini.

Additional key words: Brazil, Cerrado savanna, *Hesperocharis* group, host plant, Loranthaceae

The Neotropical fauna of Pieridae is especially distinct when compared with other regions due to the relatively high species diversity and great generic/subgeneric endemism (Braby et al. 2006; Braby & Nishida 2007). Somewhat surprisingly, information on the natural history and early stages is still lacking for many species and genera (e.g. Courtney 1986; DeVries 1987; Llorente-Bousquets & Gerardino 2007; Braby & Nishida 2007, 2010; Freitas 2008). For example, little was known about the pierine clades Aporiina and the *Hesperocharis* group (Anthocharidini) and recent studies by Braby & Nishida (2007, 2010), led to a considerable progress in our knowledge of the natural history of these groups.

The *Hesperocharis* group is a well-supported monophyletic group composed of four Neotropical genera; *Eroessa* Doubleday, *Hesperocharis* Felder, *Mathania* Oberthür, and *Cunizza* Grote (see Braby et al. 2006; Braby & Trueman 2006). The genus *Cunizza* contains a single polytypic species *C. hirlanda* (Stoll) with nine subspecies, occurring from Costa Rica to Brazil (Lamas 2004; Braby & Nishida 2007). This is the least known genus in the clade, and the only information about immature stages is a brief note on oviposition behavior and host plant record by Romero & González (2009). Even though it has been suggested that *Cunizza* could use mistletoes (Loranthaceae) as host plant (see Salazar 2004; Braby & Nishida 2007), this was only recently confirmed by Romero & González (2009).

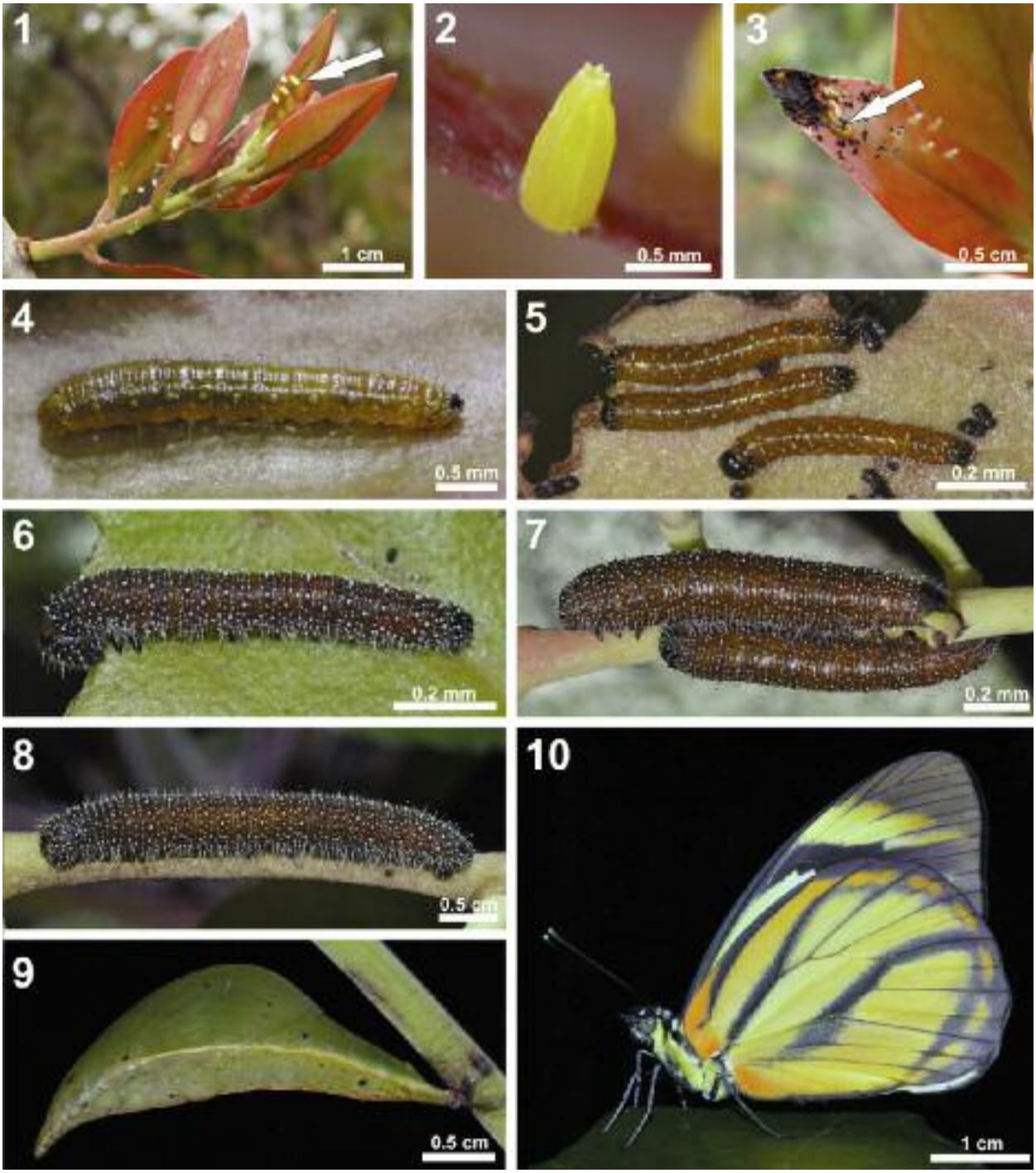
The subspecies *Cunizza hirlanda planasia* Fruhstorfer is regarded as one of the most typical butterflies of the Central Brazil Plateau, and has been recorded in several cerrado sites in altitudes from 600 to 1,350 m above sea level (Brown & Mielke 1967a, b; Emery et al. 2006, K. S. Brown Jr. unpub.). The present study describes the immature stages of *C. hirlanda planasia*, and provides a new host plant record plus general observations about natural history and behavior of this subspecies.

MATERIALS AND METHODS

Study sites. Collections were carried out in two sites of cerrado savanna in Distrito Federal (DF) and Goiás (GO) States, Central Brazil Plateau: (1) Fazenda Água Limpa (15°30'S, 47°25'W; 1000m a.s.l.), Brasília, DF; Parque Estadual da Serra dos Pireneus (15°47'S, 48°49'W; 1300m a.s.l.), Cocalzinho de Goiás, GO. In both sites the vegetation consists of a dense scrubland of shrubs and trees, classified as "cerrado *sensu stricto*" and "cerrado rupestre", respectively (see Oliveira-Filho & Ratter 2002).

Sampling and rearing of immature stages. Available mistletoe host-plants (Loranthaceae and

Viscaceae) in the study sites were visually scanned for the presence of immatures (as in Bodner et al. 2010). Plants with immatures (eggs and larvae) were collected for identification. The immatures of *C. hirlanda planasia* used for morphological description were collected in the field and reared as follows: eggs were placed in Petri dishes and observed daily until eclosion; newly hatched larvae were reared individually in transparent 500mL plastic pots under controlled conditions (25 ± 2○C; 12 h L: 12 h D). Branches of the



FIGS. 1–10. Life stages of *Cunizza hirlanda planasia* on *Passovia ovata*, from Brasília (1000m), Distrito Federal, central Brazil. **1,** loose cluster of eggs (arrow) on the new red leaf. **2,** egg in lateral view. **3,** first instar larvae on leaf, note the graze damage and accumulation of feces. **4,** first instar. **5,** second instar. **6,** third instar. **7,** fourth instar. **8,** fifth (last) instar. **9,** pupa. **10,** freshly emerged adult.

same host-plant on which each larva was found were offered *ad libitum*, and larvae were checked daily for food replacement and cleaning when necessary (following Barbosa et al. 2010). Immatures for morphological analysis were separated, fixed in Dietrich's solution, and then preserved in 70% ethanol. Shed head capsules were collected and preserved for measuring. Voucher specimens of the immature and adult stages were deposited at the Museu de Zoologia "Adão José Cardoso" (ZUEC), 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. Egg size is given as height and diameter. Head capsule width of larvae was considered to be the distance between the most external stemmata; maximum total length for both larvae and pupae corresponded to the distance from head to posterior margin of the tenth abdominal segment in dorsal view. Measurements are given as minimum–maximum values. Terminology for early stage descriptions follows Braby & Nishida (2007, 2010).

RESULTS

Description of immature stages. *Egg* (Figs 1–2). 1.16–1.20 mm high, 0.52–0.62 mm diameter (n = 5); yellow; bottle-shaped, with base flattened and similar in width to the middle portion of the egg; exochorion with about seven coarse longitudinal ribs, each terminating at micropylar end where they form small whitish protuberances, a series of finer transverse striae between longitudinal ribs. Duration ≥ 5 days (n = 10).

First instar (Figs 3–4). Maximum length 0.4 cm, head capsule width 0.38–0.42 mm (n = 9); pale orange head and body with numerous long dark brown setae inserted in sclerotized pinaculum, areas around the pinnacles are whitish; light brown prothoracic shield and dark brown anal shield. Duration 4 days (n = 10).

Second instar (Fig. 5). Maximum length 0.6 cm, head capsule width 0.62–0.74 mm (n = 7); black head; dark brown prothoracic and anal shields; orange body similar to first instar, but with primary setae smaller and arising from black conical protuberances, a few white secondary setae on each segment. Duration 4–5 days (n = 8).

Third instar (Fig. 6). Maximum length 1.0 cm, head capsule width 1.09–1.33 mm (n = 13); head dark brown, body orange-brown similar to last instar, but with white secondary setae shorter and less conspicuous. Duration 4–5 days (n = 8).

Fourth instar (Fig. 7). Maximum length 1.6 cm, head capsule width 1.72–2.03 mm (n = 13); general aspect similar to last instar. Duration 4–5 days (n = 8).

Fifth (last) instar (Fig. 8). Maximum length 3.8 cm, head capsule width 2.97–3.00 mm (n = 4); head orange, with numerous small white protuberances from which arise short white setae; body orange-brown, numerous small white protuberances on each segment from which arise short, somewhat flattened, white secondary setae bifurcated at apex; additionally, a series of larger, white conical protuberances from which arise short, spine-like white primary setae clubbed at apex; spiracles white. Duration 5–8 days (n = 11).

Pupa (Fig. 9). Maximum length 2.8 cm (n = 5); pale green, speckled with small black and white spots and a few pairs of black spots on mesothorax and abdominal segment 8; anterior region, with a rounded, slightly upturned and beak-like projection; head with whitish green eyes; wings convex ventrally, cases of forewing with a small black spot; a pale yellow, prominent lateral ridge extending from mesothorax to A10; a broad reddish mid-dorsal line extending from prothorax to A10 (including cremaster). Attached by cremaster, with silk girdle passing over A1. Duration 15–16 days (n = 2) and 101 days (n = 1).

Natural history of *Cunizza hirlanda planasia*. In the study sites several mistletoe species were checked for the presence of immatures, such as *Psittacanthus* Mart. spp., *Struthanthus* Mart. spp. (Loranthaceae), and *Phoradendron* Nutt. spp. (Viscaceae), however, eggs and larvae of *C. hirlanda planasia* were observed only on *Passovia ovata* (Pohl ex DC.) Kuijt (= *Phthirusa ovata* (Pohl ex DC.) Eichler) (Loranthaceae). Eggs were laid in small loose clusters (Figs 1–2), ranging from 2–7 eggs per cluster (n = 11 cohorts), on the upperside of new red leaf and/or petiole of the mistletoe food plant. After hatching, the newly eclosed larvae devoured most of the exochorion, before proceeding to graze the leaf surface. Early instars (first to third) are gregarious and usually covered by their pellets of excrement (Fig. 3). Like described for *Mathania* (Braby & Nishida 2007), this occurs due to the presence of numerous clear fluid droplets, to which the feces adhere, at the tips of the black forked setae. Late instars (fourth and fifth) may be found isolated, and they fed on the whole leaf. For immatures rearing at constant temperature between June–July (dry season) the development time from egg to adult lasted approximately five weeks. For immatures rearing in November (wet season) we observed pupal diapause, with one adult emerging 101 days after pupation.

DISCUSSION

The dates of collected and observed adults of *C. hirlanda planasia* (Fig. 10) in the Brazil Central Plateau indicate that this subspecies flies from late February to September, suggesting that it could be multivoltine (Brown & Mielke 1967b; E. O. Emery & K. S. Brown Jr. unpub.). Nevertheless, the long pupal period, around four months, strongly suggests that *C. hirlanda planasia* could undergo facultative diapauses during the wet season in cerrado, similar to what has been described for *Mathania* (Braby & Nishida 2007). The available data also suggest that this subspecies is highly associated with cerrado vegetation; even though *C. hirlanda planasia* was observed in gallery forests, most known records come from open cerrado areas, including all our rearing records (Brown & Mielke 1967b; E. O. Emery & K. S. Brown Jr. unpublished data). Additionally, males were observed exhibiting hilltopping behavior in Paracatu municipality (Minas Gerais State; 17°12'S,

46°54'W), a place where this species was frequently observed from February to May (K. S. Brown Jr. pers. comm.).

The use of the mistletoe *Passovia ovata* as host plant agrees with the observations of Romero & González (2009) of the subspecies *Cunizza hirlanda minturna* Fruhstorfer in Venezuela, raising two important points about the natural history of *Cunizza*. First, as discussed by Braby & Nishida (2007), *Cunizza* larvae are specialized on Loranthaceae. But more than that, our host plant records suggest that at least the subspecies *C. hirlanda planasia* is specialized on *Passovia* H. Karst. Although Romero & González (2009) have cited *C. hirlanda minturna* in *Phthirusa* sp., this host plant record must be confirmed due to recent taxonomic changes in these genera of mistletoes (see Kuijt 2011). Anyway, both genera of mistletoes are very close and have not been cited for any other mistletoe-feeding pierids (Braby & Nishida 2007, 2010). Second, larvae of *C. hirlanda planasia* are semi-gregarious or solitary, particularly in the late instars, similar to the situation described for *Mathania* (Braby & Nishida 2007).

According to Braby & Nishida (2007), *Hesperocharis* larvae are gregarious and have longer and more densely covered setae and protuberances on the body, while those of *Mathania* are semi-gregarious or solitary, presenting a smoother body surface. However, the observations made by Romero & González (2009) of the oviposition pattern of *Hesperocharis crocea idiotica* (Butler) suggest that this species, at least, does not show gregarious behavior. Thus, although knowledge of the natural history of Neotropical Pieridae has significantly increased over the last few years, much more information on their natural history is needed to enable understanding of patterns of host plant use and the evolution of gregarious behavior in this butterfly family.

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