Immature stages of *Rhinthon bajula* (Schaus, 1902) (Hesperiidae: Hesperiinae)

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Date of issue online: 18 October 2024
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.13920212

Abstract: The immature stages of the skipper butterfly *Rhinthon bajula* (Schaus, 1902) are described. The larval host plant in nature is *Canna paniculata* Ruiz & Pav. (Cannaceae). The egg is hemispherical and translucent lime green. To the naked eye, the body of the larva is smooth in all studied instars. The last instar has a plump body and is peppermint-green in color, with dorsal region whitish cream, patterned with several small circular markings. The head is dark brown with a pair of frontal white stripes. The pupa is elongated, without spines, with a long proboscis sheath, as long as the body, and is attached to the leaf by two silk girdles, one at the cremaster and the other at the thoracic region. The relationship between larval body shape and host plant use in Hesperiidae is discussed.

Key words: Atlantic Forest; Cannaceae; Hesperiini; Moncina; skipper.

Resumo: São descritos os estágios imaturos do hesperíideo *Rhinthon bajula* (Schaus, 1902). A planta hospedeira larval na natureza é o caité-do-brejo *Canna paniculata* Ruiz & Pav. (Cannaceae). O ovo é hemisférico, amarelo limão translúcido. A olho nu, o corpo da larva é liso em todos os instares. O último instar possui corpo rechonchudo e verde claro, a região dorsal é mais opaca esbranquiçada com diversas marcas circulares pequenas. A cabeça é marrom escura com um par de faixas brancas frontais. A pupa é alongada, sem espinhos, com a espirotromba com o comprimento do corpo, sendo atada à folha por dois cordões de seda, um no cremáster e outro passando pela região torácica. As relações entre a forma do corpo da larva e o uso de plantas em Hesperiidae são discutidas.

Palavras chave: Cannaceae, diabinha, Hesperiini, Mata Atlântica, Moncina.

INTRODUCTION

Skippers (Lepidoptera: Hesperiidae) comprise one of the most diverse groups of butterflies, with more than 4000 described species, with the center of diversity in the Neotropical region. Besides the remarkable diversity of the family, it is also notable for many aspects concerning its natural history being poorly studied. Moss (1949) was a pioneer in recording host plants and early stages of skippers from Pará, Brazil. Other studies describing detailed aspects of immature biology of Neotropical skippers have been published recently (Burns & Janzen, 1999, 2001; Cock, 2003, 2006, 2008, 2009; Greeney & Warren, 2003, 2004, 2009a,b; Bächtold *et al*., 2012, 2017; Moraes *et al*., 2012; Lepesqueur *et al.*, 2017; Freitas, 2018, 2020; Duerr *et al.*, 2022), but considering the richness of the family, knowledge of their early stages remains extremely scarce.

The genus *Rhinthon* Godman, 1900 belongs to the most species-rich subfamily, Hesperiinae, as part of the subtribe Moncina (Hesperiini) (Zhang *et al.*, 2022). The genus comprises seven recognized species distributed throughout the Neotropics (Mielke, 2005; Zhang *et al.*, 2022, 2023), with two species occurring in the Atlantic Forest, namely *Rhinthon bajula* (Schaus, 1902) and *Rhinthon andricus* (Mabille, 1895). *Rhinthon bajula* is not commonly observed and little is known about its behavior and bionomics. In a recent study based on molecular evidence, the genus *Rhinthon* was recovered as sister to *Cynea* Evans, 1955, and this clade is sister to a large clade composed of several genera, including *Tigasis* Godman, 1900, *Paracarystus* Godman, 1900, *Niconiades* Hübner, [1821], *Vettius* Godman, 1901 and *Thoon* Godman, 1900, among others (Zhang *et al.*, 2022).

Although the subtribe Moncina includes about 100 genera and more than 700 species, host plants and immature stages are known for only a tiny portion of the species in this subtribe. Therefore, this study contributes to filling this gap by describing the immature stages of *R. bajula* and providing general information about its natural history and behavior in constructing larval shelters.

MATERIALS AND METHODS

Adults and immatures of *Rhinthon bajula* were studied in the Serra de Mato Grosso, Saquarema municipality, Rio de Janeiro, Brazil (50 m), in November - December, 2023. All descriptions were made based on a sample of five eggs and two larvae obtained in nature. The region is covered by lowland

Atlantic Forest in the plains and submontane Atlantic Forest on the slopes of the nearby mountains. Eggs were obtained in the field and larvae were reared on potted plants of *Canna paniculata* Ruiz & Pav. (Cannaceae) by the first author. Young plants were collected in nature and planted in a sandy substrate in 1-liter plastic vases, which were kept inside a house. This method eliminates the necessity of relocating larvae due to either a shortage or drying of host plants. However, data on intermediate instars were lost during the rearing process and reliable information is available for the first and the two last instars (it is presumed that there are five instars based on data of other related taxa). Data were recorded on morphology and development time for all stages. Dry head capsules and pupal cases were retained in glass vials. Voucher specimens of the immature stages and reared adults were deposited in the Zoological Collection of the Museu de Diversidade Biológica da Unicamp (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Taxonomy is based on Mielke (2005), updated to reflect the latest changes suggested by Zhang *et al.* (2022).

RESULTS

Egg. Hemispherical, translucent lime green, diameter about 1.3 mm. A tiny black sub-central spot appears three days after oviposition, where head capsule starts to develop. Duration 5-6 days (n = 5).

First instar. Body completely smooth, tegument whitish translucent; after feeding, body becomes greenish; head capsule black and trapezoidal. Head and body tegument smooth, without scoli or projections; a black prothoracic plate dorsally on T1. Legs and prolegs pale.

Penultimate instar. Body completely smooth, tegument pale green translucent; head dark brown, trapezoidal, with a pair of rounded bumps on vertex. Legs and prolegs pale. Maximum body length 26 mm (n = 7).

Last instar. Body plump, abruptly decreasing in diameter in thoracic segments towards head, completely smooth, peppermint-green, translucent, dorsal region whitish cream, patterned with several small circular markings; head similar to previous instar but elongated, dark brown with a pair of frontal white stripes running from ocellar region to tip of bumps on vertex. Legs and prolegs pale. Head capsule width: 2.53-2.83 mm (mean = 2.722 mm, SD = 0.1018, n = 7), head capsule height 3.51-3.67 mm (mean = 3.625 mm, SD = 0.07933, n = 7); maximum body length 50 mm.

Pupa. General shape elongated, without spines; proboscis sheath same length as body. Attached to leaf by two silk girdles, one at cremaster and other at thoracic region. Inner shelter and pupa are covered by a white waxy dust. General color brown. Length: 25-28 mm (n = 7).

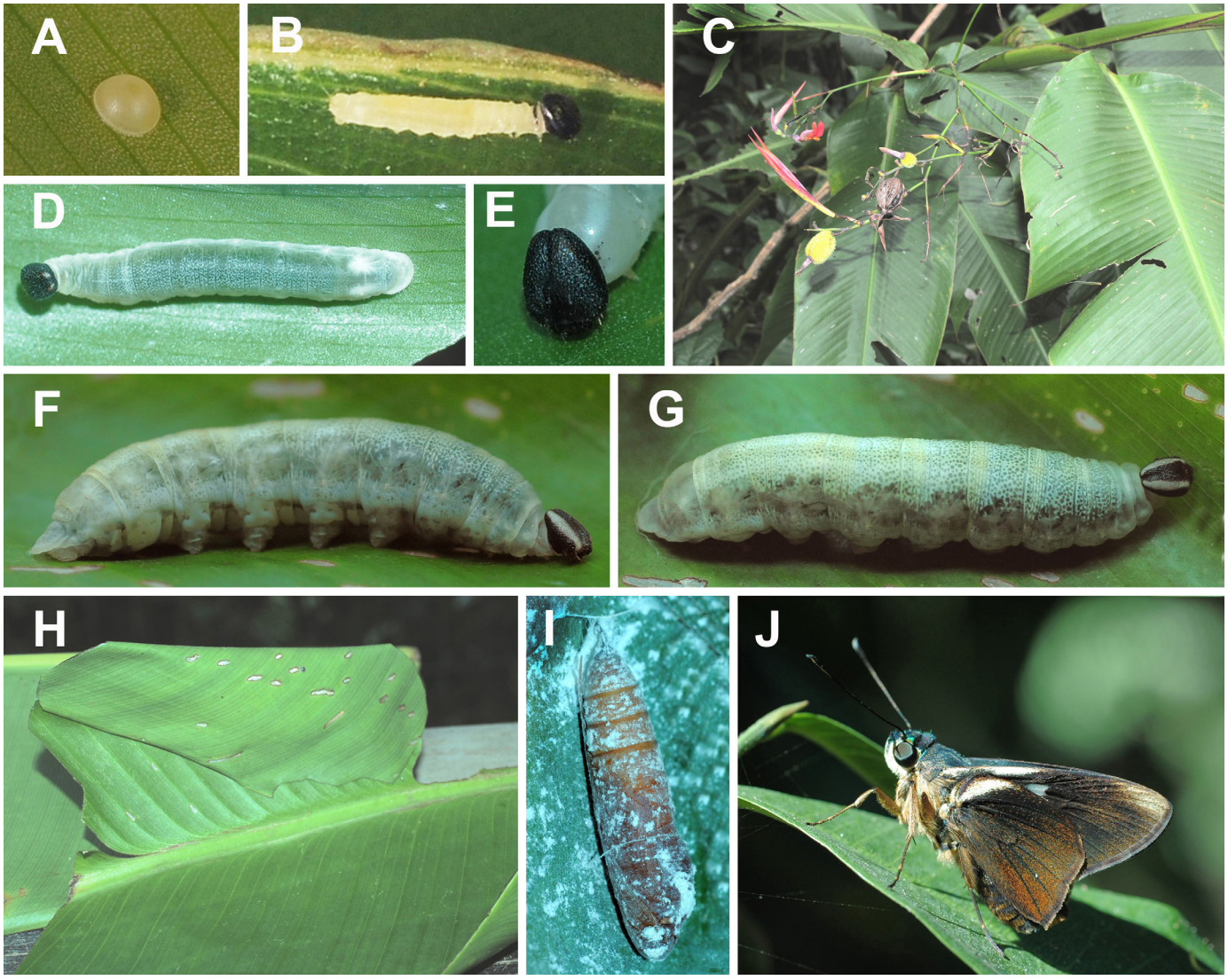

Figure 1. Life stages of *Rhinthon bajula* on *Canna paniculata* in Saquarema, Rio de Janeiro, Brazil. **A**. dorsolateral view of egg; **B**. early first instar, lateral; **C**. general view of the hostplant with flowers and fruits; **D, E**. penultimate instar, dorsal and frontal view of head; **F, G**. last instar, lateral and dorsal view, respectively; **H**. last instar shelter; **I**. pupa in lateral view; **J**. reared adult.

Behavior. Observations on oviposition were based on two separate females on two different days: egg-laying was observed around noon on plants located on the edge of a shaded trail inside the forest. The host plant in nature was identified as *Canna paniculata* Ruiz & Pav. (Cannaceae) (Fig. 1C). Although the host plant was locally common, ovipositions were observed on small plants (1-2 m tall). Female butterflies were observed flying near a group of larger plants, after which they landed on the lower leaves of the smaller plants. These females would take off again and land on the underside of the same leaf, where they laid a single egg. The egg was found to be laid around the middle point between the central vein and the leaf costa. In one instance, an isolated plant was found with a leaf containing two freshly hatched larvae and two eggs. Eggs were always laid on the underside of the leaves. After hatching, larvae ate the eggshell and moved near the leaf margin, aligning parallel to the edge where they started to eat and construct their shelter.

Shelter construction. In the first instar, the larvae positioned themselves parallel to the leaf margin and created a triangular indentation by making a transverse cut in the leaf. Then, they rolled the leaf edge using 4 to 6 silk stitches, folding the leaf and creating a small tunnel. Finally, the larvae hid under a leaf canopy that they made, remaining concealed (shelter Type 6, Group II of Greeney & Jones, 2003 and Type 4 of Greeney, 2009). The larva gradually ate the cut edge of the leaf, creating a triangular shape as it moved towards the central vein. At the same time, it attached silk to the leaf and folded it progressively. As the larva grew larger and passed through the instars, the eating process expanded the original triangular-shaped cut as it migrated down one side of the central vein along the leaf and toward the tip of the leaf. The canopy expanded as it kept accompanying the growing larva development. This methodical eating style resulted in a distinct triangular indentation that deepened as it approached the central vein. Once it reached the vein, the larva shifted its eating direction parallel to the vein and moved toward the leaf tip. In the final instar, the larva sometimes moved onto a fresh leaf and once there, the larva frequently cut halfway through the central vein at the new leaf stalk. This caused the leaf to droop down and slowly wilt over time. After the larva finished eating, it sealed both ends of the folded leaf and began to excrete a white powdery substance. After a few days, typically 2-3, the larva stopped feeding and started to pupate within the hanging and withering leaf shelter.

DISCUSSION

As previously stated, the most recent molecular phylogeny of Hesperiidae recovered *Rhinthon bajula* as a member of the subtribe Moncina, sister to *Rhinthon molion* (Godman, 1901), and the genus *Rhinthon* as sister to *Cynea* (Zhang *et al*., 2022). In fact, the immature stages of both genera are very similar, consistent with this relationship. For example, the head capsule with a pair of white stripes, the plump body abruptly decreasing in diameter towards the head, and the dorsal pattern of the last instar of *R. bajula*, are very similar to those of *Rhinthon cubana* (Herrich-Schäffer, 1865), *Rhinthon osca* (Plötz, 1882), *Rhinthon molion*, *Cynea irma* (Möschler, 1879) and also to *Cynea cannae* (Herrich-Schäffer, 1869) (Moss, 1949; Cock, 2006; Burns *et al.*, 2010; Álvarez *et al.*, 2020; Orlandin *et al.*, 2020). The pupae of both genera are also similar, with a very long proboscis, without cephalic projections, and with the production of white waxy dust (Cock, 2006; Burns *et al.*, 2010; Suênia-Bastos *et al.*, 2024). Compared to other genera of Moncina in the clade sister to *Rhinthon* + *Cynea* (see Introduction section and Zhang *et al.*, 2022), a similar plump body shape abruptly decreasing in diameter towards the head was reported only in *Vettius phyllus* (Cramer, 1777) and *Lychnuchus ponka* Evans, 1955 (Cock, 2006; Duerr *et al.*, 2022); in *Niconiades xanthaphes* Hübner, [1821], however, the larval body shape is elongated and slim, just slightly wider than the head (Cock, 2003).

It is worth noting that all plump bodies of the above listed species are associated with the use of host plants with large and soft leaves in the families Cannaceae, Heliconiaceae, Marantaceae and Zingiberaceae, a pattern previously reported for other species of Hesperiidae (Freitas, 2018, 2020). The only species using a Poaceae, *Lychnuchus ponka*, feeds on species of *Pariana* Aubl. (Poaceae: Bambusoideae: Olyreae), a plant with large and soft leaflets compared to other bamboo species (Duerr *et al.*, 2022). Curiously, *N. xanthapes*, with a slim and elongated larval body, uses *Olyra latifolia* L. (Poaceae: Bambusoideae: Olyreae), a plant with large leaflets (very similar to species of *Pariana*) (Cock, 2003).

Previous studies with Hesperiidae suggested that plump body shapes are linked to larvae feeding on plants with large and soft leaves, a pattern reported in the subtribes Thymelicina and Pericharina (see Freitas, 2018, 2020). The present study found the same pattern in a different subtribe (Moncina), reinforcing the hypothesis that leaf traits could be a key factor explaining body shape in larvae of Hesperiidae. So, we strongly encourage the descriptions of immature stages of Hesperiidae so that these data could be used in future studies mapping the evolution of body shape and host plant use in this butterfly group.

ACKNOWLEDGMENTS

To Shinichi Nakahara and and Bernard Hermier for revising and improving the submitted version of the manuscript. To Ingrid Koch who identified the host plant. AVLF thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (fellowship 304291/2020-0), and the the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (grant 2021/03868-8). RRS thanks the FAPESP (grant 2020/09595-0). The present study is registered at the SISGEN (AF3D250).

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