Immature stages of the Neotropical skipper *Saliana longirostris* (Sepp, [1840]) (Lepidoptera: Hesperiidae)

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Abstract: The early stages of the hesperiid butterfly *Saliana longirostris* (Sepp) are described. The host plant in nature is *Heliconia velloziana* (Heliconiaceae). The egg is hemispherical, pale pink, and has more than 60 vertical ribs. The body of the larva is smooth in all five of its instars. The last instar has a plump body and is salmon-pink in color, variegated with many circular dark markings, and the head is rusty brown, ellipsoidal, and with a pair of rounded bumps on the vertex. The pupa is elongated, without spines, bearing a long, thin, anteriorly-directed projection on the head and a long proboscis sheath longer than body. The immature stages are similar to those of other Calpodina, and the plump body of the later instars is similar to those of other skipper species that feed on host plants with large and soft leaves. The relationship between larval body shape and host plant use in Calpodina is discussed.

Key words: Atlantic Forest, Calpodini, Heliconiaceae, Hesperiinae

Resumo: Os estágios imaturos da borboleta *Saliana longirostris* (Sepp) (Hesperiidae) são descritos. A planta de alimento na natureza é *Heliconia velloziana* (Heliconiaceae). O ovo é hemisférico, rosado, com mais de 60 carenas verticais. A lagarta é lisa e passa por cinco instares. O último instar possui corpo rechonchudo, completamente liso, é rosado e pintalgado com marcas circulares escuras, a cabeça é marrom ferrugem, elipsoide, com um par de protuberâncias no vertex. A pupa é alongada, sem escolos, apresenta um prolongamento fino apontando para a frente na cabeça e a espirotromba mais longa que o corpo. Os estágios imaturos são similares aos da maioria dos Calpodina, com o corpo rechonchudo dos últimos instares similar ao daquelas espécies que se usam plantas hospedeiras com folhas largas e macias. A relação entre forma do corpo e planta hospedeira Calpodina é discutida.

Palavras chave: Calpodina, Heliconiaceae, Hesperiinae, Mata Atlântica

INTRODUCTION

The family Hesperiidae is the second largest among the butterflies in terms of species diversity, with more than 4000 described species in all continents. At the same time, many aspects of hesperiid biology remain poorly known, ranging from systematics to natural history, including knowledge of larval host plants and descriptions of the immature stages (Warren, 2000; Warren *et al.*, 2008). For the Neotropical species, despite the notable work of Moss (1949) and many images available on the internet (including Janzen & Hallwachs, 2018), information for host plants and immature stages are still scarce considering the taxonomic richness of the family (but see 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 and references therein).

The genus *Saliana* Evans, 1955 (Hesperiidae, Hesperiinae, Hesperiini, Calpodina) is composed of 20 species of medium-sized skippers distributed in several vegetation types from Mexico to Argentina (Evans, 1955; Mielke, 2004, 2005; Warren *et al.*, 2016). Species of *Saliana* represent a common component of most Neotropical habitats, but nevertheless there are very few data on immature stages and host plants (Genty *et al.* 1978; Brown, 1992; Cock, 2003; Beccaloni *et al.*, 2008; Janzen & Hallwachs, 2018). Larvae of *Saliana longirostris* (Sepp, [1840]) were previously illustrated by Moss (1949) and Cock (2003), but no data on the eggs, early instar larvae or pupae were provided. In addition, Moss's early stage material appears to represent two different species (see comments in Cock, 2003). Accordingly, to contribute to knowledge of the biology of Hesperiidae in general and *Saliana* in particular, the present paper describes in detail the immature stages of *S. longirostris* (Fig. 1) from southeastern Brazil.

STUDY SITE AND METHODS

A single egg was collected in "Fazenda Acaraú", a private forest reserve in Bertioga municipality, coastal São Paulo State (23°45'S, 46°2'W; 10-100 m), southeastern Brazil. The region is covered by lowland rain forest and sand forest (locally known as "restinga") (Ururahy *et al.*, 1987) with an annual rainfall

of over 3,000 mm and average annual temperature of 22.4 °C (Nimer, 1972). The larva was reared at room temperature (around 25°C and 60% humidity) in a 1000 ml plastic container with pieces of mature leaves of *Heliconia velloziana* L. Emygd (Heliconiaceae) collected in the field where the egg was found, and were offered *ad libitum*. The larva was checked daily to replace the food and to clean its container. Head capsules were collected and kept for measurements. The duration of instars and pupal development were recorded. Measurements were taken and general aspects of morphology were observed using a stereomicroscope equipped with a micrometric scale. Egg size is recorded as height and diameter. The total length of the larva and pupa were measured in dorsal view. The larval head capsule width is the distance between the most external ocelli (as in Freitas, 2018). Color patterns of immature stages *in vivo* were photographed with a digital camera. Head capsules, larval and pupal skins and the reared adult are deposited in the entomological collection of the Universidade Estadual de Campinas (Museu de Zoologia "Adão José Cardoso", ZUEC). The terminology for descriptions of early stages follows Bächtold *et al.* (2017). The host plant was identified following Lorenzi & Souza (2001) and the butterfly species was identified by comparing the adult voucher with specimens deposited at the ZUEC and with those figured in Warren *et al.* (2016).

RESULTS

Natural history

A single egg was obtained from a wild, ovipositing female. Oviposition was observed on 10 September 2005, a sunny day, at 14:00, when the temperature was above 30°C. After touching several broad leaves, while flying fast, the female landed near the edge of the upper surface of an old leaf of *Heliconia velloziana*, laying a single pale pink egg near a dry portion of the leaf (Fig. 1A). The first instar built a simple shelter by rolling a small portion of the leaf margin either onto the upper or lower surface of the leaf, forming a loose, bent shelter (Fig. 1M). In later instars and for pupation (see below), shelters were built by rolling most of or the entire leaf fragment into a tube. Frass ejection was not observed, although this behavior cannot be supposed to be absent. Pupation occurred inside a non-cut shelter consisting of the rolled apical portion of a mature leaf with the aid of two strengthened strands of silk (Fig. 1K, L). Due to potential artifacts resulting from rearing the larva in plastic pots with pieces of the host plant, shelter-building behavior could have been affected (see Greeney & Warren, 2009b). The duration from egg hatching to adult emergence was 56 days.

Description of immature stages (n=1)

Egg (Fig. 1B). Hemispherical, pale pink, with many irregular vertical ribs (more than 60); transverse ribs not visible under light microscopy; height 1.5 mm, diameter 1.1 mm. Duration: 8 days.

First instar (Fig. 1C, D). Body completely smooth, vivid red; head capsule brown, ellipsoidal, with a pair of rounded bumps on vertex. Head and body tegument smooth, without pronounced scoli or projections (possible presence and form of lenticles (Franz *et al.* 1984) could not be verified due to limited available study material for this instar); a black prothoracic plate dorsally on T1. Legs and prolegs red. Head capsule width: 0.9 mm, height 1.0 mm; maximum body length 8 mm. Duration: 5 days.

Second instar (Fig. 1E). Body completely smooth, pale reddish brown; head pale brown, ellipsoidal, with a pair of rounded bumps on vertex. Legs and prolegs pale brown. Head capsule width: 1.28 mm, height 1.36 mm; maximum body length 12 mm. Duration: 6 days.

Third instar (Fig. 1F). Body plump, completely smooth, dark reddish brown with a pair of subdorsal pale markings on A8, tracheal system visible forming a lateral white line connecting spiracles; head dark brown, ellipsoidal, with a pair of rounded bumps on vertex. Legs and prolegs reddish brown. Head capsule width: 1.86 mm, height 2.00 mm; maximum body length 23 mm. Duration: 4 days.

Fourth instar (Fig. 1G). Body plump, abruptly decreasing in diameter in thoracic segments towards head, completely smooth, dark reddish brown with a pair of subdorsal pale markings on A8, tracheal system visible forming a lateral white line connecting spiracles; head rusty brown, ellipsoidal, with a pair of rounded bumps on vertex. Legs pale brown, prolegs beige. Head capsule width: 2.77 mm, height 2.96 mm; maximum body length 29 mm. Duration: 6 days.

Fifth (last) instar (Figs. 1H-J). Early fifth instar (Fig. 1H) very similar to previous instar. Late fifth instar (Fig. 1I) body salmon-pink, variegated with many circular dark markings, tracheal system barely visible branching from each spiracle; head rusty brown. Legs pale red, prolegs beige. Presence or absence of ventral wax glands and precise form of mandibles were not recorded. Pre-pupa (Fig. 1J) whitish with rusty brown head. Head capsule width: 4.08 mm, height 4.52 mm; maximum body length 55 mm. Duration: 13 days.

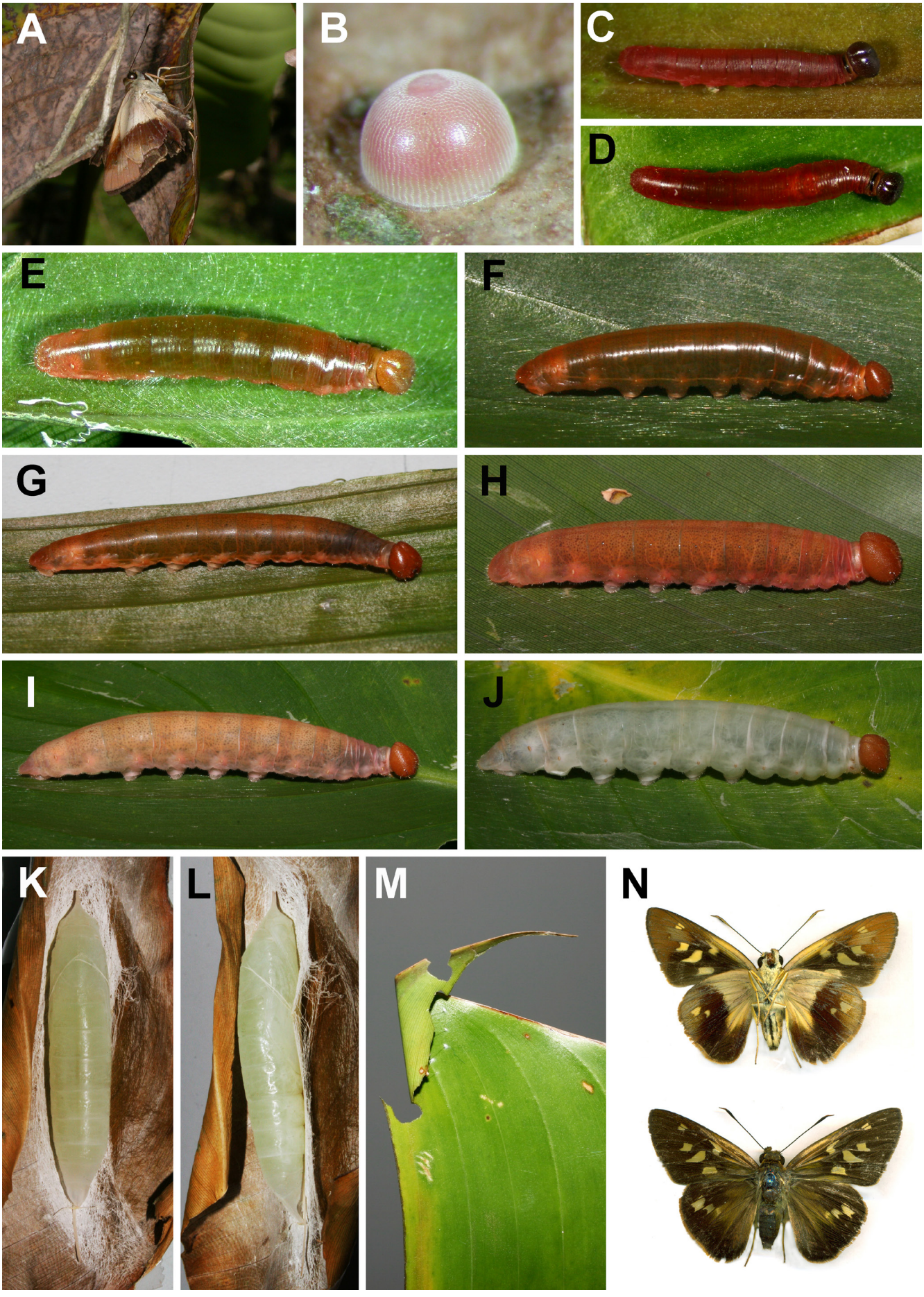
Pupa (Fig. 1K, L). General shape elongated, without spines, head bearing a long, thin, anteriorly-directed projection; proboscis sheath 7mm longer than body. Presence of setae on cremaster was not recorded. Attached to leaf by two silk girdles, one at cremaster and other at thoracic region. General color translucent white. Length 40 mm from head to cremaster (47 mm including proboscis length). Duration: 13 days.

DISCUSSION

The status and definition of the tribe Calpodini (*sensu* Evans, 1955) have been re-evaluated as a result of recent studies based on molecular evidence (Warren *et al.*, 2009; Sahoo *et al.*, 2016; Cong *et al.* 2019); former Calpodini are now distributed in at least four different clades (the subtribe Calpodina, two clades of the subtribe Carystina and in the tribe Pericharini, see Cong. *et al.* 2019 and Li *et al.* 2019). However, the taxonomic sampling is still low and the monophyly and the relationships among the putative Calpodina genera and their sister groups are still undefined (Warren *et al.*, 2009; Sahoo *et al.*, 2016; Cong *et al.* 2019).

In a recent paper on the immature stages of *Lychnuchoides ozias* (Hewitson, 1878) (Hesperiidae), a hypothesis about the evolution of hesperiid larval body shape was proposed based on the leaf traits of larval host plants (Freitas, 2018). Briefly, larvae with elongated and slim bodies were observed in species feeding on plants with slender and tough leaves and leaflets (such as Arecaceae and Poaceae), while plump bodies were found in species feeding on plants with large and soft leaves (such as Heliconiaceae, Musaceae and Marantaceae). Although

Fig. 1 (p. 83, facing page). Life stages of *Saliana longirostris* on *Heliconia velloziana* in "Fazenda Acaraú", Bertioga, São Paulo, Brazil. **A**, ovipositing female; **B**, lateral view of egg; **C-D**, early (C) and late (D) first instar, both dorsal; **E**, second instar, dorsal; **F**, third instar, lateral; **G**, fourth instar, lateral; **H**, early fifth (last) instar, lateral; **I,** late fifth (last) instar, lateral; **J**, prepupa, lateral; **K-L**, pupa in dorsal and lateral views, respectively; **M**, first instar shelter; **N**, adult reared female (ventral above, dorsal below).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this original discussion was proposed for the Calpodini (*sensu* Evans, 1955), the idea can be expanded to all Hesperiinae. The last instars of *S. longirostris* clearly fits this second group by feeding on Heliconiaceae and presenting a plump body, with its width at least twice the height of the head, abruptly decreasing in diameter in the thoracic segments towards the head (Fig. 1G-J).

While the present data agree with the above pattern, assuming this association between morphology and host plant traits to be a rule is premature considering the limited amount of data on Hesperiinae immature stages. Nevertheless, the plump body shape looks relatively stable in the genus *Saliana*. For example, besides occasionally feeding on leaflets of oil palm (*Elaeis guineensis* Jacq.) (Arecaceae) (Genty *et al.*, 1978), larvae of *Saliana severus* (Mabille, 1895) have the plump body shape present in larvae of 10 other species of *Saliana* (Janzen & Hallwachs, 2018; Cock, 2003; present study). Conversely, larvae of *Calpodes ethlius* (Stoll, 1782) are relatively slim, although feeding primarily on host plants with large and soft leaves (such as Cannaceae, Heliconiaceae, Musaceae and Marantaceae) (McAuslane, 2000; Cock, 2003; Beccaloni *et al.*, 2008; Janzen & Hallwachs, 2018). *Calpodes* Hübner, [1819] and *Saliana* are closely related genera (Sahoo *et al.*, 2016; Li *et al.*, 2019), and Zhang *et al.* (2019) suggested that they should be lumped into a single genus, *Calpodes*. The observed differences in larvae are suggestive of rather different ecological traits and could be seen as support for retaining both genera.

Finally, whether the plump body shape is a synapomorphy acquired after the switch from host plants with slender/tough to large/soft leaves or *vice versa* is an open question. Considering only the Calpodina as defined by Cong *et al.* (2019) (including four genera, namely *Calpodes*, *Saliana*, *Panoquina* Hemming, 1934, and *Zenis* Godman, 1900) and closely related groups, the use of host plants with slender (and usually tough) leaves and leaflets is more widespread and present in the putative outgroups of *Calpodes* + *Saliana*, suggesting it could be the ancestral state. Accordingly, the use of host plants with large and soft leaves could be considered a novelty of the clade *Calpodes* + *Saliana*, suggesting that the plump body shape was acquired after the host plant shift. This could be a working hypothesis to be tested in other clades where larvae feeding on large and soft leaves present similar plump body shapes (such as *Lychnuchoides* Godman, 1901, *Talides* Hübner, [1819] and *Tisias* Godman, 1901) (Freitas, 2018 and references therein). However, the absence of detailed life histories for several species and the open taxonomic issues in the group prevent a more thorough mapping of the evolution of body shape and host plant use at the present time.

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