Iheringia
Série Zoologia

e-ISSN 1678-4766
www.scielo.br/isz

Museu de Ciências Naturais

Article

Immature stages of *Nisoniades macarius* (Hesperiidae: Pyrginae: Carcharodini): biology, morphology and behavior

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Received 5 June 2021
Accepted 1 October 2021
Published 1 November 2021
DOI 10.1590/1678-4766e2021027

ABSTRACT. The biology, morphology and behavior of the immature stages of *Nisoniades macarius* (Herrich-Schäffer, 1870) are herein described from specimens collected in an anthropized area of Caatinga biome. The larva of *N. macarius* passes through six larval instars with the development time from egg to adult lasted between 31 and 35 days. Except for the first instar larva, which is easily recognized by the presence of evident primary setae on the head, there are few distinctive morphological characters among the larvae of different instars. The head capsule width proved to be the most effective character to differentiate them. Two different types of shelters are built by larvae: center-cut shelters (type 3) built by larvae from the first to the third instars and two-cut shelters (type 5) built by larvae from the fourth to the sixth instars. *Ipomoea asarifolia* (Desr.) Roem. & Schult. (Convolvulaceae) is recorded as larval host plant of *N. macarius* for the first time. A Eulophidae wasp was found as parasitoid of the earlier larval instars of *N. macarius*, this being the second record of the family as parasitoid in the genus *Nisoniades*.

KEYWORDS. Caatinga, host plant, *Ipomoea asarifolia*, parasitoid, skippers.

RESUMO. Estágios imaturos de *Nisoniades macarius* (Hesperiidae: Pyrginae: Carcharodini): biologia, morfologia e comportamento. A biologia, morfologia e comportamento dos estágios imaturos de *Nisoniades macarius* (Herrich-Schäffer, 1870) são aqui descritos a partir de espécimes coletados em uma área antropizada do bioma Caatinga. A larva de *N. macarius* passa por seis ínstares larvais com tempo de desenvolvimento variando entre 31 e 35 dias. Exceto o primeiro, que é facilmente reconhecido pela presença de cerdas primárias evidentes na cabeça, há pouca variação morfológica entre os instares larvais. A medida da cápsula cefálica mostrou-se mais eficaz na diferenciação dos ínstares. Dois tipos de abrigo são construídos pelas larvas: abrigo de corte central (tipo 3) construído pelas larvas de primeiro a terceiro ínstares e abrigo de dois cortes (tipo 5) pelas larvas de quarto a sexto ínstares. *Ipomoea asarifolia* (Desr.) Roem. & Schult. (Convolvulaceae) é registrada como planta hospedeira da larva de *N. macarius* pela primeira vez. Uma vespa Eulophidae foi encontrada como parasitoide dos primeiros ínstares larvais de *N. macarius*, sendo este o segundo registro da família como parasitoide no gênero *Nisoniades.*

PALAVRAS-CHAVE. Caatinga, planta hospedeira, *Ipomoea asarifolia*, parasitoide, hesperídeos.

The Carcharodini (Hesperiidae, Pyrginae) consists of 35 genera of skipper butterflies, most of which are restricted to the New World (Warren *et al*., 2009; Cong *et al*., 2019; Li *et al*., 2019; Zhang *et al*., 2020). The natural history of members of this tribe has been little explored, with complete descriptions of the immature stages available only for Old World species (Cock, 2016; Zhang *et al*., 2020). In the Neotropical region, the information available on the immature stages of Carcharodini is restricted to aspects of the morphology of some larval or pupal stages, records of host plants and parasitoids (Cock, 1991; Beccaloni, 2008; Janzen & Hallwachs, 2009).

Nisoniades Hübner, [1819] is the second largest genus of Carcharodini, with 33 species of wide distribution, occurring from South Texas to southern South America (Mielke, 2005). Although adults of most species of *Nisoniades* are abundant and easily collected, knowledge on their natural history is still scarce. For example, *Nisoniades macarius* (Herrich-Schäffer, 1870), the object of this study, has only a superficial description of its last larval instar and pupa (Jörgensen,1934). In addition, few authors have made references to host plants of *N. macarius*, although none of which have identified them to species level (Jörgensen, 1934; Moss, 1949; Beccaloni *et al*., 2008).

Therefore, the aim of this study is to describe more comprehensively the aspects of the biology, morphology and behavior of the immature stages of *Nisoniades macarius*, contributing to the knowledge of the natural history of the Neotropical skippers.

MATERIAL AND METHODS

Study site. The immature stages of *N. macarius* were collected at the *Centro de Saúde e Tecnologia Rural* of the *Universidade Federal de Campina Grande* (CSTR/UFCG), municipality of Patos, Paraíba state, Brazil (07°03’29”S, 37°16'36"W). The area of study is situated in the Caatinga biome, with semi-arid climate and xerophilous vegetation (Prado, 2003). The collections were made in an anthropized open area, containing native and exotic herbaceous plants (Fig. 1).

Collection and rearing of immatures. *Nisoniades macarius* eggs and larvae were collected from *Ipomoea asarifolia* (Desr.) Roem. & Schult. (Convolvulaceae) (Fig. 2), through non-systematic collections in the dry season, from July to October 2019. The leaves containing the immatures were detached and transported in plastic containers to the laboratory. To avoid dehydration, the leaf petiole was wrapped in moist paper. In the laboratory, the immatures were reared in separate, transparent plastic containers (500 ml), with the bottom lined with absorbent paper. The rearing was made in room temperature (around 27° and 30°C).

The immatures were observed daily to check the hatching of eggs, ecdysis, pupae formation, emergence of adults, shelter building, feeding behavior and presence of parasitoids. After the daily observations, the containers were cleaned and the leaves were replaced. The head capsule of each larval instar was collected and preserved for measurement. The emerging adults were prepared and deposited at the *Coleção do Laboratório de Ecologia e Interação de Insetos da Caatinga* (CLEIIC), CSTR/UFCG.

Figs 1, 2. Collection area of the immatures of *Nisoniades macarius* (Herrich-Schäffer, 1870) (Fig. 1); the host plant of *N. macarius*, *Ipomoea asarifolia* (Desr.) Roem. & Schult. (Fig. 2).

Morphological study. The morphological study and measurements of the immature were performed with the aid of a stereomicroscope with a coupled micrometric scale. The photographs were taken with a digital camera. Larval body size measurements were taken immediately after ecdysis, considering the length from the head to the distal end of the abdomen. For the measurement of the head capsule, the widest portion was considered. The color description of the immature was made from live specimens, in order to avoid changes in characteristics. Two specimens of each larval instar, pupa and egg were preserved for the study of morphology. For this purpose, the larvae were killed in hot water, fixed in Dietrich's solution for a period of 24 hours and subsequently preserved in 70% alcohol. The pupa and the egg were killed in 70% alcohol and permanently fixed in Dietrich. The parasitoids found were killed and preserved in 70% alcohol and the photograph of the adult sent to specialists for the identification. All preserved larvae, pupae, egg and parasitoids have been deposited at CLEIIC. The morphological terminology of the immatures is based on Stehr (1987) and the shelter nomenclature on Greeney (2009).

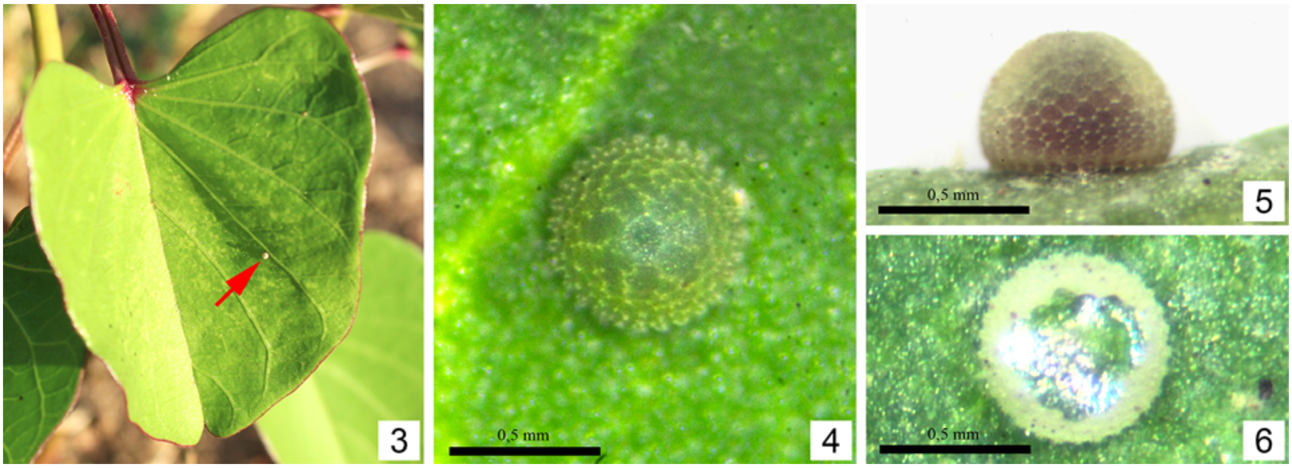
RESULTS

A total of 71 immatures of *Nisoniades macarius* were collected in *I. asarifolia*, distributed as follows: 17 eggs, 19 larvae of the first instar, 14 larvae of the second instar, eight larvae of the third instar, 11 larvae of the fourth instar and two larvae of the fifth instar. No pupae or larvae of the sixth instar were encountered in the field.

From all collected immatures, only 24 reached adult stage, 28 larvae died due to unknown causes in various instars, six were killed for storage and 13 were parasitized. Only six eggs were reared until adult stage. The development time from egg to adult lasted between 31 and 35 days, however, this time may be a little longer since it was not possible to collect eggs from the exact moment of the oviposition.

Description of immature stages. Egg (Figs 3-6): laid at adaxial portion of the leaves (Fig. 3); white to greenish (Fig. 4), darkening before hatching (Fig. 5), dome shaped; surface sculpted with hexagonal spaces that become less marked around micropylar area; area around micropyle smooth, convex and rounded; inner basal surface of the egg silvery (visible after hatching) (Fig. 6). Egg measurements: height 0.5 mm, width 0.5 mm (n=1).

First instar (Figs 7, 8): head shining black, smooth, heart shaped, with conspicuous whitish primary setae. Body narrower than the head, orange after hatching, becoming yellowish after feeding, with a greenish median longitudinal band, which results from the visualization of the gut contents through the translucent cuticle. Prothorax with black, continuous and rectangular dorsal plate. Head capsule width: 0.40–0.52 mm (n= 9). Body size: 2–3 mm (n=34). Development time: 3–8 days (n=9).



Figs 3–6. Egg of *Nisoniades macarius* (Herrich-Schäffer, 1870): 3, egg in the adaxial portion of the *Ipomoea asarifolia* (Desr.) Roem. & Schult. leaf; 4, egg in dorsal view; 5, egg in lateral view close to hatching; 6, egg after hatching.

Second instar (Figs 9, 10): head shining black, smooth, heart shaped, with numerous small, whitish secondary setae. Body shape as described for first instar. Prothorax as described for first instar but dorsal plate more developed. Head capsule width: 0.6–0.64 mm (n=12). Body size: 3.5–5 mm (n=30). Development time: 3–5 days (n=12).

Third instar (Figs 11, 12): head black, slightly rough, heart shaped, with small, whitish setae. Body yellowish, wider in the middle portion and wider than the head, with the presence of small white spots on the base of barely noticeable setae. Prothorax as described for previous instars but dorsal black plate moderately disconnected. Head capsule width: 0.85–1 mm (n=15). Body size: 4–6 mm (n=23). Development time: 2–5 days (n=18).

Fourth instar (Figs 13, 14): head as described for third instar but rougher. Body as described for third instar but narrower in relation to the head and, when viewed from side, with a longitudinal white stripe represented by the branching of the tracheal system (seen through cuticle transparency) (Fig. 14). Head capsule width: 1.27–1.55 mm (n=15). Body size: 7–9 mm (n=25). Development time: 2–6 days (n=20).

Fifth instar (Figs 15, 16): head reddish brown, extremely rough, heart shaped, with white and little evident setae. Body similar to fourth instar, except tracheal system more evident in lateral view. Prothorax with narrow, dark brown dorsal plate. Head capsule width: 1.92–2.12 mm (n=13). Body size: 9.5–15 mm (n=27). Development time: 3–6 days (n=27).

Sixth instar (Figs 17–20): head as described for fifth instar. Body fusiform, visibly wider than the head, cuticle apparently less translucent than in the previous instars, making the tracheal system less evident in lateral view. Pre-pupa robust, initially whitish, becoming darkened when close to pupa formation (Figs 19, 20). Head capsule not measured due to rupture of ecdysial line prior to pupal formation. Body size: 13–22 mm (n=34). Development time: 5–9 days (n=23).

Pupa (Figs 21–24): shining brown with yellowish aspect in the ventral surface and between the segments of the abdomen; proboscis reaching the distal portion of the wings in ventral view. Body robust and elongated, tapered towards the cremaster, with scattered short setae and almost entirely covered with a grayish mass, less abundant in the dorsal portion of the thorax. Prothorax with a pair of rounded lateral structures similar to "eyes", which consist of a central black portion surrounded by a yellow border (Fig. 24). Size: 13–15 mm (n = 6). Development time: 6–9 days (n=24).

Biology of *Nisoniades macarius*. We have only observed an adult female of *N. macarius* near the area where immatures were found, thus no oviposition behavior was recorded (Fig. 25). However, it was observed that the eggs are deposited alone in the adaxial portion of the leaf and rarely have two eggs been found on the same leaf, as well as more than one egg on different leaves of the same plant.

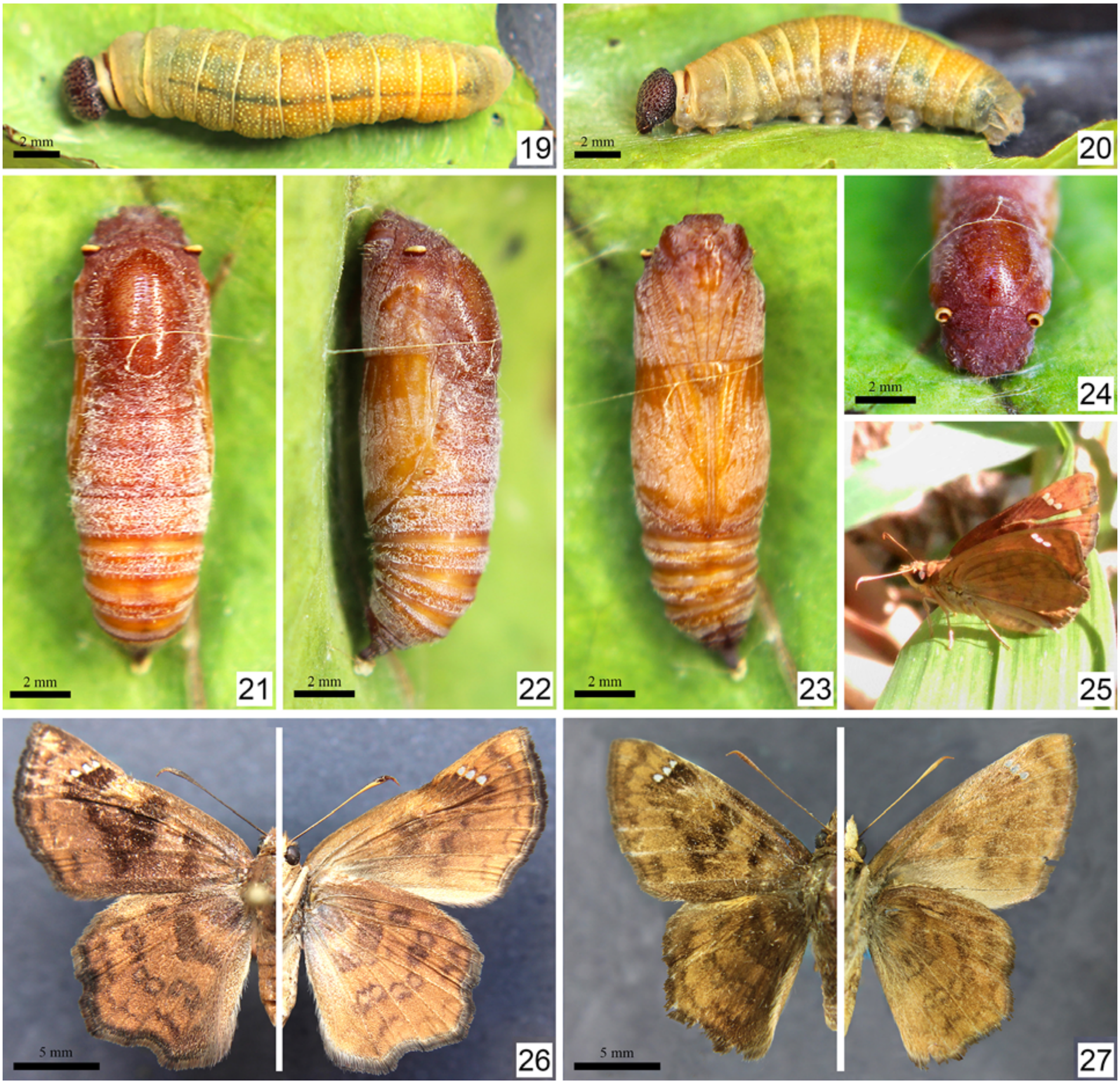
After eclosion, the first instar larva consumes a significant portion of the upper part of the chorion and then begins to build its first shelter, usually close to the hatch site. This shelter, called Type 3 or center-cut shelters by Greeney (2009), is built from a circular or oval cut of the limbus and the subsequent folding of the cut portion towards the adaxial surface of the leaf. Shelters of this type were built by larvae from the first to the third instars (Figs 28, 29). Subsequent larval instars, on the other hand, built Type 5 or two-cut shelters (Greeney, 2009). These are characterized by two rounded cuts at the leaf margins and the subsequent folding of the cut-out portion towards the surface of the limbus (Figs 30, 31).

Nisoniades macarius changed its larval feeding behavior throughout its ontogeny. In the first instar, the larva feeds through scraping, from the adaxial surface, leaving only the transparent cuticle on the opposite side of the leaf. Approximately between the final stage of the first and third instar, the larvae completely consume the limbus, leaving holes in different portions of the leaf. In the subsequent instars, the larvae start to feed from the leaf margins and consume the limb almost entirely.

Figs 7–18. Immature stages of *Nisoniades macarius* (Herrich-Schäffer, 1870) in dorsal (left) and lateral (right) views: 7, 8, first instar larva; 9, 10, second instar larva; 11, 12, third instar larva; 13, 14, fourth instar larva; 15, 16, fifth instar larva; 17, 18, sixth instar larva.

The immatures of *N. macarius* reared in this study that reached adult stage, formed their pupae in the adaxial portion of the leaf, in which they were attached through the cremaster and a silk belt wrapped around the thorax with the ends connected to the leaf substrate in both sides of the pupa (Figs 21–23).

***Nisoniades macarius* and the parasitoid**. From all collected larvae (n=71), 13 of first and third instars were parasitized with one or two larvae of a species of Eulophidae (Hymenoptera) (Figs 32–36). The parasitized larvae remained visibly paralyzed, while they were almost entirely consumed, except for the head capsule. Some pupae of the same parasitoid were also found in the field, inside the shelters of the larvae of *N. macarius*. Next to these pupae, head capsules of first and third instar larvae were found, suggesting the preference of the parasitoid for the initial larval stages of *N. macarius*. The development of the parasitoid from larva to adult was of the eight days.

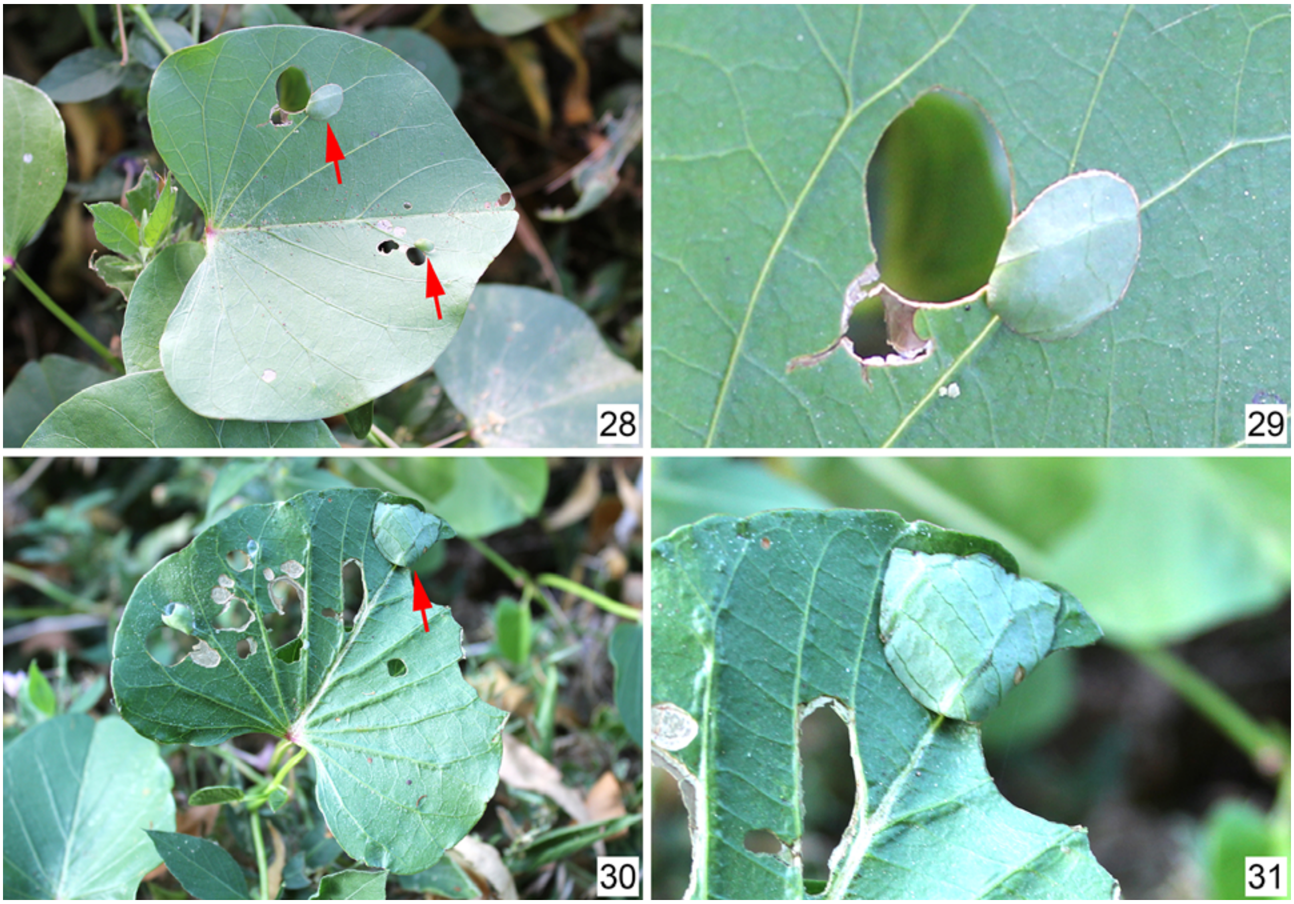


Figs 19–27. Immatures and adults of *Nisoniades macarius* (Herrich-Schäffer, 1870). Pre-pupa: 19, dorsal view; 20, lateral view. Pupa: 21, dorsal view; 22, lateral view; 23, ventral view; 24, frontal view. Fig. 25, adult female resting on grass leaf close to the host plant. Dorsal (left) and ventral (right) views of the adults raised in the laboratory: 26, female; 27, male.

DISCUSSION

In this study, larvae of *Nisoniades macarius* pass through six larval instars during their development. Although aspects of the biology of ten other species of *Nisoniades* have been recorded, no studies mention the number of larval instars, so it is not possible to compare them (Moss, 1949; Kendal, 1976; Janzen & Hallwachs, 2009). It is known, however, that the number of larval stages is quite variable between the genera of Pyrginae, occurring between five and eight instars (Greeney & Warren, 2003, 2004; Lepesqueur *et al.*, 2017).

As in most species of Hesperiidae, *Nisoniades macarius* has little morphological variation between larval instars, except for the first, which is easily recognized by the presence of relatively long and evident primary setae on the head. Thus, the differentiation between them is made mainly through body size and head capsule width (Bächtold *et al.*, 2012). This last measure proved to be extremely effective, since, although there is a certain variation in the width of the head capsule, the measurements for each instar have intervals that do not overlap with the measurements of previous or subsequent instars.



Figs 28–31. Types of shelters built by the larva of *Nisoniades macarius* (Herrich-Schäffer, 1870) in *Ipomoea asarifolia* (Desr.) Roem. & Schult: 28, 29, Type 3 shelter, or center-cut shelters; 30, 31, Type 5 shelter, or two-cut shelters. The red arrows point to the shelter's location.

Figs 32–36. Parasitoid of *Nisoniades macarius* (Herrich-Schäffer, 1870) larva: 32, parasitized first instar larva; 33, parasitized third instar larva. Pupa of the parasitoid: 34, ventral view; 35, dorsal view. Fig. 36, parasitoid in adult form. The red arrows point to the parasitoid larvae.

The construction of shelters by Hesperiidae larvae has long been documented (Weiss *et al.*, 2003) and this behavior is directly related to the protection of the larvae, increasing their survival rate (Baer & Marquis, 2021). The types of shelters vary between genera and between larval instars of the same species (Greeney & Jones, 2003). Two types of shelters were produced by the larvae of *Nisoniades macarius*: Type 3 and 5 (Greeney, 2009). These same types of shelter were registered for *Nisoniades godma* Evans, 1953 by Kendal (1976) and illustrated for *Nisoniades castolus* (Hewitson, 1878), *Nisoniades rubescens* (Möschler, 1877) and *Nisoniades pie* Steinhauser, 1989 by Janzen & Hallwachs (2009). This suggests that shelters of the Type 3 and 5 (Greeney, 2009) are probably shared by all members of *Nisoniades*, independent of the host plant used, although a detailed description for each species is necessary to identify distinctive interspecific aspects of their larval constructions.

In general, *Nisoniades* is a polyphagous genus, with larvae registered feeding on different genera and species of host plant belonging to the Convolvulaceae, Solanaceae, Asteraceae and Melastomataceae (Beccaloni *et al*., 2008; Janzen & Hallwachs, 2009). For *Nisoniades macarius*, two genera of host plants are recorded in the literature: *Solanum* (Solanaceae) (Jörgensen, 1934) and *Ipomoea* (Convolvulaceae) (Moss, 1949). In the present study, the larvae of *N. macarius* were found in *I. asarifolia* being a new host plant record for *Nisoniades*.

Parasitoids may regulate the population density of their host insects (Stireman & Singer, 2003). Janzen & Hallwachs (2009) mention a large number of parasites found in larvae, eggs and pupae of *Nisoniades*, including dipterans, nematodes, fungi and hymenopterans. From Hymenoptera, members of Braconidae are more frequently found as parasitoids of *Nisoniades*. However, the only parasitoid found by us for *Nisoniades macarius* was a Eulophidae wasp, this being the second record of this family as parasitoid of *Nisoniades* (see Janzen & Hallwachs 2009).

Acknowledgments. We thank to Dr. Diego Rodrigo Dolibaina for the valuable suggestions to the manuscript. We thank to Dr. Fernando César Vieira Zanella and Dr. André Luis Martins for parasitoid identification. We thank to Dr. Maria de Fátima de Araújo for host plant identification and Ariano Oliveira Lemos, member of the *Laboratório de Ecologia e Interações de Insetos da Caatinga*, helped in the beginning of larva rearing. Finally, we thanks to Dr. Lucas A. Kaminski and another anonymous reviewer for the valuable comments that have improved the quality of the manuscript.

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