Life cycle and ethological notes on *Burca braco braco* (Hesperiidae: Pyrginae).

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Abstract: On this study, we describe for the first time the immature stages of *Burca* species: eggs, five larval instars, prepupa, and pupa. Morfometrics means of each stage are given. According with the measures of head width, is possible the presence of non-typical individuals with more than five instars. We describe four larval shelters types, and it can be associated to particular instars. Larvae typical construct three or four shelters during larval development. We also report notes on feeding patterns and oviposition.

Keywords: *Burca*, *Croton*, Hesperiidae, skippers, larval shelters, immature stages.

Introduction

The genus *Burca* (Hesperiidae: Pyrginae: Carcharodini) was described by Bell & Comstock (1948). These butterflies are dark brown to blackish brown with the forewing rounded along the outer margin and the hindwing margin angular. Male secondary characters are variable, and palpal coloration is a useful character for species determination (Smith *et al*., 1994). *Burca* is closely related to *Nisoniades*, *Noctuana* and *Staphyllus* (Warren *et al*., 2008). It´s represented by five species endemic to Cuba, Bahamas and Hispaniola where the genus is restricted (Bell & Comstock, 1948; Smith *et al*., 1994).

On the knowledge on natural history on *Burca* species we count only with some data on geographic distribution and nectar sources (Alayo & Hernández, 1987; Smith *et al*., 1994); and recently were published the first records of host plants. *Burca b. braco* caterpillars feed on *C. lucidus* (Euphorbiaceae) (Núñez, 2001) and *C. glabellus* (Lauranzón *et al.*, 2013), while *Burca c. concolor* uses *C. sagreanus* and *C. origanifolius* (Fernández, 2004). However, immature stages and their behavior remain unknown. Knowledge on natural history in lepidopterans is necessary for ecological studies (Young, 1972).

In this paper we describe *Burca b. braco* immature stages and provide ethological notes related to larval shelters construction, oviposition behavior, and additional nectar sources.

Materials and Methods

Field observations and immature specimens were conducted on Piedra Alta (23^{0}10´ N and 81^{0}59´ W) and Boca de Canasí (23^{0}09´ N, 81^{0}46´ W). Both places are close to Cuba´s north coastline and are respectively located at 45 and 65 km E from Havana City. At Piedra Alta grows a characteristic dry coastal scrubland, while a microphyllous evergreen forest predominates at Boca de Canasí, according to Capote & Berazaín (1984), being *Croton lucidus* abundant in both places. Field observations were taken on 09:00 and 16:00 hours.

One hundred and fifty-eight eggs were taken directly from the host plant, 39 from September to November 2000, and 119 from March 2006 to April 2007. Each egg was placed alone in a Petri plates. We measured the perpendicular base diameters ($\mathbf{d_1}$ and $\mathbf{d_2}$) and height (**h**) with an ocular micrometer coupled to a stereoscope (error=50 µm). For description of the vertical ridges array of the egg, ridges were classified in three categories: complete ridges (**CR**), with one extreme on base and the other on micropyle, incomplete ridges with one extreme on basis and the other interrupted (**IRB**), and incomplete ridges with one extreme on micropyle and the other interrupted (**IRM**).

Ninety-seven caterpillars were maintained in captivity, 54 of them born from collected eggs and the others collected as larvae in nature. Larvae were provided with fresh *C. lucidus* leaves and cleaned every day to remove fecal pellets, leftovers and head capsules. Head width (**HW**) was measured for every larva and in every instar, while body length (**L**) was measured only for first and second instars. Both measurements were taken with the micrometer mentioned above. For chaetotaxy of first instar, three individuals were sacrificed and observed in microscope. We follow Hinton (1946)'s and Stehr (1987) terminology. Descriptions of larval shelters were referred to collection time in nature, after Greeney (2009).

Pupae were obtained from larvae reared in captivity. They were maintained in a 5 liters container to assure that adults could extend their wings after emergence. We measured the major thoraxic width of pupa in dorsal view (**MTW**) and the body length of pupa (**LP**) from vertex to cremaster. Both measurements were taken with a slide gauge (error=0.05 mm). We measured also the weight of pupa with a scale (error=0.001 g).

Results

Life cycle and description of immature stages

Eggs: Are light green immediately laid, then change to light gray or light orange. Infertile eggs are recognizable because they are white transparent. Vertical ridges are white. Eggs are vertical with flattened base and micropyle (Fig. 1a). Base diameters are very similar and larger than height (Table 1), so egg form is hemispherical. There is a large variation in number of ridges, and also in the three categories CR, IRB and IRM (Table 2). Egg duration was recorded in two cases: 5 and 6 days.

We couldn´t reach any larva from the first to the last instar. However, we determine that *Burca b. braco* larvae have five instars according to all measurements of HW (Fig 2). Head is rounded, robust, heart shaped and wider than prothorax in all instars (Figs. 1b, c, d, f, g). The head major width is placed around the mid region in frontal view. Body tapering and slim in the first instar. In subsequent instars the body becomes larger and robust, changing proportions between head and body.

First instar (Fig. 1b): At birth L=2.31 ± 0.27 mm (min 1.85, max 3.00 mm, N=49). HW=0.70 ± 0.05 mm (min 0.60, max 0.80 mm, N=56). The body is yellowish white and the head is bright black. Prothorax is dorsally reddish brown and ventrolaterally light red. Mouth pieces are beige. First pair of legs are light red while second and third pair of legs and prolegs with the same coloration of body. Tarsi of legs are black. Primary setae are tiny, hair like or Y shape (Fig. 3). We couldn´t feed any individual in captivity and we didn't note any changes in coloration. However, we collected in nature two individuals with dull green bodies and slightly larger: 3.50 and 4.25 mm, respectively.

Second instar (Fig. 1c): Similar to the two individuals mentioned above but its coloration is darker. There are no changes in coloration of head and prothorax. L=3.97 ± 0.49 mm (min 3.40, max 4.25, N=3). HW=1.10 ± 0.05 mm (min 1.00, max 1.15 mm, N=12).

Third instar (Fig. 1d, e, f): Body is olive green with the last three abdominal segments dark orange. Prothorax is orange. The head is black with two lines of tiny white elliptical spots, rarely absent (Fig1 e, f). There are two slight white longitudinal lines at both sides of dorsal region from mesothorax at the end of the body. HW=1.58 ± 0.09 mm (min 1.40, max 1.65 mm, N=9).

Fourth instar (Fig. 1g, h): Head with a great number of elliptical or sub-rectangular white spots in vertex, and sometimes reaches dorsally the epicranial notch and ventrally the genae. These spots are arrayed in five to seven lines. Internal spot lines run parallel to ecdysial cleavage line, while most externals are perpendiculars to frontoclypeus. Major spots are placed in the most internal line, and in every line the major spot is the lowest. The number of points by line differs between individuals. Even in the same individual, the arrays of spots from left and right sides of the head are different (Fig. 1h). The rest of head is black while prothorax is orange. The color of body is similar to third instar but lighter. In the dorsal region of the three last abdominal segments color changes gradually from orange-green to orange-brown at the end of the body. The two longitudinal white lines are more conspicuous than in third instar. Except the mid-dorsal line and prothorax, the body is uniformly covered by a large number of short white setae. The base of setae is white too, so the body seems to have tiny white dots. HW=2.39 ± 0.19 mm (min 2.00, max 2.65 mm, N=13).

Fifth instar (Fig 1i): Not quite different from fourth. Body color is lighter. Longitudinal lines and head spots are more conspicuous. Mandibules are black, while labrum varies from yellowish brown to reddish brown, sometimes both. Antennae are light gray and yellowish on base. Maxillary palpi are light gray too, but with the extremes and setae reddish brown. There are more setae on body than in the fourth instar, but so short that body looks bald. There is a dark green mid dorsal line. Legs are light greenish brown with tarsi reddish brown. Spiracles are elliptical and orange but paler than the last abdominal segments, except the bigger thoraxic which have the same color of prothorax. Prolegs are very pale, almost transparent. When larvae become prepupa gain the orange-brown coloration gradually from last abdominal segment till the mesothorax. Then the body clarifies from yellowish green to pale yellow. Prothorax now is pale orange and the head from bright black becomes dull bright. Head spots become more inconspicuous and gain a coloration like bronze. HW=3.38 ± 0.18 mm (min 3.00, max 3.80 mm, N=13).

Pupa (Fig. 1j): We never observed pupae in nature. They are greenish brown in the first hours. When the cuticle endures it becomes bright reddish brown, next to emersion it becomes dull dark brown. Labrum and the large pronounced edge of the thoraxic spiracle are black. The body seems to be bald, but thorax and abdomen are covered by tiny white setae. Frontoclypeus and eyes are covered too by tiny white setae, but a little longer that mentioned above. The only conspicuous setae are the large white ones on the cremaster. The wings totally cover the first two thoracic segments and mostly the third one and the first three abdominal segments. Frontoclypeus and vertex are much wrinkled while the rest of the body is quite smooth. LP=15.37 ± 0.77 mm (min 14.00 mm, max 16.1 mm, N=7). MTW was measured in two individuals: 5.13 and 5.35 mm, respectively. Weight measured in these two individuals was 256 and 199 mg, respectively.

Ethology

After eclosion, the larva eats the upper half of egg shell. All instars of *Burca b. braco* feed on *Croton lucidus*. Larvae construct shelters with sections of a leaf or complete leaves of the host plant modified with silk and cuts. We never observed larvae outside their shelters. These structures have been never damaged by feeding activity. On the other hand, the rest of the substrate leaf (or leaves) of shelter and/or very nearby leaves show some damage attributed to feeding activity. Architecture of shelters varies between instars. First to third instars inhabit type 5 shelters (N=40) with the two cuts in the same margin (Fig. 4a). Unlike second and third instars, first instar shelters are rarely folded to upper-leaf surface (Table 3). Larvae of second and third instars seldom construct circular type 3 shelters (N=1) (Fig. 4b). Fourth and fifth instars construct either type 5 shelters (N=12) with cuts in opposite margins (Fig. 4c) or type 2 (N=11) (two leaves joined) shelters (Fig. 4d). Sometimes we observed, close to a housing shelter, others deserted by previous instars. We never found shelters with feces accumulations. In captivity, larvae could not construct shelters as they do in nature. When they are disturbed, they react bending laterally their body to cover the head with the abdomen, or vomiting, or attacking with their mandibles.

We observed females laying eggs twice. The vast majority of the eggs observed (167) were laid singly (164) and on the upper-leaf surface (165) (Fig. 5). Adults fly typically not more than 3 m from soil and quite fast between shrubs. In resting position they extend their two pairs of wings in the same horizontal plane of the body. We recorded four nectar sources: *Croton lucidus* (Euphorbiaceae), *Duranta erecta* (Verbenaceae), *Morinda royoc* (Rubiaceae) and *Chiococca alba* (Rubiaceae).

Discussion

The typical five instars of larval development of *Burca b. braco* (see Fig. 1) are similar to the records of most Pyrginae (Scudder, 1889; Moss, 1949; Torres, 1998). However, according to our data the presence of non-typical individuals with more than five instars is possible (see Fig. 2). The intra-specific variations in number of instars, even with the existence of a typical number, are quite normal in Lepidoptera (Knopf & Habeck, 1976; Otazo *et al*., 1984; Farr, 2000; Holland, 2003; Barro, 2007). If some of our individuals is (are) non typical in the number of instars, so our estimated HW, and specially their variations, are biased. We can expect that bias is larger in major instars, and logically the first instar estimation of HW is not biased.

The descriptions of immature stages of *Burca b. braco* are the first ones for the genus. Immature morphology, color patterns, form and disposition of setae (see Fig. 1 and 3) are similar to other Pyrginae species (Scudder, 1889; Moss, 1949; Stehr, 1987). There are no cases in Pyrginae with so high variation in number of egg ridges and so complex and various arrays of CR, IRB and IRM (see Fig. 1a and Table 2). Also the complex and irregular design of white spots in the head of fourth and fifth instars is unknown for other Pyrginae in the literature we reviewed (see Fig. 1e). It is possible that both arrays are individual-specific. This could be cause of genetic depression or they are just high variable characters.

Most ethological records in this paper are similar to those for other skippers, especially Pyrginae. Several hesperiids eat the upper half of egg shell at birth (Heitzman, 1965; Young, 1985; Stehr, 1987; Torres, 1998). Construction of larval shelters is distinctive of Hesperiidae (Moss, 1949; Stehr, 1987; Scoble, 1992), and possibly it´s the most diverse family in construction patterns (Greeney & Jones, 2003). Even Pyrginae and Eudaminae are the subfamilies with more diversity because their larvae change the pattern of construction between instars (Moss, 1949; Lind *et al*., 2001; Greeney & Jones, 2003; Greeney, 2009). Larvae of *Burca b. braco* construct four to five shelters during their lifetime and of two to four different types (see Table 3 and Fig. 4), according to the classification of Greeney (2009). It seems to be usual that first instar shelters are different from second and third instars because the shelters differ in which leaf surfaces are folded, even if they belong to the same type (see Table 3). Since we never saw larvae outside their shelters, we could expect that they are nocturnal or just display low rates of activity. Hesperiid caterpillars are considered nocturnal by several authors (Scott, 1986; Stehr, 1987; Smith *et al*., 1994). It could be that *Burca b. braco* larvae don´t walk away outside their shelter. Even it´s possible that larvae spend all their lifetime in the same host plant. A similar case was experimentally demonstrated on larvae of *Epargyreus clarus* (Eudaminae) which feed closely to their shelter and spend more than 95% of daytime inside it (Lind *et al*., 2001).

Several authors report for some lepidopterans the advantages of laying eggs in clusters or colonial and subsequent larval gregariousness (Stamp, 1980; Courtney, 1983; Sillén-Tullberg & Leimar, 1988; Clark & Faeth, 1997; Denno & Benrey, 1997; Reader & Hochuli, 2003). These advantages were synchronization of displays, increase of growing rates, reinforcement of aposematic characters, and other antipredatory defenses. However, skippers like *Burca b. braco* shows a high inclination to lay solitary eggs (see Fig. 5), so larvae are solitary too and inhabit in shelters (see Fig. 4) (Stehr, 1987). It could be that shelter construction is advantageous for species with solitary displays, thus risks of predation are low (Damman, 1987; Loeffler, 1996; Eubanks *et al*., 1997; Jones *et al*., 2002). Shelter construction indirectly decrease inter-specific competition (Attlegrim, 1989, 1992), avoid chemical defenses of the host plant (Sagers, 1992; Sandberg y Berenbaum, 1989), reduces the probability of dislodgment from host plant (Cappuccino, 1993; Loeffler, 1996), and/or creates a favorable microhabitat (Loeffler, 1996). Also, it´s interesting the high inclination of *Burca b. braco* to lay eggs in the upper-leaf surface. Several authors refer that most lepidopterans prefers lay eggs in under-leaf surface to protect them from weather conditions (Moore, 1986; Tiritill & Thompson, 1988; Thompson & Pellmyr, 1991). However, some heperiids don't show preferation for any surface as oviposition substrate, like *Atrytone arogos* (Heitzman, 1965) and *Urbanus proteus* (Young, 1985). By the way, *Noctuana haematospila* (Greeney y Warren, 2004) always lays eggs in upper-leaf (Greeney y Warren, 2004). We should consider too that *Burca b. braco* shares its host plant with *Memphis verticodia echemus* (Nymphalidae: Charaxinae), which lays eggs in under-leaf surface (pers. obs.).

Except *Croton lucidus*, all nectar sources reported here are new to *Burca b. braco* and also to the genus. *Morinda royoc* and *Chiococca alba* are also the first Rubiaceae nectar sources for the genus (Smith *et al*., 1994). Other *Croton* species have been also reported as nectar sources for *B. concolor* and *B. stillmani* (Smith *et al*., 1994). *Burca concolor concolor* known hostplants also belong to *Croton*, *C. sagreanus* and *C. origanifolius* (Fernández, 2004),so is probable that *Croton*, with a large number of species in the area, be the host genus of all *Burca* species. The inclination in related lepidopterans to use related plants is well known (Gilbert y Singer, 1975), so if our suspicion is true, then immature of other species of *Burca* should be described in a near future if looking for them on *Croton*.

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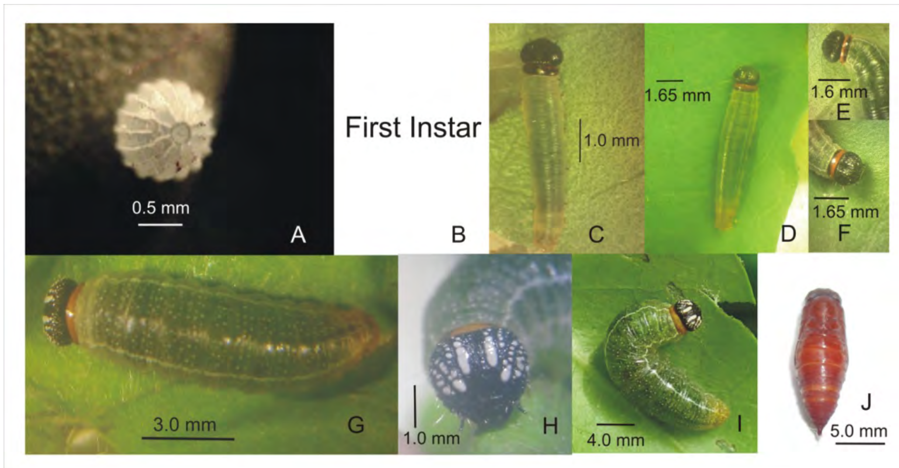


Figure 1. Immature stages of *Burca braco braco*. Egg in upper view (A), first instar (B), second instar (C) third instar (D), upper view of the head of third instar with absence of white elliptical spots (E), upper view of the head of third instar with two lines of white elliptical spots (F) fourth instar (G), upper view of the head of fourth instar (H), fifth instar (I), pupa (J).

Table1. Eggs morphometric means of *Burca braco braco*.

	N	X	SD	Min	Max
d_1 (mm)	158	1,08	0,06	0,90	1,20
d_2 (mm)	158	1,06	0,07	0,85	1,20
h (mm)	117	0,89	0,08	0,75	1,05

Table 2. Number of vertical ridges and their categories (CR=complete, IRB=incomplete with one extreme on base, IRM= incomplete with one extreme on micropyle) arraying eggs of *Burca braco braco*. Number of eggs analyzed 70.

	Mode	Min	Max
CR	6	3	10
IRB	11	6	16
IRM	5	1	6
Total	**21**	**17**	**25**

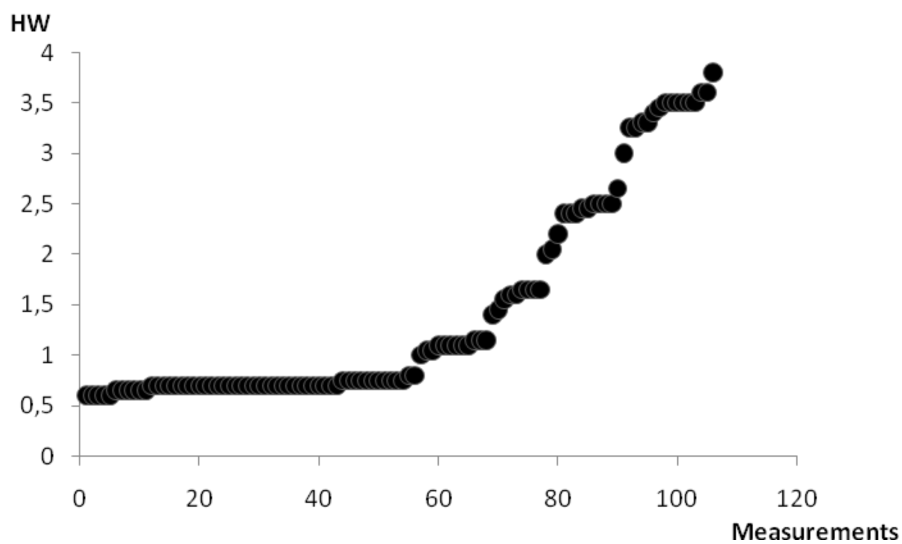
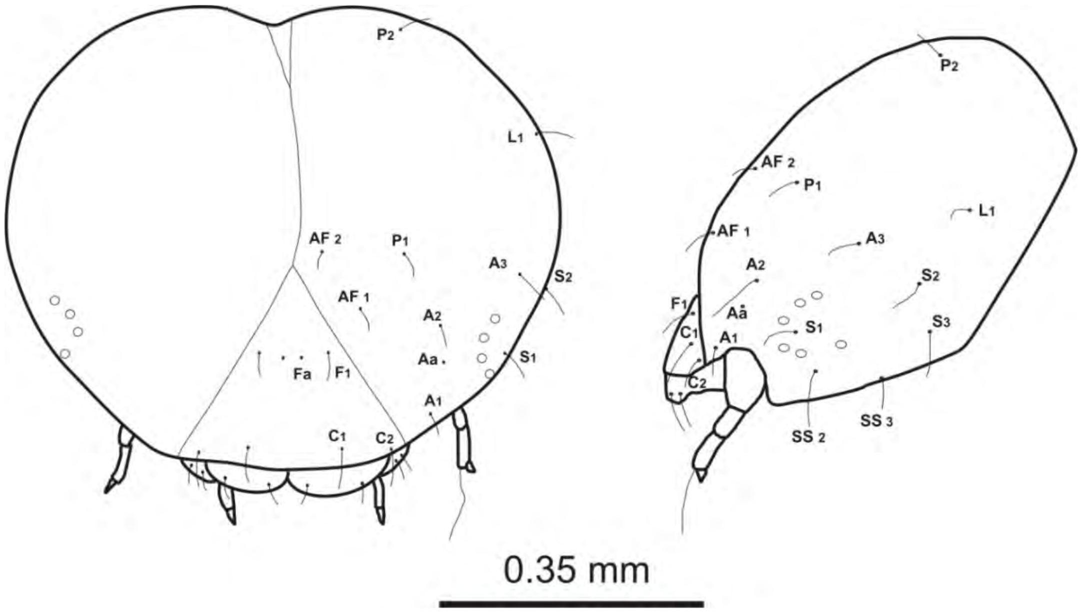


Figure 2 Dispersion of measurements of head width of *Burca braco braco* caterpillars.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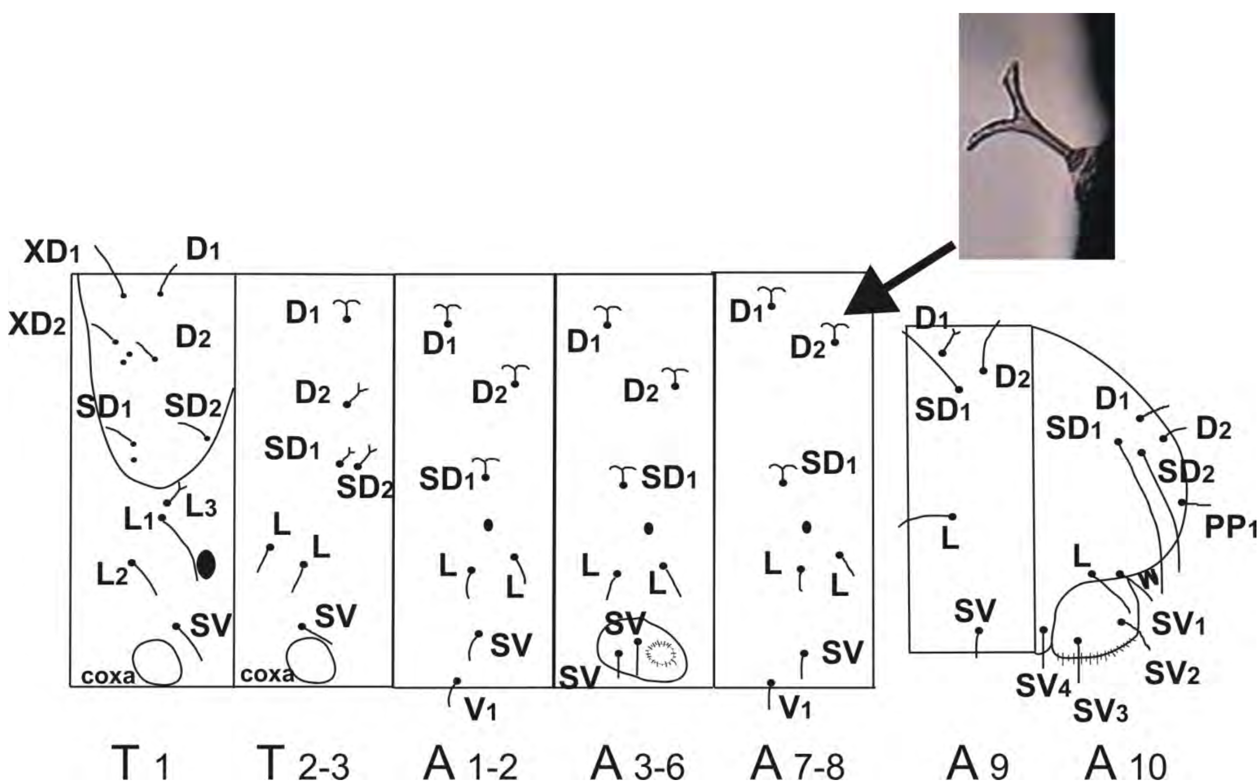
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SV2
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SV4
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A 1-2
A 3-6
A 7-8
A 9
A 10

B

Figure 3. Chaetotaxy of first instar of Burca *braco braco*. Head scheme in frontal view (left) and lateral view (right) (A), and segments of the body scheme with details of "Y" shape setae (B). T=Thoraxic segment, A=Abdominal segment.

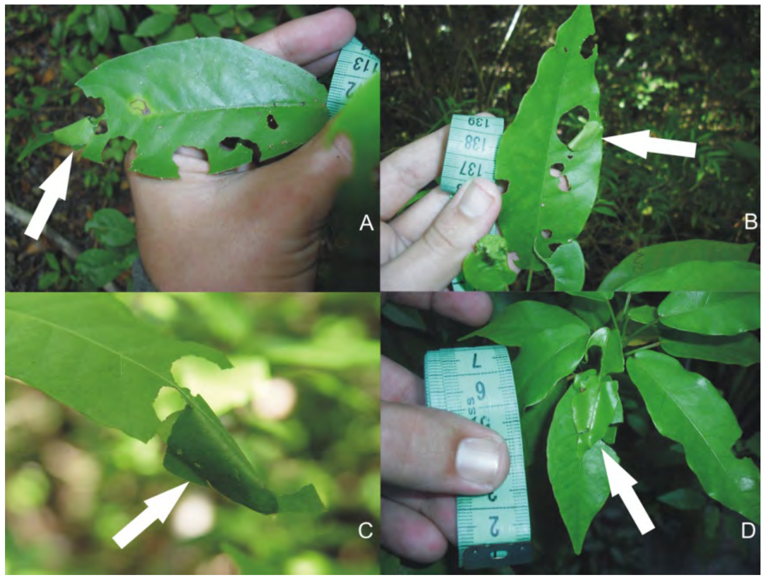

Figure 4. Larval shelters of *Burca braco braco*. Usual shelters type 5 for second and third instars (A), non usual shelters type 3 of a third instar (B), usual shelters type 5 for fourth and fifth instars (C), usual shelters type 2 for fourth and fifth instars (D). Shelter classification of Greeney (2009).

Table 3. Substrate surfaces used in shelters construction by different instars of *Burca braco braco*.

Surface/Instars	**1st**	**2nd y 3rd**	**4th y 5th**
Upper-leaf	1	11	6
Under-leaf	15	14	6
Two leaf joined	0	0	11

Figure 5. Usual way of laying eggs for *Burca braco braco*, singly and in upper-surface of leaves of *Croton lucidus*.