Biology, Ecology and Diversity

*Elbella luteizona* (Mabille, 1877) (Lepidoptera, Hesperiidae: Pyrginae) in Brazilian Cerrado: larval morphology, diet, and shelter architecture

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**Abstract**

This study examined temporal variation in the abundance of immature stages of *Elbella luteizona* (Hesperiidae) and describes the morphology and behavior of the larvae on their host plants, *Byrsonima coccolobifolia* and *Myrsine guianensis*. Five hundred sixty-eight 10 m diameter plots were searched for caterpillars in the Brazilian Cerrado over a period of one year. We inspected 5968 host plants, and found 31 eggs and 262 larvae on 244 plants. Similar numbers of immatures were found in both species of host plants. The abundance of immature stages varied monthly and was significantly higher in the dry season on both host plants, which may be due to the low density of natural enemies during that time. *E. luteizona* is univoltine, and larvae present relatively little morphological variation. However, during development, substantial changes occur in the architecture of leaf shelters that caterpillars construct. In addition, *E. luteizona* larvae develop very slowly, taking more than 300 days to complete metamorphosis.

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**Introduction**

The family Hesperiidae (Papilionoidea) is comprised of about 4000 species, 567 genera, and seven subfamilies [Warren et al., 2009; Heikkilä et al., 2011; Van Nieukerken et al., 2011]. In the Neotropics, 2365 species have been recorded, and 1165 species are known to occur in Brazil (see Mielke et al., 2008). In the Distrito Federal of Brazil, there are 335 species of Hesperiidae, which represent 14.2% of species globally and 28.8% of Brazilian Hesperiidae.

The tribe Pyrrhopygini (Pyrginae) is primarily Neotropical, being distributed from the southwest of the United States (Arizona, New Mexico, and Texas) to northern Argentina (Mielke, 1994). All known larvae and pupae of Pyrrhopygina are covered with long thin setae, a characteristic considered to be a synapomorphy of the tribe (Moss, 1949; Burns and Janzen, 2001). Larvae of Pyrrhopygina, a subtribe of which includes the genus *Elbella* Evans, 1951, is the object of the present study, have dark teguments with yellow or orange stripes (Moss, 1949; Cock, 1982; Burns and Janzen, 2001). *Elbella* includes 22 recognized species, 16 of which occur in Brazil (Mielke, 2004, 2005). In the Distrito Federal, three species of *Elbella* have been recorded (Mielke et al., 2008): *E. luteizona* (Mabille, 1877), *E. azeta giffordii* Mielke, 1994, and *E. interjecta lusca* Evans, 1951, which is endemic to the biome.

*E. luteizona* is a butterfly found also in the Brazilian states of Minas Gerais, São Paulo, Paraná, and Rio Grande do Sul (Mielke, 1994). In the Cerrado, more specifically in the Distrito Federal, the adults fly from January to April (Mielke, 1994), which is the second half of the region’s wet season that lasts from October to April. Caterpillars of the species are polyphagous, feeding on leaves from at least two plant families, the Malpighiaceae (*Byrsonima coccolobifolia* Kuntth) (Diniz et al., 2001) and Myrsinaceae (*Myrsine guianensis* Aubl.) (Kuntze) (unpublished data). However, host plant records for most of the larvae of *Elbella* species are still very scarce, except for species found in Costa Rica (Burns and Janzen, 2001). The larvae of Hesperiidae species construct and inhabit various types of leaf shelters—cuts, ties, rolls, and folds (Greeney and Jones, 2003; Greeney, 2009). The architecture of these shelters varies across genera and often between larval instars. However, shelter architecture is constant within a species (Weiss et al., 2003) and may thus be useful for species identification in the field (Greeney, 2009) as well as the evaluation of phylogenetic relationships between species (Greeney and Jones, 2003; Greeney and Warren, 2009a,b).

The larval stages of the Hesperiidae are not well studied, and more information regarding caterpillar host plant selection is required. Knowledge of immature stages is needed for species conservation and for understanding ecological interactions. Data on
caterpillar morphology may also help resolve taxonomic questions (Freitas and Brown, 2004). Thus, in this work we studied the ecology and biology of E. luteizona. We examined the temporal variation in the abundance of immature stages on B. coccobilofila and M. guianensis in the Cerrado, and describe the morphology of eggs, larvae and pupae of this species for the first time. In addition, we analyzed the behavior of different larval instars in relation to the architecture of their shelters in the field and in the laboratory.

Material and methods

Study area

This study was conducted between August 2011 and May 2013 in Cerrado sensu stricto at the Fazenda Água Limpa (FAL; 15° 55′ S and 47° 55′ W), an experimental farm belonging to the Universidade de Brasília (UnB), and at the Reserva Ecológica do Roncador of the Instituto Brasileiro de Geografia e Estatística (RECOR, IBGE; 15° 56′ S and 47° 53′ W) in the Distrito Federal (DF), Brazil. Both areas are preserved and form part of the 20,000 ha Gama e Cabeça de Veado Environmental Protection Area. The region, at an altitude of ~1050 m, has two marked seasons—dry (May to September) and wet (October to April). During the study period, the total precipitation of the dry season was 155 mm, while in the wet season it was 1381 mm.

Host plants

We searched for E. luteizona in B. coccobilofila (Fig. 1A) and M. guianensis (Fig. 1B). The first species is deciduous and widely distributed in the Cerrado (Ratter et al., 2003). Leaf production and loss are not synchronized and occur at the end of the dry season or in the dry/wet season transitions (September and October) (Morais et al., 1995). The plant has small hairy, pink leaves when young, and they become dark green and glabrous as they age. Flowering occurs from December to February (Silva Júnior, 2005). M. guianensis, an evergreen tree species with continuous growth (Lenza and Klink, 2006), is found in different vegetation types in the Cerrado. It has fleshy, concolorous, glabrous leaves which are shiny on their adaxial surface but opaque on the abaxial surface, and extrafloral nectaries (Oliveira and Leitão-Filho, 1987). The species flowering period is from September to December (Silva Júnior, 2005).

Collection and rearing

We used a standardized method for surveys and caterpillar collection, following Dyer et al. (2010), i.e., plots of 10 m in diameter. We sampled 568 temporary plots—263 in the wet season and 305 in the dry season. Plots were centered around one of the host plant species (B. coccobilofila or M. guianensis), and four strings of five meters were extended in a cross from the center plant to delineate the plot. No plant was surveyed more than once, and at least 5 m separated the plots.

In each plot, all plants of each host species 0.2–2 m tall were counted and inspected for eggs, larvae and pupae of E. luteizona. All immature stages found in the field were collected and taken to the Laboratório de Biologia de Insetos Herbívoros/UnB, where they were reared in individual plastic containers under ambient conditions. The larvae were fed on the leaves of the species upon which they were found. Every two days, the rearing containers were inspected for cleaning, new leaves were added if necessary, and egg hatching, larval instar changes, the construction of new shelters, pupation, and adult or parasitoid emergence was recorded. Concurrently, 40 individuals of M. guianensis which had 45 larvae of different instars in shelters were tagged in the field. Records of the behavior of these specimens on the host plant in the field were made every two days.

Eggs, the width of caterpillar cephalic capsules, and general morphological larval traits were analyzed using a Leica® S8APO stereomicroscope with an attached micrometric scale. Measurements are presented as minimum and maximum values. Eggs and larvae of the first instar were prepared for scanning electron microscopy (SEM) which was conducted using aJEOL® JSM 7001 F. The samples were fixed in Kahle’s solution and subsequently immersed for thirty minutes in acetone at increasing concentrations (50, 70, 90, and 100%) to dehydrate samples prior to SEM analyses. Dehydration was completed in Balzers CPD 030 after eggs and larvae were mounted on double-sided tape on metallic plates and coated with gold in a metallizer (Leica® EM-SCD 500). Morphological terminology is based on Stehr (1987). Immature stages and adult voucher specimens were deposited in the Coleção Entomológica do Departamento de Zoologia/UnB.

Results

Ecology and biology of E. luteizona

We inspected 5968 plants (5466 individuals of M. guianensis and 502 of B. coccobilofila) in 568 plots; there were on average 15.2 and 2.8 individuals of M. guianensis and B. coccobilofila per plot, respectively. Thirty-one eggs and 262 larvae of E. luteizona were found on 244 plants; only 4.1% of the plants searched were host to caterpillars at the time of sampling (Table 1). The larvae did not show any difference in host plant use in either season ($\chi^2 = 1.42, df = 1, p < 0.2$ in the wet season; $\chi^2 = 0.05, df = 1, p < 0.8$ in the dry season). The relative abundance (number of caterpillars/plot) of E. luteizona varied monthly, and it was significantly higher in the dry season on both plants ($\chi^2 = 118.1, df = 1, p < 0.05$ for M. guianensis; $\chi^2 = 14.3,$
df = 1, p < 0.05 for B. coccolobifolia). E. luteizona is a univoltine species (Fig. 2), whose larvae pass through eight instars; the developmental period from egg hatch to adult emergence lasts about 330 days. Eggs were found in the field during the transition from the wet to the dry season (March to May). No stage immature was found in the field from December to February (Fig. 3).

Eggs are laid individually on the adaxial surface of a mature leaf. On four occasions two eggs were recorded on the same plant (M. guianensis). However, no more than one egg was ever found on the same leaf. After hatching, the first instar larvae consume part of the chorion and then starts to construct its first shelter, generally on the same leaf and a short distance (sometimes less than 10 mm) from where the egg was laid.

We found that E. luteizona constructs shelters of three distinct types, during their development. The first shelter constructed by the newly hatched larvae is a center-cut shelter (Shelter Type 3, following Greeney, 2009), in which the larvae make a circular cut of 7.2–8.9 mm in diameter in the center of the leaf blade. Then the larvae spin silk so that the cut portion will be securely attached to the leaf surface (Fig. 4A–D). These shelters are usually folded onto the adaxial surface of the leaves, but in a few cases, they are folded onto the abaxial surface. The larvae do not appear to eat any portion of the leaf while building this shelter, and leaf fragments can be seen beside the cut.

In the field, the larvae remain in this shelter for as long as it provides enough space and access to food, reaching the third or fourth instar within the same shelter. After that, the larvae construct another shelter of the same type but almost twice as large as the first. In the laboratory, when the leaf dries out and is no longer a suitable source of food, the larvae leave the shelter and search for another leaf in order to build a new shelter of the same type, never returning to the previous shelter. So, more shelter can be built in laboratory than in the field during. Both in the field and in the laboratory, the larvae spin silk at the base of the leaf petiole to keep it firmly attached to the host plant. During the first and second instar, the larvae feed by scraping the leaf surface, preferably within or at the edge of the shelter. After reaching the third instar, the larvae leave the shelter to consume other portions of the leaf. We observed that the larvae eject their feces distances greater than the length of their bodies.

Fifth and sixth instar larvae construct a second type of shelter by cutting the leaf in two places (Shelter Type 5, the two-cut shelter,

### Table 1

<table>
<thead>
<tr>
<th>M. guianensis</th>
<th>B. coccolobifolia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants examined</td>
<td>Dry 2946 Wet 2520 Total 5466</td>
<td>Dry 252 249 Total 502</td>
</tr>
<tr>
<td>Plants with E. luteizona</td>
<td>Dry 204 Wet 228 Total 432</td>
<td>Dry 16 0 Total 16</td>
</tr>
<tr>
<td>Egg</td>
<td>Dry 25 Wet 3 Total 28</td>
<td>Dry 3 0 Total 3</td>
</tr>
<tr>
<td>Larva</td>
<td>Dry 216 Wet 23 Total 239</td>
<td>Dry 23 0 Total 23</td>
</tr>
</tbody>
</table>

### Fig. 2

Eiblella luteizona in the Cerrado, cycle life based on observations of field and rearing in laboratory. The period of occurrence of the adults was complemented with literature data (Mielke, 1994). L1 = first larval instar, L2 = second larval instar, and so on.

### Fig. 3

Monthly variation in relative abundance (number of immatures stages/plot) of Eiblella luteizona collected on Myrsine guianensis (light gray) and Byrsonima coccolobifolia (dark gray) in the Cerrado sensu stricto of the Distrito Federal, Brazil, from August 2011 to May 2013. The dark area in the graph corresponds to dry season in the Cerrado.
based on Greeney, 2009; Fig. 4E). The larvae make two cuts from the leaf edge toward the midrib, about 10–27 mm apart, and bend the leaf to form the cover of the shelter. The larvae continue to build shelters of this type as they grow.

When larvae reach the seventh and eighth instar, they build a third type of shelter, by tying multiple leaves together (Shelter Type 2, the multi-leaf shelter, based on Greeney, 2009; Fig. 4F). The final larval shelter is lined with silk and used for pupation.

E. luteizona and the parasitoids

Both eggs and larvae were parasitized. Approximately 20% of the eggs were parasitized by two species of Hymenoptera, Anastatus sp. (Eupelmidae: Eupelminae) and Neochrysocharis sp. (Eulophidae: Entedonina). One individual of Anastatus emerged from each egg (n = 4), and at least four individuals of Neochrysocharis emerged from each egg (n = 2). Larvae were parasitized by a solitary Casinaria sp. (Ichneumonidae: Campopleginae) (n = 5) and a gregarious Cotesia sp. (Braconidae: Microgastrinae), with up to 83 individuals emerging from a single larva (n = 3). The parasitized larvae (3%) were collected in the field in May, August, and September, the dry season in the Cerrado.

Morphology of immature stages

Egg: (Figs. 5A, B and 6). Duration: nine days. Diameter: 1.2–1.4 mm (n = 25); height = 0.7–0.9 mm (n = 14). Dome-shaped. To the naked eye, they appear grayish, but under the stereomicroscope they are white with gray spots scattered over the entire surface. The chorion is sculpted, filled with horizontal and vertical carinae forming rectangular cells (except at the base of the egg) and becomes pentagonal or hexagonal as nearer to the micropyle (Fig. 6A–D). The micropyle is located at the apex (anterior pole) of the egg. This region has elevated carinae with aeropyles occurring at the intersections (Fig. 6E).

First instar: (Figs. 4A, B, 5C, 7A–D and 8). Duration: 10–16 days (n = 9). Head capsule width: 0.9–1.0 mm (n = 45). Head: hypognathous, cordiform, brownish-red, wider than the body, marked vertical stripes, epicranial suture distinct; six stemmata, 1–4 equally spaced in semicircle, 5 near antennal base, 6 caudal of 4 and closer to 5 than 4. Head chaetotaxy (Fig. 7B): 14 pairs of tactile setae: three pairs of anterior (A) setae, one frontal (F), two adfrontal (AF), two clypeal (C), one lateral (L), two posterior dorsal (P) and three stemmatal (S) setae. Body length 2.5–4.0 mm. At hatching the body is orange-brown with light yellow transverse stripes on thoracic and abdominal segments; sparse white setae distributed on body (Fig. 5C). At the end of this instar, the body acquires a slightly darker, brown-reddish color, and the yellow rings become more evident. Prothoracic shield with sparse setae (Figs. 7A and 8). Light yellow spiracles prior to the base of yellow ring on A1–A8 round, much smaller than T1 (Fig. 7A). One lenticle dome-shaped circle subventrally conspicuous in T1 (Figs. 7A and 8). Four lenticles subventrally in A1–A2 and one in A7 and A8, all of these smaller than T1 (Figs. 7A, C and 8). Legs light yellow. Prolegs orange-brown on segments A3–A6 with biordinal crochets, arranged in complete circle; proleg on A10 with biordinal crochets, a caudal gap in an
otherwise complete circle. Anal comb visible in A10 (Fig. 7D). Anal plate same color as body. Body chaetotaxy (Fig. 8): nomenclature setal map (following Stehr, 1987) = XD only on the anterior margin of the T1 shield; setae dorsal (D); subdorsal (SD); lateral (L); subventral (SV); ventral (V). T1 with nine pairs of setae, six pairs on T2 and T3, seven pairs on segment A1–A8, six on A9 and twelve pairs of setae on A10.

**Second instar:** (Fig. 5D). Duration: 11–27 days (n = 9). Head capsule width: 1.1–1.3 mm (n = 113). Body length: 3.5–4.5 mm. Head and body general pattern as in the previous instar. Body color brown-reddish with light yellow stripes. From this instar onwards, there is an increase in the number and length of the setae of the body and head. Prothoracic shield with lines setae in anterior margin. The setae on the body remain white, but some become darker on the frontal head capsule region. The color of vertical stripes can vary from white to vibrant yellow.

**Remaining instars:** The main difference between second and remaining instars (L3–L8) is duration, head capsule width, body length (Table 2) and the body coloration whose transverse stripes may become more conspicuous in both host plants (Fig. 5E and F).

**Pupa:** (Fig. 5G and H). Duration: 14–32 days (n = 7). Length: 24.0–26.0 mm (n = 5). Pupa elongate, almost cylindrical with tapered posteriorly without any appendages or spines. The newly formed pupae are bright orange, becoming brown with a gray appearance due to the presence of numerous white setae. The pupae have transverse yellow stripes like the larva. Pupae are capable of movement, wriggling vigorously when disturbed.

**Discussion**

There are records showing the larvae of 17 genera and 28 species of Neotropical Pyrginiae (including three species of Elbella) consuming leaves of different species of Malpighiaceae (Beccaloni et al., 2008; Janzen and Hallwachs, 2009). We have recorded two other species of Elbella that occur in the Cerrado of the Distrito Federal: *E. azeta*, which was found feeding on Connaraceae and Styracaceae (unpublished data), and *E. intersecta*, which was found consuming Asteraceae and Burseraceae (Diniz et al., 2001). Our observations of *E. luteizona* represent the first record of any species of *Elbella* using a species of Myrsinaceae as a host plant. It is known that the availability and temporal variability of food resources can directly influence the foraging decisions of an herbivore and alter their diet breadth (Singer et al., 2002). Nonetheless, we did not detect a predilection for *M. guianensis* over *B. coccolobifolia*, even though *M. guianensis* was 11 times more abundant in our plots than *B. coccolobifolia*. Future studies of the nutritional quality or secondary chemistry of host plants in the Cerrado in the two seasons will help us to understand Lepidoptera diet breadth and its evolution.

The higher abundance of larvae of *E. luteizona* in the dry season is probably due to the biology of this species, given that oviposition occurs during the transition from the wet to dry season.
the dry season progresses in the Cerrado, there is a decrease in the abundance of parasitoids and predators, creating a period of reduced predation pressure (Morais et al., 1999). It is thus advantageous for vulnerable first instar caterpillars to occur at this time. Over time, mortality increases due to dehydration caused by prolonged drought (Peterson et al., 2009), which explains the lower number of caterpillars found later in the dry season. Finally, pupae and adults are most abundant in the wet season, and consequently fewer larvae are found.

E. luteizona eggs were parasitized almost seven times more frequently than larvae. However, the occurrence of Pyrrhopgygini larvae parasitized by Casinaria spp. (Ichneumonidae) and by species of Eulophidae seems to be common in Central America (Burns and Janzen, 2001). Currently, Elbella is the only genus of the Pyrrhopgygini recorded to be parasitized by Braconidae (Burns and Janzen, 2001). Although some studies show that parasitoids can detect their hosts via clues derived from shelters (e.g., Weiss et al., 2003), many others demonstrate shelters serve as protection against the attack of natural enemies (e.g., Fukui, 2001; Greeney et al., 2012). It has also been suggested that species that build shelters eject their feces, as observed in E. luteizona, in order to limit olfactory cues used by natural enemies (Weiss, 2003; Moraes et al., 2012).

The larval morphology of E. luteizona varies relatively little throughout its development. Similarly, the principle change in many Hesperiidae larvae during development is in body size rather than coloration (Cock, 2008; James, 2009; Bächtold et al., 2012). The bright yellow coloration in some caterpillars of E. luteizona is similar, for example, to E. merops (E. Bell, 1934) and E. scylla (Ménétried) presented in Burns and Janzen (2001) in dry forest. It also resembles to E. azeta, found in Cerrado, but this species differs from E. luteizona due to the white dots on base of the transversals stripes, prior to spiracles (unpublished data). However, changes in shelter architecture are also common in the Pyrginae (Graham, 1988; Miller, 1990; Greeney and Warren, 2004; Greeney and Sheldon, 2008; Greeney and Warren, 2009a,b) and appear to be the norm for Pyrrhopgygini as well (Burns and Janzen, 2001; Cock, 2008).

Some of the variation in the size and type of the shelter during larval ontogeny are related to the physical and biological requirements of the larvae. Shelters serve as protection against desiccation and natural enemies and can reflect the physical abilities of larvae of different instars to cut and bend leaves (Lind et al., 2001). Changes in the type of shelters built during larval development are considered fundamental to studies of natural history and can be informative characteristics in phylogenetic analyses (e.g., Lind et al., 2001; Weiss et al., 2003; Greeney, 2009; Greeney and Warren, 2009a,b). The differences observed in the number of shelters constructed by some larvae of E. luteizona in the field and in the laboratory, show the importance of observing this behavior in the field and emphasizes that this is the best way to accurately report the natural history of the species without human interference.

Fig. 6. Elbella luteizona, scanning electron microscopy of egg. (A) Dorso-lateral view, Mp = micropylar region; (B) horizontal and vertical carinæ forming cells, lateral view; (C) basal region, with incomplete carinæ, lateral view; (D) micropylar region, dorsal view; (E) arrow indicating aeropyles (Ae), dorsal view.
Fig. 7. *Elbella luteizona*, scanning electron microscopy of first instar larval. (A) Thoracic (T1–T3) and abdominal segments (A1) with arrow indicating subventral lenticle (*Lent.*); *Sp* = T1 and A2 spiracle, see detail; (B) head chaetotaxy, anterior view, see text for setal nomenclature; (C) abdominal segments (A1–A2), arrow indicating subventral lenticles; (D) last segment, A10, arrow indicating anal comb and proleg.

Interestingly, the first instar larvae of *E. luteizona* do not consume leaves while constructing their first shelters but feed on the egg chorion. Greeney and Warren (2004) propose that larvae consume the chorion because feeding on the leaf during this period delays the process of shelter construction and thus the larvae remain unprotected for longer. The use of silk to fix the petioles of leaves where the shelters were built is an interesting and adaptive behavior for those species with slow larval development, particularly in dry regions, as the case of *E. luteizona*. This behavior increases the likelihood of survival (Sugiura and Yamazaki, 2006).

The Pyrrhopygini includes some of the species with the longest development times of all Hesperiidae (Burns and Janzen, 2001), and *E. luteizona* larval development lasts more than 300 days. This period is about three times longer than the mean development time observed for ten other species of this tribe (Burns and Janzen, 2001). The very slow growth of *E. luteizona* larvae in the Cerrado may be linked to the scarcity of natural enemies in the dry season. Another factor that would explain this delay in the development of this species would be the diapause mechanism, in response to this period of adverse environmental conditions. In fact, the most
Hesperidae larvae pass through five instars (Greeney and Warren, 2004, 2009a,b; Moraes et al., 2012) while *E. luteizona* pass through eight instars. This life history trait, in addition to this and other behaviors, such as the ejection of feces from shelters and the use of silk to fix shelters leaves to host plants, allows larvae and pupae to remain protected inside their shelters for longer, enabling adult emergence at the beginning of the rainy season.

**Conflicts of interest**

The authors declare no conflicts of interest.

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