Immature Stages and Ecological Characteristics of Idalus lineosus Walker (Erebidae: Arctiinae)

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IMMATURE STAGES AND ECOLOGICAL CHARACTERISTICS OF *IDALUS LINEOSUS* WALKER (EREBIDAE: ARCTIINAE)

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ABSTRACT. The caterpillars of *Idalus lineosus* Walker (Arctiinae) are external folivores that specialize in eating mature leaves of *Roupala montana* Aubl. (Proteaceae) in the cerrado (Brazilian savannah). The aim of this study was to present morphological and ecological characteristics of the immature stages of *I. lineosus*. We evaluated relative abundance, seasonal variation, and parasitized proportion of *I. lineosus* in various sites of the cerrado. The caterpillars are solitary, exhibit morphological variation between instars, and present varied color patterns in the last larval instar. The relative abundance of the caterpillar on the host plant varies season- ally and spatially in the cerrado sensu stricto reliefs of two soil types (oxysoil and rocky soil).The caterpillars were parasitized mainly by a gregarious Hymenoptera; multiparasitism was also registered, with interactions between *Apanteles* sp. and *Protapanteles* sp., and *Cidaphus* sp. with *Protapanteles* sp.

Additional key words: Caterpillar, cerrado, polymorphism, *Roupala montana*, tri-trophic interaction.

Arctiinae is currently considered a subfamily of Erebidae (Lafontaine & Schmidt 2010, Zahiri et al. 2010) and includes approximately 11,000 species worldwide (Scoble 1995). Of the 2,000 species of Arctiinae estimated to occur in Brazil (Brown Jr. & Freitas 1999), 723 have been recorded in the Cerrado biome (Brazilian savannahs) (Ferro et al. 2010).

The genus *Idalus* Walker, 1855, comprises approximately 60 species (Watson & Goodger 1986); of these, four species are considered *Idalus* sensu lato (*I. veneta* Dognin, 1901; *I. arrupta* Schaus, 1905; *I. flavibrunnea* Dognin, 1906; *I. lutescens* Rothschild, 1909). Thus, *I. lineosus* Walker 1869, is considered a true *Idalus* species and occurs throughout Central and South America (http:/www.inra.fr/papillon/arct_guy/ htm). In Brazil, *I. lineosus* occurs in forests (Teston et al. 2006, Ferro & Diniz 2007) and in open areas of vegetation, as found in the Cerrado (Bendicho-López et al. 2006, Ferro et al. 2010, Oliveira 2010). There are no publications that report the occurrence of *I. lineosus* in other biomes, such as Caatinga and Pantanal; however, we cannot exclude the possibility of its occurrence.

In Distrito Federal (DF) the caterpillars of *I. lineosus* are external leaf feeders on *Roupala montana* Aubl. (Proteaceae), with certain individuals feeding on their inflorescences (Bendicho-López et al. 2006; the species *Idalus* prop *sublineata* presented by Bendicho-López et al. refers to the species *I. lineosus*, previously identified erroneously). Another species of this genus, *I. flavicostalis* Rothschild 1935, also feeds on this host plant (Bendicho-López et al. 2006).

Roupala montana is widely distributed in the Brazilian savannah, especially in the cerrado sensu stricto (Felfili & Abreu 1999), where it presents as shrubby or arboreal habit (Carvalho 2003), and in central Brazil, where *R. montana* ranges from 20 cm to 6 m in height (Oliveira 2010). This host plant may be considered evergreen because the old foliage persists on the tree until the emergence of new leaves (Franco 1998). To the best of our knowledge, the caterpillar of *I. lineosus* seems to specialize in feeding on this plant species (Diniz et al. 2001, Bendicho-López et al. 2006, Oliveira 2010).

Knowledge about the immature stages of Lepidoptera is important because it aggregates information on the biology and ecology of these organisms; it is also useful for their taxonomy and systematics (Beebe et al. 1960, Fleming 1960; Kaminski et al. 2002, Bizarro et al. 2003, Freitas & Brown Jr. 2004). Our study system comprised by *I. lineosus* and its host plant in a marked seasonal
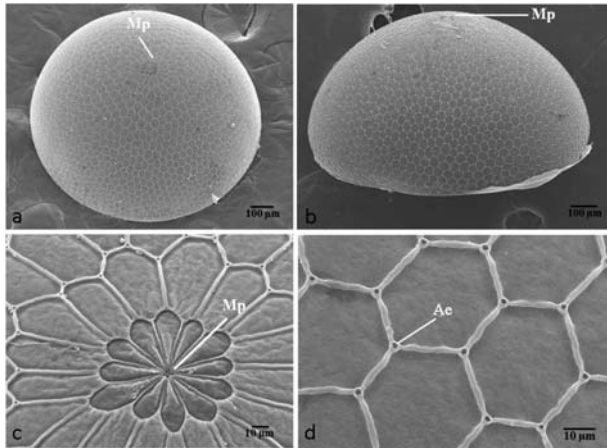
FIG. 1. Egg of *Idalus lineosus* in scanning electron microscopy. (**a**) Dorsolateral view; (**b**) lateral view; (**c**) micropylar area (Mp); (**d**) details of hexagonal cells and aeropyles (Ae).

biome, the central Brazilian cerrado, is useful for our investigation because the caterpillars of this moth species apparently consume only one species as a host plant, and this facilitates multiple comparisons, such as spatiotemporal studies. Furthermore, we can compare the relative abundance of this moth species between the two very marked climatic seasons. Thus, the objectives of this study were to characterize the immature stages of *I. lineosus*, to assess the relative abundance and seasonal variation of the caterpillars, and to verify the proportion of caterpillar parasitism of the different types of cerrado sensu stricto.

METHODS

Information on the immature stages of *I. lineosus* was obtained from three rounds of sampling at different time periods. In the three sampling methods described below, there was no duplication of any examined plants. All areas sampled are located in the Cerrado biome, which exhibits marked climatic seasonality with a dry season (May to September) and a rainy season (October to April).

In this work, we used three data sets. First, for morphological characterization, we searched for eggs, caterpillars, and pupae on the foliage of 500 plants of *R. montana* between May and June 2011 at Fazenda Água Limpa (FAL, n = 100) (altitude 1,117 m, 15°57'S, 47°55'W); Jardim Botânico de Brasília (JBB, n = 100) (altitude 1,118 m, 15°57'S, 47°55'W); and in the Ecological Reserve (RECOR, n = 300) (altitude 1,048 m, 15°56' S, 47°53' W). Second, the relative abundance of caterpillars *I. lineosus* on *R. montana* was accompanied by monthly searches on the foliage of 100 plants from May 2008 to April 2009 (n = 1,200 plants sampled), at Parque Estadual dos Pireneus (PESP), in Pirenópolis, state of Goiás (GO) (altitude 1.289 m, 15°48'S, 48°50'W), in the cerrado sensu stricto reliefs of the rocky soil. Finally, we compared the spatial relative abundance of *I. lineosus* caterpillars among sites by sampling caterpillars on 1,000 individuals of *R. montana* in May and June 2009 at each of the five cerrado sites: 1) PESP; 2) Parque Nacional Chapada dos Veadeiros
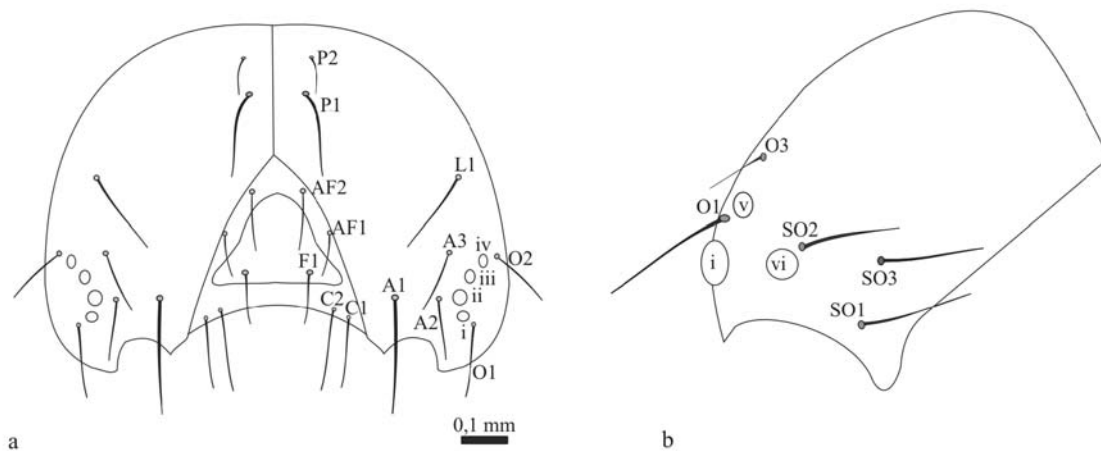

FIG. 2. Chaetotaxy of cephalic capsule of the first instar larvae of *Idalus lineosus*, (**a**) dorsal and (**b**) lateral views. Names of setae: A-anterior, AF-adfrontal, C-clypeal, F-frontal, L-lateral, O-stemmatal, SO-substemmatal; i–vi: stemmata. The small barbs on the setae and the presence of the microsetae and punctures were omitted.

(PNCV) in GO (altitude 1.034 m, 14º09'S, 47º47'W); 3) FAL; 4) JBB; and 5) Parque Nacional de Brasília (PNB) (altitude 1.085 m, 15º52'S, 47º49'W). FAL, JBB, and PNB are located in the state of DF. The study areas located in GO comprise a physiognomy cerrado sensu stricto developed in rocky soils (Felfili & Fagg 2007, Moura et al. 2007), while the other three locations comprise cerrado sensu stricto developed in oxisols (Ribeiro & Walter 2008).

All caterpillars found were individually reared in the laboratory in plastic pots protected by tulle fabric until the emergence of adults. Luminosity, humidity, and temperature were not controlled. Leaves of *R. montana*, with the petiole inserted into a small container with water to prevent drying, were added for food as needed. Two emerged adult females were kept in one cage in an attempt to obtain eggs. Observations of morphological aspects and measurements of immature stages were performed with a Leica® S8 APO stereomicroscope equipped with micrometer scale. Photographs were taken with a Canon® SX30IS digital camera.

Eggs were prepared for scanning electron microscopy (SEM), using Balzers® CPD 030 equipment for critical point drying. The eggs were then mounted on double-sided tape on a metal support and covered with gold in a Balzers® SCD 050 sputter coater. Specimens were examined in a JEOL® JSM 7001F microscope, and images were collected.

The terminology used for eggs follows Peterson (1963) and Dell'Erba et al. (2005); the general morphology of the immature stages, and the chaetotaxy of the head capsule follows Stehr (1987). Dead caterpillars were fixed in Kahle solution, and parasitoids were preserved in 70% alcohol and identified according to Wharton et al. (1997) and Gauld (1997). Species of Lepidoptera were identified by Vitor Becker, parasitoids by André R. Nascimento, and host plant species by the staff of the Herbário da Universidade de Brasília UB. Vouchers for all insect species were deposited in the entomological collection of the Department of Zoology, University of Brasília.

A circular analysis (Oriana version 4) (Kovach 2011) was used to verify the relative abundance of the caterpillars during 1 year of sampling (second data set), in which each month was converted into angles. January 2009 was chosen as 15º, and December 2008 was chosen as 345º. A Rayleigh test, available in version 4 of Oriana (Kovach 2011), was applied to determine whether the caterpillars had an aggregated or uniform temporal distribution.

A Chi-square test was used to assess spatial (PESP, PNCV, FAL, JBB, PNB) and temporal (dry season and rainy season) differences in the proportion of caterpillars collected as well as in the proportion of parasitism by Hymenoptera between the rocky soil cerrado (GO) and the oxysoil cerrado (DF). The data used for analyses of the proportion of parasitism were lumped for all caterpillars reared from the three data sets. All analyses were performed with the statistical program R 2.12.4 (R Development Core Team, 2011).

<div align="center">Results</div>

General biology of immature stages

A total of 6,700 plants of *R. montana* were inspected, and 210 caterpillars of *I. lineosus* were collected. From these, only 54 of the reared (25.6%) caterpillars did not reach the adult stage, and of these 18.5% died from parasitism and the others (44 individuals) died from unknown reasons. All caterpillars were found solitary on leaves of *R. montana*. The detailed observation of larval development was performed with 25 caterpillars obtained from the first data set. The number of caterpillars followed in each instar (n) varied because they were collected in the field at different instar stages. From these, only five caterpillars emerged as adult.

No eggs were found in the field but instead were obtained by the oviposition of two females that emerged in the laboratory. The eggs were laid singly in the rearing pot (mean 68.5 ± 10.60 eggs from the two adult females) (Fig. 3a). These eggs failed to hatch because they were not fertilized.

Idalus lineosus presented six larval instars (Fig. 3b–g) and completed its development (from first larval instar to adult), on average, in 54 ± 5.6 days (n = 5). The first instar caterpillars remained on the abaxial leaf blade, but the last instar caterpillars occurred more often on the adaxial surface. No pupa was found in the field, but in the laboratory pupae occurred on the leaves or attached to the rearing pot.

Morphological traits of immature stages

Egg (Figs. 1, 3a): diameter 1.04 ± 0.03 mm; height 0.59 ± 0.02 (n = 10). Eggs are hemispherical, slightly flattened, with sculptured chorion and a yellowish color soon after oviposition.

First instar (Fig. 3b): body length 5 ± 0.07 (n = 5), cephalic capsule width 0.6 ± 0.11 (n = 5). Head, antennae, and stemmata whitish-yellow. Cephalic capsule with 17 pairs of long setae, as seen in most Lepidoptera. Head chaetotaxy (Fig. 2) conserved in all subsequent instars. Body tegument pale yellow with sparse orange pigmentation, with fine and pale setae arising from dorsal and lateral verrucae. Both thoracic legs and prolegs yellowish white. Development time 5–6 days.
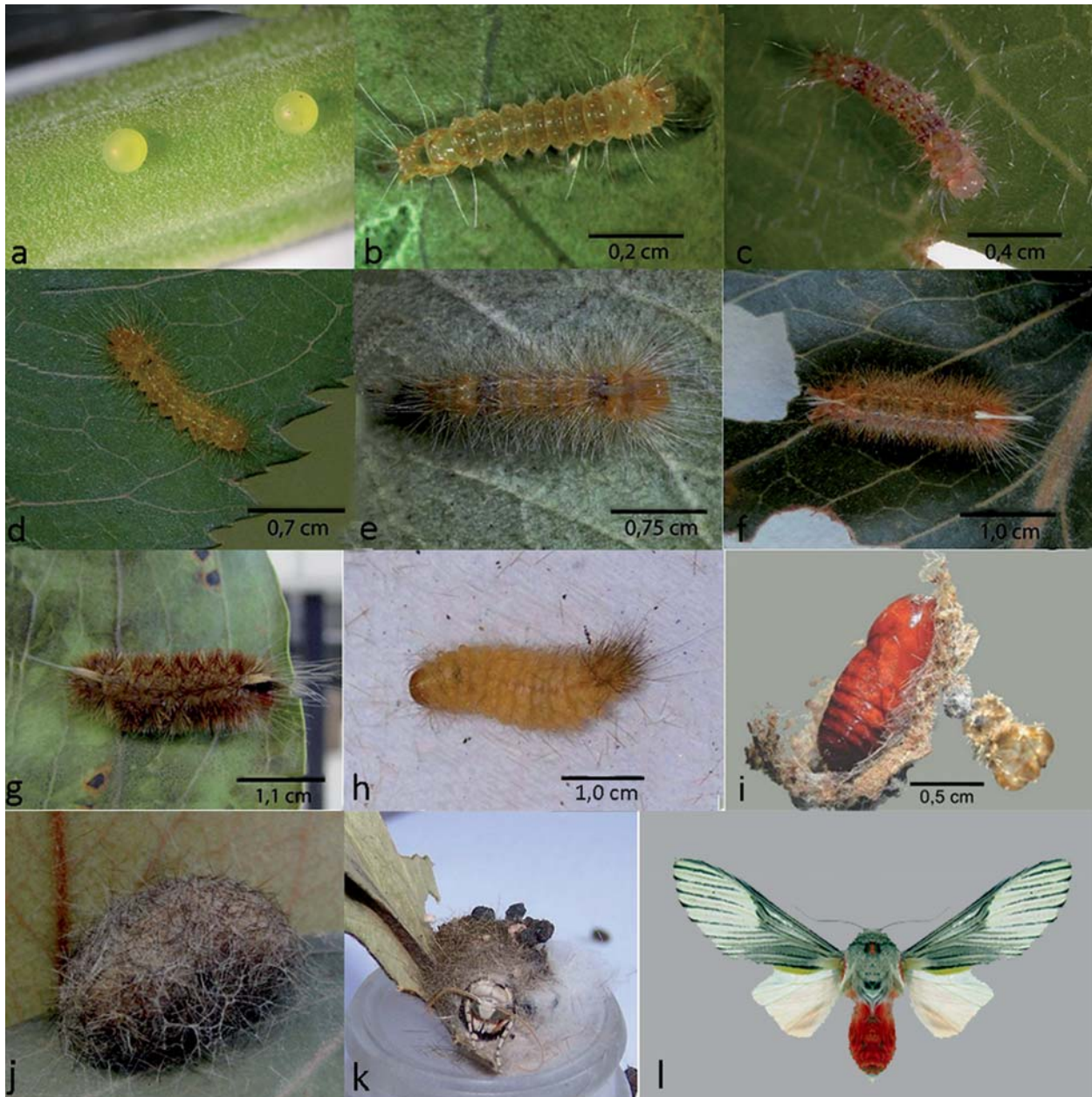
FIG. 3. Eggs, larval instars of *Idalus lineosus*, and pupa and adult of *I. lineosus*. (**a**) Eggs; (**b**) first instar; (**c**) second instar; (**d**) third instar; (**e**) fourth instar; (**f**) fifth instar; (**g**) sixth instar; (**h**) pre-pupa; (**i**) pupa; (**j**) pupal shelter; (**k**) adult emergence; (**l**) adult.

Second instar (Fig. 3c): body length 10.1 ± 0.15 (n = 6), cephalic capsule width 1.1 ± 0.04 (n = 6). Head and antennae as in the first instar. Stemmata darker than in the first instar. Body tegument pinkish with wine-colored dots all over the dorsal view, visibly darkest on abdominal segments A1 and A7. Setae larger and more densely distributed than in the first instar. Both thoracic legs and prolegs are pinkish. Development time 6–7 days.

Third instar (Fig. 3d): body length 13.1 ± 0.25 (n =

9), cephalic capsule width 1.4 ± 0.14 (n = 9). Head and antennae orange. Stemmata black. Body tegument orange, with two longitudinal light-orange stripes, and segments A1 and A7 slightly shadowed on the dorsal view. Presence of yellowish and some dark brown setae dorsally and laterally arranged. Setae are more elongated on segments A1, A2, A8, A9, and A10 than in the others. Both thoracic legs and prolegs are also orange. Development time 5–6 days.

Fourth instar (Fig. 3e): body length 16.3 ± 0.25

FIG. 4. Sixth instar caterpillar of *Idalus lineosus* showing variation in color of the setae. (**a**) whitish-yellow; (**b**) gray; (**c**) orange brown; (**d**) dark brown.

(n = 6), cephalic capsule width 2.0 ± 0.14 (n = 6). Head, antennae, and stemmata as in the third instar. Body tegument orange brownish. Dark brownish pigmentation is evident on segments A1 and A7. Presence of tufts of white setae on dorsal view of segment A1. In the remaining segments, setae are similar to those of the previous instar. Both thoracic legs and prolegs orange. Development time 6–7 days.

Fifth instar (Fig. 3f): body length 19.4 ± 0.32 (n = 5), cephalic capsule width 2.4 ± 0.12 (n = 5). Head light brown, antennae white, stemmata dark brown. Body tegument orange brownish with two longitudinal, whitish, dorsal stripes. Presence of orange and dark brownish setae dorsally and laterally arranged. Setae arising from verrucae distributed around the body tegument, and the whitish tufts of setae become more apparent on segments A1 and A7. Lateral reddish verrucae are also present. Setae arising from A1, A2, A8, A9, and A10 are longer than those arising elsewhere. Both thoracic legs and prolegs as in the previous instar. Development time 7–8 days.

Sixth instar (Fig. 3g): body length 21.8 ± 0.21 (n = 5), cephalic capsule width 3.1 ± 0.23 (n = 5). Head pale brown, antennae white, and stemmata dark brown. Body tegument is greyish with setae displaying individual variations in coloration; setae may be whitish-yellow, gray, orange-brown, or dark brown (Fig. 4). In spite of the color variation of most setae, all caterpillars show whitish setae arising in tufts on segments A1 and A7. A1 also bears a distinct blackish tuft of setae. Setae arising from dorsal and lateral verrucae are disposed all over the tegument. White setae arising from A1 and A2 segments are longer than those elsewhere. Both thoracic legs and prolegs orange. Development time 9–11 days.

Pre-pupa (Fig. 3h): mature sixth instar caterpillar exhibited reduced size and movement and lost almost all setae, which were used in constructing the pupal cocoon (Fig. 3j). Tegument becomes pale yellow, with setae restricted to the anterior and posterior ends.

Pupa (Fig. 3i): pupae (length 10.4 ± 0.15; n = 5) cylindrical, smooth, and dark brown. Development time 18 ± 2.7days (n = 5). Attached to the pupal cocoon was a deposit of setae and feces discarded by the last larval instar (Fig. 3j). The emerged and mounted adults are illustrated in Figs. 3k–l.

Seasonal and spatial variation

For the second data set, we examined 1,200 plants in the PESP in 1 year and found 32 caterpillars of *I. lineosus*. The species showed an aggregated distribution in the dry season (z = 25.88, p < 0.05), and the peak of the relative abundance of the caterpillars occurred in May, with 21 individuals (μ = 137.28°, sd_c = ±26.39°, mean = 135°) (Fig. 5).

For the third data set, we inspected 5,000 plants and collected 153 caterpillars. The relative abundance of caterpillars within each vegetation type did not differ significantly. However, the number of caterpillars was significantly higher (Z = –9.10, Z1 = 0.26, Z2 = 0.67, p <0.01) in the oxysoil cerrado (DF sites) (n = 121) than in the rocky soil cerrado (GO sites) (n = 32). The probability of finding a caterpillar in a survey of 1,000 *R. montana* plants was similar in the three oxysoil
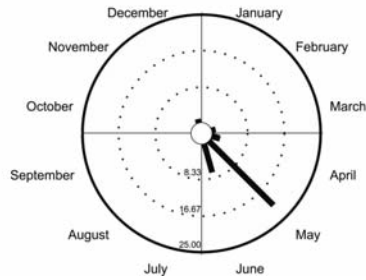
FIG. 5. Seasonal variation in caterpillar relative abundance of *Idalus lineosus* from May 2008 to April 2009, Parque Estadual dos Pireneus, Pirenópolis, Goiás. Angles and their corresponding months: 15° (January 2009), 45° (February 2009), 75° (March 2009), 105° (April 2009), 135° (May 2008), 165° (June 2008), 195° (July 2008), 225° (August 2008), 255° (September 2008), 285° (October 2008), 315° (November 2008), and 345° (December 2008).

cerrado sensu stricto sites (p ≤ 0.05) (FAL 40 caterpillars, 26%; JBB 35 caterpillars, 22.9%; PNB 46 caterpillars, 30.1%) and was also similar between the two sites in the rocky soil cerrado sensu stricto (PNCV 13 caterpillars, 8.5%; PESP 19 caterpillars, 12.4%). However, this differed significantly if all sites were lumped together to compare oxysoil (DF) and rocky soil (GO) cerrados.

Parasitism

Parasitism was responsible for only 5% (n = 10 individuals) of the total caterpillars (n = 210) collected and reared in the laboratory for all periods and sites. Eight of the parasitized caterpillars were attacked by Hymenoptera (Braconidae and Ichneumonidae), and two were killed by dipterans (Tachinidae, Exoristinae). One species of the hymenopteran parasitoids belonging to *Protapanteles* (Braconidae: Microgastrinae), a gregarious species with 49.25 ± 15.9 individuals per host (Fig. 6b), was responsible for five of the caterpillar deaths (75%). The other species were *Apanteles* sp. (Braconidae: Microgastrinae) (Fig. 6c), *Cidaphus* sp. (Ichneumonidae: Mesochorinae), and *Pristomerus* sp. (Ichneumonidae: Cremastinae) (Fig. 6d). Multiparasitism occurred in three individuals of *I. lineosus*, with interactions between *Apanteles* sp. and *Protapanteles* sp. (two occurrences in the PESP), and *Cidaphus* sp. and *Protapanteles* sp. (one occurrence in PNCV).

For a comparative analysis of the proportions of parasitism, we used our third data set in the following five areas: PESP and PNCV (rocky soil cerrado) [GO]); FAL, JBB, and PNB (oxysoil cerrado) [DF]), with equal samples and similar period by location (n = 1,000 censuses in *R. montana*). The caterpillars from the two rocky soil cerrado areas (n = 32) showed a higher species richness of hymenopteran parasitoids (n = 4 species) and higher parasitism rates (18.75%). In oxysoil cerrado areas (n = 121 caterpillars collected), only one species of hymenopteran was found parasitizing *I. lineosus*, representing only 1.65% of the caterpillars collected. A Chi-square analysis revealed that the rates of parasitism differed significantly between areas of rocky soil cerrado sensu stricto (GO) and oxysoil cerrado sensu stricto (DF) (χ^2 = 11.68, p < 0.05). The caterpillars collected in PESP (GO) had the highest parasitism rates (70% of the total parasitized caterpillars in the five areas). Parasitism differed significantly among areas (χ^2 = 7.52, p < 0.05).

DISCUSSION

Despite the high sampling effort, we found no eggs on leaves during the field work. In the laboratory, only one egg was laid on the leaf of the host plant, and the remaining eggs were laid on the plastic pot walls. This suggests that oviposition may occur on another plant structure, such as the stem or outside of the host plant (see Bernays & Singer 2002). Similar biological characteristics have been found in another species, *Idalus agastus* Dyar 1910, which has a similar egg morphology and the same number of instars (see Carlos 2011). However, the development time of *I. lineosus* from larval first instar to adult was approximately 15 days shorter than that obtained for *I. agastus* and approximately 25 days longer than that obtained for *I. admirabilis* (Cramer 1777) (Santos et al. 2006; Carlos 2011). The caterpillar of the early instars remains on the abaxial leaf, and this may be a strategy to avoid dehydration because at this time of the year the cerrado has very low rainfall and relative humidity.

The larval morphological variation found among instars of *I. lineosus* is a common feature in the caterpillars of Arctiinae (Santos et al. 2006, Pereira et al. 2007, Rodríguez-Loeches & Barro 2008). As observed in other caterpillar species, certain characters may change during their development. These variations may include the color of the tegument; density, length, and color of the secondary setae; number of verrucae (Rab Green et al. 2011), and the arrangement of the setae (Rodríguez-Loeches & Barro 2008), and in many species of Arctiinae the first instars are completely different from the last instars (Rab Green et al. 2011).

The last larval instar of *I. lineosus* presented a phenotypic variation in color, a trait that has been described for other species of Arctiinae (Wagner 2005); this is considered a common event in lepidopteran (Greene 1996, Hazel 2002; Suzuki & Nijhout 2006, Noor et al. 2008, Yamasaki et al. 2009). Several
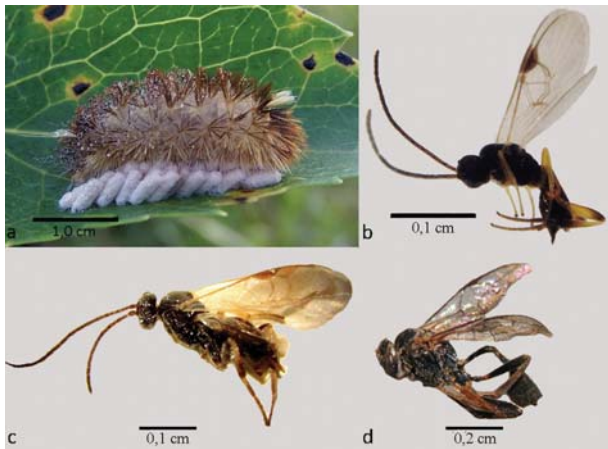
Fig. 6. (**a**) Parasitized sixth instar caterpillar of *Idalus lineosus*; (**b**) *Protapanteles* sp., the most frequent parasitoid of *I. lineosus* (Hymenoptera: Braconidae, Microgastrinae); (**c**) *Apanteles* sp.; (**d**) *Pristomerus* sp.

explanatory mechanisms for this phenomenon have been proposed. In many generalist caterpillar species, the color of the tegument varies due to the pigment present in the host plants (Greene 1989, Monteiro 1991, Ahmad 1992, Burghardt et al. 2001, Sandre et al. 2007a, Canfield et al. 2009). Other factors that may also influence this color variation include thermoregulation (Sandre et al. 2007b), seasonal hormonal regulation (Rountree & Nijhout 1995), and ontogenetic causes (Nylin et al. 2001, Grant 2007). For the *I. lineosus* caterpillars, the difference may be related to an ontogenetic process because it was found at sites with similar climate, in the same period of the year, and on a single host plant species. There appeared to be no relation to parasitism because it occurred in caterpillars of all colors, despite the proportion of parasitized caterpillars being low.

The aggregated temporal distribution of *I. lineosus* during the dry season confirms the expected pattern for caterpillars in the cerrado (Morais et al. 1999) and suggests that this species is univoltine. The convergence in the flowering times of the plants in the cerrado in the late dry season and early rainy season (Oliveira 2008) provides increased availability of food resources for adult Lepidoptera during this period. Therefore, the peak of caterpillar relative abundance in the dry season can ensure the occurrence of adults during a highly favorable period and in a probable "period free of enemies" (Morais et al. 1999).

In spite of the low overall proportion of parasitism of caterpillars, it is tempting to relate the higher proportion of parasitism and the lower abundance of caterpillars to the rocky soil cerrado. The higher proportion of hymenopteran attacks to caterpillars compared to dipteran attacks is a common pattern in the cerrado (Scherrer et al. 1997, Pessoa-Queiroz 2008), and this pattern was found for parasitism in *I. lineosus*. In the present study, the most abundant parasitoid, *Protapanteles* sp. Ashmead 1898, described as an endoparasitoid of Lepidoptera (Whitfield et al. 2002, Yu et al. 2005, Penteado-Dias et al. 2011, Souza 2012), was recorded in *I. lineosus* for the first time.

Even in the case of a single caterpillar species consuming only one species of host plant, it is known that factors other than food availability affect the size of populations, e.g., interactions with natural enemies and climatic seasonality, as corroborated in this study. In addition, we present the first biological information on the immature stages of *I. lineosus*. This information is important for understanding tri-trophic interactions (plant–herbivore–parasitoid) in the cerrado. Further descriptions of life histories in many others species whose life histories are currently unknown, it will provide results to facilitate experimental studies and phylogenetic reconstructions.

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