A study of the biology of *Epicharis (Epicharoides) picta* using emergence-traps

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Abstract

This study investigates the nesting habits of *Epicharis picta* in a nest aggregation located in a fragment of the Atlantic forest in Southeastern Brazil. Ten emergence-traps were set up in this nest aggregation to standardize data collection of phenology, natural enemies, and sex ratio. *Epicharis picta* nests were in an area of 160 m² with a density of 41 nests/m². Nest and cell architecture are described. *Epicharis picta* is a protandrous, univoltine species with its emergence in this study occurring between 28 January and 15 April. We provide direct evidence of parasitism on *E. picta* by *Rhathymus friesei*, *Tetraonyx sexguttata* and *T. aff. lycoides*. The predator *Apiomerus lanipes* was found to prey *Epicharis* for the first time. We suggest the use of emergence-traps as tools to support studies of ground-nesting bees. In addition, we compile, update, and discuss data on the nesting biology of all *Epicharis* subgenera.

Keywords

Cleptoparasitism, Emergence-trap, Ground-nesting bees, Nest architecture, Solitary bees

Introduction

Solitary bees use a wide variety of nesting substrates, digging their nests in soil or wood, constructing freestanding nest, or using pre-existing cavities (Michener 1974, 2007). In fact, most of them nest in the ground (Linsley 1958; Batra 1984; Roubik 1989; Michener 2007), which may be a plesiomorphic condition among bees.
This variety of nesting habits gives rise to a wide diversity of life cycles, nest architecture, nesting behaviors, and relationships with their natural enemies.

Centridini is a neotropical tribe of oil-collecting bees, composed of only two well-supported monophyletic genera (Moure et al. 2012; Bossert et al. 2019) of solitary bees: *Epicharis* Klug, 1807, which dig their nests in the soil; and *Centris* Fabricius, 1804, composed mostly of some species that dig their nests in the soil or sometimes in termite nests (e.g., Rozen and Buchmann 1990; Gaglianone 2001; Aguiar and Gaglianone 2003; Rozen et al. 2011) and some that use pre-existing cavities (e.g., Costa and Gonçalves 2019). Even though nesting habits tend to be constant in some species, *Centris*’ nesting behavior has some plasticity (Vinson and Frankie 1991; Martins et al. 2014).


Univoltinism is the phenological pattern observed for most species of *Epicharis* (Roubik and Michener 1980; Raw 1992; Hiller and Wittmann 1994; Gaglianone 2005; Gaglianone et al. 2015; Vivallo 2020a, b), except multivoltinism for *E. flava* (Camargo et al. 1975) and bivoltinism for *E. (Epicharis) bicolor* Smith, 1854 (Rocha-Filho et al. 2008). Adult short-term activity may be a strategy that minimizes exposure to attacks by parasitic species (Wcislo 1987).

The compilation made by Gaglianone (2005) on the data known for *Epicharis*’ nesting biology, does not point to any clear patterns among its nine subgenera. Hence there is a need for an increase in the quantity of species studied, particularly with regard to number of generations per year, presence or absence of diapause in the immature stages, type of soil used as substrate, depth of brood cells, number of cells per nest, plus other biological data that may aid us comparisons within and between subgenera of *Epicharis*. In addition, a phylogenetic approach of these characters compared to phylogenetic studies on Centridini (e.g., Martins and Melo 2016) can elucidate evolutionary aspects of these bees.

The natural enemies of *Epicharis*, include parasitoids, cleptoparasitic, and predatory insects. Bees of the genus *Rhathymus* (Apidae, Rhathymini) are known to be specialized cleptoparasites of *Epicharis*’ nests (compiled by Werneck et al. 2012),
although there are also more generalized cleptoparasites, such as those of the genus *Mesoplia* Lepeletier, 1841 (Apidae, Eriococidini) which also attack nests of *Centris* (Gaglianone 2005; Rocha-Filho et al. 2008; Rocha-Filho et al. 2009; Vivallo 2020a). Besides bees, there are records of *Epicharis* as a host for both cleptoparasitic species of Meloidae (Coleoptera) and parasitoid species of Conopidae (Diptera) and Mutillidae (Hymenoptera) (Gaglianone 2005; Rocha-Filho et al. 2008; Gaglianone et al. 2015). Despite the scarcity of information in the literature, species of Hemiptera, especially those of the genus *Apiomerus* (Reduviidae), are known to be predators of adult bees (Silva and Amaral 1973; Cane 1986; Amaral-Filho et al. 1994; Marques et al. 2003, 2006; Silva and Gil-Santana 2004).

Studies on the nesting biology, relationships with natural enemies, and phenology of *Epicharis* species have been performed using direct observations of nest aggregations. Nonetheless, there is a need for methods that provide standardization for data collection. To this aim, emergence-traps have been used in ground-nesting solitary bees and wasps studies and have shown to be effective in answering key questions on the biology of these insects (Hiller and Wittmann 1994; Sardiña and Kremen 2014; Rocha-Filho and Melo 2017; Cope et al. 2019).

*Epicharis picta* occurs in Uruguay, Paraguay, Argentina, and Brazil (Federal District and the states of Espírito Santo, Minas Gerais, Paraná, Paraíba, Rio de Janeiro, Santa Catarina, and São Paulo – Moure et al. 2012). Its life cycle is univoltine (Gaglianone et al. 2015) and it is oligoletic on Malpighiaceae (Werneck et al. 2015). However, data on its nesting behavior, nests and cell architecture, and natural enemies are scarce in the literature.

In this study, we investigated the nesting habits of *Epicharis picta* for two years and provide information on its biology, natural enemies, nest and cell architecture, sex ratio, and phenology, based on direct observations and emergence-traps data. An updated compilation of nesting biology and nest architecture data of the *Epicharis* genus is also provided.

### Material and methods

#### Study site

The nest aggregation of *Epicharis picta* studied was in a fragment of semideciduous, montane and submontane Atlantic Forest (Veloso et al. 1991), in the municipality of Viçosa, Minas Gerais-Brazil, on the access road to the Estação de Pesquisa, Treinamento e Educação Ambiental Mata do Paraíso (20°47’56”S, 42°52’07”W) (see Gaglianone et al. 2015). The climate of the region is temperate, rainy (mesothermal), with hot and rainy summers, and cool and dry winters (type Cw, according to the classification of Köppen – Kottke et al. 2006). The climatological data of the region between 2009 and 2011 are shown in Fig. 1.
Nesting biology, nest architecture, and brood cells

The fieldwork was carried out for two years. In 2010, visits took place monthly; and in 2011, daily, during the period of activity of the adult bees. The nesting habits of *E. picta* were obtained from direct observations throughout each day from 5:30 h to 19:00 h. To study the brood cells, eight excavations were made in 50 cm × 50 cm plots. The brood cells found in the soil were collected, placed in plastic pots with substrate from the nesting site, and kept in the laboratory to assess their content and dimensions. For nest architecture, as the main burrow remains open after a nest is completed, we injected plaster in five nests. Ten plots of 1 m² were randomly set up in the aggregation to estimate nest density.

Emergence-traps, natural enemies, and associated species

To standardize the sampling method when studying phenology, sex ratio, and parasite-host synchrony, 10 emergence-traps were randomly set up at the nesting site from 20 January to 20 May 2011 (Fig. 2A). The emergence-traps consisted of a pyramid-shaped wooden frame covered with thin nylon mesh, with the following dimensions: 50 cm × 50 cm base, 10 cm × 10 cm top end, and 50 cm height (Fig. 2B). On one side, a 12 cm long opening was made in the longitudinal direction to install a Velcro...
Biology of Epicharis picta

Figure 2. Emergence-traps set up on aggregate of Epicharis (Epicharoides) picta (A); Detail of emergence-trap (B).

Data on potential natural enemies and associated species that were seen visiting the nest aggregation area were collected throughout the study period. Vouchers from the specimens studied are deposited at Museu Regional de Entomologia, Departamento de Entomologia-Universidade Federal de Viçosa (MEUFV). This study follows the classification system of Moure et al. (2007), which treats the whole group of bees as a single family (Apidae).

Results

Nesting biology, nest architecture, and brood cells

Epicharis picta nests were aggregated in an area of approximately 160 m² of exposed slopes of about 45°, with an average nesting density of 41 entrances/m². Females began their activities between 6:00 h and 6:30 h and ended between 18:00 h and 18:30 h, daily. The activity peak occurred between 7:30 h and 11:30 h. The females rested inside the nests at night (Fig. 3A). Male activity period was shorter, between 6:00 h and 16:00 h. The mating male behavior is being dealt in a separate study. Every day at the beginning of activities (~6:00 h), females took about five minutes at the nest entrance before making their first flight. After their first trip, their scopae were still clean when they returned, with no evidence of oil or pollen having been collected (Fig. 3B). For digging new nests, they selected a new nest site, excavated the soil vertically, using their mandibles, anterior and middle legs, and deposited the material from the excavation around the nest entrance, forming a circular tumulus (Fig. 3C, D).
Figure 3. Nesting habits and nest architecture of *Epicharis* (*Epicharoides*) *picta* A female resting inside the nests at night B female of *E. picta* excavating nest C, D entrance of the nests showing the presence of a tumulus E, F architecture of 110 cm deep nests.
The excavated nests (\(N = 8\)) contained one to two cells each. In nests with only one cell (\(N = 6\)), there was a single tunnel (Fig. 3E, F), whereas in nests containing two cells at the end of the main tunnel (\(N = 2\)), a branch with no cell was also found. The diameter of these tunnels ranged from 10 mm to 12 mm (\(N = 20\)). The nests were perpendicular to the surface, ranging from 50 cm to 110 cm deep.

The brood cells were slightly curved (Fig. 4A–E) and built at an angle of about 45° to the ground surface. Their outer walls are rough and rigid, whereas inner walls are smooth, shiny, and hydrophobic (Fig. 4A–C). The cell cap is inserted below the apex of the cell wall and is slightly inclined (Fig. 4D). The cells size ranged from 20 mm to 27 mm (\(X = 24.57\) mm; \(N = 27\)) in length, between 10 mm and 12 mm (\(X = 11.12\) mm; \(N = 27\)) in diameter at cell cap height, and the base diameter between 12 mm and 14.5 mm (\(X = 13.74\) mm; \(N = 27\)).

Females of *Epicharis (Epicharoides) albofasciata* were observed founding nests (\(N = 14\)) in the aggregation of *E. picta*. Only one nest of *E. albofasciata* was excavated, and it consisted of a single 35 cm deep tunnel with one cell at its end. Agonistic
behavior among females of *E. picta* and *E. albofasciata* was observed when females returned from the field. *E. albofasciata* males were neither observed nor collected on the nest aggregation during fieldwork.

**Emergence-traps, phenology, and sex ratio**

Ten emergence-traps were set in the aggregation for 121 days, between 20 January and 20 May 2011. The emergence peak in the traps was from 28 January to 15 April 2011. The emergence period in the traps was from 28 January to 15 April 2011. The emergence peak, encompassing all species, occurred from 19 February to 18 March (Fig. 5A). From the 271 individuals that emerged in the emergence-traps, *E. picta* was the most abundant species (211, 78%), followed by *Rhathymus friesei* (23, 8.5%), *Tetraonyx sexguttata* (Meloidae) (18, 6.65%), *Physcephala* sp. (Conopidae) (7, 2.55%), *Tetraonyx aff. lycoides* (5, 1.85%), *E. albofasciata* (3, 1.10%), *Augochlora thalia* (Apidae, Augochlorini) (2, 0.71%), *Acamptopoeum prinii* (Apidae, Calliopsini) (1, 0.32%), and *Epinysson* sp. (Crabronidae, Nyssonini) (1, 0.32%).

*Epicharis picta* first appeared in the emergence-traps on 29 January, and males were the first to emerge (Fig. 5B), indicating protandry. From a total of 211 individuals, 111 were male (52.6%), and 100 female (47.4%). Thus, the sex ratio of *E. picta* was 1.11 males to 1 female. *Rhathymus friesei* emerged from 5 February to 18 March, peaking from 26 February to 11 March. Males were the first to emerge, on 5 February, whereas females emerged from 19 February, which also indicates protandry (Fig. 5C). From the 23 emerged individuals, 13 were males (56.53%) and 10 were females (43.47%), resulting in a sex ratio of 1.3 males to 1 female.

**Natural enemies and associated species sampled from direct observations**

In addition to species sampled from the emergence-traps, we collected another 24 species of insects found in the area, which were then identified and classified according to their association with the nesting aggregation (Table 1). *Rhathymus friesei* was the most abundant natural enemy (Fig. 6A); in some moments, up to four individuals could be seen inspecting the nest aggregation at the same time. *Tetraonyx sexguttata* was observed walking on the aggregation, landing on vegetation, and emerging from *E. picta* nests (Fig. 6B). Individuals of *Apiomerus lanipes* were observed five times near the entrances of the nests capturing females of *E. picta* when those entered or left their nest. The predator attacked the prey with its forelegs, inserting the stylet between the thorax and the head (Fig. 6C). Twenty-seven individuals of Mutillidae were collected in the aggregation. However, only one female of *Traumatomutilla* sp. was observed directly inspecting *E. picta* nests (Fig. 6D). *Physcephala* sp. specimens were also observed flying over the nest site between 5 February and 22 March.

Females of *Augochloropsis* cf. *cupreola* (Apidae, Augochlorini), *Hypanthidium nigritulum* (Apidae, Anthidini), and *Colletes petropolitanus*, (Apidae, Colletini) were observed performing inspection flights over the soil and branches of vegetation in the aggregation, but they neither nested nor interacted with *E. picta* females. *Trigona spinipes*
Figure 5. Emergence patterns in the emergence-traps set up on the nest aggregation of *Epicharis* (*Epicharoides*) *picta*. A emergence of all species sampled in the traps B emergence of the males and females of *E. picta* C emergence of the males and females of *Rhathymus friesei*. 
(Apidae, Meliponini) workers landed on the nest site and collected soil material removed by *E. picta* females during the excavation of their nests. A female of *Mesoplia rufipes* was collected on 20 March 2010. This bee flew over the aggregation and periodically approached some entrances of *E. picta* nests. However, it was not seen entering any nest.

### Brood cells collected during excavations

In the two years of studies, a total of 121 cells were collected, ranging between 30 cm and 110 cm deep. From these, 45 were already open, containing only soil in their interior. Six cells were taken by fungi, one of which contained a dead *E. picta* pupae (Fig. 7A). Another six cells containing only fungi on the food (Fig. 7B) with no evidence of dead larvae or egg, nor parasitic traces. In one cell there was an exoskeleton of *T. sexguttata* (Fig. 7C). In the 46 cells, there were 38 mature larvae (Fig. 4B) and eight pupae of *E. picta*. From the 17 cells kept in the laboratory throughout
the year, four males (Fig. 7D) and six females of *E. picta*, two *T. sexguttata*, and one *T. aff. lycoides* emerged. In the remaining four cells, there were mature larvae of *R. friesei*. Larvae of *R. friesei* were easy to identify due to the presence of their cocoon (see Rozen 1969; Camargo et al. 1975; Werneck et al. 2012).

**Discussion**

Studies reporting biological data about *E. picta* are recent in the literature (Werneck et al. 2012; Werneck et al. 2015; Gaglianone et al. 2015). These, however, do not address aspects related to their nesting habits, natural enemies, and associated species. Therefore, this present study is the first to bring such data, in addition to using emergence-traps as a model.

**Nesting biology and notes on patterns among Epicharis subgenera**

Our data on *E. picta* reinforce the hypothesis that all species of *Epicharis* nest gregariously in the soil (Vesey-FitzGerald 1939; Michener and Lange 1958; Rozen 1965;
In spite of the low quantity of species studied, it is possible to point to a pattern on the exclusive use of sandy soil for nesting by the subgenera *Epicharoides*, *Epicharis*, *Triepicharis* and *Anepicharis* (Table 2). Although *Parepicharis* also uses sandy soil, there are records of *E. metatarsalis* nesting in clay (Thiele and Inouye 2007). For *Epicharana*, there seems to be some preference over nesting in low light places, as recorded for *E. flava* (Camargo et al. 1975), *E. rustica*, and *E. elegans* (Michener and Lange 1958). Despite that, it is not possible to point to any patterns regarding nest depth, which can vary according to the characteristics of each site used for nesting (Cane 1991), nest density, and the number of brood cells per nest. A study of phylogenetic reconstruction based on nesting behavior characters encompassing the whole Centridini tribe is under way (Werneck HA, unpublished data). As a result, we expect a better understanding of the patterns and evolution of these characteristics. For a comparison of the characteristics regarding nesting habits amongst the species of *Epicharis* studied, see Table 2.

**Figure 7.** Content of *Epicharis* (*Epicharoides*) *picta* brood cells A dead *E. picta* female pupa with fungus B food in *E. picta* brood cell taken by fungus C exoskeleton of *Tetraonyx sexguttata* D male of *E. picta* emerged from brood cell maintained in laboratory.
**Table 2.** Compilation of comparative data on nesting biology of the genus *Epicharis*.

<table>
<thead>
<tr>
<th></th>
<th><em>E. (Epicharoides) picta</em></th>
<th><em>E. (Epicharoides) albofasciata</em></th>
<th><em>E. (Epicharoides) bicolor</em></th>
<th><em>E. (Epicharoides) nigritae</em></th>
<th><em>E. (Parepicharis) metatarsalis</em></th>
<th><em>E. (Parepicharis) zonata</em></th>
<th><em>E. (Triepicharis) analis</em></th>
<th><em>E. (Anepicharis) dejani</em></th>
<th><em>E. (Epicharana) flavida</em></th>
<th><em>E. (Hoplepicharis) fasciata</em></th>
<th><em>E. (Epicharitides) obscura</em></th>
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<tr>
<td><strong>Phenology</strong></td>
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<td>univoltine</td>
<td>bivoltine</td>
<td>univoltine</td>
<td>univoltine</td>
<td>univoltine</td>
<td>univoltine</td>
<td>univoltine</td>
<td>univoltine</td>
<td>univoltine</td>
<td>univoltine</td>
</tr>
<tr>
<td><strong>Nesting place</strong></td>
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<td>slanted</td>
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<td>flat</td>
<td>flat</td>
<td>flat</td>
<td>flat</td>
<td>vertical</td>
<td>vertical</td>
<td>vertical</td>
<td>flat</td>
</tr>
<tr>
<td><strong>Soil type</strong></td>
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<td>sandy soil</td>
<td>sandy soil</td>
<td>clay and sandy soil</td>
<td>sandy soil, seasonally swamp</td>
<td>sandy soil</td>
<td>sandy soil</td>
<td>basalt afloration</td>
<td>earth bank/sandy soil</td>
<td>wet soil</td>
<td></td>
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<td><strong>Nest arrangement</strong></td>
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<td>aggregated</td>
<td>aggregated</td>
<td>aggregated</td>
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<td>aggregated</td>
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<tr>
<td><strong>Nest type</strong></td>
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<td>one tunnel</td>
<td>one tunnel</td>
<td>one tunnel, branched</td>
<td>one tunnel, branched</td>
<td>one tunnel, branched</td>
<td>one tunnel, branched</td>
<td>branched</td>
<td>one tunnel, branched</td>
<td>one tunnel</td>
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<tr>
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<td>25/m²</td>
<td>12/m²</td>
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<td>1–25/m²</td>
<td>?</td>
<td>1.5/m²</td>
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<td><strong>Cell arrangement</strong></td>
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<td>isolated, end of tunnels</td>
<td>linear</td>
<td>isolated, end of tunnels</td>
<td>isolated, end of tunnels</td>
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<td>vertical</td>
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<td><strong>Cell per nest</strong></td>
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<td>1–6</td>
<td>1–2</td>
<td>1–5</td>
<td>1</td>
<td>1–7</td>
<td>2–5</td>
<td>1–10</td>
<td>1–3</td>
<td>1–2</td>
<td>1</td>
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<tr>
<td><strong>Cell length</strong></td>
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<td>15–23 mm</td>
<td>21 mm</td>
<td>20–25 mm</td>
<td>?</td>
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<td>29–36 mm</td>
<td>23–25 mm</td>
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<tr>
<td><strong>Cell diameter</strong></td>
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<td>9.5–11.5 mm</td>
<td>14.5 mm</td>
<td>20–25 mm</td>
<td>?</td>
<td>14–20 mm</td>
<td>13–18 mm</td>
<td>19–20 mm</td>
<td>13–15 mm</td>
<td>13–15 m m</td>
<td>12.5 mm</td>
</tr>
<tr>
<td><strong>Cell depth</strong></td>
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<td>16–35 cm</td>
<td>10–25 cm</td>
<td>16–60 cm</td>
<td>62–120 cm</td>
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<td>30–45 cm</td>
<td>25–140 cm</td>
<td>110 cm</td>
<td>30 cm</td>
<td>10–30 cm</td>
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*Table adapted and updated from Gaglianone (2005);

This study; †Gaglianone (2005); ‡Rozen (2016); §Rocha-Filho et al. (2008); ¶Martins et al. (2019); ¶Thiele and Inouye (2007); ¶Roubik and Michener (1980); ¶Law (1992); ¶Hiller and Wittmann (1994); ¶Dec and Vivallo (2019); ¶Camargo et al. (1975); ¶Vivey-FitzGerald (1939); ¶Laroca et al. (1993); ¶Vivallo (2020a); ¶Vivallo (2020b)

The data on *E. (Triepicharis) analis* were joined with those on *E. (Triepicharis) schrottkyi*, described by Gaglianone (2005), due to the proposal of *E. schrottkyi* as a junior synonym for *E. analis* by Vélez and Silveira (2006).

* Cited by Raw (1992) as *E. (Anepicharis) melanoxantha*. Werneck et al. (2012) reported that *E. melanoxantha* corresponds to *E. dejani*. Here, we joined the data from Raw (1992) with the available data for *E. dejani*;
Species that nest in the soil in aggregations might also build their nests in the nest aggregations of other species (Michener 1974). For instance, there are reports of *E. albofasciata* building their nests in *E. nigrita* (Gaglianone 2005) and *E. picta* (this study) nest aggregations. However, these bees can build their own nest aggregation, as described by Rozen (2016). The characteristics described by Rozen (2016), such as nest depth, presence of a tumulus around the entrance of each nest, female preference for inclined sites (about 45°), and shape and composition of the brood cells, corroborate the findings and allow us to point out that these characteristics may be diverse within *Epicharoides*.

The development of immature stages seems to be more constant within *Epicharis*. Both *E. picta* and *E. albofasciata* present the same hatching pattern, with the presence of a pharate first instar, which is also recorded for *E. flavo* and *E. nigrita* (Camargo et al. 1975; Gaglianone et al. 2015). Pharate first instar larvae were also observed for *Centris flavofasciata* Friese, 1899 (Rozen et al. 2011), in addition to other groups of solitary bees that nest in the soil, such as *Monoeca haemorrhoidalis* (Smith, 1854) (Apidae: Tapinotaspidini) (Rozen et al. 2006).

**Emergence-traps and phenology**

Emergence-traps have been effective in collecting data on species of bees and wasps nesting in the soil (Hiller and Wittmann 1994; Sardiña and Kremen 2014; Rocha-Filho and Melo 2017; Cope et al. 2019; Martins et al. 2019). In the present study, these emergence-traps were useful for sampling data on natural enemies that emerged in the nest aggregation as well as in measuring the phenology and sex ratio of *E. picta*. We were able to measure the emergence patterns of both the host and its natural enemies.

Our data from emergence-traps corroborate the hypothesis that most *Epicharis* species are univoltine (see Gaglianone et al. 2015). The beginning of the emergence of *E. picta* occurred during the period of high temperatures and rainy season. The adults remained active until the middle of April, when the temperatures decreased and the rainfall declined drastically (see Fig. 1). These abiotic factors combined with other biotic features may influence in the diapause of *Epicharis*. A discussion about the biotic and abiotic factors that can influence the diapause process, and, consequently, the phenology of *Epicharis* species, can be seen in Gaglianone et al. (2015).

The emergence of *Rhathymus friesei* occurred about one week after the beginning of the *E. picta* adult activity. As cleptoparasitic species require provisioned brood cells from their hosts to oviposit (Wcislo 1987; Rozen 2001; Michener 2007), this emergence pattern was expected. Another fact that reinforces this pattern is that *Rhathymus* females lay their eggs in cells closed by the host female (Camargo et al. 1975; Rozen 1991). The strategy of parasitizing cells closed by the host is a plesiomorphic feature among the cleptoparasitic species that might have evolved from nest-building species, which could have five independent origins within bees (Litman et al. 2013).

*Tetraonyx* species reported in this study emerged in the same period of *E. picta*. These claptoparasites, unlike *Rhathymus*, are not considered specialists of *Epicharis* species. *Tetraonyx* spp. parasitize bees that nest in the soil (Roubik 1989; Gaglianone
2005; Rocha-Filho et al. 2008; Rocha-Filho and Melo 2011; Gaglianone et al. 2015), in addition to other bees that nest in trap-nests (Morato et al. 1999; Parizotto 2019).

Augochlora thalia and Acamptopoeum prinii, and Epinysson sp. were collected in emergence-traps. These species nest in the soil and presumably were just opportunistically using the Epicharis nest site. The issue of nesting in nest aggregations of other species may be related to the strategy of inhibiting parasite attacks (Wcislo 1987; Wcislo and Cane 1996). Physocephala sp., also collected in the emergence-traps, is a parasitoid of bees and is discussed in the next section of this study.

Some natural enemies do not emerge in the same nest aggregation in which they attack their hosts. This can be a problem when the inference about the relationships between natural enemies and hosts is conducted by direct observations. Therefore, the use of emergence-traps allowed us to determine which species of natural enemies actually emerged from the nest aggregation. On the other hand, as in this study we have a mixed species nest aggregation, there were limitations to determine the type of association among some species that emerged in the emergence-traps (see Table 1).

In summary, we suggest that emergence-traps are tools that can aid in studies of solitary bees nesting in the soil. This method allows the measurement of phenology, parasite-host synchrony, parasitism rate, and sex ratio.

Natural enemies and species associated with the nest aggregation of E. picta

Many natural enemies are reported for Epicharis species, but there is direct evidence only for Rhathymus spp. and Tetraonyx spp. (Werneck et al. 2012; Gaglianone et al. 2015; this study). Indirect evidence, however, is reported for species of Mesoplia and Mesonychium (Apidae, Ericrocidini), Physocephala spp. and several species of Mutilidae (Camargo et al. 1975; Hiller and Wittmann 1993; Gaglianone 2005; Rocha-Filho et al. 2008; Luz et al. 2016). Regarding cleptoparasitism on E. picta, our data show that there is direct evidence only for R. friesei, T. sexguttata and T. aff. lycoides. Rhathymus friesei was the most abundant cleptoparasitic species observed in this study. Even though it is not possible to determine specificity relationships between Rhathymus species and Epicharis subgenera, there is specificity in the cleptoparasite-host relationship between the genera Rhathymus and Epicharis (Werneck et al. 2012). On the meloid beetles, there are data on the relationship of Tetraonyx spp. to E. dejeanii Lepeletier, 1841 (Hiller and Wittmann 1994), E. nigrita (Gaglianone 2005; Martins et al. 2019), E. bicolor (Rocha-Filho et al. 2008), and E. picta (Gaglianone et al. 2015).

Physocephala is a genus composed of parasitoid species that mainly attack adult Hymenoptera. Among neotropical bees, the host records of these Conopidae are for Bombini, Centridini, Euglossini, Megachilini, Tapinotaspidini, and Xylocopini (Rasmussen and Cameron 2004; Melo et al. 2008; Santos et al. 2008; Rocha-Filho and Melo 2011; Stuke et al. 2011; Almada et al. 2020). For Epicharis, there are records from indirect evidence that P. bipunctata may parasitize E. bicolor (Rocha-Filho et al. 2008; Santos et al. 2008). Although data from emergence-traps record Physocephala sp., our evidence is indirect, not corroborating this relationship of parasitism on E. picta.
The Mutillidae is composed of parasitic wasps that attack Hymenoptera in general, with records for bees as hosts (Brothers et al. 2000; Luz et al. 2016). Although some studies report the parasite-host relationship between these wasps and *Epicharis*, there is no direct evidence of this relationship (Rocha-Filho et al. 2008). Luz et al. (2016) compiled the data known for host bees of these wasps and these authors consider Apidae to be the main hosts of Mutillidae in the Neotropical region. For *Centris*, nine records of Mutillidae species as parasitoids are known (see Luz et al. 2016), whereas for *Epicharis*, the only record is for *Hoplomutilla myops myops* (Burmeister, 1854), considered as a potential parasitoid of *E. bicolor* (Rocha-Filho et al. 2008). In the present study, it was only possible to observe *Traumatomutilla* sp. approaching the nests of *E. picta*, although it was not seen entering nor leaving the nests.

*Apiomerus* are predators, some species being reported as common predators of bee species, such as stingless bees (Apidae, Meliponini) (Silva and Gil-Santana 2004), and *Apis mellifera* Linnaeus, 1758 (Apidae, Apini) (Silva and Amaral 1973; Amaral-Filho et al. 1994; Marques et al. 2003, 2006). In this study, we show direct evidence that this hemipteran preys on *E. picta*. Until now, no species of *Epicharis* had been associated with this predator. Data on the biology of *A. lanipes* and its mode of predation are still scarce in the literature and it will be necessary to study whether it is a predator specialized in bees.

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