Journal of Apicultural Research, 2020
https://doi.org/10.1080/00218839.2020.1717760

ORIGINAL RESEARCH ARTICLE

Nesting biology of *Centris* (*Paracentris*) *burgdorfi* (Apidae: Centridini)

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(Received 18 January 2019; accepted 26 July 2019)

We describe the nesting biology of *Centris* (*Paracentris*) *burgdorfi*, a solitary bee that nests in sandstone in northeastern Brazil. The nest consists of a shallow tunnel with access to the brood cells. Females of *C. burgdorfi* made 1–7 brood cells per nest with each cell requiring 2.58 ± 0.40 ($\overline{X}$ ± SD) days to construct. The average cell-building construction time was longer when compared to other *Centris* species. Females were larger than males, and this difference was reflected in the size of their respective emergence cells. The temperature within *C. burgdorfi* nests was lower when compared to ambient temperature. Our study is the first to report the nesting biology of *C. burgdorfi*. The detailed behavior of the female inside the nest was also described, which is unusual in the study of solitary bee nesting biology.

Keywords: foraging behavior, nesting behavior, oil-collecting bees, sex ratios, solitary bees

Introduction

Many bees within the tribe Centridini are known as oil-collecting bees, because females gather and use floral oils as resources in brood cell construction (hardened and hydrophobic cell linings) and/or high caloric and nutritious food for their larvae (Buchmann, 1987; Vogel, 1974). The genus *Centris* Fabricius comprises about 250 species distributed from Argentina to Mexico and the southwestern United States. The subgenus *Centris* (*Paracentris*) Cameron is more common in semi-arid regions, including the Sonoran Desert of northern Mexico and southwestern United States (Alcock, Jones, & Buchmann, 1976; Rozen & Buchmann, 1990), Andean regions, and the semiarid region of northeastern Brazil (Vivallo & Zanella, 2012; Zanella, 2002).

All species of oil-collecting bees are solitary, although females of some species form dense nesting aggregations. Centridine bees are important pollinators of wild and cultivated species (Freitas & Paxton, 1998; Oliveira & Schlindwein, 2009) and some plants (e.g., *Malpighia*, *Byrsonima*) depend exclusively on the pollination services of *Centris* bees because of their specialized oil-foraging behavior (Aguiar, 2003).

In Brazil, the best-studied *Centris* species are those that nest in vacant beetle burrows in dead trees, pre-existing cavities, and especially in the drilled holes of wooden trap nests, such as *C. analis* Fabricius and *C. tarsata* Smith (Garófalo, Camillo, & Serrano, 1989). Studies of ground-nesting species are less common (Aguiar & Gaglianone, 2003). In *Paracentris*, there are studies of only 6 other species (out of 59): *C. nigerrima* Spinola (Janvier, 1926) in Chile, *C. autrani* Vachal (probably *C. neffi* Moure) in Bolivia (Janvier, 1955), *C. rhodopus* Cockerell, *C. cockerelli* Cockerell, *C. caesalpiniae* Cockerell and *C. pallida* Fox (Rozen & Buchmann, 1990) conducted in Arizona, USA. In Brazil, we found a nesting aggregation of *Centris* (*Paracentris*) *burgdorfi* Friese, in northeastern Natal, in the state of Rio Grande do Norte (RN), Brazil. This species is widely distributed, occurring in other states within the northeastern region and central and south Brazil (Silveira, Melo, & Almeida, 2002; Zanella, 2002).

This study investigates the nesting biology of *C. burgdorfi* in a stabilized sandstone dune ("fossilized dunes") region of coastal Brazil. To our knowledge, there is no previously published information about the nesting behavior of *C. burgdorfi*. The mating biology and the plasticity of the trophic niche of this bee have been published elsewhere (Sabino, et al., 2018; Sabino, Silva, & Alves-dos-Santos, 2017). Here, we evaluate the activity periods and construction of nests of the species. We sought to answer the following questions: (1) How many nests do a female provision in her lifetime, and how many brood cells do these nests contain? (2) How much time do females spend on nest and cell construction? (3) How long are foraging trips for pollen and oil? (4) How long do females spend provisioning their nests? (5) What are the seasonal emergence and activity periods of the adult bees? (6) Is *C. burgdorfi* protandric with males emerging first? (7) What insect parasites are associated with this species? (8) Do the cells in which males versus females develop differ in size and shape?

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Materials and methods

Centris burgdorfi nesting behavior

This study is part of a thesis conducted in the northeastern of Brazil (Sabino et al., 2017). We located a nesting aggregation of *C. burgdorfi* within a coastal dunes region close to the city of Natal, RN (05°36.310′S, 35°14.435′W; elevation: 41 m). The average annual temperature is 26.7 °C and the rainy season extends from March to August, with an average annual rainfall of 1643.48 mm (INMET/UFRN, 2002).

The nesting season of *C. burgdorfi* is between April and July. The behavior of foraging and nesting females was observed for two successive breeding seasons, during 19 consecutive days of observation in 2014 (April 21st to May 9th—247 h of observations) and 17 consecutive days of observation in 2015 (May 25th–June 10th—208 h of observations). The fieldwork was conducted daily from 04:30 h to 17:30 h. In this location at this time of year, the sun rises at 05:15 h and sets at 17:30 h.

Centris burgdorfi females always built nests in a formation of sandstone dunes. We did not find them nesting anywhere else. The dunes exhibit sharp discontinuities in the ground surface, forming slopes reaching 50-m high (Neto, Costa, Severo, Júnior, & Scudelari, 2005) dating to between the upper Pleistocene and the Holocene (Nogueira, 1982).

Nest architecture and adult emergence

Measurements were made of nest entrances and lateral tunnels leading to brood cells. Some nests ($n = 20$) were excavated completely, and the cells were taken to the laboratory in April 2011, February 2012, and April 2014 for analyses. Brood cells were separated into individual vials and maintained in an incubator (FANEM-347 CDG) at the same internal temperature (about 28 °C) as found within outdoor nests. After all bees had emerged, their sex ratios and parasitism rates were determined. To test for any correlation between the sexes and the sizes of their brood cells, intertegular distance for 25 males and 25 females was measured (Cane, 1987). We measured the height of the cells and the cell diameters at their widest point (base) and opening for 50 cells of each sex. All measurements were performed with a digital caliper (Digimess-100.174BL).

To assess the water retardant nature and resistance of the cell wall, we wrapped ten *C. burgdorfi* cells completely in well-moistened tissue paper inside a vial for 24 h. We then unwrapped the cell and placed a drop of water on the wall inside the cells ($n = 5$) and used a dissection microscope to determine if the droplet was absorbed or not.

Nesting activity of females

The nesting behavior of 20 females that initiated nest excavations was monitored throughout the day (12 females in 2014 and 8 females in 2015). To identify individuals, females were captured and marked on their thoracic dorsa with different color combinations using non-toxic paint pens (Posca®; Tokyo, Japan). We quantified the number of cells each female constructed, as well as the foraging time and the time required to complete the construction of a cell and of a complete nest. We distinguished between floral oil and pollen-collecting trips made by females *C. burgdorfi* through observations of the material on the female scopae. We did not measure the nectar-collecting trips because this behavior is too difficult to visualize since the female can transport nectar together with other materials. Thus, it was possible to quantify the average time spent during oil-foraging trips, lining their cells with oil, the average duration of pollen-collecting trips, and the time females required to close/seal their cells. Based on these data, the total duration of cell construction times was measured, allowing comparisons of the time spent on each activity (Martins, Peixoto, & Aguiar, 2014).

To monitor female activity, we made a small hole in the lateral part of each nest ($n = 20$). The orifice was small enough (about 1.5 cm) to avoid interference in the behavior of the females, and the observation involved a quick inspection, after which the hole was re-sealed with the same sand substrate.

Another five nests were selected to record in detail the construction activity. We filmed the behavior of the nesting females and later analyzed and timed these behaviors in greater detail. These nests were not used for daily monitoring because, for video recording, it was necessary to make a larger hole at the top of the nest (about 3 cm in diameter) and, inevitably, the female abandoned her nest after this larger disturbance was made.

To evaluate the geographical dispersion of nests across the sandstone, we divided the total area into 1 m^2 quadrants (following Martins et al., 2014) and all the active nests within each quadrat were counted.

Temperature data both inside and outside the nests and the humidity of the environment were collected using a standardized digital thermo-hygrometer (Instrutemp-ITHT 2250). The external and internal nest temperatures at an average cell depth were measured every 30 min (from 05:30 h to 17:30 h) for 12 days. Nests for temperature measurements were selected randomly every day, at different heights in the sandstone dunes (lower positions near the ground, and the average middle height and the top of aggregation) to avoid a bias in the analyses. Outside ambient temperature was always measured one meter in front of the same nest that internal temperature was measured.

We also observed the presence of natural enemies, including cleptoparasitic bees, a parasitic wasp, and fly species and their behaviors in the *C. burgdorfi* nesting aggregation.

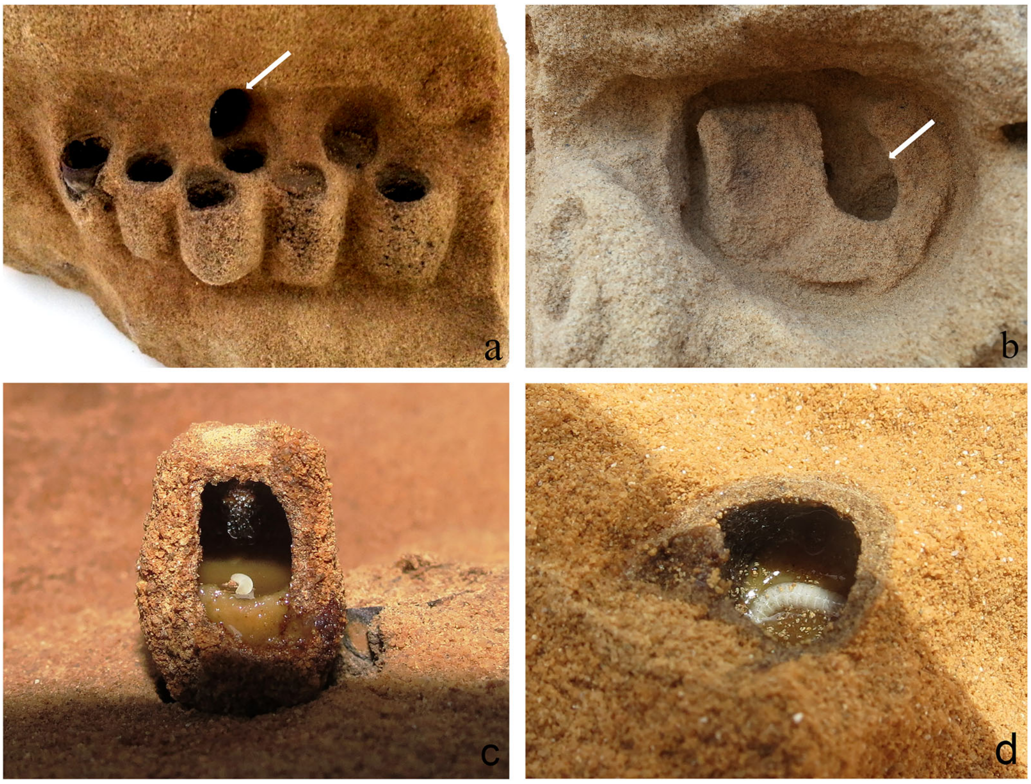

Figure 1. A *C. burgdorfi* nest in the sandstone substrate in northeastern, Brazil, showing (a) the clumped arrangement of seven brood cells constructed by the female with the access tunnel (arrow); (b) a cross section of a cell under construction, showing the cavity excavated by the female (arrow) that would subsequently be lined with oil; (c) a cross section showing the egg deposited on top of the oil and pollen; (d) removal of the cell cap showing second instar of *C. burgdorfi* larva on top of a mass of pollen and oil.

Data analysis

Continuous data are generally reported here as means ± standard errors. A Student *t*-test was used to evaluate the sizes of male versus female bees as well as possible morphological differences between male and female-destined cells.

A Chi-square test was used to assess if the proportion of males and females that emerged in the laboratory differed from a standard 1:1 Fisherian sex ratio.

A Kruskal–Wallis test with Dunn *post hoc* test was used to evaluate the three main activities of females during the construction of a cell [i.e., adding floral oil to form the cell lining, mixing pollen, and sometimes oil, to form larval food (provision) masses, and oil for "operculation"] and the duration of these behaviors inside the nest. A Mann–Whitney *U* test was used to compare the duration of pollen-collection trips with that of oil-foraging trips used for both larval food provisioning and forming the cell wall linings and cell closures (operculation). We used an ANOVA with Tukey *post hoc* test to evaluate the mean number of trips for oil collection for cell lining, for pollen collection, and for oil collection.

A repeated-measures ANOVA, with day as a factor was used, to evaluate differences in the average temperatures of the external environment with temperatures inside the nests.

Statistical analyses were performed using R (R Core Team, 2018) and Statistica 7.0 software, based on Zar (1996) and Sokal and Rohlf (1995).

Results

Architecture of the nests and emergence of individuals

Female bees built nests exclusively in the sandstone portion of the dunes, only in the vertical sides; no nests were observed in the loose sand (mobile or modern dunes). Females excavated horizontal tunnels with small circular entrance holes 1.17 ± 1.05 cm ($n = 134$) diameter leading to a relatively straight horizontal tunnel of 5.60 ± 0.96 cm in length. Immediately after digging the nest burrow the female constructed linearly aligned cells adjacent to each other (Figure 1a,b).

The emergence period extended from the end of March to the end of July 2011, and from the end of March to the beginning of August in 2012. Adults emerged from just 62% of all brood cells collected ($n = 125$), indicating a relatively high level of mortality in the immature stages. The observed male:female sex ratio of 0.825 did not differ statistically from a 1:1 sex ratio (χ^2=0.67, $p = 0.48$; 33 males and 40 females). *C. burgdorfi* is highly protandric, since in all completed nests, males in a given nest always emerged earlier than

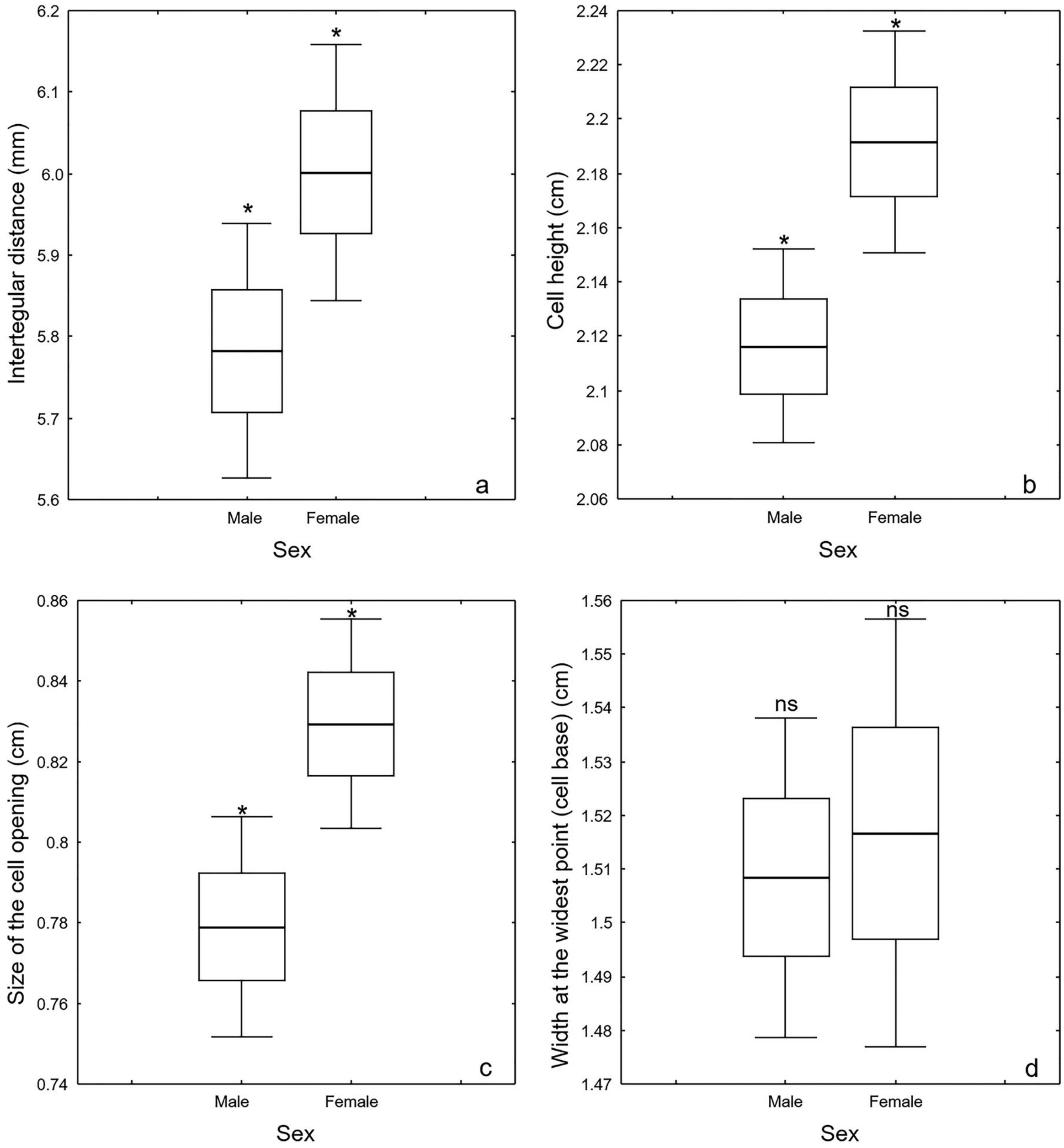

Figure 2. Morphological analysis of *C. burgdorfi* adult bees and 50 brood cells of each sex. (a) Intertegular distance of random adult bees (25 males and 25 females); (b) height of brood cells; (c) diameter of the cell opening; (d) diameter of the cell base. The main horizontal line shows the mean, boxes represent standard error, and whiskers depict 95% intervals. Asterisk symbol (*) represent significant differences and "ns" no significant difference.

females in that nest. Protandry at the level of the population was observed in the field, with only males flying in the nesting site at the beginning of the two breeding seasons.

Females were larger than conspecific males ($t = 2.04$, $p < 0.05$, d.f.=48; Figure 2a), the size difference being reflected in the size of their respective brood cells. Male-destined cells were 2.12 ± 0.13 cm in height ($n = 50$) while female-destined cells were 2.19 ± 0.14 cm in height ($n = 50$). This difference was significant ($t = 2.79$; $p < 0.05$, d.f.=98; Figure 2b). The same sexual dimorphism was observed in the size of the brood cell opening (with the inner part of the cell closure with spiral pattern), with male brood cells averaging 0.78 ± 0.1 cm in diameter ($n = 50$) and female brood cells averaging 0.83 ± 0.09 cm ($t = 2.69$, $p < 0.05$, d.f.=98; Figure 2c). However, there were no differences between the sexes in width of the brood cell at its widest point (typically, the cell base) (males = 1.51 ± 0.1 cm; females = 1.52 ± 0.14 cm; $t = 0.33$, $p = 0.77$, d.f.=98; Figure 2d).

Nesting activity of females

The period of nesting activity in Natal, northeastern Brazil, coincided with the rainy season at this location. Females initiated nest-building immediately following

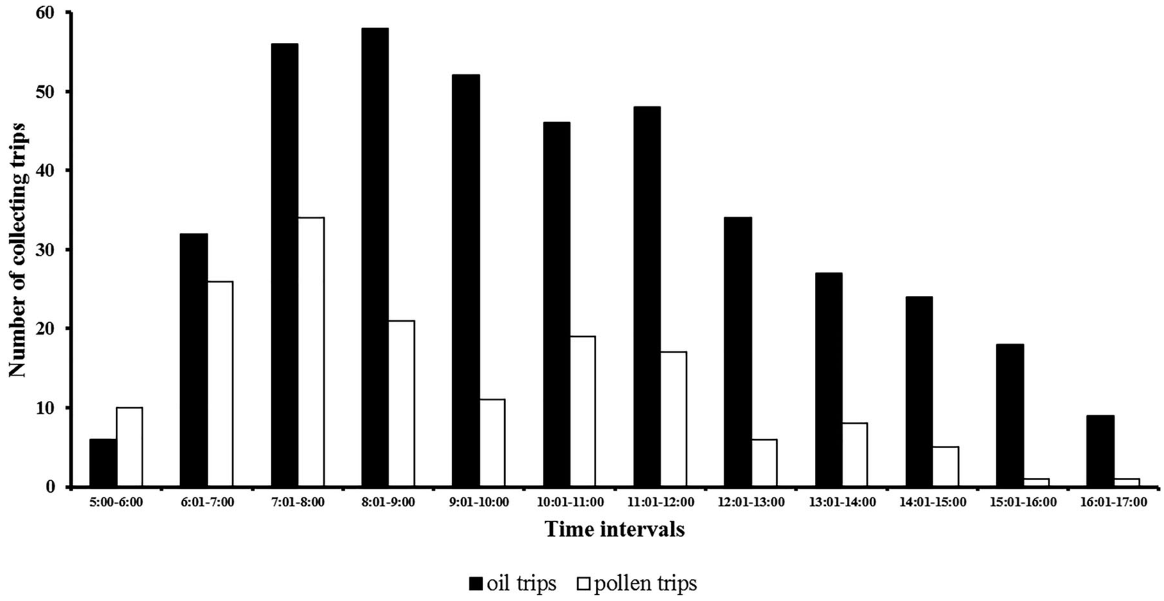

Figure 3. Number of resource collection trips made by *C. burgdorfi* females in a dune region in northeastern, Brazil, separated by time class. Data are pooled across 20 females.

mating, digging their nest. Females were observed reusing tunnels of old nests, by excavating a short lateral tunnel to the new brood cells. We commonly observed nests from other years, which had been exposed to the weather and erosion after the immatures had developed and emerged as new adults. This observation indicates that the same nesting location is being used in multiple years by this species.

The first females were observed at 05:20 h, and the last females at 16:40 h. Despite being active until almost sunset, most construction and provisioning of the nests occurred in the morning hours, between 7 and 10 h (Figure 3).

Nests were built close together in aggregations. The density of nests in these aggregations was estimated to be 9.64 nests/m^2.

The excavation of the tunnel entrance and the excavation and shaping of the first brood cell within a nest required an average of 327.8 ± 79.9 min ($n = 20$). Excavation was characterized by multiple pauses, during which the female left the nest presumably to forage for nectar (and possibly water). It was common to see females initiating an excavation in one place, then soon after, leaving that location to begin another nest somewhere else ($n = 17$). Two marked females took about 2 days to find an ideal place to start their nest excavation.

After digging the main burrow, females began to excavate and shape their brood cells. The cells were barrel-shaped with the bottoms slightly larger than the upper portions (Figure 1b).

Building a brood cell proceeded in the following manner: after digging the entrance tunnel, the female shaped the cell to be used for the floral resource provisions (pollen, nectar, and oil). Females made 9.62 ± 1.81 trips ($n = 91$) to obtain floral oils from *Krameria tomentosa* St. Hill (Krameriaceae) to line the cell walls. Upon returning from a foraging trip, the female always inspected the interior of the cell, then turned around and entered the cell from the front, using her hind legs to attach the sand grains to the top of the cell along with the collected floral oil. Females then went back into the cell and initiated a rotating movement to deposit the mixture of oil + sand (with brushes) evenly over the cell wall.

After lining the brood cell, the female began depositing her scopal pollen load, making an average of 4.91 ± 1.33 ($n = 91$) pollen-collection trips to provision one cell. Pollen deposition involved the female re-entering the cell and making rotating movements while discharging her scopal loads (Figure 4a–d). We observed that the cell linings hardened and became wax-like over a period of several days and also darkened in color with age. In our water droplet test (see Materials and methods), we observed that a droplet of water placed on the inside surface of a cell was not absorbed into the cell lining, indicating the formation of an impervious water barrier likely created by the added floral oils.

The last stage of brood cell construction was the collection and addition of additional *Krameria* floral oil used for larval feeding and construction of the operculum. The female made an average of 2.63 ± 0.77 ($n = 91$) oil-collecting trips to complete the cell. Females initially deposited and mixed a small amount of oil into the pollen mass. The oil was thoroughly mixed with the pollen provisions, resulting in a uniform texture and consistency (Figure 1c,d). After the provisioning of the brood cell, the female began the process of oviposition. The average time to lay the egg was 1.92 ± 0.21 min ($n = 5$). The female then started the operculum by supported herself on the sides of the cell and with a swift rotary

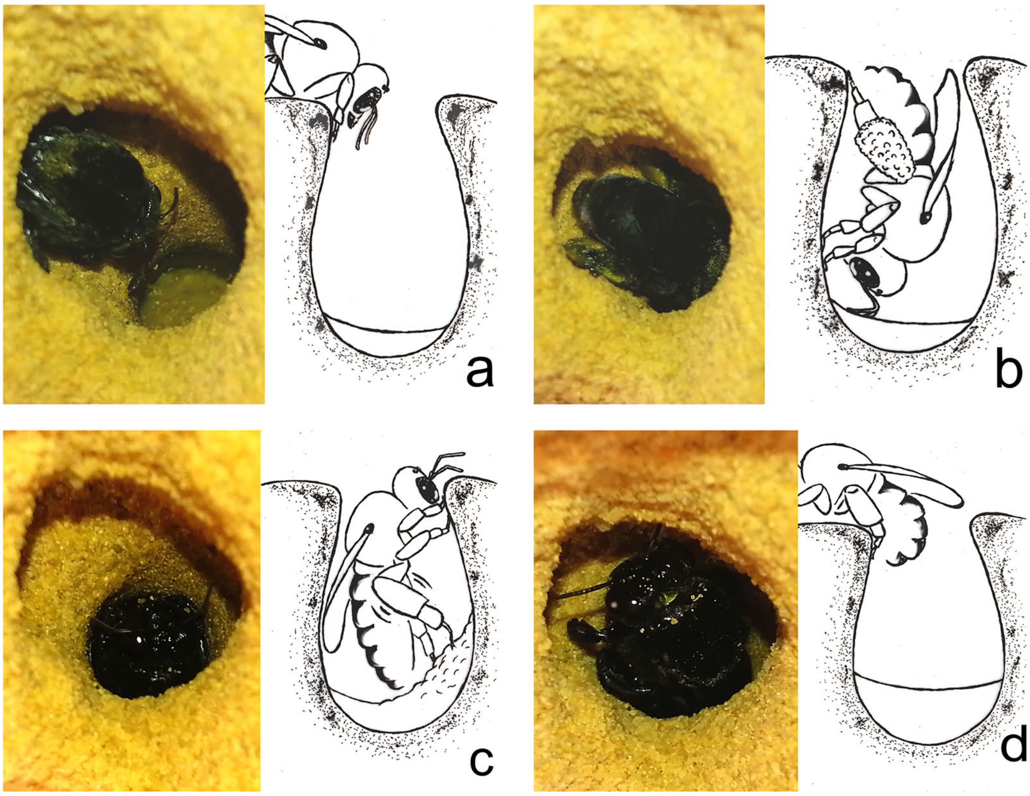

Figure 4. *Centris burgdorfi* female during the construction of a cell: (a) female returns to nest with pollen; (b) checks the cell before depositing material; (c) using rotational movements, the female arranges the material uniformly using the hind legs; (d) exits again.

Table 1. Number of days spent by each *C. burgdorfi* female to construct one brood cell in a dune region in northeastern, Brazil.

Year	Nest identification	Number of cells built	Activity days	Days/cell
2014	CB1	4	11	2.75
	CB2	6	17	2.83
	CB3	3	7	2.33
	CB4	3	10	3.33
	CB5	7	14	2
	CB6	4	9	2.25
	CB7	4	10	2.5
	CB8	5	14	2.8
	CB9	3	10	3.33
	CB10	5	12	2.4
	CB11	1	3	3
	CB12	5	9	1.8
2015	CB13	6	17	2.83
	CB14	5	15	3
	CB15	7	16	2.29
	CB16	5	15	3
	CB17	4	10	2.5
	CB18	6	16	2.67
	CB19	6	15	2.5
	CB20	2	5	2.5
	Average	4.55	11.75	2.58
	Standard deviation	1.60	4.01	0.40

motion, deposited the remaining oil on top to close the cell completely. The resulting concave surface is then flattened by the female who deposits sand above it using her front and hind legs, leaving the operculum flat [i.e., without an apical process (or nipple) characteristic of brood cells opercula in some species of *Centris*]. The number of foraging trips made to provision cells differed in relation to what was being collected and for what purpose. The mean number of trips for oil collection for cell lining, for pollen collection, and for oil collection for larval sustenance differed significantly from each other ($F_{2,220}=614.02$, $p < 0.0001$).

Females built and provisioned from 1 to 7 cells for each nest (4.55 ± 1.6; $n = 20$). No marked females were observed to build more than one nest. Each cell required an average of about two and a half days to construct (2.58 ± 0.40; $n = 20$) (Table 1). To unload oil and line the cell, females spent about 10.5 min (median; $n = 749$), while the time required to release her scopal pollen load into a cell was about 5 min (median; $n = 394$). The time necessary to use oil during operculation was also about 5 min (median; $n = 119$). Differences in time spent in these different tasks were significant ($H_{2,1262}=196.4$; $p < 0.001$). These times include both manipulation of the resource itself, as well as the time spent inside the nest in complementary activities.

The plants where females collected resources (pollen, nectar, and oil) occurred in close proximity to their nests (ca. 160 m). The duration of foraging trips to these plants by females differed according to the resources collected (oil versus pollen). Females spent more time for pollen-collection trips (73 min., median; $n = 394$) than for oil-collection trips (54 min., median; $n = 868$) [$Z(U)=7.77$; $p < 0.001$] (Figure 5).

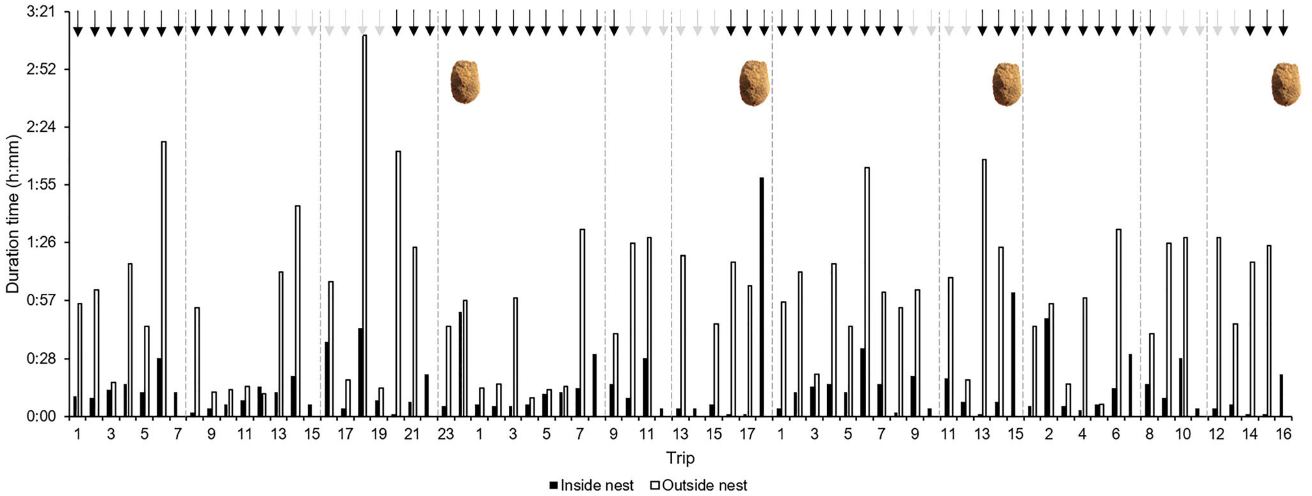

Figure 5. Time spent by one *C. burgdorfi* female (CB1—Table 1) inside and outside the nest during cell construction, provisioning, oviposition, and cell closure. Black arrows indicate oil-collecting trips, and gray arrows indicate pollen-collecting trips. Cell figure indicate the time in which one cell was completed, and the vertical dashed lines separate each day.

Natural enemies

Natural enemies, parasites, were observed around the aggregation during the nesting period. It was common to see females of mutillid wasps (*Hoplomutilla biplagiata* Mickel and *Tallium aracati* Couple) walking on the sandstone dunes searching for open nests. During these movements, the wasps antennated the ground and periodically entered open nest tunnels. When a *C. burgdorfi* female was in her nest, the bee fought with the wasp, causing the wasp fall from the surface of the dune to the ground below the nest site.

Ants of the genus *Pheidole* were also seen entering bee burrows. At sunset, there was a noticeable increase in the number of ants circulating within the nest aggregation. When *C. burgdorfi* females reached the nest in the morning they sometimes encountered tunnels full of ants. Often females did not reenter their burrows until the ants had left.

Mesoplia regalis Smith, a cleptoparasitic bee species, was also observed at the aggregation sites. These bees have a characteristic flight pattern, always close to the ground and flying very fast, making them difficult to see before landing. We observed individuals of *M. regalis* flying over the entrances, but none were observed to enter nests.

Parasitic flies in the genus *Anthrax* were also collected at the nesting site. The *Anthrax* females were always found at the base of the nesting aggregation, awaiting the departure of a female, at which time they hovered over the tunnel entrance and flicked eggs inside while still hovering about the entrances.

Of 125 brood cells maintained in the laboratory in only 8% did we observe a parasite to emerge. Three cells were parasitized by mutillid wasps: *H. biplagiata* ($n = 1$) and *T. aracati* ($n = 2$). These wasp larvae produce a fibrous cocoon within the host cocoon, which makes it easy to identify the parasitism when cells are opened. In the rest of the parasitized cells, we saw the emergence of the cleptoparasitic bee *M. regalis* ($n = 1$) and the parasitic fly *Anthrax* sp. ($n = 6$).

Temperature analysis

The temperatures inside the nest were similar to the outside ambient temperatures in the early morning, when the lowest values were measured. The internal temperature barely reached 28 °C even during the hottest periods of the day, while the outside temperatures were above 30 °C between 09:30 h and 16:00 h (peaks above 36 °C at 12:00 h), the time period in which we also recorded the lowest humidity levels on site. The difference in average temperatures between internal and external (ambient) from the nest was significantly different ($F_{1,\ 425}$=350.29, $p < 0.001$) (Figure 6).

Discussion

Our results provide detailed information about the nesting behavior of *C. burgdorfi*. The activity of the females probably follows the phenology of the plant species from which they forage. The nest substrate (sandstone dunes) provides some thermal isolation, important in an environment with high temperatures alongside the day. Following a pattern seen in many solitary bees, *C. burgdorfi* is a species that nest in aggregations. But, we observed an elevated investment of time in constructing the brood cells.

Many species of *Centris* nest in aggregations [e.g., ranging from 328 nests/m^2 in *C. caesalpiniae*, 3 nests/m^2 or less in *C. pallida* (Rozen & Buchmann, 1990), 40 nests/m^2 in *C. aenea* Lepeletier (Aguiar & Gaglianone, 2003), 708 holes/m^2 in *C. muralis* Burmeister (Cilla & Rolón, 2012), and 1.7 nests/m^2 in *C. flavifrons* Fabricius (Martins et al., 2014)]. Also, we found many old brood cells of

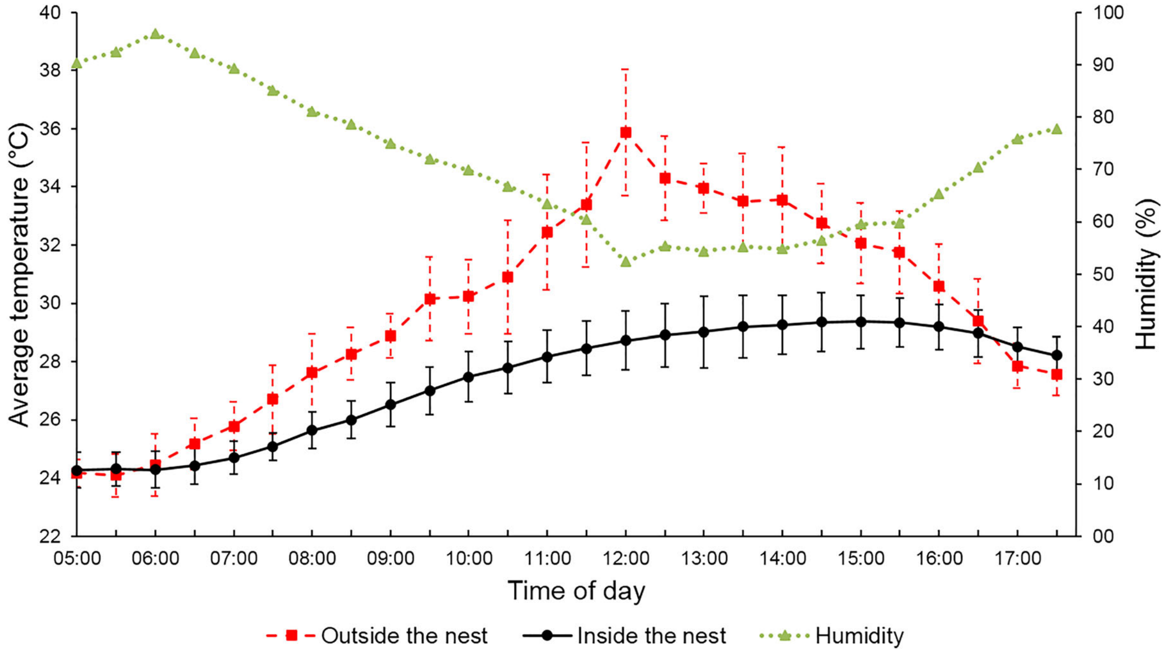

Figure 6. Average temperatures measured simultaneously inside (continuous black line) and outside (dashed red line) the *C. burgdorfi* nests throughout the day, over 12 days. The symbols represent the mean and the bars are the standard deviations of the measurements. The humidity of the environment (dotted green line) are also presented.

C. burgdorfi at the nest site that had been established in previous years. This observation suggests that nesting aggregations are often located in the same location across years. We consider the possible reasons why nests are found in aggregations and why these aggregations tend to occur in the same location across years.

First, some locations for nests may be better than others with respect to favorable edaphic conditions, close proximity to food plants, and/or reduced parasite loads. Desired locations may be limited in availability, causing nests to be closely spaced to one another. For *C. burgdorfi*, the occurrence of dense aggregations could be related to preferences for the sandy substrate chosen for nest construction. Females have a clear preference for nesting in the hard-sandy substrate (sandstone dunes), formations that are uncommon and restricted in area. The distribution of *Anthophora pueblo* Orr, another bee species that nests in similar substrates, is apparently linked to sandstone, despite an elevated cost associated with nesting in such substrate (Orr, Griswold, Pitts, & Parker, 2016). Other aggregations of *C. burgdorfi* nests were found in similar dune structures indicating such a possible preference.

Alternatively, bees might actively choose to place their nests close to where other nests are because there are benefits of aggregations that outweigh the costs. Factors that favor aggregation behavior, such as facilitating encounters by partners for mating might offset factors that disfavor such behavior, such as increased attraction of parasites or predators to these food-rich island-like targets (Michener, Lange, Bigarella, & Salamuni, 1958). The two possible determinants of nest aggregations are not mutually exclusive. Both could account for the aggregations of nests observed in these bees.

At this time, we do not know if nesting aggregations result in increased levels of predation and parasitism. Among the natural enemies of *Centris* are several species of cleptoparasitic bees in the Ericrocidini including *Mesocheira*, *Mesoplia*, and *Mesonychium* (Alves-dos-Santos, 2009; Snelling & Brooks, 1985). During studies of the nesting biology of *C.* (*Paracentris*) *caesalpiniae* in Arizona, Rozen and Buchmann (1990) observed a species of *Ericrocis* (*E. lata* Cresson) parasitizing some nests but at a very low level. However, since this genus does not occur in Brazil, other species of Ericrocidini (e.g., *Mesoplia regalis*) would be expected to parasitize *C. burgdorfi* nests. It was noted in previous studies that *C. burgdorfi* females do not spend the night inside their nests, unlike most species of *Centris* (Sabino et al., 2017) and most other bees. Not spending the night in the nest is a highly unusual behavior in all *Centris*, and most solitary bees, that could conceivably lead to more cleptoparasitic bee attacks and more predation by other insects (since females would not be present to defend their nests). The same behavior was observed recently in *Epicharis nigrita* Friese (Martins, Neto, & Cruz, 2019). Nevertheless, studies show that cleptoparasitic bees are active mainly during the day (Wcislo et al., 2004) which would not necessarily mean a greater attack rate even within unprotected nests in *C. burgdorfi*.

The choice of the nesting site probably also influences the development of the brood. The difference between internal and external temperatures inside *C. burgdorfi* nests demonstrates that the substrate acts as an excellent thermal insulator in protecting the immatures. The development of immature bees, in general, is influenced by high temperatures, which can cause high mortality, particularly in the early larval stages (Frankie, Newstrom, Vinson, & Barthell, 1993). Temperatures between 38 and 40 °C would be lethal to larvae of *C. analis* (Frankie, Vinson, Newstrom, & Barthell, 1988). Nesting females of *Nomia melanderi* Cockerell, for example, respond to hot ambient temperatures by excavating deeper nest burrows in the soil (Stephen, 1965). We do not know the critical thermal maxima for bee larvae in the genus *Centris* nor the actual temperatures inside the cells, and we do not have such data for the species. This would be an interesting avenue for future research.

The shape of the brood cells of *C. burgdorfi* resembles that of other *Centris* species (e.g., Aguiar & Gaglianone, 2003; Cilla & Rolón, 2012; Coville, Frankie, & Vinson, 1983; Martins et al., 2014; Silva, Viana, & Neves, 2001). However, in *C. burgdorfi*, the top of the operculum is concave, which differs from many other *Centris* species that add a central process or "nipple" in the operculum (cell cap), such as *C.* (*Centris*) *aenea* (Aguiar & Gaglianone, 2003), *C.* (*Centris*) *varia* Erichson (cited by Coville et al., 1983 as *C. segregate* Crawford), *C.* (*Centris*) *flavifrons* (Martins et al., 2014), and *C.* (*Hemisiella*) *transversa* Pérez (Batra & Schuster, 1977). A central process is present in other *Centris* (*Paracentris*) species, including *C. pallida*, *C. rhodopus*, *C. cockerelli* (Alcock et al., 1976) and *C. caesalpiniae* (Rozen & Buchmann, 1990). According to these authors, the central process likely allows gas exchange between the external and the intranidal environments through a pore, because gases do not diffuse as easily through the cell wall, and the tough cells are also protected against flooding and from all natural enemies except for small vertebrates (e.g., skunks or squirrels) that occasionally find, dig and eat the *Centris pallida* cells (S. Buchmann, unpublished data). We do not know exactly if differences in the operculum shape in *C. burgdorfi* mean differences in gas exchange and protection against enemies.

The brood cells were also influenced by the sex of the larva. Females of *C. burgdorfi* are larger than conspecific males and this resulted in larger cells built for female-destined larvae, and more provisioned pollen and oil per cell. Although we did not weight the pollen balls (provision masses), or the weight of fecal material, some authors have reported that adult body size is associated with the amount of food consumed by the larvae (e.g., Alcock, 1979; Kim, 1997; Klostermeyer, Mech, & Rasmussen, 1973; Krombein, 1967). In *C. analis*, Jesus and Garofalo (2000) observed behavioral differences in foraging in females according to whether they were provisioning a cell destined to be a female versus a male bee. A larger number of pollen-collecting trips were made when the cell produced a female offspring (Jesus & Garofalo, 2000). These results indicate that the production of females costs more than the production of males (Jesus & Garofalo, 2000; Peterson & Roitberg, 2006), which could affect local population sex ratios in the event of food shortages during periods of drought. Previous studies in solitary bees have shown that the ratio of males increases when food availability declines (Kim, 1999; Torchio & Tepedino, 1980).

Most activity occurs in the cooler morning hours. In the nesting location, the climate is typical of that in the equatorial belt, with little annual variation in temperature, but a pronounced division between the wet season (period where the plants are blooming in the region) and dry season. Decreases in female flight activity in the afternoon are likely related to the high ambient temperatures and possibly a decrease in the availability of floral resources (standing crop of nectar and pollen) on-site by noon. Freitas and Pereira (2004) reported a large drop in the flight activity of *C. tarsata*, and *C. bicolor* Lepeletier, around 12:00 h due to the lower availability of pollen and oil after that period.

The end of the activity period is only one of the factors that can interrupt the building of a brood cell and increase the overall time required to build it. The average time required by *C. burgdorfi* (2.59 days) females to build a cell might be considered high when compared with records for other *Centris* species. For example, *C. flavifrons* spends an average of 1.61 days to construct each cell (Martins et al., 2014). But *C. flavifrons* and *C. burgdorfi* species differ in the number of oil-collection trips required for lining their cells. *C. burgdorfi* made 9.62 oil-collecting trips while *C. flavifrons* was observed taking only three trips to collect floral oils. Similarly, *Centris aenea* was reported to make fewer oil-collection trips to line its cells (two to three trips) and females spent less time in pollen collection (33.6 min in *C. aenea* versus 68 min in *C. burgdorfi*) (Aguiar & Gaglianone, 2003). The time that *Centris* females spend gathering each type of floral resource is highly variable and likely related to the distance between the nest and the floral species that they utilize. Distance to the floral resources does not appear to be a problem for *C. burgdorfi* females since the plants used to occur in the vicinity (ca. 200 m) to the nesting site.

This study contributes to our overall understanding of the nesting biology of *Centris* bees. We successfully followed the construction of brood cells in nature step-by-step, which is rare in the study of bee nesting biology. Since *C. burgdorfi* is highly unusual in several aspects of its nesting behavior (e.g., nesting in the sandstone and females not spending the night in their burrows), it would be helpful to locate other more geographically distant nesting localities for this bee, to confirm many of the observations at the northeastern Brazil study sites. In fact, we are undertaking an expanded study of *C. burgdorfi* by searching for other nesting sites of this remarkable centridine bee.

Acknowledgments

The authors are grateful to Paulo Roberto de Castro for the help and all support in the fieldwork and to Astrid Kleinert, Celso Martins, Clemens Schlindwein, Felipe Vivallo, Maria Cristina Gaglianone and the two anonymous reviewers for comments and suggestions in the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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References

Aguiar, C. M. L. (2003). Flower visits of *Centris* bees (Hymenoptera: Apidae) in an area of caatinga (Bahia, Brazil). *Studies on Neotropical Fauna and Environment*, *38*(1), 41–45. doi:10.1076/snfe.38.1.41.14029

Aguiar, C. M. L., & Gaglianone, M. C. (2003). Nesting biology of *Centris* (*Centris*) *aenea* Lepeletier (Hymenoptera, Apidae, Centridini). *Revista Brasileira de Zoologia*, *20*(4), 601–606. doi:10.1590/S0101-81752003000400006

Alcock, J. (1979). The relation between female body size and provisioning behavior in the bee *Centris pallida* Fox (Hymenoptera: Anthophoridae). *Journal of the Kansas Entomological Society*, *52*(3), 623–632.

Alcock, J., Jones, C. E., & Buchmann, S. L. (1976). The nesting behavior of three species of *Centris* bees (Hymenoptera: Anthophoridae). *Journal of the Kansas Entomological Society*, *49*(4), 469–474.

Alves-dos-Santos, I. (2009). Cleptoparasite bees, with emphasis on the oil bees hosts. *Acta Biológica Colombiana*, *14*, 107–114.

Batra, S. W. T., & Schuster, J. C. (1977). Nests of *Centris*, *Melissodes*, and *Colletes* in Guatemala (Hymenoptera: Apoidea). *Biotropica*, *9*(2), 135–138.

Buchmann, S. L. (1987). The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics*, *18*(1), 343–369. doi:10.1146/annurev.es.18.110187.002015

Cane, J. H. (1987). Estimation of bee size using intertegular span (Apoidea). *Journal of the Kansas Entomological Society*, *60*(1), 145–147.

Cilla, G., & Rolón, G. (2012). Macroscopic and microscopic studies of the nests and the stages involved in the nesting process of *Centris muralis* Burmeister (Hymenoptera: Apidae: Centridini) bee in the adobe walls, in La Rioja, Argentina. *Biologia*, *67*(3), 573–583. doi:10.2478/s11756-012-0036-7

Coville, R. E., Frankie, G. W., & Vinson, S. B. (1983). Nests of *Centris segregata* (Hymenoptera: Anthophoridae) with a review of the nesting habits of the genus. *Journal of the Kansas Entomological Society*, *56*(2), 109–122.

Frankie, G. W., Newstrom, L., Vinson, S. B., & Barthell, J. F. (1993). Nesting-habitat preferences of selected *Centris* bee species in Costa Rican dry forest. *Biotropica*, *25*(3), 322–333. doi:10.2307/2388790

Frankie, G. W., Vinson, S. B., Newstrom, L., & Barthell, J. F. (1988). Nest site habitat preferences of *Centris* bees in the Costa Rican dry forest. *Biotropica*, *20*(4), 301–310. doi:10.2307/2388320

Freitas, B. M., & Paxton, R. J. (1998). A comparison of two pollinators: The introduced honey bee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its native range of NE Brazil. *Journal of Applied Ecology*, *35*(1), 109–121. doi:10.1046/j.1365-2664.1998.00278.x

Freitas, B. M., & Pereira, J. O. P. (2004). Crop consortium to improve pollination: Can West Indian Cherry (*Malpighia emarginata*) attract *Centris* bees to pollinate cashew (*Anacardium occidentals*). In B. M. Freitas & J. O. P. Pereira (Eds.), *Solitary bees: Conservation, rearing and management for pollination* (pp. 193–201). Fortaleza: Imprensa Universitária.

Garófalo, C. A., Camillo, E., & Serrano, J. C. (1989). Espécies de abelhas do gênero *Centris* (Hymynoptera, Anthophoridae) nidificando em ninhos-armadilha. [Bee species of *Centris* genus (Hymenoptera, Anthophoridae) nesting in trap nests]. *Ciencia e Cultura*, *41*, 799.

INMET/UFRN. (2002). *Instituto Nacional de Meteorologia/UFRN. Boletins Climatológicos: anos 1984 a Maio/2002*. [Climatological bulletins: Years 1984 to May/2002] Natal: Estação Climatológica Principal.

Janvier, H. (1926). Recherches biologiques sur les Hymenopteres du Chili. [Biological research on Hymenopteres of Chile]. *Annales des Sciences Naturelles*, *10*, 133–268.

Janvier, H. (1955). Le nid et la nidification chez quelques abeilles des Andes tropicales. [Nest and nesting of some tropical Andes bees]. *Annales des Sciences Naturelles*, *17*(11), 311–349.

Jesus, B. M. V., & Garofalo, C. A. (2000). Nesting behavior of *Centris* (*Heterocentris*) *analis* (Fabricius) in southeastern Brazil (Hymenoptera, Apidae, Centridini). *Apidologie*, *31*, 503–515.

Kim, J. Y. (1997). Female size and fitness in the leaf-cuter bee *Megachile apicalis*. *Ecological Entomology*, *22*(3), 275–282. doi:10.1046/j.1365-2311.1997.00062.x

Kim, J. Y. (1999). Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). *Behavioral Ecology*, *10*(5), 552–556. doi:10.1093/beheco/10.5.552

Klostermeyer, E. C., Mech, S. J., & Rasmussen, W. B. (1973). Sex and weight of *Megachile rotundata* (Hymenoptera: Megachilidae) progeny associated with provision weights. *Journal of the Kansas Entomological Society*, *46*, 536–548.

Krombein, K. V. (1967). *Trap-nesting wasp and Bees: Life histories nest and associates*. Washington, DC: Smithsonian Press.

Martins, C. F., Neto, V. I. D. S., & Cruz, R. D. M. (2019). Nesting biology and mating behavior of the solitary bee *Epicharis nigrita* (Apoidea: Centridini). *Journal of Apicultural Research*. doi:10.1080/00218839.2019.1584963

Martins, C. F., Peixoto, M. P., & Aguiar, C. M. L. (2014). Plastic nesting behavior of *Centris* (*Centris*) *flavifrons* (Hymenoptera: Apidae: Centridini) in an urban area. *Apidologie*, *45*(2), 156–171. doi:10.1007/s13592-013-0235-4

Michener, C. D., Lange, R. B., Bigarella, J. J., & Salamuni, R. (1958). Factors influencing the distribution of bees' nests in earth banks. *Ecology*, *39*(2), 207–217. doi:10.2307/1931865

Neto, O. F., Costa, F. A. A., Severo, R. N. F., Júnior, O. F. S., & Scudelari, A. C. (2005). Estudo da dinâmica de Falésias do município de Tibau do Sul – RN [Study of the Dynamics of Cliffs of the city of Tibau do Sul - RN]. In *Anais do 11 Congr. Brasil.* Florianópolis: Geologia de Engenharia e Ambiental.

Nogueira, A. M. B. (1982). O Cenozoico continental da região de Natal-RN. *Coleção de Textos Acadêmicos da UFRN*, *2*(284), 1–117.

Oliveira, R., & Schlindwein, C. (2009). Searching for a manageable pollinator for acerola orchards: The solitary oil-collecting bee *Centris analis* (Hymenoptera: Apidae: Centridini).

Journal of Economic Entomology, *102*(1), 265–273. doi:10.1603/029.102.0136

Orr, M. C., Griswold, T., Pitts, J. P., & Parker, F. D. (2016). A new bee species that excavates sandstone nests. *Current Biology*, *26*(17), R792–R793. doi:10.1016/j.cub.2016.08.001

Peterson, J. H., & Roitberg, B. D. (2006). Impacts of flight distance on sex ratio and resource allocation to offspring in the leafcutter bee, *Megachile rotundata*. *Behavioral Ecology and Sociobiology*, *59*, 589–596. doi:10.1007/s00265-005-0085-9

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rozen, J. G., & Buchmann, S. L. (1990). Nesting biology and immature stages of the bees *Centris caesalpiniae*, *C. pallida* and the cleptoparasite *Ericrocis lata* (Hymenoptera: Apiodea: Anthophoridae). *American Museum Novitates*, *2985*, 1–30.

Sabino, W. O., Alves-dos-Santos, I., Queiroz, E. P., Faria, L. B., Papaj, D. R., Buchmann, S. L., & Silva, C. I. (2017). *Nesting biology of the bees Centris (Paracentris) burgdorfi Friese and Centris (Paracentris) pallida Fox (Apidae: Centridini)- Chapter 2* (Dissertation theses). Universidade de São Paulo.

Sabino, W. O., Alves-dos-Santos, I., & Silva, C. I. (2018). Versatility of the trophic niche of *Centris* (*Paracentris*) *burgdorfi* (Apidae, Centridini). *Arthropod-Plant Interactions*, *13*, 227–237. doi:10.1007/s11829-018-9654-5

Sabino, W. O., Silva, C. I., & Alves-dos-Santos, I. (2017). Mating system and sleeping behaviour of the Male and Female *Centris* (*Paracentris*) *burgdorfi* Friese (Apidae, Centridini). *Journal of Insect Behavior*, *30*(1), 103–118. doi:10.1007/s10905-017-9600-x

Silva, F. O., Viana, B. F., & Neves, E. L. (2001). Biologia e arquitetura de ninhos de *Centris* (*Hemisiella*) *tarsata* Smith (Hymenoptera, Apidae, Centridini). [Biology and nest architecture of *Centris* (*Hemisiella*) *tarsata* Smith (Hymenoptera, Apidae, Centridini. *Neotropical Entomology*, *30*(4), 541–545. doi:10.1590/S1519-566X2001000400005

Silveira, F. A., Melo, G. A. R., & Almeida, E. A. B. (2002). *Abelhas brasileiras: Sistemática e identificação* [Brazilian bees: Systematics and identification]. Belo Horizonte: Fundação Araucária.

Snelling, R. R., & Brooks, R. W. (1985). A review of the genera of cleptoparasitic bees of the tribe Ericrocini (Hymenoptera: Anthophoridae). *Natural History Museum of Los Angeles County*, *369*, 1–34.

Sokal, R., & Rohlf, F. J. (1995). *Biometry—The principles and practice of Statistics in biological research*. New York, NY: W. H. Freeman and Co.

Stephen, W. P. (1965). Temperature effects on the development and multiple generations in the alkali bee, *Nomia melanderi* Cockerell. *Entomologia Experimentalis et Applicata*, *8*(3), 228–240. doi:10.1111/j.1570-7458.1965.tb00856.x

Torchio, P. F., & Tepedino, V. J. (1980). Sex ratio, body size and seasonality on a solitary bee, *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). *Evolution*, *34*(5), 993–1003. doi:10.1111/j.1558-5646.1980.tb04037.x

Vivallo, F., & Zanella, F. C. V. (2012). A new species of Centris (*Paracentris*) Cameron, 1903 from northeastern Brazil, with a key for the *Centris* species of the Caatinga region (Hymenoptera: Apidae). *Zootaxa*, *3298*(1), 1–16. doi:10.11646/zootaxa.3298.1.1

Vogel, S. (1974). Ölblumen und ölsammelnde Bienen. [Oil flowers and oil-collecting bees]. *Tropische und subtropische Pflanzenwelt*, *7*, 285–547.

Wcislo, W. T., Arneson, L., Roesch, K., Gonzalez, V., Smith, A., & Fernández, H. (2004). The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): An escape from competitors and enemies? *Biological Journal of the Linnean Society*, *83*(3), 377–387. doi:10.1111/j.1095-8312.2004.00399.x

Zanella, F. C. V. (2002). Sistemática, filogenia e distribuição geográfica das espécies sul americanas de *Centris* (*Paracentris*) Cameron, 1903 e de *Centris* (*Penthemisia*) Moure, 1950, incluindo uma análise filogenética do "grupo *Centris*" sensu Ayala, 1998 (Hymenoptera, Apoidea, Centridini). [Systematics, phylogeny and geographical distribution of South American species of *Centris* (*Paracentris*) Cameron, 1903 and *Centris* (*Penthemisia*) Moure, 1950, including a phylogenetic analysis of the "*Centris* group" sensu Ayala, 1998 (Hymenoptera, Apoidea, Centridini. *Revista Brasileira de Entomologia*, *46*, 435–488. doi:10.1590/S0085-56262002000400001

Zar, J. H. (1996). *Biostatistical analysis* (3rd ed.). Englewood Cliffs, NJ: Prentice Hall.