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Nest Structure, Seasonality and Female Behavior of *Epicharis* (*Anepicharis*) *dejeanii* Lepeletier (Hymenoptera, Apidae, Centridini) in a Restinga Ecosystem, in Southern Brazil

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Abstract

We investigated the nesting behavior of females of *Epicharis dejeanii* and the architecture of their nests, in a large aggregation in a Restinga area, on Ilha do Superagui, southern Brazil. Surveys were carried out intermittently through the warm-wet seasons from different years between 2013 and 2017. The nest aggregation occupied an area of approximately 2,000 m^2 and was situated on a sand bank and on flat sandy soil. Each nest consisted of a long unbranched tunnel, averaging 1.45 ± 0.35 m (N = 8), connected to a single brood cell with a mean length of 3.13 ± 0.2 cm (N = 13) and mean diameter of 1.2 ± 0.1 cm (N = 11). On average, females carried out 4.0 ± 2.4 foraging trips per day (N = 109) to collect floral resources for provisioning brood cells. Similar times were spent by females in their foraging trips for: only pollen (15.8 ± 14.3 min, N = 72), oil (22.5 ± 15.7 min, N = 45), or both resources (17.0 ± 15.1, N = 63). Our findings reveal that some variation in both nesting architecture and female behavior of *E. dejeanii* during nesting activities can occur in different locations from the same region.

Introduction

Epicharis Klug, 1807 (Hymenoptera, Apidae, Centridini) is an exclusively Neotropical genus of bees representing about 35 species, which exhibit solitary behavior, the habit of digging their nests in the ground, usually in large aggregations (Roubik & Michener, 1980; Hiller & Wittmann, 1994; Gaglianone, 2005; Thiele & Inouye, 2007; Rocha-Filho et al., 2008; Rozen, 2016; Dec & Vivallo, 2019). *Epicharis* belongs to a group of approximately 400 species known as oil-collecting bees, due to the female behavior of collecting floral oils, which are used for both cell construction and larval provisions (Alves-dos-Santos et al., 2007; Gaglianone et al., 2011).

Aspects of the nesting biology for different species of *Epicharis*, including details on the nest architecture and female behavior during nest construction and cell provisioning, have been studied in seven out of nine subgenera belonging to the genus (see Gaglianone, 2005). In general, the different species and subgenera of *Epicharis* studied so far construct the brood cells deep down in sandy soils (Gaglianone, 2005; Thiele & Inouye, 2007; Rocha-Filho et al., 2008; Rozen, 2016; Dec & Vivallo, 2019). Detailed nest descriptions have also been used in assessments on the evolution of nest habitat and architecture in this genus (Thiele & Inouye, 2007).

Epicharis (*Anepicharis*) *dejeanii* Lepeletier, 1841, the only member of this subgenus (Moure et al., 2012), is a univoltine species that constructs its nests in aggregations in sandy soil (Hiller & Wittmann, 1994; Dec & Vivallo, 2019). This species occurs in South America (Moure et al., 2012). In Brazil, *E. dejeanii* is widely distributed, occurring from the northern (Amazonia) to the southern region (Atlantic Forest) (Hiller & Wittmann, 1994; Steiner et al., 2010; Moure et al., 2012).

To date, although information on the nest architecture of *E. dejeanii* has already been published by Hiller and

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Wittmann (1994) and, more recently, by Dec and Vivallo (2019), some aspects related to nest structure of this species, such as number of cells per nest, are divergent in both studies. Concerning this subject, specifically in the preliminary study carried out by Hiller and Wittmann (1994), the authors inferred the number of brood cells per nest, reporting a number of 52 brood cells found in an area containing only five nests; suggesting thus, approximately 10 cells per nest (Hiller & Wittmann, 1994; reviewed by Gaglianone, 2005). However, in a new publication on nesting biology of *E. dejeanii* a very distinctive number of brood cells (varying from 1 to 2) per nest was identified (Dec & Vivallo, 2019). Since both studies were carried out in southern Brazil, a provocative question that emerges at this point is: could the number of cells constructed by females per nest vary considerably, revealing a plasticity in nesting behavior for *E. dejeanii*?

Another relevant aspect related to studies on bee nest aggregations concerns the length of time of these aggregations. Although Michener (1974) states that nest aggregations are sometimes very long-lived, few reports on nest aggregations of *Epicharis* (e.g. Gaglianone, 2005; Thiele & Inouye, 2007; Martins et al., 2019) monitored the aggregation for more than one or two years (Roubik & Michener, 1980; Hiller & Wittmann, 1994; Rocha-Filho et al., 2008; Werneck, 2012; Dec & Vivallo, 2019).

Therefore, in the current study we bring more information on nesting biology of *E. dejeanii*, mainly concerning the number of cells constructed by females per nest, on the persistence of nest aggregation along the time and on behavior of females during the nest construction activity. To achieve our aims successfully, we have focused on the following questions:

i) How long is the burrowing phase? ii) How many cells are constructed per nest? iii) How long is the provisioning phase? iv) How many pollen flights/pollen loads are necessary to provision one cell? v) Does pollen collection occur throughout the day or mainly in the morning/evening? vi) How long is the activity phase of the aggregation? vii) How long does the aggregation last?

Material and Methods

Study area

The studied nest aggregation of *E. dejeanii* was first identified in December 2013, in a Restinga area of the Ilha do Superagui (IS), about 1 km from the study base of the Chico Mendes Institute for Biodiversity Conservation (ICMBio), 25°27.51.4"S - 48°14.07.1'W. The area belongs to the Parque Nacional do Superagui (PNS), located in the Environmental Protection Area (APA) of Guaraqueçaba (ICMBio, 2016), in the state of Paraná, southern Brazil. The landscape of the islands includes the Atlantic Forest (AF), comprising the Serra do Mar Corridor, one of the most diverse areas of the AF (Aguiar et al., 2005).

The climate of the region is classified as subtropical humid (Köppen classification Cfa). The average annual temperature is 21°C. Annual rainfall is higher than 2500 mm. Mean temperature for the coldest month is lower than 18°C, and for the warmest, higher than 22°C (IPARDES, 2001; Giangarelli & Sofia, 2018).

The vegetation comprises a combination of Dense Ombrophilous Forest and pioneer formations. Among these is the Restinga, which covers 30% of the total area of the island, being gradually replaced by forest as the distance from the sea increases (Schmidlin et al., 2005). In the current study, the area where the aggregation was located showed herbaceous and grassy vegetation scattered among the nests.

Data Collection

Data of this study correspond to a set of surveys conducted intermittently in the study area in different months from different years, since December 2013 until November 2018. However, in November 2014 and 2018 the study area was visited only for checking if the aggregation was in activity in the area. Although desirable, unfortunately these limitations are due to some difficulties of accessing the study site (e.g., limited number of boat crossing times available, site isolation, many rainy days from November to January etc), samplings could not to be carried out through an entire warm season, when the studied species is active (see Hiller & Wittmann, 1994; Dec & Vivallo, 2019). Thus, the findings presented here result from data sets gathered over different years.

All dates of field studies in IS, where samplings were conducted, are shown in Table S1 (real time, not Brazilian summertime). In total, we carried out 29 days and approximately 249 hours of field work. The behavior of *E. dejeanii* females was observed for approximately 160 h.

Observations on the nesting behavior of bees were based on Gaglianone (2005) and Rocha-Filho et al. (2008), with some modifications. With exception of rainy days, the bee behavior observations were carried out mostly from 6:00 am to 11:00 am and from 1:00 pm to 6:00 pm; thus, with an interval of two hours between 11:00 am to 1:00 pm (Table S1). This procedure was adopted to avoid the excessive tiredness of the collectors due to the strong collection effort usually under high temperature conditions. However, aiming to fill the gap on the information regarding the period between 11:00 am to 1:00 pm, we decided to conduct five samplings of 12 hours, from 6:00 am to 6:00 pm; these observations were conducted from 31 October 2017 to 04 November 2017 (Table S1). In addition, complementary observations were conducted to identify possible twilight activity of the bees in December 2016 from 5:00 to 7:00 am and from 6:00 to 8:00 pm. Nest excavation activities were conducted in December 2013, from 7:00 to 12:00 am, and in February and December 2016, from 7:00 am to 7:00 pm and from 8:00 am to 6:00 pm, respectively (Table S1). During the period of study (from November to February), the sunrise in the region is at approximately 05:15 am and the sunset at 07:00 pm.

Behaviors of female bees were observed by two observers, who simultaneously observed females from different nests, from a distance between 40 cm and 100 cm from the entrance of the nests marked with tape and styrofoam numbered plates, fixed on wooden rods (Figure 1b). The following activities of the bees were recorded: i) the behavior of females during the nest building, including all the nest excavation process. In this latter case, after a few centimeters of tunnel depth, the behavior of the bee could only be deduced based on the removal of the sand; ii) type of floral resource present in the scopae (pollen, oil, oil + pollen), which was detected through visual observations when females returned to the nest; iii) time spent during the foraging trips for collection of the pollen and oil resources;; iv) time spent inside the nest after each foraging trip v) the number of fights performed by females during the period of observation. The duration of activities was recorded with a chronometer. All these parameters have also been evaluated and described in different studies on *Epicharis* nesting biology (Gaglianone, 2005; Rocha-Filho et al., 2008; Martins et al., 2019).

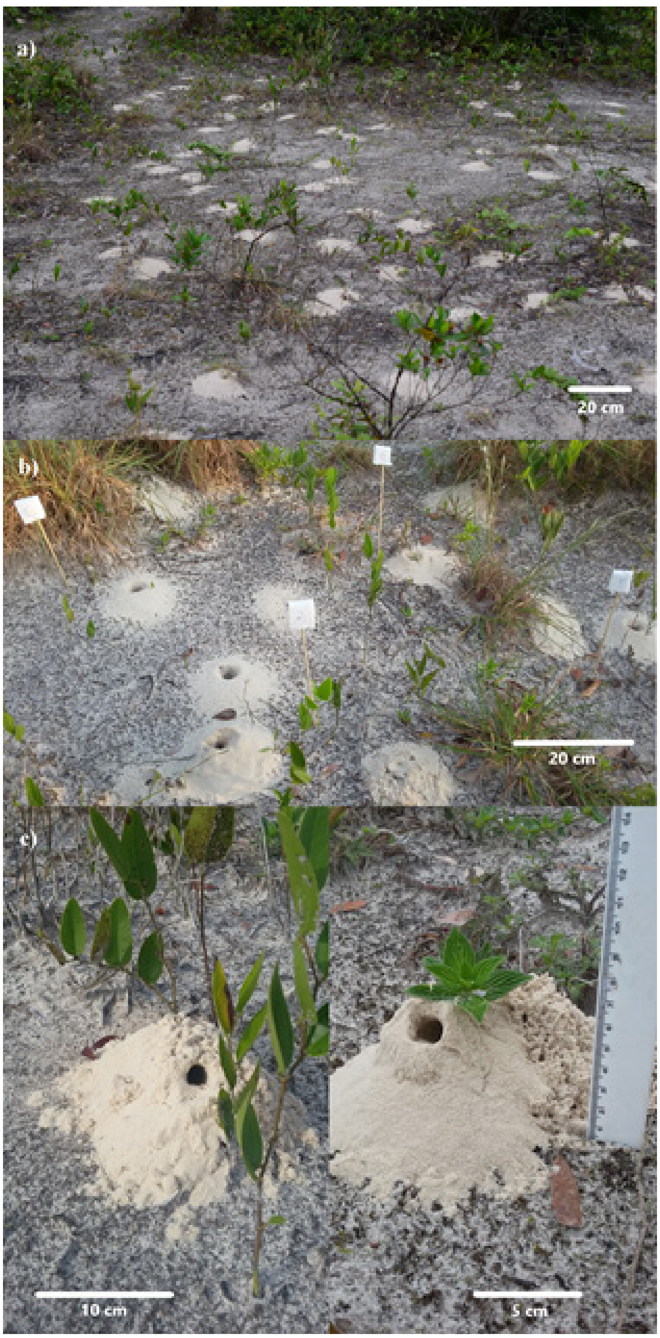

Fig 1. (a) General view of *Epicharis dejeanii* nest aggregation in the study area. (b) A view of several tumuli around the nest entrances and styrofoam plates positioned next to the nest entrances for identification. (c) Detail of the tumulus.

The density of the nests was calculated by random marking of four quadrants of 4 m × 4 m (i.e., 16 m^2 each) along the aggregation. In these quadrants, the nests found with open and closed entrances were counted during the months of samplings, in order to compare the density of nests/m^2 during the months of activity of the species. For each month the number of nests in all quadrants was summed up, divided by 64 and expressed as nests/m^2.

To study the architecture of the nests, a mixture of plaster and water was poured into the nest entrances of 17 nests with a funnel, until the tunnel was filled (Michener, 1964). After a few hours when the plaster was dry and hard, the soil around the entrance of the nest and along the burrow was excavated in search of the brood cells. With a tape or ruler, the length and depth of the tunnels and cells connected to them were measured. During excavations, brood cells not connected to the burrow (i.e., belonging to other nests) were found (N = 5), and their depth and size cells were also measured, totaling eight cells analyzed. Throughout this stage, photographic records were made. Some casts of tunnels and all cells found were taken to the Laboratory of Genetics and Animal Ecology (LAGEA) of the State University of Londrina (UEL).

Data analysis

The Kruskal-Wallis rank sum test, followed by the post-hoc Dunn test, when necessary, was used to test for possible statistical differences in the time spent by females on foraging trips for different types of feeding resources (i.e., pollen, oil or both pollen and oil). This comparison was performed on two sets of data: i) between the types of floral resources, considering all females together; ii) to check for possible differences among females, considering each floral resource. The statistical analysis was carried out on R software, version 3.2.3 (R Development Core Team, 2015), and values were considered significantly different where $p < 0.05$.

Results

Features of the nest aggregation and nesting period

The aggregation area was in sandy soil, typical of Restinga, and had a total extension of approximately 2000 m^2. The nests were distributed in part along a gully and in part in flat ground. Nests were easily detected due to the mound of sand, called tumulus, which forms outside the entrances from the excavated sand. The tumulus has a lighter color than the surrounding soil (Figure 1a-c). The nests were often established near the roots of the Restinga vegetation (Figure 1a-c). Of the 27 nests studied for observing female behavior, 18 were found in this situation.

Individuals of *E. dejeanii* were active for a period of three months (November to January). In 2015, although we started to conduct our observation on 11 November, previous visits to the study area showed that females first appeared on 08 November, when they started to dig the first burrow. On November 8, we also observed the activity of males flying over the place where the nests would be built later.

In 2017, the first two nests were constructed on 31 October, therefore one week earlier than in 2015. In both years, new females were observed burrowing tunnels on the following days. A nest density peak was observed in December 2016, showing 0.9 open nests per m^2 (or 17.5 and 14.7 nests/quadrant). Although in January 2016, 1.1 nests built/m^2 was registered, only 0.6 were in activity (open), (Figure 2). It is also noticeable that the decline in nest construction started in January. Nesting activities of *E. dejeanii* ended in February, since in this month the number of open nests dropped drastically (Figure 2). In addition, during this month, no bee activity was observed at the aggregation site during the sampling days.

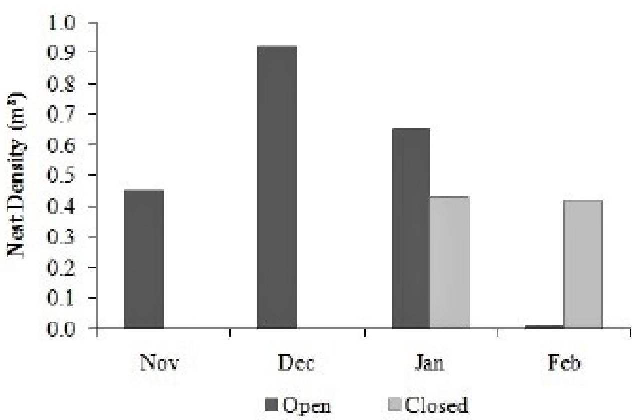

Fig 2. Density (nests/m^2) of open (in activity) and closed nests per month in an aggregation of *Epicharis dejeanii* on Ilha do Superagui (IS) during the warm-wet season, when the nests are constructed, showing data of November 2015 (N =29), January 2016 (N = 70), February 2016 (N = 28) and December 2016 (N = 59). The representation of density of the open nests in December 2016 is shown in different color (dark gray) to highlight that the nests from this month were surveyed in the following reproductive season.

Nest architecture

The entrance of nests is circular, measuring approximately 1.5 cm in diameter. The nest is formed by a single narrow tunnel, which extends vertically (in some cases, making small curves) into the ground, ending in a single brood cell. No marks indicating branches or tunnel connections were visible on the plaster. The tunnels had the same diameter as the entrances of the nests, slightly wider than the body of the bee, which measures approximately 1 cm in diameter. The tumuli ranged from 3 to 8 cm in height (`X = 5.8 ± 1.7; N = 24), and 9.5 to 19 cm (`X = 15.5 ± 3.3; N = 24) in diameter at the base (Figure 1a-c). From a total of 17 nests filled with the plaster and water mixture, we succeeded in excavating only eight thoroughly, with tunnel and cell (Figure 3a). These eight nests were in depths ranging from 64 cm to 184 cm (`X = 145 ± 35 cm; N = 8). However, due to the fact that several excavated tunnels were not linear in their trajectories, from the nest entrance to the brood cell, showing some curves, the total length of nests ranged from 71 cm to 216 cm (`X = 160 ± 39 cm; N = 8).

Only one cell per nest was found. The total length of these varied from 2.90 to 3.5 cm (`X = 3.1 ± 0.19; N = 13). Cells were oval and had a rough, hard, and resistant outer layer (Figure 3b, c), composed of sand particles which were clearly visible and detectable by touch, but the sand was darker than that around the tunnel. The inner surface of the cells was smooth, dark and a bit shiny. The brood cells, in the end of each tunnel, were aligned in different directions in relation to the surface.

Nesting behaviors and female foraging activities

The results on nesting behavior and foraging activities of *E. dejeanii* females reported herein are based on different periods of observation and, therefore, on behaviors and activities from different groups of females during our study.

In November 2015, we observed the complete sequence of three nest foundations by distinctive females and, three phases have been clearly identifiable: i) nest site selection; ii) burrowing and cell construction and iii) brood cell provisioning. When choosing a nesting site, the females flew in circles over the ground at a height of approximately 10 cm over a large area and then in smaller circles, focused on a smaller region, until selecting the site to land and start construction (Figure S1a, b). In those nests, the females excavated with their front legs, then transferred the sand to their middle and hind legs respectively, so that, as they dug, they pushed the sand to the surface. The removed sand was accumulated on the outside, forming a growing tumulus, which was arranged quickly with the hind legs every time they returned, coming up abdomen first. In addition, the distal tergal was used to compact the soil, probably to avoid collapse of the tunnel (vide-not included due to size). On rainy days, the tumulus collapsed. When the nest was closed for some reason, like rain for example, females returning from their trips dug and re-opened the entrance burrow (Figure S2).

The females carried out the nest excavation for periods ranging from 14 to 166 min (`X = 66.6 min ± 38.1; N = 12). The excavation was interrupted by a few brief flights, ranging from 13 to 27 min (`X = 19.7 min ± 5.1; N = 7; data not shown), after which they returned to the nests without any floral resources in their scopae and, re-started digging.

The females started digging in the morning and, at the end of the day, at 6:00 pm, they were still building the nest (probably the tunnel). In two out of the three nests observed

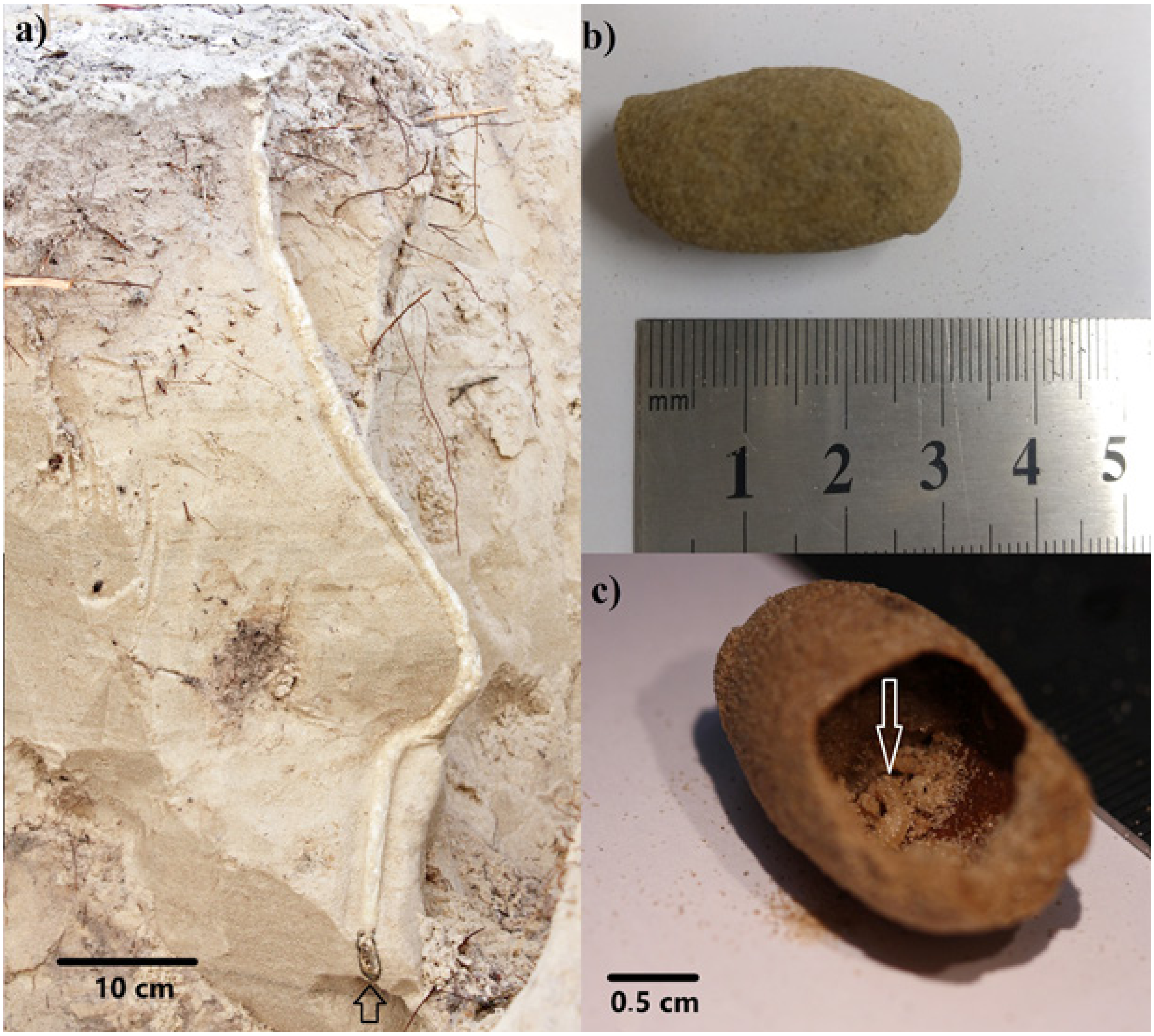

Fig 3. (a) Detail of an excavated nest, showing the main tunnel and the cell (arrow) connected to it. (b) The outer view of the cell shown laterally. (c) Longitudinal view of an opened cell with larva inside it (arrow).

for nesting construction, the females started the nest digging at 7:30 am (nest 1) and at 9:02 am (nest 2), while the nest 3 excavation began in the end of the morning (at 10:23 am). Therefore, the nest excavation lasted all day for nest 1, totaling at least 630 min (> 10 h) of digging. As already mentioned, from a certain tunnel depth, we were only able to infer that females were still excavating, since they emerged from time to time, pushing sand with their abdomen, accumulating it in the tumulus. We also observed that although nest excavations occurred mostly in the morning, nests could be initiated any time during the day. For instance, in November 2015, we registered females from other two nests nearby starting the excavation late in the afternoon (after 5:00 pm).

In the morning of the following day, at 6:00 am, no more excavation activities were observed for the three nests; at this time, the bees were already performing foraging trips, returning to their nests. Therefore, the three females observed during the nest building phase took from 540 min to 630 min throughout the day and, at least, part of the night performing the excavation. However, it is not possible to state with certainty how many hours the females spent in the total phase of nest construction, since they remained inside their nests after the sunset and we do not have data on their nocturnal activity.

After the nest burrowing and cell construction phase, the female starts the brood cell provisioning phase, carrying out foraging trips to collect oil and pollen. It is important to highlight that although the nests were frequently very close to each other, no antagonistic interaction was observed between the females in the aggregation during all period of observation.

During the period of study, observations on foraging activities of females from 27 nests revealed that the they spent most of their time, from early morning to early evening, outside the nest on foraging trips for oil, pollen or both resources, with usually short intervals inside the nest for unloading (Figure 4). They stayed most of the time, up to 10 minutes inside the nests after their trips and approximately one hour outside the nests foraging (Figure 4). The average time spent inside the nests after arriving was: with pollen`X = 15.8 ± 14.3 min (N = 72); with pollen and oil `X = 1517.0 ± 15.1 min (N = 63), and with oil `X = 22.5 ± 15.7 min (N = 45), (Figure 5); no significant differences were found among these values. In November 2017, for five days, from 6:00 am to 6:00 pm, we registered the period of foraging activity, mean number of foraging trips and number of trips for pollen, oil and for both pollen and oil carried out by females from three nests (Table 1). In all these nests females had already initiated the foraging trips when the observations started, which means that the nests were in the cell provisioning phase. As shown in Table 1, during the period of study, the mean number of foraging trips performed per day by the three females varied from 2.40 (± 1.52; N = 3) to 4.00 (± 1.83; N = 3). Although the mean number of foraging trips for both pollen + oil (`X = 2.33 ± 1.15; N = 6) was below those registered for only pollen (`X = 5.33 ± 3.21; N = 16) or only oil (`X = 5.00 ± 2.00; N = 15). These measures did not differ statistically (H = 3.56, df = 2; N = 3).

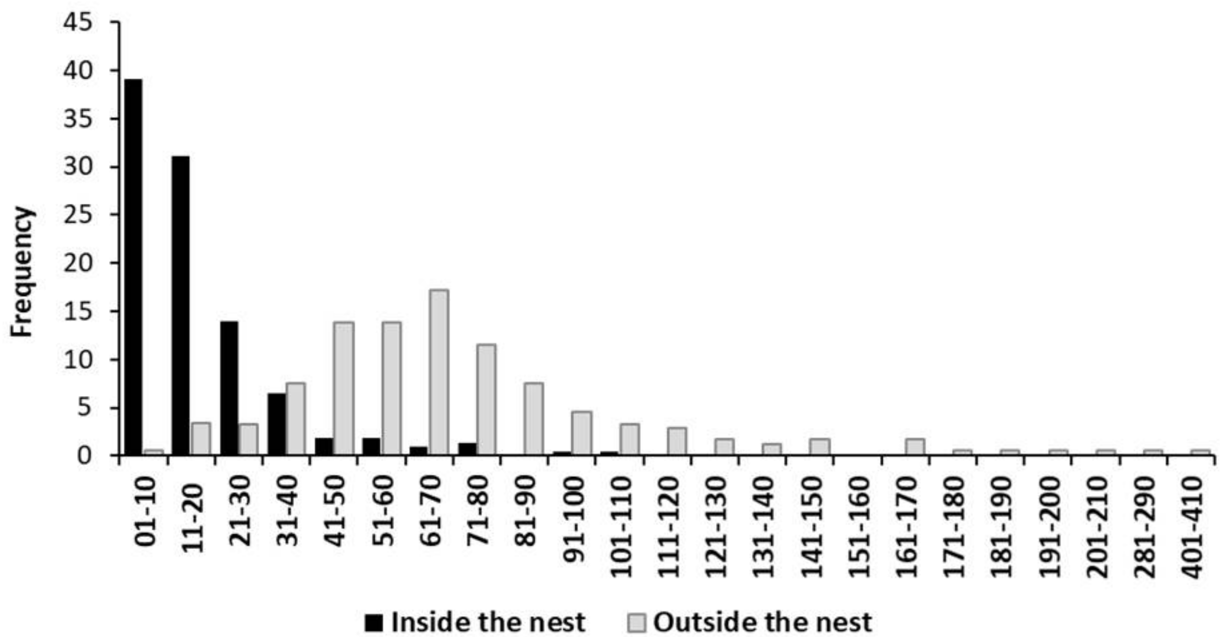

Fig 4. Time spent by *Epicharis dejeanii* females inside the nest on cell provisioning (N = 215 records) and outside the nest on foraging trips (N = 174 records) during the observation period. Results are shown in 23 frequency distribution classes (in intervals of 10 min). Observations were carried out at the entrance of 27 nests in total, on November 15, January 16, December 16 and November 17.

Observations conducted from 08 to 14 January 2016 on foraging activities of females from 13 nests revealed that the average number of trips carried out was`X = 23.38 (± 4.41; N = 13), ranging from 23 to 35. Distinctively, the number of foraging trips carried out by females in November 2015 was`X = 2.67 ± 1.23 (N = 5), ranging from 1 to 5 per day, while in November 2017 was`X = 3.14 ± 1.70 (N = 3), varying between 1 and 6. In January 2016, the females performed a statistically superior average number of foraging trips compared to those females from November of both years (H = 27.02, df = 5, P < 0.0001).

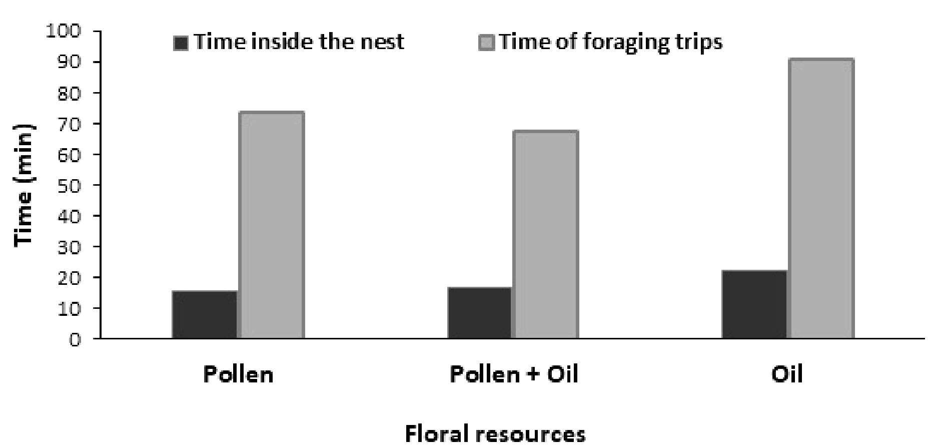

Fig 5. Average time spent by *Epicharis dejeanii* females inside the nest, after each foraging trip, and on foraging trips for collecting floral resources (only pollen, pollen + oil and only oil).

In the observations conducted during a week, in January 2016, estimates of the time spent by the 13 females during the collection of the resources showed that the pollen trips lasted from 8 to 114 min (`X = 64.34 ± 27.29; N = 38), oil trips between 30 to 103 min (`X = 60.18 ± 20.57; N = 34) and trips where females carried pollen and oil together in the scopae lasted 25 to 118 min (`X = 64.71 ± 21.42; N = 35). Females of these nests foraged for the three types of resources along the day (Figure 6). The number of foraging trips increased after 7:00 am and remained stable throughout the day, with a small peak in the period between 1:00 pm to 3:00 pm. However, since we only had a few samplings between 11:00 am and 1:00 pm, this period of the day was not considered. Thus, we are not able to state what the female activity was like during this period.

Table 1. Period of activity (in days) by females of *Epicharis dejeanii* in November 2017, during a survey conducted, from 6:00 am to 6:00 pm, at Ilha do Superagui, south of Brazil, in a nest aggregation. It is also shown the total number of foraging trips along the study period, mean number of foraging trips per day (± sd) and number of foraging trips for different types of resources (oil, pollen and oil + pollen). The observations were carried out at the entrance three nests, by two observers. In all nests, when the observations started, the females had already initiated the cell provisioning activities.

Nest	Period of activity in days	Total number of foraging trips*	Mean number of trips per day (± ds)	Number of foraging trips with only pollen	Number of foraging trips with only oil	Number of foraging trips with pollen and oil
3A	4	16	4.00 (± 1.83)	9	5	1
4A	5	16	3.20 (± 1.60)	3	7	3
5A	5	12	2.40 (± 1.52)	4	3	3
All	$\bar{X}$ = 4.67	$\bar{X}$ = 14.67 (± 2.31)		$\bar{X}$ = 5.33 (± 3.21)	$\bar{X}$ = 5.00 (± 2.00)	$\bar{X}$ = 2.33 (± 1.15)

* The total number of trips registered was superior to the sum of the numbers of trips for the different resources, since for some foraging trips it was not possible to identify for sure the type of resource in the scopae of the females when they returned to the nest.

For these 13 nests, we also registered that the females performed for up to 6 days of foraging trips, varying from 4 to 6 days. Moreover, when we interrupted our observations on 14 January, most females had not finished the foraging trips yet nor abandoned their nests. Only in two out of the 13 nests under observation, females stopped the foraging trips before the sixth day. In this case, females left their nests on the fourth and the fifth days and did not return. In these 13 nests, the mean number of foraging trips per day ranged from 3.8 (± 2.0; N = 23) to 5.8 (± 3.5; N = 35).

In additional observations to confirm the period females ceased their activities outside the nest, we registered that they ended near twilight, when the last bee was seen entering the nest at 7:18 pm. Lastly, since no bee emerged from cells taken to our laboratory, this result is not shown in our study.

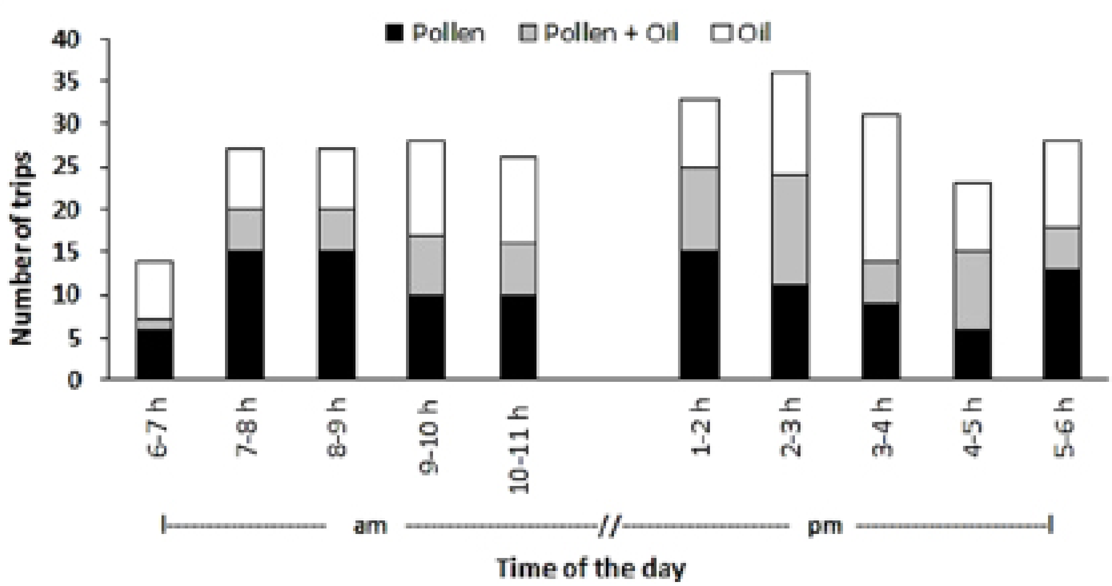

Fig 6. Number and diurnal distribution of trips carried out by females of *Epicharis dejeanii*, at Ilha do Superagui, Brazil, to collect the three classes of resources: pollen, pollen + oil and oil. // = period without observations on bee behavior.

Discussion

Nest architecture

Our results on *E. dejeanii* agree in great part with those of Hiller and Wittmann (1994) as well as Dec and Vivallo (2019), who also studied the nest architecture of this species in southern Brazil. The aggregations studied by these authors, as in our study, were constructed in sandy soil and nests were similar in their general structure, including the characteristics of the brood cell and diameters of tunnel, nest entrance and tumulus surrounding the entrance. However, some differences can be pinpointed here. For example, in the aggregation at IS we never detected the occurrence of more than one entrance giving access to the nest as reported in both studies (Hiller & Wittmann, 1994; Dec & Vivallo, 2019). In addition, all eight nests excavated in our study presented a descending tunnel, predominantly vertical, with only slight curves (or inclinations) going down in the soil, until it found the brood cell (as shown in Figure 3). This finding differs from those reported by Dec and Vivallo (2019), who found a conspicuous heterogeneity in the tunnels from 15 dug nests. These authors frequently found nests presenting a vertical tunnel, which after a slope ended in a horizontal tunnel, a condition we never found.

Concerning the number of brood cells per nest, our results are more closely in line with the findings of Dec and Vivallo (2019), who reported one or two brood cells per nest. However, considering that the latter excavated a higher number of nests (N = 15) than the current study (N= 8), this divergence might be reflecting the difference in sampling efforts. Despite this, both results by Dec and Vivallo (2019) and ours support the fact that the number of brood cells per nest of *E. dejeanii* is inferior to those estimated in Hiller and Wittmann (1994). In this case, either the higher number of cells per nest found by Hiller and Wittmann (1994) is a result of a plasticity in behavior of *E. dejeanii*, as already reported for *E. nigrita* (Martins et al., 2019) or it could be due to the difference in the methodology employed in the preliminary study on *E. dejeanii* (Hiller & Wittmann, 1994). In fact, considering that digging deep nests seems to be an energetically expensive task to solitary bees, by producing several brood cells per nest rather than just one, a female could optimize its digging effort, having reproductive advantage with this behavior. (Martins et al., 2014). It has also been suggested that, among species that show variation in number of cells built per nests, the low production is environmentally mediated (Michener, 1974), and that it is possible that perturbation by other nesting females or parasites may cause the nesting female to close the nest and start another one (Martins et al., 1994). However, in our study, the number of cells per nest was invariably one, despite most of nests surveyed are deep in the ground and independently of the number of days used for provisioning the nests by females. Thus, other factors could be mediating the construction from one (this study) to more than one brood cell per nest (Hiller & Wittmann, 1994; Dec & Vivallo, 2019) in *E. dejeanii*, which we are not able to point out for sure here.

The mean depth of nests excavated in the aggregation at IS was higher than those already described for *E. dejeanii* (Hiller & Wittmann, 1994; Dec & Vivallo, 2019). In fact, our results revealed the deepest nests so far described for *Epicharis* (e.g. Gaglianone, 2005; Rocha-Filho et al., 2008; Werneck, 2012; Dec & Vivallo, 2019). The construction of cells deep in sandy soils is common in *Epicharis*, and probably represents an important feature to avoid parasites (Gaglianone, 2005). It should be highlighted that although in our study the deepest cell was found at 185 cm deep, the total length of the tunnel excavated by the female, reached more than 210 cm, suggesting great effort of the bee in digging down the soil.

Many reports in the literature have shown that ground-nesting bee aggregations can persist for a long time, even decades, in the same area (Michener, 1974; Rozen & Michener, 1988; Cane, 2008; Danforth et al., 2019; Martins et al., 2019). In fact, the persistence of bee nest aggregations for years or even decades in a same area may be more common than we know (Cane, 2008). In our study, we observed that the large aggregation of *E. dejeanii* at IS persisted in activity for at least six years (from 2013 to 2018) in the same area. A combination of different appropriate factors, including soil texture and plenty and stable food resources have been pointed out as affecting the long-term persistence of such aggregations (Danforth et al., 2019). In addition, the habit of build nests in aggregations shown by a number of species of bees seems to have some advantages, including, for example, both high chances of encounters between partners for mating (Pina et al., 2020) and detections of cleptoparasites by the host bees (Litman, 2019).

Nesting and foraging behavior

Our results also agree with findings that pointed out *E. dejeanii* as an univoltine and a seasonal species, active only during the warm and wet season (Hiller & Wittmann, 1994; Dec & Vivallo, 2019). Since the three studies on nesting biology of *E. dejeanii* were carried out in the south of Brazil, in areas located between the following geographical coordinates: 25°27.51.4"S - 48°14.07.1'W (current study) and 29°22'33" - 51°06'43"W (Hiller & Wittmann, 1994), under subtropical climate, we could expect a similarity in the period of activity of the studied species. Whereas our results concerning the period of activity of *E. dejeanii* showed a complete overlapping with that described by Dec & Vivallo (2019), who also reported the activity of bees from November to January, the activity of this species started a little later in a region located farther south (Hiller & Wittmann, 1994). Therefore, even within a limited range of latitude and longitude, some variation can be found in the period of activity of *E. dejeanii*. The seasonal activity and univoltine cycle detected for *E. dejeanii* seems to be almost a pattern in *Epicharis*, having been reported for different species of this genus (Roubik & Michener, 1980; Gaglianone, 2005; Gaglianone et al., 2015; Martins et al., 2019), although exception for univoltine life cycle was reported to the species *Epicharis bicolor* Smith, 1958 (Rocha-Filho et al., 2008).

Concerning the period of diurnal activity of *E. dejeanii* at Ilha do Superagui (IS), as reported by Hiller and Wittmann (1994), we also observed that this species began its activity very early in the morning, at about daybreak, particularly between 5:00 am and 5:30 am in IS, and one hour earlier compared to the findings of Dec and Vivallo (2019) in the state of Santa Catarina. Our results at the end of the diurnal activities of *E. dejeanii* corroborate that the females ceased their external activities at dusk as reported by Hiller and Wittmann (1994). The behavior of spending the night inside the nest herein described was also observed in the two previous study with *E. dejeanii* (Hiller & Wittmann, 1994; Dec & Vivallo, 2019). By spending more time away from the nest, the female bee leaves it more vulnerable to predators and parasites (Kline & Joshi, 2020). Despite the activity of parasites and predators of bee nests is more intense during the day (Kelber et al., 2005), the behavior of females in spending the night inside the nest could be related to a more effective strategy in protecting their nests.

Diversely from the brief breaks for nectar uptake, the foraging trips for collecting oil, pollen or both usually took longer, most of which between 30 and 90 min, averaging about 60 min. Our results also revealed that the time of the foraging trips carried out by females of *E. dejeanii* at IS were very superior than those reported by Dec and Vivallo (2019), for this species in Ilha das Flores in Santa Catarina state. Thus, it is possible to infer that the higher time of foraging trips at Ilha do Superagui is probably a consequence of the longer distance to the food plants. In fact, at IS, although we walked around the study area looking for females of *E. dejeanii* foraging on flowers nearby, we only registered this species in shrubs of *Tibouchina* spp, suggesting that the females foraged for plants located far from the nest aggregation. Surely, the availability of food resources in abundance close to the study area favors shorter foraging trips. Gaglianone (2005) observed that *E. nigrita* performed shorter foraging trips (20 – 40 min), during the peak of the nesting activity, precisely when the main plant used as resource by this species was in the peak of flowering in the studied area. On the other hand, the foraging trips carried out by *E. nigrita* lasted up to two hours, probably because of the scarcity of food resources.

The intense activity by *E. dejeanii* carrying out foraging trips all day, starting soon after dawn and only ceasing the trips at dusk, was reported to *E. nigrita* by Gaglianone (2005). Particularly for females of solitary and univoltine bees, this intense activity in nest tasks is expected, since they usually live for only a few weeks (Willmer & Stone, 2004; Danforth et al., 2019) and have only a short time during the year to carry out the nesting activities to raise their offspring.

As shown by Gaglianone (2005) for *E. nigrita*, we also detected that, after returning to the nest from foraging trips, most of the times (@70%), females of *E. dejeanii* took less than 20 min to unload the different types of resources carried in their scopae. Our findings are also in line with those from Dec and Vivallo (2019), who reported a mean time of 23 min (± 3.55; N = 20) for *E. dejeanii* inside the nest after the foraging trips. Thus, at least for *E. dejeanii* and *E. nigrita* (Gaglianone, 2005) the time for unloading and possibly handlining the plant resources collected was similar between these two species, as well as didn't vary among different locations.

A conspicuous difference between our findings and those reported by Dec and Vivallo (2019) is related to the period in days that females worked in their nests. According to these authors, the total period ranging from the nest excavation and the abandonment of the nests occurred in 3 to, rarely, 4 days. However, in our study, this period was usually longer, since in January 2016, for example, we observed 13 nests for a week, when all these nests were already in the provisioning phase. In addition, when observations were stopped after a week, 11 out of 13 females had not abandoned their nests and were carrying out foraging trips, indicating that they still had not finished their activity for brood cell provisioning. Hiller and Wittmann (1994), on the other hand, estimated a period of five days of female activity. A reasonable explanation to the longer period performing nesting tasks detected for *E. dejeanii* at Ilha do Superagui, could be the high rainfall rates (> 2,500 mm per year) in the region where this island is located, with the highest incidences of rain coinciding exactly with the period of activity of this species. In fact, we observed that *E. dejeanii* had to frequently interrupt its external activities due to unfavorable weather conditions.

Based on our results on behavior of *E. dejeanii* females during their nesting activities and comparing our findings with two previous studies (Hiller & Wittmann, 1994; Dec & Vivallo, 2019), we can conclude that in spite of a number of similarities found among these studies, a variation or plasticity in nesting behavior of this species can occur in different locations from the same region. Together, these three studies contribute with complementary information to more detailed knowledge on the nesting biology of *E. dejeanii*, until date, the only species representing the *Anepicharis* subgenus.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Authors' Contributions

N. Uemura: conceptualization, methodology, investigation, formal analysis, writing
S.H. Sofia: conceptualization, methodology, formal anlysis, writing
A.L. Gobatto: investigation, data curation, formal analysis
W.C. Pina: investigation
R.H. Ono: investigation
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Supplementary Material

Table S1. Dates of samplings conducted at Ilha do Superagui, state of Paraná, southern Brazil, during some months in warm-wet season, from December 2013 to November 2017, types of activities carried out by study team in each date and total time spent in each sampling day.

Date	Period of sampling	Total sampling time	Type of activity
22/12/2013	7:00 – 12:00 am	5 h	Nest excavation
23/12/2013	7:00 – 12:00 am	5 h	Nest excavation
11/11/2015	6:00 – 11:00 am 1:00 – 6:00 pm	10 h	Bee behavior observation
12/11/2015	6:00 – 11:00 am 1:00 – 6:00 pm	10 h	Bee behavior observation
13/11/2015	6:00 – 11:00 am 2:00 – 3:00 pm*	6 h	Bee behavior observation
14/11/2015	6:00 – 11:00 am 2:00 – 4:00 pm*	7 h	Bee behavior observation
15/11/2015	6:00 – 8:00 am*	2 h	Bee behavior observation
16/11/2015	6:00 – 11:00 am 1:00 – 6:00 pm	10 h	Bee behavior observation
17/11/2015	6:00 – 11:00 am 1:00 – 5:00 pm*	9 h	Bee behavior observation
18/11/2015	6:00 – 11:00 am 1:00 – 6:00 pm	10 h	Bee behavior observation
08/01/2016	6:00 – 11:00 am 1:00 – 6:00 pm	10 h	Bee behavior observation
09/01/2016	6:00 – 11:00 am 1:00 – 4:00 pm*	8 h	Bee behavior observation
11/01/2016	7:00 – 11:00 am* 1:00 – 6:00 pm	9 h	Bee behavior observation
12/01/2016	1:00 – 6:00 pm*	5 h	Bee behavior observation
13/01/2016	6:00 – 11:00 am 1:00 – 6:00 pm	10 h	Bee behavior observation
14/01/2016	6:00 – 11:00 am*	5 h	Bee behavior observation
13/02/2016	7:00 am – 7:00 pm	12 h	Nest excavation
14/02/2016	7:00 am – 7:00 pm	12 h	Nest excavation
15/02/2016	7:00 am – 7:00 pm	12 h	Nest excavation
16/02/2016	7:00 am – 7:00 pm	12 h	Nest excavation
13/12/2016	5:00 – 10:00 am 6:00 – 8:00 pm	5 h 2 h	Bee behavior observation
14/12/2016	5:00 – 6:00 am 6:00 – 8:00 pm	1 h 2 h	Bee behavior observation
15/12/2016	8:00 am – 6:00 pm	10 h	Nest excavation
31/10/2017	6:00 am – 6:00 pm	12 h	Bee behavior observation
01/11/2017	6:00 am – 6:00 pm	12 h	Bee behavior observation
02/11/2017	6:00 am – 6:00 pm	12 h	Bee behavior observation
03/11/2017	6:00 am – 6:00 pm	12 h	Bee behavior observation
04/11/2017	6:00 am – 6:00 pm	12 h	Bee behavior observation
TOTAL		**249 h**	

* = samplings interrupted due to bad weather conditions (rain)

Figure S1. (a) Detail of a female of *Epicharis dejeanii* flying over the ground at a height of approximately 2 cm; (b) on the ground, beginning the construction of the nest.

Figure S2. Sequence of images (A-F) showing moments of a nest re-excavation by a female of *Epicharis dejeanii* after a rainy period, which caused the obstruction of the nest entrance.