Revista Ciência Agronômica, v. 54, e20228348, 2023
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Nesting and foraging behaviour of the solitary bee *Epanthidium tigrinum* (Schrottky, 1905) bred in trap nests[1]

Comportamentos de nidificação e forrageio da abelha solitária *Epanthidium tigrinum* (Schrottky, 1905) criada em ninhos-armadilha

Vitória Inna Mary de Sousa Muniz[2]*, Larysson Feitosa dos Santos[2], Pedro de Assis de Oliveira[2], David Rezende da Silveira[2], Breno Magalhães Freitas[2]

ABSTRACT - The bee *Epanthidium tigrinum* is native to Brazil and shows potential for use in pollinating agricultural crops. However, the little information available concerning its reproductive and feeding habits has prevented any rational breeding or management of these bees. The aim of this study was to investigate their acceptance of trap nests, and their nesting and foraging behaviour, with a view to understanding the potential of the species for rational breeding. Trap nests were offered, and the females were observed for 16 months to monitor the establishment, building and closing of nests, as well as their architecture and the behaviour of the bees when searching for the resources used to build and provision the nests. The results showed that *E. tigrinum* performs well in trap nests; prefers cavities with a diameter of around 0.45 cm; depends on other plant resources besides pollen and nectar, such as leaves, fibres and resins, to build its nest; and remains active throughout the year. The study concluded that the species has the potential for use in agricultural pollination programs; however the observed nesting requirements must be taken into account, both in conservation efforts and in zootechnical exploitation when breeding the bee for agricultural pollination, as both can influence the number of constructed nests and cells, and consequently, the size of the pollinator population.

Key words: Beekeeping. Nest-building. Floral resources. Non-floral resources. Seasonality.

RESUMO - A abelha *Epanthidium tigrinum* é nativa do Brasil e apresenta potencial para uso na polinização de culturas agrícolas. No entanto, a pouca informação disponível sobre seus hábitos reprodutivos e alimentares tem impedido qualquer criação ou manejo racional dessas abelhas. O objetivo deste estudo foi investigar a sua aceitação de ninhos-armadilha, e o seu comportamento de nidificação e forrageamento, com vista a compreender o potencial da espécie para a criação racional. Ninhos-armadilha foram oferecidos, e as fêmeas foram observadas por 16 meses para monitorar o estabelecimento, construção e fechamento dos ninhos, bem como suas arquiteturas e o comportamento das abelhas na busca dos recursos utilizados para a construção e provisão dos seus ninhos. Os resultados mostraram que *E. tigrinum* tem um bom desempenho em ninhos-armadilha; prefere cavidades com diâmetro em torno de 0,45 cm; depende de outros recursos vegetais além do pólen e do néctar, como folhas, fibras e resinas, para construir seu ninho; e permanece ativa durante todo o ano. O estudo concluiu que a espécie tem potencial para uso em programas de polinização agrícola; no entanto, as exigências de nidificação observadas devem ser levadas em consideração, tanto nos esforços de conservação quanto na exploração zootécnica quando criando a abelha para polinização agrícola, pois ambas as situações podem influenciar o número de ninhos e células construídas e, consequentemente, o tamanho da população de polinizadores.

Palavra-chave: Criação de abelha. Construção de ninhos. Recursos florais. Recursos não florais. Sazonalidade

---

DOI: 10.5935/1806-6690.20230031
Editor-in-Article: Profa. Andrea Pereira Pinto - deiapp@hotmail.com
*Author for correspondence
Received for publication on 09/02/2022; approved on 14/10/2022

[1] Part of the Master dissertation presented to the Graduate Course in Animal Science, Federal University of Ceará (UFC), funded by a cooperation agreement between BAYER AG. (Germany) and the Federal University of Ceara (Brazil)

[2] Department of Animal Science, Bee Research Unit, Federal University of Ceará (UFC), Fortaleza-CE, Brazil, vitoriamuniz63@hotmail.com (ORCID ID 0000-0002-3387-6688), laryssonfeitosa17@gmail.com (ORCID ID 0000-0002-3735-9637),daviddasilveira5@gmail.com (ORCID ID 0000-0002-1873-6951), pedromanari@hotmail.com (ORCID ID 0000-0001-9960-9278), freitas@ufc.br (ORCID ID 0000-0002-9932-2207)

INTRODUCTION

There are approximately 20,000 described species of bees, of which around 85% have solitary habits (DANFORTH *et al*., 2019; MICHENER, 2007). The solitary way of life is characterised by a single female building nests without the cooperation of other individuals. As a result, floral resources for provisioning the cells are sought independently, and shortly after oviposition, the nests are closed and abandoned with no overlap between generations (DANFORTH *et al*., 2019; MICHENER, 2007; NEFF, 2008). Thus, much of the life of solitary female bees is dedicated to searching for nesting sites, building brood cells, and collecting larval food. As the nests of these bees are difficult to locate in natural environments, for most solitary bee species, especially tropical species, there is a lack of information regarding nesting habits or foraging to provision the nest (COSTA; GONÇALVES, 2019; PARIZOTTO; MELO, 2015; PARIZOTTO; URBAN; MELO, 2022). This lack of information hinders conservation efforts and the sustainable use of these pollinators (GALETTO *et al*., 2022; BRASIL *et al*., 2019).

Epanthidium tigrinum (Schrottky, 1905) (Hymenoptera: Megachilidae) is one of those species for which little information is available. This small solitary bee (1.97 cm) is widely distributed, occurring from Brazil to Bolivia, Paraguay and the north of Argentina (URBAN; MOURE, 2012), where there are reports of it visiting and potentially pollinating wild plants and also cultivated plants such as the bean (*Phaseolus vulgaris*), cowpea (*Vigna sinensis*) and cashew (*Anacardium occidentale*) (ALVES-DOS-SANTOS, 2004; CAMAROTTI-DE-LIMA; MARTINS, 2005; FREITAS *et al*., 2014; GOMES *et al*., 2020). The few studies with this species show that it accepts linear trap nests, where it builds four to five cells using resin mixed with plant material (CAMAROTTI-DE-LIMA; MARTINS, 2005; GOMES *et al*., 2020; MESQUITA; AUGUSTO, 2011). However, there is still little information about its breeding habits, seasonal nesting patterns or use of floral resources. Furthermore, as these observations were made at a small number of nests or over a short period, and since the species is active throughout the year, there is the possibility of the information being relevant to that period and those climate conditions only (GOMES *et al*., 2020). On the other hand, the use of trap nests allows bionomic data on the females to be collected when establishing their nests, such as the process of nest selection, identification of the type of food stored in the cells, nest architecture, and the time taken to build the cells and nests, in addition to investigating the relationship with other insects associated with the nests (kleptoparasites) (COSTA; GONÇALVES, 2019).

The aim of this study, therefore, was to obtain information on the nesting and foraging behaviour of *E. tigrinum* in trap nests over a long period in order to understand the nesting habits and requirements of the bee over time, such as its behaviour when choosing and building the nest, foraging activity and the resources used, the internal architecture of the nests, and interactions with other insects that may also be attracted to the trap nests. The idea is to create a knowledge base for future studies on breeding and managing the species for use in agricultural pollination, which can contribute to our knowledge of the way solitary tropical bees adjust their reproductive cycles according to the season and availability of resources.

MATERIAL AND METHODS

The study was conducted from May 2019 to November 2020, in the Bee Unit of the Department of Animal Science, on the Pici Campus, Federal University of Ceará, Fortaleza, Ceará (03°43’02” S, 38°32’35” W, altitude 16 m) (INSTITUTO DE PESQUISA E ESTRATÉGIA ECONÔMICA DO CEARÁ, 2017). According to Köppen (1918), the climate in the region is classified as Aw’: sub-humid hot tropical, with a rainy season from January to May. The average annual rainfall is 1338.0 mm, with an average temperature of 26.9 °C. The average annual minimum and maximum temperature is 23.6 °C and 30.4 °C, respectively. The average relative humidity is 78%, with insolation of 2,856.5 hours per year and average wind speed of 3.7 m/s (INSTITUTO DE PESQUISA E ESTRATÉGIA ECONÔMICA DO CEARÁ, 2017). During the experimental period, these parameters were monitored by the Weather Station of the Federal University of Ceará, located just 500 m from the study site. The data showed that the maximum temperature reached 29.1 °C in November 2020, while the minimum recorded temperature was 25.8 °C in July of the same year, with the hottest times of the day between 10:00 and 15:00, and the coldest between 02:00 and 05:00. The maximum humidity reached 83.4% in April, with a minimum of 63.1% in September, both in 2020. The wind speed ranged from 0.52 m/s in March 2020 to 3.40 m/s in September 2019. Each of these values is within the historical mean of the parameters under evaluation.

In the present study, trap nests (TN) were set up 130 cm above the ground in an open shed, 10 m long and 3 m wide, with an asbestos roof for protection against the sun and rain. The trap nests comprised forty-two wooden boards, each with 10 semi-circular openings, so that overlapping the boards formed holes for the insertion of cardboard tubes closed at the end with the wax from *Apis mellifera*, in adaptation of the trap-nest technique proposed by Krombein (1967) (Figure 1a). The cardboard tubes were 12 cm long, and divided into seven diameter classes: 0.35 cm, 0.40 cm, 0.45 cm, 0.5 cm, 0.55 cm, 0.6 cm and 1.0 cm, giving a total of 420 trap nests for the bees. In addition, 45 trap nests made from transparent plastic tubes installed inside a closed wooden box were made available to the bees in order to allow observation of the interior of the nests (Figure 1b). Only the entrance of each nest was open to the external environment, ensuring a dark environment inside the box, necessary for the bees to nest (KROMBEIN, 1967).

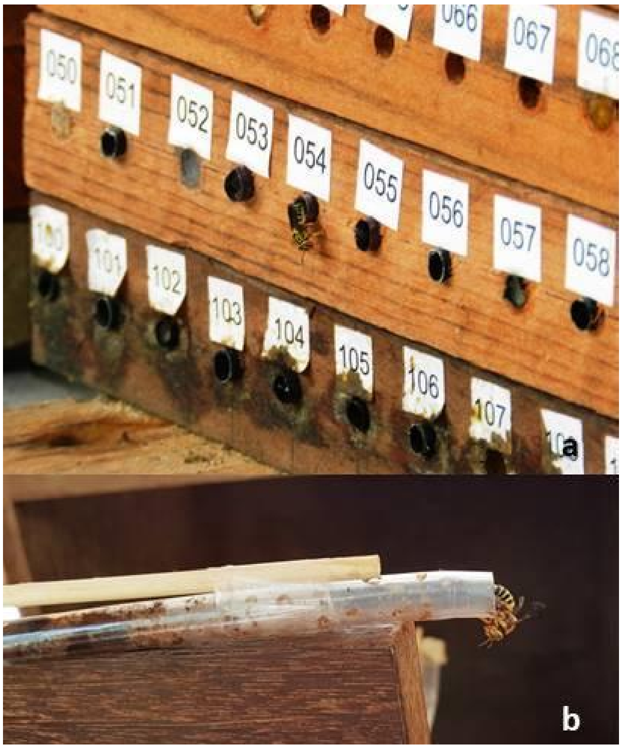
The bees were observed throughout the study while searching for a nesting site and building nests. The activities of females that nested in the TN were monitored individually, from when they started to inspect the trap nests, including their choice of TN, to when they started building the nest until its conclusion, and even later, in the case of establishing other nests. These observations were made throughout the time of day that the bee was active, usually from 05:00 to 18:00. To make it possible to monitor each bee individually, as soon as a young female had chosen a TN, it was captured at the entrance with the aid of a Falcon tube and placed in a freezer at -4.0 ºC for three minutes until it fell asleep. The bee was then marked on the thorax with a distinct pattern that allowed it to be identified. The markings were made with non-toxic paint, using a model D and Z 3378 ball-brush for decorating nails.

The bees were also monitored for their foraging behaviour (time, collected resources, materials used to build the nest, closing the nest, fighting, pillaging, warm-up flight, etc.), and for cell-construction time, as well as for information about the number of nests established by each female and the presence of natural enemies. For this, the females of *E. tigrinum* were monitored visually and the information recorded over 75 days from July to September 2020. The observations were made continuously from dawn (05:00) to dusk (18:00).

Nest-building was observed in both the cardboard trap nests (external aspects) and the transparent trap nests (internal aspects). In addition, following the emergence of the hatchlings from the cardboard nests, the internal architecture of 86 randomly chosen nests was investigated based on the following parameters: number of cells per nest, presence or absence of vestibule cells, total length of the nests, and length of each cell. The shape of the cells and their arrangement and orientation in relation to the cavity, partitions and opercula were also described. Measurements inside the nests were taken with the aid of an MTX model 316119 digital calliper.

The search for nesting sites; building the nest and the number of nests built; the number and time of the trips to collect pollen, nectar, resin and other resources; the length of the constructed cells; the time spent constructing the cells and building the nests; and the nesting behaviour were analysed using descriptive statistics. Differences between the busiest times of the day searching for such resources as pollen, nectar and resin were submitted to ANOVA after transforming the data, and the mean values were compared *a posteriori* by the Student-Newman-Keuls (SNK) test at 5% confidence. To assess a possible preference of *E. tigrinum* females for a nest diameter of any of the classes under study, the nests were grouped by diameter and counted. The Chi-square test was used at a level of 5% to investigate a possible ‘preference’ in relation to the diameter of the TN and the type of cell arrangement most used by *E. tigrinum*. The mean value and respective standard deviation were calculated for each of the parameters under evaluation.

Figure 1 - Types of trap nests available in the *Epanthidium tigrinum* nesting experiment: a – breeding nests made of black cardboard tubes inserted into wooden blocks; b – nests for interior observation, made of transparent plastic tubes placed in a dark environment



RESULTS AND DISCUSSION

E. tigrinum searched for a nesting site between 09:00 and 16:00, but especially at 15:00, with 23.52% (n = 83) of the 353 bees under observation preferring this time. The choice of a place to build the nest began with the female inspecting various cavities in the blocks of TN, entering quickly and leaving shortly after (Figure 2a). This behaviour was monitored 53 times throughout the study period, and showed that the bees visited from 2 to 18 (5.92 ± 4.13 SD) cavities to choose a TN for nesting. After inspecting the cavities, the female selected one and remained inside it, exhibiting guard-bee behaviour at the entrance (Figure 2b) for a period that could vary from 28 to 344 minutes (115.31 ± 82.95, n = 38). Nest-building would begin immediately following this period, or the following day if the choice of nest had been made close to sunset.

The observed behaviour when choosing a nesting site was generally similar to that previously described for other members of Megachilidae (ALVES-DOS-SANTOS, 2004; SANTOS *et al*., 2020), and for *E. tigrinum* itself (GOMES *et al*., 2020), with the bees initially evaluating various cavities until choosing the most pleasing. This is because such aspects as the type of cavity, protection against light, excessive heat or humidity, parasites and predators are important criteria for the bees when choosing a cavity (BOSCH; KEMP, 2002; BOSCH *et al*., 2021). Despite all the cavities in the trap nests being similar in the present study, *E. tigrinum* bees generally spent several hours visiting dozens of cavities before choosing one, showing that the females can be demanding regarding a nest-building site when given a choice.

During the experiment, the bees built 131 nests in the 420 trap nests in each of the diameters offered, representing an occupancy of 31.19%. Despite nesting in TN with diameters that ranged from 0.35 to 1.0 cm, the bees showed a significantly greater preference ($X^2 = 571.94$, $p = 0.002$) for nests with a diameter of 0.45 cm (Table 1). This is probably due to the smaller diameters not allowing the bees to work with the resources they collected, while the larger diameters increase competition with other species (bees and wasps) and require far more resources to line the cavity and build the nest (CHUI; KELLER; LEONHARDT, 2021; COSTA; GONÇALVES, 2019; GOMES *et al*., 2020). This information is important for developing a breeding system for *E. tigrinum*.

After choosing the TN, nest-building began with the search for plant resin that the bees collected from plants close to the nesting site. The individuals collected the resin and deposited it in lumps inside the nest, close to the entrance, so that they could be moulded at a later time. This behaviour, which was better observed when the TN was dissected to study the architecture (Figure 2c), explains how the bees are able to continue building the nest on days they clearly bring no resin back from the field (CHUI; KELLER; LEONHARDT, 2021). For example, it is assumed that storing and later handling the resin are related to the hottest hours of the day, i.e. the short time this resource is malleable and can be collected from plants and flowers (GOMES *et al*., 2020; TÖLKE *et al*., 2020). In addition, storing the resin can save the bee from flying day after day searching for this resource, or risking it not being available later (CHUI; KELLER; LEONHARDT, 2021; COLLETT; CHITTKA; COLLETT, 2013; DANFORTH *et al*., 2019; ROUBIK, 1992). This may be a strategy for ensuring the existence of resources to build the nest even when they are no longer available in the field, whether temporarily or permanently.

To building the brood cells, the female *E. tigrinum* would choose a location in the innermost part of the cavity, usually between 10 to 12 cm from the entrance, and begin to line the interior wall of the cavity with resin. In some cases, plant fragments of unidentified origin that resembled leaves, petals and fibres were added to the resin (Figure 2d). Furthermore, when the females established nests in previously occupied cavities or in the deactivated nests of *Centris analis*, *Tetrapedia diversipes* and wasps that also took advantage of the available TN, the resin might include the sand and/or clay previously used by these insects in building their nests. The use of resins, plant fibres, flower buds, sand and clay was already well known in other members of Anthidiini (MELLO; GAGLIANONE, 2019; MESQUITA; AUGUSTO, 2011; MICHENER, 2007), in addition to having been recently reported for *E. tigrinum* (GOMES *et al*., 2020). However, the observations reported in the present study expand our knowledge of how *E. tigrinum* females acquire and manipulate some of the materials used to construct the cells, and their opportunistic behaviour in taking advantage of materials previously used by other tenants but which they themselves do not collect, such as sand and clay.

Table 1 - Number and percentage of nests built by *Epanthidium tigrinum* in 420 trap nests of different diameters, from May 2019 to September 2020, in the Bee Unit of the Department of Animal Science at the Federal University of Ceará

Trap-nest diameter (cm)	Number of nests built	(%)
0.35	17 b	12.97
0.40	10 bc	7.63
0.45	62 a	47.33
0.50	19 b	14.51
0.55	18 b	13.74
0.60	04 c	3.06
1.00	01 c	0.76
Total	131	100.00

Figure 2 - Choosing and building the nest in *Epanthidium tigrinum*: a – female inspecting trap nests before choosing the nesting site; b – female guarding the entrance of the trap nest, to establish possession after choosing; c – resin stored inside the nest; d – material of plant origin (fibres, leaves, flowers) also used to line the nest; e – *E. tigrinum* egg on a mass of pollen shortly after oviposition; f – finished nest, showing the closing wall

After preparing the cell, the female made several trips (5.54 ± 3.45, n = 94) to the field in search of pollen and nectar for provisioning. Once the desired amount had been collected, the pollen and nectar were agglutinated and moulded into a uniform circular mass on which the female laid a single, proportionally large egg in relation to her own size (Figure 2e). Then, she closed the cell with a layer of resin and plant fragments. This layer predominantly of resin delimited the end of that cell and served as the basis for constructing the next one, thereby forming a partition between the brood cells. This process was repeated successively in later cells until the final cell was finished, completing the nest with a structure called the closing wall (Figure 2f).

The female *E. tigrinum* took 24 to 120 hours (1 to 5 days) to complete a cell (n = 60), and 299.5 ± 19.46 hours (12.47 days) on average to complete a nest (n = 115). The shortest time seen for a female to build a nest was only 24 hours, recorded when the female completed the nest after building a single cell (n = 3). On the other hand, some females built two to three nests simultaneously (n = 5), which increased the time to complete any one of these nests; the longest time taken to complete a nest was 1,416 hours (59 days).

On average, the *E. tigrinum* bees started foraging at 08:31 ± 1.10 h. But, on warmer days, some bees were seen to start their activities around 06:00, and on cloudy days or with rainfall in the early morning, the bees would start foraging around 10:00 (Figure 3a). The females collected pollen more frequently during the morning, gradually reducing the collection of this resource throughout the day (Figure 3b).

The time spent in the field on pollen collection ranged from 8 to 170 minutes, with 331 trips to collect pollen seen between 09:00 and 12:00 during the study period (Figure 3b). Such variation is usually related to the accessibility of the resources, as well as their availability and location (FARZAN; YANG, 2018; KLEIN; STEFFAN-DEWENTER; TSCHARNTKE, 2004). There were important pollen sources for *E. Tigrinum* close to the trap nests, such as *Byrsonima crassifolia* and *Vigna sinensis*, which might explain the occasions on which the collection of these resources was quick, while collections that took more time were probably due to the need to search for more-distant pollen sources when closer sources were not in flower, especially during the time of the year when there is little flowering. The proximity of floral resources to the trap nests can be advantageous, since a prolonged search for food is costly in terms of time and energy, and can lead to a lower rate of reproductive success (KLEIN; STEFFAN-DEWENTER; TSCHARNTKE, 2004; SANTOS *et al*., 2020).

Upon arriving from the field with pollen, *E. tigrinum* would immediately enter the nest with its head facing inwards, then leave the nest with its abdomen facing outwards and the pollen still in the scopae, then, without taking flight, turn around in the entrance to the nest. The bee would then re-enter the nest, but this time with its abdomen facing inwards. After exhibiting this behaviour, the females would provision the cells with the pollen. Sometimes they would repeat this process of entering and leaving the nest once or even twice, before finally concluding the ritual and depositing all the pollen. This ‘dancing’ around the nest usually took place when the bee arrived with pollen, but was occasionally carried out when returning from trips to collect resin and other plant material (Figure 4).

The entire process, from the first entry after arriving from the field and the final entry before unloading all the pollen, took from one to ten minutes (3.20 ± 2.79 minutes, n = 45). Sometimes the bees would be unable to perform the ‘dance’. In this case, after several attempts, they would start visiting other cavities, perhaps looking for a new nesting site, or trying to deposit some of the collected resources in another cavity and then more easily enter the original nest. Such behaviour was recorded seven times (Figure 3a).

Resin collection also took place throughout the day, starting in a very small way at 08:00 and continuing until 16:00 (Figure 3b). During the afternoon, from 12:00 onwards, the number of trips to collect resin was greater, especially between 13:00 and 15:00. At the peak of resin collection (15:00), the bees were seen to make 112 trips to collect resin during the study period (Figure 3b).

The trips to collect resin took between 10 and 60 minutes (29.45 ± 13.43 minutes, n = 153), and were easily distinguished from trips in search of pollen or nectar, as the bees would return to their nests with lumps of resin trapped in their mandibles (Figure 5a). The resin generally had an amber or light-yellow colour, or was transparent. In the present study, the botanical origin of the resins collected in the field was not investigated.

In addition to collecting resin, the bees also collected plant material, which consisted of petals, leaves and fibres that were also used in constructing the cell walls and the plug that seals the nests. A total of 15 trips (n = 15 bees) to collect this type of resource were observed at peak time (13:00) during the study period (Figure 3b).

The bees also made trips from which they returned with no visible pollen or resin, suggesting that they were looking for liquids, such as nectar, which is used to moisten and provide energy for the mass of food prepared for the larvae, or water, which can also be used to increase the humidity of the food and/or to increase the humidity of the air inside the nest. Nectar and water were also collected throughout the day, from 09:00 to 15:00, but far less frequently than the pollen and resin, as they are needed in far smaller amounts (Figure 3b). The outside activities of the bees generally ended at dusk, around 16:00, although on one occasion, one bee ended its activities at 17:00 (Figure 3a).

In general, the availability of resources, the type of resource used, variations in the environment, and the intrinsic differences between individuals within species, influenced the time of foraging activity in *E. tigrinum*, corroborating preliminary reports from studies of shorter duration and with a smaller number of observations of both this and other species of Megachilidae (GOMES *et al*., 2020; ROUBIK, 1992; SANTOS *et al*., 2020). Reliable information on the foraging patterns of *E. tigrinum* is a prerequisite that should be taken into account when developing breeding sites for implementing conservation or pollination programs for plants of interest.

Other behaviour shown by the females of *E. tigrinum*, albeit recorded less frequently, included direct fight with other individuals of the same species, with males and females of *C. analis* and *T. diversipes*, and with natural enemies (ants), always when defending their nests against appropriation by those individuals (Figure 5b); warming up the thorax muscles before the first morning flight; a short flight, followed immediately by entering the nest, which was probably to clean the nest or defaecate; and pillaging other nests, which was recorded 27 times during the period under observation (Figure 3a). In 2019, perhaps as a result of a year of low rainfall and few resources available in the field, the bees clearly displayed this pillaging behaviour more frequently. There was even one case in June 2019 of an individual that never went into the field, and built its nest only by pillaging the resources collected by females nesting in the vicinity of its own nest (Figure 5c).

After finally collecting pollen to provision a cell, the female would lay an egg and then start building a partition between the cells. The time of oviposition, and of closing one cell and beginning a new cell, was clearly evident by the change in the behaviour of the female, which had been collecting pollen, and now began to collect resin. As the nest was built, the female successively alternated the collection of resin and pollen each time one cell was finished and another started, until finally completing the partition of the last brood cell in the nest, when in most cases, she built the closing wall (Figure 2f). The building of these structures in the nests was followed in detail over 45 events carried out by several females during the period under observation (Figure 6).

Closing the nest occurred during the hottest time of the day (10:00 to 15:00), probably because the resin was easier to handle (Figure 3a). After closure, some females remained active around the blocks of trap nests and looked for other cavities to build new nests (Figure 5d). Sometimes these females would build a new nest and return to the previously closed nest to inspect the area of closure with

Figure 3 - Frequency of nesting and foraging behaviour (a) and the resources used (b) from 06:00 to 17:00 for *Epanthidium tigrinum* in trap nests from July to September 2020, in the Bee Unit of the Department of Animal Science at the Federal University of Ceará

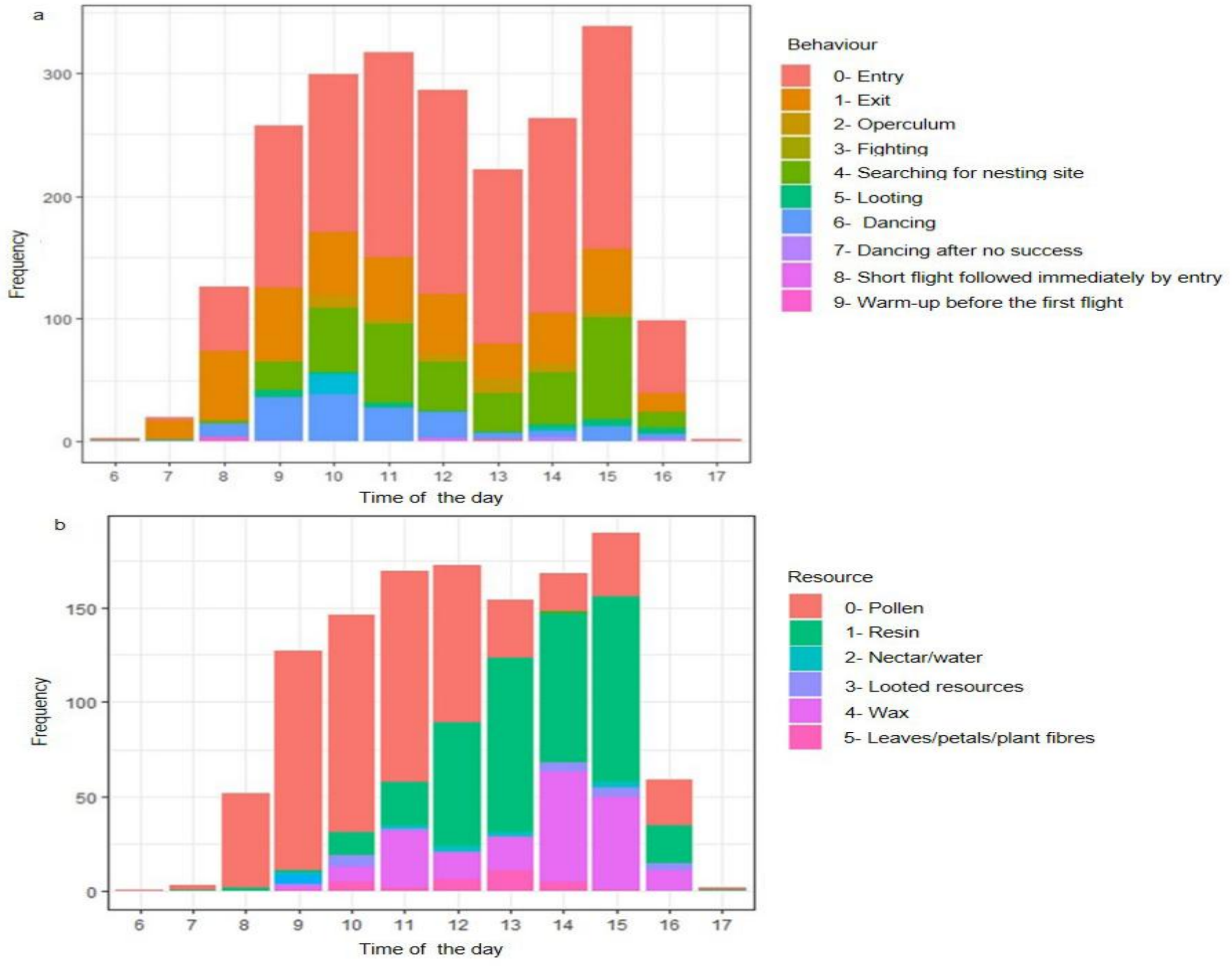
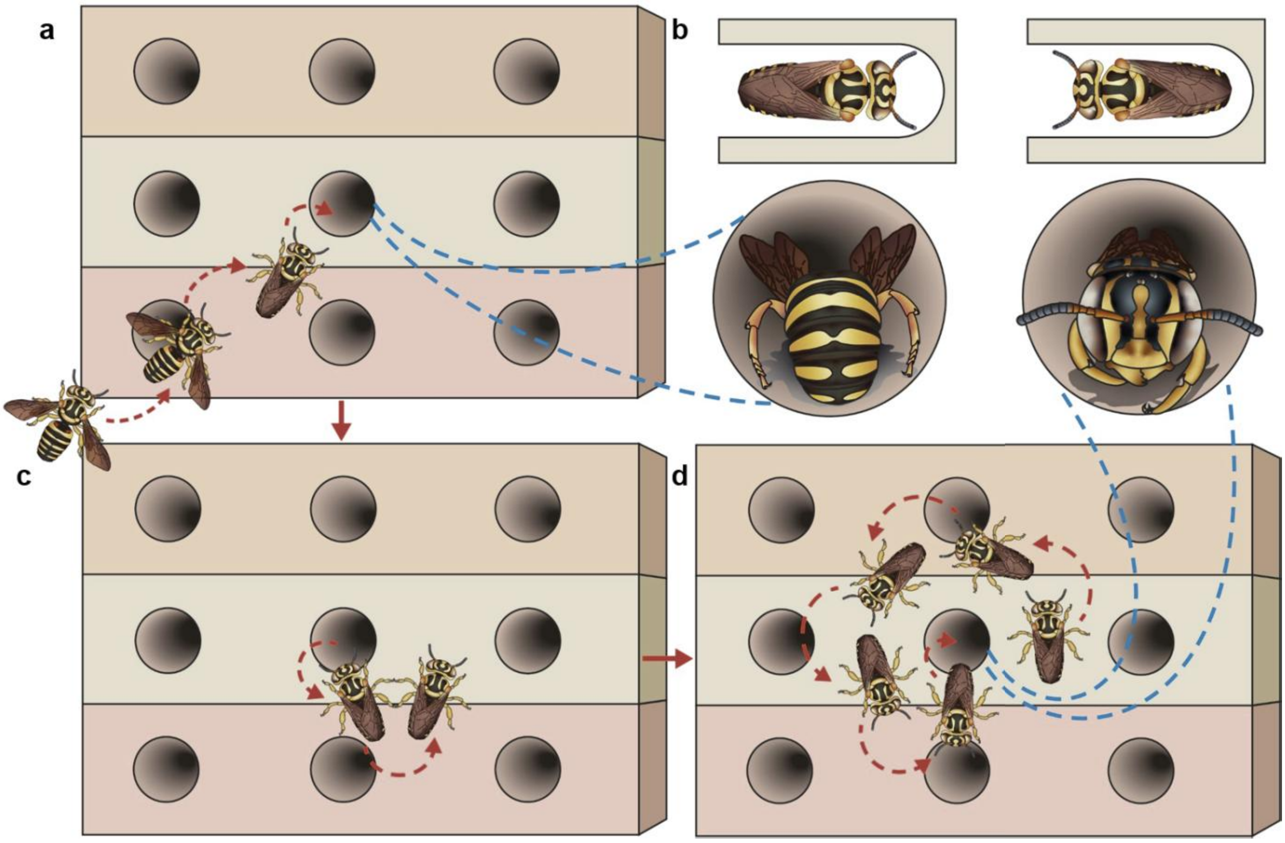


Figure 4 - Schematic representation of the ‘dancing’ behaviour displayed by *Epanthidium tigrinum* at the entrance of the trap nests after returning from the field



(a) Arriving from the field, *E. tigrinum* enters the nest directly with the head turned inwards as shown in the drawings on the left side of Figure b; (c) It then leaves the nest backwards, with the abdomen facing outwards and pollen still present in the scopae; (d) Without taking off, the bee circles around the entrance of the nest and then re-enters, only this time with the abdomen facing the interior of the nest, as shown in the drawings on the right side of Figure b

their mouthparts, evaluating the state of the resin and/or the possible removal of small portions for the construction of new nests. Resources were also seen to be divided between nests built simultaneously by the same female.

In studying the architecture of the nests, a total of 86 of the 131 nests were analysed and their main internal structures were measured and described (Table 2). The number of cells per nest ranged from 1 to 10 (4.55 ± 2.30, n = 86), the maximum number being higher than that found by Gomes *et al*. (2020) and Mesquita and Augusto (2011). This suggests that this factor varies considerably for the species, and is probably related to the greater or lesser ease in obtaining the necessary resources, and the time spent to build each nest. The length of the cells ranged from 0.26 to 3.53 cm, and the overall mean length of the brood cells was 1.05 ± 0.41 cm (Table 2), similar to the values found by Gomes *et al*. (2020).

The nest of *E. tigrinum* generally consists of a linear series of cells that may be oriented horizontally or transversally in the cavity and is separated by walls (partitions). The linear arrangement of the cells, grouped and in random rows, is reported for *E. tigrinum* and other species of Megachilidae (GOMES *et al*., 2020; MELLO; GAGLIANONE, 2019; MESQUITA; AUGUSTO, 2011; SANTOS *et al*., 2020).

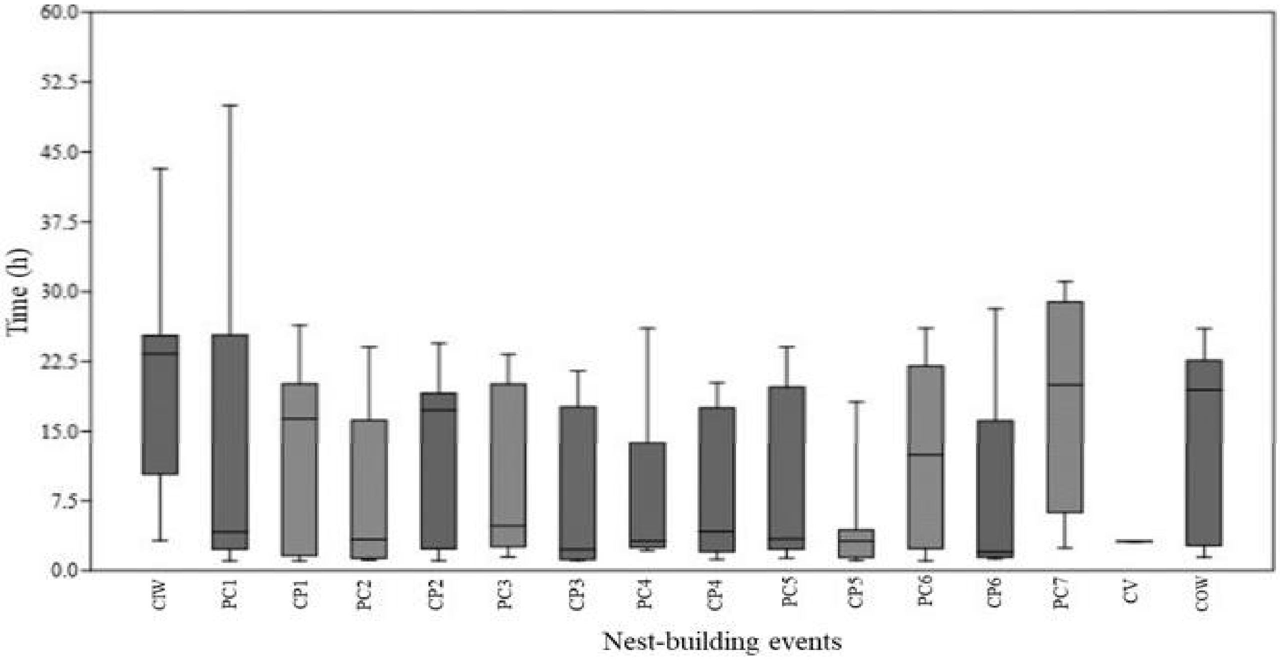
For *E. tigrinum*, all the cells initially had similar architecture (horizontal cells). However, when cavities of larger diameter (0.45 to 1.00 cm) were available, the construction of transverse cells was sometimes seen. This type of architecture occurred in nests with a diameter of 0.45 to 1.00 cm, and was more frequent at a diameter of 0.55 cm (Figure 7).

The average number of cells per nest with transverse architecture was 5.7 ± 2.21, while with linear architecture it was 4.44 ± 2.25. However, there was no significant difference ($p < 0.05$) between the average number of cells for the type of architecture used. The only time ten cells were seen in one nest was when transversal architecture was used. However, the horizontal type of architecture (n = 77) was significantly more common

Figure 5 - Behaviour of females of *Epanthidium tigrinum* nesting in trap nests

a – carrying resin in their mandibles; b – marked female trying to expel a male of *Centris analis* that came to rest in the nest while the female was away; c – pillaging material from nearby nests; d – inspecting places to build a new nest (note the worn wings indicating an older bee)

Figure 6 - Average time spent (hours) by females of *Epanthidium tigrinum* on each stage of nest-building in trap nests, from May 2019 to September 2020, in the Bee Unit of the Department of Animal Science at the Federal University of Ceará

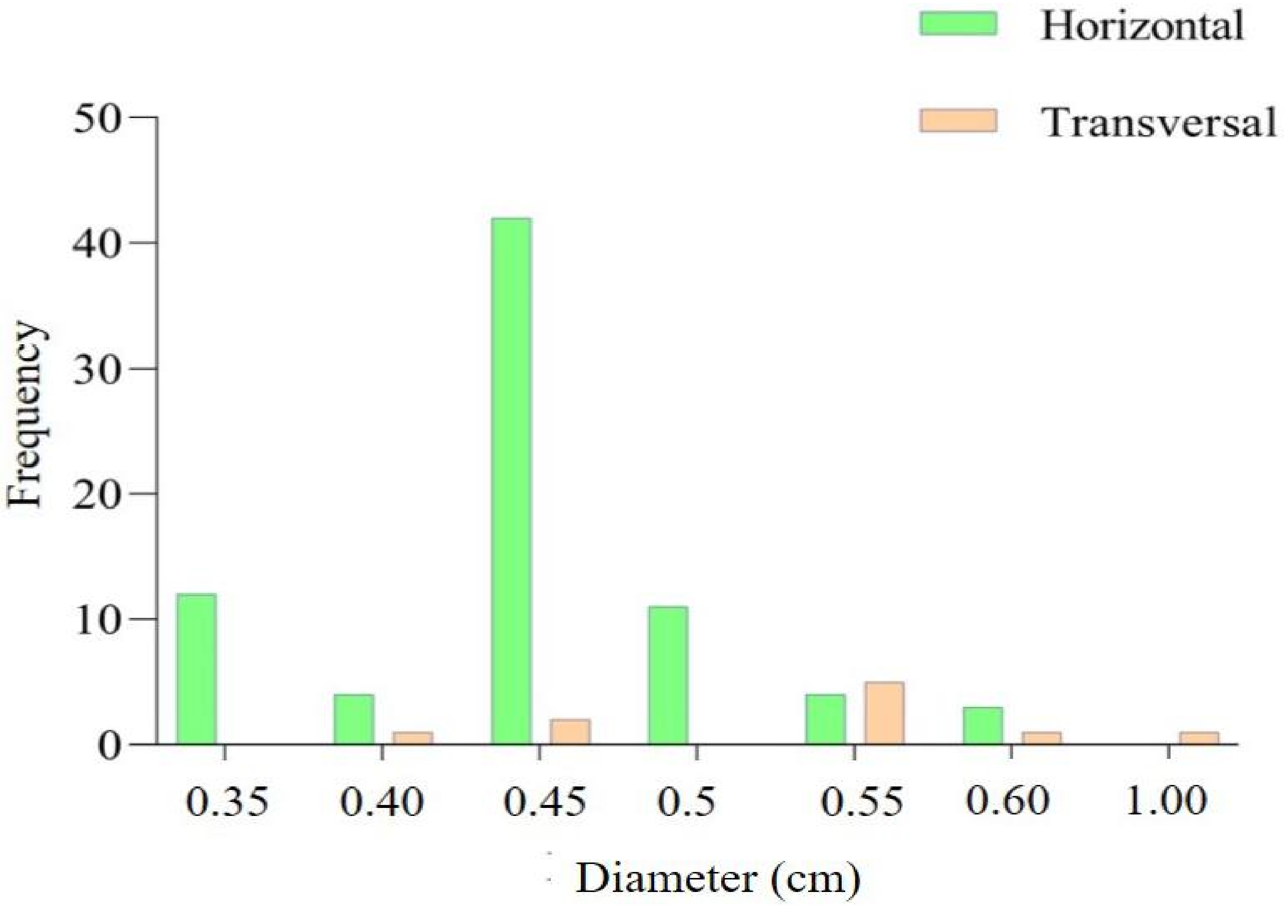


CIW – Construction of the Initial Wall; CP – Construction of the Partition; PC – Provisioning the Cell and oviposition; CV – Construction of the Vestibular Cell; COW – Construction of the Operculum Wall

Table 2 - Measurements (cm) of the main internal structures of the nests of *Epanthidium tigrinum*, measured in trap nests from May 2019 to September 2020, in the Bee Unit of the Department of Animal Science at the Federal University of Ceará N= number of observations, SD = standard deviation of the mean

Nest structure	N	Variation(cm)	Mean ± SD (cm)
Total length of the nest	86	2.35-12.07	8.27 ± 2.52
Length of the brood cells			
1stCell	86	0.26-3.23	1.10 ± 0.38
2ndCell	78	0.54-3.4	1.11 ± 0.47
3rdCell	66	0.53-1.99	1.04 ± 0.28
4thCell	66	0.53-1.99	1.00 ± 0.28
5thCell	42	0.4-3.53	0.93 ± 0.61
6thCell	31	0.57-1.71	0.92 ± 0.21
7thCell	16	0.35-2.2	0.95 ± 0.38
8thCell	16	0.53-1.42	0.90 ± 0.23
9thCell	4	0.61-0.77	0.70 ± 0.06
10thCell	1	0.79	0.79
Length of the vestibule cell	76	0.71-8.6	3.37 ± 1.92
Width of the initial wall	13	0.05-1.08	0.39 ± 0.35
Width of the closing wall	19	0.07-0.86	0.37 ± 0.27

Figure 7 - Frequency of orientation of the brood cells (horizontal and transversal) in *Epanthidium tigrinum* relative to the diameter class of the cavity, in the Bee Unit of the Department of Animal Science at the Federal University of Ceará

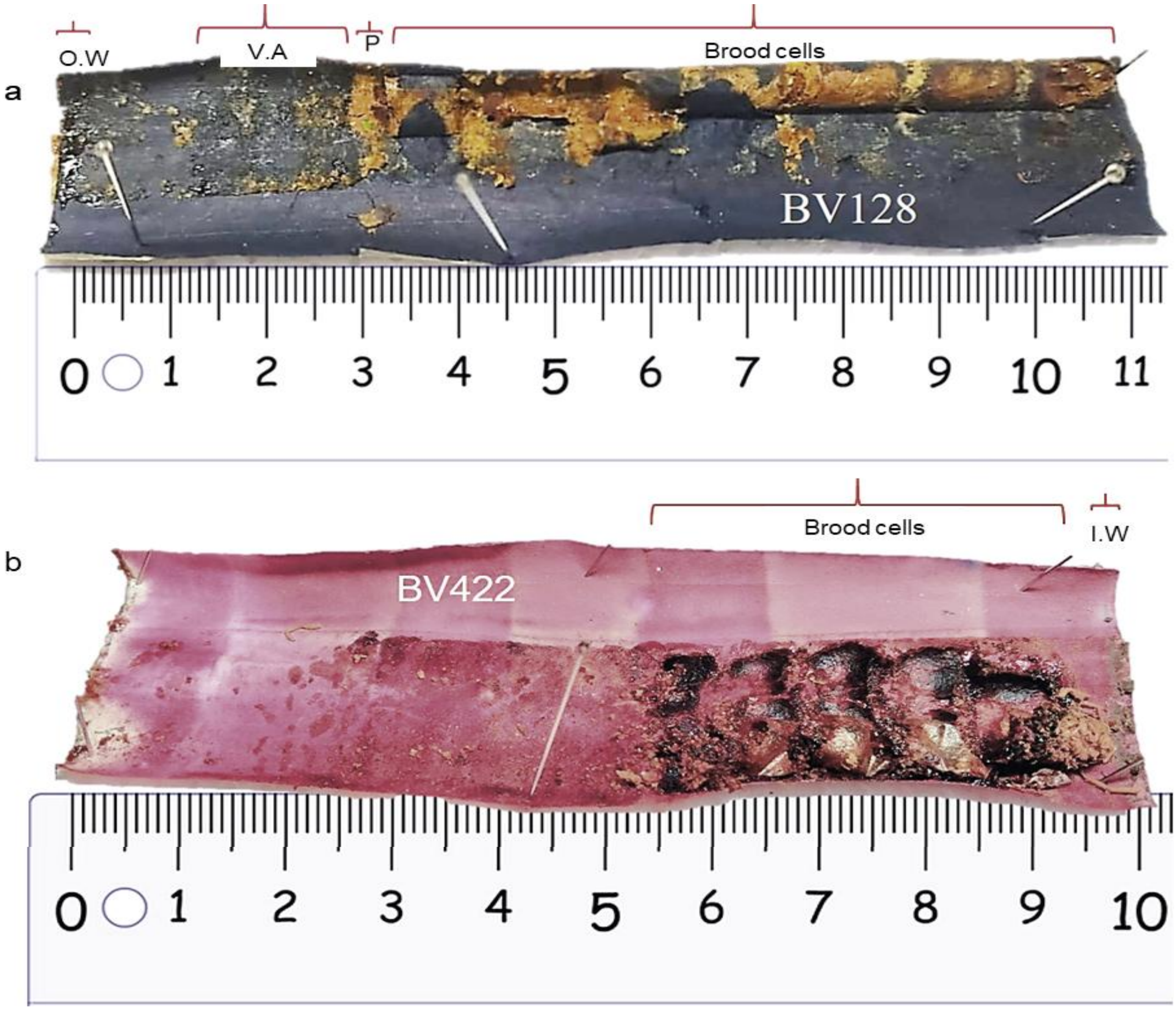


than the transversal type (n = 9) ($X^2 = 53.767$; $p < 0.01$). Until now, cell orientation in the nests of *E. tigrinum* has been described as horizontal. However, in this study the transverse orientation of the cells was also seen. Some species of family Megachilidae build heteromorphic cells, where the arrangement and orientation are related to the type of cavity available (MELLO; GAGLIANONE, 2019; MICHENER, 1964). Furthermore, for family Megachilidae, there is a tendency for some species to build clustered cells, where different orientations can be seen for cells that are built in cavities of different diameters (MELLO; GAGLIANONE, 2019; PARKER, 1986; VANDENBERG, 1995). Based on this information, when considering a rational breeding system for pollination using *E. tigrinum*, a more-thorough investigation is needed into the relationship between the architecture used to build the cells and the size of the available cavity, and the way that this influences production of the hatchlings, since these aspects can directly affect the productive potential of the bees.

In each brood cell, the cocoons had a cylindrical shape and smooth texture, and were composed of a thin, fragile and translucent material similar to cellophane, of which the basal portion (back end) was darker, with a brownish colour due to the presence of faeces (Figures 8 and 9).

The space that was effectively occupied by the nest varied between 2.35 and 12.37 cm and had an average

Figure 8 - Internal architecture of the nests of *Epanthidium tigrinum* showing the brood cells oriented horizontally (A) and transversally (B); the partitions (P); the vestibule area (V.A); the operculum wall (O.W) and the initial wall (I.W), collected in the Bee Unit of the Department of Animal Science at the Federal University of Ceará



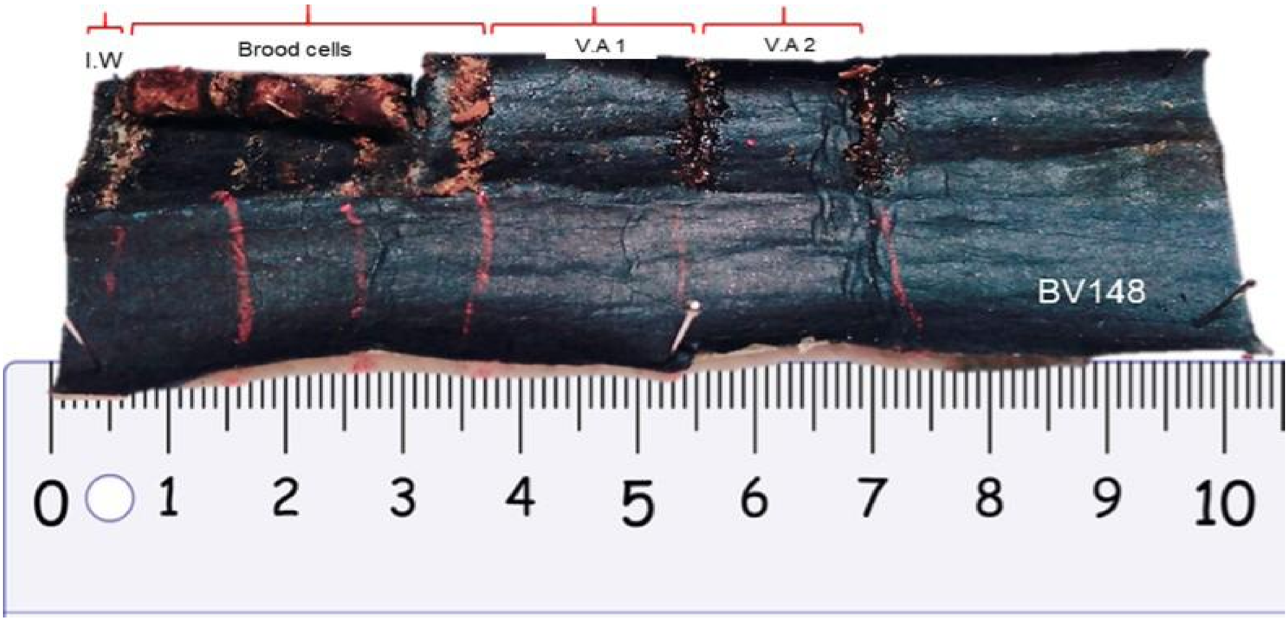
length of 8.27 ± 2.52 cm. This variation is mainly due to the size of the cells of the nest itself, so that the average length of the earliest cells, at the bottom of the nest, was greater than that of the later cells, which were closer to the exit (Table 2), a fact also seen in other species of Megachilidae (ALVES-DOS-SANTOS, 2004; SANTOS *et al.*, 2020). This pattern can be explained if it is assumed that for solitary bees in general, males are reared in the cells closer to the exit of the nest, while females are produced in the first brood cells of the nest, where the mother bee allocates more resources for the production of females, which are generally larger than the males (MICHENER, 2007; ROUBIK, 1992; SANTOS *et al*., 2020).

In this study, the exact position of the offspring was not investigated; however, for *E. tigrinum* there are reports of the male offspring being located at the bottom of the cavities, while the female offspring are closer to the exit of the nest (GOMES *et al*., 2020). This needs further investigation. When the final cell is completed, the bees usually build a wall at the entrance to the nest, called the closing wall. This wall was present in 61.84% of the collected nests. The thickness of the closing wall ranged from 0.07 to 0.86 cm (Table 2). The presence of a vestibule cell (empty cell between the closing wall of the nest and the last provisioned cell) was recorded in 89.53% of the nests under analysis, and in some cases can be found twice in the same nest (n = 3) (Figure 9).

The average length of the vestibule cells was 3.37 ± 1.92 cm, and ranged from 0.71 to 8.6 cm (Table 2). This type of cell is common in several species of solitary bee (COSTA; GONÇALVES, 2019; MICHENER, 2007; ROUBIK, 1992). Empty cells can be found in different positions in the nest, and it is common for more than one cell of this type to be present. It is assumed that these cells are used as a defence strategy against the attack of parasites and predators, and that they occur when the activity of parasites is more intense (SANTOS *et al*., 2020). However, despite the presence of vestibule areas, no parasitised nests of *E. tigrinum* were recorded during the experimental period, even with a high frequency of females of *Coelioxys* sp. (kleptoparasitic bee) seen near the nests. *Coelioxys* sp. was generally seen parasitising the nests of *C. analis* that were close to the trap nests of *E. tigrinum* (personal observation).

Finally, the present study, conducted over 16 months and including a large number of observations, is the first to investigate the nesting and foraging behaviour of these bees over a long period, under varying circumstances and with a large number of nests, minimising the effect of individual or seasonal trends. In fact, the results showed variations between individuals and in the materials used in the construction and architecture of the nests, the number of cells per nest, the number of nests built, the time spent looking for resources, etc., throughout the study period. As such, the information obtained in this study is important when considering conservation efforts for *E. tigrinum* and/or a production and management system for this pollinator.

Figure 9 - Internal architecture of a nest of *Epanthidium tigrinum* showing the presence of two vestibule areas (V.A 1; V.A 2), collected in the Bee Unit of the Department of Animal Science at the Federal University of Ceará



CONCLUSIONS

1. This study leads us to conclude that although *E. tigrinum* is an opportunistic species, and shows flexible behaviour when it comes to nesting and foraging for resources in general, it performs well in trap nests, which qualifies it for consideration in programs of agricultural pollination;
2. The species shows a preference for cavities of around 0.45 cm in diameter; depends on other floral resources besides pollen and nectar, such as plant fibres and resins to build its nest; and remains active throughout the year. All this information must be taken into account, both in conservation efforts and in zootechnical exploitation when breeding the bee for agricultural pollination, since both can influence the number of nests and cells built, and consequently, the size of the pollinator population.

ACKNOWLEDGEMENTS

Vitória I. M. S. Muniz thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001 for a M. Sc. scholarship. Breno M. Freitas thanks CNPq for Research Productivity Fellowships (#308358/2019-8).

CONFLICT OF INTEREST STATEMENT

This study results from a cooperation agreement between BAYER AG. (Germany) and the Federal University of the State of Ceara (Brazil) on the project “Breeding, multiplication and handling of native pollinators for crop pollination in Brazil”. The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

REFERENCES

ALVES-DOS-SANTOS, I. Conhecimento e criação de abelhas solitárias: um desafio. **Revista Tecnologia e Ambiente**, v. 10, n. 2, p. 99-113, 2004.

BOSCH, J. *et al*. Use of a managed solitary bee to pollinate almonds: population sustainability and increased fruit set. **Insects**, v. 12, n. 1, p. 56, 2021.

BOSCH, J.; KEMP, W. P. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. **Bulletin of Entomological Research**, v. 92, n. 1, p. 3-16, 2002.

BRASIL, M. de O. G. *et al*. Occupation and emergence of solitary bees in different types of trap nests. **Sociobiology**, v. 66, n. 2, p. 316-326, 2019.

CAMAROTTI-DE-LIMA, M. F.; MARTINS, C. F. Biologia de nidificação e aspectos ecológicos de *Anthodioctes lunatus* (Smith) (Hymenoptera: Megachilidae, Anthidiini) em área de tabuleiro nordestino, PB. **Neotropical Entomology**, v. 34, n. 3, p. 375-380, 2005.

CHUI, S. X.; KELLER, A.; LEONHARDT, S. D. Functional resin use in solitary bees. **Ecological Entomology**, 10.1111/een.13103, 2021.

COLLETT, M.; CHITTKA, L.; COLLETT, T. S. Spatial memory in insect navigation. **Current Biology**, v. 23, n. 17, p. R789-R800, 2013.

COSTA, C. C. F.; GONÇALVES, R. B. What do we know about Neotropical trap-nesting bees? Synopsis about their nest biology and taxonomy. **Papéis Avulsos de Zoologia**, v. 59, e20195926, 2019.

DANFORTH, B. N. *et al*. **The solitary bees**: biology, evolution, conservation. New Jersey: Princeton University Press, 2019. 488 p.

FARZAN, S.; YANG, L. H. Experimental shifts in phenology affect fitness, foraging, and parasitism in a native solitary bee. **Ecology**, v. 99, n. 10, p. 2187-2195, 2018.

FREITAS, B. M. *et al*. Forest remnants enhance wild pollinator visits to cashew flowers and mitigate pollination deficit in NE Brazil. **Journal of Pollination Ecology**, v. 12, n. 4, p. 22-30, 2014.

GALETTO, L. *et al*. Risks and opportunities associated with pollinators’ conservation and management of pollination services in Latin America. **Ecología Austral**, v. 32, n. 1, p. 55-76, 2022.

GOMES, A. M. S. *et al*. Bionomy and nesting behavior of the bee *Epanthidium tigrinum* (Schrottky, 1905) (Hymenoptera: Megachilidae) in trap-tests. **Sociobiology**, v. 67, n. 2, p. 247-255, 2020.

INSTITUTO DE PESQUISA E ESTRATÉGIA ECONÔMICA DO CEARÁ. **Perfil Municipal 2017**. Fortaleza, 2017. Disponível em: https://www.ipece.ce.gov.br/wp-content/uploads/sites/45/2018/09/Fortaleza_2017.pdf. Acesso em: 20 jul. 2019.

KLEIN, A. M.; STEFFAN-DEWENTER, I.; TSCHARNTKE, T. Foraging trip duration and density of megachilid bees, eumenid wasps and pompilid wasps in tropical agroforestry systems. **Journal of Animal Ecology**, v. 73, n. 3, p. 517-525, 2004.

KOPPEN, W. Klassification der Klimate nach Temperatur, Niederschlag und Jahreslauf. **Petermanns Geographische Mitteilungen**, v. 64, p. 193-203, 1918.

KROMBEIN, K. V. **Trap-nesting wasps and bees**: life histories, nests and associates. Washington: Smithsonian Institution Press, 1967. 570 p.

MELLO, B. N. S.; GAGLIANONE, M. C. Nesting biology of sympatric species of Megachilidae bees in a conservation area in Brazilian Atlantic Forest. **Sociobiology**, v. 66, n. 1, p. 52-60, 2019.

MESQUITA, T. M. S.; AUGUSTO, S. C. Diversity of trap-nesting bees and their natural enemies in the Brazilian savanna. **Tropical Zoology**, v. 24, n. 2, p. 127-144, 2011.

MICHENER, C. D. Evolution of the nests of bees. **American Zoologist**, v. 4, n. 1, p. 227-239, 1964.

MICHENER, C. D. **The bees of the world**. 2. ed. Baltimore: JHU Press, 2007. 953 p.

NEFF, J. L. Components of nest provisioning behavior in solitary bees (Hymenoptera: Apoidea). **Apidologie**, v. 39, n. 1, p. 30-45, 2008.

PARIZOTTO, D. R.; MELO, G. A. R. Nests of bees of the anthidiine genus *Ananthidium* Urban (Hymenoptera, Apidae, Megachilinae). **Journal of Hymenoptera Research**, v. 47, p. 115-122, 2015.

PARIZOTTO, D. R.; URBAN, D.; MELO, G. A. Phylogeny and generic classification of the Anthidiini bees from the Neotropical region (Hymenoptera: Apidae). **Zoological Journal of the Linnean Society**, v. 194, n. 1, p. 80-101, 2022.

PARKER, F. D. Nesting, associates, and mortality of *Osmia sanrafaelae* Parker. **Journal of the Kansas Entomological Society**, v. 59, n. 2, p. 367-377, 1986.

ROUBIK, D. W. **Ecology and natural history of tropical bees**. New York: Cambridge University Press, 1992. 514 p.

SANTOS, A. A. *et al*. Nesting biology and flower preferences of *Megachile (Sayapis) zaptlana*. **Journal of Apicultural Research**, v. 59, n. 4, p. 609-625, 2020.

TÖLKE, E. D. *et al*. Diversity of floral glands and their secretions in pollinator attraction. *In*: MÉRILLON, J. M.; RAMAWAT, K. **Co-evolution of secondary metabolites**. New York: Springer, 2020. cap. 28, p. 709-754. Reference Series in Phytochemistry.

URBAN, D.; MOURE, J. S. Anthidiini Ashmead, 1899 *In*: MOURE, J. S.; URBAN, D.; MELO, G. A. R. **Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version**. 2012. Disponível em: http://moure.cria.org.br/catalogue. Acesso em: 20 jul. 2019.

VANDENBERG, J. D. Nesting preferences of the solitary bee *Osmia sanrafaelae* (Hymenoptera: Megachilidae). **Journal of Economic Entomology**, v. 88, n. 3, p. 592-599, 1995.