

Nesting behavior of the wool carder bee *Anthidium florentinum* (Fabricius, 1775) (Hymenoptera; Megachilidae) in artificial nests in the region of Constantine

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ABSTRACT: During the summer of 2021, the nesting biology of *Anthidium florentinum* (Megachilidae) was investigated in artificial nests made of wooden cylinders and tie holes in Constantine (northeastern Algeria). The findings from the observation of 26 nests indicate that nests were multicellular (two to seven cells per nest), linear in structure, and made of plant fibers surrounding the nest or old woolly material from pre-existing cavities. The majority of female-occupied nests measured 0.8 to 2.2 cm in diameter. The partitions of the analyzed nests had accumulated wool caps, which is a behavior unique to Megachilid bees. The analysis of brood cell content revealed that *A. florentinum* females prefer Lamiaceae pollen grains for their broods.

KEYWORDS: Nesting biology, nest structure, wool carder bee, floral hosts

Bees are among the most important pollinators (Tschartke *et al.*, 2005; Winfree *et al.*, 2008). They are classified into seven families, with approximately 20,000 known species. Megachilidae is one of these families, with over 4000 species (Michener, 2007). They have specific pollen-collecting structure under their abdomen (Westerkamp, 1987; Westrich, 1989; Wcislo and Cane, 1996; Thorp, 2000). They are solitary and exhibit a variety of nesting behavior. Many species in this family build their nests above ground, in pre-existing vacant cavities, wood and rock crevices, or empty snail shells, which provide an ideal microclimate for egg incubation. They also prefer to nest in areas that have been altered by humans, particularly in urban habitats (Westrich, 1989; Michener, 2007; Sedivy, 2012).

Anthidiini is an important and diverse Megachilidae tribe in Algeria, with eight genera, thirteen subgenera, and 33 reported species (Aouar-sadli *et al.*, 2012; Aguib, 2014; Kasperek *et al.*, 2024). The majority of *Anthidium* females use hair and fuzzy plant needles to build a woolly nest. However, many species exhibit a second type of nesting behavior, which involves the use of pre-existing cavities, stems, or synthetic substrates

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(Krombein, 1967; Eickwort *et al.*, 1981; Michener, 2007). These insects separate their brood cells by accumulating woolly layers. The majority of *Anthidium* individuals have yellow or red maculation on certain parts of their bodies and a tripod-shaped apical tergum (Kasperek, 2017). They are most active during the summer, June, July, and August. The majority of species in the genus are polylectic generalists that are considered effective pollinators of certain plants, such as *Lavandula officinalis* L. in Algeria (Benachour, 2017).

Anthidium florentinum is an interesting species that was reported in Algeria for the first time in 2010 (Aguib, 2010). This species is found throughout the Eurasian and Mediterranean regions (Warncke, 1980; Gogala, 2012). In Algeria, it has been suggested that it be limited to Constantine, Mila, and Guelma (Aguib, 2014). The species is univoltine, meaning it emerges around the same time as its host plants. Its flight season lasts from June to August, with peak summer activity (Aguib, 2014). This study aims to fill a gap in the literature by gathering information on the nesting behavior of these bees. Ecological observations on *A. florentinum* in artificial trap nests and attachment holes, including details of nest architecture, established activity patterns, abundance and floral host preferences in an urban environment, are presented.

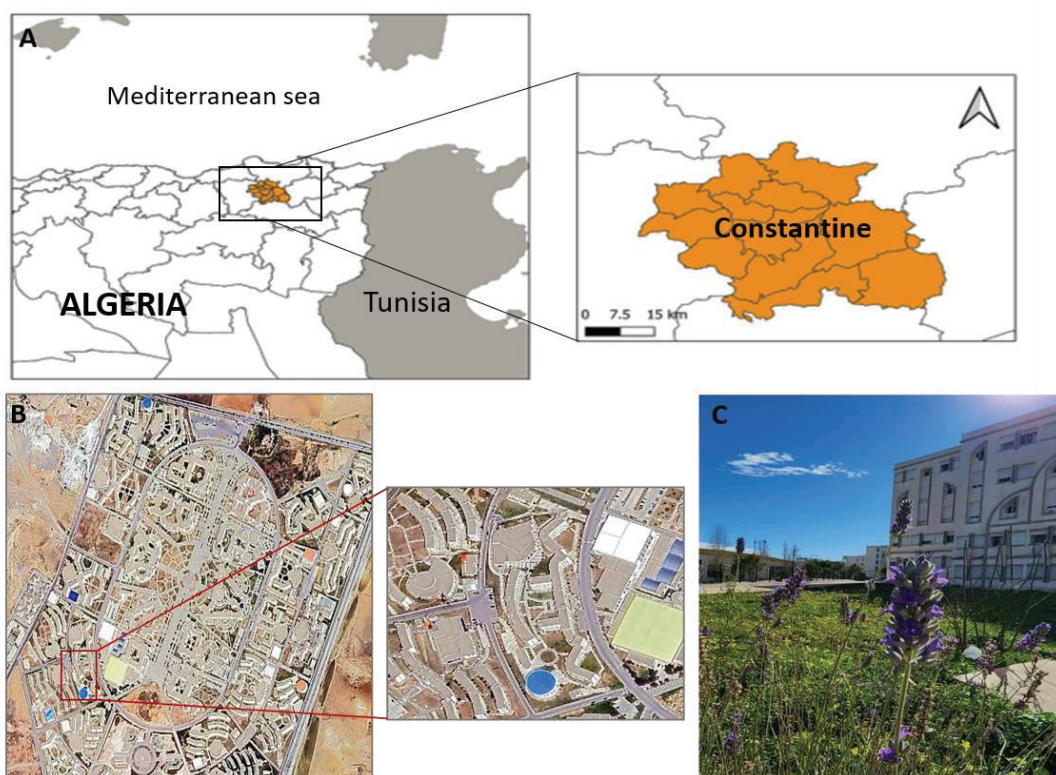


Figure 1. A) Map of Constantine province; B) Satellite image of Salah Bounider University 3 indicating with a red rectangle the location of the campus of Ain El Bey 9 (Source: Google Earth); C) Campus of Ain El Bey 9.

MATERIALS AND METHODS

Study site

This study was conducted in Ali Mendjli, a new city in the southern suburbs of Constantine (36°15'47"N 6°35'37"E), at the campus of Ain El Bey 9 located at Salah Boubnider University 3 (36°16'37"N 6°35'04"E). The climate in the region is Mediterranean semi-arid. It is distinguished by hot summers and cold winters (<https://climatedata.org/>).

Preparing and installing artificial trap nests

Bamboo cylinders were used to form artificial trap nests. More than thirty cylinders, ranging in diameter from 0.5 to 1.8 cm and length from 4.5 to 29 cm, were placed in two wooden boxes measuring 20 × 20 × 30 cm. They were installed in May 2021 at a height of 11 meters on the edge of a room's window in the university campus of Ain El Bey 9 (Fig. 2), facing northeast and exposed to the morning sun. The entrance of the bamboo cylinders was checked four to five times per week to ensure female occupation.



Figure 2. Boxing with the cylinders installed at the edge of the window of the university room (campus of Ain El Bey 9).

Tie holes

Plastic cylinders were used to form these tie holes, which stretched across a 20 × 3 m concrete wall. They were 30 cm long and 1.1 – 2.2 cm in diameter. The wall had 96 tie holes, ranging in height from 25 cm to 2 m (Fig. 3). The wall was facing east and exposed to the morning sun.



Figure 3. A) An overview of the concrete wall; B) Tie holes on the wall; C) Enlarged photo of a tie hole.

Identification of the bee

After installing the trap nests, we noticed female bees interested in both trap nests and tie holes in the concrete wall. These females were observed while foraging, and individuals were captured and identified as females of *Anthidium florentinum*.

Nesting activity

Female *A. florentinum* nesting activity was observed and monitored for 14 days in both nests between June and July, 2021. Females were observed constructing their nests and foraging from morning to late afternoon or early evening.

Nest structure

Five nests of each type were recuperated to describe the nest structure, including cell count, arrangement, and partition measurements. We extracted the nests from the tie holes using a metal wire while bamboo cylinders were split lengthwise in the lab.

Females host plants

To identify the host plants of *A. florentinum*, we first gathered a reference collection of pollen grains from the surrounding area. Pollen grains from 68 identified wild and cultivated flowers (found on campus and primarily represented by Asteraceae, Rosaceae, and Boraginaceae) were extracted and mounted between a slide and coverslips in fuchsine-colored glycerin jelly. The content of 16 brood cells was recovered. Each sample was fragmented with a mounting pin and distributed on the slide with droplets of distilled water to melt and spread the contents, followed by droplets of absolute ethanol to degrease the pollen grains; this process was repeated three times, and the slides were rinsed after the ethanol had evaporated. Finally, pollen grains were covered with colored glycerin jelly and identified using reference slides.

Table 1. Pollen rate and composition of brood cells' content of *Anthidium florentinum* in % n=15 brood cells.

		Lamiaceae		Boraginaceae	Fabaceae		Asteraceae	
		<i>Lavandula dentata</i>	<i>Lavandula officinalis</i>	<i>Anchusa italica</i>	<i>Medicago sativa</i>	<i>Hedysarum coronarium</i>	Unknown	<i>Crepis capillaris</i>
Nest 1	B.C 1	39.06	11.71	2.34	46.87	0	0	0
	B.C 2	41.93	6.45	4.83	46.77	0	0	0
	B.C 3	49.15	5.08	6.77	38.98	0	0	0
	B.C 4	44.94	2.24	0	33.7	2.24	16.85	0
Nest 2	B.C 1	35.76	3.64	1.45	40.14	0	13.13	5.83
	B.C 2	34.07	2.96	2.22	38.51	0	19.25	2.96
	B.C 3	46.66	3.33	0	26.66	0	23.33	0
	B.C 4	38.57	4.28	0	35.71	0	21.42	0
	B.C 5	64.51	17.20	0	12.9	5.37	0	0
Nest 3	B.C 1	42.17	3.4	6.8	39.45	0	8.16	0
	B.C 2	48.07	7.69	5.76	27.21	0	9.61	0
	B.C 3	43.24	0	14.41	14.41	27.02	7.2	0
	B.C 4	29.21	0	22.47	33.7	5.61	8.98	0
	B.C 5	24.11	9.41	28.23	25.29	0	12.94	0
	B.C 6	13.92	11.39	31.64	24.05	0	18.98	0

B.C: Brood cell

Table 2. Mortality rate, emerged adults' sex and date of first emergence of *A. florentinum* in bamboo cylinders and tie holes.

Nest	Bamboo cylinders					Tie holes				
	I	II	III	IV	V	VI	VII	VIII	IX	X
Number of cells	2	2	4	4	6	6	3	7	4	3
Mortality rate	50%	50%	50%	25%	0%	16%	0%	28%	25%	33%
Emerged adults' sex	1f	1f	2f	1m	4f 2m	2f 3m	2f 1m	3f 2m	2f 1m	2f
First emergence (2022)	18/III	15/IV	21/IV	29/III	28/III	15/V	28/V	06/V	15/V	12/VI

*Distribution of sex is ordered.

Adult emergence

To determine the mortality rate of each life stage and adult emergence of *A. florentinum*, cells from five nests from each type (bamboo cylinders and tie holes) were collected and placed in plastic vials. After the pupation stage was observed, the cocoons were moved to boxes to observe the emergence of adults. Plastic vials were labeled with the number and type of nest. The date of first emergence and sex ratio in both nest types were also recorded (Table 2). The tie holes' plugs were daily checked outside. Laboratory temperatures ranged from a minimum of 20 °C to a maximum of 27.3 °C, while relative humidity varied between 30% and 44%.

RESULTS

Anthidium florentinum females occupied twenty tie holes and six bamboo sticks in the summer of 2021. Other arthropods, including Megachilidae, Araneae, and Eumeninae, already represented 28% of the tie holes (Fig. 4D), while 12% were filled with old *A. florentinum* nests, which were used as source of building material. The rest of the tie holes remained empty. Four nests of *Megachile* sp. were found on bamboo sticks.

Nesting activity

Females of *A. florentinum* started building nests in June and finished by the end of July. The number of nests and the level bee's activity varied. Peak activity can last until early August. Nesting activity took place every day between sunrise and sunset for an average of 8 hours. The observed number of nesting females in tie holes ranged from 6 to 15. The mean number of individuals was 9.92 ± 3.33 per day ($n = 14$ days). Nesting activity in both types of nests began around 7 am when the average temperature was 26 °C. Females reach their peak activity between 1:00 and 3:30 pm when the temperature rises to 36 °C. The latest activity was observed around 7 pm, with an average temperature of 33 °C. Females collect nesting materials from plant fibers, seed heads, and pre-existing cavities and transport them to the nest using their developed mandibles (Fig. 4F). They alternately collect material from numerous pre-existing cavities. Two females were observed constructing more than one nest in tie holes.

Wool was loaded from pre-existing cavities in 25.28 ± 10.48 seconds ($n = 30$ specimens), while partition construction took 3.75 ± 1.75 minutes. Females took 28.72 ± 2.8 hours ($N = 18$) to build one brood cell, which can take two to three days. Nourishment was obtained from wild or cultivated flowers (nectar and pollen) around the nest. *A. florentinum* was observed foraging *Anchusa italica* Retz. and *Lavandula dentata* L., 1753 which were discovered 30 m and 18 m from the tie holes and trap nests, respectively. Fuzzy material was harvested from the stems and pedicles of specific plants, such as *L. dentata*, *L. officinalis* (Lamiaceae), *A. italica* (Fabaceae), and *Dittrichia viscosa* (L.) Greuter 1973 (Asteraceae). This last was the primary sources of seed heads. Female bees spend the night inside the cavities, their heads facing the bottom of the nest (Fig. 4H). During the study period, two tie holes were observed to be home to two peaceful female bees; however, during the day, we observed aggressive behavior when one female attempted to access another's nest.

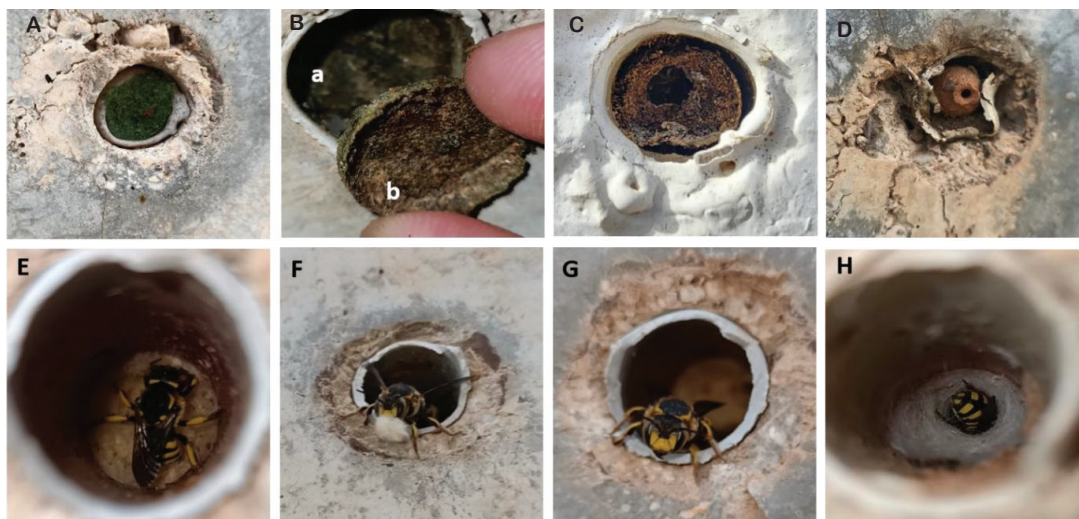


Figure 4. A) Nest plug; B) Tie hole nest shows two plugs (a and b); C) Dug nest entrance indicating adult emergence; D) Eumeninae nest; E) A female *Anthidium florentinum* building the separating wall inside the tie hole; F) *Anthidium florentinum* carrying wool from a pre-existing nest; G) *Anthidium florentinum* leaving the tie hole to retrieve building material; H) *Anthidium florentinum* spending night inside the tie hole.

Nest and brood description

The nest had a linear structure covered in a thin, woolly layer, making the cells easily identifiable (Fig. 6E). The number of cells ranged from two to seven (4.1 ± 1.7), and they were more abundant in tie holes compared to bamboo cylinders. They were cylindrical and approximately 1 cm long, separated by a woolly structure cap made up of numerous concave layers of plant fibers, seed heads, and mattress sponge (thought to have been collected from discarded campus equipment) (Fig. 4E). The length of a cap ranged from 0.1 cm to 4.5 cm, depending on the number of layers (Fig. 5). Correlation coefficients revealed no relationship between the number of cells and the length of the target cavity ($r = 0.1$, $df = 24$, $p < 0.05$).

Foraged nourishment was found at the bottom of each cell. The final cap (between the last cell and the plug) was made thicker. Its length can exceed 19.3 cm, which is equivalent to 15 layers. Orifices in the nest were closed with a plug made primarily of vegetal debris (Fig. 4A). This primarily accounts for the nest's external appearance, which becomes concave when dried (Fig. 4C). Three of the hosted nests contained two plugs (Fig. 4B). However, four nests lacked any closing material. Microscopic examination of the nest plug revealed that it was densely packed with chewed plant debris, plant fragments, soil, trichomes, and small amounts of pollen grains. All elements had been masticated and transformed into a solid material. Old nests from previous generations were discovered behind five newly constructed nests in tie holes, with cap layers that were noticeably darker and the cocoon casing fragile.

The larva has a noticeably robust, curved, yellow body (Fig. 6A). Cocoons are ovate and dark brown, with a nipple at the edge for air exchange (Fig. 6F). Before spinning the cocoon, the larva ejects fecal pellets along the cell wall. Before defecating for the final time, the larva positions its posterior to face the exit of the nest and pushes excrement toward the anterior part of the cell. Following that, the larva turns and positions its anterior region toward the exit, signaling the start of cocoon formation. We found no parasitic or predation interactions within the nests or at their entrances.

Diameter of occupied nests

The occupied cavities of both tie holes and bamboo sticks revealed that females nested in cavities with diameters ranging from 0.8 cm to 2.2 cm. Females (N = 10) preferred and occupied cavities with a diameter < 1.5cm, followed by those with diameters < 1 and < 2.5 cm. These were chosen less frequently, with seven occupied cavities each. Finally, only two occupied cavities had a diameter of less than 1 cm.

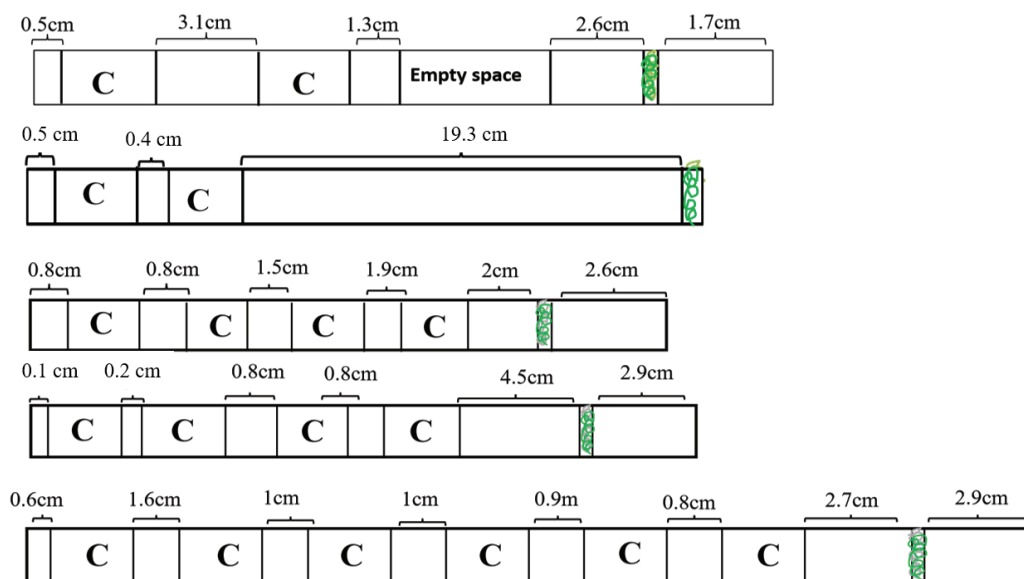
Pollen hosts

Microscopic examination of the contents of 15 brood cells extracted from various nests revealed unequal amounts of pollen from several plant species. We identified pollen grains by family and species, with a significant number of unknown species. Pollen grains from all extracted contents were dominated by two major species: *L. dentata* and *Medicago sativa* L., 1753. French lavender (*L. dentata*) was significantly represented, accounting for 40.35% of the total number of pollen grains. This was followed by Alfalfa (*M. sativa*) pollen grains, which made up 31.01%. These findings indicate that Lamiaceae and Fabaceae are the primary host plants of female *A. florentinum*. The undefined species (Asteraceae) and *A. italica* (Boraginaceae) accounted for 10.23% and 9.56% of the contents, respectively. True lavender (*L. officinalis*) accounted for 5.68% of the contents, followed by *Hedysarum coronarium* L. (2.57%) and *Crepis capillaris* L., which was found in a few nests in very small amounts (0.56%).

Adult emergence

Nests of bamboo cylinders and tie holes relocated to the laboratory appeared earlier than those left outside. Pupation took place over 20 days, from late March to April. Female *A. florentinum* emerged on average 12 days before their male counterparts (Table 2). Dissected nests indicated mortality rate of *A. florentinum* among bamboo cylinders and tie holes.

Bamboo cylinders



Tie holes

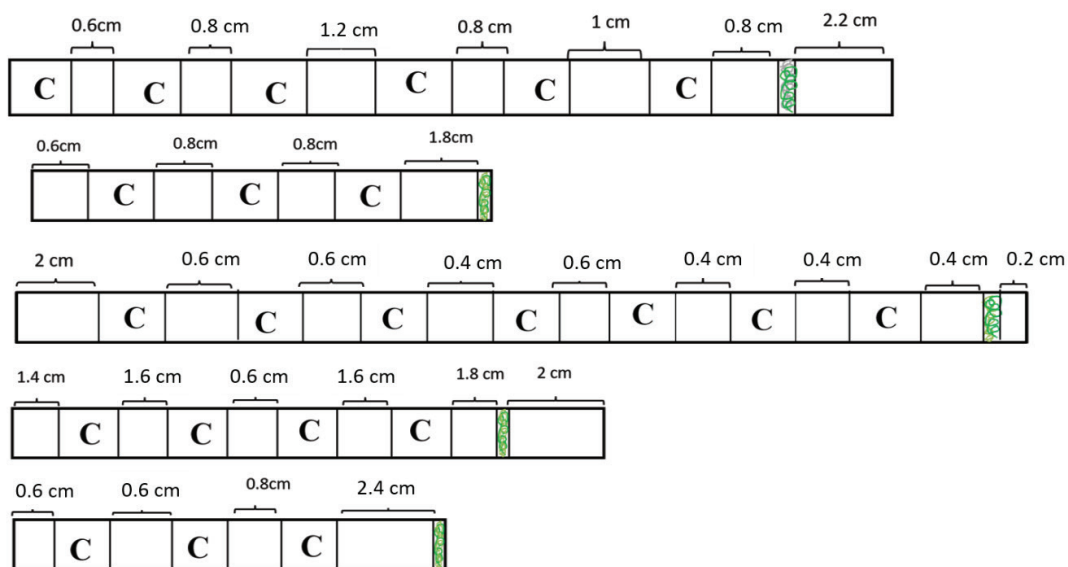


Figure 5. Diagram of the inside of the nest showing brood cells arrangement and partitions' measures.

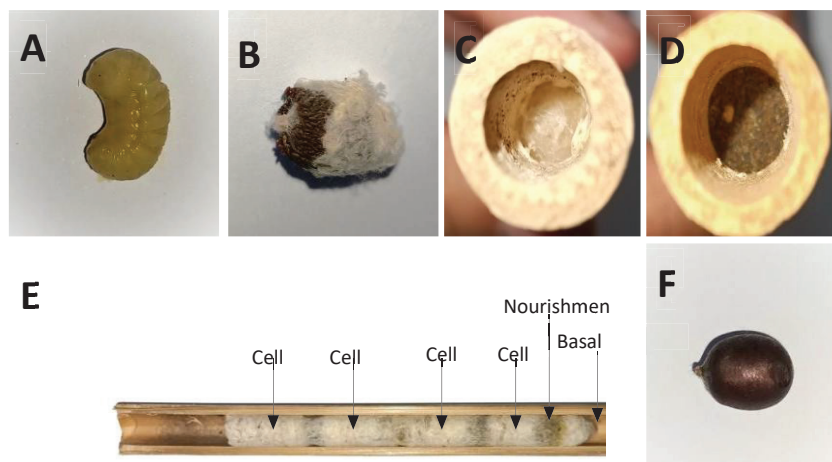


Figure 6. A) Larva; B) Cocoon with fecal matter covering its surface; C) Base of the nest; D) Nest plug; E) Nest containing four cells in trap nest; F) Cocoon.

DISCUSSION

Despite a similar number of prospective nesting sites in tie holes and installed bamboo cylinders, tie holes hosted more nests and appear to be preferred by *A. florentinum* females. Doroshina (1990) reported that *A. florentinum* builds nests in structures near host plants. This finding explains the preference observed in the current study, in which females preferred to nest in tie holes in the concrete wall surrounded by host plants. Furthermore, this preference is strongly correlated with the appropriate substrate and tie hole size. Bamboo cylinders, on the other hand, had varying dimensions and a high position, which may have influenced female nesting activity. The majority of *Anthidium* species are univoltine, with the exception of *A. andinum*, which nests twice a year and is probably bivoltine (Kurtak, 1973). During our research, one generation of *A. florentinum* appeared before winter.

Temperature fluctuations dominated *A. florentinum* females' daily activity. The activity begins in the morning and increases significantly until it reaches a peak at midday, when the temperature is at its highest, before decreasing throughout the night as the temperature drops. Females return to their nests to spend the night. They spend varying amounts of time collecting fuzzy nesting material and foraging, but they tend to spend more time in the nests depositing resources or building the cap layers. Females provide nourishment and development for their offspring by masticating pollen and nectar placed at the bottom of the brood cell.

In our current study, we observed hostile behavior when one female tried to enter another's nest. Previous research has also shown that some anthidiini females exhibit aggressive behavior at nest entrances. Ansel (2011) reported that *A. manicatum* females circle each other in aggressive spiral flights when one tries to enter a nest already occupied by another.

The arrangement of brood cells in all obtained nests followed a linear pattern along the cavities. The number of brood cells in the tie holes and trap nests fluctuated. The findings indicate that *A. florentinum* nests in bamboo cylinders and tie holes with varying lengths and diameters.

The nesting behavior of *Anthidium florentinum* females in two types of artificial nests was observed during the summer of 2021. We noted that the tie holes were more occupied by females than the wooden cylinders.

Although, tie holes had greater number of brood cells, the structure of longer cavity revealed the absence of a positive relationship with the number of brood cells. In the current study the abundance of nearby resources significantly reduced trip time and thus increased the number of brood cells. Additionally, the majority of the pubescence plants' resources were found on the upper surface of the concrete that contained tie holes. Zurbuchen *et al.* (2010) declared that the number of brood cells in some species decreases by almost 46% when foraging distance and trip time increase. The diameters of the cavities revealed that *A. florentinum* prefers diameters ranging from 1 cm to 1.5 cm in both types of cavities, which is consistent with the size of their robust bodies, where thorax width (0.54 ± 0.04 cm) ($N = 32$). As confirmed in recent nesting studies (Vitale, 2017) of the Anthidiini, such as *A. decaspilum* and *A. rubripes*, it was revealed that some species prefer 0.5 cm diameter cavities, whereas larger species, such as *A. manicatum*, prefer 0.8 cm diameter. Nonetheless, differences in diameter preference have no effect on the number of released brood cells in both types of artificial nests.

Unlike most Megachilidae species, the cell walls of *A. florentinum* nests were entirely composed of accumulated layers of the wool cap rather than vegetal material partitions (Fig. 4C). This fluff acts as a "buffer" between the cells, forming a smooth and compact layer on the inner surface and a raw, brittle layer on the outer surface (Fortunato, 2013).

One of the bamboo cylinder nests, had the longest final wool cap, measuring 19.3 cm. The layers of this final wool cap were not combined and were spaced 1 to 2 mm apart. The primary function of this final cap is to deter predators and danger and provide additional protection to the developing insect. Previous studies on *Anthidium* species nesting behavior, by Kurtak (1973) and Severinghaus *et al.* (1981), revealed that females of a similar species (*A. manicatum*) build their nests primarily high above ground. Doroshina (1990) particularly found that *A. florentinum* nests in above-ground cavities. This behavior was regarded as a preventive action (Ansel, 2011). All detected and hosted nests in our current study were placed above ground, reducing the exposure to predators and parasites. Besides, Vitale (2017) believes that the nests' proximity to construction material, such as old nests and host plants, provides a significant advantage in reducing the risk of brood cell attacks during construction.

When reusing a previously occupied nest, most cavity-nesting bees remove old nesting materials and replace them with new ones. In the current study, some of the nests examined contained fragments of old brood cells and polluted cap layers from previous generations. We propose that the reason for nesting in the previous generation's cavities without pushing out the old material, could be female bees' inability to unload the accumulated wool left deep inside cavities.

Anthidiini are typically summer species; their abundance peaks in mid-June, coinciding with the peak flowering of their primary host plants (Aguib, 2014). Their flights are scheduled throughout the summer months of June, July, and August, and they frequently extend into early September. *Anthidium florentinum* is a polylectic species that inhabits a variety of plant families. This includes many plants identified in previous studies: *Lotus corniculatus* (Fabaceae), *Linaria* sp. (Scrophylariaceae), *Centaurea solstitialis* (Asteraceae) (Özbek and Zanden, 1979), *Vitex agnuscastus* (Verbenaceae), *Melissa officinalis* (Lamiaceae) (Özbek and Zanden, 1993), *L. officinalis* (Lamiaceae) (Aguib, 2014), *Scabiosa columbaria* (Dipsaceae), *Lythrum salicaria* (Lythraceae), *Buddleja davidii* (Buddlejaceae), and *Rubus* sp. (Rosaceae) (Fortunato, 2013).

This study documented the species foraging on various plants, including fabaceous crops, *Cicer arietinum* L., 1753, *M. sativa*; Lamiaceae such as *L. dentata*, *L. officinalis*, and Boraginaceae. *Anchusa italica* and Lamiaceae were the favored sources of both pollen and fibrous material. The current study established the consistency and quantities of foraged plants by correlating pollen grains with their corresponding reference samples. The lavender served as an appealing host plant. It was the predominant plant species among those identified (Table 2). Previous Algerian agricultural studies indicated that *A. florentinum* is a principal pollinator species of *Lavandula* spp. (Benachour, 2017). The integral association between host plants and bees accounts for the significant quantity of *Lavandula dentata* pollen grains present in all brood cells. Müller (1996) reported that Lamiaceae plants display a nototribic pollination, which deposits pollen on the head, back or bill of the visitor; bees have evolved morphological adaptations to gather pollen from nototribic flowers. Gonzalez *et al.* (2012) documented that both new world (NW) and old world (OW) *Anthidium* species exhibit two different types of morphological specializations: modified hairs on the clypeus or supra clypeus. These structures facilitate obtaining pollen grains from nototribic flowers, which explains the preference of *A. florentinum* for lavender.

Other studies (Taggar *et al.*, 2021) identified *Anthidium* species as prevalent pollinators of Fabaceae. This finding aligns our study, which demonstrated a substantial presence of Fabaceae pollen grains in most of the examined brood cells' contents. This plant family was predominantly present in the study area.

The maturation and emergence of laboratory-incubated adults occurred more rapidly and earlier than those exposed to outdoor conditions. This is strongly attributable to the disparity in temperature conditions and the external instability compared to the laboratory environment. Temperature significantly influenced the breaking process of prepupal diapause in specific Megachilid bees (Kamel *et al.*, 2019). *Megachilidae* species often require a period of cold temperatures to complete diapause, as noted by Bosch, Sgolastra, and Kemp (2010). Females matured and emerged prior to males in both nests obtained from tie holes and bamboo sticks, as males possess the capacity to mate with the same female multiple times or with various females (Wirtz *et al.*, 1992). They remain in the natal nest for an extended duration before emerging subsequently.

CONCLUSION

Megachilid bees are known for their highly varied nesting biology. This is the inaugural study on the biology of *A. florentinum* in Algeria. It sought to facilitate forthcoming initiatives to reveal additional nesting behavior of this family. Multiple factors contribute to bee decline, with habitat loss being a significant consideration. The utilization of artificial nesting materials may significantly contribute to the protection and conservation of bees. Our findings advocate for the utilization of tie holes in the conservation of *A. florentinum* and are likely applicable to other species within the same genus. The nests could facilitate pollination services provided by *A. florentinum* and other bee species, consequently benefiting various industries related to seed production and food security.

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