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Nesting patterns of bees (Apidae) in brick walls in southern Brazil

Padrões de nidificação de abelhas (Apidae) em paredes de tijolos no sul do Brasil

Andressa K. G. dos **SANTOS**[1]; Manuel **WARKENTIN**[1]; Juliane V. da **SILVA**[1];
Rogério N. **BARBOSA**[1] & Denise M. D. S. **MOUGA**[1,2,3]

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ABSTRACT
Aiming to verify the nesting pattern of solitary bee species, a study was performed (October 2012 to March 2013) in Joinville, SC, southern Brazil, along a brick wall. There were found the following species *Melitoma segmentaria* (Emphorini), *Caenohalictus incertus* (Halictini) and *Leiopodus lacertinus* (Protepeolini). 147 nests showed activity in the sampling period (118 of *C. incertus*, 29 of *M. segmentaria)*, 98% located in the masonry. For *M. segmentaria*, 29 active nests were observed (density of 4.53/ m^2), with an average distance of 12.22 cm between them, 17 nests between the ground and 90 cm high, distributed in aggregated form (R = 0.004, p <0.02). The nests opening measured on average 0.9 cm in diameter (n = 29), two of them with a mud tower entrance 0.6 cm high, with a smooth inner wall and a outer, rough. For *C. incertus*, 118 active nests were observed (density of 18.43/ m^2), with a mean distance of 8.18 cm from each other, 98 nests between the ground and 70 cm high, distributed in aggregated form (R = 0.066, p <0.09). Individuals of *L. lacertinus* were noticed in all observations days, at the entrance of nests of *M. segmentaria*. *Anthrax* cf. *virgo* emerged from *M. segmentaria* nests.
Keywords: Behavior; *Caenohalictus incertus*; *Leiopodus lacertinus*; *Melitoma segmentaria*; solitary bees.

RESUMO
Visando verificar o padrão de nidificação de abelhas solitárias, um estudo foi realizado (outubro 2012 a março 2013) em Joinville, sul do Brasil, em uma parede de tijolos. As espécies *Melitoma segmentaria* (Emphorini), *Caenohalictus incertus* (Halictini) e *Leiopodus lacertinus* (Protepeolini) foram encontradas. No período 147 ninhos mostraram atividade (118 de *C. Incertus* e 29 de *M. segmentaria*), 98% localizados na massa. Para *M. segmentaria*, 29 ninhos ativos foram observados (densidade 4,53/ m^2), com distância média de 12,22 centímetros entre eles; destes 17 ninhos entre o solo e 90 cm de altura, estando agregados (R = 0,004, p <0,02). A abertura dos ninhos possuía, em média, 0,9 centímetro de diâmetro (n = 29); dois deles com uma torre de barro na entrada (0,6 cm de altura), de parede interna lisa e externa, áspera. Para *C. incertus*, 118 ninhos ativos foram achados (densidade 18,43/ m^2), com distância média de 8,18 centímetros entre eles; destes 98 ninhos entre o solo e 70 cm de altura, estando agregados (R = 0,066, p <0,09). Indivíduos de *L. lacertinus* foram verificados em todos os dias de observações, na entrada de ninhos de *M. segmentaria*. *Anthrax* cf. *virgo* emergiu de ninhos de *M. segmentaria*.
Palavras-chave: Abelhas solitárias; *Caenohalictus incertus*; comportamento; *Leiopodus lacertinus*; *Melitoma segmentaria*.

1 Laboratório de Abelhas (Label), Universidade da Região de Joinville (Univille), Joinville, SC, Brasil.
2 Departamento de Ciências Biológicas, Univille, Rua Paulo Malschitzki, n. 10, CEP 89219-710, Joinville, SC, Brasil.
3 Autor para correspondência: dmouga@terra.com.br.

INTRODUCTION

Building of nests for bees is constant, from the solitary species to the social, having this importance of having a prepared site, which serves as a shelter, where the eggs are laid, immature develop, there is food storage and social interactions take place (MICHENER, 2007). The nesting habits are rather varied and characteristic at family or genus level, these are part of the knowledge about the bionomics and have descriptive importance in legal protection devices to bees and assessment of the conservation status (SILVEIRA *et al.*, 2002). Bees gather approximately 20.000 species, with variable patterns of sociability, spread practically all over the world (MICHENER, 2007). Nowadays, 1678 names are valid for the species in Brazil (RAFAEL *et al.*, 2012), with estimates of more 3.000 species to be described (ALVES-DOS-SANTOS, 2002).

The tribe Emphorini (no corbiculated Apidae) includes, in the neotropical region, 10 genera and 89 species (MOURE *et al.*, 2012) and is characterized by being uniquely American, spreading from Argentina and Chile to Canada, more diverse in temperate regions of South America and less diverse in the tropics. Robust species, small to large, which are usually specialized in collecting pollen of specific groups of plants, constitute this group (SILVEIRA *et al*., 2002). All species of this tribe are solitary and build their nests on the ground or in earth banks (MICHENER, 2007). In terms of behavior and nesting, there have been studies done with *Ptilothrix plumata* Smith, 1853 (PICK *et al.*, 2006) and *Ptilothrix fruitful* (Holmberg, 1903) (OLIVEIRA & SCHLINDWEIN, 2010). The genus *Melitoma* occurs from the United States to Argentina, gathers about 10 species, for which some studies were carried out in terms of behavior and nesting (ROZEN, 1984; MAMEDE FILHO *et al.*, 1990; CAMILLO *et al.*, 1993; CORTOPASSI-LAURINO *et al.*, 2010).

The tribe Halictini includes 22 genera and 132 species in the Neotropics (MOURE *et al.*, 2012), is represented by many species on every continent and is more diverse in the Palearctic region and less in the Neotropics (SILVEIRA *et al.*, 2002). This group includes solitary, communal and many primitive eusocial species (SILVEIRA *et al.*, 2002). For behavior and nesting, few studies were conducted for Halictini (MICHENER & LANGE, 1958; BATRA, 1964; MICHENER, 2007; ALBERT & PACKER, 2013). The genus *Caenohalictus* usually consists of bees of bright green, rarely red, black or non-metallic color, and the species are especially abundant and diverse in the Andean countries (from the province of Chubut / Argentina to the Mexico) and about 55 species are known (MICHENER, 2007). Nests of some species are similar to those of *Habralictus* (Caenohalictina), with lateral excavations taking, each, to a single horizontal cell (MICHENER & LANGE, 1958). Other species, however, build cells organized more or less in horizontal agglomerates in a cavity, suggesting nests of certain Augochlorini (MICHENER *et al.*, 1979). These nests were illustrated by Claude-Joseph in 1926 and copied by Sakagami & Michener (1962) but incorrectly associated with the genus *Caenoaugochlora*, according to Michener (2007).

To contribute to information about the nesting behavior of no social bee species, in southern Brazil, a study was conducted in Joinville/SC, aiming to check the species living in brick walls, the pattern of spatial distribution of the nests and the developed activities.

MATERIAL AND METHODS

The study was conducted from October 2012 to March 2013 in Joinville, SC (26°10'34.44" S 48°55'076" W) in Pirabeiraba district, in a rural property (*Sitio Kersten*), inserted in the protected environmental area *Serra Dona Francisca,* with vegetation cover of dense lowland rain forest, 38 m altitude, climate after Koeppen CFa (mesothermal moist without dry season) and average rainfall of 2418.0 mm (EPAGRI, 2003). The study was divided into two phases, the first for the choice and recognition of t was selected he study location and the second for the observation. In the first phase, a built covered area was selected(ranch), where there were numerous nests of bees, wasps and spiders and where a wall was chosen, with lots of occupied holes, easily visible (figure 1). The wall measured 4 m x 3,10 m (total area 12.4 m^2), 12 cm thick, and had been built in May 1978, with massive red brick clay, seated with masonry of clay and water, materials from a nearby river. From the study wall, an area of 6.4 m^2 with numerous nests was set, which were all numbered and registered in sketch. Individuals from several nests were collected to identify the species. In the second phase, there were carried

out 11 continuous days of daily views of the behavior of the wall inhabitants (169 hours of sampling effort). There were measured the heights of the nest openings in relation to the ground as well as the diameters of the openings and their external construction was verified. Distances between nests in activity were measured to determine the level of aggregation, by the method of the nearest neighbor (CLARK & EVANS, 1954), in Excel program. The activities undertaken by individuals at the entrance of the nests were observed.

Figure 1 – Wall of study, with the nests numbered.

RESULTS AND DISCUSSION

THE NESTING BEE SPECIES IN BRICK WALLS

Bees sampled in the brick wall come from the species *Caenohalictus incertus* (Schrottky, 1902) (Halictini, Halictinae), *Melitoma segmentaria* (Fabricius, 1804) (Emphorini, Apinae) and *Leiopodus lacertinus* (Smith, 1854) (Protepeolini, Apinae). Also sampled was the fly *Anthrax* cf. *virgo* Egger, 1859, Diptera) (figure 2). It was verified, on the wall study, the presence of 305 holes, of which 158 (52%) showed no activity in the sampling period and 147 showed activity in the sampling period, belonging 118 (39%) to *C. incertus* and 29 (9%) to *M. segmentaria*.

In relation to the input of all nests found (active and inactive), the majority (298/ 98%), without distinction of species, were located in the masonry between one brick and another, wherein the masonry varies between 1 and 1.5 cm (figure 3). Only seven (2%) nests were directly in brick. Michener *et al.* (1958b) report that the bees' nests were more abundant in horizons C1 and C2 (made of decomposed gneiss, relatively soft, usually without cracks) near the bottom and that they found only *Melitoma* nests above C1 horizon (that was composed of two layers: layer A-dark brown, with granular surface, ratio SiO_2/Al_2O_3=2.4, percentage of humus=3.2 and layer B-brown eluvial, with coarse granular structure, percentage of humus=1.4). The authors mention that the association of bees found in the earth banks is different from that found in flat ground.

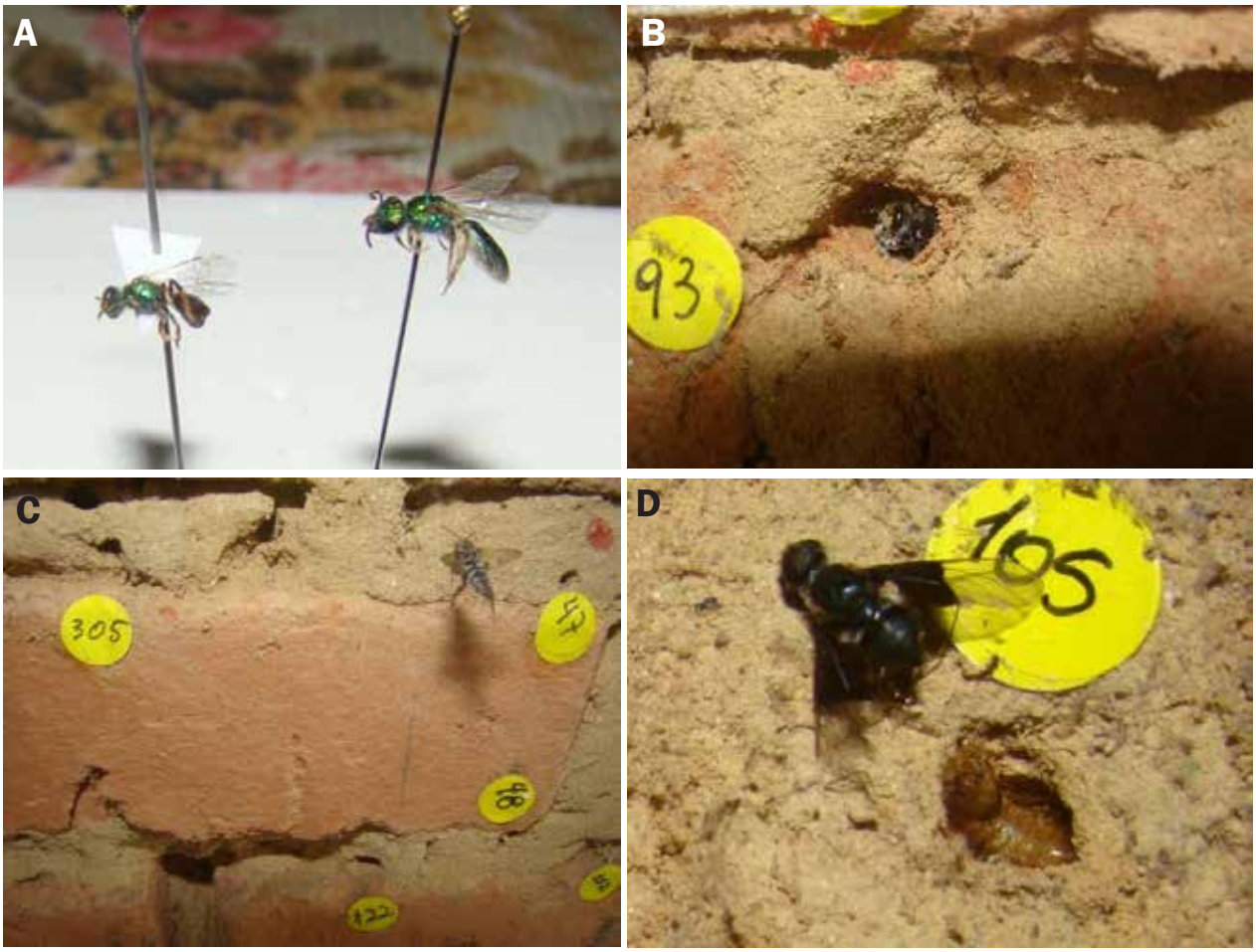

Figure 2 – The sampled species nesting in the brick wall. A) *Caenohalictus incertus*; B) *Melitoma segmentaria*; C) *Leiopodus lacertinus*; D) *Anthrax* cf. *virgo*.

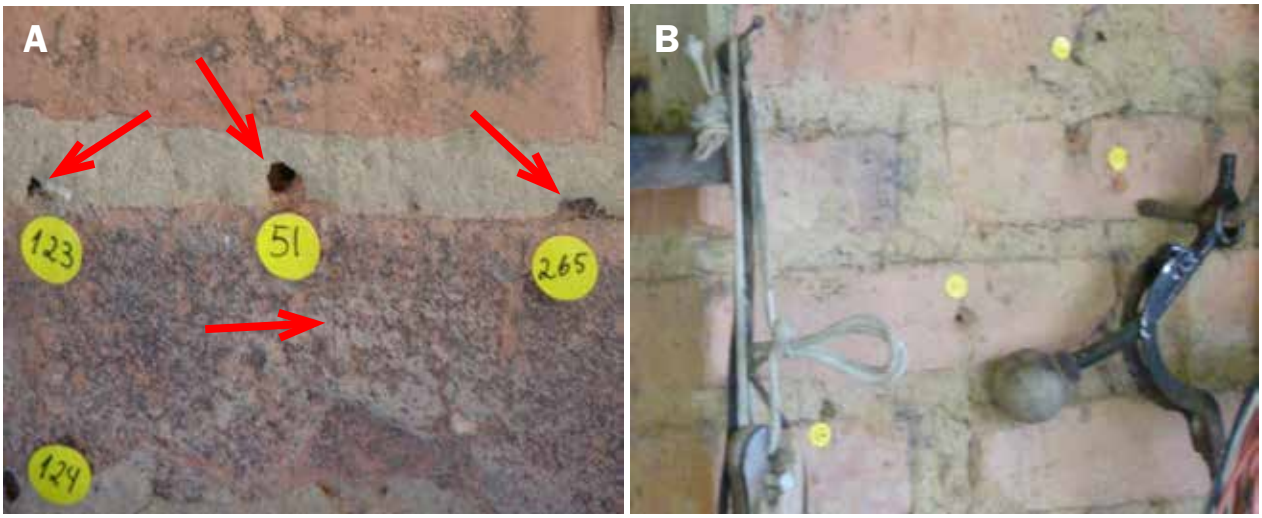

Figure 3 – Location of the nests in the wall. A) entrances in the masonry; B) entrances in the brick.

Melitoma segmentaria

For *M. segmentaria*, 29 active nests were observed, in a density of 4.53/ m^2. Pick *et al.* (2006) studied the aggregation density and architecture of the nests of *Ptilothrix plumata* Smith, 1853 (Emphorini), in ground devoid of vegetation in the valley of Catimbau, Pernambuco, Brazil, and found an aggregation of 328 nests that occupied an area of 48 m^2, thus in an aggregation density of 6.8 nests/ m^2. The nests of *M. segmentaria* were at an average distance of 12.22 cm between them (table 1), with 17 nests (59%) in a range between the ground and 90 cm high and 41% from 70 cm until 2 m.

Table 1 – Distances between the nests of *Melitoma segmentaria.* Legend: N = number of the nest; N + P = number of the nearest nests; D = distance between both in centimeters.

	N	N+P	D (cm)		N	N+P	D (cm)
1	24	27	9	16	115	32	4
2	27	24	9	17	116	60	4,5
3	32	115	4	18	133	60	61
4	51	305	15	19	141	275	7
5	60	116	4,5	20	201	24	10,5
6	64	78	12	21	207	275	8,5
7	65	66	6	22	217	291	5,5
8	66	287	2	23	257	217	10,5
9	78	89	9	24	275	207	8,5
10	80	81	11,5	25	287	66	2
11	81	80	11,5	26	291	217	5,5
12	83	81	15,5	27	292	133	18
13	89	78	9	28	293	83	49
14	102	257	11,5	29	305	51	15
15	104	102	15,5	Total			354,5

The nests are aggregated (R = 0.004, p <0.02). According to Michener (2007), in the genus *Melitoma*, various species form aggregations in hard clay banks or adobe walls. Mamede Filho *et al.* (1990) studied *M. segmentaria* nests on the ground and in an abandoned termite nest, where the nests were distant from each other, on the soil surface, at least 10.2 cm and at most 45 cm and, and, inside the earth, at least 1 cm and at most 20 cm. Cortopassi-Laurino *et al.* (2010) observed *M. segmentaria* nests in an artisan bread oven and verified the internal architecture of the nests. These works, however, did not check numerically the pattern of detachment of the nests.

The nests of *M. segmentaria* showed an opening measuring on average 0.9 cm in diameter (n = 29). Two nests showed a tower (figure 4) of 0.6 cm height at the nest entrance. The tower at the entrance was apparently built with mud left over from the interior nest excavation and had a smooth inner wall and the outer was rough. Camillo *et al.* (1993) also report nests with a tower of about 1 cm high and a diameter ranging from 0.6 to 0.7 cm. Pick *et al.* (2006) described a tower (2 mm high) surrounded with soil pellets around the entrance of the nest of *Ptilothrix plumata*, built with hard substrate material (ground), moistened with water.

Figure 4 – Nest of *M. segmentaria* with tower at the entrance.

Caenohalictus incertus

For *C. incertus*, 118 active nests were observed, in a density of 18.43/ m^2, with a mean distance of 8.18 cm from each other (table 2), 98 nests (83%) being in a range between the ground and 70 cm high (figure 4) and 17% from 70 cm until 2 m.

Table 2 – Distances between the nests of *Caenohalictus incertus*. Legend: N = number of the nest; N+P = number of the nearest nest of the same species; D = distance between both in centimeters.

	N	N+P	D (cm)		N	N+P	D (cm)
1	01	300	22,5	60	167	168	7
2	02	229	11	61	168	169	3,5
3	03	261	15	62	169	168	3,5
4	04	290	4	63	170	100	7
5	05	273	14	64	171	135	16
6	07	249	15,5	65	204	28	9
7	19	28	49	66	208	72	3
8	28	204	9	67	212	215	6
9	41	42	8,5	68	214	302	5
10	42	43	6,5	69	215	212	6
11	43	282	5,5	70	219	62	11
12	46	47	3,5	71	221	304	14
13	47	46	3,5	72	228	273	19
14	48	152	3	73	229	129	4,5
15	49	142	6,5	74	231	238	27,5

	N	N+P	D (cm)		N	N+P	D (cm)
16	52	162	4,5	75	232	211	27,5
17	57	298	1	76	235	149	4,5
18	58	264	4,5	77	237	164	8
19	61	169	7,5	78	238	231	27,5
20	62	219	11	79	239	02	18
21	63	164	7,5	80	242	125	8,5
22	69	300	7,5	81	246	242	10,2
23	70	82	7	82	249	158	9
24	72	208	3	83	252	253	4,5
25	74	253	8,5	84	253	286	1,5
26	79	70	8	85	254	267	6,5
27	82	70	8	86	258	148	10
28	91	142	3,5	87	259	212	7,5
29	92	113	8,5	88	261	262	1
30	94	140	34,8	89	262	263	0,8
31	98	268	5,5	90	263	262	0,8
32	99	269	2	91	264	298	4
33	100	170	7	92	265	114	4
34	103	129	9	93	266	279	4,5
35	105	106	8,5	94	267	268	3
36	106	105	8,5	95	268	267	3
37	112	282	5	96	269	99	2
38	113	92	8,5	97	272	276	5
39	114	265	4	98	273	05	14
40	118	119	3	99	274	279	5,5
41	119	118	3	100	276	272	5
42	122	48	3,5	101	277	278	14
43	123	125	6	102	278	135	8,4
44	125	301	5	103	279	274	4,5
45	128	243	11	104	282	112	5
46	129	229	4,5	105	285	264	5,5
47	135	278	8,4	106	286	253	2
48	138	139	18,5	107	288	07	20
49	139	138	18,5	108	290	04	4
50	140	294	2	109	294	140	2
51	142	91	3,5	110	295	82	20
52	147	91	4,5	111	298	264	5
53	148	258	10	112	299	42	8
54	149	235	4,5	113	300	69	7,5

	N	N+P	D (cm)		N	N+P	D (cm)
55	152	48	3	114	301	159	4
56	158	265	7,3	115	302	214	5
57	159	160	1	116	303	169	5
58	160	159	1	117	304	221	14
59	164	63	7,5			total	957,2

The nests of *C. incertus* are aggregated (R = 0.066, p <0.09). Aggregation of nests in Halictinae may stem from the behavior of return to the birthplace by the founder of the nest (philopatry) or optimal substrate conditions for deployment of the colonies, leading thereof to the concentration (MICHENER & LANGE, 1958). Eickwort (1969) stated that *Caenohalictus* belongs to the *Agapostemon* group, in which females frequently build cells in burrows constructed by other females, usually of the same species. According to Alves-dos-Santos (2002), some species of solitary bees can construct their nests aggregated, with several nests of the same species disposed in the same place.

The nests of *C. incertus* showed a circular opening measuring on average a diameter of 3 mm, with no tower. It seems to be excavated and is smoothed inside, with no special coating (wax or resin lining). It is very friable to the examination. Michener & Lange (1958) report the difficulty of following the deep, slender burrows.

THE OBSERVED ACTIVITIES

Melitoma segmentaria

During the period of observation, the external movement began at 04:30 a.m. The local was artificially illuminated from this hour. Reports of bees in external movement without the presence of light were made by Franco *et al.* (2007) while Mamede Filho *et al.* (1990) reported the start of *M. segmentaria* activities around 06:30 a.m. Almut *et al.* (2006) report that bees left the nest extremely early, before astronomical twilight began, when light levels in the forest were lower than 0.00001 cd m^2 and that in all these cases, bright artificial light had been used at the nest site (observers' headlights, a flashlight purposely pointed directly toward the nest entrance).

During the study period, only female individuals were collected in external movement. The outputs had an average duration of three minutes, the returns occurring with white loadings in *scopa* (*tibia* and *basitarsus*) and stays, on average, of one minute within the nest. The outputs with external charging extended until 09:00 a.m. Thereafter, when a bee without pollen load entered the nest (without apparent swollen abdomen), it remained there for six seconds to one minute. After, it went out and took an average of 55 seconds to return. After an average sequence of 9 inputs and 9 outputs, the bee remained about three minutes in the nest and then went backwards (abdomen) or frontwards, carrying or pushing with the jaws, the clay of the excavation till the nest entrance. When reaching the entrance, the bee pushed this material out, without leaving the nest, and returned to the inside, this movement being repeated several times each day. This sequence was observed over several days of observation in various nests. At this stage, it was noticed the appearance of damp soil below the entrance of the nests and the smoothing of the internal duct, done with the movement of the jaws and abdomen, which suggests digging the earth, softened by water brought to the nest. Michener (2007) reported that some species of *Ptilothrix* and *Melitoma* nest in earth banks or into the flat ground of hard soil and carry water from ponds and puddles to soften the soil for excavation and construction of cells.

Cortopassi-Laurino *et al.* (2010) observed a single active bee, collected in the afternoon while returning to the nest, that regurgitated water, and also found that, at 6: 50 a.m. (23º C, 88% RH), this female has returned from the field with pollen. Mamede Filho *et al*. (1990) found that the flight time ranged from one to 26 minutes and the stays of the bees in the nests varied from one to 40 minutes. In this work, the end of the external activity occurred, on average, around 05:00 p.m. In the study of Mamede Filho *et al.* (1990), the activities ended around 03:15 p.m. There was closure of 14 nests (of

29) between 8 and 10 a.m. that, in subsequent days, some were reopened. Only one of the 29 active nests showed activity until the end of the sampling period.

Caenohalictus incertus

Despite the fact that the nests of *C. incertus* were very close to those of *M. segmentaria*, the beginning of the activities of *C. incertus* was never seen in the dark, as it was performed by *M. segmentaria*. According to Almut *et al.* (2006), a crepuscular or noctural lifestyle has evolved in bees several times independently, probably to explore rewarding pollen sources without competition and to minimize predation and nest parasites but, despite these obvious advantages, only a few bee species are nocturnal.

Males and females in external movement were collected (start between 7:08 a.m., return from 12:30 p.m. until 04:30 p.m.). Dalmazzo & Roig-Alsina (2012) studied the nest structure and social behavior of *Augochlora amphitrite* (which nests in decaying wood) and found that foraging activity began 15-20 minutes after the sunlight hit the entrances (around 11:20 a.m.) and continued for approximately 4 hours until no more sunlight bathed the nests (around 03:00 p.m.).

Observations were daytime and there was no observation of foundation or excavation of new nests for this species but it was noted that there was loose earth accumulations below the entries. According to Batra (1964), most species of Halictinae dig specially at night and portions of the excavated soil are found in the entries of the nests in the early morning. Dalmazzo & Roig-Alsina (2012) mention that species which nest in the soil may be less constrained by the substrate and can fully express their behavioral capabilities.

On the way out, the individuals showed no loading and, in returning back, some were seen with visible load. Dalmazzo & Roig-Alsina (2012) observed that females leaving and entering nests in March-April did not carry pollen loads.

Leiopodus lacertinus

There was observed one to two individuals of *Leiopodus lacertinus*, bee parasite, in all observation days, usually in the afternoon, in waiting position (time from 20 seconds to 2 hours), at the entrance of 14 nests of *M. segmentaria*. When the nest occupant left, the individual of *L. lacertinus* entered and remained there during an average time of one minute. Only once, when *L. lacertinus* was in waiting position, very close to the nest entrance number 171, the occupant *M. segmentaria* of this nest left the nest, came flying up the parasitic bee, touched its thorax with one jaw, the individual of *L. lacertinus* did not move and *M. segmentaria* bee returned to the nest. There were collected only females of *L. lacertinus*. In Roig-Alsina & Rozen (1994) and Michener (2007), the genus *Leiopodus* is cited as cleptoparasitic in Emphorini nests of the genera *Diadasia, Melitoma* and *Ptilothrix.*

Anthrax cf. *virgo*

At the entrance of three nests of *M. segmentaria*, in five occasions, it was observed the moving of a pupae (from the interior of the nest to the entrance) from which emerged the fly *Anthrax* cf. *virgo*. There were no observions flies in waiting positions outside. After the left of the flies, there were observed individuals of *M. segmentaria* in these nests. Mamede Filho *et al.* (1990) observed the fly *Anthrax luctuosus* in a guarding position at the nest entrance of *M. segmentaria*, and this last one, in any moment, attacked or rushed away the fly, indicating that this dipteran is not recognized as an enemy by the bee. In the study of Yeates & Greathead (1997), the genus *Anthrax* is cited as an ectoparasite of larvae and pupae of bees and wasps.

Although the number of studied nests is high, the information recovered needs to be improved. Further studies with the females of *C. incertus* needs to be done in order to ascertain the sociality of the species as the social behavior within *Caenoalictus* genus is probably more variable than previously thought.

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