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Nesting biology of three *Megachile* (Hymenoptera: Megachilidae) species from Eastern Amazonia, Brazil

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ABSTRACT

Megachile Latreille is a conspicuous genus of solitary bees distributed worldwide. However, the biology of tropical species is still little known. We present data on biology of *Megachile brasiliensis* Data Torre, *Megachile sejuncta* Cockerell and *Megachile stilbonotaspis* Moure found in two remnants of eastern Amazonian forest in northeastern Brazil. The study was conducted using the trap-nest methodology in two different areas during four periods. We collected a total of 24 nests of *M. brasiliensis*, 26 of *M. sejuncta* and 28 of *M. stilbonotaspis*. The differential abundance of collected nests may reflect the population size in each sampled place. The nesting activity was concentrated mainly between July and January and species presented a multivoltine pattern, except for *M. sejuncta*, which was partly univoltine. Assessed pollen use showed a predominant use of *Attalea* sp. (Arecaceae) and, for *M. stilbonotaspis*, *Tylesia* sp. and *Lepidaploa* sp. (Asteraceae). Babassu is a very common palm in the studied areas and the studied species seem to have a strong link with it. We also reported change of pollen use by *M. sejuncta*, probably due to competition with *M. brasiliensis*, which may have influenced the biased sex ratio observed in *M. sejuncta* toward males. Parasites reported here were also recorded for other *Megachile* species, such as *Coelioxys*, *Brachymeria*, Meloidae and Pyralidae species. Mites were observed in association with *M. stilbonotaspis*. The data presented here set up a background that encourages new studies on the ecology of these three Amazonian species, providing tools for proper biodiversity management and conservation.

Introduction

Megachile Latreille (Hymenoptera: Megachilidae) is a large genus of solitary bees, with about 2645 species distributed around the world (Michener, 2007; Raw, 2007). Approximately 524 species are distributed in the Neotropics, but this number may surpass 600 species (Raw, 2007). Due to the group diversity, many problems exist relative to species position among the several subgenera, hindering identification of specimens collected on specific level, and thus the available data on their nesting biology (Raw, 2007).

Nesting habits of *Megachile* are conspicuous among solitary bees, mainly due to how they build their nests: using leaves, leaf fragments and/or petals to line their brood cells (Eickwort et al., 1981; Michener, 2007; Torretta et al., 2014). However, there is great variability of how such nests are built, and several types of materials may be employed such as clay, sand, chewed leaves, vegetal matter, man-made cavities, etc. (Krombein, 1967; Michener, 2007; Sheffield, 2017). This knowledge is important since the material used to build nests may provide clues about female morphological functionality, especially their mandibles (Laroca et al., 1992). Therefore, nest architecture may provide useful characters for taxonomy and phylogeny of those species, contributing to a better understanding about the evolution of the genus (Gonzalez, 2008).

Substrates used to build nests may be cavities in the ground, pre-existing cavities in wood or even living plant stems (Eickwort et al., 1981; Michener, 2007; Orr et al., 2015). The behavior of nesting in pre-existing cavities associated with the use of the trap-nest methodology (Krombein, 1967) facilitates obtaining solitary bee specimens. Trap-nests also allow research on biology, life cycle and associated organisms. Moreover, we can infer on plant hosts used to provision for immature larvae (Buschini et al., 2009; Torretta et al., 2014).

The trap-nest methodology also allows research on pollination, since some solitary bee species that nest in pre-existing cavities are pollinators of native plants, and in some cases are the foundation for bee management on economically important crops, such

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as *Megachile rotundata* Fabricius (Pitts-Singer and Cane, 2011), although few studies report pollen content used by bees, especially in the Neotropical region (Buschini et al., 2009; Strickler et al., 1996; Rocha-Filho and Garófalo, 2016; Torretta and Durante, 2011; Torretta et al., 2014). In Brazil, *Megachile* seems to prefer Fabaceae and Asteraceae (Garófalo et al., 2004) and has great potential for management in crops of economic interest (Pitts-Singer and Cane, 2011).

Megachile species are better studied in the south of Brazil, whereas few studies were conducted in the Amazonian region (Morato, 2003; Pinheiro-Machado et al., 2002). Regarding this knowledge gap, the aim of this study was to describe nesting biology of three *Megachile* species in Amazonian forest remnants and Restinga from Maranhão Island and forest areas at "Baixada Maranhense", Maranhão State, Northeastern Brazil.

Material and methods

The study was conducted in four different areas in the Maranhão State, Brazil: in Maranhão Island, at São José de Ribamar municipality, in "Sítio Aguahy" (2° 38′ S 44° 08′O) belonging to "Quercegen Agronegócios I LTDA" (henceforth referred to as "SA"); and in "Área de Proteção Ambiental da Baixada Maranhense"(henceforth referred to as "APABM"), in three municipalities: "Peri-Mirim" ("Parque Agroecológico Buritirana" – 2°38′S 44°50′O), "Penalva" ("Canadá Farm" – 3°17′S 45°07′O) and "Vitória do Mearim" ("Coque Farm" – 3°32′S 44°57′O) (Fig. 1). The three areas sampled in the APABM will be henceforth referred to as "PM", "PE" and "VM", respectively. All areas are part of the Amazonian biome.

SA comprises about 600 ha of transitional vegetation, merging remnants of Amazonian forest, mangrove and restinga. Some forest remnants are better preserved, being called primary forest, whereas other forest remnants are called secondary forest, due to the high number of trails and crops, constituting a mosaic of environments. Restinga area is characterized by vegetation adapted to high salinity, with plants found in herbaceous, shrubby, and arborous layers (Serra et al., 2016).

APABM is characterized by a transition zone between "Cocais forest" to the south, Amazonian forest to the west and marine systems to the north, all of these with *Attalea speciosa* Mart. Ex Spreng palms (Arecaceae). It is located in the largest set of lake basins of the Brazilian Northeastern region and is subjected to seasonal flooding (Pinheiro et al., 2010). PM harbors secondary forests of *A. speciosa* mainly and herbaceous fields that flood only partially. PE also has secondary forests, but these are surrounded by flooded fields of herbaceous plants during the rainy season. VM is the best preserved area, and has primary forests with low human interference, besides gallery forests typical of those with springs and small water streams.

Following Peel et al. (2007), climate at SA is characterized as type Awh, with high temperatures throughout year and a rainy season concentrated during January to July; the annual average rainfall reaches 1900 mm. At BM, the climate varies between Am and Aw and the annual rainfall regime varies from 1000 to 2000 mm, concentrated from February to May. Rainfall data from each area during all periods were provided by "Laboratório de Meteorologia do Núcleo Geoambiental da Universidade Estadual do Maranhão".

We used the trap-nest methodology (henceforth "traps") to sample the study areas (Krombein, 1967). During the SA phase, traps made using Tatajuba wood with diameters of 8, 10, 12, 14 and 16 mm were set in groups of ten inside polyethylene terephthalate (PET) bottles, two of each diameter, each set considered a sampling unit (SU). At APABM, traps with diameters of 4, 6, 8, 10, 13 and 15 mm were used, two for each diameter, comprising 12 traps per SU placed in rectangular tubes of polyvinyl chloride (PVC). The tunnel of each trap was 11 cm deep and the SUs were placed hanging from three branches at 1.5 m off the ground. The number of SUs in field and the period of study in each area are summarized in Table 1.

Each SU was inspected monthly and traps occupied were replaced by empty traps and brought to laboratory to be monitored until adult emergence, when we registered date and order of emergence. Adults were sacrificed in jars with Ethyl Acetate, sexed, and pinned. Voucher specimens were deposited in the LESPP Collection

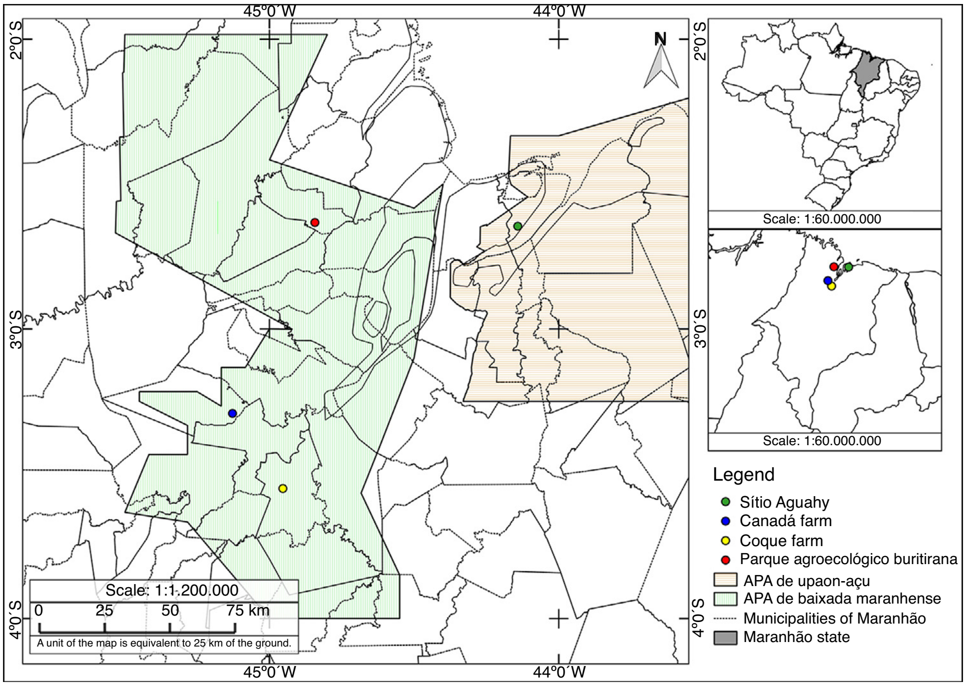

Fig. 1. Study areas and sampling locations in the Maranhão state, Brazil.

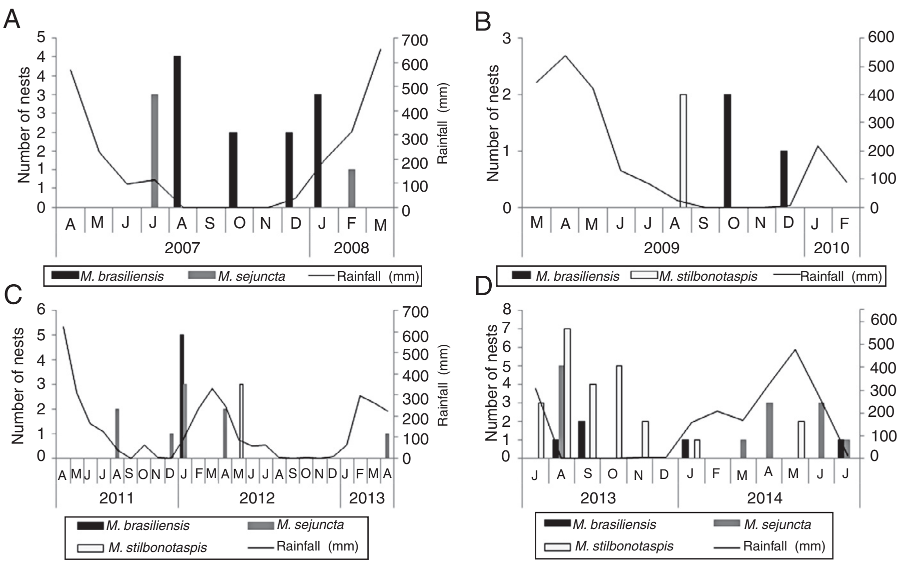

Fig. 2. Recorded rainfall and number of sampled nests during the study in SA and APABM: (A–C) nests collected and rainfall recorded in SA; (D) nests collected and rainfall recorded in APABM.

Table 1
Arrangement of sampling units (SU) in both areas during the four study periods.

Sample periods	Sítio Aguahy			APABM			Total
	PF	SF	RE	VM	PE	PM	
Period I (Mar/2007–Feb/2008)	20	30	–	–	–	–	500
Period II (Feb/2009–Jan/2010)	20	20	–	–	–	–	400
Period III (May/2011–Apr/2013)	15	15	12[a]	–	–	–	420
Period IV (Jul/2013–Jul/2014)	–	–	–	18	18	18	540

PF, primary forest; SF, secondary forest; RE, restinga; VM, Vitória do Mearim; PE, Penalva; PM, Peri-Mirim.

[a] Only 20 traps of 16 mm were used during this period.

of the Biology Department at "Universidade Federal do Maranhão", in the Entomological Collection of the Taxonomic Collections at "Universidade Federal de Minas Gerais" and in the Padre Jesus Santiago Moure Collection at "Universidade Federal do Paraná".

Nests where adults had emerged or died were used to describe architecture. Measurements of the nest and brood cells were taken. The process of nest analysis was recorded through photographs. This study is a data compilation of three studies carried out at different periods and with different traps as showed in Table 1. Collects occurred during periods I and II were meant to perform a survey of solitary Hymenoptera species which nest in pre-existent cavities. The aim of collects during Period III was to study of *Megachile* species biology, then development time of bees were recorded and data presented is considering this period and period IV. Collects during Period IV aimed to evaluated aspects of vegetation cover and nesting activity of solitary Hymenoptera (DBM, unpublished data). For building activity we followed the methodology described by Cardoso and Silveira (2003) to infer the span of minimum and maximum development time of bees. For the first span we considered the time of development as being from the day the nest was collected up to the emergence of the adult bee in the laboratory; for the second span of development time we considered that the first bee was originated from an egg laid the day after the last survey of nests until the emergence of the adult bee in laboratory. Regarding bee mortality, brood cells were opened a year after nests were collected, in the case of Periods III and IV. Nests of Period I and II had their brood cells analyzed during Period III when bees did not emerge.

Acetolysis process was used to analyze the residual pollen content and larval feces from brood cells following protocols by Erdtman (1943) modified by Lieux (1980). Three slides were made from each nest and 400 pollen grains were counted whenever possible. We followed Michener (2007) classification in order to classify bees according to pollen contents. Statistical tests were run in the programming package R version 3.2.2 (R Development Core Team, 2015). Data normality was assessed using the Shapiro–Wilk test. Correlation between rainfall and nesting was evaluated through Fisher's exact test; sex-ratio of emerged bees and the influence of diameter in trap-nest use was assessed using the Chi-square test. Occupancy rate (OR) of the trap-nests was calculated by dividing the total of nests occupied during the study in each sampled period by the total of nests offered in the field (Table I, supplementary material). Confidence intervals used in tests were $p < 0.05$.

Results

Nest abundance and nesting activity

We collected 78 nests of *Megachile* in the studied areas: 24 nests of *M.* (*Melanosarus*) *brasiliensis* Dalla Torre, 26 of *M.* (*Austromegachile*) *sejuncta* Cockerell, and 28 of *M.* (*Pseudocentron*) *stilbonotaspis* Moure. Occupancy rate of traps at SA was 3.75% during period I, 1.25% in period II and 4.04% in period III; at APABM, occupancy rate was 7.77% considering all the areas together in APABM (but see Table I of the supplementary material, for detailed occupancy rates). The most abundant species were *M. brasiliensis* at SA and *M. stilbonotaspis* at APABM. Detailed number of nests by species and area, as well as emerged specimens are shown in Table 2.

Megachile brasiliensis and *M. sejuncta* nested mainly between July and January in both areas (Fig. 2). Due to few *M. stilbonotaspis* nests collected at area A we cannot infer any nesting pattern, as this species founded nests sparsely during the study period. Only *M. stilbonotaspis* nesting activity was correlated with rainfall at area B (Fisher's exact, $p = 0.003$).

The estimates for minimum and maximum development time of bees are summarized in Fig. 3. At SA, *M. brasiliensis* was multivoltine; *M. stilbonotaspis* could not have its emergence pattern assessed because some individuals emerged before the next survey of nests. *Megachile sejuncta* had a population partially univoltine; some individuals ($n = 4$) from two nests collected in August/2011

Table 2
Data of emerged adults (excluding parasites) from *Megachile* at Sítio Aguahy and APABM (Penalva, Peri-Mirim and Vitória do Mearim) in Maranhão state, Brazil.

Species	Sítio Aguahy				Penalva				Peri-Mirim				Vitória do Mearim			
	Nests		ES		Nests		ES		Nests		ES		Nests		ES	
	N	Ø (*n*)	*N*	%	*N*	Ø (*n*)	*N*	%	*N*	Ø (*n*)	*n*	%	*N*	Ø (*n*)	*N*	%
M. brasiliensis	19	8 (8) 10 (2) 12 (4) 14 (3) 16 (2)	55	59.1	–	–	–	–	4	8 (2) 6 (2)	16	59.2	1	10 (1)	1	1.7
M. sejuncta	13	8 (2) 10 (9) 12 (1) 14 (1)	30	32.4	2	6 (1) 10 (1)	3	10.7	1	10 (1)	1	3.7	10	8 (4) 10 (4) 13 (1) 15 (1)	34	56.6
M. stilbonotaspis	5	10 (2) 12 (2) 14 (1)	7	7.5	9	6 (4) 8 (3) 10 (2)	25	89.3	4	6 (2) 10 (2)	10	37.1	11	8 (6) 10 (2) 13 (3)	25	41.7
Total	37		92		11		28		9		27		22		60	

Ø, trap diameter; (*n*), absolute number of traps used or emerged specimens; ES, emerged specimens.

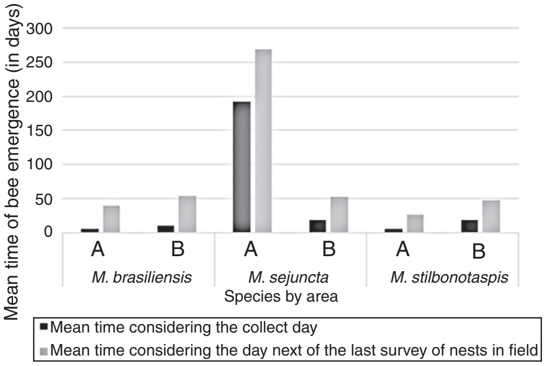

Fig. 3. Estimatives of minimum and maximum time development of *Megachile* bees in SA and APABM.

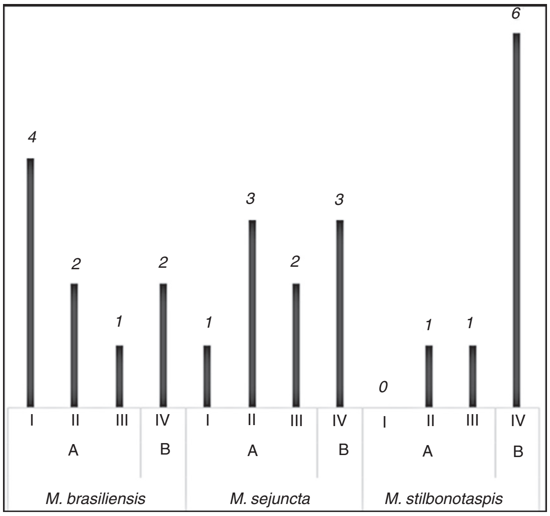

Fig. 4. Estimated number of generations of *Megachile* bees in SA and APABM. The number on the columns refer to the number of generations in each period (roman numerals) by each area sampled (A) "Sítio Aguahy"; (B)"Área de Proteção Ambiental da Baixada Maranhense".

and two nests collected in April/2012 (*n* = 4) had their development delayed and took more than one year in average to emerge (Fig. 3). At APABM, all species had a multivoltine pattern. Number of generations by species and local studied are presented in Fig. 4.

Table 3
Pollen types used by *Megachile* species at Sítio Aguahy, Maranhão Island, Maranhão State, Brazil.

Family	Pollen type	*M. brasiliensis*	*M. sejuncta*	*M. stilbonotaspis*
Arecaceae	*Attalea* sp.	89.23%	59.09%	34.05%
Asteraceae	*Tilesia* sp.	0.34%	–	34.05%
	Lepidaploa sp.	–	–	31.90%
Euphorbiaceae	*Croton* sp.	0.59%	0.52%	–
	Phyllanthus sp.	1.79%	0.11%	–
Gentianaceae	*Coutoubea* sp.	0.57%	–	–
Lamiaceae	*Hyptis* sp.	–	0.18%	–
Myrtaceae	*Myrcia* sp.	–	28.88%	–
Rubiaceae	*Spermacoce* sp.	4.28%	–	–
Onagraceae	*Ludwigia* sp.	3.20%	0.07%	–
–	Type 1 sp.	–	11.15%	–

Table 4
Pollen types used by *Megachile* species at APABM, Maranhão State, Brazil.

Family	Pollen type	*M. brasiliensis*	*M. sejuncta*	*M. stilbonotaspis*
Arecaceae	*Attalea* sp.	99.97%	96.48%	54.43%
Asteraceae	*Lepidaploa* sp.	–	–	8.53%
Asteraceae	*Tilesia* sp.	–	–	16.26%
Asteraceae	*Vernanothura* sp.	–	–	0.90%
Convovulaceae	*Ipomea* sp.	–	–	0.97%
Euphorbiaceae	*Croton* sp.	–	–	0.89%
Fabaceae	Cesalpinoidea sp.1	0.03%	–	–
Fabaceae	Type 1	–	0.07%	–
Fabaceae	*Bahunia* sp.1	–	0.01%	–
Lamiaceae	*Hyptis* sp.	–	–	0.64%
Myrtaceae	*Myrcia* sp.	–	2.85%	
Onagraceae	*Ludwigia* sp.	–	–	5.92%
Passifloraceae	Type 1	–	0.59%	–
Phyllanthaceae	*Phyllanthus* sp.	–	–	2.52%
Pontederiaceae	Aff. *Eichornia* sp.	–	–	3.37%
Solanaceae	Type 1	–	–	0.78%
Unidentified	Type 2	–	–	3.08%
Unidentified	Type 3	–	–	1.72%

Pollen use

We performed acetolysis on pollen residuals and bee feces from ten *M. brasiliensis* nests, eight *M. sejuncta* nests and four *M. stilbonotaspis* nests from SA; from BM, we analyzed pollen residuals and bee feces of three *M. brasiliensis* nests, six *M. sejuncta* nests and thirteen M. *stilbonotaspis* nests (Tables 3 and 4).

M. brasiliensis was classified as oligolectic, using mainly pollen of type *Attalea* sp. (Arecaceae) (Fig. 5A). However, we observed one nest at SA whose pollen content varied greatly, with pollen types of *Spermacoce* sp., *Ludwigia* sp., *Phyllantus* sp., *Cotobea* sp. and *Tilesia* sp. At APABM, almost 100% of pollen grains counted belonged to *Attalea* sp.

Pollen types of *Attalea* sp., *Tilesia* sp. (Fig. 5B) and *Lepidaploa* sp. (Fig. 5C) were counted at all areas studied for *M. stilbonotaspis*, classified as narrowly polylectic. At PE and VM, the pollen type *Attalea* sp. was not present in August/2013 and May/2014 and pollen types of *Tilesia* sp. and *Lepidaploa* sp. were abundant on the slides analyzed. *M. sejuncta* was classified as narrowly polylectic at SA.

Nests of *M. sejuncta* presented pollen types of *Attalea* sp. and *Myrcia* sp. in the nest slides of February and *Attalea* sp. as predominant in April and July. An unidentified pollen type was present in nests collected in August/2011 (*n* = 1) and January/2012 (*n* = 3). Type *Myrcia* sp. (Fig. 5D) was predominant in samples of nests collected in February/2008 (*n* = 1) and December/2011 (*n* = 1). At area B, *M. sejuncta* used mainly type *Attalea* sp. (Table 4).

Sex ratio and diameter cavity occupancy

Sex ratio did not differ from 1:1 in *M. brasiliensis* and *M. stilbonotaspis*. For *M. sejuncta*, sex ratio was significantly biased toward

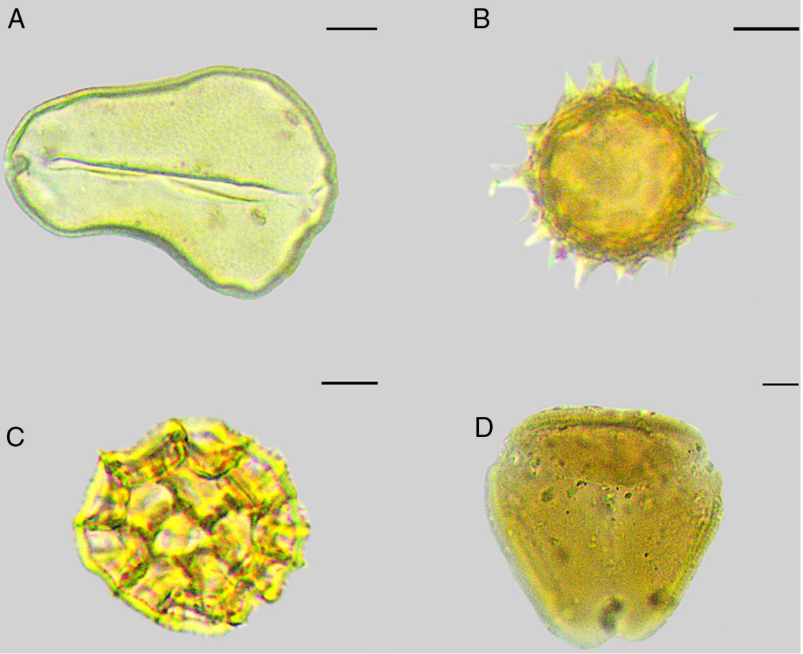

Fig. 5. Main pollen types identified on *Megachile* slides from larvae feces: (A) type *Attalea* sp.; (B) type *Tilesia* sp.; (C) type *Lepidaploa* sp.; (D) type *Myrcia* sp. Scale bar of figures A - C 10 μm; figure D: 20 μm.

Table 5
Sex ratio and χ^2 values of *Megachile* emerged from nests in SA and in APABM.

Species	SA				APABM			
	(♂:♀)	SR	χ^2	*p*-Value	(♂:♀)	SR	χ^2	*p*-Value
M. brasiliensis	26:25	1.08:1	0.0196	0.886	07:10	0.7:1	0.52941	0.4669
M. stilbonotaspis	–	–	–	–	30:29	1.03:1	0.0169	0.8964
M. sejuncta	19:05	3.03:1	5.761	0.016	14:26	0.53:1	3.6	0.0577

♂, males; ♀, females; sex ratio, SR.

Table 6
Nest architecture data taken from *Megachile* nests at area A and B. Measures were taken in centimeters.

Species	Area	Nests (*n*)	ML ± SD	BC (*n*)	BC/*N*	ML ± SD
M. brasiliensis	AS	12	6.6 ± 1.60	41	1–5	0.97 ± 0.20
	APABM	3	7.65 ± 2.69	17	3–7	1.07 ± 0.91
M. sejuncta	SA	11	5.17 ± 1.85	23	2–5	1.02 ± 0.16
	APABM	10	7.69 ± 1.85	37	2–6	1.20 ± 0.18
M. stilbonotaspis	SA	4	5.04 ± 0.39	12	3	1.20 ± 0.12
	APABM	11	6.80 ± 1.17	51	3–6	1.16 ± 1.99

ML, mean length; SD, standard deviation; BC, Brood cells; BC/*n*, amplitude of brood cells by nests.

males at SA, and toward females at APABM, although the latter was only marginally statistically significant (Table 5).

Nests of 8 and 10 mm diameter were used the most by *M. sejuncta* in SA ($\chi^2 = 20.462$; $p < 0.001$) and in APABM ($\chi^2 = 12.385$; $p < 0.05$) (Table 2). The same trend was observed for *M. stilbonotaspis* in APABM where 6, 8 and 10 mm were used the most ($\chi^2 = 24.5$; $p < 0.001$) (Table 2).

Nest architecture: general aspects

Nests of all three species were characterized by having brood cells lined and covered with layers of interwoven leaf fragments; nests had a cylindrical shape (Fig. 6). No vestibular cell was accounted for; neither were any intercalary cells or any gaps between the first and last cells built (Table 6).

Each brood cell in a line series was snugly fitted, so that the apex of one brood cell faced the bottom of the next one (except for the innermost brood cell, which faced the bottom of the trap, and outermost brood cells, which faced the nest's opercula). The brood cells were covered with a batch of leaf fragments, making it difficult to observe how many cells were in the whole nest; but most nests were easily disassembled, and brood cells were separated without cell damage (Fig. 6, but see *M. sejuncta* description below). In all nests analyzed, feces of the immatures were adhered on the brood cell walls. Leaf fragments used to build the nests had indentations around the edges, giving them a crenulated outline. The leaf fragments used to build the brood cell walls were cut through the mid vein of the leaves and pressed together by the adult females, using their mandibles.

The nest of Megachile brasiliensis

Nests of this species did not differ between areas (Fig. 6A and B) and were typically covered by elongated subtrapezoidal pieces of leaves, with one side of the fragment much more rounded than the other (Fig. 6C). The sharp apex of the pieces points upwards, giving a scaly aspect to the nest's surface. The leaf fragments forming the walls of the brood cells were tightly fitted on the sides, forming a strongly fastened cup (although no trace of any gluing substance was found); those leaf fragments above the brood cell walls were partially overlapped, forming a projection that fits to the bottom of the next cell. The bottom of the cup was formed by the posterior side of bent leaf fragments (Fig. 7C). The cap of the brood cell was formed by several rounded pieces of leaves, which were bigger in diameter than the cup, so that when the brood cell was closed, these leaf fragments acquired a convex shape. After the last cell was built, several rounded pieces of leaves were stuck to the cavity floor, forming the nest opercula.

Two nests in a cavity of 16 mm had each two series of lined brood cells in the same tunnel (Fig. 6A). We also found another nest with two cells, from which three bees emerged, and with an abnormally big outermost cell (Fig. 7D), from which two males emerged. A nest of *M. brasiliensis* built in a 14 mm cavity had another nest of *Tetrapedia rugulosa* Friese (Hymenoptera: Apidae) built in front of it (Fig. 7A), evidencing nest supersedure. Another cavity was shared by *M. brasiliensis* with another Hymenoptera species, which

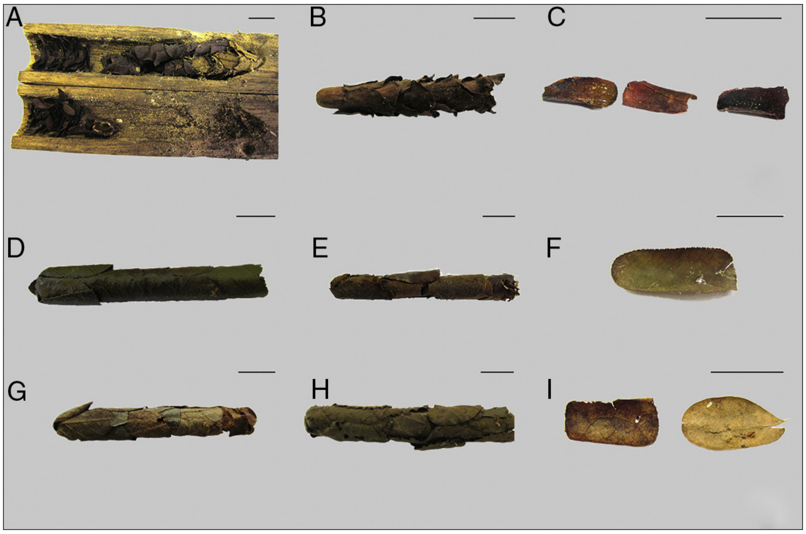

Fig. 6. Photographs of nests from the three *Megachile* species: (A)–(C) *M. brasiliensis*; (D)–(F) *M. sejuncta*; (G)–(I) *M. stilbonotaspis.* Nests A, D and G were collected at SA, whereas nests B, E and H were collected at APABM. Scale bar = 1 cm.

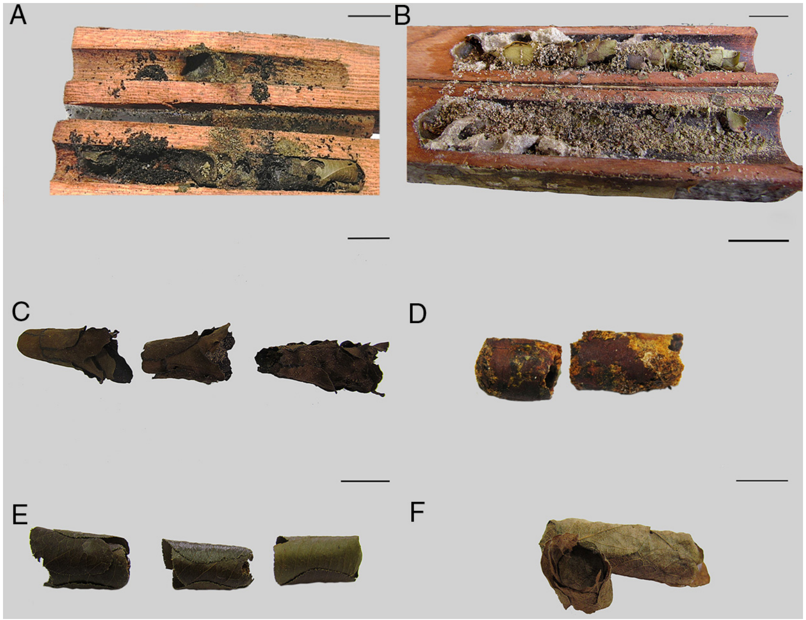

Fig. 7. Brood cells of *Megachile* species: (A) nest supersedure of Tetrapedia rugulosa over a *Megachile brasiliensis* nest; (B) cavity sharing by *M. brasiliensis* and a unidentified species of Centris; (C) unattached cells of *M. brasiliensis*; (D) normal and big-sized cell of *M. brasiliensis*; (E) *M. sejuncta* brood cells; (F) *M. stilbonotaspis* brood cells. Scale bar = 1 cm.

we were unable to identify due to the fact that the larvae did not survive until adulthood. However, we suspect it may be *Centris* sp. because of the nest architecture (Fig. 7B).

The nest of Megachile sejuncta

Nests of this species varied between the two sites regarding types of leaf fragments used to build brood cells (Fig. 6D and E; Fig. 7E). The leaves used in area A were more coriaceous, while in area B leaves were coriaceous (apparently the same species used in area A) and others were hirsute. Leaf fragments were overlapped and not tightly fastened, so that when removing the nest from the trap, some brood cells got detached. The nest opercula had several leaf fragments arranged from the last cell built up to the entrance of the boring.

Leaf fragments used to construct the brood cell walls and the upper layers of the nest walls were elongated, the longer sides cut in a straight line, while the shorter sides had a more rounded cut. The brood cells were formed by leaf fragments fastened by their sides, forming the cell cup; the bottom of the nest is formed by leaf fragments bent to adjust the bottom nest structure to the substrate. The cap of the cell is formed by several rounded leaf fragments and has a bigger diameter than the nest structure, sealing it with a convex shape.

The nest of Megachile stilbonotaspis

This species presented noticeable variation in its nest structure, with two kinds of leaf material used to construct nests: leaf fragments and leaflets (Fig. 6G and H). The leaflets were hirsute and cut out at the petiole, so that the whole structure was used to build

Table 7
Mortality rate, parasites and pathogens recorded for the *Megachile* nests at SA and three municipalities of APABM.

Hosts	Mortality cause	Sítio Aguahy			Penalva	Peri-Mirim	Vitória do Mearim
		P I	P II	P III	P IV	P IV	P IV
M. brasiliensis	*Brachymeria* sp.1	6	–	–	–	–	–
	C. tolteca	1	–	–	–	–	–
	C. otomita	–	–	3	–	1	–
	Pyralidae	–	–	2	–	–	–
	Fungi	–	–	6	–	–	–
	Unknown causes	–	–	3	–	1	–
	% BC mortality	15.9	0	58.3	0	11.76	0
M. sejuncta	*Brachymeria* sp.2	1	–	–	–	–	–
	Pyralidae	–	–	2	–	–	–
	Unknown causes	6	–	–	–	–	–
	% BC mortality	58.3	0	14.28	0	0	0
M. stilbonotaspis	*C. tolteca*	–	1	–	–	–	–
	C. simillima	–	–	–	1	–	–
	C. otomita	–	–	–	–	1	–
	Meloidae	–	–	–	–	–	1
	Unknown causes	–	–	–	3	–	–
	% BC mortality	0	20	0	17.39	9	5

Brood cells, BC.

the cup of the brood cells. Leaf fragments varied regarding cut patterns ranging from almost rectangular shaped, to oblong (Fig. 6I). The bottom of each cell was built by bending the tip of the leaflet or the leaf fragment. The cap of the brood cells was built with rounded leaf fragments and seemed to be made from leaflets (Fig. 7F). The diameter of such rounded fragments was almost the same as the nest diameter, fitting snugly with the entrance of the cell. The nest opercula had several leaflets and leaf fragments arranged from the last built cell and the entrance of the boring. The pattern of leaves used did not vary between areas; however, the leaflets seemed to be from two different species of Fabaceae. Some of the leaflets had cut marks around the edges, probably made by the bee to better fit them into the nest.

Mortality and associated species

Data on general and specific mortality rates are summarized in Table 7, and in Table II of the supplementary material for detailed number of brood cells. The highest mortality rate recorded, considering all periods sampled, was for *M. brasiliensis* at SA by cleptoparasitism. *Brachymeria* sp. 1 and *Coelioxys tolteca* Cresson parasitized nests of *M. brasiliensis* in periods I and II. In period III, *Coelioxys otomita* and Pyralidae (Lepidoptera) were recorded. At APABM, *C. otomita* Cresson was the only parasite of *M. brasiliensis* and one bee died of unknown causes as an adult, but only fragments of the body was found.

We recorded cleptoparasitism in *M. sejuncta* only in period III at SA by *Bachymeria* sp.2 and Pyralidae (Lepidoptera). At APABM, only deaths by unknown causes were reported, all in larval stage.

Regarding *M. stilbonotaspis*, *C. tolteca* was recorded at a secondary forest in SA; at APABM, we recorded Meloidae (Coleoptera), *Ceolioxys simillima* Smith, *C. tolteca* and *C. otomita*, besides unknown mortality causes. We also observed the occurrence of mites on the bodies of *M. stilbonotaspis* specimens emerged from Restinga nests of SA. They were found at the forewings, propodeum and first tergite of the bees.

Discussion

We have here provided data on nests of three species of *Megachile* for the first time. Previously, two species of bees from the subgenus *Melanosarus* had their biology described: *M.* (*Melanosarus*) *xylocopoides* Smith (Krombein, 1967) and *M.* (*Melanosarus*) *nigripennis* Spinola (Marques and Gaglianone, 2013). For the subgenus *Austromegachile*, there is data provided by Morato (2003) and notes on a wild nest found in an Araucaria forest by Laroca et al. (1987) in Brazil. Regarding the *Pseudocentron* subgenus, there is data from Torretta et al. (2012) for *M.* (*Pseudocentron*) *gomphrenoides* Vachal, Laroca (1991) for *M.* (*Pseudocentron*) *apicipennis* Schrottky.

In this study, occupancy rate differed between sampled areas, where SA showed low nest abundance compared with APABM, even though SA was sampled for a considerably longer period of time. This may be explained by the fact that SA is under more anthropic pressure and thus can only harbor smaller populations of the studied species. Occupancy rate of nests in APABM was higher than previously recorded in a similar study (Morato, 2003), but among APABM areas, VM was the area where we found the highest number of nests. This area is considered the best preserved area we sampled at APABM and the higher number of nests may be linked to the availability of resources for nest building for *Megachile* species, such as suitable substrates and material to build nests, besides pollen and nectar for feeding larvae (Michener, 1953; Stefan-Dewenter and Schielle, 2008; Sheffield et al., 2011). Other researchers have previously found a positive correlation with the presence of nesting bees and the vegetation cover (DBM, unpublished data), which might be linked with presence of resources bees use for nesting.

Regarding the places chosen for nesting, it is important to point out that *M. sejuncta* was collected only in the more preserved areas in this study (primary forest in SA and the municipality of Vitória do Mearim in APABM), suggesting that this species probably has little tolerance when it comes to disturbed areas, which was also observed for some *Austromegachile* species (Laroca et al., 1987; Morato, 2003). For *M. brasiliensis* and *M. stilbonotaspis*, we could not find any pattern in the nesting places used. Few studies address factors that influence nesting activities of solitary bees in the Amazon forest biome. Morato (2003) and Morato et al. (1999) observed no correlation of rainfall with nesting activities of *Megachile* and *Centris* Fabricius, respectively, in the Amazonian region. In this study, only the nesting activity of *M. stilbonotaspis* had its peak in months with low rainfall records, and although not detected by the Fisher's test, *M. brasiliensis* showed the same trend. The lack of patterns recognizable in SA may be due to low abundance of nests, despite intense sampling efforts. Solitary bees' activity pattern seems to vary among and within genera throughout the tropics. Becker et al. (1991) found a positive correlation between rainfall and flowering seasons and activity peaks for *Euglossa* Latreille species, while studies focused on other taxa failed to show this association (Morato, 2003; Sabino and Antonini, 2017).

Another factor that may determine nesting activity is pollen availability, as observed by Cardoso and Silveira (2012). For *M. brasiliensis* and *M. sejuncta*, the pattern of nesting activity may be correlated with the peak of *Attalea* sp. bloom, since this was the predominant floral resource used by these two *Megachile* species. Bernini et al. (2007) noted peaks of *A. speciosa* bloom during dry and wet season in Roraima (north of Brazil, deep into the Amazonian region) and this peak variation may be related to pollinator visits during the year and probably the quantity of fruits produced. However, there was some variation in pollen use in SA by *M. sejuncta*, which also used Myrtaceae and an unidentified pollen type, what may be related to nesting activity during low blooming of *Attalea* sp. or competition for food with *M. brasiliensis*. This flexibility of pollen use was observed in other Megachilidae, such as *Megachile brevis* Say (Michener, 1953), *Osmia* Panzer (Williams, 2003) and species of the genus *Chelostoma* Latreille (Sedivy et al., 2008). Nesting activity of the species we studied matched with the flowering season of *Attalea* in Maranhão and Mato Grosso states (Anderson et al., 1988; Fava et al., 2011; Pinheiro, 2011). On the other hand, *M. stilbonotaspis* was found predominantly using Asteraceae pollen in SA; in APABM, the resource used was mainly *Attalea* sp., what may reflect pollen use according with one or other available source, and thus explain the bigger number of nests of this species in APABM, since *Attalea* stands are abundant in the area.

It is important to point out that previous studies on *Attalea* (Babassu palm trees) pollinators have never recorded any *Megachile* species, only social bees such as *Trigona spinipes* Fabricius and Coleoptera species (Anderson et al., 1988; Fava et al., 2011; Pinheiro, 2011). Furthermore, only one Arecaceae species, *Sabal palmetto* (Walt.) Lodd, was recorded for *Megachile* (Raw, 2007). There are very few studies focused on pollen use by solitary bees in the tropics (Cardoso and Silveira, 2012), and very little is known about the pattern of pollen use in brood cells for those species. *Attalea* species are widespread in several parts of the Neotropics (May et al., 1985) and their distribution may play a role in explaining some *Megachile* species' distribution, since Arecaceae seems to be an important food resource.

Oligolecy was observed for *M. brasiliensis*. Buschini et al. (2009) cite *Ludwigia* as a pollen source for *M. brasiliensis* based on observations from Schlindwein (2004), which contradicts our results from the analyses of mounted brood cell slides. To our knowledge, only one study of *Ludwigia sericea* reports *M. brasiliensis* visiting its flowers sporadically in the state of São Paulo (Sazima and Santos, 1982). However, flower visitation does not guarantee its effective pollination, as only direct contact with reproductive parts of the plant will ensure pollen collection. Indirect evidence of pollination (Michener, 1953; Schlindwein, 2004) is also possible by analyzing the content of brood cells, as provided in this study. *M. sejuncta* and *M. stilbonotaspis* may be classified as strictly polylectic (Michener, 2007) due to usage of a several unrelated pollen types, considering data from both studied areas. No pollen data exists for any species of the *Austromegachile* subgenera; regarding for *Pseudocentron* subgenus, Torretta et al. (2012) report the Asteraceae along with other families as pollen hosts for another co-subgeneric species, *Megachile gomphrenoides* Vachal in Argentina. The difference of nest abundance between the two areas may be explained by the pollen resources available, as *M. stilbonotaspis* used a wider range of pollen types and was highly abundant in APABM.

The voltinism pattern found for *Megachile* species, considering nesting period and developmental time span, was of at least two yearly generations. However, for *M. sejuncta* in SA we observed a two delayed emergence pattern in part of the population. From the data we collected, those specimens emerged around the same time as their nests were built in the following season. However, pollen provisions were different for each observed period (Supplementary material). *M. sejuncta* population in SA had a strongly biased sex ratio toward males and three patterns of emergence: direct development, delayed emergence with individuals emerging in April/May from the past April/May and individuals emerging in August from the past August. The population from APABM was multivoltine, had a marginally statistically significant, biased sex ratio toward females and the pollen used remained constant all season. This pollen use stability may be linked with the nesting activity rate and biased sex ratio toward females.

Although the data gathered do not allow us to make general assumptions in SA, we believe two factors may be influencing the emergence patterns observed in *M. sejuncta*: females may be spawning more males to increase their fitness (sons do not require a father and are cheaper than daughters, so the genetic load of the offspring comes only from the mother; this theory is explored by Buschini and Bergamaschi, 2010), since those males will emerge only in the subsequent season, and mate with the females emerging by that time. However, we did not find females in the same proportion as males, and we could speculate females are being produced elsewhere, an opposite situation to the one observed by Buschini and Bergamaschi (2010) for *Trypoxylom opacum* Brèthes. The offspring from nests collected in April, and emerged in the subsequent period of April/May, benefit from pollen of Type I sp. and *Myrcia* when Babassu palms were in low bloom, which could be explained by the difference in pollen load observed in the slides. The pollen grains used to provision those nests may not be able to sustain female development due to its quality, which may explain the male-biased sex ratio, a pattern observed by Brockmann and Grafen (1992) for *Trypoxylon politum* Drury.

However, in light of our data for the *Megachile* species studied here, the topic remains speculative, because different patterns of emergence are known for other Hymenoptera groups, such as *Trypoxylon* (Brockmann, 2004; Brockmann and Grafen, 1992; Buschini and Bergamaschi, 2010), and the authors of those studies enumerated several factors that might influence sex ratio and emergence time, such as latitudinal variation, and environmental cues such as prey availability (Brockmann, 2004). Many emergence patterns, as well as varied sex ratios, were found for megachilid bees in different biomes of the Neotropical region (Cardoso and Silveira, 2003; Marques and Gaglianone, 2013; Rocha-Filho and Garófalo, 2016; Sabino and Antonini, 2011; Zilikens and Steiner, 2004), in compliance with this study. The sex ratios and emergence patterns may vary under ecological factors such as nest width, parasites and competition for food (Rocha-Filho and Garófalo, 2016; Tepedino and Torchio, 1989), but we did not record high parasitism rates and males were reared from wide cavity nests as well; it is worth noting that genetic factors may also play a role here (Buschini and Bergamaschi, 2010).

The nest architecture of the studied species fits the *Megachile* pattern (Krombein, 1967; Michener, 2007). All three species studied here used round pieces of leaves to build the nest and brood cells opercula; furthermore, the bottom of the nests was made of elongated pieces of leaves with a bent posterior side, forming the cup of the nest. These features were already pointed out by Gonzalez (2008) as being phylogenetically informative for *Megachile* lineages.

M. brasiliensis nests were similar to *M. nigripennis*' (Marques and Gaglianone, 2013), including the behavior of building more than one cell series in wider traps, which shows the independence of confining walls that align their brood cells. They were also similar in shape and arrangement of leaf pieces to nests of *M. xylocopoides*, described by Krombein (1967). The remarkable characteristic of these nests was the tip of the leaf fragments that faced the nest plug with a distinct upward tip, giving the nest a scaly aspect, and the nest opercula with rounded leaf fragments glued to the trap's floor (this study and Marques and Gaglianone, 2013), although according to the description and images provided by Krombein (1967), we cannot state the same for *M. xylocopoides*.

We observed some variation in the structure of leaves used to build nests of *M. stilbonotaspis* in two sites in SA (secondary forest and restinga), i.e. the use of leaf fragments and entire leaflets. These two areas are 1.5 km apart from each other and the use of leaf fragments and leaflets may reflect the scarcity of resources used for nest building in the area (Cardoso and Silveira, 2012) combined with a small foraging range (Gathmann and Tscharntke, 2002). Cardoso and Silveira (2012) also observed similar variation in nest building, but with smaller distances (about 40 m). Gathmann and Tscharntke (2002) evaluated the foraging range of solitary bees and observed that on plant-species-poor sites the effort for finding suitable nest material seems to be greater than on plant-species-rich sites, although other factors may influence the foraging range, such as bees body size (Greenleaf et al., 2007; Zurbuchen et al., 2010).

Females of *M. sejuncta* also showed variation between nests regarding the type of leaves used to build nests in the primary forest from SA, indicating that several plant species were used. However, we noted that each type of leaf fragments was put together in different parts of the nest, evidencing that the bees targeted and collected specific types of materials while those were available to them. This leaf collecting pattern was also observed for *M. brevis* by Michener (1953) who stated that such behavior enables a rapid gathering of plant material. We also observed nests of *M. sejuncta* with a nest building pattern similar to another co-subgeneric species, *Megachile habilis* Mitchell (Laroca et al., 1987). These results agree with the observations from Kambli et al. (2017).

Regarding nests of *Megachile* species in general, nests of *M. brasiliensis* and *M. stilbonotaspis* were more similar to each other than to nests of *M. sejuncta*. According to Gonzalez (2008), species placed into *Melanosarus* and *Pseudocentron* subgenera belong to the *Pseudocentron* lineage, and data on nest architecture seem to corroborate his phylogenetic hypothesis. This means that the features of nests described here may be of interest for future phylogenetic research.

Mortality rates observed in this study were not as high as in other studies, where more than 50% of bees did not reach adulthood (Marques and Gaglianone, 2013; Rocha-Filho and Garófalo, 2016; Torretta and Durante, 2011). Parasitism by Lepidoptera (Pyralidae) has been recorded in the literature only for *M. rotundata* Fabricius and *Macrozamia concinna* Smith (Raw, 2007). Parasitism by *Coelioxys* is common (Cardoso and Silveira, 2012; Raw, 2007) and only *M. sejuncta* was not a host for any *Coelioxys* species recorded in this study. Nesting in pre-existing cavities in wood may facilitate attacks from parasites such as Chalcidoidea (Grissell, 2007), as recorded for *M. sejuncta* and *M. brasiliensis* in this study. Within Chalcidoidea, Raw (2007) describes Leucospidae and Eurytomidae as parasites of *Megachile*. All parasites reported here are new records for the studied species.

Besides parasites, other species were associated with *Megachile*. We observed cavity supersedure of *T. rugulosa* over *M. brasiliensis*, although it was unclear if it occurred due to competition for the cavity or if the *T. rugulosa* female took over the cavity to build its nest. It seems to be a common process among trap-nesting solitary Hymenoptera (Krombein, 1967). Another unidentified Hymenoptera also shared a cavity with a *M. brasiliensis* whose immatures died before emergence, hindering identification, but as seen from its nest architecture it was probably a *Centris* species. Nest sharing also appears to be common and cavities may be shared by bees, whether they belong to the same species or not (Cardoso and Silveira, 2012; Krombein, 1967). However, nest architecture of some species may be incompatible with another bee's nests, which is the case for Euglossini species (Zilikens and Steiner, 2004) due to different material employed to build nests.

Another interspecific association observed was the occurrence of mites on some *M. stilbonotaspis* specimens that emerged from nests at the restinga in SA. Those mites were attached to the body of the adults and probably in their phoretic life stage. These mites probably did not feed on the provisions, since the bees completed their development, although observations during the bee life cycle must be made to examine the interaction between the immature bee and the mites in the brood cell. Associations like these occur in solitary (Cordeiro et al., 2010; Klimov and O'Connor, 2007) and social bees (Sammataro et al., 2000), and they may be commensal, mutualistic or predatory/parasitic.

Authors contributors

DM and GA conceived this research. DM and DBM collected the data. DM analyzed data and wrote the manuscript. GA contributed revising it critically for intellectual content. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2018.03.002.

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