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Pollen Spectrum and Trophic Niche Width of *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) in Highly Urbanized and Industrialized Sites

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Abstract

The floristic composition of an environment is important to ensure the trophic niche of bee species. *Melipona scutellaris* Latreille, is a typical stingless bee of Atlantic rainforest sites in northeastern Brazil, a region widely established in meliponaries for honey and pollen production. *M. scutellaris* is reared (meliponiculture) in rural and urban areas, where the species depends on the availability of different plants for nectar and pollen collection. In this study, we estimated food niche width, equitativity, and similarity between different colonies of *M. scutellaris* in highly urbanized and industrialized sites of the Metropolitan Region of Salvador, Bahia State, Brazil. We analyzed pollen spectrum of 58 honey samples from six meliponaries, during 12 months. We identified 111 pollen types distributed in 28 plant families. The Fabaceae family showed the highest diversity in pollen types (33.33% of the total) and *Mimosa caesalpiniifolia* was the most frequent pollen type, found in 100% of the samples. *M. scutellaris* concentrated its foraging activity on a few trophic resources (H’ = 2.69 and J’ = 0.01) indicating a few melittophilous plant species belonging to the genera *Eucalyptus*, *Mimosa*, *Protium*, *Serjania* and *Tapirira*, should be managed on a regional scale to favor meliponiculture with this native bee species.

Introduction

Meliponiculture is practiced throughout Latin America and honey is the main product marketed. The rearing of stingless bees offers valuable and profitable resources, especially for family agriculture, as meliponiculture is a sustainable activity of low-cost as well as beneficial to the environment, such as pollination and reproduction of many agricultural and native plant species (Wilms & Wiechers, 1997; Venturieri et al., 2012; Quezada-Euán et al., 2018). The rearing of stingless bees stands out in the northeastern Brazil, mainly of *Melipona scutellaris* Latreille (Silva & Paz, 2012). This species plays an important role in maintaining natural vegetation, as well as helping to generate income for family farming (Alves et al., 2012).

Products of stingless bees, especially honey, have a wide use in different industries, namely food, cosmetic, pharmaceutical and medical (Guez et al., 2013). Compared to honey from Africanized bees (*Apis mellifera* L.), *Melipona* species produce an economically valuable honey, often from mixed sources of native floral (Camargo et al., 2017), with distinct physical, chemical and biological features (Abadio Finco et al., 2010).

The pollen analysis (melissopalynology) is an important tool for quality control of honey and management of honeybees and stingless bees colonies, since it allows to identify pollen types and plant species that contribute to the diet of these bees (Barth et al., 2013; Estevinho et al., 2016; Souza et al., 2018). The pollen analysis is also a valuable tool for ecological studies on trophic niche and food preferences of social bees

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(Kleinert et al., 2012; Hilgert-Moreira et al., 2013). Studies in Brazil have evaluated the niche width of stingless bees in the Atlantic and Amazonian forests (Wilms & Wiechers, 1997; Ramalho et al., 2007; Ferreira & Absy, 2017; Freitas et al., 2017; Matos & Santos, 2017), in the caatinga (Santos et al., 2013; Novais et al., 2014), Cerrado (Calaça et al., 2018), dunes (Viana & Kleinert, 2006), rocky fields (Franco et al., 2009), and agricultural areas (Lucas et al., 2017).

This study is the first carried out on *M. scutellaris* trophic niche in an urbanized and industrialized site. We analyzed the pollen spectrum of regional honey samples to identify the main nectar sources for this species of stingless bee and evaluate variation in food niche width and similarity between colonies and meliponaries. We also aimed to generate data for the management of the regional melitophilous flora and thus contribute to meliponiculture development.

Material and methods

Study site and Samples

The samples were collected from five colonies of meliponaries (n=6) in highly urbanized and industrialized sites in the Metropolitan Region of Salvador (MRS), Bahia, Brazil: Salvador (I): S'12°51'32.4'' and W'038°27'9.9''; Salvador (II): S'12º49'58.7'' and W'038º22'27.4''; Salvador (III): S'12º51'28.3'' and W'038º21'54.3''; Simões Filho (IV): S'12º43'55.5'' and W'038º23'51.6''; Lauro de Freitas (V): S'12º50'38.1'' and W'038º21'12.1''; and Dias D'Ávila (VI): S'12º32'28.0'' and W'038º21'42.3''). All meliponaries were inserted into a fragment of the disturbed Atlantic Forest, in areas near and far from urban-industrial development such as: the landfill of Salvador, the Petrochemical Complex of Camaçari and the Industrial Center of Aratu.

The honey samples were collected monthly, from June 2015 to May 2016, totalizing 58 honey samples. We collected samples (approximately 200 g) from closed honey pots with disposable syringe. Each sample was placed in plastic tubes, conditioned in thermal bags and sent for laboratorial analyses.

Sample preparation

The honey samples were prepared using the method of Louveaux et al. (1978), following modifications proposed by Jones and Bryant Jr. (2004). To process each sample, 10 g of honey was weighed and dissolved in 10 mL of distilled water and 50 mL of 95% ethyl alcohol (ETOH). The mixture was centrifuged (3000 rpm for 5 min) and the supernatant liquid was discarded. After centrifugation, the pollen sediment was dehydrated in glacial acetic acid and then subjected to the acetolysis process proposed by Erdtman (1960). The resulting pellet was mounted between slides and cover slips with glycerinated gelatin (Kisser, 1935), sealed with paraffin for further identification and pollen grain counts under the microscope.

To identify pollen types in the samples, images were captured using an Olympus® E-330 digital camera coupled to the Olympus CX41 microscope. Afterwards, pollen grains were compared with the collection of references of the Federal University of Recôncavo of Bahia and specialized literature, such as Barth (1989), Roubik and Moreno (1991) and Punt et al. (2007).

Pollen analyses

After identifying the pollen types, we counted up to 1,000 pollen grains per sample. The frequency class of pollen types proposed by Louveaux et al. (1978) was used: predominant pollen - PP (> 45% of total grains), secondary pollen - SP (16 to 45%), important minor pollen - IMP (3 to 15%) and minor pollen - MP (< 3%). We then established the relative frequency (%) of each pollen type between samples using the equation: $F = (n_{ij}/N_j) \times 100$ where, F = relative frequency of pollen type i in sample j; ni = number of pollen grains of the pollen type i in the sample; N = total number of pollen grains in sample j.

Ecological indices
Trophic Niche Width and Similarity

We calculated the trophic niche width using the diversity index of Shannon (1948) ($H' = - \Sigma\ pK \times \ln pk$), where pK is the proportion of each pollen type in the *M. scutellaris* diet. Equitativity (or evenness) of pollen types was calculated using the index of Pielou (1969) ($J' = H'/H'max$). The richness index (S) was obtained from the total number of pollen types identified in the honey samples and similarity between samples with the Jaccard coefficient of similarity (CS). The analyses were performed using PAST software (Paleontological Statistics), version 2.17c (Hammer et al., 2001).

Results

We identified 111 pollen types distributed in 28 botanical families and 25 types did not have their pollen affinity determined (Tables 1 and 2). In all meliponaries in the MRS, Fabaceae showed the highest number of pollen types in honey samples, representing 33.33% of the total, followed by Anacardiaceae (8.97%), Arecaceae (8.97%) and Euphorbiaceae (6.41%) (Table 1).

The relative frequency of pollen types of the samples also reveals the importance of some plant species for the study site. In meliponary Salvador (I), pollen types *M. caesalpiniifolia*, *Miconia* and *Solanum* Type were the most frequent among the samples, present in 100%, 89% and 78% respectively. In Salvador (II), the most frequent pollen types were *Acacia*, *Eucalyptus* and *M. caesalpiniifolia*, present in 100% of the samples. For meliponary Salvador (III) *M.*

Table 1. Pollen types identified in honey samples by *Melipona scutellaris* from highly urbanized and industrialized sites in the Metropolitan Region of Salvador, Bahia, Brazil.

Family	Pollen Types	Monthly Samplings											
		01	02	03	04	05	06	07	08	09	10	11	12
Anacardiaceae	*Spondias*				SP (5)								
Anacardiaceae	*Tapirira*	SP (5)	PP (1); SP (6)	PP (2,5); SP (6)									
Burseraceae	*Protium*			SP (3,6)	SP (1,4)								
Combretaceae	*Terminalia*		SP (6)										
Euphorbiaceae	Euphorbiaceae Type	SP (6)											
Fabaceae	*Acacia*			SP (6)	SP (2,6)	SP (2)							
Fabaceae	*Mimosa caesalpiniifolia*	SP (1,2)	PP (2,5); SP (6)		SP (1,2)	SP (1,3)	PP (3,6); SP (4)	PP (1,2,3,6); SP (4)	PP (3,4,6); SP (1,2,5)	PP (1); SP (3,4,6)	SP (5,6)	PP (5)	PP (5)
Fabaceae	Fabaceae Type 2				SP (6)								
Lauraceae	*Persea americana*						SP (3)						
Malpighiaceae	*Byrsonima*		SP (3)	SP (4)									
Melastomataceae	*Miconia*			PP (1)									
Melastomataceae	*Tibouchina*	PP (4); SP (2,6)	SP (2,4)	SP (3,4)	PP (5); SP (2,4)	PP (6); SP (5)	SP (6)		SP (6)	SP (4)	SP (6)		
Myrtaceae	*Eucalyptus*						SP (4)	SP (4)		SP (3)			
Myrtaceae	*Eugenia*									SP (5)			
Myrtaceae	*Myrcia*	SP (1)				SP (6)	SP (2)		SP (1,5)	SP (1)	PP (3)		
Myrtaceae	*Psidium*	SP (5)	SP (5)			SP (5)	SP (6)	SP (1,2,5)	SP (2,6)	SP (6)			
Rubiaceae	*Borreria*	SP (2)	SP (3)										
Rubiaceae	*Mitracarpus*			SP (2)									
Sapindaceae	*Cupania*		SP (3)			SP (5)							
Solanaceae	*Solanum* Type	SP (1)			SP (6)	PP (1)	PP (1,5)	SP (5)	SP (1,5)	SP (5,6)	SP (5)		
Solanaceae	*Solanum paniculatum*				PP (3); SP (4)	PP (3,4); SP (2)	PP (2)	SP (2,4)	SP (2,4)	SP (3)			

PP – Predominant Pollen (PP>45% of total pollen grain); **SP** – Secondary Pollen (45%>SP>16%); Meliponary: **1** – Salvador (I), **2** – Salvador (II), **3** – Salvador (III), **4** – Simões Filho (IV), **5** – Lauro de Freitas (V) and **6** – Dias D'Ávila (VI).

caesalpiniifolia (90%), *Serjania* (70%) and *Tapirira* (60%) were the most frequent. In Simões Filho (IV), the most frequent pollen types among the samples were *Acacia* Type, *Leucaena* Type, *M. caesalpiniifolia*, *Psidium*, *S. paniculatum* and *Tibouchina*, all present in 100% of the samples. In meliponary Lauro de Freitas (V), pollen types *M. caesalpiniifolia* (83%) and *Solanum* Type (75%) were the most frequent, while in Dias D'Ávila (VI), *M. caesalpiniifolia* and *Tibouchina* were the most frequent types, both representing 90% of the total, followed by *Protium* (80%). *Mimosa caesalpiniifolia* was frequent in the samples of all meliponary evidencing the importance of this plant species for the reared of *Melipona scutellaris*.

Table 2. Number of honey samples of *Melipona scutellaris* per meliponary and associated pollen types and plant families, in highly urbanized and industrialized sites in the Metropolitan Region of Salvador, Bahia, Brazil.

Meliponary / sites	Geographical coordinates	Number of samples	Pollen types		Family
			Identified	Not identified*	Identified
Salvador (I)	S'12°51'32.4"; W'038°27'9.9"	09	42	6	17
Salvador (II)	S'12º49'58.7"; W'038º22'27.4"	08	38	5	15
Salvador (III)	S'12º51'28.3"; W'038º21'54.3"	10	32	6	14
Simões Filho (IV)	S'12º43'55.5"; W'038º23'51.6"	09	35	4	16
Lauro de Freitas (V)	S'12º50'38.1"; W'038º21'12.1"	12	27	4	11
Dias D'Ávila (VI)	S'12º32'28.0"; W'038º21'42.3"	10	28	2	14

*Botanical affinity not identified.

Analyzing the meliponaries separately, we found that values of the trophic niche width ranged from H' = 2.02 to H' = 2.40, while equitativity ranged from J '= 0.55 to J' = 0.72 (Table 3). The richness index (S) was higher than 25 pollen types for all meliponaries. The highest richness values of pollen types were found in Salvador (II) and Salvador (III) meliponaries, with 42 and 38 pollen types (Tables 1 and 2). Similarity between meliponaries was often low and below 0.3. The highest similarity index (CS = 0.37) occurred between Salvador (I) and Lauro de Freitas (V), while the lowest index (CS = 0.18) occurred between Salvador (III) and Simões Filho (IV) (Table 4).

Table 3. Trophic niche width (H'), equitativity (J') and pollen type richness (S) in honey samples of *Melipona scutellaris* from highly urbanized and industrialized sites in the metropolitan region of Salvador, Bahia, Brazil.

Indices	Meliponaries					
	Salvador (I)	Salvador (II)	Salvador (III)	Lauro de Freitas (IV)	Simões Filho (V)	Dias D´Ávila (VI)
S	42	38	32	35	27	28
H´	2.04	2.33	2.28	2.02	2.22	2.40
J´	0.55	0.64	0.66	0.61	0.62	0.72

Discussion

The pollen analysis of honey samples from the Metropolitan Region of Salvador, Bahia, showed that *M. scutellaris* visits many plant species, represented in the pollen spectrum of the samples studied. Meliponaries farther from the urban perimeter (Simões Filho IV, Lauro de Freitas V and Dias D'Ávila) presented higher diversity of pollen types explored by *M. scutellaris*; conversely meliponaries in the urban environment (Salvador I, II and III) showed lower diversity of pollen types (Table 1 and 2). This is possibly related to landscape fragmentation and consequent reduction of flora in large urban centers (Elmqvist et al., 2016).

The families Anacardiaceae, Arecaceae, Euphorbiaceae and Fabaceae were the most representative in our study. Other authors (Oliveira et al., 2009; Ferreira & Absy, 2015; Souza et al., 2015; Matos & Santos, 2017) have also reported that species of these plant groups are important for cultivation of stingless bees. Plant species of these families should receive more attention by beekeepers and researchers of stingless bees to identify and conserve species of these taxonomic groups, important suppliers of trophic resources for stingless bee.

The pollen types of Fabaceae family (Mimosoideae) was the most diverse in honey samples of MRS. This was similar to results reported in other studies comparing floral resources used by *M. scutellaris* (Matos & Santos, 2017;

Table 4. Similarity matrix (coefficient of similarity of Jaccard, CS) between meliponaries based on pollen types identified in *Melipona scutellaris* honey from highly urbanized and industrialized sites in the Metropolitan Region of Salvador, Bahia, Brazil.

	SSA (I)*	SSA (II)	SSA (III)	Lauro de Freitas (IV)	Simões Filho (V)	Dias D' Ávila (VI)
SSA (I)	-	0.23	0.27	0.28	0.18	0.25
SSA (II)	-	-	0.29	0.35	0.21	0.22
SSA (III)	-	-	-	0.37	0.29	0.30
Lauro de Freitas (IV)	-	-	-	-	0.29	0.31
Simões Filho (V)	-	-	-	-	-	0.21
Dias D'Ávila (VI)	-	-	-	-	-	-

*Sample data by location are available in Table 2. SSA = Salvador

Lucas et al., 2017), *Melipona subnitida* Ducke (Meliponini) and *A. mellifera* (Almeida-Murandian et al., 2013).

In the samples of all meliponaries studied, pollen type *M. caesalpiniifolia* occurred as predominant pollen (PD). *Mimosa caesalpiniifolia* Benth is considered an important source of nectar and pollen for bees and a mellitophilous plant species (Carvalho, 2007; Lima et al., 2008) thus it has potential to be used in beekeeping in the Metropolitan Region of Salvador, due to its presence in the pollen spectrum of the honeys analyzed. This reinforces the use and conservation of this species in apicultural pasture of the studied region and other municipalities of Bahia, as well as in other Brazilian states.

Among the most frequent pollen types in the honeys of MRS, *Eucalyptus*, *M. caesalpiniifolia*, *Protium* and *Serjania* stand out, and some species belong to these genera described as nectariferous plants (Nascimento et al., 2015; Matos & Santos, 2017). Thus, the conservation of species of these genera which are part of diet of *M. scutellaris* diet, is important for the success of meliponiculture in the MRS, as conservation of these species can increase the range of the trophic niche of *M. scutellaris* in the studied region contributing to flora and fauna conservation in this urban environment. In addition to the plants diversity, floral resources can increase stability of these pollinators in a given environment (Venjakob et al., 2016).

Tibouchina was another very frequent pollen type in the samples and was identified and classified in different frequency classes (Table 1). According to Pirani and Cortopassi-Laurino (1993), many *Tibouchina* species are pollen and nectar sources used by stingless bees (Meliponini) and *A. mellifera*. The *Tibouchina* genus (Melastomataceae) is distributed mainly in tropical and subtropical regions of the Americas and encompasses approximately 350 species, 129 native to Brazil, which occur naturally in the states of Bahia, Minas Gerais, Rio de Janeiro and São Paulo. *Tibouchina granulosa* (Desv.) Cogn. is the most common species of this genus in Brazilian cities (Agostini & Sazima, 2003; Pirani & Cortopassi-Laurino, 1993). An important fact regarding the use of species of the *Tibouchina* genus by honeybees is its flowering period, which occurs practically throughout the year. The *Tibouchina* pollen type was identified in honey samples (Table 1), indicating that this pollen type corresponds to a species with potential for use by *M. scutellaris* colonies settled in these places, because these plants are considered components of mellitophilous plant species for the maintenance of colonies.

The identification of the *Solanum* type and *S. paniculatum* type in the pollen spectrum of the analyzed honey samples does not present contribution to nectar volume of honey composition. The presence of these pollen grains in honey is possibly due to an involuntary addition, considering that bees visit several plant species and pollen grains attached to their bodies can be deposited in honey pots along with the nectar. According to Dórea et al. (2017), Solanaceae species are frequently found in urban areas, and are used as pollen sources by solitary and social bees. Pollen types *Solanum* and *S. paniculatum* have also been found in the pollen spectrum of Meliponini honey in recent studies, such as Barth et al. (2013), Nascimento et al. (2015), Souza et al. (2015) as well as Matos and Santos (2017).

The index richness (S) reflects the amount of plant species visited during the foraging activity of *M. scutellaris*. Meliponary Salvador (III), although inserted into an urban perimeter, had the highest richness index (42 pollen types) (Table 3), indicating that the colonies may have been more exposed to variations in flowering (Ramalho et al., 2007). Another factor to be considered is the abundance of plant individuals of the same blooming species visited by the bees (*M. scutellaris*) to collect trophic resources in the area surrounding the meliponary. The bees may choose to collect trophic resources in certain preferential floral sources or search for resources in higher number of species to supply their energy and protein needs (Silva et al., 2007; Filipiak et al., 2017). On the other hand, meliponary Lauro de Freitas, far from the urban perimeter, had the lowest richness index (27 pollen types), indicating that bees concentrated foraging on local floral resources available throughout the year, which sufficiently supplied their food requirements.

Environmental factors affect the flowering periods (Freitas et al., 2013) and in the foraging activity of bees (Nascimento et al., 2012; Oliveira et al., 2012). Oliveira et al. (2009) observed in the Amazon region that the frequency of rains promoted a diversification of pollen types due to low flowering. In this sense, diversity of pollen types explored by *M. scutellaris* in the MRS also suffers direct influence of the climatic factors, besides the factors related to landscape changes in the urban environment.

The variation in trophic niche width and equitativity values for the samples of each site studied (Table 3) may be related to the number of samples and reveals the importance of the sampling period, which shows variation of the trophic niche in a given period. The largest range of the trophic niche was recorded for samples from meliponaries Salvador (II and III) and Simões Filho (Table 3). In general, the trophic niche width values found in our study is similar to results recorded for other bee species (Carvalho et al., 1999; Aguiar et al., 2013; Sabino et al., 2016).

There was a diversity of pollen types and oscillation in *M. scutellaris* trophic niche width in the different sites (meliponaries). In addition, meliponaries with higher diversity in pollen types in their samples also had a greater trophic niche width. However, some pollen types were more representative among samples such as *M. caesalpiniifolia* and *Tibouchina*, indicative of relative concentration in the collection of floral resources by *M. scutellaris* in some plant species, which can be considered key species for the diet of these bees in the studied site.

The low similarity in the resources explored by *M. scutellaris* in the MRS may have been influenced by the diversity of plants visited by the bees in different sites, thus evidencing the generalist foraging behavior of *M. scutellaris*, as described by Ramalho et al. (2007).

Vegetation with several floral species is a determinant factor in the foraging activity of bees, when they do not have floral selectivity or preferences to resources in the environment (Ramalho et al., 2007; Vaudo et al., 2015; Adgaba et al., 2017). Velikova et al. (2000) report that a high density of resources in tropical habitats leads stingless bees (*Melipona*) to forage in distances shorter than other bees do, with a maximum radius of 500 m. Therefore, the pollen analysis of honey revealed diversity of plant species that can be explored by *M. scutellaris* for honey production. The contribution of the Fabaceae family was remarkable in the results, as this family was the most frequent with higher richness of pollen types in the honey samples produced by *M. scutellaris* from MRS, showing that species of this family can be considered key plants for local meliponiculture, for example, *M. caesalpiniifolia*.

The trophic niche width reveals that *M. scutellaris* bees explored various floral resources available in the environment, despite showing preference for *M. caesalpiniifolia*. Based on the ecological indices of all meliponaries, *M. scutellaris* concentrated its foraging activity on a few trophic resources. Therefore, we suggest the management on a regional scale of a few melittophilous plant species, belonging to the genus *Eucalyptus*, *M. caesalpiniifolia*, *Protium*, *Serjania* and *Tapirira*, to favor meliponiculture with this native bee species.

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