Paubrasilia **Artigo Original**
https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Pollen Spectrum of Honey Produced by *Scaptotrigona nigrohirta* (Apidae: Meliponini) in the Amazon Region

Espectro Polínico do Mel produzido por Scaptotrigona nigrohirta *(Apidae: Meliponini) na Região Amazônica*

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Palavras-chave
recursos florais, melissopalinologia, grãos de pólen, abelhas sem ferrão, polinizadores tropicais

Keywords
floral resources, melissopalynology, pollen grains, stingless bees, tropical pollinators

Recebido em: 09/02/2026
Aceito em: 18/04/2026

Editor: Jaílson S. Novais (UFSB)

eISSN: 2595-6752

Resumo

As abelhas na Amazônia desempenham um papel essencial além da produção de mel, pólen e própolis, atuando como polinizadoras-chave da flora nativa. Compreender os recursos florais utilizados por *Scaptotrigona nigrohirta* apoia a meliponicultura, uma atividade regional sustentável. Uma análise melissopalinológica foi realizada em doze amostras de mel para determinar a origem botânica do pólen no mel produzido por essa espécie e para subsidiar estratégias de conservação. As amostras foram processadas pelo método de acetólise, com a contagem de pelo menos 500 grãos de pólen por amostra. Sessenta e cinco tipos de pólen foram identificados, pertencentes a 24 famílias, 34 gêneros e 21 espécies. Anacardiaceae, Fabaceae e Myrtaceae foram as famílias mais representativas. Esses resultados auxiliam no manejo de plantas melíferas, indicando quais espécies vegetais devem ser priorizadas, protegidas e incorporadas em estratégias de restauração ou plantio, de acordo com sua importância como recursos florais ao longo do ano. Essa abordagem apoia a manutenção das colônias de *Scaptotrigona nigrohirta* e contribui para o desenvolvimento da meliponicultura.

Abstract

Bees in the Amazon play an essential role beyond honey, pollen, and propolis production, acting as key pollinators of native flora. Understanding the floral resources used by *Scaptotrigona nigrohirta* supports meliponiculture, a sustainable regional activity. A melissopalynological analysis was performed on twelve honey samples to determine the botanical origin of the pollen in honey produced by this species and to support conservation strategies. Samples were processed using the acetolysis method, with at least 500 pollen grains counted per sample. Sixty-five pollen types were identified, belonging to 24 families, 34 genera, and 21 species. Anacardiaceae, Fabaceae, and Myrtaceae were the most representative families. These results help guide melliferous plant management by indicating which plant species should be prioritized, protected, and incorporated into restoration or planting strategies according to their importance as floral resources throughout the year. This approach supports the maintenance of *Scaptotrigona nigrohirta* colonies and contributes to the development of meliponiculture.

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Introduction

Bees play a vital role for the planet, contributing to 15 of the 17 Sustainable Development Goals and generating wide-ranging socioeconomic and environmental benefits (Giampieri et al., 2022; Patel et al., 2021). Although Brazil's bee fauna comprises approximately 2,000 species (Ascher & Pickering, 2025), including diverse groups such as stingless bees (Meliponini) and honeybees (Apini) (Engel et al., 2023), less than 10% of the world's 20,000 species are managed for pollination or production (Ayala & Ortega, 2018). Within this context lies meliponiculture, a name attributed to the creation of wild bees that are native to Brazil. Meliponiculture is a very widespread activity in the Northern and Northeastern regions of the country, with honey being the most valuable product (Nogueira-Neto, 1953; R. M. O. Alves, 2007).

Brazilian ecosystems, especially the Amazon, present many conditions that favor bee creation, including warm climate, flora rich in species that supply nectar, pollen, resin, and flowering distributed throughout the year (Venturieri, 2008). In the Amazon, bees are not only valued for their production of honey, pollen and propolis, but are also primary pollinators of the native flora, making them essential for the reproduction of plants and consequently for sustainable production (F. F. Oliveira et al., 2013; Kerr et al., 2001).

In the group of stingless bees stands the genus *Scaptotrigona* (Moure, 1942), which comprises 51 described species, distributed in seven subgenus (Engel et al., 2023). Their nests are usually found in cavities of large tree trunks. Nest entrances are characterized by a cerumen tube, a mixture of wax and resins that ends near the breeding cells, with tubes varying in length and shape, depending on the species (Camargo & Pedro, 2013). The species of this genus stand out for their efficiency as pollinating agents and travel long distances (Leão, 2014; Nogueira-Neto, 1997).

When visiting flowers in search of trophic resources offered by plants, bees involuntarily transport pollen grains throughout their bodies, which could be why it is possible to find pollen grains in honey (S. D. Silva & Absy, 2000). Therefore, pollen analysis of honey helps determine its geographical and botanical origins, as well as indicates the important pollen types for stingless bees feeding (Louveaux et al., 1978).

The study of honey analysis of this native bee from the Amazon region, *Scaptotrigona nigrohirta* Moure, 1950, was performed because some authors have evidenced its outstanding importance in the productive potential of honey and pollen (K. B. Costa, 2010). For this reason, we think it is important to know the plants visited by this bee and thus contribute to the meliponiculture, that constitutes a sustainable alternative for the region.

Therefore, a melissopalinological analysis was performed to characterize the botanical origin of the pollen resources used by the bee *Scaptotrigona nigrohirta* Moure, 1950 (popularly called the "canudo" bee) from the São Francisco Community of the Lago Grande Agroextractive Settlement Project, Arapiuns River, Santarém-PA, to obtain results that support sustainable conservation of these bees in this region of the Amazon. The research also evaluates the temporal dynamics of floral diversity and analyzes the structure of interaction networks to better understand how resource fluctuations affect local biodiversity over time. Furthermore, this species of native bee is widely managed in the Lower Amazon, thus this study could contribute to the socioeconomic development of this Amazonian region.

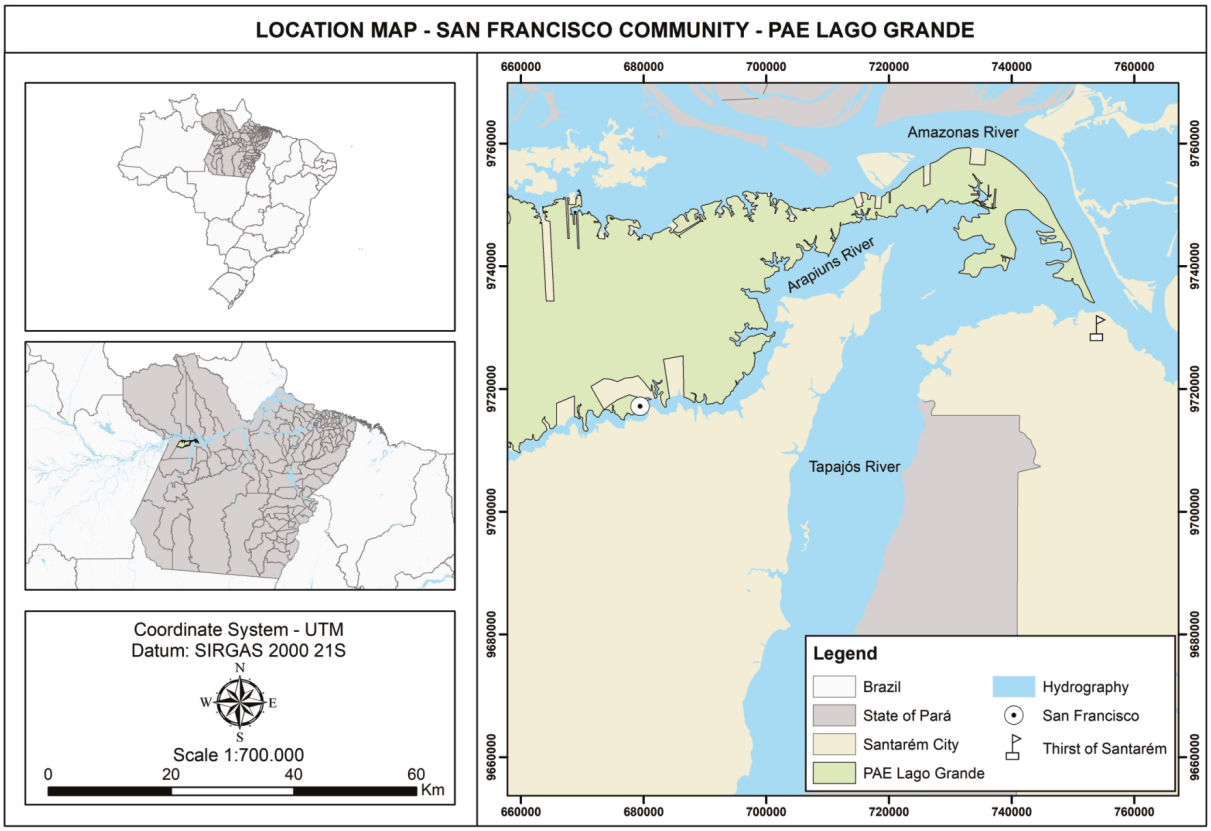
Materials and Methods

Study area

The study was developed at the São Francisco Community Meliponary (25°28'48"S, 55°17'58"O) which is located in the rural area of the Santarém municipality-PA, on the left bank of the Arapiuns River, which is part of the Lago Grande Agroextractive Settlement Project. This settlement was created on November 28, 2005 by the National Institute of Colonization and Agrarian Reform (INCRA) (Figure 1).

Figure 1

Map of the São Francisco Community of the Lago Grande Agroextractive Settlement Project, Arapiuns River, Santarém-PA



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On solid ground, the vegetation cover is represented by species that follow the relief conditions of the terrain, where the Amazonian Equatorial Forest is located, alternating with smaller species and secondary or "capoeiras" formations in several stages (Fundação Amazônia de Amparo a Estudos e Pesquisas [FAPESPA], 2016). According to the Instituto Nacional de Meteorologia [INMET] (2018) and A. F. Silva et al. (2013), the climatic characteristics of the Santarém municipality are Am-type, according to Köppen classification, with constantly high air temperatures and annual average of 25 °C. Regarding relative humidity of the air, it presents values above 80% in almost all months of the year and precipitation approaches 2,000 mm per year, however, with some irregularities throughout the year.

Honey collection and bee identification

Honey samples (approximately 10 mL) from *Scaptotrigona nigrohirta* bees were collected monthly from December 2017 to November 2018, totaling 12 collections. These samples were always obtained from the same bee boxes belonging to the meliponary. Materials used for collections were Pasteur pipettes and sterile plastic jars. The samples were properly identified and kept refrigerated at about 10 °C until processing began.

For bee identification, 10 individuals were collected with an entomological net and placed in a death chamber. The specimens were assembled and labeled according to entomological standards. The samples were sent to the Laboratory of Bionomy, Biogeography and Insect Systematics (BIOSIS) at the Federal University of Bahia (UFBA), for identification by the specialist Dra. Favízia Freitas de Oliveira.

Analysis of honey samples

For honey dilution, the methodology recommend by Jones & Bryant Jr (2004) and Novais et al. (2013), using 95% ethanol (ETOH). Subsequently, each sample was submitted to the acetolysis process according to the Erdtman method (1952, 1960). After the acetolysis process, at least three slides from each sample were prepared using glycerin gelatin (Salgado-Labouriau, 1973), sealed with paraffin and deposited in the pollen collection of the Botany and Palynology Laboratory (LABOP) at the Institute of Biodiversity and Forests (IBEF) of UFOPA for further microscopic analysis.

Pollen was identified based on morphological characteristics, mainly: the ornamentation of the wall, the number and type of openings and the pollen unit (Correia, 2016; Erdtman, 1952; Punt et al., 2007; Roubik & Moreno, 1991). All pollen characteristics can be used to identify families, genera and species.

The method used to count pollen grains followed Louveaux et al. (1978) (minimum of 500 grains/sample) using an optical microscope with 40x lens. The frequency classes were established based on the percentage of each pollen type, which were: predominant pollen (>45%), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen (<3%) (Louveaux et al., 1978). Pollen grains of each pollen type were measured with a Zeiss optical microscope (Primo Star) and ruler attached to the ocular, measuring 5 grains of each pollen type and calculating the arithmetic mean afterwards.

For the taxonomic characterization of pollen grains, the concept of "pollen type" proposed by de Klerk & Joosten (2007) and Joosten and de Klerk (2002) was adopted. The results were presented in a table prepared in Microsoft Excel® 2010. The images were captured directly from the Zeiss microscope (Primo Star) at 100x magnification (immersion) using a coupled digital camera. Palinograms were elaborated in the Corel Draw®X7.

Ecological classification of floral resources used by *Scaptotrigona nigrohirta*

Brito et al. (2006), Marchant et al. (2002), Modro et al. (2011), Queiroz (2009), R. F. Alves & Santos (2014), as well as information available on the Online Catalog Network (Rede de Catálogos Polínicos Online [RCPol], 2025) website and on the Flora e Funga do Brasil (2025), were used to identify the floral resources provided by the plant species analyzed.

Data analysis

The pollen-type composition among the honey samples of *Scaptotrigona nigrohirta* was compared using the Jaccard similarity coefficient, which is based on presence–absence data. The analysis was performed in PAST (Paleontological Statistics Software Package for Education and Data Analysis), version 4.03 (Hammer et al., 2001).

A qualitative interaction network was constructed based on the adjacency matrix between pollen types and samples (honey from the pots) for each month analyzed. This matrix was populated with presence (1) and absence (0) data for the pollen types identified in the food pots. Network construction and metric calculations were performed in R (R Core Team, 2022) using the packages bipartite (Dormann et al., 2008) and igraph (Csárdi et al., 2024).

The diversity of pollen types in each honey sample was estimated using the Shannon–Wiener index (H), calculated as $H = \Sigma - pi * \ln(pi)$, where pi corresponds to the proportion of pollen grains of type i, and ln represents the natural logarithm (Magurran, 2004). The evenness in the distribution of the identified types was assessed using Pielou's equitability index ($J = H / \ln S$), derived from the Shannon index, in which S indicates the richness of pollen types; its values range from 0 to 1, reflecting the degree of homogeneity in the representation of types (Pielou, 1966). All diversity index calculations were performed in the software PAST (Paleontological Statistics Software Package for Education and Data Analysis), version 4.03 (Hammer et al., 2001).

Results

A total of 65 pollen types were found in honey samples from *Scaptotrigona nigrohirta*, assigned to 24 families, of which only two were classified to the family level (Poaceae pollen types), 34 to the genus level and 21 were identified to the species level. Only eigth pollen types could not be identified, among which two occurred in November (Table 1). The families with the highest richness in number of pollen types were: Fabaceae (12 pollen types), Myrtaceae (7 pollen types); Anacardiaceae (6 pollen types). The other families presented one (1), two (2) or tree (3) pollen types (Table 1 and Table 2).

Among the pollen types identified, only five (5) were classified as predominant pollen (>45%), namely: *Byrsonima basiloba* (60%) (Figure 3G), *Psidium guajava* (54.4%) (Figure 3J), *Humiria balsamifera* (50%) (Figure 3F), *Tapirira guianensis* (49.6%) (Figure 3B) and *Casearia* type (45.8%) (Figure 3L) (Figure 2). Therefore, these species greatly contribute to the characterization of this honey. Pollen types classified as secondary pollen (16 to 45%) were a little more abundant, and were distributed in nine (9) families: Anacardiaceae, Araliaceae, Fabaceae, Humiriaceae, Melastomataceae, Myrtaceae, Poaceae, Rhamnaceae, and Salicaceae (Figure 2) (Table 1).

One of the families with expressive pollen types classified as secondary pollen was Anacardiaceae, which despite having only two (2) pollen types with this classification, presented a favorable distribution over the months, being: *Astronium obliquum* (Figure 3A) in two different months (with 16% in March and 20% in June) and *Tapirira guianensis* (Figure 3B) in three different months (with 39.2% in December; 27.1% in August and 16.5% in November). After Anacardiaceae, most pollen types classified as secondary pollen belonged to Fabaceae, namely: *Aeschynomene* type (Figure 3E) (with 27% in August and 32.4% in October), *Bauhinia* type 1 (up 16.7% in May) and *Copaifera martii* (Figure 3D) (with 44% in June) (Table 1) (Figure 2).

Other pollen types also classified as secondary pollen were found in: Araliaceae (*Schefflera* type with 28.2% in November) (Figure 3C); Humiriaceae (*Humiria balsamifera* with 27.3% in July) (Figure 3F); Melastomataceae (*Bellucia* type (Figure 3H) with 22.2% and *Miconia impetiolaris* with 16.7%, both in May) (Figure 3I); Myrtaceae (*Psidium guajava* with 18.8% in July) (Figure 3J); Poaceae type 1 (with 17% in May); Rhamnaceae (*Ziziphus* with 45% in February and 17.6% in April) (Figure 3K) and Salicaceae (*Casearia* type with 29.7% in March and 28% in August) (Figure 3L) (Figure 2) (Table 1).

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Table 1

Pollen Types and Frequency Classes (%) of Honey Samples From December 2017 to November 2018 of the Bee Scaptotrigona nigrohirta *(Apidae: Meliponini) in the Amazon Region*

Family/Pollen types			2017					2018						
	Code	FR	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Anacardiaceae														
Anacardium occidentale	P01	P/N		1.0					4.0					
Astronium obliquum	P02	P/N	10.0		2.0	16.0	11.8		20.0		11.5			
Mangifera indica	P03	P/N			1.0								5.4	
Spondias mombin	P04	P		1.0	2.0		12.0							1.0
Tapirira guianensis	P05	P/N	39.2	49.6	7.2	6.6	12.0		4.0		27.1			16.5
Thyrsodium type	P06	P	2.0			10.0								
Annonaceae														
Annona type	P07	P	0.8											1.0
Apocynaceae														
Aspidosperma type	P08	P					6.0							
Aquifoliaceae														
Ilex type	P09	P/N			1.0									
Araliaceae														
Schefflera type	P10	P/N							4.0				5.4	28.2
Boraginaceae														
Tournefortia type	P11	N						5.5						
Burseraceae														
Protium heptaphyllum	P12	P/N		1.0										8.2
Commelinaceae														
Commelina type	P13	P/N								9.1				
Convolvulaceae														
Merremia type	P14	P/N				0.4					5.2	10.0		
Euphorbiaceae														
Alchornea type	P15	P			1.0						2.0			
Croton type	P16	P/N			3.2	4.3	6.0				11.5			
Euphorbia type	P17	P/N			2.7									
Fabaceae														
Aeschynomene type	P18	P/N	5.0	1.0		8.5	12.0				27.0		32.4	
Bauhinia type 1	P19	P/N						16.7						
Bauhinia type 2	P20	P/N									1.0			
Clitoria type	P21	P/N		1.0										
Copaifera martii	P22	P/N						1.0	44.0		4.0			
Desmodium type	P23	P	11.0											
Inga type	P24	P/N						1.0			4.0			
Mora type	P25	P/N		10.8		9.0								
Schizolobium amazonicum	P26	P/N	1.0	1.0	1.0									
Sclerolobium paniculatum	P27	P				2.0	6.0							
Stryphnodendron type	P28	P/N									9.0			
Tamarindus indica	P29	P											8.1	
Humiriaceae														
Humiria balsamifera	P30	N	1.0							27.3	4.0	50.0		
Lythraceae														
Cuphea type	P31	N			3.0									
Malpighiaceae														
Byrsonima basiloba	P32	P	6.0		2.0			11.1			3.5	60.0		

Continued

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Table 1 (Continued)

Family/Pollen types	2017							2018						
	Code	FR	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Malvaceae														
Ceiba type	P33	N					6.0							
Eriotheca gracilipes	P34	P/N	1.0	0.6		0.6								
Waltheria type	P35	N	8.2	0.2	0.5									
Melastomataceae														
Bellucia type	P36	P		1.0	1.0			22.2		9.1	2.1			
Miconia impetiolaris	P37	P	2.0	2.0	1.0	2.0		16.7	8.0		1.0			
Myrtaceae														
Eugenia pitanga	P38	P/N	1.0	1.0										
Eugenia type 1	P39	P/N	5.0											
Eugenia type 2	P40	P/N		1.0	5.3									
Myrcia type	P41	P/N					11.8							
Psidium cattleyanum	P42	P	1.0										3.0	
Psidium guajava	P43	P	2.0	2.0	54.4	6.0			12.0	18.8		10.0		
Syzygium malaccense	P44	P/N		1.0										1.2
Poaceae														
Type 1	P45	P						17.0						
Type 2	P46	P										10.0		
Polygonaceae														
Antigonon type	P47	P/N			0.5	1.2								
Coccoloba type	P48	N		1.0		4.0							8.0	
Rhamnaceae														
Ziziphus type	P49	P/N	2.2	2.0	45.0		17.6		4.0			10.0		9.4
Rubiaceae														
Faramea type	P50	N		1.0										
Morinda citrifolia	P51	N			1.0									1.2
Salicaceae														
Casearia type	P52	P/N	9.2	45.8	12.4	29.7	6.0			9.1	28.0			
Ryania type	P53	N	2.0	2.0		1.0								
Sapindaceae														
Matayba type	P54	P/N				1.5								
Serjania type 1	P55	P/N		1.0							2.0	5.0		
Serjania type 2	P56	P/N			1.0									
Solanaceae														
Solanum leucocarpon	P57	P									2.0			
Undetermined				0.2				11.1	9.0		1.0	10.0	5.4	17.5
Total			19	23	21	16	11	9	9	5	18	8	7	10

Note. FR = Flora resources. P/N = Pollen/Nectar.

As for pollen types classified as important minor pollen, representatives were observed in almost all families, except Annonaceae, Aquifoliaceae, Rubiaceae, and Solanaceae. The pollen types classified as occasional were not observed in the following families: Apocynaceae, Araliaceae, Boraginaceae, Commelinaceae, Lythraceae, and Poaceae (Table 1).

Analyzing the total number of pollen types, including the undetermined ones, the highest number was observed between December and March, ranging from 16 to 23 pollen types, with an unexpected increase in August (18 pollen types) (Table 1). The first months of the year correspond to the rainy season in the Amazon region. From May onwards, rainfall started to decrease, and the dry season began from June onwards, with an increase in rainfall from November, when the rainy season begins

Table 2

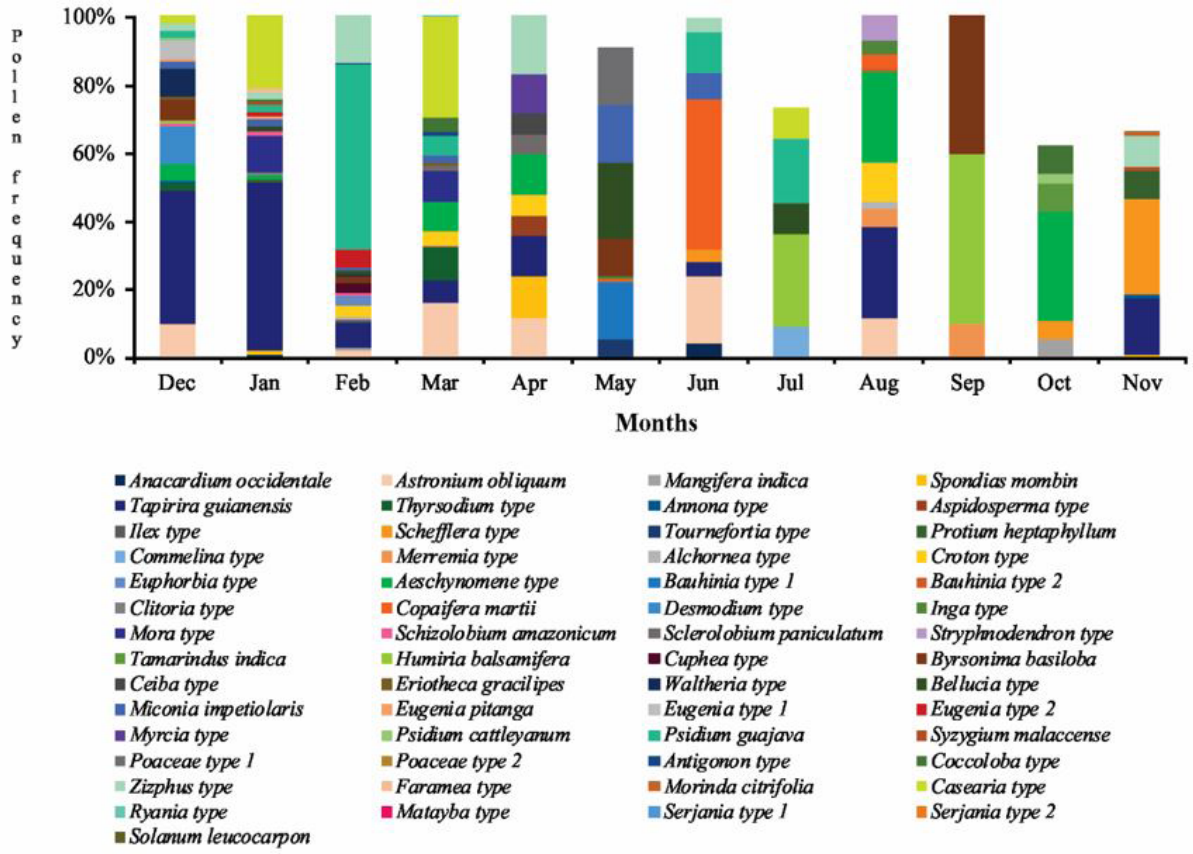
Indices of Diversity of Honey Samples From December 2017 to November 2018 of the Bee Scaptotrigona nigrohirta *Nogueira & Santos-Silva, 2022 From the São Francisco Community of the Lago Grande Agroextractive Settlement Project, Arapiuns River, Santarém-PA*

Diversity indices	Values (%)
Shannon-Weaver Index	3.18
Pielou's Evenness Index	0.78
Chao-1	57

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Figure 2

Frequency of Pollen Types (Except Undetermined) Found in Honey Samples of the "Canudo" Bee, Scaptotrigona nigrohirta*, From the São Francisco Community Meliponary of the Lago Grande Agroextractive Settlement Project, Arapiuns River, Santarém-PA, From December 2017 to November 2018*



in the Amazon region. This seasonal pattern, with higher richness during the rainy season and a reduction in the dry period, is consistent with the Shannon diversity index ($H' = 3.18$), which reflects an overall high diversity of pollen types across the year, despite temporal fluctuations. In addition, the Pielou evenness index ($J = 0.78$) indicates that this variation in richness is not accompanied by strong dominance, suggesting that pollen sources are relatively evenly used even when the number of available types decreases.

Through the results of honey samples collected over 12 months from a Scaptotrigona nigrohirta colony, we observed a high richness of pollen types, indicating that this species exhibits polylectic behavior, foraging on a wide variety of plant species. In addition, the honey samples were predominantly classified as heterofloral, reflecting the absence of a single predominant pollen type in most months. However, occasional increases in the relative frequency of certain pollen types were observed, although not sufficient to characterize the honey as monofloral.

Regarding the floral resources provided by the plant species identified, most of them offered both pollen and nectar to *Scaptotrigona nigrohirta*, totaling 32 occurrences, followed by pollen alone (16) and nectar alone (9) (Figure 4).

The analysis of the qualitative interaction network (Figure 5) constructed from honey pots collected from *Scaptotrigona nigrohirta* colonies over twelve months revealed an average of 2.14 links per month, along with a connectance value of 2.16. This pattern does not necessarily indicate that *S. nigrohirta* relies on a limited set of floral resources, but rather reflects a higher frequency of certain pollen types in honey samples. Considering that melissopalynological data primarily represent nectar foraging, these results should be interpreted with caution, as they do not fully capture pollen resource use. Given the large colony size of *S. nigrohirta* and its known generalist behavior, it is likely that this species exploits a broad spectrum of floral resources throughout the year, with some plant species being more prominently represented in honey due to nectar availability and foraging dynamics. The most frequent pollen types recorded in the study area include *Astronium obliquum* (P02), *Tapirira guianensis* (P05), *Aeschynomene* type (P18), *Zizyphus* type (P49), and *Casearia* type (P52), which provide both nectar and pollen, as well as *Byrsonima basiloba* (P32), *Bellucia* type (P36), *Miconia impetiolaris* (P37), and *Psidium guajava* (P43), which are mainly pollen sources. Regarding nectar resources, *Humiria balsamifera* (P30) was the most prominent.

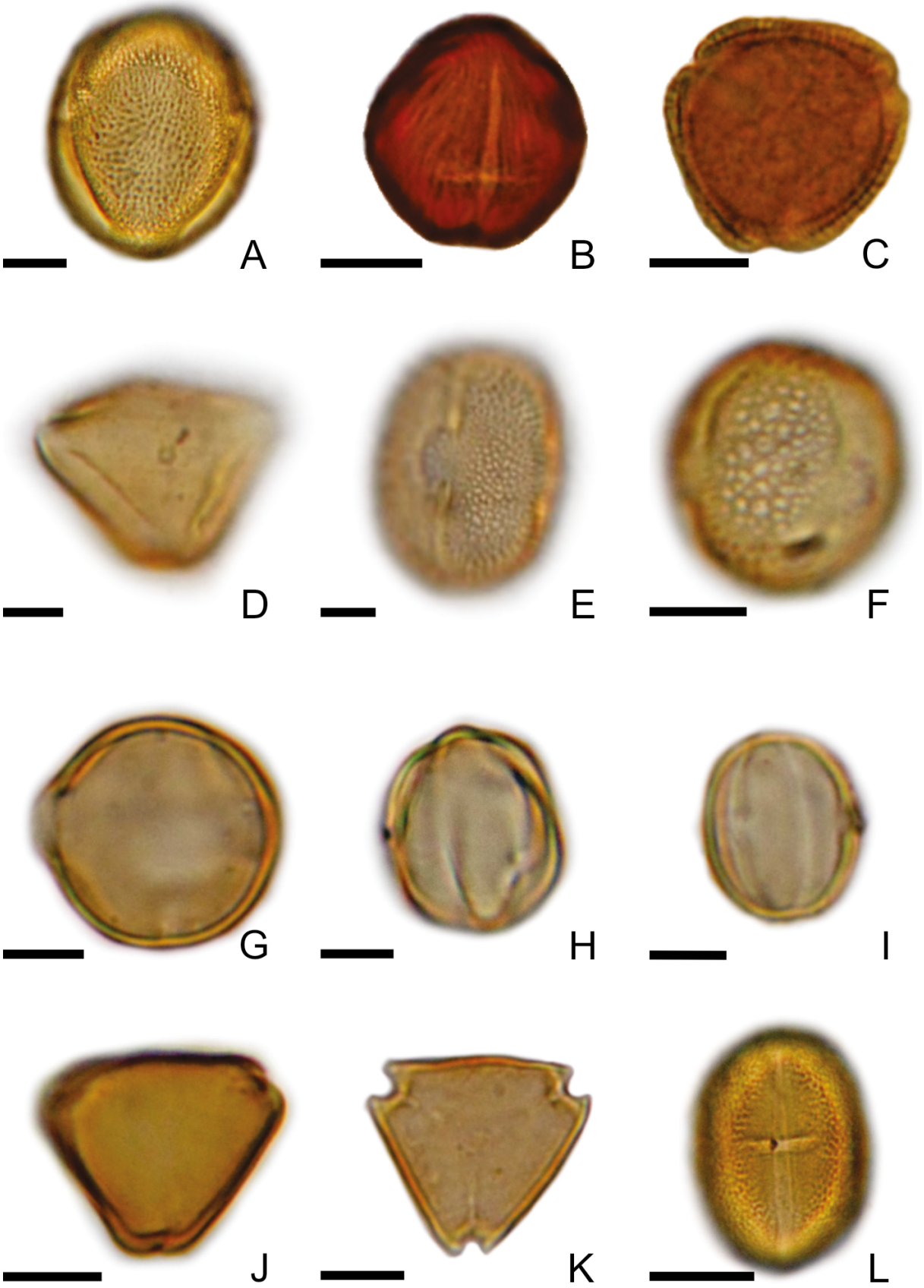
The interaction network showed low modularity ($M = 0.36$). Within this structure, the *S. nigrohirta* honeys collected over the twelve months were grouped into seven distinct modules. Module 01 consisted of November 2018 and included seven pollen types (e.g., members of the Anacardiaceae family: *Spondias mombin* (P04), and *Tapirira guianensis* (P05)). Module 02 corresponded to February 2018 and contained seven pollen types (e.g., members of the Euphorbiaceae family: *Alchornea* type (P15) and *Euphorbia* type (P17)). Module 03 comprised the month of April 2018 and included six pollen types (e.g., *Ceiba* type (P33) and *Myrcia* type (P41)). Module 04 represented the months of May 2018, August 2018, and September 2018 and included fourteen pollen types (e.g., members of the Fabaceae family: *Bauhinia* type 1 (P19), *Bauhinia* type 2 (P20), *Copaifera martii* (P22), *Inga* type (P24), and *Stryphnodendron* type (P28)). Module 05 represented the month of October 2018 and included six pollen types (e.g., *Schefflera* type (P10) and *Aeschynomene* type (P18)). Module 06 was formed by the grouping of December 2017, January 2018, March 2018, and June 2018, and included fifteen pollen types (e.g., members of the Fabaceae family: *Clitoria* type (P21), *Desmodium* (P23), Mora type (P25), and *Schizolobium amazonicum* (P26)). Finally, Module 07 was formed by July 2018 and contained two pollen types: *Commelina* type (P13) and *Casearia* type (P52) (Figure 6).

In Figure 7, it is possible to observe that exclusive pollen types occurred in the samples collected each month. In December 2017, the exclusive types were *Desmodium* type (P23) and *Eugenia* type 1 (P39). In January 2018, the exclusive types were *Clitoria* type (P21) and *Faramea* type (P50). In February 2018, the exclusive types were *Ilex* type (P09), *Euphorbia* type

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Figure 3

Most Representative, Predominant and Secondary Pollen Types in Honey Samples From the "Canudo" Bee, Scaptotrigona nigrohirta



Note. a-b. Anacardiaceae – a. *Astronium obliquum*; b. *Tapirira guianensis*; c. Araliaceae – *Schefflera* type; d-e. Fabaceae – d. *Copaifera martii*; e. *Aeschynomene* type; f. Humiriaceae – *Humiria balsamifera*; g. Malpighiaceae – *Byrsonima basiloba*; h-i. Melastomataceae – h. *Bellucia* type, i. *Miconia impetiolaris*; j. Myrtaceae – *Psidium guajava*; k. Rhamnaceae – *Ziziphus* type; l. Salicaceae – *Casearia* type. Bar = 10 μm.

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Figure 4

Floral Resources Associated With the Pollen Types Found in Honey Samples of the "Canudo" Bee, Scaptotrigona nigrohirta

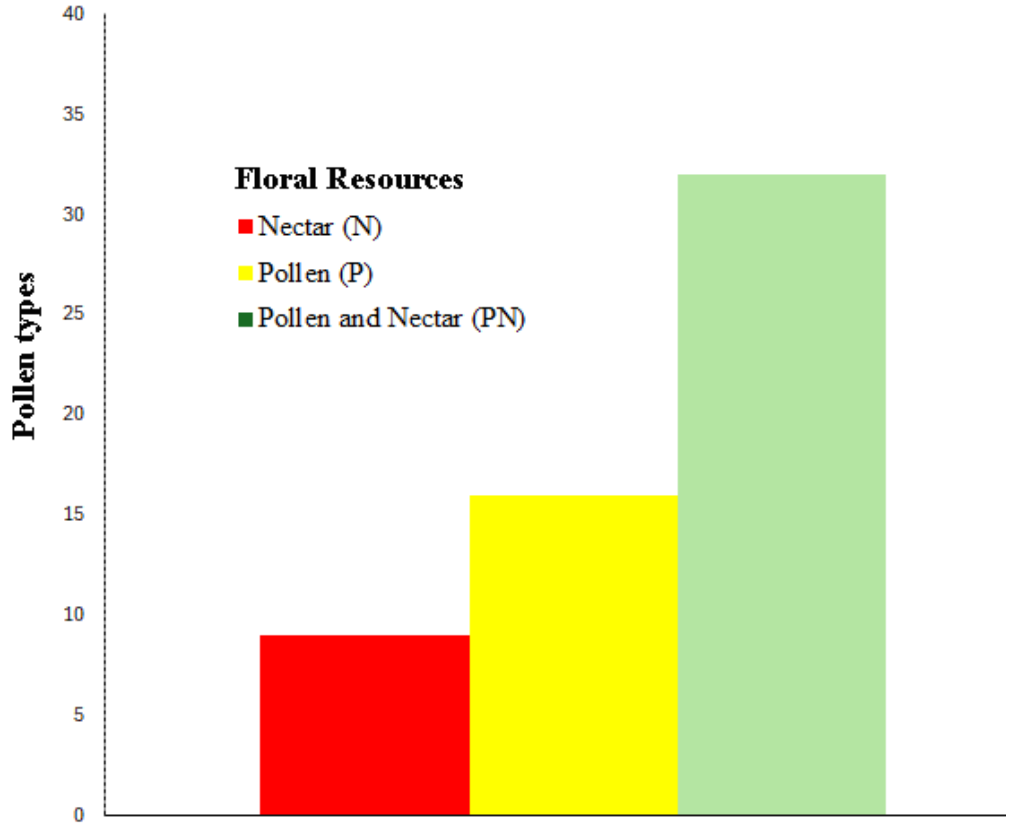
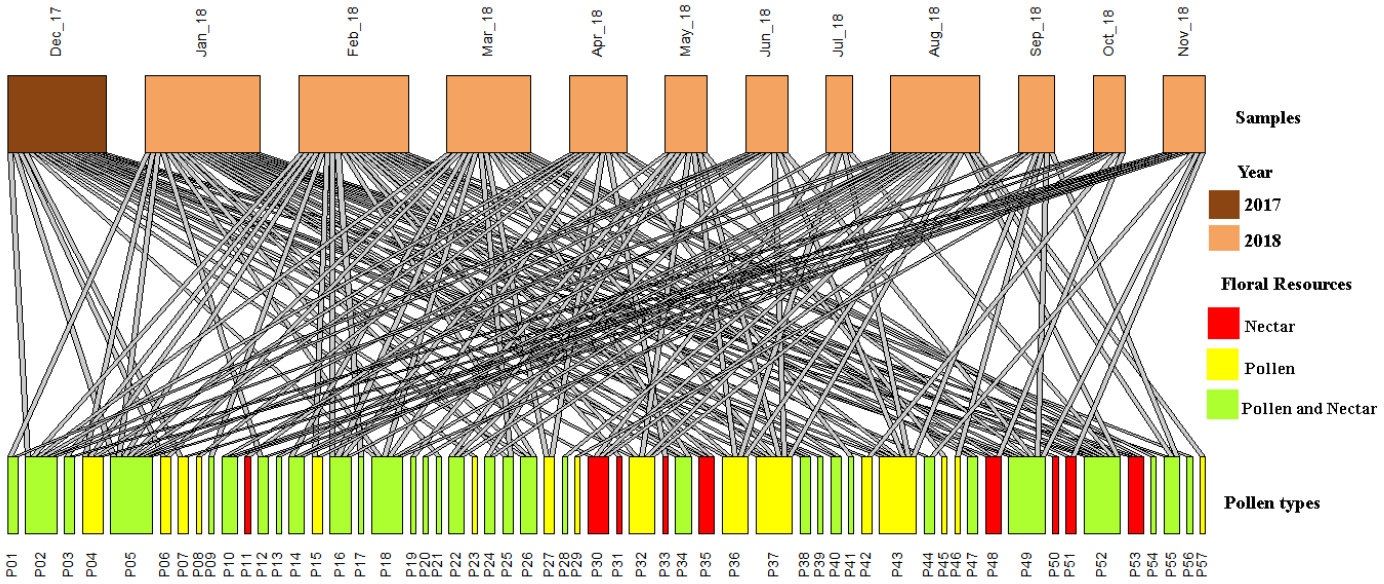


Figure 5

Bipartite Interaction Network Between Honeys Collected From Scaptotrigona nigrohirta *Honey Pots Over Twelve Months (Circles) and the Pollen Types Identified in These Honeys (Squares)*

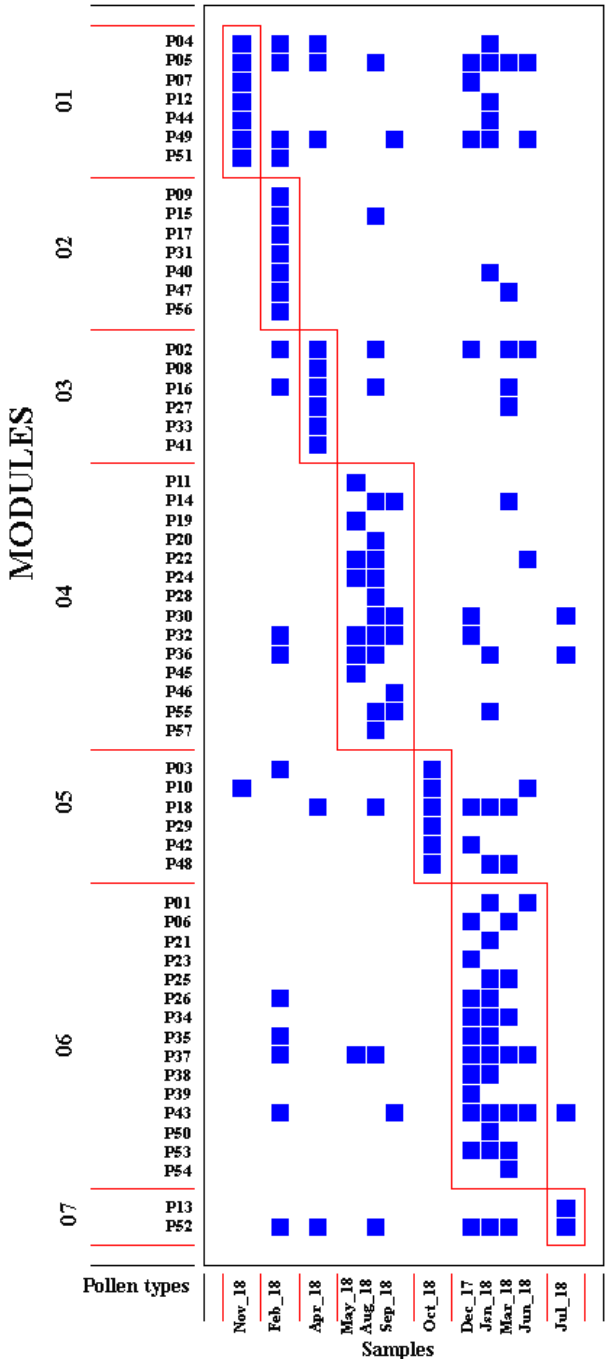


Note. Codes are listed in Table 1.

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Figure 6

Modular Interaction Network Between Honeys Collected From Scaptotrigona nigrohirta *Honey Pots Over Twelve months and the Pollen Types Identified in These Honeys*



Note. Codes are listed in Table 1.

(P17), *Cuphea* type (P31), and *Serjania* type 2 (P56). In March 2018, the exclusive type was *Matayba* type (P54). In April 2018, the exclusive types were *Aspidosperma* type (P08), *Ceiba* type (P33), and *Myrcia* type (P41). In May 2018, the exclusive types were *Tournefortia* type (P11), *Bauhinia* type 1 (P19) and Poaceae type 1 (P45). In July 2018, the exclusive type was *Commelina* type (P14). In August 2018, the exclusive types were *Bauhinia* type 2 (P20), *Stryphnodendron* type (P28), and *Solanum leucocarpon* (P57). In September 2018, the exclusive type was Poaceae type 2 (P46) and in October 2018, the exclusive type was *Tamarindus indica* (P29).

The occurrence of exclusive pollen types in each month indicates a clear temporal variation in the floral resources used by *Scaptotrigona nigrohirta*. This pattern suggests that the species does not rely on a fully stable set of plants throughout the year but instead adjusts its foraging activity according to seasonal fluctuations in flower availability. Months with a higher number of exclusive pollen types, such as February, may reflect periods of increased floral diversity in the landscape, while months with only one exclusive type suggest lower turnover in available resources or greater dependence on a core set of plants. The presence of these unique pollen types reinforces the importance of maintaining a diverse and temporally continuous floral supply in the surrounding vegetation, as the loss of seasonally restricted plant species could disproportionately affect the diet and colony functioning of *S. nigrohirta*.

The similarity dendrogram, constructed using the Jaccard coefficient based on the presence/absence matrix of pollen types identified in the honey pots collected from *Scaptotrigona nigrohirta* colonies over twelve months, revealed the formation of a main cluster (Cluster A). This group shows approximately 30% similarity and comprises the samples from December 2017, January 2018, February 2018 and March 2018, which share four pollen types: *Tapirira guianensis* (P05), *Miconia impetiolaris* (P37), *Psidium guajava* (P43) and *Casearia* type (P52) (Figure 8). Within this cluster, a closer relationship is observed between the samples from December 2017 and January 2018, which reach approximately 40% similarity and share eleven pollen types, including *Aeschynomene* type (P18), *Schizolobium amazonicum* (P26), *Eriotheca gracilipes* (P34) and *Waltheria* type (P35). This pattern suggests that during this period there was greater stability in the composition of floral resources exploited by the colonies, possibly reflecting similar availability of pollen sources in the environment.

Regarding the diversity indices, the following values were obtained: Shannon–Wiener (*H'*) of 3.18, Pielou's evenness (*J'*) of 0.78 and a Chao-1 richness estimate corresponding to 57%. The relatively high H' value indicates a pollen community with high diversity, while the evenness value of 0.78 suggests a moderately uniform distribution of pollen types among samples, indicating that no few types strongly dominated the collection. The Chao-1 estimate shows that approximately 57% of the expected total richness was recorded in the samples, suggesting that the floral resource community exploited by the colonies is even more diverse than what was directly observed (Table 2).

Discussion

In the present study, five species were considered important for *Scaptotrigona nigrohirta* feeding, represented as predominant pollen. Marques-Souza et al. (2007) described a similar pattern when studying the characteristics of pollen collected by the Amazon bee *Scaptotrigona fulvicutis* which collected pollen grains from 97 species distributed in 36 families, while only six families made up the majority of annual feeding for this Meliponini.

The species *Byrsonima basiloba*, with 60% representativeness in September, was one of the five species classified as predominant pollen. According to Lorenzi (2020), this species has a shrub habit and can be found in several phytophysiognomies. Its floral resources are available throughout the year, especially during the summer, according to Silva et al. (2012), its pollination occurs through bees.

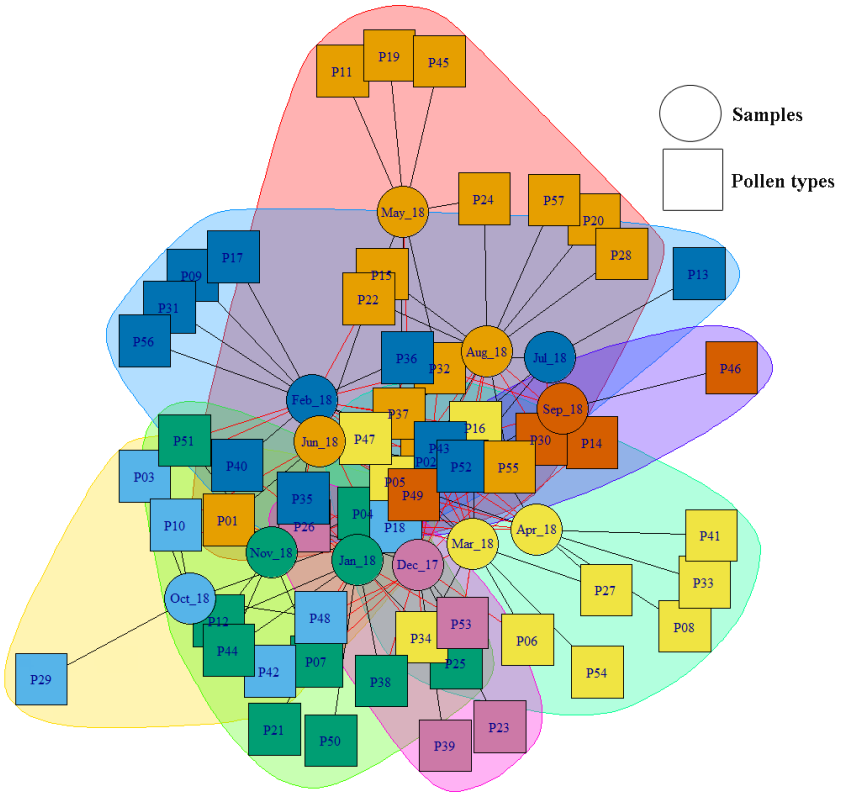
We observed that the *Scaptotrigona nigrohirta* used the resources of *Byrsonima basiloba* at distinct times in both rainy and dry seasons. In the studies carried out by P. O. Silva et al. (2016), the emission of floral buds of *B. basiloba* was seasonal, occurring closer to the rainy season, but the authors also recorded flower bud formation in both dry and rainy seasons. According to P. E. A. M. Oliveira (2008), the distinct flowering times of *Byrsonima* were influenced by the competition between its pollinating agents, as Malpighiaceae species depend exclusively on bees, which share the same floral resources.

Following *Byrsonima basiloba*, we found *Psidium guajava* with 54.4% expressiveness. This species belongs to the Myrtaceae family, which besides having this representative classified as predominant pollen, was also one of the families with the highest number of pollen types (seven). The foraging of bees in Myrtaceae species can be explained by the floral structure of their representatives, which are attractive to bees as they usually present white flowers with numerous extruded stamens, and their resources are easily accessible (Joly, 1993).

https://doi.org/10.33447/paubrasilia.2026.e0230 2026, Vol. 9, e230

Figure 7

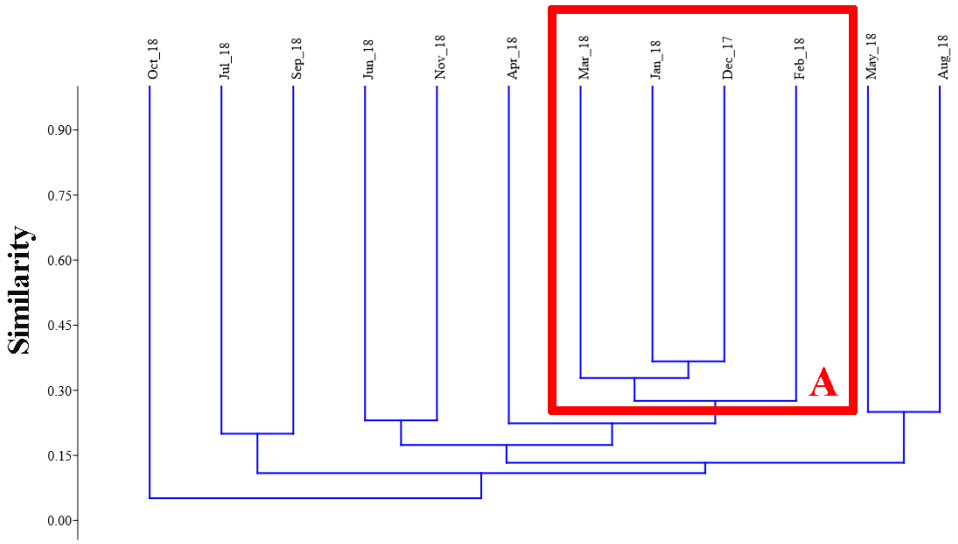
Bipartite Interaction Network Between Honeys Collected From Scaptotrigona nigrohirta *Honey Pots Over Twelve Months (Circles) and the Pollen Types Identified in These Honeys (Squares)*



Note. Codes are listed in Table 1.

Figure 8

Similarity (Jaccard Coefficient) Between Honeys Collected in Scaptotrigona nigrohirta *Pots Over Twelve Months*



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Myrtaceae is always presented in research about plants used by bees to collect pollen and/or nectar, and has been mentioned, along with Fabaceae, as the family most frequently used by bees (Marques-Souza et al., 2007; Wilms & Wiechers, 1997). In addition, the agroforestry yard where this Meliponary is located contains Myrtaceae species close to the bee boxes, e.g., red guava (*Psidium cattleyanum*), guava (*Psidium guajava*) and Malay rose apple (*Syzygium malaccense*).

After *Psidium guajava*, *Humiria balsamifera* was classified as predominant pollen, with 50%. This species has a subshrub to arboreal habit and can be found in different environments (Cuatrecasas 1961). According to J. A. S. Costa & Ramalho (2001), native bees are considered effective pollinators of this species. Furthermore, Rocha & Miranda (2009) found that bees prefer feeding on *H. balsamifera* throughout its flowering cycle because this species offers both nectar and pollen. Therefore, it is recommended for meliponiculture pasture as it helps maintain hives, provides a honey with pleasant flavor and aroma, and produces fruits that can be consumed by beekeepers as well as the local fauna. Although *Humiria balsamifera* continuously flowers throughout the year, the month with the greatest activity was September, which Vianna (2016) also observed for this same species. Furthermore, it was pollinated by social bees, showing that its resources were best used in this period.

Another predominant species was *Tapirira guianensis*, with 49.6 % representativeness. This species belongs to the Anacardiaceae family, which, like Myrtaceae, presented many pollen types (six). The Anacardiaceae family presents small flowers that are not very showy, hermaphroditic or unisex, longitudinal anthers dehiscence and its fully exposed pollen increases its attractiveness to pollinators (Judd et al. 2009). Tapirira guianensis Aubl. was the most abundant pollen foraged by *Scaptotrigona nigrohirta*, occurring more frequently in December and January during the rainy season. In the works by Carreira et al. (1986) and F. P. M. Oliveira et al. (1998), performed in Pará State, this pollen type was also predominant in the sampled months, especially in periods with high rainfall.

Fernandes et al. (2012) and Lenza & Oliveira (2005) presented remarkable characteristics of *T. guianensis*: the species presents annual flowering and also rapid flowering peaks called "pulsed bang", managing to recruit a larger number of pollinators in these peaks by offering pollen and nectar as a floral reward to its visitors. As for its size, *T. guianensis* is a tall tree with rapid growth that can be used for reforestation purposes, and because it presents persistent flowering, it can help manage meliponiculture pasture.

When studying the behavior of the Amazonian bee *Scaptotrigona fulvicutis*, Marques-Souza et al. (2007) also identified Anacardiaceae pollen types, among them *Anacardium* was the most significant from August to November, with September being the most representative month. On the other hand, in the present study, Anacardiaceae pollen types were more abundant in the rainy season, from December to April. From these results, we understand that *Tapirira guianensis* pollen and other pollen types belonging to the Anacardiaceae family are some of the main protein sources for *Scaptotrigona nigrohirta* and the visitation patterns to species of this family show that these plants are seasonal with short flowering periods.

Finally, *Casearia* type pollen was also classified as predominant, with 45.8 %, and was present in almost all months sampled, especially during the rainy season, with greater representativeness in February. According to Melo et al. (2004), species of the genus *Casearia* are considered strategists, as they maintain their energy stores according to their reproduction, seeking to preserve their individuals.

In studies by Imatomi et al. (2009) and Marquete & Mansano (2016), the genus *Casearia* shows flowering with great variation throughout its area of distribution, which is due to its genetic variability and phenotypic plasticity that allows the taxon to survive in any environment. *Scaptotrigona nigrohirta* showed strong interest in *Caesaria* because of its flowers, which according to Pott & Pott (1994) have aromatic nectar and fully reflex sepals, commonly called honey perfume, which is an important honey source with high pollen production that attracts meliponine bees, as well as has important medicinal properties.

Regarding the pollen types classified as secondary pollen, which were frequently found in the sampling period, the Fabaceae family presented the most pollen types (12). However, despite this significant number, there were no predominant representatives. In surveys of meliponiculture flora, Aleixo et al. (2013), Marques-Souza (2010), M. E. C. Oliveira et al. (2008) and Novais et al. (2009) also highlighted high numbers of pollen types from the Fabaceae family. This is because the species in this family are widely distributed and their representatives in the study area are remarkable, as they occur in various habitats in the Amazon, where they are abundant, and their representatives are constantly recorded in surveys of plants visited by bees.

The works of F. P. M. Oliveira et al. (2009), Martins et al. (2010) and M. I. B. Oliveira & Sigrist (2008) demonstrated the importance of Fabaceae in feeding stingless bees, because it offers pollen as well as blooms all year round. Resources can differ for bees depending on the soil and climatic conditions, making inventories of the regional flora important, since species that are considered excellent nectar/pollen producing in one region may not be present in another.

According to Carvalho & Marchini (1999) and Estigarribia et al. (2017), Fabaceae is a representative and regionally important family, as it is the third largest in number of species, and provides bees with pollen, nectar, resins and nesting sites. Lorenzon et al. (2003) corroborate the high representativeness of Fabaceae in the honey samples of *Scaptotrigona* sp., stating that bee preference is not related to the resources offered by Fabaceae, but by the high abundance of blooms throughout the year.

Freitas et al. (2010) emphasize that the families Fabaceae, Euphorbiaceae, Melastomataceae, Myrtaceae and Solanaceae are among the most frequent pollen types found in honey samples of stingless bees in Brazil. Bawa (1990) points out that Euphorbiaceae, Fabaceae and Melastomataceae are exclusively pollinated by bees. The families Euphorbiaceae, Melastomataceae and Solanaceae were also present in the honey samples of *Scaptotrigona nigrohirta*, but at low frequencies, being mostly classified as important or minor pollen.

According to Barth (1989), pollen types classified as secondary are those that occur between 16% and 45% of the total grains found in the sampled honey, indicating samples devoid of predominant pollen. Secondary pollen plays an important role in the diet of bees, contributing intermediately due to the great floristic diversity of the studied region. This same importance was verified by J. I. Silva (2014), who frequently found secondary pollen in their samples, which contributed significantly to bee feeding.

Ziziphus Mill., which belongs to the Rhamnaceae family, was classified as secondary pollen that reached a high representativeness, with 45% in the February honey sample and was present in both wet and dry seasons in the Amazon. *Ziziphus* is a genus with fruit species that are economically and ecologically important (Lorenzi, 2021; Prado & Gibbs, 1993).

Dantas et al. (2014) described that *Ziziphus joazeiro* develops in different soil types, remains green throughout the year, flowers and fruits in dry and rainy periods, respectively, and nectar is its main resource collected by floral visitors, including native bees. Nadia et al. (2007) found that the flowers of *Z. joazeiro* were often visited by bees of the meliponini tribe, including *Scaptotrigona* sp. It has been found that *Z. joazeiro* uses the strategy of excessive flower production to attract its pollinators, with the species being an important food resource for meliponine bees as well as for the maintenance of their colonies.

The species *Ziziphus mauritiana* Lam. is the only one from the genus reported in the North of Brazil, according to Lima et al. (2025). It is a tree species that is popularly known as Black Mary (Santos et al., 2020) and presents morphology composed of tirsoid inflorescences, with crass nectariferous discs in its flowers (Lima et al., 2025).

Regarding the total number of pollen types, it is clear that the number of pollen types present in the *Scaptotrigona nigrohirta* honey samples reduced from April onwards, resulting in a large amount of pollen grains classified as important minor and minor.

The work by F. P. M. Oliveira et al. (2009) with four stingless bee species showed a different pattern, revealing the influence of climatic factors on meliponine bee resource collection. Therein, it was reported that bees were more active in June, which coincides with the end of the rains, and August, which is the period of greatest flowering, contributing to the increased collection of different resources found herein. In the study conducted by Macieira & Proni (2004), the bee *Scaptotrigona postica* showed high resistance to temperature variations during the six-hour experiments. This result may explain why the bees in the present study were able to efficiently forage in the rainy season.

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Regarding the other families, most of the species identified presented very low frequencies, therefore their pollen types were classified as important minor pollen or minor pollen. According to Barth (1970), Brazilian honeys are characterized by large amounts of minor pollen. Barth (1989) also states that pollen considered occasional (minor) has little importance as to the amount of nectar supplied for bee health, although it may be interesting for determining geographical origin. Generally, pollen grains with very low frequencies could enter the honey composition accidentally.

However, according to Vanderhuck (1995), plants that provide few trophic resources and plants that provide many resources for a short period of time are both important for sustaining bees. To understand the relationship between bees and plants, it is important to study pollinators and develop strategies for the rational exploitation of natural resources (Nogueira-Ferreira and Augusto 2007).

Thus, the richness of pollen types found in honey samples of *Scaptotrigona nigrohirta* from the São Francisco Community Meliponary revealed that the most representative families, in terms of predominant pollen classification, were: Anacardiaceae, Humiriaceae, Malpighiaceae, Myrtaceae and Salicaceae. The families with the highest number of pollen types were: Anacardiaceae, Fabaceae and Myratcaeae.

We conclude that this bee presented a generalist behavior in the use of food sources, with honey samples being classified as heterofloral. In the rainy months (December to April), a high number of pollen types were observed, indicating that *Scaptotrigona nigrohirta* bees are very resistant to temperature variations. Consequently, information about bee floral preferences makes it possible to manage this species around the melioponary and is an important tool for conserving *Scaptotrigona nigrohirta* and the local flora, favoring biodiversity conservation. Therefore, the importance of local supply and behavior in the food profile of native bees is relevant to the beekeeper, who uses meliponiculture as a family livelihood. Thus, works like this, of melissopalynology, enrich not only the increase in production and maintenance of biodiversity, but also generate quality of life for the population who depend on these bees for their livelihood.

Conclusions

The study of the pollen morphology of *Scaptotrigona nigrohirta* honey confirms its diverse foraging and classifies the honey as heterofloral, given the high richness of 65 pollen types, distributed across 24 families. The most important families for the diet were Fabaceae, Myrtaceae and Anacardiaceae, with the predominant pollen types acting as essential markers of the local flora. The analysis revealed high pollen diversity, but also a relative dependence on a limited set of key resources throughout the year, suggesting bee selectivity or availability constraints. This adaptive strategy is evidenced by the temporal fluctuation of resources, with unique pollen types occurring monthly and greater resource similarity observed between December and March. Pollen types such as *Tapirira guianensis*, *Astronium obliquum*, and *Casearia* type are crucial, as they provide both nectar and pollen, which are essential for the colony. Therefore, *S. nigrohirta* honey is a record of the rich and seasonal floral diversity of the Amazon, reinforcing the need to conserve a continuous and diverse floral supply for the sustainability and functioning of the species, as this study provides fundamental data on ecological importance, conservation, and meliponiculture, as it details the trophic network of *S. nigrohirta* in the Amazon over an annual cycle, identifying the floral resources (nectar and pollen) essential for the colony's survival.

Acknowledgments

We are grateful to Mrs. Edilena Cristina Teixeira de Oliveira, who contributed greatly to this research by allowing us to collect from her Meliponary, and to Dr. Favízia Freitas for identifying the bees.

Funding

The authors declare no funding sources to report.

Author Contributions

V.H.R.A. and R.R.F. collected the data and conducted data analyses. V.H.R.A., V.R.M. and M.L.A. contributed to the design of the study, aided in interpreting the results, worked on the manuscript, wrote the draft of the manuscript, discussed the results, and commented on the manuscript and the proof outline.

Conflict of Interest

The authors declare no conflicts of interest to report.

Data Availability Statement

The complete datasets analyzed during the current study are presented within the manuscript.

Ethical Compliance

Not applicable.

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How to Cite This Article:
Como Citar Este Artigo:

(ABNT)
ABREU, V. H. R.; FONSECA, R. R.; MATOS, V. R.; ABSY, M. L. Pollen spectrum of honey produced by *Scaptotrigona nigrohirta* (Apidae: Meliponini) in the Amazon region. **Paubrasilia**, Porto Seguro, v. 9, e230, 2026. DOI: 10.33447/paubrasilia.2026.e0230

(APA)
Abreu, V. H. R., Fonseca, R. R., Matos, V. R., & Absy, M. L. (2026). Pollen spectrum of honey produced by *Scaptotrigona nigrohirta* (Apidae: Meliponini) in the Amazon region. *Paubrasilia*, *9*, e230. https://doi.org/10.33447/paubrasilia.2026.e0230