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Research Article

Melissopalynological and chemical analyses of propolis from *Scaptotrigona nigrohirta* Nogueira & Santos-Silva, 2022 (Apidae - Meliponini) in the Lower Amazon River region

Análises melissopalinológica e química da própolis de Scaptotrigona nigrohirta Nogueira & Santos-Silva, 2022 (Apidae - Meliponini) na região do Baixo Amazonas

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ABSTRACT

The chemical composition of propolis is highly variable and complex and is related to the phytogeography of a region. Therefore, the ecology of the local flora is directly related to its chemical composition. The aim of this study was to identify the pollen types and chemical substances of the hexane extracts present in *Scaptotrigona nigrohirta* propolis from a meliponary in Santarém, Pará state, in the Lower Amazon River region, in order to obtain data that can be used for the sustainable conservation of the bee species and flora in this Amazonian region. Pollen analyses were performed on 11 propolis samples collected at the meliponary and chemical analyses on only two (wet and dry seasons). For the melissopalynology, the propolis was diluted in acetone and then applied to acetolysis. For a chemical evaluation, hexane extractions of propolis samples from April and August 2018 and gas chromatography-mass spectrometry analyses were performed. A multivariate analysis was performed on pollen and chemical data. Seventy-seven pollen types were found, defining the propolis as heterofloral, with the families Fabaceae, Anacardiaceae and Myrtaceae being the most significant in the pollen spectrum of the samples. The most frequent species were: *Copaifera martii*, *Eugenia brasiliensis*, *Mitracarpus strigosus*, *Protium heptaphyllum* and *Tamarindus indica*. The hexane extracts of propolis had a 35.75% yield. Sixteen compounds were found and cycloartenol, lupeol acetate and lupenone were identified as major constituents.

KEYWORDS: Bees. Fabaceae. Pollen grains. Triterpenes.

RESUMO

A composição química da própolis é altamente variável e complexa, estando relacionada com a fitogeografia da região. Portanto, a ecologia da flora local está diretamente relacionada à sua constituição química. Assim sendo, o objetivo deste estudo foi identificar os tipos polínicos e as substâncias químicas dos extratos de hexano presentes na própolis de *Scaptotrigona nigrohirta* de um meliponário em Santarém, estado do Pará, na região do Baixo Amazonas, a fim de obter resultados que contribuam para a conservação sustentável dessa espécie de abelha e da flora nessa região da Amazônia. As análises polínicas foram feitas em 11 amostras de própolis coletadas no meliponário e a química em apenas duas (período chuvoso e seco). Na metodologia aplicada para melissopalinologia, a própolis foi diluída em acetona e depois aplicada a acetólise. Para uma avaliação química foram realizadas as extrações hexânicas das amostras de própolis de abril e agosto de 2018 e análises por cromatografia gasosa acoplada à espectrometria de massa. Foi feita uma análise multivariada com os dados polínicos e químicos. Foram encontrados 77 tipos polínicos, definindo a própolis como heterofloral, sendo as famílias Fabaceae, Anacardiaceae e Myrtaceae as mais significativas no espectro polínico das amostras. Os tipos polínicos mais frequentes foram: *Copaifera martii*, *Eugenia brasiliensis*, *Mitracarpus strigosus*, *Protium heptaphyllum* e *Tamarindus indica*. O extrato hexânico da própolis teve um rendimento de 35,75%. Foram encontrados dezesseis compostos. O cicloartenol, acetato de lupeol e lupenona foram identificados como constituintes predominantes.

PALAVRAS-CHAVE: Abelhas. Fabaceae. Grãos de pólen. Triterpenos.

INTRODUCTION

The physical-chemical composition of propolis is highly variable and complex, being related to the phytogeography of the region. Therefore, the ecology of the local flora is directly related to its composition (BANKOVA 2005, CASTRO *et al.* 2007, SOUSA *et al.* 2007, BURIOL *et al.* 2009). We can mention, e.g., the differences in the chemical composition of geopropolis from the same bee species (*Melipona fasciculata* Smith) collected in different municipalities in Maranhão State, Brazil, where the samples presented distinct chemical profiles in their compositions (BATISTA *et al.* 2016).

Among the metabolites that are present in propolis samples we highlight flavonoids, aromatic acids and esters, aldehydes and ketones, terpenoids and phenylpropanoids, steroids, amino acids, polysaccharides, hydrocarbons, fatty acids and other compounds in small quantities (ABD EL HADY & HEGAZI 2002, BITTENCOURT *et al.* 2015).

Among the techniques used to determine the chemical profile of propolis is liquid and gas chromatography coupled with mass spectrometry (FERNANDES-SILVA *et al.* 2013).

In addition to the chemical composition, propolis contains pollen grains transported by bees or wind (SODRE *et al.* 2008), as well as a combination of resinous substances that bees collect from plants (BURDOCK 1998, ALENCAR 2002, MATOS *et al.* 2014). Consequently, this product is protective for bees, acting as a thermal insulator and preventing intruders from entering the hives (PARK *et al.* 2005, FREITAS *et al.* 2010). On the other hand, humans widely use propolis in folk medicine due to its diverse biological products (MARCUCCI & BANKOVA 1999).

Therefore, in studies about plant-pollinator interaction, melissopalynology involves pollination, identification of the floral origin of honeys, knowledge about the diet and food preferences of pollinators, as well as competition between them, especially bees (MICHENER 2007). For this reason, studying the pollen types that bees collect is very important for determining the relationships they have with plants in a given area.

Hence, insect-plant relationships can be studied through melissopalynology rather than direct observations. Such analyses have helped beekeepers, since the results generate a calendar with the species or pollen types that these producers can use to enrich their meliponiculture pasture.

In Brazil several studies have been conducted with pollen analysis of propolis and geopropolis from stingless bees and mainly *Apis* sp., but studies involving the genus *Scaptotrigona* are rare. We can cite some of these works, which are very important for Brazil, such as: BARTH 1998, 2004, 2006; BARTH & FREITAS 2015; BARTH & LUZ 2003, 2009; BARTH et al. 1999, 2013; CITÓ et al. 2020; FREITAS & BARTH 2003; FREITAS et al. 2010, 2011, 2012; LUZ et al. 2009; MATOS & SANTOS 2017; RIBEIRO *et al.* 2013, 2018.

The chemical analysis of extract from propolis can be used as another indicator type of botanical origin when compared to the plant source. So, such data contributes to the sustainable conservation of this species and the flora that it visits, especially since *Scaptotrigona nigrohirta* is a native species and has been poorly studied. Furthermore, this species is the most used by beekeepers in the lower Amazon River region, which makes this research an important contribution for the socioeconomic development of the region.

Therefore, the objective of this study was to identify the pollen types found in propolis over the course of a year and the chemical substances present in the hexanic extract of *Scaptotrigona nigrohirta* propolis from a meliponary located in the lower Amazon region, Santarém, Pará, Brazil.

MATERIAL AND METHODS

Study area

The research was carried out from a meliponary in Santarém, Pará state, at the Federal University of Western Pará (UFOPA), on the banks of the Tapajós River, from the lower Amazon River (coordinates 55º 10'02 7” W longitude and 2º 40'59 9” S latitude) (Figure 1).

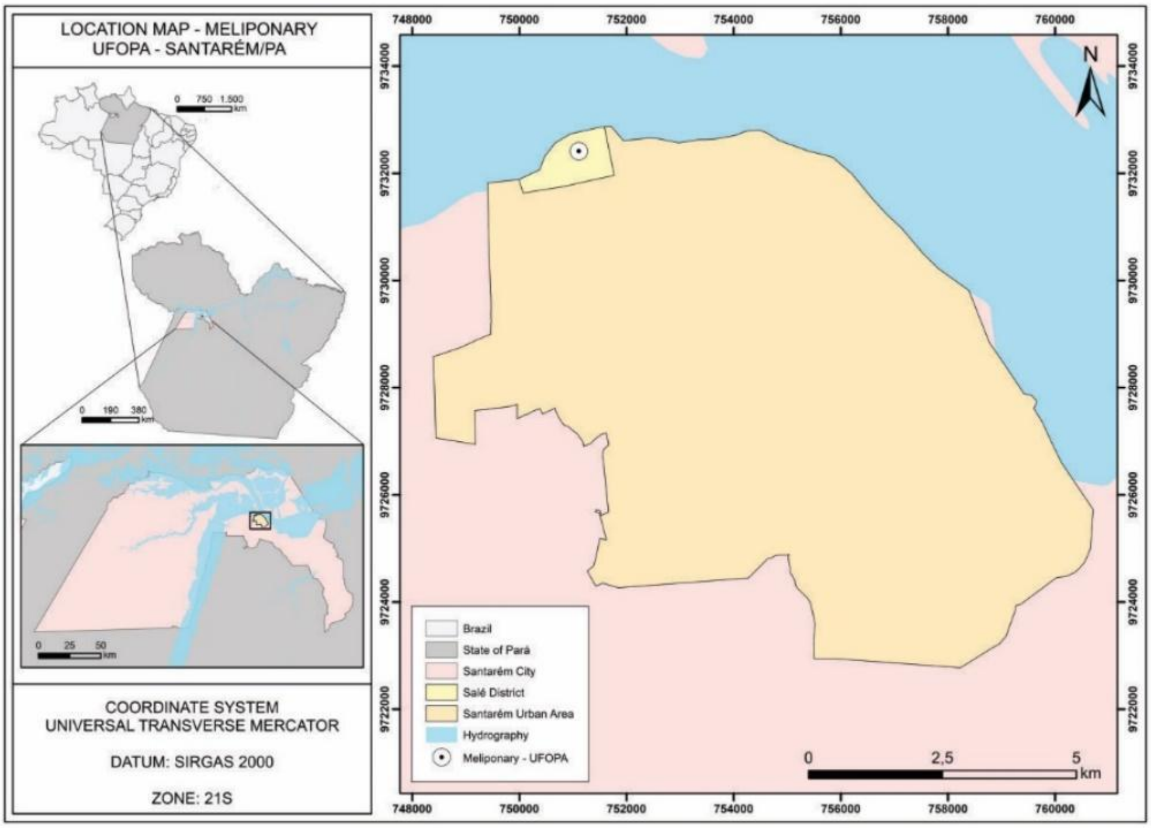

FIGURE 1. Federal University of Western Pará (UFOPA) and meliponary.

The study area comprises about 12ha and has vast vegetation with native (mostly) and exotic herbaceous, shrub and tree species, with more than 100 species distributed in about 50 botanical families (SOUZA *et al.* 2019). This study area is adjacent to the archaeological site listed by IPHAN, the Porto/Vera Paz site, with an area of about 30,000 m^2 (SCHAAN & LIMA 2010).

Propolis and bee collection and identification

Propolis was collected monthly, from April 2018 to March 2019, excluding May 2018, as it was not possible to open bee boxes due to the abundance of rain in the region. However, for the chemical analyses only two months were chosen (Apr/Aug), a rainy Amazonian period and a dry one. Propolis samples were always collected from the same bee boxes.

To identify the bees, 10 individuals were collected using an entomological net. The bees were stored in plastic containers with lids, fixed and labeled according to entomological standards, and sent to the Laboratory of Bionomy, Biogeography and Insect Systematics (BIOSIS) at the Federal University of Bahia (UFBA), for identification by Professor Doctor Favízia Freitas de Oliveira, who is a specialist in the area.

Preparation and analysis of the material for melissopalynology

To analyze pollen grains from propolis, the methodology of MATOS et al. (2014) was used, with modifications, instead of EtOH (95%) we used acetone P.A. (as it facilitated the dilution of propolis due to its fatty acids). Thus, propolis samples (approximately 5g) were grounded and stored in PA acetone for 24 hours. Preparations were centrifuged (10 min, 2.500 rpm) in order to gather solid residues. Sediments were treated by KOH solution 10% (20 mL), boiling for 10 min. At room temperature, preparations were centrifuged in order to concentrate solid residues. After centrifugation, sediments were treated by acetolysis methodology (ERDTMAN 1952).

After the acetolysis process, at least three slides of each sample were prepared using Kisser glycerin gelatin (SALGADO-LABOURIAU 1961 *apud* 1973) and sealed with paraffin (J. Müller modified in ERDTMAN 1952). After the analysis, the slides were deposited in the pollen collection at the Botany and Palynology Laboratory (LABOP) of the Institute of Biodiversity and Forests (IBEF) of Federal University of Western Pará (UFOPA).

Following the methodology of LOUVEAUX *et al.* (1978), at least 500 pollen grains were counted under optical microscopy. After microscopic analysis, photomicrographs were taken (immersion 100x) under Zeiss microscope with a AxioCamc5s camera and pollen grains were measured with a ruler attached to the eyepiece and Zen 2012 software, which was also used to make the measurements.

Subsequently, pollen types were grouped into four relative frequency classes: dominant pollen (≥45%), accessory pollen (15 to 44%), important isolated pollen (3 to 14%) and occasional isolated pollen (<3%) (LOUVEAUX *et al.* 1978).

To identify pollen types, comparisons were made based on specialized bibliographic references and on the LABOP pollen collection, being classified to family, genus and/or species. For the taxonomic characterization of pollen grains, the concept of "pollen type" proposed by JOOSTEN & KLERK (2002) and KLERK & JOOSTEN (2007) was used.

The terminology adopted follows BARTH & MELHEM (1988) and PUNT *et al.* (2007). For better visualization, results were presented in tables made in Microsoft® Excel® 2010. Palynograms were made using the Corel DRAW18® program.

Preparation of hexanic extract from propolis

In each month, April and August 2018, about 14 grams of propolis were collected from *Scaptotrigona nigrohirta*. After collection, the propolis was refrigerated and taken to the Laboratory of Research and Development of Bioactive Natural Products (PDBio) of Federal University of Western Pará (UFOPA). The low polarity compounds were extracted from propolis by hexane extraction at 40ºC, with the material in contact with the hexane for 4 hours under a closed system so the solvent did not evaporate. After this process, the material was filtered and immediately passed through a rotary evaporator to obtain the crude hexanic extract. We followed the extract preparation methodology according to MACÊDO *et al.* (2020), with adaptations.

Chromatographic analysis

An amount of 100 milligrams of hexanic extract were sent for chromatographic analysis in CG-MS to the Laboratory of Organic and Pharmaceutical Chemistry at the Center for Chemical, Biological and Agronomic Research - CPQBA- UNICAMP. For this analysis, a 1 μL aliquot of the hexane extract was submitted to gas chromatographic analysis coupled to mass spectrometry under the following conditions: CG-MS Agilent, HP-6890 model coupled to selective mass detector, HP-5MS capillary column (30m x 0.25mm x 0.25 μm). Temperatures: injector = 220° C, detector = 250°C, column = 60° C, 3°C min^{-1}, 240°C (20 min) and carrier gas = He 1.0 $mL.min^{-1}$ (MACÊDO *et al.* 2020). The components of hexanic extracts were identified using the NIST library by comparing the retention rates calculated with those in the literature (ADAMS 2007).

Multivariate analysis

A multivariate analysis was performed with the relationships among the volatile compounds, pollen types, and the months analyzed (April and August) using Nearest Neighbor and Euclidean (Pythagorean) similarity coefficients. A Cluster Analysis Dendrogram and a Principal Component Analysis (PCA) plot were generated. These analyses were performed using PC-ORD Version 5.31 software (McCUNE & MEFFORD 2011).

RESULTS

Melissopalynological analysis

Analysis of the propolis slides from the *Scaptotrigona nigrohirta* bee revealed 6,013 pollen grains. Among them, there were 63 pollen types, distributed across 22 families (Table 1).

The Fabaceae family represented 38.84% of this total, while Anacardiaceae represented 19.24% and Myrtaceae 14.23% (Figure 2). Together, these families represent 72.31% of all pollen collected by *Scaptotrigona nigrohirta* between April 2018 and March 2019, and are therefore the most abundant families. Fabaceae was represented by 27 pollen types, while Anacardiaceae and Myrtaceae were represented by 6 and 4 pollen types, respectively (Table 1).

Table 1. Frequency of pollen types found in propolis samples of *Scaptotrigona nigrohirta.* Dominant Pollen (≥45%); Accessory Pollen (15-44%); Important Isolated Pollen (3-14%) and Occasional Isolated Pollen (<3%).

Families	Pollen types	Percentage of the months April to March										
		Apr	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Amaranthaceae												
	Alternanthera type	-	-	-	-	0.17	0.17	-	-	2.28	-	-
Amaryllidaceae												
	Amaryllidaceae type	-	-	1.10	-	-	-	-	-	-	-	-
Anacardiaceae												
	Anacardiaceae type 1	18.79	-	-	-	-	-	-	-	-	-	-
	Anacardiaceae type 2	-	-	22.05	-	-	-	-	-	-	-	-
	Anacardiaceae type 3	-	-	0.91	10.70	8.02	-	-	8.10	1.71	4.35	3.75
	Mangifera indica	-	9.88	-	-	-	-	23.49	10.49	-	-	-
	Spondias mombin	-	31.55	7.90	-	-	17.08	7.84	3.86	4.95	-	4.12
	Tapirira guianensis	-	-	-	-	-	-	-	-	6.09	-	-
Arecaceae												
	Arecaceae type 1	-	-	-	-	-	2.51	-	-	-	-	-
	Arecaceae type 2	-	-	-	-	-	-	-	9.57	-	-	-
	Arecaceae type 3	-	-	-	-	-	-	-	-	-	2.57	-
Asteraceae												
	Tridax procumbens	-	-	-	-	-	-	-	-	0.19	-	-
Araliaceae												
	Schefflera type	-	-	-	5.35	-	1.61	-	-	3.42	-	2.81
Bignoniaceae												
	Handroanthus type	-	3.04	-	-	-	-	-	-	-	-	-
Burseraceae												
	Protium heptaphyllum	3.78	1.71	3.12	6.04	2.26	1.43	1.82	1.47	2.47	1.18	2.06
Combretaceae												
	Terminalia catappa	-	0.76	-	-	-	0.17	-	-	-	-	-

Commelinaceae												
	Commelinaceae type	-	-	-	-	-	-	0.36	-	-	-	-
Euphorbiaceae												
	Alchornea type	0.51	-	-	-	-	-	-	-	-	-	-
	Euphorbiaceae type 1	-	-	-	-	-	-	1.55	-	-	-	-
Fabaceae												
	Aeschynomene type	-	-	-	-	-	-	-	-	-	-	0.37
	Bowdichia virgilioides	1.03	-	-	10.88	-	-	0.91	-	-	-	-
	Clitoria fairchildiana	0.17	-	-	-	-	-	-	-	-	-	-
	Copaifera martii	4.64	4.18	7.72	2.59	23.19	17.98	30.02	19.33	17.71	17.62	18.01
	Desmodium type	-	-	0.36	-	-	-	-	-	-	-	-
	Diplotropis purpurea	-	0.38	-	-	-	-	-	-	-	-	-
	Fabaceae type 1	-	4.75	-	-	-	-	-	-	-	-	-
	Fabaceae type 2	-	-	4.59	-	-	-	-	-	-	-	-
	Fabaceae type 3	-	-	-	0.69	-	-	-	-	0.57	-	1.68
	Fabaceae type 4	-	-	-	-	11.16	-	-	0.55	-	-	-
	Fabaceae type 5	-	-	-	-	4.18	-	-	-	-	-	-
	Fabaceae type 6	-	-	-	-	1.39	-	-	-	-	-	-
	Fabaceae type 7	-	-	-	-	1.74	-	-	-	-	-	-
	Fabaceae type 8	-	-	-	-	-	-	-	4.23	-	-	-
	Fabaceae type 9	-	-	-	-	-	-	-	1.47	-	-	-
	Fabaceae type 10	-	-	-	-	-	-	-	2.94	8.57	-	-
	Fabaceae type 11	-	-	-	-	-	-	-	-	-	8.57	-
	Fabaceae type 12	-	-	-	-	-	-	-	-	-	2.37	-
	Fabaceae type 13	-	-	-	-	-	-	-	-	-	4.75	-
	Fabaceae type 14	-	-	-	-	-	-	-	-	-	-	1.87
	Fabaceae type 15	-	-	-	-	-	-	-	-	-	-	5.06
	Inga type	0.17	0.19	0.18	-	0.52	-	-	-	-	0.39	-
	Mimosa tenuiflora	0.51	-	-	-	0.17	-	-	-	-	-	2.06
	Papilionoideae type	-	-	-	-	-	-	-	-	-	2.17	-
	Schizolobium amazonicum	-	-	-	-	-	-	-	-	-	-	4.31
	Stylosanthes type	0.68	-	2.02	1.55	-	-	-	-	-	-	-
	Tamarindus indica	15.49	27.94	15.99	26.42	13.08	20.50	12.95	11.04	5.90	9.50	10.88
Gentianaceae												
	Gentianaceae type	-	-	-	-	-	-	-	-	3.80	-	-
Malpighiaceae												
	Byrsonima type	18.93	-	-	-	-	-	-	9.20	-	-	-
	Malpighiaceae type 1	-	-	-	9.32	-	-	-	-	-	-	-
Malvaceae												
	Ceiba type	-	-	-	-	-	0.17	-	-	-	-	-
Myrtaceae												
	Eugenia brasiliensis	-	3.42	9.37	1.76	9.59	16.00	7.29	9.94	5.52	-	0.93
	Myrcia splendens	20.66	-	-	-	-	-	-	-	-	9.90	13.13

	Psidium cattleyanum	0.51	6.69	-	-	0.17	0.53	4.92	1.11	2.28	7.52	1.50
	Psidium guajava	-	-	-	-	-	-	8.85	-	11.99	-	-
Polygona-ceae												
	Triplaris weigeltiana	-	-	-	-	-	2.87	-	-	1.90	-	-
Poaceae												
	Poaceae type	-	-	-	-	-	-	-	-	-	-	3.75
Rubiaceae												
	Borreria verticillata	2.23	-	1.65	7.59	1.91	0.82	0.54	0.55	0.76	0.59	0.56
	Ixora type	-	-	-	-	-	-	-	-	-	7,12	
	Mitracarpus strigosus	11.90	-	18.19	8.29	2.74	12.23	1.64	2.02	2.66	12.47	1.31
Salicaceae												
	Casearia type	-	-	-	-	-	-	-	2.94	7.76	1.98	8.63
Sapinda-ceae												
	Serjania type	-	-	0.18	-	-	-	1.45	0.18	-	-	-
	Sapindaceae type 1	-	-	-	-	-	0.17	-	-	9.47	-	-
Solanaceae												
	Solanaceae type	-	-	-	-	0.52	-	-	-	-	-	-
Indetermi-nate		0	1	0	0	3	1	2	1	0	2	3
Indetermi-nate (%)		0	5.51	0	0	19.19	5.75	3.10	0.92	0	6.95	13.21
	Total (%)	100	100	100	100	100	100	100	100	100	100	100

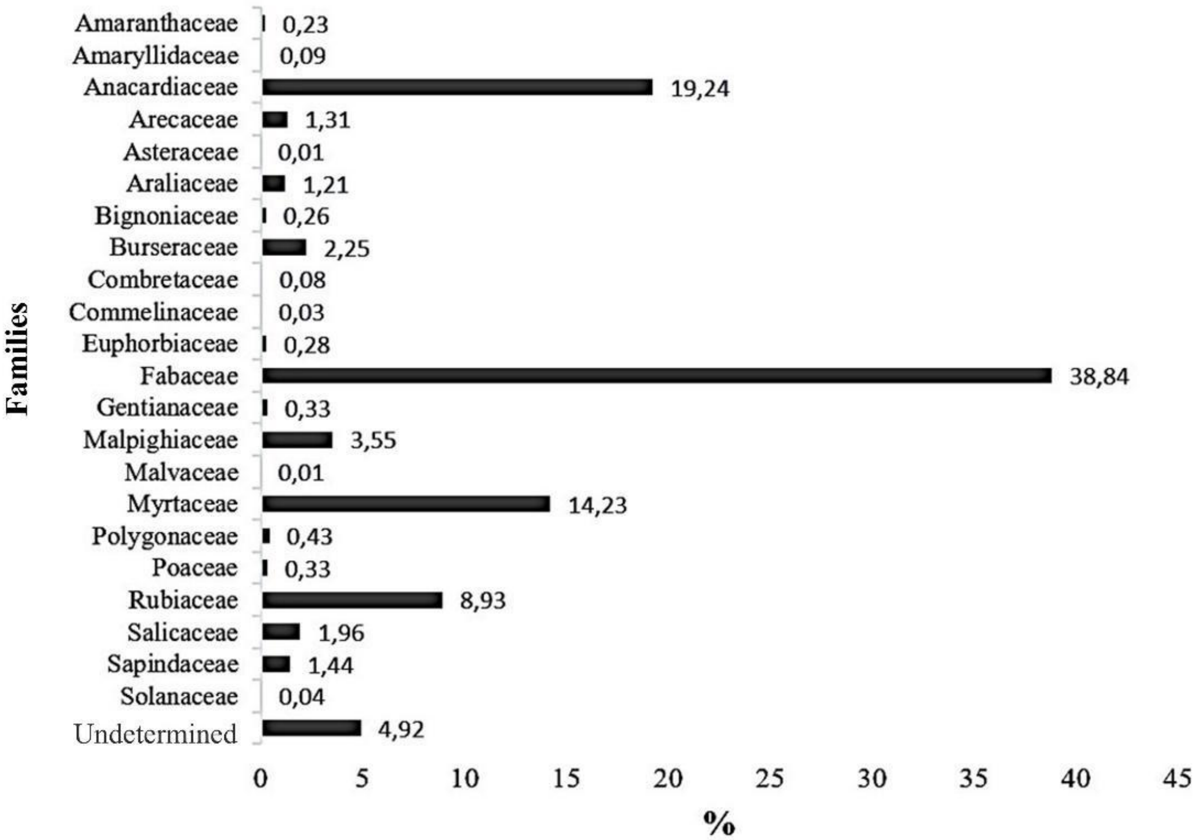

FIGURE 2. Percentage of families found in the propolis samples of *Scaptotrigona nigrohirta* from the meliponary of the Tapajós Campus of the Federal University of Western Pará (UFOPA) from April 2018 to March 2019.

Among pollen types, 36 of them appeared only once, that is, the equivalent of only one (1) per month (Table 1), while only 3 occurred during all months: *Copaifera martii*; *Protium heptaphyllum* and *Tamarindus indica*. The Poaceae family appeared in small numbers and was the only one with anemophilous pollination found in the March sample (3.75%). Bignoniaceae ("Ipê") was only found in June (3.04%) (Table 1).

The species with the highest quantities and frequencies stood out from the others due to their proximity to the meliponary, never exceeding 500 meters, which were: *Tamarindus indica*, *Copaifera martii*, *Mitracarpus strigosus*, *Eugenia brasiliensis* and *Protium heptaphyllum*.

No dominant pollen types were found (≥45%). Nevertheless, an abundance of accessory pollen (15 to 44%) was observed, e.g., *Spondias mombin* (Figure 3k) in June with 31.55% and October with 17.08%. The species *Copaifera martii* (Figure 3d-f) presented high amounts from September to March and a more significant result in November with 30.02% (Table 1).

Other accessory pollen included: *Tamarindus indica* (Figure 3l), which had significant results from April to October, except in September, and the highest result in June (27.94%), followed by *Mangifera indica* (Figure 3h) in November (23.49%); Anacardiaceae type 2 (Figure 3b) in July (22.05%); *Myrcia splendens* in April (20.66%) (Figure 3i); *Byrsonima* type (Figure 3c) in April (18.93%); Anacardiaceae type 1 (Figure 3a) in April (18.79%); *Mitracarpus strigosus* (Figure 3j) in July (18.19%) and *Eugenia brasiliensis* (Figure 3g) in October (16%).

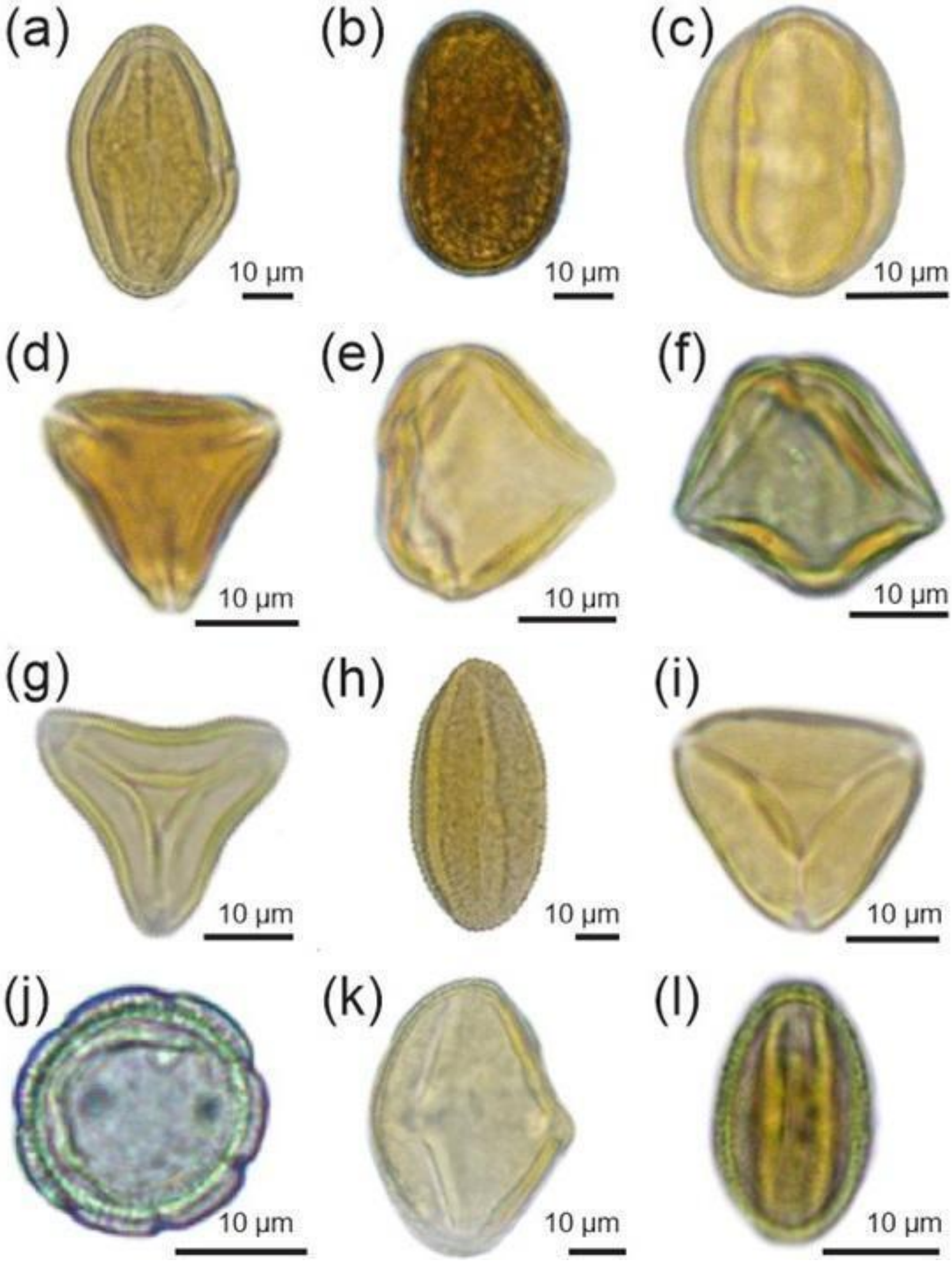

FIGURE 3. Pollen types found in *Scaptotrigona nigrohirta* propolis.

Regarding the important isolated pollen (3 to 14%), many pollen types were found, with *Psidium guajava* standing out in January (11.99%) and *Bowdichia virgilioides* in August (Table 1).

Chemical analysis

Concerning chemical analysis, the yield of the hexane extract was 35.75% for the propolis sample of April. Sixteen compounds were found and eleven were identified (Table 2). Among the most relevant metabolites were triterpenoids structures, e.g., cycloartenol (~ 41%), lupeol acetate (~ 24%) and lupenone (~ 7%).

Table 2. Compounds identified in the hexanic extract from propolis of *Scaptotrigona nigrohirta*.

Compounds number	RT (min)	RI	Substance	Area %	
				EH-April	**EH-August**
1	15.257	898	.(8Z)-14-methyl-8-hexadecen-1-ol	0.60	0.23
2	20.685	1348	γ-palmitolactone	0.409	0.088
3	23.947	1427	muscalure	0.301	0,661
4	24.912	1451	6-tridecyl tetrahydro-2h-pyran-2-one	0.482	0.150
5	29.896	1505	α-bulnesene	-	2.205
6	30.256	1598	3-pentadecylphenol	-	3.692
7	33.122	1788	3-(4,7-heptadecadienyl) phenol	-	10.932
8	40.324	1874	olean-12-one	1.432	1.668
9	40.943	1893	β-amirine	4.668	7.281
10	41.165	1899	lanosterol	1.641	2.271
11	41.564	1912	lupenone	7.905	5.426
12	42.303	1935	cycloartenol	41.302	38.375
13	43.747	1980	3β-3methoxy-olean-12-ene	4.118	7.604
14	45.368	2032	lupeol acetate	24.417	3.273
15	50.327	2199	lupeol	7.025	1.340
16	55.018	2369	lupan-3-yl acetate	4.718	1.144
Total				**99.018**	**86.340**

According to the similarity analysis between volatile compounds and pollen types, a dendrogram was generated with 27.46% clustering. Cluster 2 showed the similarity between compound 3-(4,7-heptadecadienyl) phenol and Anacardiaceae type 3. In this cluster there was also the similarity of 3-(4,7-heptadecadienyl) phenol with Malpighiaceae type 1 (Figure 4).

In the third cluster we observed the similarity between 3-pentadecylphenol and *Schefflera* type. This cluster also generated a similarity between α-bulnesene and *Eugenia* type. In the fourth cluster there was similarity between compounds olean-12-one and lanosterol with *Stylosanthes* type (Figure 4).

In the fifth cluster the highest similarity observed was between the compounds β-amyrin and 3β-3methoxy-olean-12-ene with the *Protium heptaphyllum* type. And also with the compounds lupeol and lupan-3-yl acetate with the *Copaifera martii* type (Figure 4).

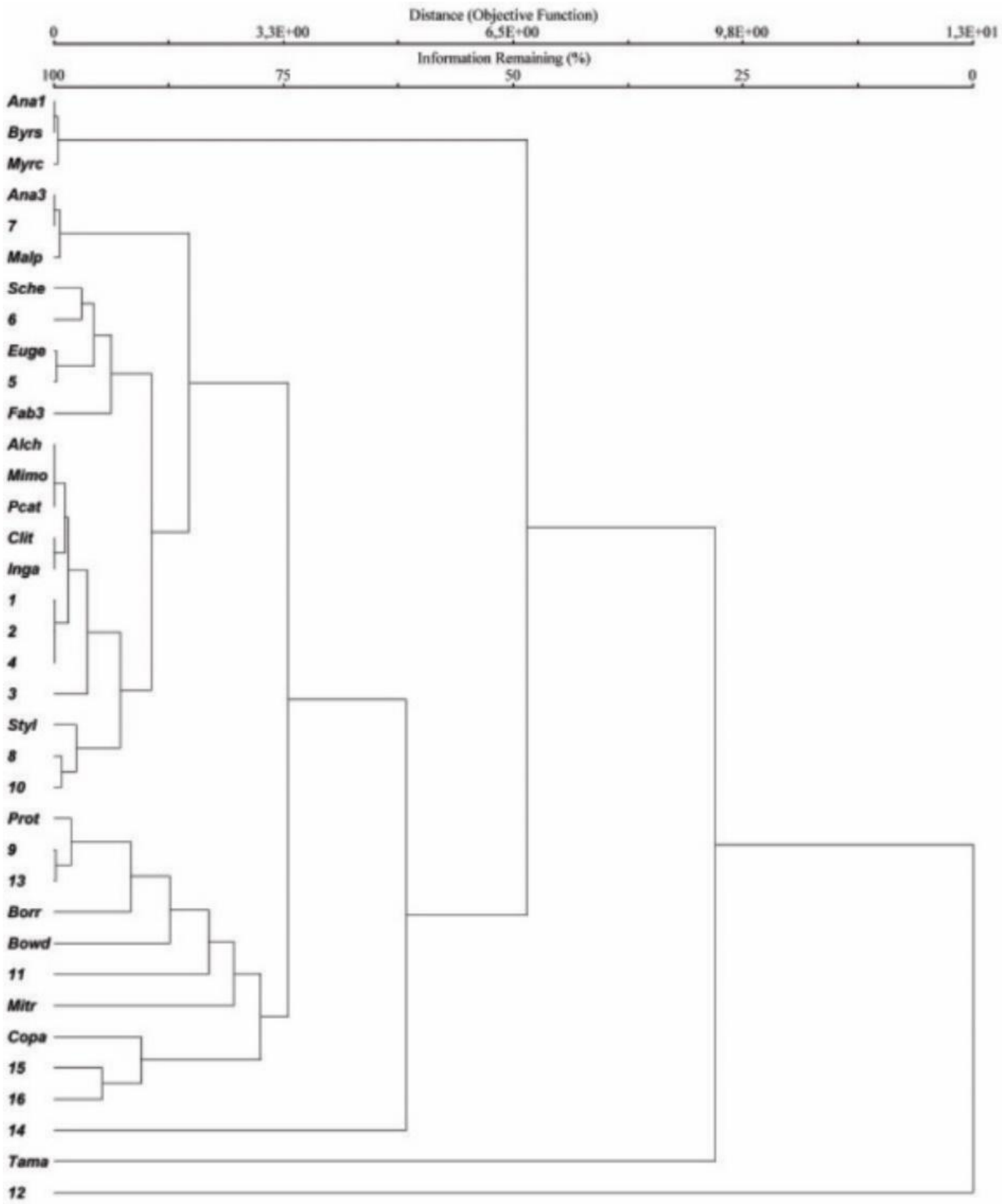

FIGURE 4 Hierarchical cluster analysis of the volatile compounds and pollen types from April and August (2.018) according to quantitative variables. Abbreviations: The volatile compounds are numbered and follow according to the order in Table 2, while the pollen types are formed with their first 4 letters according to Table 1.

The presence of steroids in propolis may be related to the species that serve as a resource for resin collection in the place where the meliponary is located. Therefore, the plants are the sources of these substances and the main responsible for the variations in the constitution of propolis.

Although no similarity of the substances (8Z)-14-methyl-8-hexadecen-1-ol, γ-palmitolactone and 6-tridecyl tetrahydro-2h-pyran-2-one has been found with the pollen types, this grouping with high Euclidean similarity may be related to the structural skeleton of the substances, since they resemble each other by having long-chain hydrocarbon groups.

According to the PCA analysis, we can observe the variables that best represent the pollen types and volatile compounds, such as: *Tamarindus indica*, cycloartenol, *Byrsonima* type, Anacardiaceae type 1, *Myrcia splendens* and lupeol acetate (Figure 5).

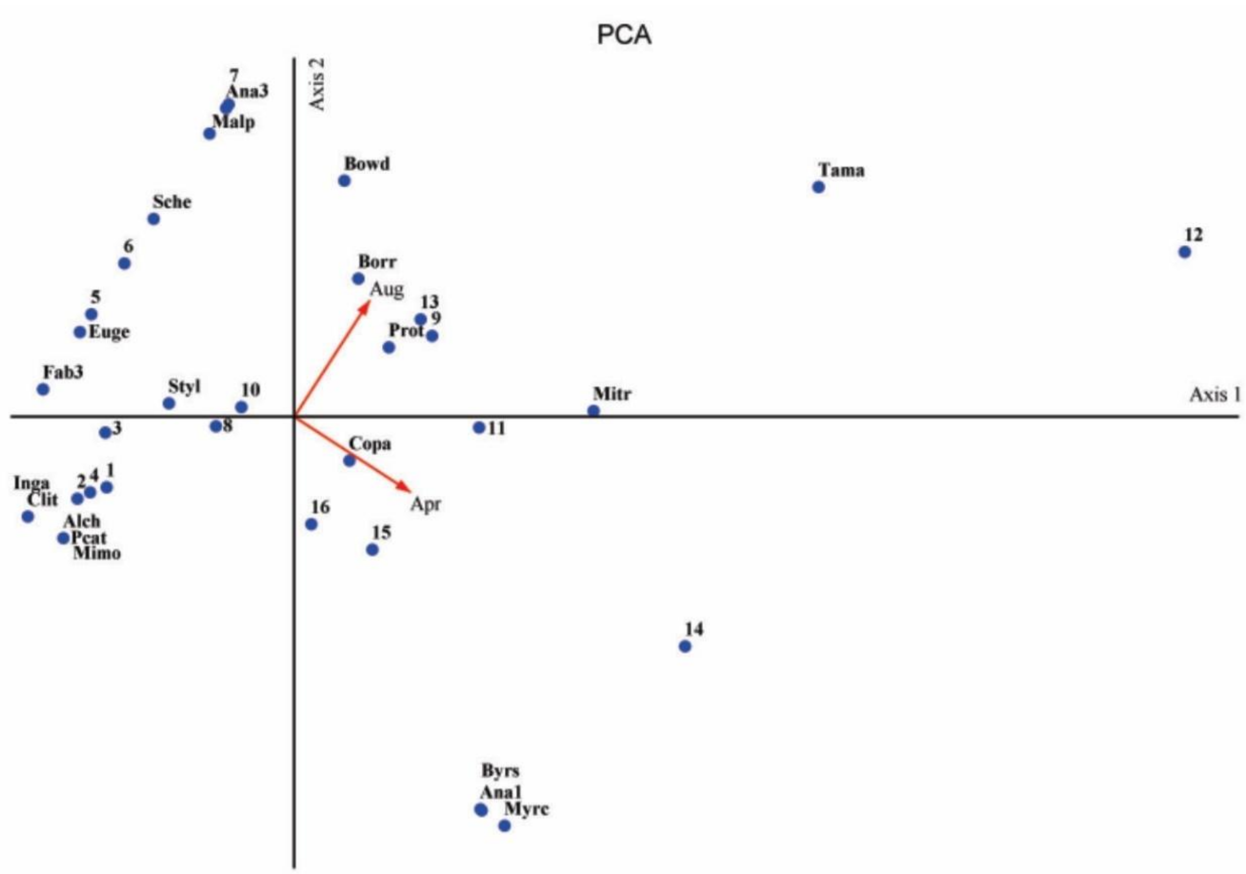

FIGURE 5 Principal component analysis performed with the volatile compounds and pollen types of April and August (2018) according to quantitative variables. The blue points are volatile compounds and pollen types and red arrows are variables. Abbreviations: The volatile compounds are numbered and follow according to the order in Table 2, while the pollen types are formed with their first 4 letters according to Table 1.

DISCUSSION

Melissopalynological discussion

Fabaceae presented the highest number of pollen types, which can be explained by the fact that this family is geographically well distributed in the Amazon and is the third largest botanical family regarding number of species (SOUZA *et al.* 2021). Such data was confirmed by CANTUÁRIA *et al.* (2017), who found the highest number of Fabaceae species and the second highest frequency of specimens (21.4%) in the Environmental Protection Area (APA) of Fazendinha in Macapá - AP.

For the Anacardiaceae family, with 19.24% of the total pollen types, meliponines bees are essential to promote fruiting through pollination due to their size and behavior that are appropriate for the floral structure of these plants, which makes them effective pollinators. One example of this insect-plant interaction of Anacardiaceae species is *Astronium urundeuva* and *Schinopsis brasiliensis*, which stingless bees visit and play an important role in the pollination process (KIILL *et al.* 2010).

BARRETO *et al.* (2006) considered stingless bees as pollinators of *Spondias tuberosa*, which belongs to the same genus as *Spondias mombin* identified herein, with 31.55% in June and the most abundant in this month.

The fruit trees of the genus *Psidium* stand out because guava and *araçás* (*P. guajava* and *P. cattleyanum*) are widely distributed throughout Brazil (CAMPOS 2010). Pollen is the major resource offered by Myrtaceae flowers. The petals and/or stamens are visual attractants to pollinators, but the stamens are usually the most noticeable structures in an open flower. The sweet aroma also plays an important role in attracting pollinators (NIC LUGHADHA & PROENÇA 1996, GRESSLER *et al.* 2006).

In a study conducted in Barra do Corda, Maranhão, by SOUZA *et al.* (2015) with the propolis samples of *Scaptotrigona* aff. *postica* Latreille, 1807, 94 pollen types belonging to 35 families were identified. Of this total, the most frequent pollen types in the samples were: Myrtaceae (38.37%), Rubiaceae (34.18%), Fabaceae (20.4%) and Anacardiaceae (7.7%). And regarding the flight area, a flight area of 750m was found for Scaptotrigona postica (NOGUEIRA-NETO 1997). As observed around the studied meliponary there is a similar radius, which serves as pasture for the bees.

The large number of entomophilic pollen types (pollination by insects) found in these propolis samples occurred due to the contact of bees with flowers of various species and by the fact that bees of the Meliponini tribe have polylectic behaviors, i.e., collect pollen or nectar from flowers of several plant species (DIAS 2015). An important role is found in the interaction of bees with plants when they use floral resources and in pollination itself, favoring the maintenance of bee colonies (SOUZA 2015).

The pollen types that appeared only once a month may indicate seasonal characterization for the propolis production of *Scaptotrigona nigrohirta* in relation to the place where the meliponary is installed, thus making it possible to manage production. If seasonal variation and phytogeographic origin determine the chemical and physical composition of propolis and its biological activities, this could be an important factor (AGUERO *et al.* 2010, SIMÕES-AMBROSIO *et al.* 2010).

The small percentages of Poaceae and Bignoniaceae found were probably caused by contamination of propolis samples through the air (BARROS *et al.* 2013), because pollination is anemophilous for Poaceae, while Bignoniaceae flowers have tubular-infundibuliform corollas (LORENZI & MATOS 2002). Nevertheless, these bees do not tend to collect from flowers with this long corolla shape, because they tend to specialize to collect nectar from short corolla flowers, making it easier for bees to collect nectar (SANTANA & MACHADO 2010) because they have small size.

On the other hand, the genus *Ixora*, despite having extremely tubulous flowers, has free stamens and facilitates collection by bees. In species of the Solanaceae family in which the anther opening is poricidal, bees such as *Trigona spinipes* collect pollen by inserting their proboscis through the pores to remove pollen or drilling holes in the anthers and then removing the pollen with the proboscis (SILVA *et al.* 2010).

Chemical discussion

The major substances found in the hexanic extract of the propolis were exclusively from plants, confirming the interspecific animal-plant interaction, with many triterpenes and steroids (phytohormones) that naturally occur in vegetables. Phytohormones are known to promote plant growth and development, therefore, they actively participate in plant cells, pollen grains and their tissues, and consequently in all plant organs.

Regarding chemical analyzes, the triterpenes β- amyrin and lanosterol were found by CG/MS in propolis of the "Jataí" bee from southern Brazil (VELIKOVA *et al.* 2000) and ethanolic extract from propolis from São Paulo (MARCUCCI 1998), which were also recorded herein. Lupeol and lupenone also were encountered in dichlorometan extract from propolis samples from Minas Gerais (PEREIRA *et al.* 2002). In ethanolic extracts from Egyptian propolis, the presence of β- amyrin, lanosterol, cycloartenol and lupeol was reported, corroborating the findings of this work (ABD EL HADY & HEGAZI 2002). Another substance found in a small amount in the hexanic extract was muscalure (0.301%), also known as Z-9-trichoene, which is a sexual pheromone that has already been detected in housefly (*Musca domestica*) (VERBA *et al.* 1985).

Triterpenes are substances that have anti-inflammatory (REYES *et al.* 2006), antiulcerogenic and antimicrobial activities (ANDRADE *et al.* 2008). Cycloartenol is a triterpene naturally found in plants and a precursor in the biosynthesis of stanols and sterols, possessing anti-inflammatory activities, while lupeol acetate is classified as a pentacyclic triterpene with anti-inflammatory properties (RAMIREZ *et al.* 2004, LIMA *et al.* 2007, SUDHAHAR *et al.* 2008). This may also support the anti-inflammatory properties attributed to propolis.

Muscalure is reportedly a pheromone widely used in communication between bees. Pheromones are often used by bees to coordinate the activities of hive members (THOM *et al.* 2007). The sense of smell of these insects plays an important role in communication, being limited to aromas acquired from the environment and on route to the floral food sources (FARINA *et al.* 2005).

The metabolite 3-(4,7-heptadecadienyl) phenol is a lipid phenolic structure commonly found in resins from genera of the Anacardiaceae family, also called anacardic acid derivatives, detected in *Apis mellifera* propolis (SILVA *et al.* 2008). Studies have already reported the presence of cardinois in apolar extracts originating from *Scaptotrigona postica* propolis (NEGRI *et al.* 2019). According to BUCHMANN (1987), among the plants of importance to the Meliponini tribe are the species of the Malpighiaceae family.

Reports of triterpenes of the oleane, ursane and euphane series present in the oily sap of the genus *Protium* are common (BANDEIRA *et al.* 2002). The triterpene β-amirine is commonly found in both the resin and leaves of this genus, in addition to lupeol and lupenone (BANDEIRA *et al.* 2002, GUIMARÃES & SIANI 2007). There are few chemical reports in the literature for *Copaifera martii*. However, lupeol and lupeol acetate have already been found in leaf extracts of one species of this genus, uggesting that other *Copaifera* species may be sources of these triterpenes (CARVALHO *et al.* 2019).

Chemical studies on leaf, bark and fruit extracts of *Tamarindus indica* have already shown the detection of triterpenes and steroids in these extracts (UKWUANI & HASSAN 2014, ABDALLAH & MUHAMMAD *2018*). In the chemical study proposed by KHANZADA *et al.* (2008), cycloartanol was identified and can be considered as a precursor of cycloartenol.

Studies with chloroform extracts of a species of the genus *Byrsonima* have already led to the identification of the triterpenes lupenone, b-amyrin and lupeol, and the fractionation of the ethanolic extract of the leaves of *Byrsonima gardneriana* led to the isolation of the lup-20(29)-en-3-ol a molecule precursor for lupeol acetate (ROLIM *et al.* 2013), which may suggest the correlation found.

In the Anacardiaceae family there are reports on the presence of the triterpene lupeol (CHAVES *et al.* 2010), a molecule that is also a precursor for lupeol acetate. As for the triterpenes found in *Myrcia*, we have the derivatives of betulinic acid (CASCAES *et al.* 2015), which are structures analogous to lupeol acetate.

CONCLUSION

Overall, the bee *Scaptotrigona nigrohirta* is a generalist species that visits several plant species, making its propolis well diversified. We observed higher percentages of pollen from *Spondias mombin*, *Mangifera indica*, *Copaifera martii* and *Myrcia splendens*, and from the Fabaceae, Anacardiaceae and Myrtaceae families. Chemical analyses showed that the major constituents of propolis were cycloartenol, lupeol acetate and lupenone triterpenes that are naturally found in plants. Therefore, this study contributes to the physicochemical knowledge of the propolis of this bee, meets our expectations regarding the objectives and will help the socio-economic activities of producers in the lower Amazon region.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, Graciene Conceição dos Santos, Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira; software and validation, Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira; investigation, Erlison Silva Souza; Graciene Conceição dos Santos; Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira resources and data curation, Erlison Silva Souza; Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira; writing-original draft preparation, Erlison Silva Souza and Ana Beatriz Fonseca de Oliveira; writing-review and editing, Graciene Conceição dos Santos; Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira; visualization, Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira x, x and x; supervision, Vanesa Holanda Righetti de Abreu; project administration, Graciene Conceição dos Santos; Vanesa Holanda Righetti de Abreu and Kelly Christina Ferreira; funding acquisition, (This work was not supported by any funding agency). All authors have read and agreed to the published version of the manuscript.

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Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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