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The presence of Fabaceae in the pollen profile of propolis produced in northeastern Brazil

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ABSTRACT

Propolis is a resin-like substance composed mainly of resin, wax, essential oils, pollen grains and specific plant parts collected by the honeybee *Apis mellifera*, which mixes them with fluids they secrete. The components and chemical properties of propolis vary among regions. Therefore, in order assess variation in the botanical composition of propolis, 26 samples of propolis (brown, red, and green) produced throughout northeastern Brazil were analyzed by acetolysis specifically adapted for propolis. In total, 196 pollen types were recorded, representing 123 genera and 47 families, with types of Fabaceae and Rubiaceae being present in 100 % of the samples. Fabaceae was the richest group with 49 pollen types, followed by Malvaceae (10 types), particularly related to the high frequencies of *Mimosa pudica* (84.62 %), *Alternanthera*, *Borreria verticillata*, and *Myrcia* (80.77 %). Remarkably, 34 % of the pollen types with frequencies above 50 % belonged to Fabaceae, even though this family has been traditionally regarded as less important with regard to propolis production, given that most of its included taxa are classified as polliniferous or nectariferous. Similarity analyses revealed clusters of propolis samples that share pollen types associated with plants having apiculture potential.

Keywords: Apiculture, *Apis mellifera*, beekeeping, palynology, pollen grains

Introduction

Pollen analysis is used to establish the botanical origin of propolis. In general, propolis is composed of 50-60 % resins and balsams, 30-40 % wax, 5-10 % essential oils, and 5 % pollen grains, along with microelements such as aluminum, calcium, strontium, iron, copper, manganese, and small amounts of vitamins B1, B2, B6, C, and E (Matsuno 1995; Pietta *et al.* 2002; Funari & Ferro 2006).

The presence of pollen grains in propolis is usually related to their transportation via wind and by their adherence to vegetal resins that represent the major component of propolis. Therefore, the identification of the pollen grains in propolis is regarded as an indirect indicator of both its botanical and geographic origin (Borges *et al.* 2006).

Brazil is the third largest producer of propolis worldwide, reaching up to 150 tons/year. About 75 % of this total is exported mainly to Japan, followed by USA, Germany, and China, creating an income of around R$300 million per year (Braga 2009). Indeed, the demand for propolis in the international market has been increasing annually as a result of its popularization as a natural medicine by several studies that have attributed to it antioxidant, antitumor, antimicrobial, and antiulcer properties (Braga 2009).

Determining the geographic and botanical origin and the chemical composition of propolis is important inasmuch as these parameters assure quality control of Brazilian

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propolis in the marketplace (Alencar 2002; Teixeira *et al.* 2003). The propolis produced in Brazil is very diverse, putatively because of the presence of distinct biomes with unique and diverse flora, according to regional samples. Therefore, 13 types of propolis are recognized according to their physicochemical traits and biological properties (Park *et al.* 2002; Alencar *et al.* 2005).

Nonetheless, beekeepers discriminate only three basic types of propolis based on their coloration. Green propolis is produced mainly from *Baccharis dracunculifolia* (Asteraceae), and is rarely found in northeastern Brazil. Red propolis is mainly associated with *Dalbergia ecastophylum* (Fabaceae), and is produced mostly in the states of Sergipe and Alagoas in northeastern Brazil. Brown propolis is the most common type, and is produced from several vegetal species (Alencar *et al.* 2005). Both brown and red propolis account for most of the propolis productivity in northeastern Brazil (Park *et al.* 2000).

Several recent studies have employed distinct techniques with the aim of effectively identifying the origin of propolis produced in tropical areas. Pollen analysis has found species of the family Fabaceae to represent a major and ubiquitous component of pollen grains (Matos *et al.* 2014; Matos & Santos 2017), since this family is well represented in regional ecosystems.

Given the significant presence of the family Fabaceae in the flora of northeastern Brazil, particularly throughout the semiarid region (Queiroz 1999), and the abundance of pollen types associated with this family in propolis samples, the goal of the present study was the analyze the pollen grains in propolis samples produced in northeastern Brazil, with particular emphasis on the family Fabaceae, which includes several species useful to indicate vegetation.

Materials and methods

A total of 26 propolis samples produced from June 2016 to July 2017 by small producers and apiculture cooperatives were analyzed. These samples comprised the main types of propolis produced in northeastern Brazil and include 22 samples of brown propolis, three of red propolis and one of green propolis (Fig. 1).

The samples were obtained from several states in northeastern Brazil, including Alagoas (municipalities of Delmiro Gouveia, and Marechal Deodoro), Bahia (municipalities of Alagoinhas, Araci, Caetité, Nilo Peçanha, Paripiranga, São Domingos, and Teofilândia), Ceará (municipalities of Campos Sales and Mombaça), Maranhão (municipalities of Caxias and Codó), Paraíba (municipality

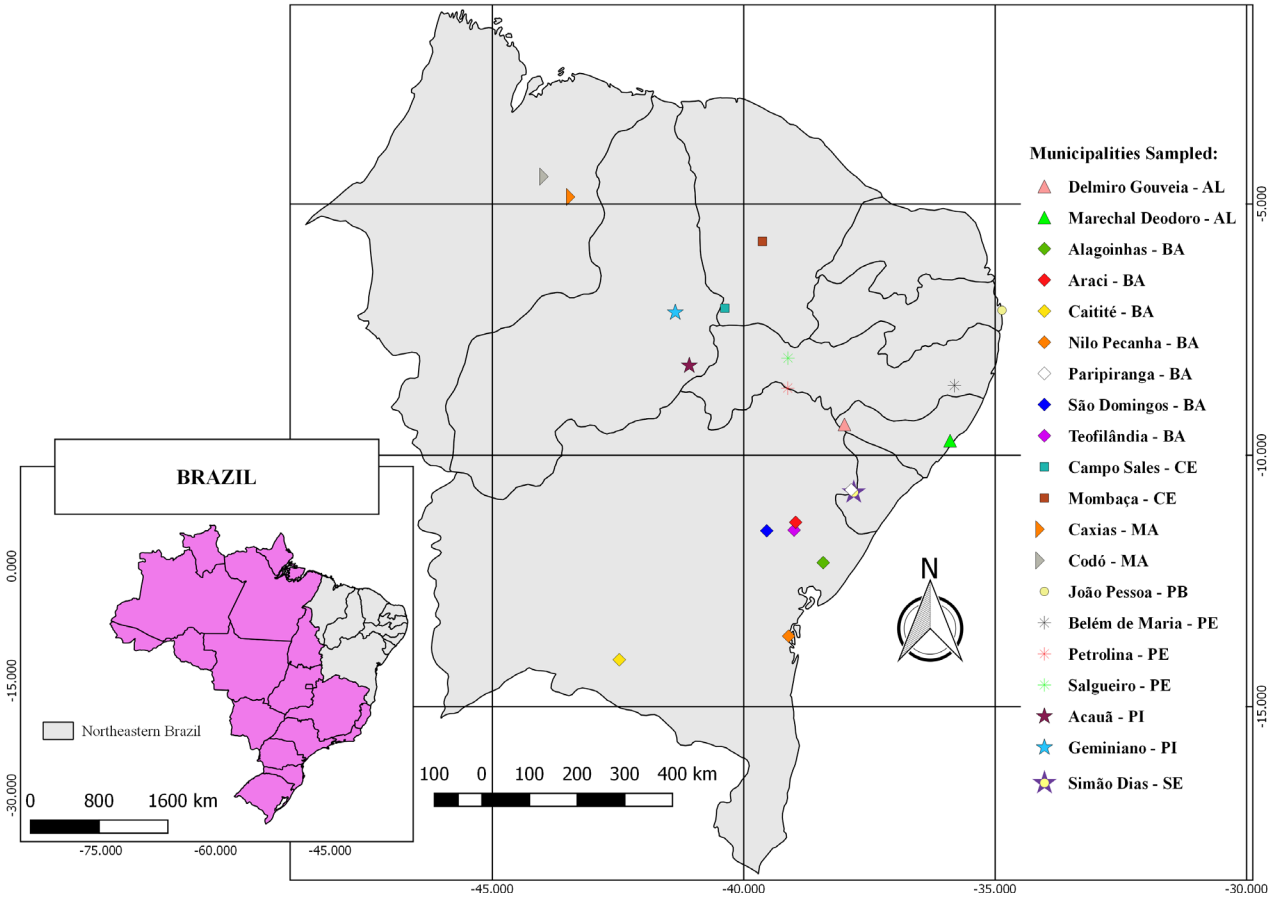

Figure 1. Map showing the location of the propolis sample collection in Northeastern Brazil.

of João Pessoa), Pernambuco (municipalities of Belém de Maria, Petrolina, and Salgueiro), Piauí (municipalities of Acauã and Geminiano) and Sergipe (municipality of Simão Dias).

The main criterion for the collection of propolis samples was the location of apiaries in areas of distinct phytophysiognomies that comprise the biomes of northeastern Brazil, with a minimum distance of 100 km between areas.

The samples were collected by shaving the internal walls of boxes, borders, nests, and roofs of beehives, and then stored hermetically in tagged containers for further analyses at the Plant Micromorphology Laboratory (LAMIV) at Universidade Estadual de Feira de Santana (UEFS).

Pollen analysis followed the procedure reported by Matos *et al.* (2014), in which a subsample of 0.5 grams of propolis was separated for dilution for at least 24 hours in 10 ml of absolute ethanol. The preparations were then centrifuged (2500 rpm for 10 min) and the resulting pellets boiled in 10 % KOH for 5 minutes in a moist chamber, followed by another centrifugation and a wash in distilled water. The material was then filtered twice by discarding the water and placed in 5 ml of glacial acetic acid were it was kept for 2 to 12 hours. The pellets were subsequently submitted to acetolysis in a moist chamber at up to 80 °C for nearly 2 min (Erdtman 1960). The preparations were then washed in distilled water, centrifuged and stored for 1 to 24 hours in a 50 % glycerin aqueous solution. The pollen sediment was mounted on slides (five per sample), with glycerin jelly, for microscopic analysis.

In order to determine the frequency distribution of pollen types (taxa) in the collected samples, we followed the parameters established by Jones & Bryant (1996), as follows: >50 % = "very frequent"; 20-50 % = "frequent"; 10-20 % = "slightly frequent", and <10 % = "rare". The relative frequency of pollen types in each sample was based on counts of at least 500 grains of pollen per sample, according to Santos (2011).

The pollen grains were identified by comparisons with slides stored in the pollen collection of the Plant Micromorphology Laboratory (LAMIV), pollen atlases and literature reports (Roubik & Moreno 1991; Colinvaux *et al.* 1999; Carreira & Barth 2003). The pollen types followed those recommended by Santos (2011).

Analysis of floristic similarity among samples was performed by constructing a dendrogram using the software PAST-Palaeontological Statistics, ver. 1.89 (Hammer *et al.* 2001) with Jaccard's similarity coefficient, since it disregards shared absences as similarities.

Results

Pollen analysis of propolis samples revealed 196 pollen types. Of this total, 172 types were taxonomically identified and comprised 123 genera distributed among 47 botanical families (Tabs. 1-3). The most frequent (≥50 %) pollen types included: *Mimosa pudica* (84.62 %); *Alternanthera*, *Borreria verticillata* and *Myrcia* (80.77 %); *Mimosa tenuiflora* (76.92 %); *Eucalyptus* and *Schinus terebinthifolius* (69.23 %); *Angelonia*, *Cecropia* and *Cocos nucifera* (65.38 %); *Mikania,* Poaceae and *Spondias tuberosa* (61.54 %); *Mimosa acutistipula*, *Serjania* and *Syagrus coronata* (53.85 %); and *Hypenia* and *Hyptis* (50 %).

The taxonomic affinities of pollen types could not be determined for 12 of the propolis samples (Tabs. 1, 2). These undetermined types (n = 24) exhibited low frequencies in each sample, and were most conspicuous in propolis from Mombaça – CE (MOM), which had a high frequency (5.4 %) of three distinct pollen types (Tab. 1).

Eleven out of the 47 families identified were present in the all types of propolis (brown, red, and green): Anacardiaceae, Arecaceae, Asteraceae, Euphorbiaceae, Fabaceae, Myrtaceae, Poaceae, Rhamnaceae, Rubiaceae, Sapindaceae, and Urticaceae. The most representative family was Fabaceae, with 49 pollen types, followed by Malvaceae with 11 pollen types. Moreover, 34 % of pollen types with frequencies above 50 % corresponded to the family Fabaceae. The pollen grains of species from this family played a major role in the pollen spectrum of the analyzed propolis, ranging from 4% in the sample from João Pessoa — sample 2 (JOP2) — to 78 % in the sample from Delmiro Gouveia (DEG) (Fig. 2). On average, representatives of Fabaceae accounted for 40.1 % of the pollen grains in the analyzed samples.

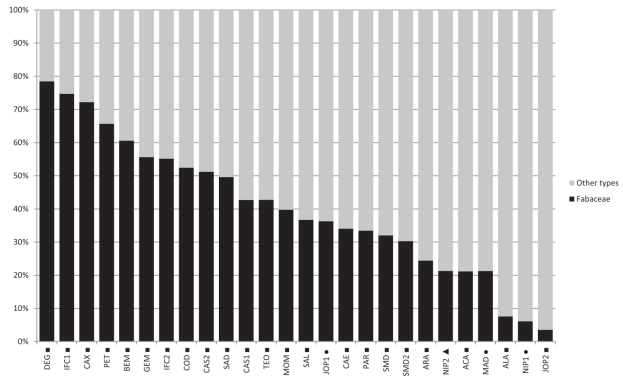

Figure 2. The contribution of Fabaceae pollen (black column) to the pollen spectrum of propolis samples from northeastern Brazil. Sampled municipalities: SMD = Simão Dias – Sergipe; MOM = Mombaça – Ceará; TEO = Teofilândia – Bahia; SMD2 = Simão Dias (sítio do cavaco)- Sergipe; JOP2 = João Pessoa (sample 2) – Paraíba; BEM = Belém de Maria – Pernambuco; PAR = Paripiranga – Bahia; SAD= São Domingos – Bahia; CAE = Caetité – Bahia; ALA = Alagoinhas – Bahia; ARA = Araci – Bahia; SAL = Salgueiro – Pernambuco; PET = Petrolina – Pernambuco; CAS1 = Campos Sales (sample 1) – Ceará; CAS2 = Campos Sales (sample 2) – Ceará; GEM = Geminiano – Piauí; ACA= Acauã – Piauí; DEG= Delmiro Gouveia – Alagoas; CAX = Caxias – Maranhão; COD = Codó – Maranhão; IFC1 = Instituto Federal MA (sample 1) Codó – Maranhão; IFC2 = Instituto Federal MA (sample 2) Codó – Maranhão; NIP1 = Nilo Peçanha (sample 1) – Bahia; JOP1= João Pessoa (sample 1) – Paraíba; MAD = Marechal Deodoro – Alagoas; NIP2 = Nilo Peçanha (sample 2) – Bahia. Propolis types: ■ = brown, ● = red, ▲ = green.

Table 1. Frequency (%) of pollen types in samples of brown propolis produced in northeastern Brazil.

Pollen Types (%)	SMD	MOM	TEO	SMD2	JOP2	BEM	PAR	SAD	CAE	ALA	ARA	SAL	PET	CAS1	CAS2	GEM	ACA	DEG	CAX	COD	IFC1	IFC2
Acanthaceae																						
Justicia														0.18								
Amaranthaceae																						
Alternanthera	0.35	3.80	5.70	7.19		4.28	0.78	7.94	0.68		0.40	10.3	2.29	0.55	0.92	0.17	0.57	1.87	7.16	1.62	4.66	3.80
Gomphrena			0.40									2.14										
Anacardiaceae																						
Anacardiaceae								0.72														
Anacardium occidentale			0.20	2.53																		
Myracrodrum														4.20								
Schinus terebinthifolius	1.38		2.30	0.80		2.50		3.79	36.9	17.74		9.98	2.94	36.68	28.28	0.50	7.01		2.21	4.50		2.34
Spondias tuberosa			0.50	1.60	1.42	0.18		2.17				1.43	0.49	0.91			2.84	3.37		0.36	0.18	1.75
Tapirira	0.35		1.50		8.01		5.83	5.05		1.04	8.75				6.10							
Apiaceae																						
Apium																0.66						
Apocynaceae																						
Apocynaceae								4.15														
Forsteronia			0.90																			
Aquifoliaceae																						
Ilex			0.20							0.70												
Arecaceae																						
Cocos nucifera			0.20	0.93	4.98		0.97	1.26		2.26		0.53	3.43		0.55			0.37		3.60	0.18	5.84
Elaeis oleifera					1.96					1.57											0.18	0.73
Syagrus coronata			1.40	0.67			0.58	3.07		0.17		3.21	0.82		0.37	0.17		0.56	0.68	0.18		
Asteraceae																						
Baccharis							3.11															
Eupatorium				7.19		0.53			0.51			1.60								0.36		
Mikania	0.87	0.70	1.00	0.27	5.34	1.25	0.78	0.18		3.48	4.17		0.49	0.18						0.72		
Mikania II					2.49							0.18	0.49									
Mikania III														0.36								
Vernonia		0.30	0.80	3.46	0.89	0.36				1.22						0.17				1.26		0.15
Vernonia II																1.16		0.19				
Bignoniaceae																						
Arrabidaea			0.30																			
Bignonia					0.36																	
Piriadacus										2.96												
Tabebuia	0.35				0.36												0.19			0.36		
Bromeliaceae																						
Bromeliaceae			0.20																			
Catopsis								0.36														
Boraginaceae																						
Cordia												0.89								0.18		
Heliotropium																				0.18		
Burseraceae																						
Protium heptaphyllum															0.18							
Cactaceae																						
Cereus			0.10															0.56				
Capparaceae																						
Capparis								1.26					2.78									
Clusiaceae																						
Symphonia					49.11					1.04												
Commelinaceae																						
Commelina				0.13	0.53					0.17			0.33						0.34	0.18	0.18	0.15
Convolvulaceae																						
Evolvulus		0.10	0.90					0.72	0.34			0.18										
Jacquemontia																		0.37				
Curcubitaceae																						

Table 1. Cont.

Pollen Types (%)	SMD	MOM	TEO	SMD2	JOP2	BEM	PAR	SAD	CAE	ALA	ARA	SAL	PET	CAS1	CAS2	GEM	ACA	DEG	CAX	COD	IFC1	IFC2
Cayaponia				0.13																		
Posadaea			0.10																			
Euphorbiaceae																						
Cnidoscolus				1.86			0.19				2.58							0.19		0.90		
Croton			1.30	2.13					0.51								0.19	0.19				
Croton II	0.52							4.51	1.19	0.17		1.60	0.33	1.46	0.92			0.37			0.18	1.46
Manihot								1.62				0.89		0.36	0.37		0.19					
Fabaceae																						
Acacia		0.70	2.30	3.86					10.4	0.17	0.60	0.36			0.18					1.62		0.88
Anadenanthera																	0.76					
Bauhinia																						4.82
Caesalpinia microphylla													0.36									
C. pyramidalis							1.08															
Caesalpinia I		0.10																				
Caesalpinia II			0.30				8.54						0.82									
Caesalpinia III			0.10																			
Centrosema			1.70																			
Chamaecrista		0.30											0.16									
Copaifera	0.69					0.53							0.65		0.37				0.34			
Crotalaria										0.17												0.44
Dalbergia										0.70												
Delonix regia					0.36							0.18										
Desmanthus					0.53																	
Desmodium							2.72				0.80										0.18	
Dioclea			0.90					0.18														
Fabaceae IV		0.40																				
Fabaceae V			0.10																			
Fabaceae VI			0.70																			
Fabaceae VII							9.32					2.67										
Fabaceae VIII									2.05	0.87												
Fabaceae IX													0.82									
Fabaceae X		0.60																				
Fabaceae XI																			0.51			
Galactia												0.18										
Inga		0.90	0.10					0.90								0.50						
Leucaena																			0.68			
Lonchocarpus																		5.62				
Macroptilium		1.70			0.36			6.86														
Mimosa acutistipula			2.10	0.27		11.41		4.51			2.19		7.35	9.12	25.51	27.2			41.1	40.9	59.14	41.61
Mimosa caesalpinifolia		7.70										3.03								3.42	2.69	
Mimosa invisa										0.70			2.78			17.9						
Mimosa misera				0.27				3.97														
Mimosa pudica	24.2	7.50			1.07	19.79	5.83	0.54		4.87	5.17	2.67	4.90	3.83	5.18	4.65		0.56	15.8	5.77	12.72	6.28
Mimosa quadrivalvis			14.10	10.39		4.99	0.19	10.47	2.05							5.32	1.70	1.50	3.75	0.18		
Mimosa tenuiflora	6.92	17.00	13.20	13.58	0.36	18.00	6.80	20.76	18.9		12.3	11.9	39.2	26.46	17.19		18.4	41.2	9.37	0.18		
Mimosa ulbrichiana													1.63	1.46								
Mimosa ursina			1.40																			
Piptadenia	0.17	0.40	0.20	0.80							2.39		0.33	0.36	0.55							
Plathimenia			0.90								0.99											
Senna			2.70	2.40		5.88						15.7	6.37	1.09	2.22		0.38	29.6	0.51			
Senna II					0.89																	
Zollernia																						1.17
Zornia																				0.36		
Flacourtiaceae																						
Casearia											0.60											
Krameriaceae																						

Table 1. Cont.

Pollen Types (%)	SMD	MOM	TEO	SMD2	JOP2	BEM	PAR	SAD	CAE	ALA	ARA	SAL	PET	CAS1	CAS2	GEM	ACA	DEG	CAX	COD	IFC1	IFC2
Krameria								0.36														
Lamiaceae																						
Eriope		2.50	1.60							0.70												
Hypenia		1.60	1.30	0.27		0.53	2.72				0.99					2.33	27.3		1.70	5.41	1.61	3.07
Hyptis		9.00							3.07			2.67	0.16	6.75	4.07	6.98	28.6	0.19	0.51	2.70	0.36	3.21
Rhaphiodon			10.90						3.75													
Loranthaceae																						
Psittacanthus		1.30	0.20				0.78						0.33									
Psittacanthus II		1.00																				
Lythraceae																						
Cuphea					0.18	0.18			0.17	0.35									0.68			
Malpighiaceae																						
Barnebya		0.10																				
Byrsonima																					3.23	
Malvaceae																						
Ceiba			0.10																			
Erioteca					2.85																	
Herissantia			0.20									0.36										
Malvaceae										0.35												
Pachira									0.17													
Pavonia										0.17										0.36		
Pseudobombax									0.34													
Triumfetta																						0.73
Sida		0.40												1.09	0.18							
Waltheria		0.30	0.20						0.51				0.16			0.50						
Melastomataceae																						
Clidemia		4.20																				
Miconia		9.70			4.98	1.25						0.71			0.18	2.33			1.36	4.86		
Meliaceae																						
Meliaceae			0.30																			
Trichilia			1.30							0.70										1.44		
Monocotyledoneae																						
Monocotyledoneae								1.81														
Moraceae																						
Perebea				2.93																		
Myrtaceae																						
Eucalyptus	2.25		0.40	0.27		2.32	0.58		1.19	24.35	0.60		1.80			0.83		1.87	0.17	1.44		0.44
Eucalyptus II									0.85			0.36										
Myrcia	52.8		5.20	2.40	1.07	9.98	39.03	2.89	3.07	14.26	33.8	4.81	11.1			1.99		4.31	0.17	4.50	3.58	2.04
Myrcia II								0.72											0.17			
Myrcia III											7.55									2.34		
Myrcia ovata			1.90								3.18											1.17
Psidium	7.27						1.75		0.17		0.40										0.18	
Nyctaginaceae																						
Boerhaavia		0.10																0.75				
Nymphaea								0.36														
Phytolacaceae																						
Microtea			0.10	0.13				0.18					1.14		0.18			0.19	0.85	0.18		
Plantaginaceae																						
Angelonia	0.17	0.10	0.30	0.13		0.53				1.04	5.77	17.3	2.78	0.91	0.74		5.68	3.18	0.17	3.96		
Poaceae																						
Poaceae	0.17				2.31			1.81	0.17	2.61	2.19	0.53		0.36	0.92	1.33	0.19	0.56	0.51		0.18	1.75
Poaceae II				0.27																		
Poaceae III				0.80																		
Polygonaceae																						
Polygonum		0.10																				

Table 1. Cont.

Pollen Types (%)	SMD	MOM	TEO	SMD2	JOP2	BEM	PAR	SAD	CAE	ALA	ARA	SAL	PET	CAS1	CAS2	GEM	ACA	DEG	CAX	COD	IFC1	IFC2
Rhamnaceae																						
Ziziphus joazeiro			1.70	1.20		0.36	2.14	2.53	5.80	1.91	3.18				0.92							
Rubiaceae																						
Borreria verticillata	0.52	16.00	4.00	18.51	1.96	3.74	1.17			5.91	0.99	0.36	0.65	2.37	2.03	15.8			6.81	3.06	0.72	9.93
Borreria I												1.78	0.33	0.18	1.29		2.84			0.18		1.31
Diodia			0.70			0.36						0.89	0.49			8.97	3.22		0.34			
Guettarda			0.20						0.85													
Mitracarpus		0.60	3.20					0.54	0.68	2.61	0.40		0.16					1.50			0.18	
Richardia grandiflora			0.20		0.18		0.58		0.51									0.19				
Rutaceae																						
Citrus			0.50																			
Zanthoxylum								0.18														
Sapindaceae																						
Cardiospermum			0.20									0.18	0.49					0.19				
Cupania															0.18							0.73
Paullinia												0.18								0.18		0.44
Serjania		4.50	1.40	0.40	0.18	0.89	0.39	0.54	0.34					0.55	0.37	0.17						
Serjania II			0.30	0.13	0.18							0.18										
Solanaceae																						
Cestrum								1.26		0.87												
Solanum	0.87			2.13		0.71			1.71										0.34			
Turneraceae																						
Turnera																				0.54		
Ulmaceae																						
Celtis		0.10	0.20																0.51			0.44
Trema									0.34													
Urticaceae																						
Cecropia				0.27	6.94	9.45	5.05		1.02	2.43				0.18		0.33		0.56	3.07	1.98	9.68	3.36
Indetermined		5.40 (3t)	3.0 (6t)		0.18 (1t)		0.19 (1t)	0.72 (1t)	1.71 (1t)	1.74 (2t)			0.98 (1t)						0.17 (1t)			

Codes: SMD = Simão Dias – Sergipe; MOM = Mombaça – Ceará; TEO = Teofilândia – Bahia; SMD2 = Simão Dias (sítio do cavaco) - Sergipe; JOP2 = João Pessoa (sample 2) – Paraíba; BEM = Belém de Maria – Pernambuco; PAR = Paripiranga – Bahia; SAD = São Domingos – Bahia; CAE = Caetité – Bahia; ALA = Alagoinhas – Bahia; ARA = Araci – Bahia; SAL = Salgueiro – Pernambuco; PET = Petrolina – Pernambuco; CAS1 = Campos Sales (sample 1) – Ceará; CAS2 = Campos Sales (sample 2) – Ceará; GEM = Geminiano – Piauí; ACA = Acauã – Piauí; DEG = Delmiro Gouveia – Alagoas; CAX = Caxias – Maranhão; COD = Codó – Maranhão; IFC1 = Instituto Federal MA (sample 1) Codó – Maranhão; IFC2 = Instituto Federal MA (amostra 2) Codó – Maranhão; t = types.

The most frequent families within the samples of brown propolis were: Fabaceae and Rubiaceae (100 %), Anacardiaceae (95 %), Amaranthaceae (91 %), Lamiaceae (86 %), Myrtaceae and Asteraceae (82 %), Euphorbiaceae (77 %), Poaceae and Sapindaceae (73 %), Arecaceae (68 %), Urticaceae (59 %) and Rhamnaceae (41 %). For red propolis the most represented families were: Anacardiaceae, Arecaceae, Fabaceae, Myrtaceae, Rubiaceae, Sapindaceae and Urticaceae (100 %); Amaranthaceae, Asteraceae and Poaceae (67 %); and Euphorbiaceae, Lamiaceae and Rhamnaceae (33 %).

The families with the greatest number of pollen types found simultaneously in the three propolis types were Asteraceae (*Eupatorium*, *Mikania*, and *Vernonia*), Fabaceae (*Acacia*, *Caesalpinia* I and *M. pudica*), and Rubiaceae (*B. verticillata*, *Borreria* I and *Mitracarpus*), with three pollen types each. These were followed by the families Anacardiaceae (*S. terebinthifolius* and *S. tuberosa*), Arecaceae (*C. nucifera* and *Elaeis oleifera*), Myrtaceae (*Eucalyptus* and *Myrcia*), and Sapindaceae (*Cupania* and *Serjania*) with two pollen types each. The families Euphorbiaceae (*Croton*), Poaceae (type Poaceae), Rhamnaceae (*Ziziphus joazeiro*) and Urticaceae (*Cecropia*) were represented by a single pollen type in all propolis types.

Considering the 172 pollen types identified, 109 belong to groups that offer pollen as floral resources (Tab. 3). Other pollen types belonging to groups that produce resins or oils were also found in analyzed samples (Tab. 4), with remarkable representation of species of the family Anacardiaceae, as expected since they play a major role in the production of the resin that is the main constituent of propolis.

Regarding the vegetation used for foraging by *Apis mellifera*, tree-like plants are highlighted in the production of propolis since 36 % of the pollen types were of arboreal species while 34 % were of shrub-like groups. Herbaceous

Table 2. Frequency (%) of pollen types in samples of red and green propolis produced in northeastern Brazil.

Propolis	Red			Green	Propolis	Red			Green
Pollen Types	NIP1	JOP1	MAD	NIP2	Pollen Types	NIP1	JOP1	MAD	NIP2
Amaranthaceae					Fabaceae III			5.96	
Alternanthera		0.18			*Mimosa acutistipula*		5.27		
Gomphrena			9.04		*Mimosa paraibana*		1.76		
Anacardiaceae					*Mimosa pudica*	2.94	6.68	0.77	20.74
Astronium				0.15	*Mimosa quadrivalvis*			3.27	
Myracrodrum	0.69				*Mimosa tenuiflora*	0.35	21.09		
Schinus terebinthifolius			5.77	10.52	*Senna*		0.53	0.58	
Spondias tuberosa	3.11	0.88		0.44	**Lamiaceae**				
Tapirira	24.91	10.37	0.77		*Hypenia*	0.17			
Apocynaceae					*Hyptis*				
Apocynaceae				0.44	*Rhaphiodon*				
Forsteronia			4.42		*Loranthaceae*				
Forsteronia II			0.19		*Psittacanthus*		0.35		
Aquifoliaceae					*Psittacanthus* II				
Ilex	4.33		2.31		**Lythraceae**				
Ilex II				0.30	*Cuphea*	1.04	3.34		
Araliaceae					**Malpighiaceae**				
Didymopanax	0.17				*Barnebya*				
Arecaceae					*Byrsonima*				
Arecaceae	0.52				**Malvaceae**				
Cocos nucifera	0.35	0.88	0.58	0.30	*Erioteca*			2.88	
Elaeis oleifera	30.10	0.35	2.31	40.74	*Helicteres*		0.35		0.15
Syagrus coronata	0.35	0.53			*Waltheria*			3.65	
Asteraceae					**Melastomataceae**				
Baccharis	2.94				*Clidemia*				
Conocliniopsis	0.87				*Miconia*		1.76	0.77	
Eupatorium	0.52		0.38	3.11	**Meliaceae**				
Eupatorium I	0.35				*Trichilia*		0.35	6.35	
Holocheilus			0.77		**Moraceae**				
Mikania	0.52		0.19	2.22	*Brosimum*	9.17		0.19	0.30
Mikania II			0.19		*Ficus*			4.42	
Vernonia	0.52		1.92	0.59	*Myrtaceae*				
Vernonia II			0.58		*Eucalyptus*	3.81	2.46	1.54	0.59
Bignoniaceae					*Myrcia*	0.17	10.54		6.07
Arrabidaea			0.38		**Phyllanthaceae**				
Burseraceae					*Phyllanthus*				0.15
Protium heptaphyllum		2.11			**Piperaceae**				
Capparaceae					*Piperonia*			2.50	
Capparis		0.18			**Plantaginaceae**				
Clusiaceae					*Angelonia*	0.17	1.41		
Symphonia	0.17		1.90		**Poaceae**				
Commelinaceae					Poaceae				0.30
Commelina			0.58		Poaceae II	1.04	1.58		0.44
Euphorbiaceae					**Rhamnaceae**				
Alchornea				0.15	*Ziziphus joazeiro*		1.23		0.89
Croton		0.53			**Rubiaceae**				
Croton II		0.53		0.59	*Borreria verticillata*		12.65	0.58	0.74
Fabaceae					*Borreria* I		0.88	0.38	0.44
Acacia			1.73	0.44	*Coffea*			2.00	
Centrosema	2.08			0.15	*Guettarda*	1.04			
Chamaecrista			1.92		*Mitracarpus*		1.05	0.58	0.44
Delonix regia		0.35			*Richardia grandiflora*		0.35	7.12	
Desmodium	0.52				**Sapindaceae**				
Fabaceae			4.23		*Allophylus*		0.18		
Fabaceae II			1.92		*Cupania*	0.17			0.15

Table 2. Cont.

Propolis	Red			Green	Propolis	Red			Green
Pollen Types	NIP1	JOP1	MAD	NIP2	Pollen Types	NIP1	JOP1	MAD	NIP2
Serjania	0.17		3.00	0.15	*Trema*		0.18		
Serjania II	0.17				**Urticaceae**				
Solanaceae					*Cecropia*	2.94	5.80	7.10	8.30
Cestrum		1.58			Indetermined				
Ulmaceae					Indetermined	3.63(3t)	1.76(2t)		0.30(2t)

Sample codes: NIP1 = Nilo Peçanha (sample 1) – Bahia; JOP1 = João Pessoa (sample 1) – Paraíba; MAD = Marechal Deodoro – Alagoas; NIP2 = Nilo Peçanha (sample 2) – Bahia; t = types.

Table 3. Summary of pollen types in analyzed samples of propolis produced in northeastern Brazil.

Pollen Types	DF	Ha	FR	Pollen Types	DF	Ha	FR	Pollen Types	DF	Ha	FR
Acanthaceae				*Tabebuia*	LF	A	N	*Desmanthus*	R	A/H	N/P
Justicia	R	A	N/P	**Bromeliaceae**				*Desmodium*	LF	A/H	N/P
Amaranthaceae				Bromeliaceae	R	S/H/L	N	*Dioclea*	R	L	N
Alternanthera	VF	H	N	*Catopsis*	R	H	N	*Fabaceae*	R	A/S/H	N/P
Gomphrena	LF	H	P	**Boraginaceae**				Fabaceae II	R	A/S/H	N/P
Anacardiaceae				*Cordia*	R	A/S	N	Fabaceae III	R	A/S/H	N/P
Anacardiaceae	R	A/S	N/R/O	*Heliotropium*	R	H	N	Fabaceae IV	R	A/S/H	N/P
Anacardium occidentale	R	A	R/O	**Burseraceae**				Fabaceae V	R	A/S/H	N/P
Astronium	R	A	R/O	*Protium heptaphyllum*	R	A	N/R	Fabaceae VI	R	A/S/H	N/P
Myracrodrum	R	A	R/O	**Cactaceae**				Fabaceae VII	R	A/S/H	N/P
Schinus terebinthifolius	VF	A	N/P/R	*Cereus*	R	A/S	N/P	Fabaceae VIII	R	A/S/H	N/P
Spondias tuberosa	VF	A	N/R	**Capparaceae**				Fabaceae IX	R	A/S/H	N/P
Tapirira	F	A	N/R	*Capparis*	LF	A/S	N/P	Fabaceae X	R	A/S/H	N/P
Apiaceae				**Clusiaceae**				Fabaceae XI	R	A	P
Apium	R	H	O	*Symphonia*	LF	A	N/R	*Galactia*	R	A/H	N/P
Apocynaceae				**Commelinaceae**				*Inga*	LF	A	N/P
Apocynaceae	R	A/S/L	N/P	*Commelina*	F	H	N	*Leucaena*	R	A	N/P
Forsteronia	R	A/S/L	N/P	**Convolvulaceae**				*Lonchocarpus*	R	A	N
Forsteronia II	R	A/S/L	N/P	*Evolvulus*	LF	H	P	*Macroptilium*	LF	A/H	N
Aquifoliaceae				*Jacquemontia*	R	L	N	*Mimosa acutistipula*	VF	S	P
Ilex	LF	A/S	N/R	**Curcubitaceae**				*Mimosa caesalpinifolia*	LF	A	N/P
Ilex II	R	A/S	N/R	*Cayaponia*	R	L	N/P	*Mimosa invisa*	LF	S	N/P
Araliaceae				*Posadaea*	R	L	N/P	*Mimosa misera*	R	H	P
Didymopanax	R	A/S	N/P	**Euphorbiaceae**				*Mimosa paraibana*	R	S	N/P
Arecaceae				*Alchornea*	R	A	P	*Mimosa pudica*	VF	H	P
Arecaceae	R	A	N/P	*Cnidoscolus*	LF	A/S	N	*Mimosa quadrivalvis*	F	H	P
Cocos nucifera	VF	A	N/P	*Croton*	F	S	N/P	*Mimosa tenuiflora*	VF	S	P
Elaeis oleifera	F	A	N/P	*Croton* II	F	S	N/P	*Mimosa ulbrichiana*	R	S	P
Syagrus coronata	VF	A	P	*Manihot*	LF	S	N/P	*Mimosa ursina*	R	S	P
Asteraceae				**Fabaceae**				*Piptadenia*	F	S	N/P
Baccharis	R	S	N/P	*Acacia*	F	A	N/P	*Plathimenia*	R	A	N
Conocliniopsis	R	S	N/P	*Anadenanthera*	R	A	P	*Senna*	F	S	P
Eupatorium	F	S	N/P	*Bauhinia*	R	A/S	N/P	*Senna II*	R	S	P
Eupatorium II	R	S	N/P	*Caesalpinia microphylla*	R	S	N	*Zollernia*	R	A	P
Holocheilus	R	A/S/H	N/P	*Caesalpinia pyramidalis*	R	A	N	*Zornia*	R	H	N
Mikania	VF	C	P	*Caesalpinia* I	R	A/S	N	**Flacourtiaceae**			
Mikania II	LF	C	P	*Caesalpinia* II	LF	A/S	N	*Casearia*	R	A/S	P
Mikania III	R	C	P	*Caesalpinia* III	R	A/S	N	**Krameriaceae**			
Vernonia	F	S/H	N/P	*Centrosema*	LF	L	N	*Krameria*	R	S/H	O
Vernonia II	LF	S/H	N/P	*Chamaecrista*	LF	A/S	P	**Lamiaceae**			
Bignoniaceae				*Copaifera*	LF	A	N/P	*Eriope*	LF	S/H	N/P
Arrabidaea	R	L	N	*Crotalaria*	R	S	N/P	*Hypenia*	VF	S/H	N
Bignonia	R	L	N	*Dalbergia*	R	A	N/P	*Hyptis*	VF	S/H	N
Piriadacus	R	S	N	*Delonix regia*	LF	A	P	*Rhaphiodon*	R	H	N

Table 3. Cont.

Pollen Types	DF	Ha	FR	Pollen Types	DF	Ha	FR	Pollen Types	DF	Ha	FR
Loranthaceae				*Brosimum*	LF	A	N/P	*Ziziphus joazeiro*	F	A	N
Psittacanthus	LF	H	N	*Ficus*	R	A	N/O	**Rubiaceae**			
Psittacanthus II	R	H	N	*Perebea*	R	A	P	*Borreria verticillata*	VF	H	N/P
Lythraceae				**Myrtaceae**				*Borreria* I	F	H	N/P
Cuphea	F	S/H	N/P	*Eucalyptus*	VF	A	N/P	*Diodia*	F	H	N
Malpighiaceae				*Eucalyptus* II	R	A	N/P	*Guettarda*	LF	A	N/P
Barnebya	R	A	O	*Myrcia*	VF	A	N/P	*Mitracarpus*	F	S	N
Byrsonima	R	A	P/O	*Myrcia* II	R	A	N/P	*Richardia grandiflora*	F	H	N
Malvaceae				*Myrcia* III	R	A	N/P	**Rutaceae**			
Ceiba	R	A	N	*Myrcia ovata*	LF	A	N/P	*Citrus*	R	A	N/P
Erioteca	R	A	N/P	*Psidium*	LF	A	P	*Coffea*	R	S	N/P
Helicteres	R	S	N/P	**Nyctaginaceae**				*Zanthoxylum*	R	A/S	N/P
Herissantia	R	S/H	P	*Boerhaavia*	R	H	N	**Sapindaceae**			
Malvaceae	R	A/S/H	N/P	*Nymphaea*	R	H	N/P	*Allophylus*	R	A/S	N/P
Pachira	R	A	N	**Phyllanthaceae**				*Cardiospermum*	LF	L	N/P
Pavonia	R	S/H	N/P	*Phyllanthus*	R	A/H	N	*Cupania*	LF	A/S	N
Pseudobombax	R	A	N/P	**Phytolacaceae**				*Paullinia*	LF	A	N
Triumfetta	R	S/H	N	*Microtea*	F	S	N	*Serjania*	VF	L	N
Sida	LF	H	N/P	**Piperaceae**				*Serjania* II	LF	L	N
Waltheria	F	S/H	N/P	*Piperonia*	R	H	P	**Solanaceae**			
Melastomataceae				**Plantaginaceae**				*Cestrum*	LF	S	P
Clidemia	R	S	P	*Angelonia*	VF	A/H	N/O	*Solanum*	LF	A	P
Miconia	F	A/S	P	**Poaceae**				**Turneraceae**			
Meliaceae				Poaceae	VF	H	P	*Turnera*	R	S/H	N/P
Meliaceae	R	A	N/P/O	Poaceae II	LF	H	P	**Ulmaceae**			
Trichilia	LF	A	N/O	Poaceae III	R	H	P	*Celtis*	LF	A	P
Monocotyledoneae				**Polygonaceae**				*Trema*	R	A/S	P
Monocotyledoneae	R	H	N/P	*Polygonum*	R	S	N	**Urticaceae**			
Moraceae				**Rhamnaceae**				*Cecropia*	VF	A	N/P/R

DF (distribution frequency): R = rare; LF = low frequent; F = frequent; VF = very frequent. Ha (habit): A = arboreal; S = shrubby; H = herbaceous; L = liana; C = climber. FR (floral resource): N = nectar; P = pollen; R = resin; O = oil.

Table 4. Resiniferous and oleiferous pollen types identified in samples of propolis from northeastern Brazil.

Propolis	Resiniferous types	Oleiferous types
Brown (n = 22)	*Cecropia*, *Ilex*, *Myracrodruon*, *Protium heptaphyllum*, *Schinus terebinthifolius*, *Spondias tuberosa*, *Symphonia* and *Tapirira*	type Anacardiaceae, *Anacardium occidentale*, *Angelonia*, *Barnebya*, *Byrsonima*, *Krameria*, *Myracrodruon*, type Meliaceae and *Trichilia*
Red (n = 3)	*Cecropia*, *Ilex*, *Myracrodruon*, *Protium heptaphyllum*, *Schinus terebinthifolius*, *Spondias tuberosa*, *Symphonia* and *Tapirira*	*Angelonia*, *Myracrodruon* and *Trichilia*
Green (n=1)	*Astronium*, *Cecropia*, *Ilex* II, *Schinus terebinthifolius* and *Spondias tuberosa*	*Astronium* and *Trichilia*

plants represented 23 % of pollen types in the analyzed samples of propolis.

The analysis of similarity among propolis samples based on the pollen types of resinous and oleiferous groups revealed two main clusters (Fig. 3) and two isolated samples that formed individual branches in the dendrogram. The group AR is composed of ten samples (TEO, JOP2, NIP1, ALA, MAD, JOP1, PAR, ARA, CAS2, and SMD). The pollen types that stand out in this cluster refer to *Tapirira*, *Angelonia* and *S. terebinthifolius*, which were found in nine, seven, and six samples, respectively. The group BR encompassed 14 samples (CAE, GEM, CAX, IFC2, SAL, PET, ACA, SMD2, BEM, CAS1, COD, DEG, NIP2, and IFC1), and was characterized by the presence of pollen types from *S. terebinthifolius* in 12 samples, *S. tuberosa* in 11 samples and *Angelonia* as well as *Cecropia* in ten samples. The propolis sampled from São Domingos (SAD) and Mombaça (MOM) were placed apart from each other as individual branches because of the presence of the exclusive pollen types of Anacardiaceae and *Krameria*, and *Barnebya*, respectively.

On the other hand, three clusters were formed in the dendrogram when the palynological similarity among propolis samples was based only on the pollen types of the family Fabaceae (Fig. 4). The group AF (ALA, IFC2, NIP2 and MAD) was characterized by pollen types from *Acacia* and *M. pudica*. The group BF shared several pollen

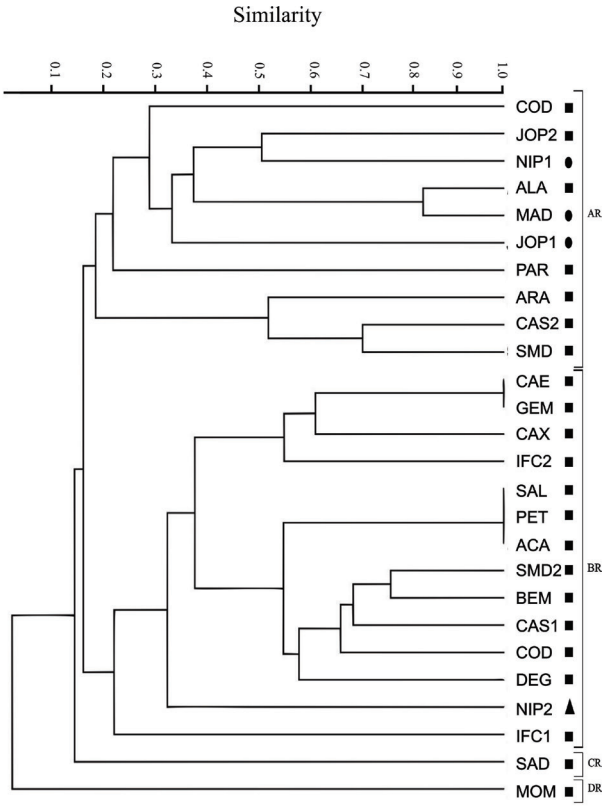

Figure 3. Similarity dendrogram, based on Jaccard's coefficient for resinous and oleiferous pollen types, comparing propolis samples produced in northeastern Brazil. Four sample groups were formed: AR, BR, CR, and DR. See Figure 2 for sample codes.

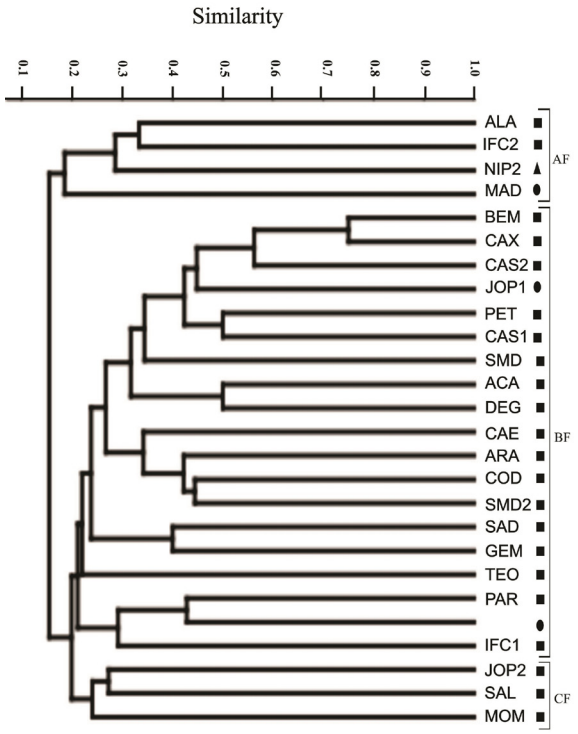

Figure 4. Similarity dendrogram, based on Jaccard's coefficient for pollen types of Fabaceae, comparing propolis samples produced in northeastern Brazil. Three sample groups were formed: AF, BF, and CF. See Figure 2 for sample codes.

types of Fabaceae and encompassed most of the analyzed samples (n=19). The group CF (JOP2, SAL, and MOM) shared the types *M. pudica* and *M. tenuiflora*, while in the group AF, the three propolis types (red, brown, and green) were clustered together.

Considering the entire pollen spectrum (172 identified pollen types), the similarity dendrogram revealed three main clusters (Fig. 5). The group AP (SMD2, BEM, COD, GEM, CAX, PAR, ARA, and SMD) shared the pollen types of *Alternanthera*, *B. verticillata*, *Eucalyptus*, *M. pudica*, *M. tenuiflora* and *Myrcia*. The group BP (SAL, PET, CAS1, CAS2, ACA, DEG, IFC1 and IFC2) was characterized by pollen types of *Croton* and *Hyptis* (100%); *M. pudica*, Poaceae and *S. tuberosa* (88%); and *Angelonia*, *B. verticillata*, *Borreria* I, *C. nucifera*, *M. tenuiflora*, *S. terebinthifolius* and *Senna* (75%). The group EP comprised six samples (JOP2, MAD, NIP1, ALA, JOP1 and NIP2) that shared the pollen types *Cecropia*, *C. nucifera*, *E. oleifera* and *M. pudica* (100%); *B. verticillata*, *Eucalyptus*, *Mikania*, *Myrcia*, *Tapirira* and *Vernonia* (83%); and *Cuphea*, *Mitracarpus*, *Serjania* and *S. tuberosa* (67%). A small group named FP was represented by only two samples (TEO and MOM) that shared 16 pollen types. In this analysis, two samples were isolated in the dendrogram by the presence of unique pollen types; one of propolis obtained in Caetité (CAE) with the pollen types *Pachira* and *Pseudobombax*; and

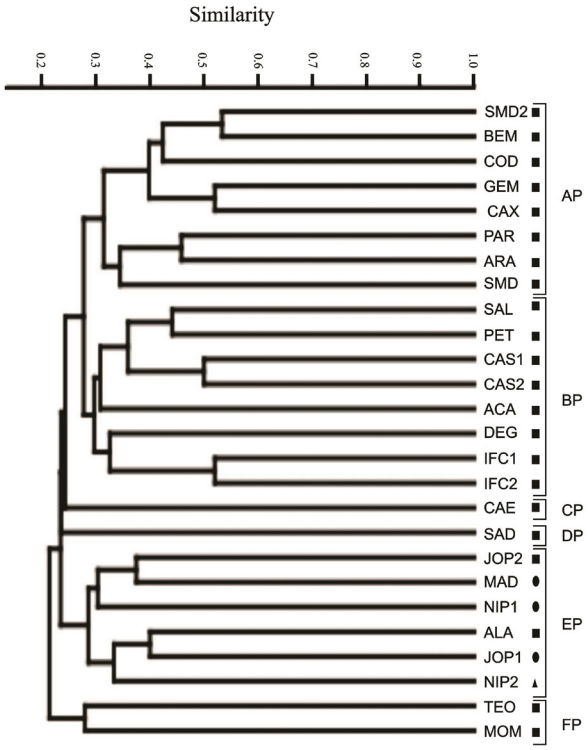

Figure 5. Similarity dendrogram, based on Jaccard's coefficient for all pollen types, comparing propolis samples produced in northeastern Brazil. Six sample groups were formed: AP, BP, CP, DP, EP, and FP. See Figure 2 for sample codes.

one from São Domingos (SAD) characterized by pollen types of Anacardiaceae, *Catopsis*, *Caesalpinia pyramidalis*, *Krameria*, Monocoyledoneae, *Nymphaea*, *Senna* II and *Zanthoxylum*.

The sample from Mombaça (MOM) was differentiated in all three similarity analyses by the occurrence of several exclusive pollen types: *Caesalpinia* I, Fabaceae IV and X (Fabaceae), *Psittacanthus* II (Loranthaceae), *Barnebya* (Malpighiaceae), *Clidemia* (Melastomataceae) and *Polygonum* (Polygonaceae).

Discussion

The pollen types of the studied samples of propolis were mostly from species that play a key role in the maintenance of bee colonies. Thus, pollen types such as *Alternanthera*, *Mimosa pudica, Borreria verticillata*, and *Myrcia*, were distinguished because they are polliniferous and nectariferous plants, and thus important sources of carbohydrates and proteins for colonies.

The most representative pollen type (84.62 %) in the analyzed propolis samples was *M. pudica* (Fabaceae), a polliniferous and herbaceous species. This pollen type was found in all samples of red and green propolis and in 18 (82 %) of the brown propolis samples. These data are in agreement with previous reports by Matos & Santos (2017), who analyzed 22 pollen samples from nine municipalities along the northern coast of the state of Bahia and found 59 pollen types with *M. pudica* and *Mikania* being present in 100 % of the samples. According to beekeepers, *M. pudica* produces high amounts of pollen, and thus holds great potential for apiculture, particularly in northeastern Brazil where this species is widespread (Queiroz 2009).

The pollen types of *B. verticillata* (Rubiaceae), which are recognized as herbaceous nectariferous/polliniferous plants, and *Myrcia* (Myrtaceae), an arboreal nectariferous/polliniferous group, were also commonly present among the analyzed samples, but in low frequencies in the samples of brown, red, and even green propolis.

In previous studies by Barth & Luz (2009), *Borreria*-type pollen appeared in all samples of red propolis from the states of Bahia, Alagoas, and Paraíba. Accordingly, Freitas *et al*. (2010) and Matos & Santos (2017) reported that the pollen type for *B. verticillata* was widely distributed among the samples analyzed, but at low frequencies, similar to the pattern found in the present study. The taxa related to this pollen type are poor producers of the resin that represents the main component of propolis, and so the presence of these pollen types are likely related to other activities in beehives such as feeding.

Regarding resin production, there are three pollen types that are considered very frequent and thus important for the production of propolis in northeastern Brazil because they represent plant groups that are widely distributed in the region. Among these types are *Schinus terebinthifolius* (Anacardiaceae), *Spondias tuberosa* (Anacardiaceae) and *Cecropia* (Urticaceae). This result indicates that the species related to these pollen types are potential suppliers of resin for the propolis of *Apis mellifera*, as corroborated by other recent reports (Matos *et al*. 2014; Matos & Santos 2017).

Considering the habit of the plants associated with the present pollen analysis, tree-like plants represented a large proportion of the pollen types (49.4 %), followed by shrubs (45.3 %); vines represented the least frequent pollen types in propolis samples, accounting for just 1.7 % of the pollen types. According to Pereira *et al*. (2004), the arboreal stratum is the main supplier of resources (nectar, pollen, oil, and resin) for most bee species.

The pollen types *Cecropia* and Poaceae were quite relevant, as also reported by Barth & Luz (2009) and Matos & Santos (2017). These genera comprise anemophilous species, which might also explain their conspicuous presence in the studied samples, especially those from more open areas.

The dendrogram based only on the pollen types associated with resinous and oleiferous taxa revealed the formation of groups that differed in pollen types, which might be related to the generalist behavior of foraging by bees. Nonetheless, *S. terebinthifolius* and *S. tuberosa* were highly represented in the three types of propolis, reinforcing their importance in the supply of resins for the production of propolis, as previously mentioned.

The pollen types *Acacia* and *M. pudica* shared by the samples in group AF are closely related to typical plants of the Atlantic Forest (Freitas *et al*. 2010; Matos & Santos 2017) that may produce pollen as floral resources. On the other hand, the presence of the types *M. pudica* and *Mimosa tenuiflora* in the samples of the CF group are associated with herbaceous and shrubby plants that offer pollen as the main floral resource. The samples from the largest cluster in this analysis (BF, with 19 samples) were not characterized by particular pollen types of Fabaceae, but instead encompassed a large assemblage of pollen types from local flora.

The similarity analysis considering the entire pollen spectrum revealed a close relationship between propolis samples and the physiognomies of each area. For instance, the samples from groups AP, EP, and FP included pollen types belonging to taxa that diverge both in habit and in the types of resources offered to bees. Nevertheless, some sample groups shared pollen types belonging to similar taxa in spite of being derived from physiognomically distinct areas, such as the occurrence of pollen types *Croton* and *Hyptis,* which are recognized as nectariferous and shrub-like plants, in all samples of the BP group. It should be pointed out that species of the genera *Croton* and *Hyptis* are important resources for the survival of bee colonies because of their production of nectar in semiarid conditions (Santos *et al*. 2006). The samples from Caetité (CAE) and São Domingos (SAD) were set apart from each other in the dendrogram because of their unique pollen types that appeared at low frequencies, possibly indicating particularities of the local flora, as reported by Freitas *et al*. (2010).

The present analyses revealed a large representation by the family Fabaceae, which contributed the highest number of pollen types to the production of propolis by *Apis mellifera* in northeastern Brazil. It is worth mentioning that most taxa of Fabaceae are not directly responsible for the production of propolis, but to the maintenance of colonies since they are mostly polliniferous or nectariferous, and thus assure the health of bees that produce this important product.

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