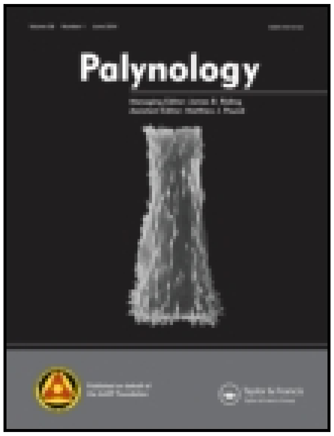

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Pollen content of marmeleiro (Croton spp., Euphorbiaceae) honey from Piauí State, Brazil

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Pollen content of marmeleiro (*Croton* spp., Euphorbiaceae) honey from Piauí State, Brazil

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Samples of honey, called marmeleiro honey by its producers, from the state of Piauí, Brazil, were analysed to study their pollen contents. Samples were dissolved in water, alcohol was added to the solution and the samples were subjected to acetolysis. The absolute concentration of pollen grains in the samples was established using an exotic marker. Multivariate cluster analysis was performed to determine the similarity amongst samples. A total of 158 pollen types were identified from 48 families and 103 genera of plants. The most prevalent families in the pollen spectra were Leguminosae, Myrtaceae, Rubiaceae and Euphorbiaceae. The pollen types *Mimosa caesalpiniifolia* and *Pityrocarpa moniliformis* were predominant in 12 samples. Other notable pollen types were *Borreria verticillata*, *Combretum* and *Mitracarpus salzmannianus*. Similarity analysis did not distinguish samples produced on different vegetation types or in the Piauí microregions. Only three samples were considered monofloral. In contrast to beekeepers' designations, only two of the samples studied can potentially be considered monofloral marmeleiro honeys (*Croton* spp.). Our results stress the need for further studies on the pollen/nectar contribution to honey by the *Croton* species in this semi-arid region and demonstrate the importance of melissopalynology in investigating the botanical origin of honeys.

Keywords: beekeeping; species of *Croton* honey; melissopalynology; semi-arid; Brazil; Euphorbiaccae; marmeleiro honey

1. Introduction

Pollen content analyses are used to infer the geographical and botanical origin of honeys and are considered to be the most reliable methodology for such investigations (Ruoff & Bogdanov 2004; Aronne & Micco 2010). They are indispensable for the determination of the authenticity of the honey origin and characteristics (Von der Ohe et al. 2004). Since the very first contact between humans and bees, humans have observed bee behaviour and the plants they visit. Beekeepers closely observe their bees to understand their behaviour and identify which plants they visit to collect nectar; they then use this information to infer the botanical origins of their honeys. Melissopalynological studies make it possible to determine the nectar and pollen sources used by bees for honey production and to more precisely indicate the geographical origin of these sources (Maurizio 1976; Jones & Bryant 2004; Corbella & Cozzolino 2008).

Marmeleiro honeys are widely recognised and appreciated for their clove flavour (Bezerra 2004). *Croton blanchetianus* Baill. (Euphorbiaceae) is a shrub or small tree that is commonly found in northeastern Brazil and is important in beekeeping, the timber industry and popular medicine in the region. Additionally, this plant is the main source of nectar for marmeleiro honey production (Santos et al. 2005). However, other plant species are also popularly known as marmeleiro plants (Torres 2009). According to Santana (2009), *Croton blanchetianus* Baill. is one of the most common species in floristic surveys conducted in the caatinga vegetation, which occupies a large area in northeastern Brazil (Giulietti et al. 2002) and is known for its high botanical diversity, with high rates of endemic and rare species, in addition to its aridity (Giulietti et al. 2003).

The production of marmeleiro honey is popular in the state of Piauí, which exports this product to various countries. In addition to the caatinga biome, Piauí has other vegetation types, such as the cerrado (savannas), babassu forests (palm forests of *Attalea speciosa* Mart.), coastal vegetation and transitional areas (ecotones), which are characterised by enclaves of different interpenetrating vegetation types (Castro 2007), all with high honey production potential.

Our study compares the beekeepers' designations of the botanical origin and pollen profile of their honey with those based on melissopalynological analysis.

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2. Methodology

2.1. Study area

The state of Piauí is located in northeastern Brazil, and more than 50% of its area is in a semi-arid region (Figure 1), where a large volume of honey is produced. As the second largest producer of honey in Brazil, Piauí's beekeeping activity utilises its native flora, resulting in quality speciality honeys that do not taste like honeys produced from agricultural crops or *Eucalyptus* plantations. Piauí's flora is relatively diverse and can be divided into five biogeographical units by vegetation type (Castro 2007) as follows: caatinga, cerrado (savanna), transitional areas (ecotones), babassu forests and coastal vegetation. The flora is very rich in species (approximately 1630 species) and has a large number of endemic species, such as *Thyrsacanthus microphyllus* A.Côrtes & Rapini (Acanthaceae), *Lepidaploa luetzelburgii* (Mattf.) H.Rob. (Asteraceae) and *Aeschynomene matosii* Afr.Fern. (Leguminosae).

Of these vegetation types, the following three are discussed here because of their involvement in the production of marmeleiro honey: caatinga (10 samples), transitional areas (11 samples) and coastal vegetation (one sample).

2.2. Processing

The samples were obtained through an agreement with Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)-Meio Norte (Teresina, Piauí, Brazil), a company that specialises in the chemical and physical analysis of honey in northeastern Brazil. The samples were from the micro-regions of the Alto Médio Canindé (PI1–4), Baixo Parnaíba (PI5), Campo Maior (PI6–9), Chapadas do Extremo Sul (PI10–11), Littoral (PI12–13), Picos (PI14), São Raimundo Nonato (PI15–20) and Valença do Piauí (PI21–22).

All the samples were labelled marmeleiro honey (*Croton* spp.) by their producers with the exception of samples PI2 and PI3, which are marmeleiro honey with angico-de-bezerro honey (*Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson, Leguminosae), and PI21, which is marmeleiro with bamburral honey (*Hyptis suaveolens* (L.) Poit., Lamiaceae). Samples PI1–4, PI14–18 and PI20 were produced in areas of the caatinga, PI5–12, PI19 and PI21–22 were produced in transitional areas and sample PI13 was produced in an area of coastal vegetation.

Palynological processing followed the method of Louveaux et al. (1978) with modifications suggested by Jones and Bryant (2004). First, 10 g honey was dissolved in 10 mL of water, and 50 mL of ethanol was added to minimise the loss of pollen grains. The resulting pellet was subjected to acetolysis (Erdtman 1960). The quantification of the number of pollen grains per sample was done in accordance with Moar (1985), and at least 500 pollen grains were counted using the frequency classes of Louveaux et al. (1978) as follows: P (predominant pollen) S (secondary pollen), I (important minor pollen), and m (minor pollen). The term trace pollen (t) was added to indicate the pollen types with frequencies lower than 1%.

An exotic marker of *Lycopodium clavatum* L. (Stockmarr 1971) was introduced (18583 spores) to establish the total number of pollen grains by volume of honey. The classes adopted by Maurizio (1976) were used to establish relationships amongst samples through the distribution frequency of their pollen types.

The identification of pollen types was conducted by comparing the reference collection of the Laboratory of Plant Micromorphology (LAMIV) of the Universidade Estadual de Feira de Santana (UEFS), Bahia, Brazil, with its representative pollen collection of semi-arid flora. Identification was performed based on the recommendations of Santos (2011) and consulting Chávez et al. (1991), Roubik & Moreno (1991), Carreira & Barth (2003), Melhem et al. (2003), Silva (2007) and, for *Mimosa* spp., Lima et al. (2008).

2.3. Statistical analysis

PAST (Palaeontological Statistics) version 2.15, freely-available data analysis software originally created for palaeontological analysis, was used for the statistical analysis. Multivariate cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm was performed to obtain similarity amongst samples (Hammer et al. 2001).

3. Results

Pollen spectra of marmeleiro honeys yielded 158 pollen types (Table 1) from 48 families and 103 genera with some indeterminate types. In addition, some pollen types were labelled as 'present', meaning that they were not counted during pollen counts but were recorded (Table 1).

The average number of pollen types per sample was 21 ± 10, with samples PI7, PI10 and PI13 having 40, 33 and 33 pollen types, respectively, and samples PI3, PI6 and PI8 having 8, 8 and 10 types, respectively. Five different types of *Croton* pollen were found in the analysed samples (Plate 1), and these pollen grains represented all of the frequency classes, with the exception of the predominant pollen class. The most abundant pollen types (Plates 2 and 3) were from plants belonging to the Leguminosae, Myrtaceae, Rubiaceae and Euphorbiaceae.

Leguminosae was the most dominant family, with 31 pollen types. *Mimosa caesalpiniifolia* (Plate 3, figure 1) and *Pityrocarpa moniliformis* (Plate 3, figure 3) were abundant in a total of 12 different samples

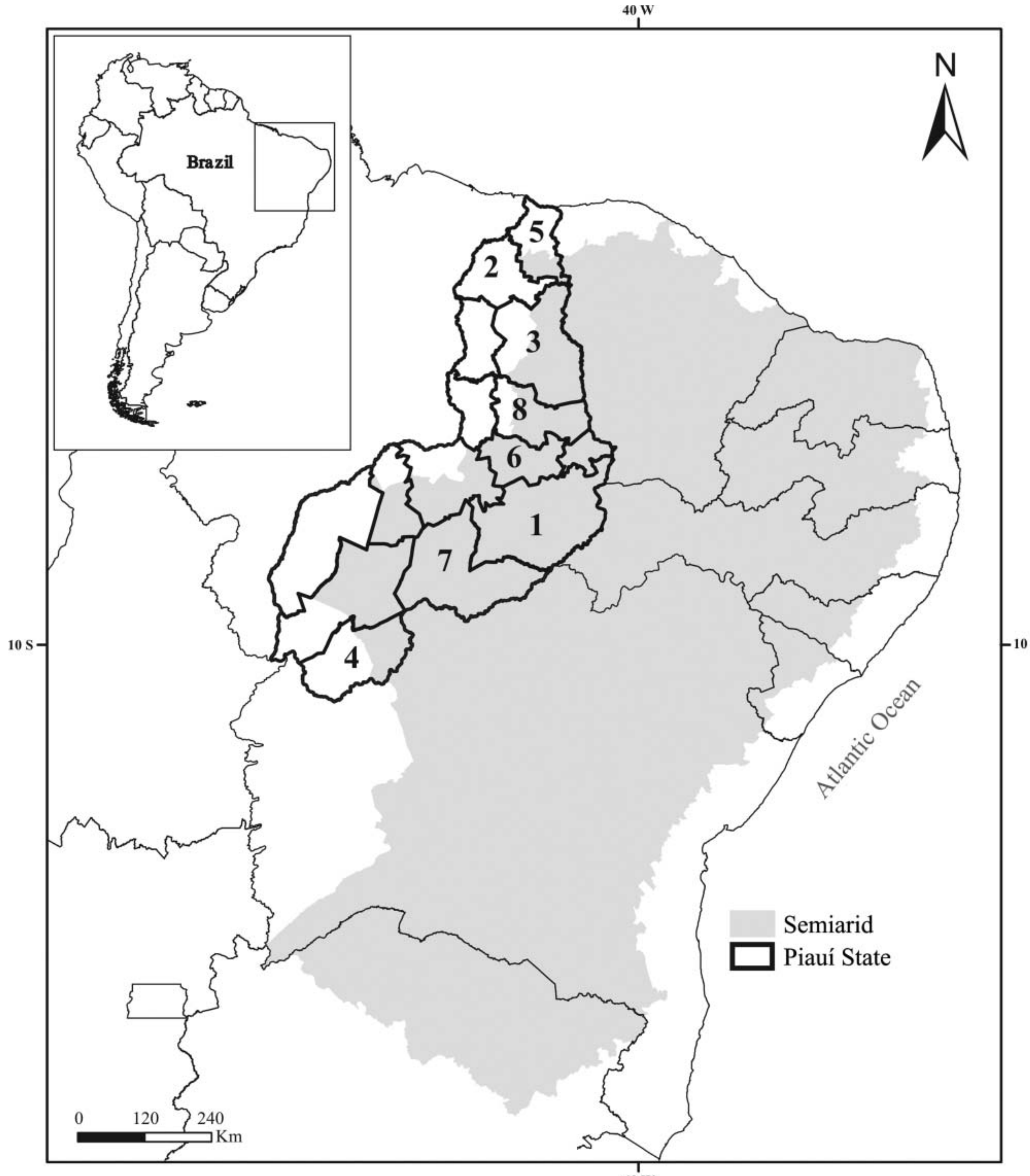


Figure 1. Northeast Brazil with the State of Piauí and its micro-regions. The micro-regions sampled are numbered as follows: 1 – Alto Médio Canindé, 2 – Baixo Parnaíba, 3 – Campo Maior, 4 – Chapadas do Extremo Sul, 5 – Littoral, 6 – Picos, 7 – São Raimundo Nonato, 8 – Valença do Paiuí.

(six samples each), followed by *Borreria verticillata* (Plate 3, figures 8–9)*,* *Combretum* sp. (Plate 2, figures 7–9) and *Mitracarpus salzmannianus* (Plate 3, figures 10–12), with dominance in one sample each. Samples PI7, PI10, PI11, PI17 and PI19–21 did not have a predominant pollen.

Considering secondary pollen types, Leguminosae was also the most represented family, with five pollen

Table 1. Frequency of pollen types in the marmeleiro honey samples from Piauí State, Brazil. Frequency classes: P – predominant pollen (> 45%), S – secondary pollen (16–45%), I – important minor pollen (>3–15%), m – minor pollen (1–3%), t – trace pollen (< 1%), • – pollen types only present, but not included at the pollen count. Concentration: I (< 20.000), II (20.000–100.000), III (100.000–500.000), IV (500.000–1.000.000) and V (> 1.000.000 pollen grains/10 g of honey).

		Honey samples																					
Pollen types		PI1	PI2	PI3	PI4	PI5	PI6	PI7	PI8	PI9	PI10	PI11	PI12	PI13	PI14	PI15	PI16	PI17	PI18	PI19	PI20	PI21	PI22
	Concentration	II	V	II	III	II	I	II	I	II	II	II	III	II	II	I	I	I	II	III	I	I	II
Acanthaceae																							
Justicia				•																			
Amaranthaceae																							
Alternanthera		m	•				t			t	t		t				t		m	I	t	•	
Amaranthus		m					t							t					t	t			
Gomphrena		t									•												
Anacardiaceae																							
Schinus				I	t		t	t		t	I			t				•			m		
Spondias							t													t			
Anacardiaceae							t										t						
Annonaceae																							
Rollinia														t									
Apiaceae																							
Apiaceae																						•	
Arecaceae																							
Chamaedorea					t																		
Elaeis					t																		
Syagrus							m			t				t				t	•				
Asteraceae																							
Aspilia																		t	t				
Bidens													t					•				t	
Emilia										m													
Eupatorium								t														t	
Gochnatia									t														
Lepidaploa											•							t			I		
Vernonia 1		t					t												m	m	I		
Vernonia 2																					t		
Asteraceae																					t		
Bignoniaceae																							
Piriadacus erubescens		t																					
Spatodea										•													
Boraginaceae																							
Heliotropium	•																						
Tournefortia										t													
Varronia										t												t	
Boraginaceae										t													
Burseraceae																							
Protium													t										

(*continued*)

Table 1. (*Continued*)

Pollen types		PI1	PI2	PI3	PI4	PI5	PI6	PI7	PI8	PI9	PI10	PI11	PI12	PI13	PI14	PI15	PI16	PI17	PI18	PI19	PI20	PI21	PI22
		Honey samples																					
Bromeliaceae																							
Bromeliaceae												t											
Cactaceae																							
Cereus							•																
Cactaceae																	t						
Cannabaceae																							
Celtis iguanaeus													t				t						
Trema micrantha								t															
Caryophylaceae																							
Caryophylaceae							t						t										
Combretaceae																							
Combretum	P	I				I	I			t	t	I	t	m			t				m	•	
Combretaceae 1	S				t		t		t				m										
Combretaceae 2	m			m																			
Commelinaceae																							
Commelina erecta L.	t	t					t												m	t	t		
Convolvulaceae																							
Evolvulus glomeratus		t							t					t									
Jacquemontia		•		t									t										
Cucurbitaceae																							
Cucurbitaceae														•									
Elaeocarpaceae																							
Elaeocarpaceae										m													
Euphorbiaceae																							
Bernardia													t										
Croton 1		t	m		t		m	•		S			m			t		•	S	t			
Croton 2					t		t			I			m	t								t	
Croton 3							I			m	t		m	t	t								
Croton 4										m			m										
Croton 5										m									I				
Euphorbia													t										
Pedilanthus							t																
Poinsetia													•										
Ricinus communis		•							t				t			t							
Sapium										I	t												
Euphorbiaceae													m		m								
Gentianaceae																							
Schultesia		•				t	t			t						t	m		I				
Lamiaceae																							
Hyptis		•	•	m			t				t		•					t	m	m	I		
Raphiodon														t									
Salvia		t		t																	I		

(*continued*)

Table 1. (*Continued*)

Pollen types		Honey samples																					
		PI1	PI2	PI3	PI4	PI5	PI6	PI7	PI8	PI9	PI10	PI11	PI12	PI13	PI14	PI15	PI16	PI17	PI18	PI19	PI20	PI21	PI22
Lauraceae																							
Ocotea cernua																t							
Leguminosae																							
Acacia angustissima																		t					
Aeschynomene					m								t	m				t				t	
Anadenanthera colubrina										t			t	t					t				
Bauhinia																		•	•			•	
Caesalpinia							t							t									
Chamaecrista 1			•				t					t		t								t	
Chamaecrista 2																					t		
Copaifera martii													•	m			t					•	
Desmanthus										•		•		t				•					
Galactia remansoana												t						t					
Inga quaternata																	t						
Inga 1		•	•										•		m	t	t						
Machaerium		m	m				t			•	m	•	t				t	m		t			
Mimosa caesapiniifolia	I	I	P	P	m	P	I	P	P	m	m	P	I	m		t	t	I	S	S	S	t	
Mimosa lepidophora					t													m					
Mimosa misera		t	t	m	m		m			t	t		t	t	m		m	S	m		I	t	
Mimosa pithecelobioides	m																						
Mimosa pudica				t	m	I	m	t	t		t	I	t	t	m	t	m		S		m		
Mimosa serrulata																			t				
Mimosa tenuiflora			m	m		t				t	I	I	t	I	S	I	S	t	m	m	I	•	
Mimosa ursina										t													
Mimosa xiquexiquensis		t			t						t	t				I	I		•		t		
Parapiptadenia zenthneri					t																		
Piptadenia stipulaceae	t																	t					
Pithecelobium rufescens														t	t								
Pityrocarpa moniliformis	I	m	t		P	I	I	t	S	S	I	t	P	P	P	P	S	S	t	t	I	P	
Senegalia piauhiensis	t	t					•	•		t	m	t						t				•	
Senna undulata															t								
Senna 1		I	t								t		t						I	t			
Senna 2													t						t				
Zornia							I				t			t				•					
Leguminosae 1												t		t									
Leguminosae 2								t															
Malpighiaceae																							
Banisteriopsis							t								t	t				t	t		
Byrsonima					t						t											t	
Malpighia emarginata																	m						

(*continued*)

Table 1. (*Continued*)

Pollen types		Honey samples																					
		PI1	PI2	PI3	PI4	PI5	PI6	PI7	PI8	PI9	PI10	PI11	PI12	PI13	PI14	PI15	PI16	PI17	PI18	PI19	PI20	PI21	PI22
Malvaceae																							
Helicteris eichleri										t								t					
Heliocarpus															t								
Herissantia tiubae		•	•							I				•	t		t						
Luehea							m																
Melochia/Waltheria			•				t			t							t						
Sida					t									•	t		t						
Malvaceae				t																			
Melastomataceae																							
Clidemia																		t					
Huberia																			•				
Melastomataceae		•																					
Myrtaceae																							
Aulomyrcia																			•				
Eugenia	t				t		t			I	I		t	t				m					
Myrcia fosteri	t																						
Myrcia laurotteana							t												t				
Myrcia multiflora																		t					
Myrcia 1					m						I				I	t	S	I				t	
Myrcia 2									t						t		I						
Myrcia 3											I						t						
Myrcia 4								t									m	m					
Myrciaria																	t		•				
Psidium								t		I													
Myrtaceae 1			•								m							t					
Myrtaceae 2					t						t							I					
Myrtaceae 3					m	m	S			m			t					t					
Nyctaginaceae																							
Boerhavia																			•				
Piperaceae																							
Peperomia									t														
Piper	t						I	m			t										m		
Phytolacaceae																							
Microtea	m	•			t		I			t	t			m				t					
Poaceae																							
Poaceae		t	•	t			m		t	t	t	t	I	t	t	t	t	t	t		t		
Polygalaceae																							
Polygala																		t	•				
Polygonaceae																							
Polygonum																		•					
Portulacaceae																							
Portulaca							•			•													
Proteaceae																							
Roupala													•										

(*continued*)

Table 1. (*Continued*)

Pollen types		Honey samples																					
		PI1	PI2	PI3	PI4	PI5	PI6	PI7	PI8	PI9	PI10	PI11	PI12	PI13	PI14	PI15	PI16	PI17	PI18	PI19	PI20	PI21	PI22
Rhamnaceae																							
Ziziphus joazeiro		•							t	t	I		m					t	t				
Rubiaceae																							
Borreria densiflora																					t		
Borreria latifolia					t		t			t											I		
Borreria ocimoides																	t						
Borreria verticillata	t	I	t	S			t	•	t	m	t	•	•	m	m	t	t	I	t	P	I	•	
Borreria 1	m																						
Chomelia																				t			
Diodela radula																		•			t		
Faramea													•										
Mitracarpus albomarginatus				I					•														
Mitracarpus baturitensis																		t					
Mitracarpus salzmannianus	t	P																		t			
Richardia grandiflora		•					t						•	t									
Rubiaceae	t																						
Rutaceae																							
Citrus										t													
Salicaceae																							
Casearia arborea															m								
Sapindaceae																							
Paullinia										m			t										
Serjania															t			t					
Sapindaceae 1																	m						
Sapindaceae 2							t																
Sapotaceae																							
Pouteria										t	t												
Scrophulariaceae																							
Angelonia					m		t			m													
Solanaceae																							
Capsicum																t							
Solanum					t					m	t			t									
Solanaceae										t					t			•	•				
Turneraceae																							
Turneraceae												•											
Urticaceae																							
Cecropia						t	I				t		t			I	I	t			t		
Urera													t										
Verbenaceae																							
Lippia																			•				
Violaceae																							
Violaceae							•																

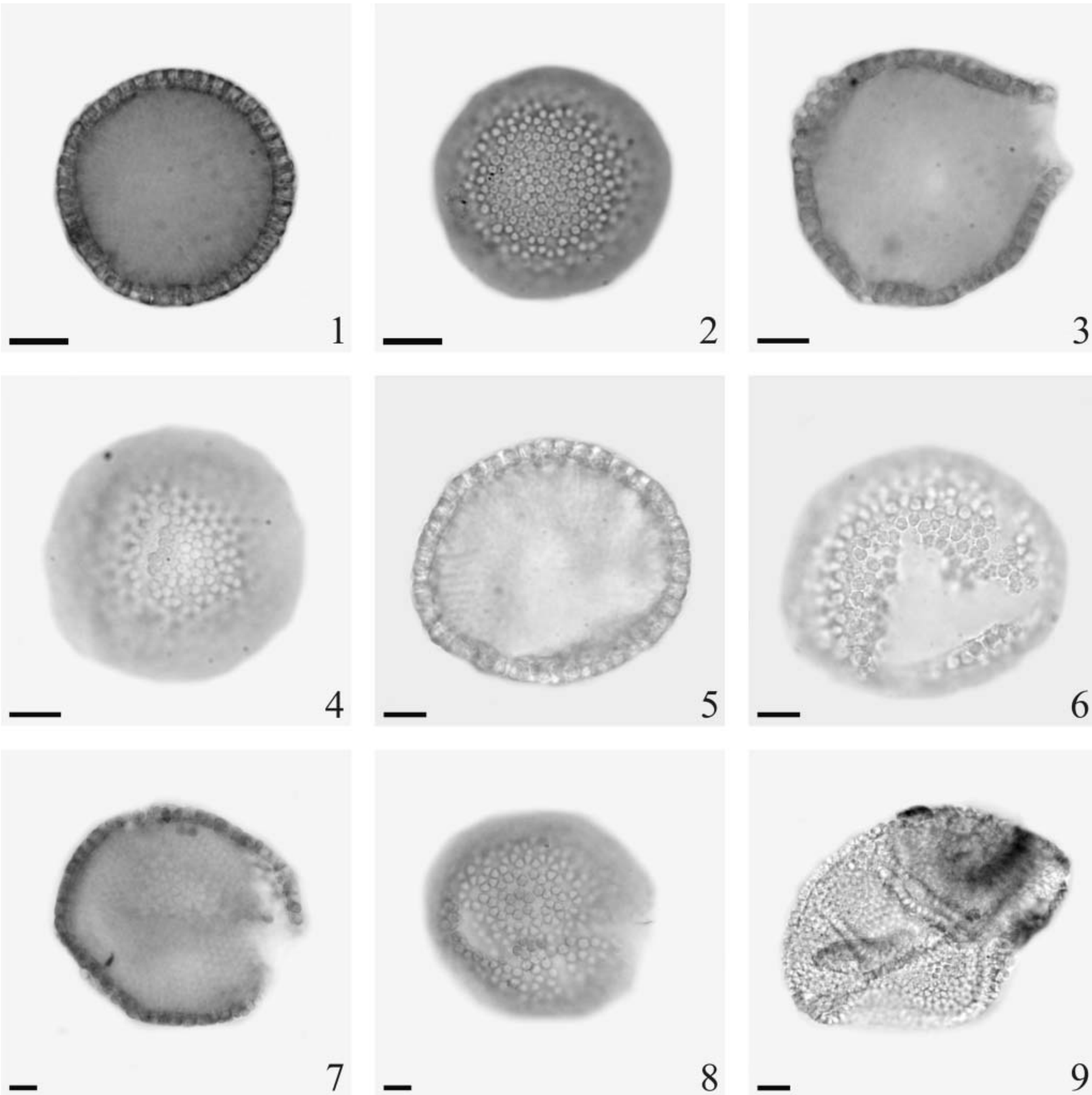


Plate 1. *Croton* (Euphorbiaceae) pollen types present in marmeleiro honey from the State of Piauí, Brazil. Some pollen grains are damaged or folded. 1–2. *Croton* 1; 3–4. *Croton* 2; 5–6. *Croton* 3; 7–8. *Croton* 4; 9. *Croton* 5. Scale bars = 10 μm.

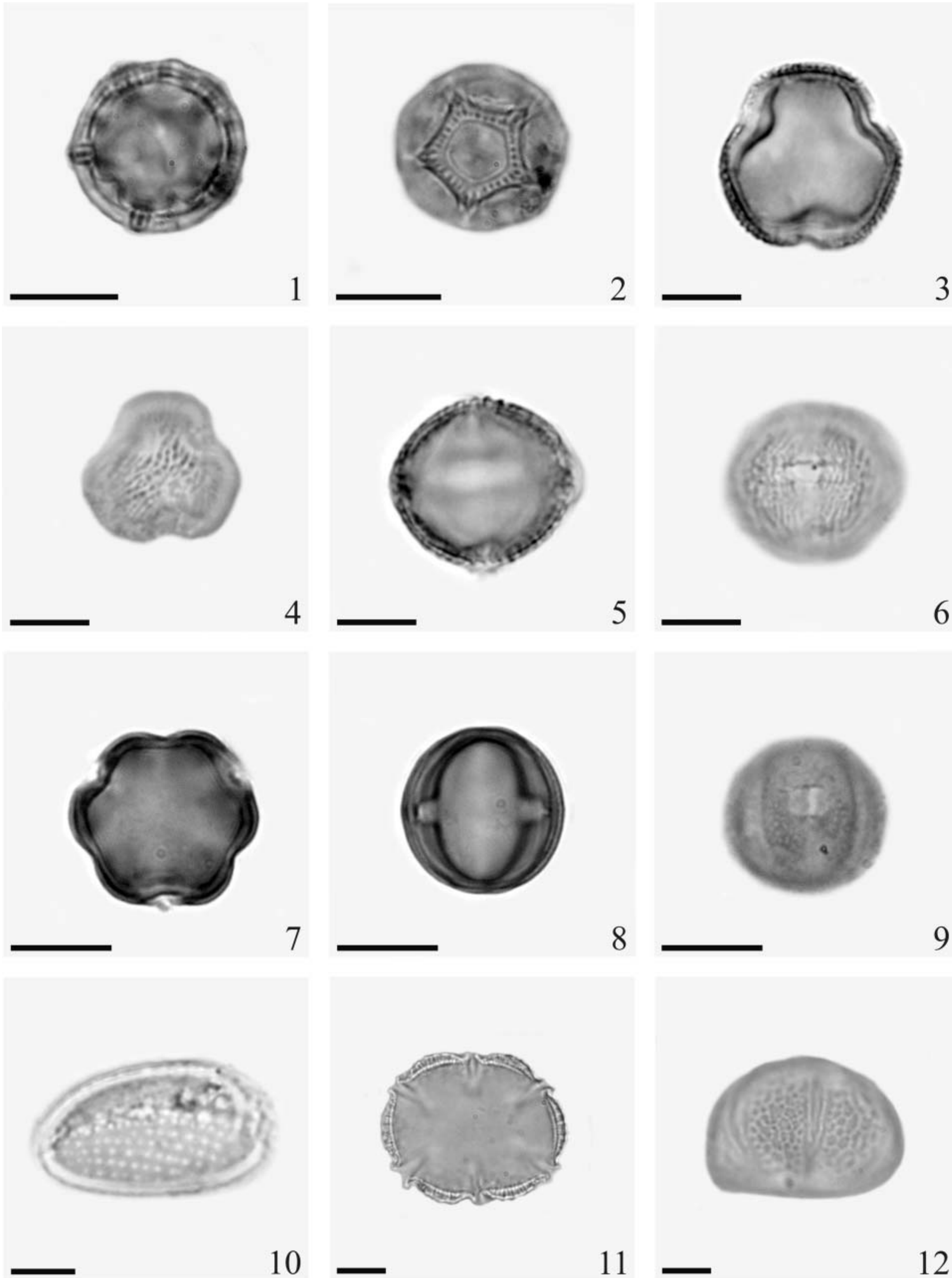


Plate 2. Main pollen types present in samples of marmeleiro honey from the State of Piauí, Brazil. Amaranthaceae: 1–2. *Alternanthera*; Anacardiaceae: 3–6. *Schinus*; Combretaceae: 7–9. *Combretum*; Commelinaceae: 10. *Commelina*; Lamiaceae: 11–12. *Hyptis*. Scale bars = 10 μm.

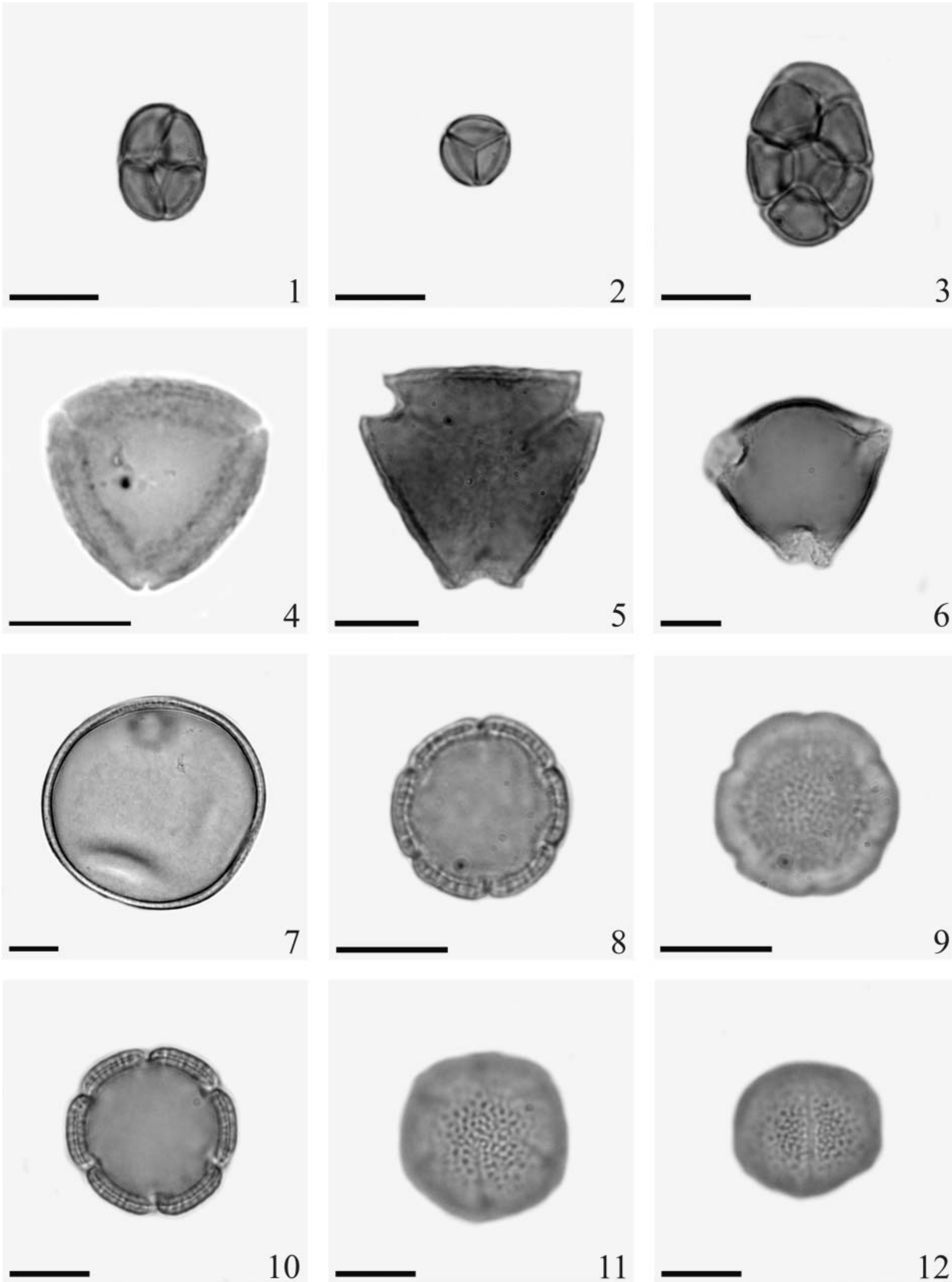
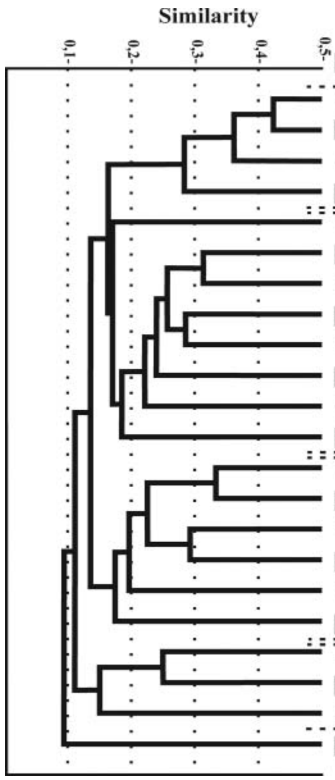


Plate 3. Main pollen types present in samples of marmeleiro honey from the State of Piauí, Brazil. Leguminosae: 1. *Mimosa caesalpiniifolia*; 2. *M. pudica*; 3. *Pityrocarpa moniliformis*; Myrtaceae: 4. *Eugenia*, 5. *Myrcia* 1; 6. Myrtaceae 3; Poaceae: 7. Poaceae; Rubiaceae: 8–9. *Borreria verticillata*; 10–12. *Mitracarpus salzmannianus*. Scale bars = 10 μm.



Sample	Municipalities	Veg.	Pollen types	Frequency 1	Frequency 2	Croton	
PI 2	São João do PI	C	20	*Mitracarpus salzmannianus*	*Borreria verticillata, Combretum*	0.2%	I
PI 20	São Braz do PI	C	16	-	*Alternanthera, M. caesalpiniifolia*	0.4%	
PI 19	Caracol	E	18	-	*Croton* 1, *M. caesalpiniifolia, M. pudica*	25.8%	
PI 3	João Costa	C	8	*M. caesalpiniifolia*	-	1.4%	
PI 4	Jaicós	C	13	*M. caesalpiniifolia*	*Borreria verticillata*	-	II
PI 7	Castelo do PI	E	40	-	Myrtaceae 3, *P. moniliformis*	4.32%	
PI 21	Inhuma	E	26	-	*Borreria latifolia, M. caesalpiniifolia*	-	
PI 10	Avelino Lopes	E	33	-	*Croton* 1, *P. moniliformis*	30.22%	
PI 11	Curimatá	E	30	-	*Myrcia* 1, *Myrcia* 3, *Ziziphus joazeiro*	0.2%	
PI 14	Picos	C	29	*P. moniliformis*	-	0.56%	
PI 13	Buriti dos Lopes	L	33	*P. moniliformis*	*M. caesalpiniifolia*	6.18%	
PI 18	São Raimundo Nonato	C	29	-	*M. caesalpiniifolia,* Myrtaceae 2, *P. moniliformis*	•	
PI 6	Castelo do PI	E	8	*M. caesalpiniifolia*	-	-	III
PI 16	Coronel José Dias	C	16	*P. moniliformis*	-	0.6%	
PI 15	São Raimundo Nonato	C	20	*P. moniliformis*	*M. tenuiflora, Myrcia* 1	0.2%	
PI 17	São Raimundo Nonato	C	30	-	*M. tenuiflora, Myrcia* 1, *P. moniliformis*	-	
PI 9	Sigefredo Pacheco	E	12	*M. caesalpiniifolia*	*P. moniliformis*	-	
PI 12	Piracuruca	E	12	*M. caesalpiniifolia*	*Combretum, M. pudica*	-	
PI 5	Brasileira	E	24	*P. moniliformis*	-	1%	IV
PI 22	Lagoa do Sitio	E	11	*P. moniliformis*	-	0.193%	
PI 8	Pedro II	E	10	*M. caesalpiniifolia*	-	•	
PI 1	Itainópolis	C	17	*Combretum*	Combretaceae 1, *P. moniliformis*	-	

Figure 2. Pollen similarity analysis of marmeleiro honey from the State of Piauí, Brazil. I, II, III and IV are groups formed by pollen similarity. Vegetation type (Veg.): C = caatinga, E = ecotones (transitional areas), L = littoral (coastal vegetation). Pollen types (number of pollen types excluding unidentifiable types). Frequency 1 (pollen types with frequency of more than 45%); Frequency 2 (pollen types with frequency between 10% and 45%); Croton (frequency of the *Croton* type in the samples); • = present, but not included in the counts.

types in seven samples. *P. moniliformis* and *M. caesalpiniifolia* were present in four (PI9, PI10, PI17, PI18) and three samples (PI19–21), respectively; followed by *Croton* 1 (PI10 and PI19) and *Mimosa tenuiflora* (PI15 and PI17), which were each found in two samples, and *Borreria verticillata* (PI4), Combretaceae sp. 1 (PI1), *Mimosa misera* (PI18), *Mimosa pudica* (PI19), *Myrcia* 1 (PI17) and Myrtaceae sp. 3 (PI7), which were all found in one sample. Other pollen types were represented as important minor pollen (I), minor pollen (m) or trace pollen (t).

Of the plant families identified, 30.76% were present with low frequency in only one sample, and most of them were classified as trace pollen; 25.64% of the families were present in over 40% of the samples, with Combretaceae, Leguminosae and Rubiaceae being the most representative in all of the categories described by Louveaux et al. (1978). The most frequent pollen types were *M. caesalpiniifolia* and *P. moniliformis*, which were found in 95% of the samples, and *B. verticillata*, *M. misera, M. pudica*, *M. tenuiflora* and Poaceae, which were found in 68% of the samples.

Leguminosae was present in all samples and had the greatest number of pollen types in the three vegetation areas, with 25 in caatinga, 23 in transitional areas and 10 in coastal areas. Seventy-three percent of Euphorbiaceae pollen types were present in sample PI13 (coastal areas).

Even though they were designated marmeleiro honeys by their producers, the pollen profile of PI1, PI4, PI6, PI12, PI17 and PI21 had no pollen types associated with *Croton* spp. In samples PI8 and PI18, some pollen grains related to *Croton* spp. were recorded as 'pollen present' (Table 1). In other samples, *Croton* spp. were recorded in classes S, I, and m (Table 1), with prominence for *Croton* 1, which was found in 20.6% of sample PI10 and in 22.4% of sample PI19. All samples, excluding PI21, were obtained from January to April, when *Croton* species are blooming (Souza et al. in press).

Similarity analysis (Figure 2) produced four major groups, designated I, II, III and IV, with samples from different vegetation types. Group I was represented by samples PI2, PI20 and PI3, from caatinga vegetation, and sample PI19 from the transitional areas. Group II was characterised by samples from all three vegetation types. Group III included samples from the caatinga and transitional areas, while Group IV contained samples only from the transitional areas.

Using an exotic marker, the concentration of pollen grains in 10 g of honey was calculated, and the samples were grouped into categories I, II, III and V (Table 1), in accordance with Maurizio (1976). Sample PI17 showed the smallest number of pollen grains per 10 g of honey, with 2044 pollen grains, while sample PI02 showed the largest, with 1,548,583 pollen grains.

4. Discussion

The great richness of pollen types found in the samples analysed is explained by the fact that it was a study of samples from different localities and from different vegetation types (Shubharani et al. 2012), reflecting the plant diversity in the state of Piauí. Studies using large samples from many localities tend to identify a large number of pollen types as observed in Sajwani et al. (2007), Oliveira et al. (2010) and Shubharani et al. (2012).

The diversity of pollen types per sample, when comparing samples PI7, PI10 and PI13 with samples PI3, PI6 and PI8, did not correlate with the vegetation types because both the caatinga and the transitional areas had samples with high and low richness of pollen types per sample. The difference in the diversity of the pollen types is also not explained by the climatic conditions, given that the samples were obtained between January and April when the average index of monthly rainfall in Piauí is greater than 100 mm, facilitating continuous exuberant flowering.

The high representation of Euphorbiaceae, Leguminosae, Myrtaceae and Rubiaceae pollen in the samples is because all of these families are well represented in the Brazilian semiarid region, with the largest number of species in the caatinga and cerrado (Queiroz et al. 2006). Leguminosae, which is widely found in Brazilian semiarid regions, especially the caatinga (Giulietti et al. 2002; Queiroz et al. 2006), showed the greatest number of pollen types for this type of vegetation, with 25 types, and was present in 23 types in the transitional areas. This result is not surprising because many of these species are good nectar and pollen producers.

The similarity between the transitional areas and the caatinga is because the transitional areas in Piauí, according to Castro (2007), are characterised by interpenetrating vegetation types of the cerrado, caatinga and carrasco (deciduous spineless vegetation). According to Queiroz et al. (2006), Leguminosae is present in caatinga and cerrado vegetation, representing 18.4% and 8.29% of the species, respectively. Its high frequency is also supported by Barth (1989), who identified *Pityrocarpa moniliformis* as an indicator of honeys from Piauí when predominant, and by Sodré et al. (2007, 2008) who studied samples from Piauí. Other studies in the Brazilian semiarid region also suggest that legumes are frequently present in various bee products (Novais et al. 2006, 2009; Oliveira 2009; Oliveira et al. 2010; Nascimento 2011), and indicate the importance of these plants in the maintenance of honey bee colonies.

Santos-Filho (2009) conducted a survey of the coastal vegetation of the State of Piauí and found 15 species of Euphorbiaceae, five of which were *Croton*. These data may explain the high pollen type diversity of Euphorbiaceae in sample PI13.

The cluster analysis results did not assign prominence to any of the three vegetation types, but some inferences may still be made. Although Group I is composed of three samples from the caatinga and one sample from the transitional areas, their locations are geographically close, which may have contributed to the similarity in their pollen spectra. Group II is represented by samples from the caatinga (PI4, PI14 and PI18), coastal areas (PI13) and transitional areas (PI7, PI10, PI11 and PI21) and is divided into smaller groupings, as in the case of [PI10 + PI11], with samples from neighbouring municipalities, and that of [PI7 + PI21], which, although geographically distant, belong to the same vegetation type. This grouping, even with the diversity of vegetation types, can be explained by the floristic composition of ecotones of Piauí, which are marked by the presence of species typical of both the cerrado and caatinga (Castro 2007).

Group III is characterised by two smaller clades, [PI6 + PI16] and [PI15 + PI17], and by PI9 and PI12 individually. Samples PI6 and PI16 seem to be grouped based on the low number of shared types (six). PI15 and PI17 are from the same municipality, São Raimundo Nonato, and have several pollen types in common, such as *Herissantia tiubae*, *Sida* and *Myrcia* 2, which were found in only a few studied samples. Group IV is representative of the transitional areas only, with PI5 and PI22 the most interrelated samples. These samples share *Aeschynomene* and *Byrsonima* pollen types, which are rarely shared in other samples, and both have *Pityrocarpa moniliformis* as the predominant pollen type.

Beekeepers' designation of the botanical origin of their honey is based on field observations and their belief that bees visit only one type of resource at a time (Todd & Vansell 1942). However, our study and other studies comparing beekeepers' labelling with the results of pollen analyses (Moar 1985; Bryant & Jones 2001) contradict beekeepers' opinions. As previously shown by Todd & Vansell (1942), Maurizio (1976), Louveaux et al. (1978), Moar (1985) and Bryant & Jones (2001), it is necessary to evaluate more than just the pollen assemblages and instead combine this information with those about floral sources of nectar.

For example, *Croton blanchetianus* (one of the species know as a marmeleiro plant) has low nectar production (Alves et al. 2005), but, because of its wide distribution with individuals in large numbers

(Carvalho et al. 2001; Santana 2009) and the large number of flowers per inflorescence, it is an important source of nectar and pollen.

Furthermore, according to Torres (2009), other species of *Croton* are also popularly known as marmeleiro, demonstrating that non-scientific knowledge recognizes marmeleiro as not just one species, but a complex of different species. According to Torres (2009), amongst the *Croton* species that occur in the semiarid region of northeast Brazil, those known as marmeleiro include *Croton andrade-limae* A.P. Gomes & M. Sales, *Croton blanchetianus* Baill., *Croton nepetifolius* Baill., *Croton* aff. *triangularis* (Müll. Arg.) Kuntze and *Croton urticifolius* Lam. All of these species have very similar pollen grains, which are characterised by exine ornamentation consisting of an array of pila in small overlapping circles (the *Croton* pattern).

It should be noted that other species of Euphorbiaceae in the semiarid region have pollen grains of the *Croton* pollen type, such as species of *Cnidoscolus* Pohl and *Julocroton* Mart. (Salgado-Labouriau 1973), as well as other *Croton* species with the same pollen type that are not popularly known as marmeleiro (Oliveira & Santos 2000), thus extending the limits of this species complex even further.

The high number of species designated as *Croton* type can also be observed in the number of pollen types in the samples analysed, the five types related to this genus. Taking into account the whole '*Croton* complex', i.e., considering all *Croton* types as one, to designate marmeleiro honey, sample PI10 would be 30.03% *Croton*, while PI19 would be 25.8% *Croton*, which could indicate monofloral marmeleiro honeys, as is the case in other under-represented pollen types, such as *Citrus* spp., *Medicago* spp. and *Tilia* spp. (Maurizio 1976; Louveaux et al. 1978). However, in terms of the pollen concentration, samples PI10 and PI19 should be considered heterofloral.

The pollen profile of sample PI1 was 45.6% *Combretum* pollen type and could be considered monofloral (Santos et al. 2005). Quirino & Machado (2001) studied the floral biology of three species of *Combretum*, two from the caatinga (*C. leprosum* Mart. and *C. pisonioides* Taub.) and one from the Atlantic forest (*C. fruticosum* (Loefl.) Stuntz), and noted that the caatinga species can have inflorescences with up to 45 flowers with the nectar production of ca. 5 μL per flower and sugar concentrations of up to 21%. These data show that the former species are good producers of nectar and confirm our diagnosis of sample PI1.

Sample PI8 was considered monofloral, with 93.3% *Mimosa caesalpiniifolia* because, as noted by Maurizio (1976), honeys in which the predominant pollen type is from species with over-represented pollen exceeding 90% can be considered monofloral.

Pityrocarpa moniliformis (Benth.) Luckow & R.W. Jobson pollen was present in samples PI5, PI13–PI16 and PI22 in percentages ranging from 52.4% to 96.13%. This species is also considered an important source of nectar and pollen (Santos et al. 2005). Despite its relatively low nectar production, ca. 1 μL per 10 flowers, individuals of *P. moniliformis* have ca. 200 flowers per inflorescence (Ferreira 2009) and are therefore a major nectar producer, making the cited honey samples potentially monofloral.

Samples PI2 and PI20, in which *Mitracarpus salzmannianus* and *Borreria verticillata*, respectively, were dominant, were not considered monofloral. No studies of the characteristics of nectar produced by *Mitracarpus salzmannianus* have been performed; its nectar volume and sugar concentration are unknown, and very little is known about its floral visitors. This is also true of *Borreria verticilatta* G. Mey, which is considered by Santos et al. (2005) to be a nectar producer, but no studies on its characteristics are available. Although the above samples were not considered monofloral, these two bee plants are likely to be the main nectar providers for those honeys.

Louveaux et al. (1978) showed that the absolute number of pollen grains per 10 g of honey varies between 20,000 and 100,000 in most flower honeys, corresponding to Class II, with our own data confirming this classification by placing most samples in Class II. The same authors found Class I honeys to be generally monofloral and poor in pollen grains. That finding was not confirmed in our study because only two of the seven samples (PI8 and PI22) were considered to be monofloral.

5. Conclusions

Contrary to beekeepers' labelling of their honey as marmeleiro (*Croton* spp.), only two of the samples examined showed evidence of a possible monofloral status in their pollen spectra, with the amount of pollen grains of the *Croton* type compatible with other under-represented types (e.g., *Citrus*, *Medicago* and *Tilia*), and, even though the percentage of pollen grains belonging to these types were below 45%, they may still be considered monofloral.

However, more studies are needed to support such claims because the relative contribution (pollen/nectar) of the *Croton* species to honey production remains unknown. Because of the great diversity in vegetation in the Brazilian semi-arid areas and insufficient knowledge about the behaviour of bees, foraging observations alone are not sufficient to establish the true contribution (of the nectar/pollen) to the composition of honeys of the native flora, and melissopalynological studies are essential to the accurate identification of the botanical sources in such honeys. Our results show the importance of pollen studies in the determination of plant sources of honeys, and strengthen the argument that melissopalynology is an efficient method for such studies.

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