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Pollen analysis in honey samples (*Apis mellifera* L.) from the main honey-producing microregion in Brazil

Análise polínica de amostras de mel de *Apis mellifera* L. provenientes da principal microrregião produtora do Brasil

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Abstract: In Piauí, beekeeping has become one of the most important economic activities in the state, particularly in the semi-arid region, where the Alto Médio Canindé microregion stands out as the largest honey producer in the country. Honey derived from the flowering plants of the Caatinga vegetation, exhibits distinct sensory characteristics, and understanding the plant species responsible for honey production in Piauí can be a valuable tool for enhancing the product's value. In this context, the aim of this study was to identify the main pollen types present in honey samples from the Alto Médio Canindé microregion, Piauí. To achieve this, 50 samples were collected from municipalities within the territory. The identification of the pollen spectrum in the samples was performed by counting and determining the frequency of the most representative pollen grains. As a result, 36 pollen types were found. Of these, 16 types were identified as belonging to 10 botanical families, with the Fabaceae family showing the highest representation. The pollen types with the highest grain frequency were *Mimosa caesalpiniifolia, Sida maculata, Piptadenia retusa, Combretum leprosum,* and *Croton*. The presence of pollen types from *Mimosa caesalpiniifolia, Croton, Jatropha*, and *Sida maculata* confirmed the honey's origin as being from the Caatinga biome, as these genera include a high number of endemic species.

Key-words: bee flora; beekeeping; melissopalynology.

Resumo: No estado do Piauí, a apicultura consolida-se como uma das atividades econômicas mais importantes do Estado, especialmente no território semiárido, com destaque para a microrregião Alto Médio Canindé, maior produtora nacional. O mel proveniente das floradas do bioma Caatinga apresenta características sensoriais diferenciadas e o conhecimento relativo às espécies vegetais responsáveis pela produção apícola no Piauí pode ser um importante instrumento para a valorização do produto. Nesse sentido, o objetivo desse estudo foi identificar os principais tipos polínicos presentes em amostras de mel provenientes da microrregião Alto Médio Canindé, Piauí. Para tanto, foram adquiridas 50 amostras junto aos apicultores dos municípios da referida microrregião. A identificação do espectro polínico foi realizada por meio da contagem e do estabelecimento de frequências para os grãos de pólen presentes nas amostras. Como resultado, encontrou-se 36 tipos polínicos. Destes, foram identificados 16 tipos que pertencem a 10 famílias botânicas, sendo que a família Fabaceae apresentou a maior representatividade. Os tipos polínicos em maior frequência foram: *Mimosa caesalpiniifolia, Sida maculata, Piptadenia retusa, Combretum*

leprosum e *Croton*. Concluiu-se que a presença de grãos de pólen de *Mimosa caesalpiniifolia, Croton, Jatropha e Sida maculata* atestaram a origem do mel como proveniente do Bioma Caatinga, sendo gêneros com elevado número de espécies endêmicas.

Palavras-chave: apicultura; flora apícola; melissopalinologia.

1. Introduction

Piauí, the third-largest state in the Northeast of Brazil [1], is located between the Amazonian sub-humid and the Northeastern semiarid regions, forming one of the largest ecotonal zones in the country, with a diversity of landscapes consisting of different soil and climate profiles[2,3]. These traits, combined with the adaptability of the Africanized honeybee (*Apis mellifera* L.), especially to semiarid climates[4], contribute to making this region responsible for the highest amount of honey produced in the country, particularly the Alto Médio Canindé microregion, the largest national producer[5]. Thus, beekeeping strengthens the local economy and enables producers and their families to remain in rural areas[6] through the sale of honey, which is primarily intended for export. It is important to note that, according to the Ministry of Industry, Foreign Trade, and Services[7], Piauí was the country's leading honey exporter in 2023.

Although other beehive products have received increasing attention from consumers and the scientific community in recent years, honey remains the most widely produced and marketed product worldwide[8]. In Piauí, most beekeepers are concentrated in the Caatinga biome[9], characterized by rich plant species diversity and a high degree of endemism[10,11,12,13]. Thus, honey produced from the blooms in this ecosystem has a unique composition, with specific traits mainly related to flavor, aroma, and color[14], such as honey derived from the Aroeira tree (*Myracrodruon urundeuva* Allemão)[15,16,17].

The floristic diversity of the region is reflected in the pollen grains found in honey samples. In this context, melissopalynology emerges as a methodology that allows researchers to infer the origin of bee products through the identification and counting of pollen grains[18], and determine their phytogeographic origin[19]. Thus, considering the high degree of endemism in the Caatinga, the presence of pollen types associated with endemic plant species can confirm the geographical origin of the honey, adding value to this important regional product. In this context, the aim of this study was to identify the main pollen types present in honey samples from the Alto Médio Canindé microregion.

2. Material and methods

The state of Piauí covers an area of 251,756.515 km^2 and is divided into four mesoregions, which are further subdivided into microregions [1]. In the Alto Médio Canindé microregion, the main honey producer in Piauí [5], the following municipalities were selected for honey sample collection from beekeepers' associations (Figure 1): Vera Mendes (341.974 km^2), Caridade do Piauí (498.793 km^2), Patos do Piauí (801.403 km^2), Paulistana (1,941.111 km^2), Jacobina (1,333.796 km^2), Massapê do Piauí (530.169 km^2), Acauã (1,280.838 km^2).

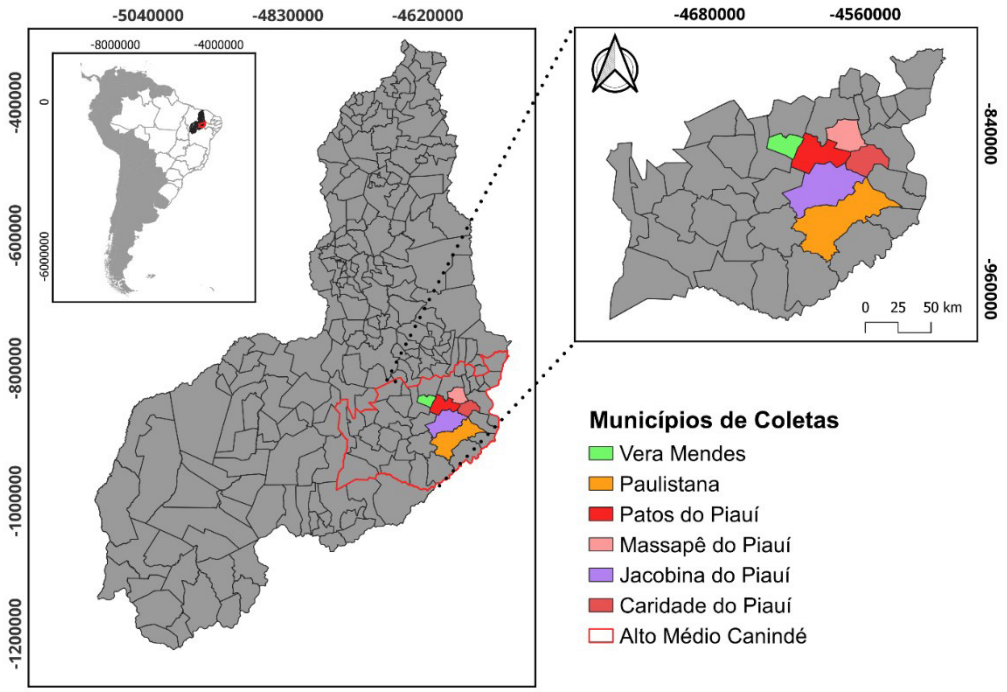

Figura 1. Municipalities where samples were collected in the Alto Médio Canindé microregion, Piauí.

This area lies within the Caatinga domain, with a BSh climate—hot and semiarid—according to the Köppen classification. The rainy season occurs during the summer, with an average annual rainfall of approximately 600 mm. Relative humidity is around 60%, dropping significantly during the dry season[20].

2.1 Collection of honey samples

Honey samples were collected from beekeepers' associations in six municipalities (Figure 1). Collections were conducted only during the rainy season (between December and April), which corresponds to the region's main honey harvest period. A total of 50 samples were collected and submitted to pollen analysis. Beekeepers were instructed to set aside a 300g sample from each harvest and label each plastic storage container with the collection date and the predominant blooming plants in the area surrounding their apiaries. This information was used solely to guide the identification of pollen types in the samples and not as data for the discussion. It is important to note that the samples were stored for six months in the laboratory at room temperature and protected from direct light exposure.

2.2 Honey sample processing

Melissopalynological analyses were conducted at the Laboratory of Bee and Apicultural Products Research (LEAPI) at the Federal University of Piauí. Each sample was homogenized and processed following the protocol by Louveaux, Maurizio, and Vorwol [19], while the sediments were acetolyzed according to Erdtman [21]. For each sample, pollen slides were prepared in triplicate using glycerin jelly to fix the material.

2.3 Pollen type identification and predominance analysis

To identify the pollen types present on the slides, the ZEN 2.3 SP1 microscopy software was used in conjunction with an image capture camera (Axiocam 105) coupled to a Zeiss optical microscope. The

polar and equatorial diameters of up to 25 pollen grains of each pollen type found in the samples were measured at 400x magnification (0.65).

This process allowed us to establish the shape and size of the pollen grains, which enabled the identification of pollen types (family, genus, or, whenever possible, botanical species). Identification was achieved by comparing the pollen grains with material deposited and described in the palinotheca of the Laboratory of Bee and Apicultural Products Studies, the Online Pollen Catalog Network (RCPol), and other available pollen catalogs [22, 23, 24].

Pollen frequency classes were determined based on the classification proposed by Louveaux, Maurizio, and Vorwohl [19]: dominant pollen (DP): > 45% of the total pollen grains, accessory pollen (AP): 16% to 45%, important isolated pollen (IIP): 3% to 15% and occasional isolated pollen (OIP): < 3%.

3. Results

Although 50 samples were collected, one did not contain any pollen grains and was therefore excluded from the study. According to the established categories [19], 39 samples were classified as monofloral, showing predominance (over 45%) of a single pollen type (Table 1).

Table 1. Pollen types found in honey samples from the Alto Médio Canindé microregion and their predominance percentages.

Family	Pollen types	DP%	AP%	IIP%	OIP%
Amaranthaceae	*Alternanthera tenella*	-	-	4.08	2.04
Combretaceae	*Combretum leprosum*	2.04	10.20	8.16	4.08
Euphorbiaceae	*Croton*	2.04	10.20	12.24	4.08
	Jatropha	-	2.04	6.12	2.04
Fabaceae	*Mimosa caesalpiniifolia*	55.10	24.48	8.16	-
	Piptadenia retusa	4.08	6.12	6.12	6.12
	Anadenanthera colubrina	-	2.04	-	-
	Pityrocarpa moniliformis	-	6.12	4.08	2.04
	Cenostigma nordestinum	-	-	4.08	2.04
Lamiaceae	*Mesosphaerum suaveolens*	-	-	4.08	2.04
Malvaceae	*Sida maculata*	10.20	8.16	10.20	8.16
Myrtaceae	Myrcia	-	-	-	2.04
Nyctaginaceae	*Boerhavia difusa*	-	2.04	-	2.04
Oxalidaceae	*Oxalis*	-	-	2.04	2.04
Rubiaceae	*Borreria verticillata*	-	-	6.12	2.04
	Rubiaceae	-	-	-	2.04
NR*	-	6.12	8.16	40.81	18.36

Dominant pollen (DP); Accessory pollen (AP); Important isolated pollen (IIP); Occasional important pollen (OIP); Not reported (NR)*.

The pollen spectrum of the honey samples from the study region comprised 36 pollen types. Of these, 16 were identified and are distributed among 10 botanical families (Table 1 and Figure 2). The presence of pollen types associated with endemic Caatinga species such as *Mimosa caesalpiniifolia*, *Pityrocarpa moniliformis*, and *Cenostigma nordestinum*, is highlighted.

In relation to the diversity of pollen types present in the analyzed samples, March had the greatest number of plant species used by the bees (Table 2).

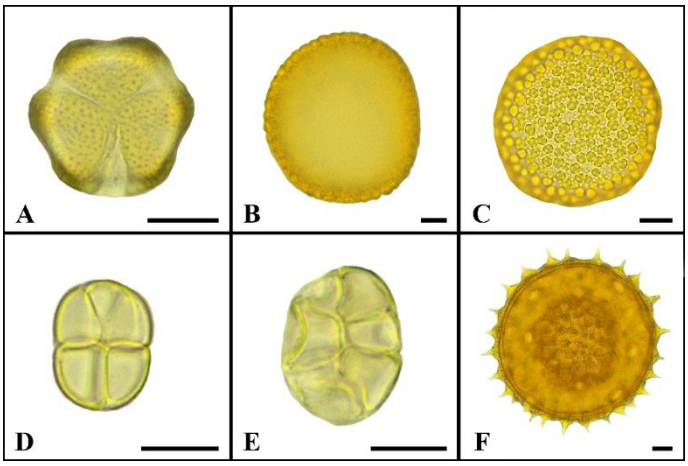

Figure 2. Dominant pollen types found in the honey samples. *Combretaceae*: A – *Combretum leprosum*. *Euphorbiaceae*: B and C – *Croton. Fabaceae*: D – *Mimosa caesalpiniifolia*. E – *Piptadenia retusa. Malvaceae*: F – *Sida maculata*.

Table 2. Dominant and accessory pollen types identified in honey samples from the Alto Médio Canindé microregion, according to the month of production.

Month/2022	Dominant Pollen (% of samples)	Accessory Pollen (% of samples)
December (n = 1)	*Combretum leprosum* (2.04%)	-
January (n = 11)	*Mimosa caesalpiniifolia* (10.20%) *Sida maculata* (4.08%) NI** (2.04%)	*Mimosa caesalpiniifolia* (6.12%) *Anadenanthera colubrina* (2.04%) *Sida* (2.04%) *Croton* (4.08%) *Boerhavia* spp. (2.04%)
February (n= 5)	*Mimosa caesalpiniifolia* (4.08%) *Piptadenia retusa* (2.04%) *Croton* (2.04%)	-
March (n = 13)	*Mimosa caesalpiniifolia* (18.36%) *Sida maculata* (2.04%) *Piptadenia retusa* (2.04%)	*Mimosa caesalpiniifolia* (8.16%) *Piptadenia retusa* (4.08%) *Pityrocarpa moniliformis* (4.08%) *Sida maculata* (4.08%) *Jatropha* (2.04%) *Croton* (4.08%) *Combretum leprosum* (2.04%) NI** (2.04%)
April (n = 6)	*Mimosa caesalpiniifolia* (8.16%)	*Mimosa caesalpiniifolia* (2.04%)
May (n = 3)	*Mimosa caesalpiniifolia* (4.08%)	-
Not reported (n = 11)	*Mimosa caesalpiniifolia* (10.20%) *Sida* (4.08%) NI** (4.08%)	*Mimosa* (2.04%)

Not reported: NR**.

4. Discussion

Given that the Fabaceae family contains the largest number of plant species in the Caatinga [25], their pollen types were the most abundant in our samples. This aligns with Aleixo *et al.* [26], Moraes *et al.* [27], and Bendini *et al.* [9], who studied the melliferous flora of the Alto Médio Canindé microregion and observed that Fabaceae species predominate among plants visited by Africanized bees.

Sodré *et al.* [28] identified a predominance of pollen types associated with Fabaceae taxa, such as Piptadenia, Mimosa caesalpiniifolia, and M. verrucosa, in honey samples from the municipality of Picos. Similarly, Borges *et al.* [29] recorded the consistent presence of Fabaceae pollen types in all analyzed samples from the municipality of Simplício Mendes (Alto Médio Canindé microregion), thereby confirming the importance of this botanical family for honey production in the region.

In this study, Mimosa caesalpiniifolia was the predominant pollen type present in the samples. According to Jesus *et al.* [30], Borges *et al.* [29], and Sodré *et al.* [28], Mimosa pollen types frequently occur in samples from Piauí. These authors attribute honey production in the state to the high nectar potential of these species.

On the other hand, Silveira [31] questions determining pollen type predominance solely based on frequency counts in samples. The author suggests that pollen grain volume should also be used to assess the effective contribution of botanical species to honey production.

Pollen grains from Mimosa species vary in size from very small to medium [24]. Therefore, based on Silveira's study [31], it can be inferred that the predominance of Mimosa grains ma not accurately reflect their real contribution to honey production. According to Marcazzan *et al.* [32], this reinforces the need to use combined melissopalynology, physicochemical, and sensory analyses to determine the product's origin.

Borges *et al.* [29] also highlight that the diversity of pollen types found in honey is related not only to the presence of flowering botanical species but also to the foraging behavior of bees. Therefore, studies on the floral resource availability of Mimosa species are necessary to elucidate their real contribution to honey production in Piauí.

In Piauí, species of the genus Croton are recognized as major contributors to honey production [27]. However, in the Alto Médio Canindé microregion, the Croton pollen type was dominant in only 2.04% of the samples and appeared as accessory pollen in 10.2% of all honey samples analyzed in this study. It is important to emphasize that, according to Bendini *et al.* [9] and Moraes *et al.* [27], the main Croton species in the region (*Croton betaceus* Baill., *Croton campestris* A. St.-Hil., *Croton mucronifolius* Müll. Arg. Shr, *Croton jacobinensis* Baill.) bloom between January and February. According to Souza[33], this period is also when most beekeepers in Piauí are populating their apiaries using bait hives, which often prevents them from fully exploiting the nectar-producing potential attributed to Croton species.

Bendini *et al.* [9] studied honey production dynamics and apicultural plants in the region of Simplício Mendes, Alto Médio Canindé, and observed that February and March showed the greatest diversity of flowering botanical species, corroborating the results obtained in the present study. This underscores the importance of synchronizing beekeeping management with floral resource availability to optimize the use of flowering areas by local beekeepers.

The presence of pollen types attributed to *Mimosa caesalpiniifolia, Pityrocarpa moniliformis,* and *Cenostigma nordestinum* in the samples suggests that the honey from the Alto Médio Canindé microregion originates in the Caatinga Biome. These species are recorded as belonging to this phytogeographic domain and, according to Giulietti *et al.* [10], are part of groups with a high degree of endemism in the Caatinga. Additionally, the Flora and Funga of Brazil platform also recognizes their distribution as characteristic of this biome [34, 35, 36]. Therefore, melissopalynological analysis has proven to be a useful tool for inferring the geographical origin of honey.

5. Conclusion

It was concluded that Fabaceae was the most representative botanical family among the apicultural species. The *Mimosa caesalpiniifolia* pollen type predominated in the samples considered monofloral. Other dominant pollen types in the samples analyzed were *Sida maculata, Piptadenia retusa, Croton,* and *Combretum leprosum*. The species representing these taxa deserve special attention from local beekeepers since they ensure honey production and the traceability of products originating in the Caatinga biome.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

Data will be made available on request to the corresponding author.

Author contributions

Conceptualization: A. L. S. Campos and J. do N. Bendini. Formal analysis: A. L. S. Campos and K. M. L. Borges. Visualization: A. L. S. Campos, K. M. L. Borges, M. C. de Abreu, and J. do N. Bendini. Supervision: J. do N. Bendini. Writing – Original Draft: A. L. S. Campos, K. M. L. Borges, and J. do N. Bendini..

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