Tracing botanical origins of unifloral Aroeira honey with Geographical Indication: Anacardiaceae pollen characterization from northern Minas Gerais' Seasonally Dry Tropical Forest and Cerrado.

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Abstract

Many species of Anacardiaceae from the Seasonally Dry Tropical Forest biome and Cerrado, an ecoregion of the Brazilian Tropical Savanna biome, are of great importance as a source of nectar and pollen. They are trees that massively bloom and attract several insects as floral visitors, especially honeybees and stingless bees. We aim to provide a practical tool for use in melissopalynological analyses of honeys, especially those produced in the northern region of Minas Gerais, Brazil. Here, a palynological description of six species of Anacardiaceae occurring in both vegetation types is provided using light and scanning electron microscopy. Statistical analyses were performed with the morphometric data to test the relevance of pollen morphology for defining pollen types and assisting in the certification of the botanical origin of honeys through melissopalynology. The most important metric variables for identifying the analyzed species were a combination of pollen grain size and width of the colpus margo, whereas pollen shape and other variables were less informative due to great intraspecific variation. *Spondias tuberosa*, with the largest pollen grains, stood out from the other species. The margo of the colpus wide contributed to the distinction of *Schinopsis brasiliensis* pollen grains. Qualitative traits analysis based on the pollen wall ornamentation contributed greatly to revealing two distinct groups: suprastriato-inframicroreticulate [*Astronium fraxinifolium*, *A. graveolens*, *A. urundeuva*, *Schinopsis brasiliensis*] and striate-perforate [*Schinus terebinthifolia, Spondias tuberosa*]. The palynological variation found among the studied species reinforces the importance of pollen morphology for the correct certification of botanical origin of Brazilian Aroeira honey.

Introduction

Belonging to order Sapindales, Anacardiaceae is a widely distributed cosmopolitan family with 81 genera and around 800 mostly tropical species (Pell et al. 2011). There are 13 genera of Anacardiaceae in Brazil, with 57 species including 17 endemics (Silva-Luz and Pirani 2020). These species are resinous, woody and have pale flowers with a distinct floral nectariferous disk (Pirani and Silva-Luz 2018).

Several species of Anacardiaceae are of huge socioeconomic importance for beekeepers since bees intensely visit their nectariferous flowers to produce honey (Cesário and Gaglianone 2008; Pell et al. 2011; Luz 2011; Fernandes et al. 2012; Calaça et al. 2018; Vieira et al. 2018). The presence of an intrastaminal nectary in the flowers of many species of Anacardiaceae favors the fall of pollen grains into the nectar during collection by bees, which is known as “honey contamination by pollen grains” (Barth 1989).

In the Seasonally Dry Tropical Forest (SDTF) as well as the Cerrado (a savanna-like Brazilian ecoregion) in Brazil, there are a number of melliferous species of Anacardiaceae that bloom during the dry season, when they dominate floral resources and attract *Apis mellifera* L. and stingless bees (Cesário and Gaglianone 2008; Luz 2011; Fernandes et al. 2012; Calaça et al. 2018).

The SDTF, popularly known as “Mata Seca” (dry forest) includes Caatinga (a type of deciduous, xeric shrubland) and semideciduous and deciduous forest (Veloso et al. 1991; Joly et al. 1999; Fiaschi and Pirani 2009). Cerrado *s.lat.* includes one forest physiognomy (Cerradão), several savanna physiognomies that may include small and tortuous trees (Cerrado *s.str.*, Parque Cerrado, Vereda, Palmeiral) and grassland formations (Campo Sujo, Campo Limpo, Campo Rupestre), in addition to the deciduous, semideciduous and perennial forests from other biomes occurring along riverbanks and small streams (Ribeiro and Walter 1998).

Seasonal stress caused by xeric conditions during the dry season occurs in both the SDTF and Cerrado (Pennington et al. 2000). Aspects of climate determine the distribution of SDTF’s, such as a well-defined rainy and dry season, with the latter lasting up to 5–6 months, and low water availability (500–1800 mm annual rainfall) with erratic and short rainy seasons (Gentry 1995; Pennington et al. 2009). Mean annual rainfall for areas of the SDTF in Brazil located near the transition between Cerrado and Atlantic Forest (in restinga vegetation) is more than 1000 mm/year, while near the Caatinga domain it is around 800 mm/year (Santos et al. 2012). Although the climate of Cerrado is more humid (1200 to 2000 mm/year) than the SDTF, its trees are frequently tolerant of fire, and component tree species are sclerophyllous due to nutritional limitation of the soil and its acidic pH (Ratter et al. 1997; Ruggiero et al. 2002).

Most SDTF species shed their leaves during the severe dry season, and the herbaceous stratum disappears and only some trees flourish (Pereira et al. 2011; Santos et al. 2012). Among these are some particularly abundant SDTF species of Anacardiaceae, such as *Astronium urundeuva* (M. Allemão) Engl. (Syn. *Myracrodruon urundeuva* Allemão)], small to tall trees ranging 2.5–20 m in height, popularly known as “aroeira, aroeira-do-sertão” (Santin and Leitão 1991; Caetano et al. 2008; Santos et al. 2012; Silva-Luz et al. 2020a). This deciduous species flowers between May and October and also occurs in dry formations inside the Cerrado, with stony soils (Fleig 1989; Prado and Gibbs 1993; Santos et al. 2008; Santin and Leitão 1991; Nunes et al. 2008). Furthermore, it is a dioecious species that when flowering produces more staminate than pistillate flowers (Kiill et al. 2010; Calaça 2019), but both flower genders produce nectar (Santin and Leitão 1991).

Another characteristic species of the SDTF and Cerrado is *Schinopsis brasiliensis* Engl. (Prado and Gibbs 1993; Mendonça et al. 1998; Silva-Luz et al. 2020b). This species is popularly known as “braúna, pau-preto, baraúna, chamacoco”, and is dioecious with deciduous trees ranging 12–20 m in height that bloom during the dry season as well (Carvalho 2009; Kiill et al. 2010; Silva-Luz et al. 2020b).

Known as “ambu, imbu, ombu, umbu” *Spondias tuberosa* Arruda is a small deciduous tree ranging 4–7 m in height (Barreto and Castro 2010; Epstein 1998) endemic to the Caatinga of Northeast Brazil, but well adapted to semi-arid regions as well (Prado and Gibbs 1993; Mertens et al. 2017). It frequently has hermaphroditic flowers, but some trees are bisexual or andromonoecious with staminate and hermaphroditic flowers (Nadia et al. 2007; Mitchell and Daly 2015). The flowering period is variable according to the local climate conditions, but the reproductive phase begins at the end of the dry season (Lima Filho 2007; Machado et al. 1997; Mertens et al. 2017) and enters the rainy season from November to February (Epstein 1998; Nadia et al. 2007).

There are some species of Anacardiaceae that are rare in the SDTF but common in the Cerrado and also bloom during the dry season, such as *Astronium fraxinifolium* Schott, a tree of 8–18 m in height popularly known as “ubatan, aroeira, gonçalo-alves”; *Astronium graveolens* Jacq. also, a tree of 8–18 m in

height, known as “guaritá, aroeira”; and *Schinus terebinthifolia* Raddi, a tree of 3–4 m in height called “aroeira-mansa, aroeira-vermelha, pimenta-rosa”. *Astronium fraxinifolium* and *A. graveolens* have similar vegetative and reproductive characteristics, both being deciduous species that are widely distributed in several biomes in Brazil, but the former is especially frequent in Cerrado and the latter in Dense Ombrophilous and Semideciduous forests (Luz 2011; Silva-Luz et al. 2020). Both *Astronium* species are dioecious with unisexual flowers. *Astronium fraxinifolium* blooms between June and August, while *A. graveolens* blooms from August to September and in December (Santin 1989; Leite 2002; Santos et al. 2008; Luz 2011). *Schinus terebinthifolia* is a native and widely distributed species in Brazil (Silva-Luz et al. 2019) and is frequently used as ornamental plant in Florida and Hawaii (USA), where it is considered as one of the most harmful invasive species (Schmitz et al. 1997). This species is monoecious and can have two flowering periods per year, which are influenced by the local environment (Fleig 1989; Santos et al. 2008; Luz 2011).

In the state of Minas Gerais (Southeast Brazil) there is a number of different physiognomies of the Cerrado and SDTF (Sano and Almeida 1998). Honey samples produced in areas of Cerrado in Minas Gerais showed multifloral nectariferous sources, with a major contribution of *Astronium* Jacq. and *Schinus* L. nectar (Bastos et al. 2003). On the other hand, Calaça et al. (2018) showed a high percentage of pollen grains of *Astronium urundeuva* [referred to as *Myracrodruon urundeuva* (M. Allemão) Engl. by the authors] in more than 60 samples of the dark amber unifloral honey of aroeira produced during the dry season in the SDTF belt in northern Minas Gerais. Furthermore, these authors reported that honeybees have a preference for staminate flowers, with a tenfold greater frequency of visits than to pistillate flowers. These results can be related to the massive blooming of this species and the high density of trees in the SDTF. Kill et al. (2010) reported that the massive blooming of *Astronium urundeuva* and of *Schinopsis brasiliensis* temporarily dominate floral resources during the dry season in Caatinga and attract *Apis mellifera* and stingless bees. Species of stingless bees were considered the pollinators of the trees due to the compatibility between the bee’s body size and the flowers, their behavior and their frequency of flower visits.

The study of pollen morphology is a prerequisite to correctly indicating the botanical or geographical origins of honeys. Furthermore, pollen identification has been useful in the prevention of the spread of bee pathogens and in helping inspections by Brazilian health authorities attempting to prevent the illicit import of contaminated bee products (Luz et al. 2018a; Teixeira et al. 2020).

The Aroeira honey produced from *Astronium urundeuva* in Northern Minas Gerais was widely rejected in the past due to its less sweet taste and dark amber color, which reduced its economic value. However, studies have shown that this honey is a unique and differentiated natural food with therapeutic properties that strengthen the immune system, exhibiting antioxidant, anti-inflammatory, and antibiotic actions. These properties add significant potential for the use of honey in drug development (Viana et al. 2018; Gardoni et al. 2022). The geographical indication certification of the aroeira honey produced by *Apis mellifera* Linnaeus in 64 cities of the northern region of the State of Minas Gerais was registered by the Brazilian National Institute of Industrial Property in 2022 (RPI 2665, 1st of February 2022). However, for a proper labeling that indicates the high content of phenolic compounds present in this honey, it is necessary to perform melissopalynological analysis to confirm the predominance of at least 60% of *A. urundeuva* pollen in the product. Thus, we aim to provide a practical tool for use in melissopalynological analyses of aroeira honeys, especially those produced in the northern region of Minas Gerais. The palynological study of these six species that occur in this region will contribute to controlling the origin and quality standard of unifloral aroeira honey.

Some pollen grains of Anacardiaceae can be discriminated by exine ornamentation, among other characteristics, but several studies group them in the same “pollen type *Schinus*” or even “Anacardiaceae pollen type” (Barth 1990). Therefore, to correctly identify the botanical origin of bee products originating from areas of the SDTF and Cerrado, a detailed palynological study, as presented herein, is necessary.

The pollen morphology of *Astronium fraxinifolium* was described by Anzótegui (2002), Luz et al. (2018b), Assis (2018), Assis and Saba (2018) and Assis et al. (2021); pollen grains of *A. graveolens* by Roubik and Moreno (1991), Sánchez-Dzib et al. (2009), Ybert et al. (2016), Lorente et al. (2017), Assis (2018), Assis and Saba (2018) and Assis et al. (2021); pollen grains of *A. urundeuva* (referred to as *Myracrodruon urundeuva*) by Markgraf and D’Antoni (1978), Silva et al. (2014), Lorente et al. (2017), Assis (2018), Assis and Saba (2018), Silva et al. (2020) and Assis et al. (2021).

The pollen grains of *Spondias tuberosa* was characterized by Silva (2007), Silva et al. (2016), Assis (2018), Assis and Saba (2018), Silva et al. (2020) and Assis et al. (2021). Pollen morphology of *Schinopsis brasiliensis* was documented by Assis (2018), Assis and Saba (2018) and Assis et al. (2021). *Schinus terebinthifolia* (sometimes referred to as *Schinus terebinthifolius*) is the most studied species with regard to palynology, beginning with Erdtman (1952) followed by Anzótegui (1992), Gonçalves-Esteves and Ferreira (1994), Cruz-Barros and Granito (1997), Barros et al. (1999), Takeda et al. (2000), Anzótegui (2001, 2002), Willard et al. (2004), Silva et al. (2014), Bitencourt (2016), Ybert et al. (2016), Lorente et al. (2017), Assis (2018), Assis and Saba (2018) and Assis et al. (2021). However, most of these studies are only descriptive, and without a multivariate analysis that could indicate which pollen morphological variables can be used to segregate taxa and contribute as tools for the certification of botanical origin of bee products. Furthermore, there are few photomicrographs and electron microscopy images that could help distinguish patterns of exine ornamentation and apertures of the pollen grains of different species.

Thus, our aim was to describe the pollen grains of six melliferous species of Anacardiaceae, using multivariate analysis, as well as images from light and electronic microscopy, which will aid in determining the botanical origin of bee products from native areas of the Seasonally Dry Tropical Forests and Cerrado in Minas Gerais, Brazil, contributing to their preservation.

Materials and methods

Reproductive period of the studied species

Due to the great variability of the flowering period of the six studied Anacardiaceae species that we found in literature, we downloaded collection data from *SpeciesLink* (CRIA 2020) and Reflora (2020) databases. We used only collection data with geographical coordinates and that were collected in the SDTF and Cerrado areas of North Minas Gerais state (Brazil), with exception for the *Schinus terebinthifolia* specimens that were only collected from the southern region

of Bahia state, in some areas borders to the Minas Gerais state. Based on these data, we inferred the most likely reproductive period of these species in our studied site (Fig. 1).

Pollen morphology studies

Pollen morphology of *Astronium fraxinifolium*, *A. graveolens*, *A. urundeuva*, *Schinopsis brasiliensis*, *Schinus terebinthifolia* and *Spondias tuberosa*, represented by 15 herbarium specimens, were surveyed (Appendix: Specimens investigated). The acronyms are in accordance with the Index herbariorum (Thiers et al. 2023, continuously updated). Mature buds were removed from dried voucher specimens from the Universidade Federal de Minas Gerais (BHCB and Herbário do Jardim Botânico of the Fundação de Parques Municipais e Zoobotânica (BHZB) (Brazil).

Pollen grains were acetolysed following Erdtman (1960) and were measured up to three days after the preparation (Salgado-Labouriau et al. 1965), using an Olympus OSM-4 (10×) micrometre drum coupled to the eyepiece of an Olympus BX50 light microscope, with 1200× magnification. Their length axis averages were derived from measurements of 25 pollen grains per sample. Measurements of thickness of the exine layers measured at the mesocolpium were based upon ten pollen grains per sample. In all cases two or three herbarium specimens per taxon were investigated to identify any possible intraspecific variation. Statistical analyses include arithmetic mean (x), average standard deviation (sx), standard deviation of the sample (s), coefficient of variability (V%) and 95% confidence interval (CI) (Vieira 2011).

The classification for shape (P/E) and pollen grain size, followed Erdtman (1952). The classification of the index of polar area (IAP) in a zonocolpate pollen grain was taken by the ratio of the distance between the apices of the two adjacent colpi, and the largest width of the pollen grain in polar view (equatorial axis in polar view), followed Iversen and Troels-Smith (1950) and Faegri and Iversen (1966).

The index of colpus width (CWI) was calculated dividing the equatorial axis in equatorial view by the width of the colpus. Thus, an index ≤ 5.00 corresponds to very wide colpi, while ‘wide colpi’ from 5.00 to 7.00 and ‘narrow colpi’ are ≥ 7.00 follow Gasparino et al. (2013).

The relationships among the studied species through the quantitative data were explored by a principal component analysis (PCA) using the software FITOPAC (Shepherd 1996) for conversion of the metric measurements of the pollen grains by natural logarithm (log [x + 1]) and PC-ORD version 7 (McCune & Mefford 1999) for ordination from the covariance matrix. For this analysis, we used eleven metric variables of all specimens: Polar axis in equatorial view (P), Equatorial axis in equatorial view (E), index of pollen shape (P/E), colpus length (CL), colpus width (CW), colpus margo width (MAR), endoaperture length (EL), endoaperture width (EW), nexine layer thickness (N), sexine layer thickness, i.e., tectum and columella (S) and the polar area index (IAP).

For light microscopy (LM) observations, the photomicrographs were produced by using an Olympus BX 50 microscope with a video camera and the Olympus software Cell-Sens 1.5. For scanning electron microscopy (SEM), the non-acetolysed pollen grains for each species were washed with distilled water in a water bath at 60 ºC for 1 min, five to seven times, for *pollenkitt* removal (O.M. Barth, pers. comm., 24 May 2022), and thereafter dehydrated with ethanol 70% (Johansen 1940), spread directly on the microscope stubs and sputtered with a ~ 20 nm thick gold layer, for 120 seconds. Scanning electron microscope images were obtained using a JEOL JSM-6010 Plus/LA scanning electron microscope of Fundação Ezequiel Dias (FUNED), Minas Gerais.

The pollen descriptions followed the sequence proposed by Barth and Melhem (1988). Terminology follows Punt et al. (2007) and Hesse et al. (2009). The microscope slides with the pollen material are deposited in the palynotheca of the Laboratório de Palinologia of the Instituto de Pesquisas Ambientais, São Paulo.

Results

Reproductive period of the studied species

The *SpeciesLink* (CRIA 2020) and Reflora (2020) data compilation showed that the reproductive period of the six Anacardiaceae species in the studied area of SDTF and Cerrado is mostly during the dry season, from May to September (*Astronium graveolens*, *Astronium urundeuva*, *Schinopsis brasiliensis*). However, the reproductive phase of *Astronium fraxinifolium* is at the end of the dry season, that of *Spondias tuberosa* is at the beginning of the rainy season, and that of *Schinus terebinthifolia* is at the end of the rainy season (Table 1).

Table 1

Reproductive period according to literature and our dataset[2] of the six species of Anacardiaceae from SDTF and Cerrado, Brazil.

Species	N[1]	January	February	March	April	May	June	July	August	September	October	November	December
Astronium fraxinifolium	31								*	*	*		
Astronium graveolens	15						*	*	*				
Astronium urundeuva	53					*	*	*	*				
Schinopsis brasiliensis	57					*	*	*					
Schinus terebinthifolia	225			*	*	*							
Spondias tuberosa	56									*	*	*	*

Notes: N[1] = Number of specimen's collection data downloaded from *SpeciesLink* (CRIA 2020) and Reflora (2020) database. We used only data with geographical coordinates and that were collected in the SDTF and Cerrado areas of North Minas Gerais State and southern Bahia State. For *Schinus terebinthifolia* we used the data from southern region of Bahia State that borders Minas Gerais, Brazil. Reproductive period according to literature and our dataset[2] = Bloom period based on literature: Santin 1989, Santin and Leitão 1991, Machado et al. 1997, Epstein 1998, Leite 2002, Nadia et al. 2007, Nunes et al. 2008, Santos et al. 2008, Carvalho 2009, Kiill et al. 2010, Almeida et al. 2011, Luz 2011, Mertens et al. 2017, Santos et al. 2018, Coradin et al. 2018. (*) Shows the flowering period according to our survey from *SpeciesLink* (CRIA 2020) and Reflora (2020) database.

Pollen grains general description

The pollen grains are monads, of small to large size (Table 2), radially symmetric, isopolars, oblate-spheroidal to prolate, amb subcircular or subtriangular (obtusus angles, convex sides), 3-zonocolporate, with long and narrow colpi with tapering ends and the aperture membrane sparsely granulate. The psilate colpi margines are narrow or wider, and the colpi margines are prominent over the endoapertures, forming a protrusion, creating a constriction in the middle of the colpi on both sides of the touching margines, noted both in light and scanning electron microscopies. The space/cavity formed below the domed sexine is devoid of nexine, since the nexine delimiting the boundary of the endoapertural area. The endoapertures are lalongate of three types (rectangular, in "H", elliptical). Ectoapertures (colpi) and endoapertures without costae or fastigium. Sexine semitectate, with muri simplicolumellate. Sexine much thicker than the nexine, both layers with homogeneous thickness all over the pollen grains. Two ornamentations on the surface of pollen grains were observed: 1) suprastriato-inframicroreticulate, with sinuous striae, of high muri, branched in some areas, predominantly continuous and long at mesocolpium, oriented in the direction of the polar axis, in some areas juxtaposed with some transverse striae, mainly in the apocolpium, with irregular lumina of infrareticulum, with a variety of shapes and sizes; 2) striate-perforate, with striae formed by low muri, comparatively less sinuous striae, at mesocolpium tightly or less tightly connected striations in the direction of the polar axis, with some transverse striae mainly in the apocolpium, striae short and very branched or predominantly long and continuous (less branched).

Astronium fraxinifolium (Figs. 2A–H, 6A) — Pollen grains of small to medium size, prolate-spheroidal to subprolate, amb circular. Narrow colpus margo. Rectangular endoapertures, in "H" endoapertures, or elliptical with tapering ends endoapertures. Exine thickness 1.56–1.63 µm, suprastriato-inframicroreticulate sexine in SEM. In LM, microreticulate-striate, with microreticulum more pronounced in the apocolpium.

Astronium graveolens (Figs. 2I–O, 3A, 6B) — Pollen grains of small to medium size, prolate-spheroidal, amb circular. Narrow colpus margo. Narrow elliptical with tapering ends endoapertures. Exine thickness 1.35–1.52 µm, suprastriato-inframicroreticulate sexine in SEM. In LM,microreticulate-striate, with microreticulum more pronouced in the apocolpium.

Astronium urundeuva (Figs. 3B–K, 6C-E) — Pollen grains of small to medium size, oblate-spheroidal to subprolate, amb subcircular or subtriangular. Narrow colpus margo. Rectangular endoapertures, in "H" endoapertures, or elliptical endoapertures with rounded ends endoapertures. Exine thickness 1.51–1.58 µm, suprastriato-inframicroreticulate sexine in SEM. In LM it is microreticulate-striate, with microreticulum more pronouced in the apocolpium.

Schinopsis brasiliensis (Figs. 3L–O, 4A-C, 6F-H) — Pollen grains of small to medium size, prolate- spheroidal to subprolate, amb subcircular or subtriangular. Wider colpus margo. Narrow rectangular endoapertures. Exine thickness 1.40–1.69 µm, suprastriato-inframicroreticulate sexine in SEM. In LM, microreticulate-striate, with microreticulum more pronouced in the apocolpium.

Schinus terebinthifolia (Figs. 4D-K, 6I-K) — Pollen grains of small to medium size, prolate- spheroidal, amb subcircular or subtriangular. Narrow colpus margo. Elliptical with rounded ends endoapertures. Exine thickness 1.26–1.29 µm, striate-perforate sexine in SEM, with narrow striae, predominantly long and continuous (less branched), tightly connected striations. In LM, striato-microreticulate, with microreticulum more pronounced in the apocolpium, whose low muri gives a subtle aspect to the striated ornamentation in the first visualization focus.

Spondias tuberosa (Figs. 4L-O, 5A-F, 6L-O) — Pollen grains of medium to large size, presence of abnormal size pollen grains (much smaller than the average size of the species) (Fig. 5F), subprolate to prolate, amb circular. Narrow colpus margo. Rectangular endoapertures or elliptical endoapertures with rounded ends endoapertures. Exine thickness 1.51–1.67 µm, striate-perforate sexine in SEM, with broader striae, predominantly shorter and very branched, tightly

connected striations, in the apocolpium the striae are more juxtaposed transversely. In LM striato-microreticulate, with microreticulum more pronounced in the apocolpium, whose low muri gives a subtle aspect to the striated ornamentation in the first visualization focus.

Table 2
Measurements (in micrometers) of Anacardiaceae pollen grains in equatorial view through light microsc

Specimens (PCA code)	P			E			P/E	DA/EAPV (*x*)	IAP	Colpus		CWI	Wid colp
	Cli – Cif (x ± sx)	s	V%	Cli - Clf (x ± sx)	s	V%				Length	Width		
Astronium fraxinifolium BHCB27601 (Afra01)	30.03–31.74 (30.89 ± 0.40)	2.79	9.05	25.23–27.88 (26.55 ± 0.61)	4.33	16.32	1.16 (Subprolate)	2.82/28.47	0.09	24.38	0.92	28.85	
Astronium fraxinifolium BHCB114116 (Afra16)	21.72–23.14 (22.43 ± 0.30)	2.99	13.35	19.59–20.83 (20.21 ± 0.26)	2.62	12.98	1.10 (Prolate spheroidal)	5.05/21.20	0.24	16.91	0.81	24.95	
Astronium graveolens BHCB146262 (Agra62)	24.67–25.69 (25.18 ± 0.23)	1.86	7.39	23.23–24.30 (23.77 ± 0.24)	1.95	8.20	1.05 (Prolate spheroidal)	2.23/22.79	0.09	17.95	1.00	23.77	
Astronium graveolens BHCB24631 (Agra31)	29.59–30.85 (30.22 ± 0.28)	2.30	7.62	28.45–29.71 (29.08 ± 0.28)	2.31	7.96	1.03 (Prolate spheroidal)	2.59/30.03	0.08	23.16	0.93	31.26	
Astronium urundeuva BHCB43894 (Auru94)	24.18–24.88 (24.53 ± 0.16)	1.29	5.25	19.81–20.79 (20.30 ± 0.22)	1.81	8.92	1.21 (Subprolate)	2.60/21.12	0.12	18.03	0.90	22.55	
Astronium urundeuva BHCB27154 (Auru54)	26.71–27.82 (27.26 ± 0.25)	2.03	7.43	24.87–25.69 (25.28 ± 0.18)	1.49	5.90	1.08 (Prolate spheroidal)	2.99/26.53	0.11	20.21	0.86	29.39	
Astronium urundeuva BHZB11912 (Auru12)	27.75–29.32 (28.53 ± 0.37)	2.41	8.45	28.81–29.99 (29.40 ± 0.28)	1.80	6.13	0.97 (Oblate spheroidal)	6.80/29.59	0.22	24.75	2.55	11.53	
Schinopsis brasiliensis BHZB11000 (Sbra00)	29.34–30.45 (29.89 ± 0.26)	1.71	5.73	27.16–27.87 (27.52 ± 0.17)	1.09	3.97	1.08 (Prolate spheroidal)	5.25/25.16	0.21	23.05	1.00	27.52	
Schinopsis brasiliensis BHCB145605 (Sbra05)	26.03–26.57 (26.30 ± 0.12)	0.98	3.75	20.89–21.54 (21.22 ± 0.15)	1.18	5.58	1.23 (Subprolate)	2.38/19.58	0.12	19.75	0.92	23.06	
Schinopsis brasiliensis BHCB109961 (Sbra61)	26.58–27.23 (26.91 ± 0.15)	1.19	4.41	23.29–24.24 (23.77 ± 0.21)	1.73	7.28	1.13 (Prolate spheroidal)	2.76/25.21	0.11	19.61	0.84	28.29	
Schinus terebinthifolia BHCB2881 (Ster81)	24.62–25.05 (24.83 ± 0.10)	0.78	3.19	24.17–23.43 (23.80 ± 0.17)	1.36	5.71	1.04 (Prolate spheroidal)	3.91/23.48	0.16	18.39	0.85	28.00	
Schinus terebinthifolia BHCB146450 (Ster50)	25.87–27.06 (26.46 ± 0.27)	2.19	8.29	23.57–24.56 (24.07 ± 0.22)	1.82	7.56	1.09 (Prolate spheroidal)	3.22/25.01	0.13	20.31	0.77	31.26	
Spondias tuberosa BHZB11911 (Stub11)	47.53–50.11 (48.82 ± 0.61)	3.96	8.12	38.42–39.87 (39.15 ± 0.34)	2.23	5.71	1.24 (Subprolate)	8.68/38.42	0.22	42.68	1.73	22.63	
Spondias tuberosa BHCB7116 (Stub16)	42.26–43.91 (43.08 ± 0.37)	3.03	7.02	29.65–31.12 (30.39 ± 0.33)	2.69	8.84	1.42 (Prolate)	7.52/36.00	0.21	36.17	0.72	42.20	
Spondias tuberosa BHCB64829 (Stub29)	40.00–42.39 (41.19 ± 0.54)	4.37	10.61	31.55–33.21 (32.38 ± 0.37)	3.03	9.36	1.27 (Subprolate)	8.95/35.64	0.25	33.70	0.87	37.21	

Note: Arithmetic mean (x), mean standard deviation (s_x), standard deviation of the sample (s), coefficient of variability (V%) and 95% confidence interval probability of the lowest sample values (CIi) and highest sample values (CIf). Polar axis in equatorial view (P), Equatorial axis in equatorial view (E), Index of pollen shape (P/E) (n = 25). Length and width of colpus and endoaperture, thickness of the nexine layer (N), thickness of the sexine layer (S), Equatorial axis in polar view (EAPV), distance of the extremities of the two adjacent apertures (DA) (n = 10). Polar area index (IAP) – 'no polar area' [0.00]; 'very long colpus' [< 0.25]; 'long colpus' [0.25–0.50]; 'short colpus' [0.50–0.75]; 'very short colpus' [> 0.75]. Colpus width index (CWI) – 'very wide colpus' [≤ 5.00]; 'wide colpus' [5.00–7.00]; 'narrow colpus' [≥ 7.00]).

Principal component analysis (PCA)

The first two axes of the PCA represented 88.16% of the total variability according to the pollen metric variables (Fig. 7). The first axis summarized 69.90% of the variation with the highest correlated variables being the polar axis in equatorial view (P), equatorial axis in equatorial view (E), the length of the colpus (CL), the length and width of the endoaperture (EL and EW), the index of polar area (IAP) and the colpus margo width (MAR) (Table 3). The *Spondias tuberosa* specimens with the largest pollen grains, highest values of colpi length (highest IAP) and broader endoapertures, were ordinated on the negative side (on the left) of the first axis. In contrast, the other specimens of the remaining species with the smallest pollen grains, appears very close together on the positive side (on the right) of this axis (except *Astronium urundeuva* BHZB11912). The second axis contributed less to the ordination (18.26%) were the most relevant variables for the species grouping were the index of pollen shape (P/E), the colpus width (CW), nexine (N) and sexine (S) layer thickness (Table 3). The highest values of width of colpus (CW) were the most important variable for the placement of the *Astronium urundeuva* BHZB11912 along the positive side of the second axis. The specimen *Schinopsis brasiliensis* BHZB11000 was ordinated according its wider colpus margo, being the largest among all pollen grains analysed.

Table 3
Principal Component Analysis loadings for pollen grains metric variables of the first two axes of ordination for the specimens of Anacardiaceae.

		Principal components	
Acronyms	Variables (meaning)	Axis 1	Axis 2
P	Polar axis in equatorial view	-0.3636	-0.3439
E	Equatorial axis in equatorial view	-0.3073	-0.0870
P/E	Index of pollen shape	-0.0724	-0.2834
CL	Colpus length	-0.4422	-0.3159
CW	Colpus width	-0.3097	0.8010
EL	Endoaperture length	-0.6023	0.1816
EW	Endoaperture width	-0.3364	-0.1325
N	Nexine layer thickness	-0.0095	0.0101
S	Sexine layer thickness	-0.0105	-0.0163
IAP	Index of polar area	-0.0131	-0.0013
MAR	Colpus margo width	-0.0189	-0.0381

The PCA with all specimens of the six species confirms the importance of the pollen grain size to well separated *Spondias tuberosa* from the others. As all the remaining species fall together on the same side of the ordination, a second PCA was performed from the average measurement values of the same metric variables from each species, excluding *Spondias tuberosa* specimens (Fig. 8).

The first two axes of the PCA without *Spondias tuberosa* specimens represent 89.33% of the total variability according to the pollen metric variables (Fig. 8). The first axis explains the greatest variation in the data, summarized by 61.75% of the variation with the highest correlated variables being CL, CW and S (Table 4). The *Astronium urundeuva* pollen grains, with the longest and wider colpi and broader endoapertures among all species, were ordinated on the negative side of the first axis. In contrast, *Schinus terebinthifolia* presents the smallest CL and CW, placing it in the opposite direction, on the positive side of the graph. The second axis contributed less to the ordination (27.57%), being P, E, P/E, EL, EW, N, IAP and MAR the most relevant variables. *Astronium graveolens* with the largest pollen grains among these five species, and with the smallest P/E, appears on the positive side of this axis. The highest values to P/E contributed to the placement of *Astronium fraxinifolium* and *Schinopsis brasiliensis* species very close to each other on the same side of the graph. *Schinopsis brasiliensis* was ordinated according their wider colpi margines near to the MAR variable.

Table 4
Principal Component Analysis loadings for pollen grains metric variables of the first two axes of ordination for the specimens of Anacardiaceae, excluding *Spondias tuberosa* specimens.

		Principal components	
Acronyms	**Variables (meaning)**	**Axis 1**	**Axis 2**
P	Polar axis in equatorial view	0.0092	0.1916
E	Equatorial axis in equatorial view	-0.0723	0.3215
P/E	Index of pollen shape	0.0191	-0.2121
CL	Colpus length	-0.0844	0.0696
CW	Colpus width	-0.8802	0.1707
EL	Endoaperture length	-0.4460	-0.5312
EW	Endoaperture width	0.0748	-0.7009
N	Nexine layer thickness	0.0025	0.0037
S	Sexine layer thickness	-0.0589	-0.0380
IAP	Index of polar area	0.0020	-0.0246
MAR	Colpus margo width	0.0671	-0.0746

Pollen types

Two pollen types have been recognized based on pollen exine ornamentation (LM, SEM) and the dimensions of the longest axis (values of polar axis, minimum and maximum). Four pollen subtypes were additionally recognized, based on the width of the colpus margo.

Pollen type 1 — Pollen with suprastriato-inframicroreticulate exine ornamentation, with sinuous striae of high muri that are branched in some areas, small to medium sized [P. min = 21.72 µm, P. max = 31.74 µm].

Subtype 1a — With margo of the colpus narrow, prevailing medium sized, occasionally small sized (*Astronium fraxinifolium, A. graveolens, A. urundeuva*).

Subtype 1b — With margo of the colpus wide, only medium sized (*Schinopsis brasiliensis*).

Pollen type 2 — Pollen with striate-perforate exine ornamentation, with comparatively less sinuous striae formed by low muri, small to large sized.

Subtype 1c — With margo of the colpus narrow, narrow striae, prevailing medium sized, occasionally small sized [P. min = 24.62 µm, P. max = 27.06 µm] (*Schinus terebinthifolia*).

Subtype 1d — With margo of the colpus narrow, broader striae, prevailing medium sized, occasionally large sized [P. min = 40.00 µm, P. max = 50.11 µm] (*Spondias tuberosa*).

Discussion

The obtained results showed that *Schinus terebinthifolia* was the species most represented in collections, followed by *Schinopsis brasiliensis*, *Spondias tuberosa*, *Astronium urundeuva*, *A, fraxinifolium* and lastly *A. graveolens*.

Data compiled from online databases and consulted literature demonstrated that, in the study area, the reproductive period of *Astronium graveolens*, *A. urundeuva* and *Schinopsis brasiliensis* is mostly during the dry season (Santin 1989, Santin and Leitão 1991, Machado et al. 1997, Leite 2002, Nunes et al. 2008, Carvalho 2009, Santos et al. 2008, Kiill et al. 2010, Luz 2011, Santos et al. 2018, Coradin et al. 2018). For *Astronium fraxinifolium* and *Spondias tuberosa* this period is during the beginning of the rainy season, and for *Schinus terebinthifolia* it is during the end of the rainy season (Santin 1989, Machado et al. 1997, Epstein 1998, Leite 2002, Luz 2011, Nadia et al. 2007, Santos et al. 2008, Almeida et al. 2011, Mertens et al. 2017, Santos et al. 2018, Coradin et al. 2018). As there were no occurrences of specimen collections of *Schinus terebinthifolia* in the study area, collections from the southern region of the state of Bahia were chosen.

Data compiled from the online databases was not always in agreement with the phenology data for these species presented by the consulted literature — either the beginning of the flowering period was anticipated, or the species started flowering late. The greatest disagreements were for species with broad geographic distributions, even though they are related to the Cerrado and to the proximity of the Caatinga Biome region, or even with those that do not show synchronism in the reproductive period within the same population and even in the same plant (*e.g.*, in *Astronium graveolens*).

Pollen morphology and standardization of terminology - a mini review

Anacardiaceae is characterized as eurypalynous, meaning that some palynological features are widely variable, especially concerning size, shape, apertures and exine ornamentation (Erdtman 1952). However, the six species studied here are morphologically very similar concerning the 3-colporate apertures and the presence of striae on the sexine, which characterizes them as stenopalynous. Also, the colpi are narrow and the colpi margines are prominent over the endoapertures, forming a protrusion noted both by LM and SEM. The space/cavity formed below the domed sexine is devoid of nexine, since the nexine forms the boundary of the endoapertural area. There is no separation of the inner part of the exine (nexine) from the domed sexine in the region of the endoaperture, meaning that there is no *fastigium* (vestibulum), as Ybert et al. (2016) observed for the species *Astronium graveolens* and Assis (2018) and Assis et al. (2018) for *Schinopsis brasiliensis*. Likewise, no thickening of the nexine close to the apertures (costae) was observed in any of the studied species, as reported by Assis et al. (2018) and Assis (2018) for *Astronium fraxinifolium*, by Assis et al. (2018), Assis (2018) and Ybert et al. (2016) for *Astronium graveolens*, and for *Schinus terebinthifolia* by Barros et al. (1999) and Ybert et al. (2016).

Pollen shape showed great intraspecific variation with specimens of the same species showing different P/E classifications. The same occurred with colpus width and length, and of sexine and nexine thickness, and so they also were not considered important characters for pollen discrimination.

Assis (2018) and Assis et al. (2021) used the superior and inferior limits of lalongate endoapertures, and its parallel or concave contour, as attributes that could corroborate taxonomic classification. In the present study, the three types of lalongate apertures (rectangular, “H”-shaped or elliptic) showed wide intraspecific variation and, therefore, were not sufficiently consistent to segregate taxa. Only Ybert et al. (2016) observed endoapertures as an “H” in some of the species of Anacardiaceae they studied; this, however, was not mentioned by Assis (2018). It is worth mentioning that the endoapertures of the pollen grains of *Astronium urundeuva* and *Spondias tuberosa* are wider while those of *Astronium graveolens* are narrower.

On the other hand, some measurements obtained, excluding qualitative data, were important for characterizing some genera or species through pollen morphology. The most important variables, especially in combination, were pollen grain size and the width of the margo of the colpus. These two variables were explained by both the PCA with all the studied species and the PCA with the average dimensions obtained for the pollen grains of each species but excluding *Spondias tuberosa*. The widest colpus margo was observed in *Schinopsis brasiliensis* and the largest pollen grains were those of *Spondias tuberosa*, which stood out from the rest. When *S. tuberosa* was excluded from the multivariate analysis, the highest averages for P and E were for *Astronium graveolens* and the smallest for *Schinus terebinthifolia*.

Characterization of exine ornamentation under SEM was essential for distinguishing the suprastriato-inframicroreticulate pollen grains of Pollen type I (in all species of *Astronium* and in *Schinopsis brasiliensis*) from the striate-perforate pollen grains of Pollen type II (*Schinus terebinthifolia* and *Spondias tuberosa*). The sinuous striae of the first group have predominantly continuous, long and high muri spaced between them in the direction of the polar axis, which are also branched and juxtaposed transversely in some areas, mainly in the apocolpium, with irregular infrareticulum and lumina of varying shapes and sizes.

The striae of the second group are comparatively less sinuous, with low muri that are shorter and branched (*Spondias tuberosa*) or longer and less branched (*Schinus terebinthifolia*), disposed very close together in the direction of the polar axis, with less transverse striae in the apocolpium than in Pollen type I. Under LM, the surface ornamentation of the pollen grain was characterized as microreticulate-striate, with a pronounced microreticulum in the region of the apocolpium (*Astronium fraxinifolium, A. graveolens, A. urundeuva* and *Schinopsis brasiliensis*), or striate-microreticulate (*Schinus terebinthifolia* and *Spondias tuberosa*), with low muri that give a subtle appearance of a striate pattern in the first viewing focus.

Palynologically, the pattern of sexine ornamentation of Pollen type I, together with the morphological similarities of its pollen grains, were important characteristics to reinforce the taxonomic proximity of species inside the tribe Rhoeae, represented in our study by the species of *Astronium* and *Schinopsis brasiliensis*. *Schinus terebinthifolia*, which also belongs to the tribe Rhoeae, was an exception since its pollen grains were more similar to Pollen type II, along with *Spondias tuberosa* from the tribe Spondieae. Therefore, the studied species of the tribe Rhoeae showed great diversity of striae organization, as shown by Assis (2018) and Assis et al. (2021) for some species also studied here, and by Suarez et al. (2019) for other species of *Schinopsis*.

The pollen morphology observed for the taxa of the present study differed from some pollen descriptions in the literature.

One of the two specimens of *Astronium fraxinifolium* had medium-sized pollen grains, and P and E values similar to those reported by Luz et al. (2018), yet bigger than those reported by Anzótegui (2002), Assis (2018) and Assis et al. (2021). The other specimen had small-sized pollen grains that were similar to those of the previous cited authors. Our description of the suprastriato-inframicroreticulate sexine ornamentation, observed via SEM analysis, differed from the striate-perforate sexine ornamentation showed by Assis (2018) and Anzótegui (2002), and the striae observed here were high rather than shallow as described by Assis (2018). Our morphological descriptions based on LM also differed from Assis (2018), since we observed microreticulate-striate ornamentation with a pronounced microreticulum in the apocolpium while they reported striato-microreticulate ornamentation. Our descriptions made under LM were similar to the description of reticulate in the apocolpium and reticulate-striate in the mesocolpium of Luz et al. (2018b), differing only in the resolution of the size of the lumina (less than 1 µm in the present study). It is worth mentioning that interpretations of ornamentation under LM can vary among authors, since what is important for these kinds of descriptions is what is predominantly seen in the first focus of visualization, the striae or (micro-) reticulum, raising doubts due to the subtlety of the coarse and fine adjustments in the different levels of focus. Unlike Assis (2018) and Assis and Saba (2018), there was no proeminence on the sexine formed by a gradual increase in height of the columellae in the aperture region. Nor does the nexine become gradually thicker in the equatorial region as described by these authors.

The average values for P and E for *Astronium graveolens* pollen grains were similar to those reported by Roubik and Moreno (1991), Lorente et al. (2017), Assis (2018) and Assis et al. (2021), yet smaller than those of Sánchez-Dzib et al. (2009) and Ybert et al. (2016). The microreticulate-striate ornamentation of its pollen grains, as described herein under LM, with the microreticulum being more evident at the apocolpium, differed from the striato-reticulate description of Roubik and Moreno (1991) and Lorente et al. (2017), from the reticulate description of Sánchez-Dzib et al. (2009) and from the striato-microreticulate description of Assis (2018), although it was similar to the reticulate-striate to reticulate description of Ybert et al. (2016). A gradual increasing in the height of

the columellae in the region of the aperture, as described by Assis (2018), as well as a thinner nexine at the poles becoming gradually thicker in the equatorial region, were not observed in the present study. These authors also pointed out other characteristics that were not seen by us, such as the presence of costa in the colpus (Roubik and Moreno 1991), circular endoapertures (Sánchez-Dzib et al. 2009), a *fastigium* and costae in the endoapertures (Ybert et al. 2016).

The most significant difference from the P and E limits found here for the pollen grains of the three specimens of *Astronium urundeuva* were the larger limits reported by Silva et al. (2020). Our results for P and E limits were similar to those of Silva et al. (2014), Lorente et al. (2017), Assis (2018) and Assis et al. (2021). The ornamentation considered by us under LM as microreticulate-striate with more pronounced microreticulum in the apocolpium region, differed from the striato-reticulate of Silva et al. (2014) and Lorente et al. (2017), the striato-microreticulate of Assis (2018) and the striate of Silva et al. (2020). Pollen analysis under SEM showed that the exine of the species is suprastriato-inframicroreticulate, as also described by Markgraf and D'Antoni (1978) and Assis et al. (2021). The description of Assis (2018) under SEM was as striate-perforate, but the images of the mesocolpium shown by the author reveal the ornamentation to be equal to our description for this species. In one of the images of Assis (2018) we can even see a lot of *pollenkitt* covering the apococolpium, which can lead to misinterpretation of perforation due to the clogging of striae and muri of the microreticulum. Assis (2018) and Assis and Saba (2018) described in optical (cross-) section a gradual increase in columellae height in the apertural region, which was not seen in the present study. Lorente et al. (2017) described the presence of an ectannulus in polar view, which does not seem correct, since annulus is a term used for porate pollen grains, which is not the case for this species.

Assis (2018), Assis and Saba (2018) and Assis et al. (2021) reported on the pollen morphology of *Schinopsis brasiliensis* and argued that its pollen grains were one of the smallest among their studied species, which differs from what was found by the present study. The pollen surface was described by these authors, under both LM and SEM, as striato-reticulate or striato-microreticulate, which is in agreement with the exine suprastriato-inframicroreticulate that we found under SEM. However, it diverged from the microreticulate-striate exine observed under LM in all the three specimens studied here. Our SEM results for *Schinopsis brasiliensis* are in consonance with those reported by Suaréz et al. (2019) for pollen grains of six other species of *Schinopsis* from Argentina, which were characterized as predominantly suprastriato-inframicroreticulate. Assis (2018), Assis and Saba (2018) and Assis et a. (2021) indicated that pollen grains of *S. brasiliensis* have comparatively thicker colpi margines than other species of Anacardiaceae. The colpi margines for the specimens studied here also stand out, not for thickening, but for having greater width, since both the height of the columellae and the tectum of the sexine, as well as the thickness of nexine, showed constant thickness throughout the pollen grain.

The P and E values for the two specimens of *Schinus terebinthifolia* analyzed here were very close to the values reported by Erdtman (1952), Assis (2018) and Assis et al. (2021), smaller than those reported by Gonçalves-Esteves and Ferreira (1994), Cruz-Barros and Granito (1997), Barros et al. (1999), Silva et al. (2014) and Ybert et al. (2016), and greater than those found by Anzótegui (1992), Takeda et al. (2000), Willard et al. (2004), Bitencourt (2016) and Lorente et al. (2017). The striato-perforate ornamentation observed under SEM was only cited by Assis (2018) and Assis et al. (2021) and differs from the striato-reticulate ornamentation cited by Barros et al. (1999) and Bitencourt (2016), and the microreticulate ornamentation cited by Anzótegui (1992). On the other hand, the specimens studied here were characterized as striato-microreticulate under LM, coinciding with Assis (2018) and Assis et al. (2021), and similar to the striato-reticulate description of Gonçalves-Esteves and Ferreira (1994), Silva et al. (2014), Ybert et al. (2016) and Lorente et al. (2017), yet differing from the finely reticulate (microreticulate) presented by Erdtman (1952), Anzótegui (1992) and Willard et al. (2004), and the reticulate ornamentation described by Cruz-Barros and Granito (1997). There are different interpretations of the surface ornamentation of pollen grains among palynologists even for same plant species, and especially when talking about what is considered a reticulum, microreticulum or perforation. This may be due to a lack of SEM analysis, or a lack of using international recognized glossaries such as Punt et al. (2007) that standardize palynological terminology. These differences could also be due to natural variability of species with broad geographic distributions (Cruz-Barros and Granito 1997).

The P and E limits among the three analyzed specimens of *Spondias tuberosa* varied widely, ranging from medium to large-sized pollen grains, considering the confidence interval. Smaller pollen grains were reported for this species by Silva (2007), Silva et al. (2016), Assis (2018), Silva et al. (2020) and Assis et al. (2021). Assis (2018) described it as having striato-microreticulate ornamentation based on SEM analysis, which differs from our observation of a striate-perforate exine surface. The images shown by Assis (2018) had a large amount of *pollenkitt* on the surface of the grains surface, which could have influenced their evaluation of exine ornamentation. Later, Assis et al. (2021) revaluated the description and cited it as striato-perforate under SEM. Under LM analysis the ornamentation is striato-microreticulate with low muri giving a subtle aspect to the striae at the first viewing focus, which coincides with the descriptions of Silva (2007), Silva et al. (2016) and Assis (2018), while differing from the microreticulate ornamentation described by Silva et al. (2020). We did not observe a gradual increase in the height of the columellae in the region of endoapertures, nor bridges in the colpus forming a protrusion, as observed and described in optical (cross-) section and SEM by Assis (2018) and Assis and Saba (2018). What we observed were the margines of the colpi forming protrusions over the endoapertures, but without having a thicker sexine in these areas nor the presence of bridges, since the margines do not connect from side to side, dividing the colpus into two parts, which is what defines a "bridge" according to Punt et al. (2007). None of the cited authors mentioned abnormally sized pollen grains, as seen here.

The proposal by Anzótegui (2001) and Suaréz et al. (2019) for only one pollen type for the family Anacardiaceae named "*Schinopsis balansae*" is not supported by the results of the present. study. We described two pollen types for the six species analyzed here, using differences in ornamentation and pollen size. Furthermore, the literature indicates more than one pollen type for Anacardiaceae.

Conclusions

Implications for certification of Anacardiaceae botanical origin of honey

Undoubtedly, pollen morphology analysis of Anacardiaceae is quite important for the certification of the botanical origin of honeys produced in areas of SDTF and Cerrado in Brazil, especially since several species undergo massive blooming, some of them flowering together, with striking climatic seasonality during

the dry season.

It can be emphasized that, in general, knowledge of the reproductive period of species of Anacardiaceae based on online information from the herbarium collections of *SpeciesLink* (CRIA 2020) and Reflora (2020) will assist in the certification of the botanical origin of honeys that may contain nectar of their flowers.

Regarding the stenopalynous genus *Astronium*, the species *A. fraxinifolium* starts flowering in August in the study area, a month that is construed as the end of the reproductive period of two other species of the genus (*A. graveolens* and *A. urundeuva*). However, the same timing cannot be used at other locations in Brazil since there were many disagreements in the literature about the beginning of the flowering periods of these three species. These three species are palynologically similar to *Schinopsis brasiliensis*, and the flowering periods of *A. graveolens* and *A. urundeuva* overlap with that species, and so much attention is needed to differentiate the pollen grains of these species in honey samples.

Species of the tribe Rhoeae had the highest and sinuous striae and not so tightly connected striations, with the presence of an inframicroreticulum, described under LM as microreticulate-striate pollen grains. *Schinus terebinthifolia* was the exception with lower and less sinuous striae and tightly connected striations, with perforations between striae, described under LM as striato-microreticulate pollen grains.

Some characteristics of the endoapertures were important for the recognition of *Astronium graveolens* and *A. urundeuva*. The pollen grains of *Astronium fraxinifolium* and *Schinopsis brasiliensis* were very similar, but the wider colpus margo of the latter, the largest among all the analyzed pollen grains, was important for its distinction.

Spondias tuberosa, the only species studied of the tribe Spondiaea, stood out for the size of its pollen grains and the striate-perforate ornamentation under SEM analysis, with lower and less sinuous striae, compared to the tribe Rhoeae, and not so tightly connected striations, which was described as striato-microreticulate sexine under LM analysis.

Features of exine ornamentation, pollen grain size, endoaperture shape and colpus margo width were variable among the six studied species and allowed the recognition of two pollen types and four subtypes among them. Differences in the organization, spacing and height of striae were very important, as was the presence of an inframicroreticulum or perforations between striae.

Knowledge of the variability of pollen morphology of Anacardiaceae reinforces the importance of knowing palynological characteristics for the correct certification of the botanical origin of bee products from areas of SDTF and Cerrado. We hope that the palynological data provided here will contribute to improving bee product certification in Brazil.

Declarations

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Figures

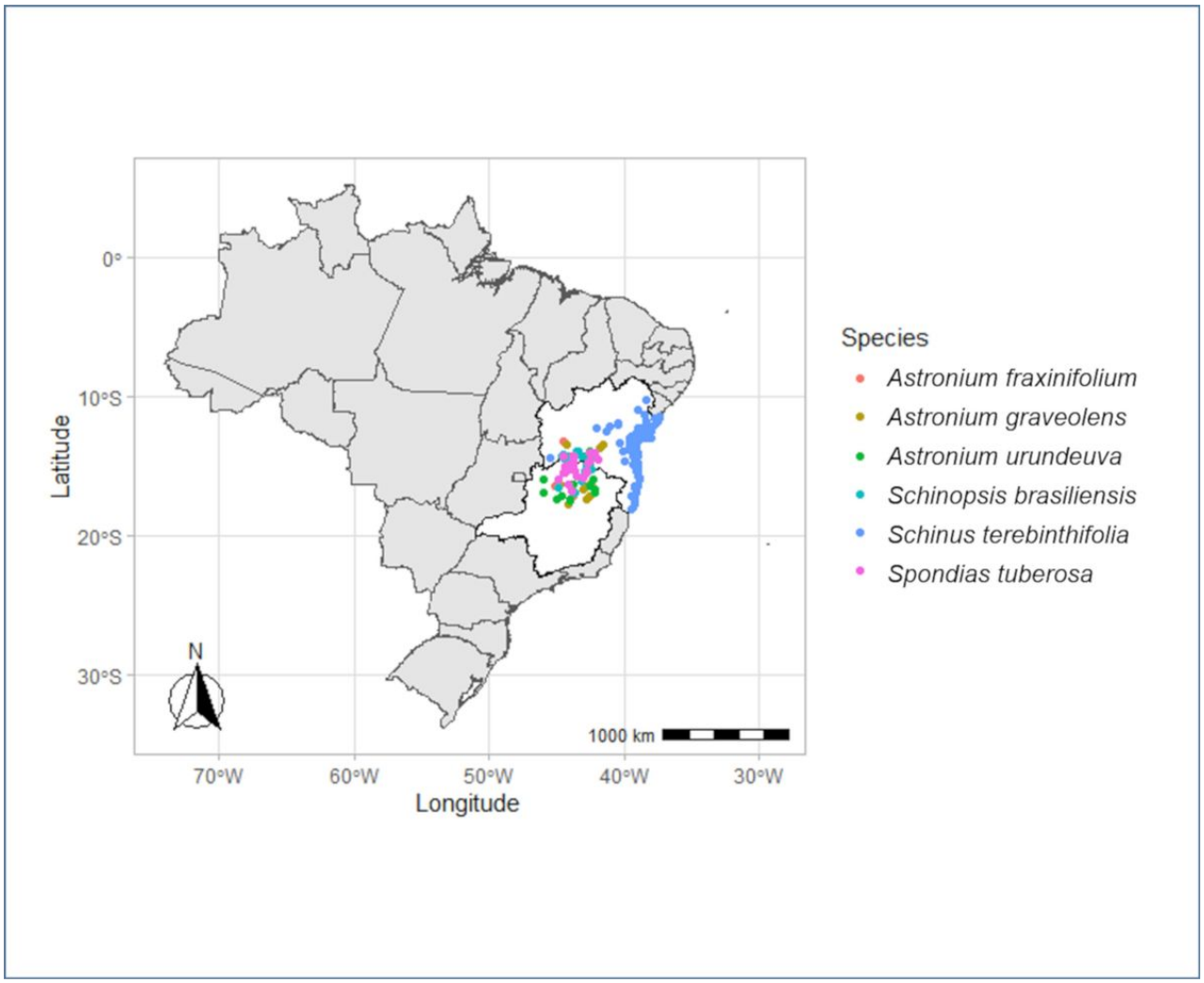

Figure 1

Map showing the compilation of locations data of the Anacardiaceae species based on *SpeciesLink* (CRIA 2020) and Reflora (2020) databases.

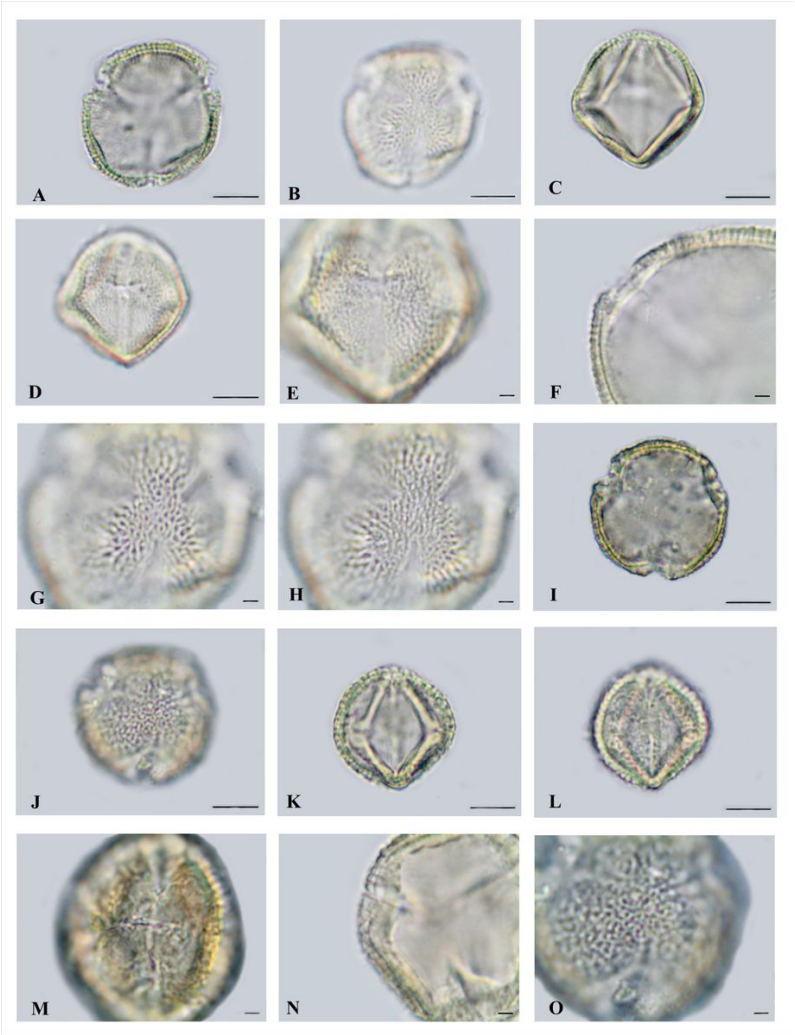

Figure 2

Light microscopy (LM) images of Anacardiaceae pollen grains. **A-H.** *Astronium fraxinifolium* Schott. **A.** Polar view, optical (cross-) section. **B.**Polar view, surface. **C.** Equatorial view, optical (cross-) section. **D.**Equatorial view, colporus. **E.** Endoaperture details. **F.** Optical (cross-) section details showing the domed sexine in the region of the endoaperture. **G.** LO1 (high focus). **H.** LO2 (low focus). **I-O.** *Astronium graveolens* Jacq. **I.** Polar view, optical (cross-) section. **J.** Polar view, surface. **K.** Equatorial view, optical (cross-) section. **L.** Equatorial view, colporus. **M.** Endoaperture details. **N.** Optical (cross-) section details showing the domed sexine in the region of the endoaperture. **O.** LO1 (high focus). Scale bars – 10 µm (A-D, I-L), 2 µm (E-H, M-O).

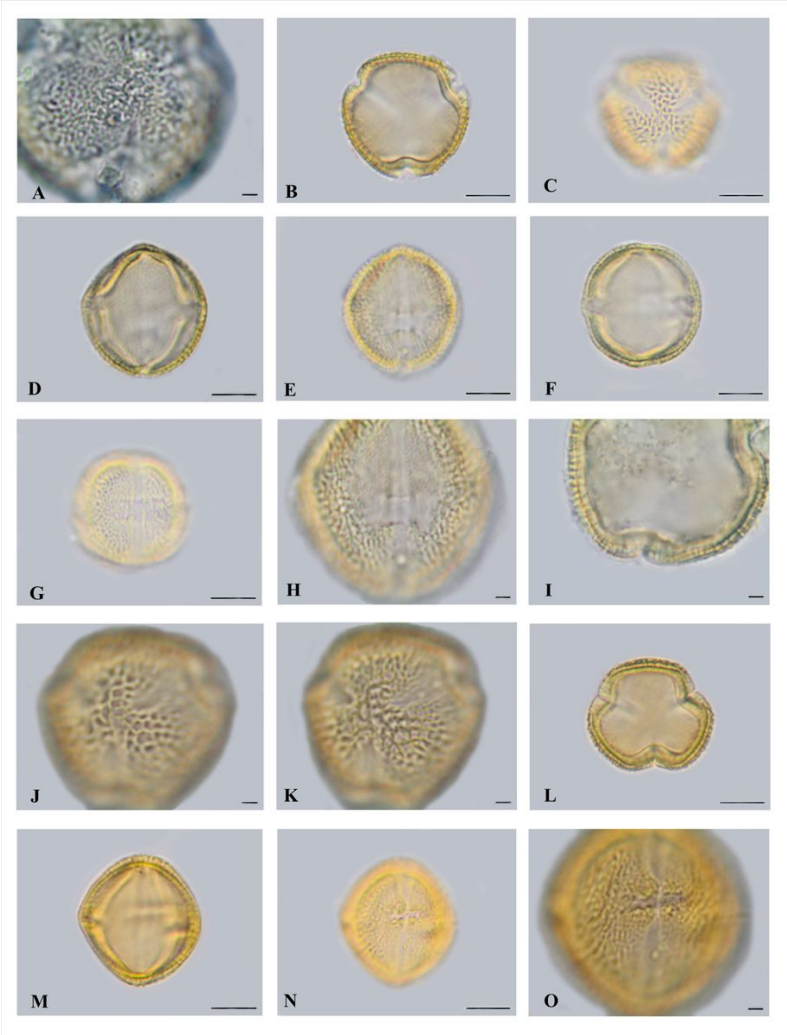

Figure 3

Light microscopy (LM) images of Anacardiaceae pollen grains. **A.** *Astronium graveolens* Jacq., LO2 (low focus). **B-K.** *Astronium urundeuva*(M. Allemão) Engl. **B.** Polar view, optical (cross-) section. **C.**Polar view, surface. **D.** Equatorial view, optical (cross-) section, subprolate pollen grain. **E.** Equatorial view, colporus, subprolate pollen grain. **F.** Equatorial view, optical (cross-) section, oblate spheroidal pollen grain. **G.** Equatorial view, colporus, oblate spheroidal pollen grain. **H.** Endoaperture details. **I.** Optical (cross-) section details showing the domed sexine in the region of the endoaperture. **J.**LO1 (high focus). **K.** LO2 (low focus). **L-O.** *Schinopsis brasiliensis* Engl. **L.** Polar view, optical (cross-) section. **M.**Equatorial view, optical (cross-) section. **N.** Equatorial view, colporus. **O.** Endoaperture details. Scale bars – 10 µm (B-G, L-N), 2 µm (A, H-K, O).

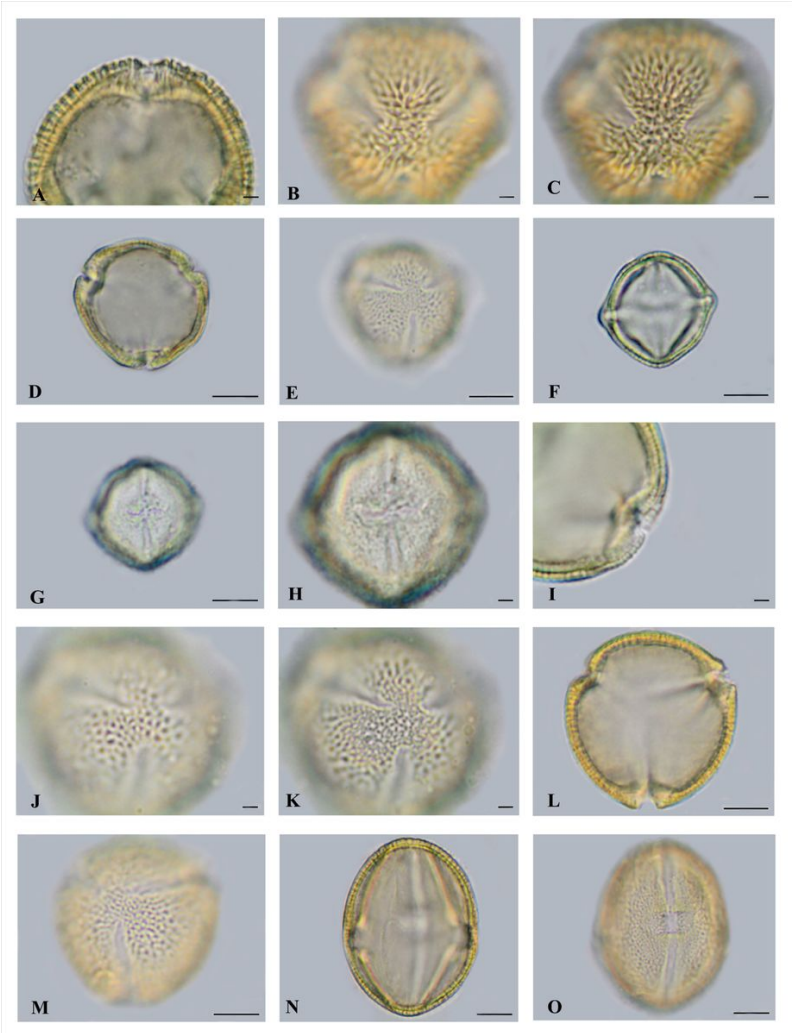

Figure 4

Light microscopy (LM) images of Anacardiaceae pollen grains. **A-C.** *Schinopsis brasiliensis* Engl. **A.** Optical (cross-) section details showing the domed sexine in the region of the endoaperture. **B.** LO1 (high focus). **C.**LO2 (low focus). **D-K.** *Schinus terebinthifolia* Raddi. **D.**Polar view, optical (cross-) section. **E.** Polar view, surface. **F.**Equatorial view, optical (cross-) section. **G.** Equatorial view, colporus. **H.** Endoaperture details. **I.** Optical (cross-) section details showing the domed sexine in the region of the endoaperture. **J.**LO1 (high focus). **K.** LO2 (low focus). **L-O.** *Spondias tuberosa*Arruda. **L.** Polar view, optical (cross-) section. **M.** Polar view, surface. **N.** Equatorial view, optical (cross-) section. **O.** Equatorial view, colporus. Scale bars – 10 µm (D-G, L-O), 2 µm (A-C, H-K).

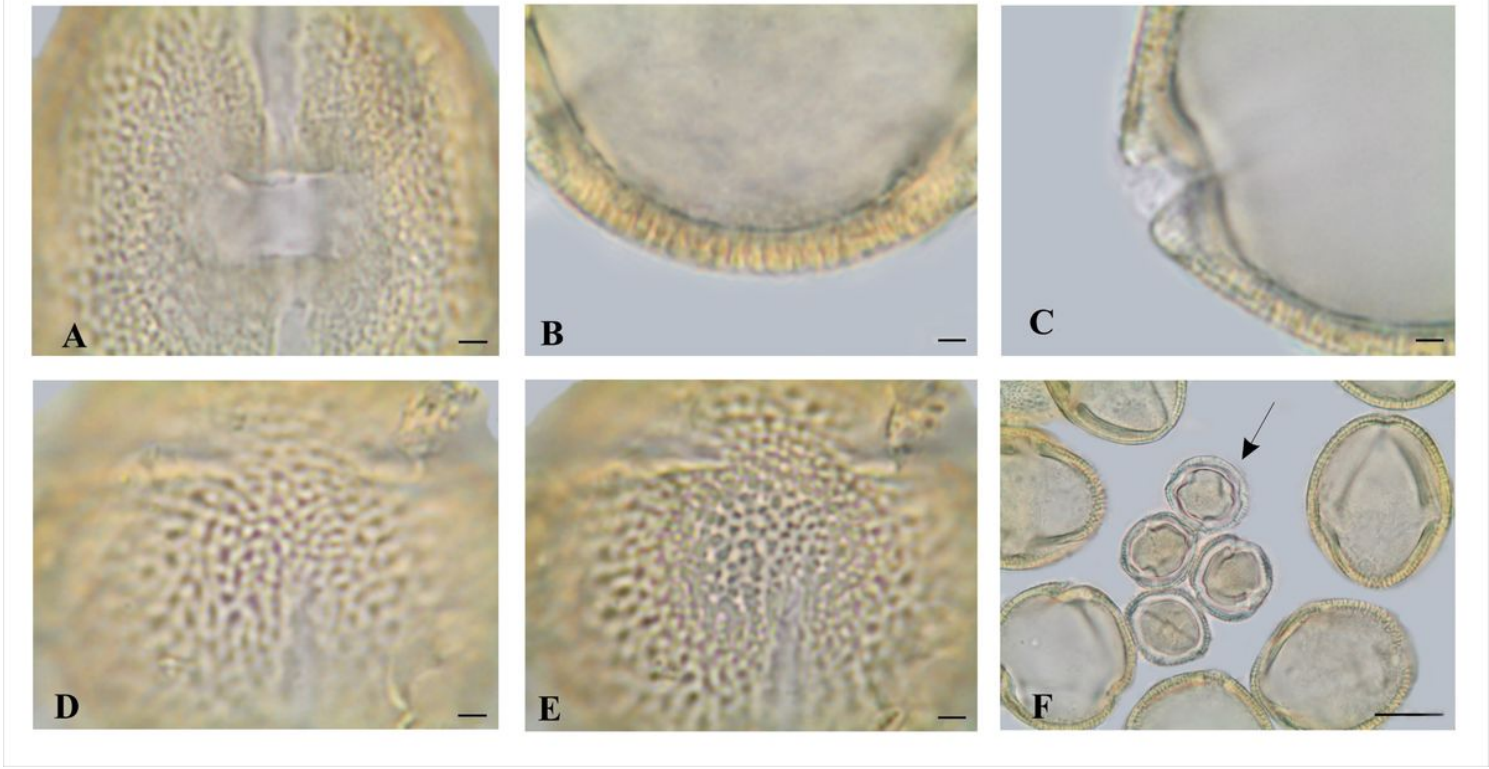

Figure 5

Light microscopy (LM) images of Anacardiaceae pollen grains. **A-F.** *Spondias tuberosa* Arruda. **A.** Endoaperture details. **B.** Optical (cross-) section. **C.** Optical (cross-) section details showing the domed sexine in the region of the endoaperture. **D.** LO1 (high focus). **E.** LO2 (low focus). **F.** Abnormal pollen grains (arrow). Scale bars – 20 µm (F), 2 µm (A-E).

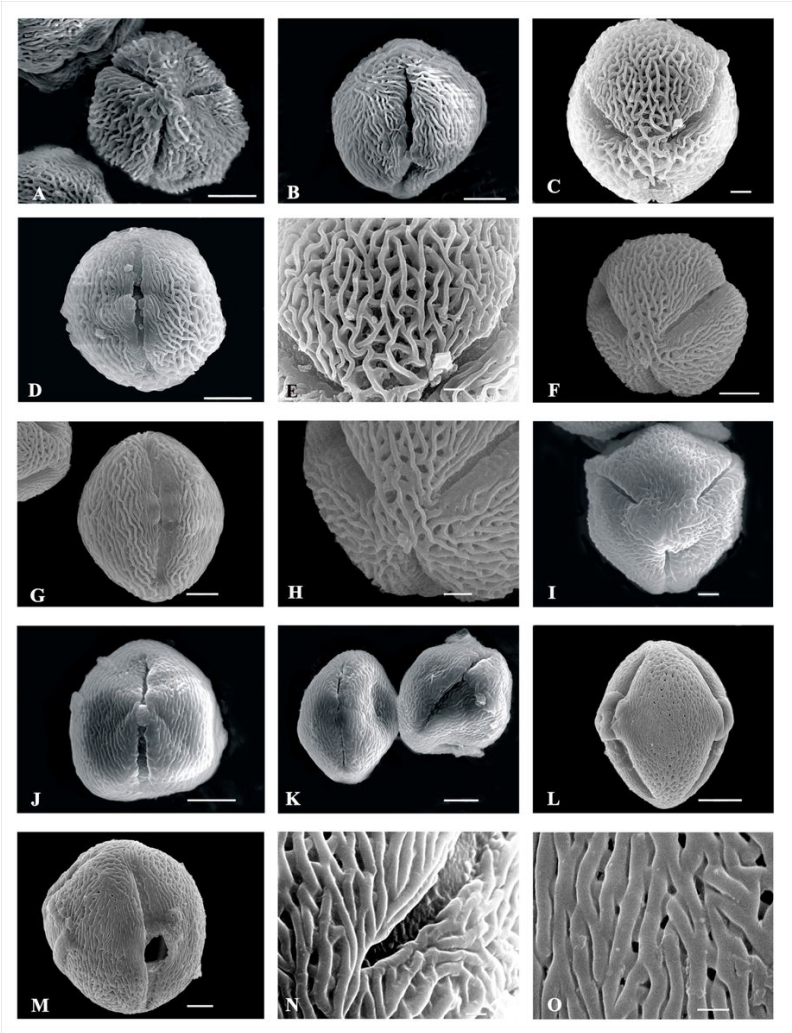

Figure 6

Scanning electron microscopy (SEM) images of Anacardiaceae pollen grains. **A.** *Astronium fraxinifolium* Schott., polar view. **B.** *Astronium graveolens* Jacq., equatorial view. **C-E.** *Astronium urundeuva* (M. Allemão) Engl. **C.**Polar view. **D.** Equatorial view. **E.** Sexine detail in the apocolpium region. **F-H.** *Schinopsis brasiliensis* Engl. **F.**Polar view. **G.** Equatorial view. **H.** Sexine detail in the apocolpium region. **I-K.** *Schinus terebinthifolia* Raddi. **I.** Polar view. **J.**Equatorial view. **K.** Equatorial view, two pollen grains. **L-O.** *Spondias tuberosa* Arruda. **L.** Equatorial view, mesocolpium region. **M.**Equatorial view, colporus. **N.** Detail in the colporus membrane. **O.**Scale bars – 10 µm (L), 5 µm (A-B, D, F-G, J-K, M), 2 µm (C, H-I), 1 µm (E, N-O).

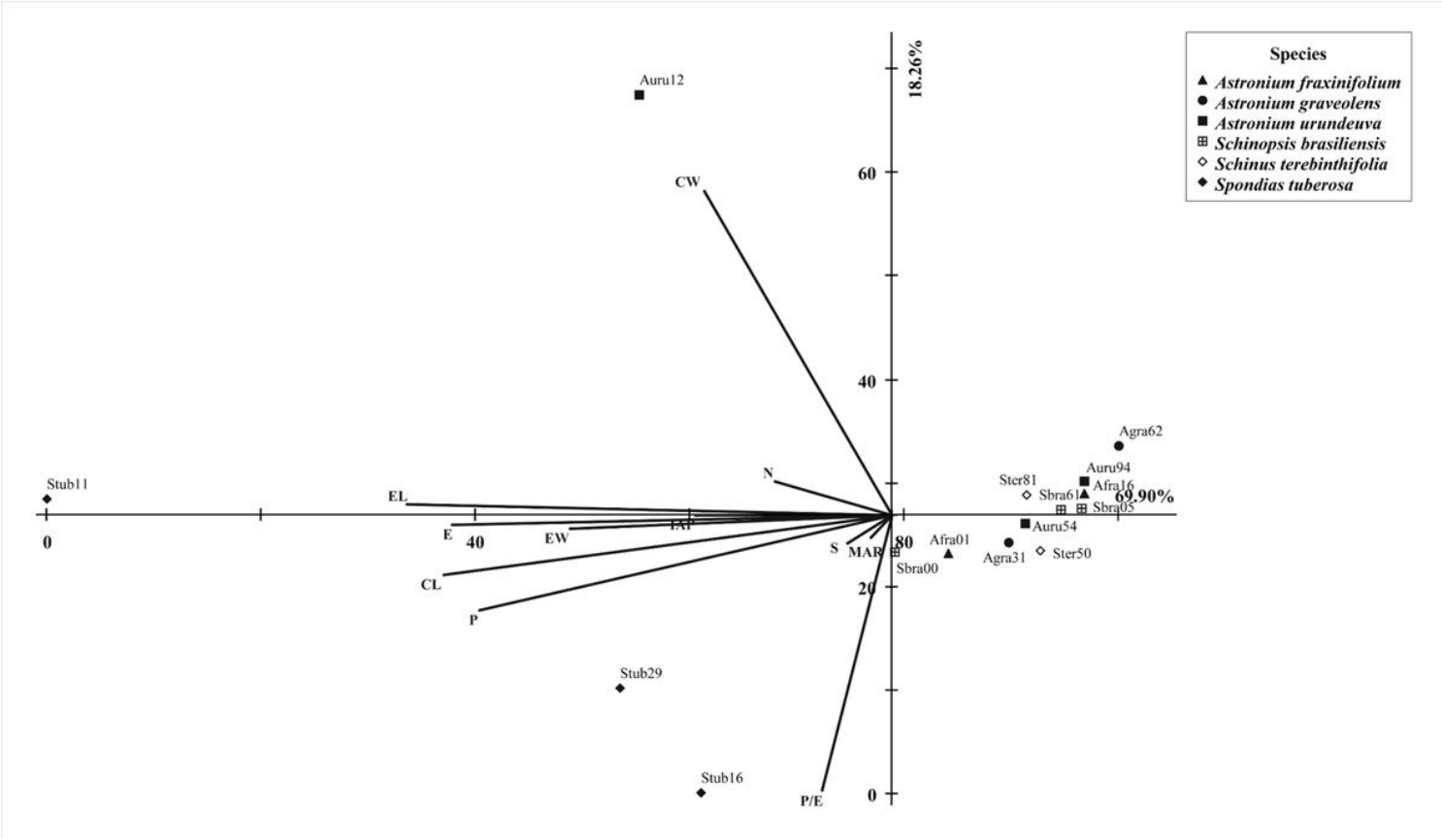

Figure 7

Ordination by principal component analysis of the specimens of Anacardiaceae, correlating the metric variables of the pollen grains (see Table 2 for species' codes).

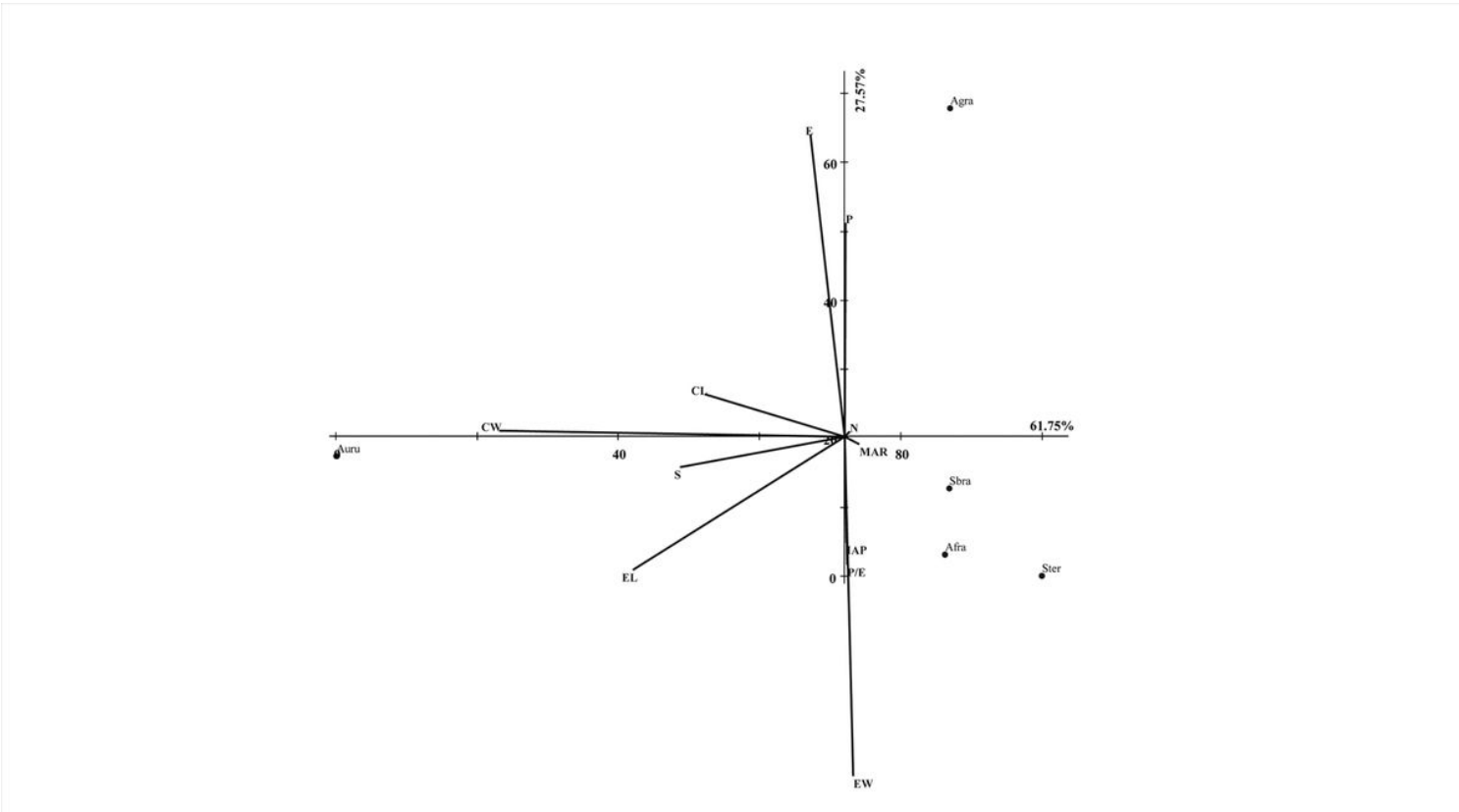

Figure 8

Ordination by principal component analysis of the species of Anacardiaceae, correlating the metric variables of the pollen grains, excluding *Spondias tuberosa*(see Table 2 for species' codes).