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Original Article

Red propolis produced on the island of San Andrés, Colombia: Palynological profile

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ABSTRACT

Propolis primarily comprises resins, waxes, pollen, and several compounds collected by honeybees and mixed with secreted fluids. Globally, red propolis production is limited to a few regions, and San Andrés Island (Colombia) is one of these locations. This study aims to outline the palynological profile of red propolis produced by *Apis mellifera* on San Andrés Island, Colombia, to identify its relationship with the local vegetation. Red propolis samples were collected for the production year 2022 from two apiaries located on the island. Red propolis samples were initially treated with ethanol and KOH for pollen grain analysis, followed by the standard acetolysis techniques. After the palynological analysis and botanical identification, 76 pollen types were identified, associated with 32 plant families, 61 genera, and 42 species. Leguminosae and Asteraceae were the botanical families with the most representative, each with seven pollen types. Pollen types such as *Mimosa pudica*, *Cecropia peltata*, and *Byrsonima crassifolia* exhibited the highest importance values within the pollen spectrum. Most pollen types were associated with arboreal and shrubby taxa, predominantly distributed in forest formations and vegetation characteristic of anthropic areas. The analyses highlighted the distribution and diversity of pollen types within red propolis samples from San Andrés Island.

Keywords: *Apis mellifera*, Colombian Caribbean, honeybee products, melissopalynology, red propolis, resinous plants.

Introduction

Propolis is a natural resinous product made by different genera of bees from plant secretions (Salamanca-Grosso, 2017). It exhibits color schemes that vary from brown, dark brown, reddish, green, and, in some cases, black, depending on its botanical and geographical origin (Salamanca-Grosso, 2017; Sforcin *et al.*, 2017). The most well-known propolis is produced by *Apis mellifera* L., which serves as a thermal insulator and crack sealer and helps to isolate the hive (Matos & Santos, 2017). Furthermore, it is an antimicrobial substance, inhibiting the growth of many bacteria, yeasts, and fungi, most of which are associated with several degrees of pathogenicity in humans (Meneses *et al.*, 2009). It also possesses anti-inflammatory, healing, immunomodulatory, and antitumor activities (Salatino, 2018).

Although it is not easy to establish a general classification for its composition, it is estimated that 40-50% consists of resins, 8-12% of waxes, and 3-5% of volatile compounds, microelements, and pollen grains (Salamanca-Grosso, 2017). The latter can come from intentional honeybee collection or airborne pollen (anemophilous) that accidentally adheres to their bodies (Matos *et al.*, 2014). Thus, it is important to recognize them because they allow the identification of the plants that *Apis mellifera* L. uses to produce propolis (Matos & Santos, 2017). Therefore, identifying the plants visited

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by honeybees is of utmost importance to beekeepers, as it provides valuable information about their food sources. Moreover, understanding the composition and structure of the landscape expands the knowledge about their foraging habits, which is crucial since the landscape significantly influences honeybees, especially regarding pollen harvesting. This aspect is pivotal for implementing management and conservation programs (Alves & Santos, 2014; Piroux *et al.*, 2014; Casas-Restrepo *et al.*, 2021).

In Colombia, there has yet to be official data on propolis production. Still, according to López-Patiño (2011), the volume produced is low because beekeepers are unaware of its benefits and there is a lack of standardized methods for its collection. Nevertheless, significant studies on its characterization, especially those related to its physicochemical properties, have been carried out (Bastos *et al.*, 2011; Martínez *et al.*, 2012; Rodríguez *et al.*, 2012; Viloria *et al.*, 2012), along with research on biological activity against different bacteria and fungi (Meneses *et al.*, 2009; Samara-Ortega *et al.*, 2010; Bastos *et al.*, 2011; Campo *et al.*, 2018; Martínez *et al.*, 2012; Herrera *et al.*, 2012). However, palynological studies are scarce, with only one research focused on samples of red propolis from San Andres Island whose analysis of the pollen spectrum evidenced a predominance of pollen types such as *Cyperus haematodes*, *Avicennia germinans*, *Conocarpus erectus*, *Dalbergia brownei*, among others (Salamanca-Grosso & Osorio-Tangarife, 2019).

Therefore, this research aims to outline the palynological profile (through identification of pollen types) of the red propolis produced by *Apis mellifera* L. on San Andrés Island, Colombia, to infer the main plant species used by the honeybees for red propolis production.

Materials and Methods

Study Area

San Andrés Island is in the department of the Archipelago of San Andrés, Providencia, and Santa Catalina, in the Caribbean Sea, at 619 km northwest of the northern coast of Colombia and 150 km east of the coast of Nicaragua (Fig. 1). It is situated in the intertropical zone, presenting a hot and humid climate, and an average annual temperature ranging between 27.5 °C on the coast and 26.5 °C in the mountainous area of the island (Gamboa *et al.*, 2012). The average yearly precipitation is 1,950 mm, exhibiting mixed rainfall (Ochoa & Escovitch, 2023).

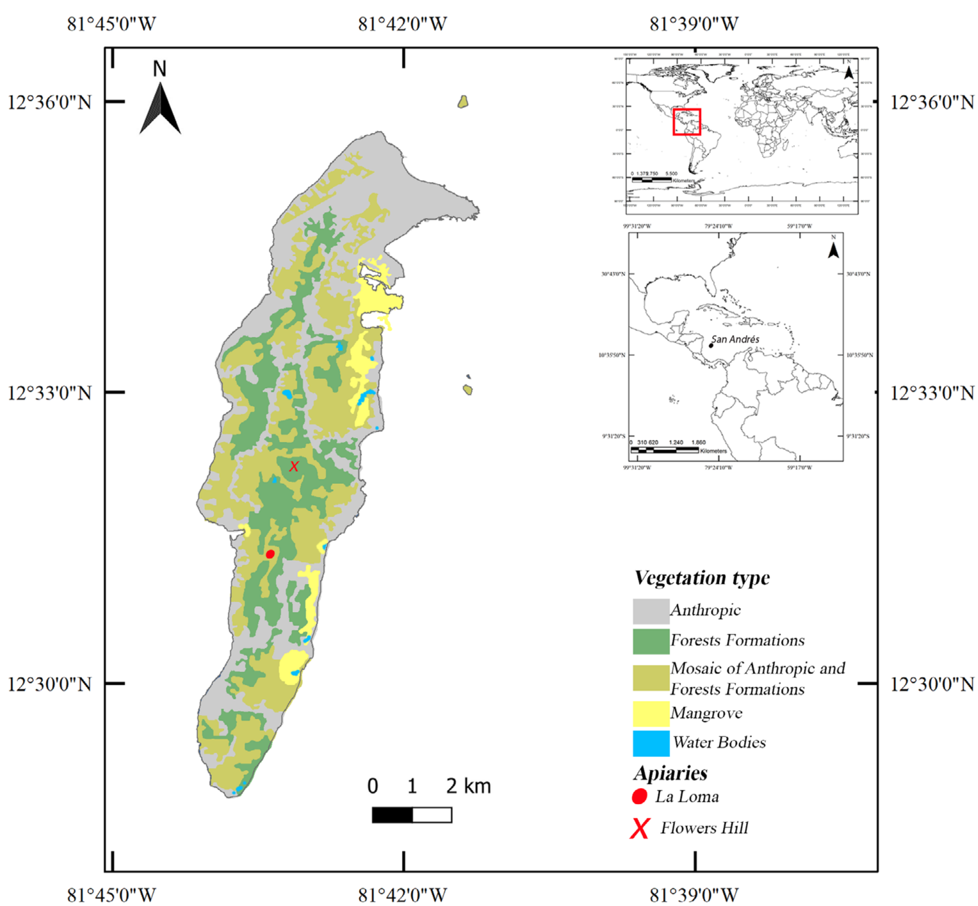

Figure 1. Location and types of vegetation present on San Andrés Island, Colombia.

The island's landscape consists of a system of hills up to 100 m.a.s.l in the central part of the island and a coastal flatland composed of emerged coral terraces (Cortés, 1982; Urrego *et al.*, 2010). The type of vegetation on the island's hills was initially described as a combination of dry and wet forest (Lowy, 2000; Urrego *et al.*, 2010; Tobar-Vargas & Kondo, 2015). However, it currently lacks primary vegetation, and it is dominated by secondary formations composed of trees, shrubs, and vines (Urrego *et al.*, 2010). The coastal flatland area comprises four landscape units: sandy coast, rocky coast, mangroves, and the transition zone between mangrove and terrestrial vegetation (Murcia *et al.*, 2012). Beach vegetation and mangrove forests dominate these flatland areas (Urrego *et al.*, 2010). Furthermore, the beach vegetation is predominantly herbaceous, while in the mangrove forests, *Rhizophora mangle* L. is the dominant species (Murcia *et al.*, 2012).

Currently, more than 50% of the island's territory is occupied by anthropized land covers, with a predominance of urban areas, pastures, and crops, including coconut plantations (*Cocos nucifera* L.) and other types of crops such as Noni (*Morinda citrifolia* L.), cassava (*Manihot esculenta* Cranz), and plantains (*Musa* spp.) (Bolivar & Vélez, 2004; Urrego *et al.*, 2010; Velásquez, 2014). Regarding the natural land covers, secondary vegetation is predominant, with dense forests and some water bodies formed during the rainy season (Corporación Ambiental CORALINA & Fundación ALMA, 2015).

Secondary vegetation, grasslands, urban areas, dense forests, and swamps cover the vegetation near the apiaries. In the sectors of La Loma (LL) and Flowers Hill (FH), are found species such as *Avicennia germinans* (L.) Stearn, *Cecropia peltata* L., *Chrysobalanus icaco* L., *Cocos nucifera* L., *Conocarpus erectus* L., *Ficus* spp., *Laguncularia racemosa* (L.) C.F. Gaertn, *Leucaena leucocephala* (Lam.) de Wit, *Mangifera indica* L., *Mimosa pudica* L., *Paspalum paniculatum* L., *Psidium guajava* L., *Rhizophora mangle* L., *Spondias mombin* L., *Terminalia catappa* L., etc. (Corporación Ambiental CORALINA & Fundación ALMA, 2015).

Sample collection

A total of nine (9) samples of red propolis were collected from two apiaries (Flowers Hill – FH and La Loma – LL) for the production year 2022. The samples were collected by scraping the inner walls of the hives with the help of local beekeepers. Afterward, propolis samples were stored in amber jars and sealed for analysis.

Pollen analysis

The analysis process of the red propolis samples followed the procedure established by Matos *et al.* (2014). Initially, 5g of the sample was dissolved in 95% ethyl alcohol, and the resulting sediment was subjected to a hot solution of 10% KOH. The residue was then dehydrated with glacial acetic acid, subjected to the acetolysis method proposed by Erdtman (1960), mounted between slides and coverslips with glycerin gelatin, and sealed with paraffin for observation using light microscopy (LM). Pollen grains were counted (minimum 500 per sample) and microphotographed using a Leica ICC50 W light microscope.

Pollen identification

Our study is based on the use of morphological characteristics of each pollen type to identify the botanical origin of red propolis samples by linking those pollen types to a specific taxon (*e.g.*, a species, a group of species, a genus, or a family) (De Klerk, 2002; De Klerk & Joosten, 2007). Scientific names were verified using the International Plant Names Index (IPNI, 2024). The identification of pollen grains was carried out as described by Santos (2011), through comparison with reference slides from the pollen library of the Plant Micromorphology Laboratory (LAMIV/UEFS) and specialized palynological literature (Roubik & Moreno, 1991; Villanueva, 2002; Luz *et al.*, 2007; Silva & Santos, 2009; Mambrín *et al.*, 2010; Gosling *et al.*, 2013; Jones & Pearce, 2015; Souza *et al.*, 2016; Stanski *et al.*, 2016; Antonio-Domingues *et al.*, 2018a; Antonio-Domingues *et al.*, 2018b; Corrêa *et al.*, 2018; Rakarcha *et al.*, 2018; Adeleye *et al.*, 2020; Dutra *et al.*, 2020; Souza *et al.*, 2020; Assis *et al.*, 2021; Vovides *et al.*, 2021; Lopes *et al.*, 2022; Xiaoyan *et al.*, 2023).

To recognize the vegetation on the island, data reported by Lowy (2000), Tobar-Vargas *et al.* (2013) and Tobar-Vargas and Kondo (2015) was considered, which also provided information about the type of vegetation in which the taxa related to the pollen types are distributed. However, the types of vegetation were reclassified as follows, considering the classifications already proposed by the cited authors: forest formations (FF), which include transitional vegetation, secondary vegetation, and subxerophytic vegetation; anthropic areas (An): including crops, ruderal species, and ornamental species; wet areas (WA): gallery and swamp vegetation; psammophilous vegetation (Ps); and mangroves (MG).

Based on Bernal *et al.* (2016a, 2016b) and different herbarium databases provided information about each taxon's growth habit. Similarly, information about each species' vegetal resources for *Apis mellifera* was obtained from various specialized literature (Table 1).

Data analysis

We compare the pollen type composition variability between samples based on a Non-Metric Multidimensional Scaling (NMDS) and a dendrogram using the PAST-Palaeontological Statistics software, ver. 4.17 (Hammer *et al.,* 2001). Additionally, a multivariate analysis was conducted to evaluate the differences in pollen type composition of the red propolis samples according to the apiary's location using a Permutational Analysis of Variance (Anderson, 2001). The Jaccard similarity coefficient was employed in all cases, as it does not consider shared absences as similarities.

Table 1. Frequency (%) of pollen types, growth habit, offered resource, and vegetation type of plants associated with pollen types registered in red propolis samples produced by *A. mellifera* on San Andrés Island, Colombia.

Pollen type	Sample									GH	Vegetation type	Plant resource	
	LL1	LL2	LL3	LL4	FH1	FH2	FH3	FH4	FH5			Type	Reference
Acanthaceae													
Avicennia germinans	0.20	0.36	0.57	0.58	1.39	1.08	1.36	1.23	1.59	T	MG	N	Almeida *et al.* (2021)
Dicliptera sexangularis			0.38							H	WA	N	Matos & Santos (2017)
Justicia carthagenensis					0.20	0.18				H	WA	N	Chicangana *et al.* (2023)
Ruellia tuberosa				0.19		0.36	0.19		0.35	H	An		
Amaranthaceae													
Alternanthera sessilis			0.19					0.53		Sb	WA	P	Ghugal *et al.* (2018)
Anacardiaceae													
Anacardiaceae 1	2.61	0.36	0.19	0.19	0.80	2.33	0.39	3.52	0.53	T	FF	N/P/R/O	Avelino & Santos (2018); Reis *et al.* (2023)
Metopium brownei	1.20	2.18	0.76	3.11	3.18	1.26	3.10	3.35	3.36	T	FF	N	Santos *et al.* (2000)
Spondias mombin	5.01	3.45	0.57	0.58	1.19	3.41	2.71	10.56	5.30	T	FF	N/P	Oliveira *et al.* (2020)
S. purpurea	4.81		5.48	3.11						T	FF	N/P	Cristóbal-Pérez *et al.* (2022)
Apocynaceae													
Mandevilla subcarnosa			0.19							S	An/FF		
Arecaceae													
Cocos nucifera	6.41	3.27	6.05	3.88	8.55	9.16	4.65	4.75	3.00	T	An/FF/Ps	N/P	Free *et al.* (1975)
Sabal mauritiiformis		0.36			0.80	1.08		1.41	0.18	T	FF	N/P	Durán-Escalante *et al.* (2023)
Asteraceae													
Bidens pilosa	1.00	0.18		0.78						H	An	N/P	Velandia *et al.* (2012)
Cyanthillium cinereum	1.40	1.63	0.19	0.19	2.39	1.44	0.19	1.23	2.12	Sb	An	N/P	Apolito *et al.* (2010)
Eclipta alba					0.60		0.39	0.53	0.53	H	An	P	Brandão *et al.* (1984)
Emilia sonchifolia			0.19		0.60			0.18		H	An	N/P	Medabalimi *et al.* (2017)
Erigeron canadensis		0.54			0.40	0.36	0.39	0.18	0.71	H	An		
Pluchea carolinensis		0.36	0.19	0.19	0.99	0.36	0.78	0.70	0.71	S	An/Ps	N/P	González-Suárez *et al.* (2020)
Verbesina	0.60			0.19						S	An/FF	N/P	Chamorro (2016)
Bignoniaceae													
Dolichandra unguis-cati			0.57	0.39		0.18				L	FF		

Table 1. Cont.

Pollen type	Sample									GH	Vegetation type	Plant resource	
	LL1	LL2	LL3	LL4	FH1	FH2	FH3	FH4	FH5			Type	Reference
Bromeliaceae													
Tillandsia dasyliriifolia	1.80	0.73	0.38		1.79	0.90	0.97	1.76	1.77	Ep	FF	N	Ramírez-Morillo *et al.* (2004)
Burseraceae													
Bursera simaruba	0.60	0.36	0.38	1.55		0.36	1.16		1.59	T	FF	N/R	Barrance *et al.* (2003)
Cannabaceae													
Trema micrantha			1.51	0.39						T/S	FF	P	Avelino & Santos (2018)
Combretaceae													
Combretum indicum	4.01	1.63	1.70	2.52		1.80	2.71		2.65	C	An/WA	N/P	Gobatto *et al.* (2021)
Conocarpus erectus		0.73	1.51	1.36	2.58	1.08	3.68	3.35	3.36	T/S	MG	N	Diniz *et al.* (2020)
Laguncularia racemosa	3.01	2.36	1.32	1.17	2.19	2.15	1.16	0.18	1.59	T/S	MG	N	Diniz *et al.* (2020)
Terminalia		0.36	0.76	1.94			0.97		2.65	T	An/FF	N/P	Villalpando-Aguilar *et al.* (2022)
Commelinaceae													
Tradescantia		0.36	0.76		1.99	1.97		1.41	3.18	H	An	N/P	Koch *et al.* (2020)
Connaraceae													
Rourea glabra	1.20	1.27	0.38	0.19	1.59	2.33	3.68	2.82	1.06	S	FF		
Convolvulaceae													
Ipomoea 1	0.40		0.19	0.19		0.36			0.71	H/Sb/L/C	FF/Ps	N/P	Galetto & Bernardello (2004); Salis *et al.* (2015)
Ipomoea 2									0.35	H/Sb/L/C	FF/Ps		
Cycadaceae													
Cycas		0.18			0.99	0.90	0.39	0.70	0.18	S	An		
Cyperaceae													
Scleria gaertneri	1.40			0.19						H	An/WA		
Euphorbiaceae													
Cnidoscolus aconitifolius							0.19	0.35		S	An	P	Munguía-Rosas & Jácome-Flores (2020)
Fabaceae													
Aeschynomene americana	1.00	0.18	0.19	0.97	1.59	0.54	1.36	1.41	0.71	Sb	FF/WA	N	Giraldo *et al.* (2011)
Chamaecrista glandulosa		2.36	1.13	1.36	6.76	3.95	4.26	4.58	1.59	Sb	An/FF	N/P	Campbell *et al.* (2018)
Dalbergia brownei	6.21	4.36	2.08	12.04	2.19	3.77	1.36	1.41	1.94	C	WA	N	Real-Luna *et al.* (2021)
Leucaena leucocephala	0.40	0.36	0.76	0.19	0.80	0.36	0.39	0.18	0.18	S	An/FF	P	Chávez (2011)
Mimosa 1		0.18	0.19	1.55				0.53					

Table 1. Cont.

Pollen type	**Sample**									**GH**	**Vegetation type**	**Plant resource**	
	LL1	**LL2**	**LL3**	**LL4**	**FH1**	**FH2**	**FH3**	**FH4**	**FH5**			**Type**	**Reference**
M. pudica	7.01	13.97	25.14	8.74	9.15	6.28	18.22	11.27	11.84	H	An	N/P	Velandia *et al.* (2012)
Pithecellobium				0.19	0.99	0.54		0.35	0.35	T/S	An/FF/WA	N/P	Real-Luna *et al.* (2021); Villalpando-Aguilar *et al.* (2022)
Lamiaceae													
Ocimum 1						0.18		0.18		H	An	N/P	Abrol *et al.* (2017); Almeida *et al.* (2021)
Ocimum 2			0.19	0.19						H	An		
Malpighiaceae													
Byrsonima crassifolia		11.62	6.81	7.38	10.74	5.92	9.11	4.23	8.66	T/S	FF/WA	O/P	Sousa (2021)
Stigmaphyllon				0.19						S	WA	O	Matos & Santos (2017)
Malvaceae													
Ceiba pentandra	1.00	0.18	0.76	0.19	0.40	0.36	0.19			T	FF	N/P	Almeida *et al.* (2021)
Corchorus		1.45	0.57	0.97	0.20	2.15	1.36	1.58	2.12	S/H	An/FF	P	Reis *et al.* (2023)
Gossypium	0.40		0.19	0.19					0.18	S	An	N/P	Buchmann & Shipman (1990)
Triumfetta 1		0.36		0.19	0.40	1.26	0.97	0.53	0.71	S/Sb/H	An/FF/WA	N/P	Velandia *et al.* (2012)
Triumfetta 2			0.57	0.19		1.26		1.41	0.35	S/Sb/H	An/FF/WA		
Meliaceae													
Trichilia		0.18	0.76	0.58	1.59	1.80	0.19	1.23	0.35	T/S	FF/WA	N/P	Maldonado & Méndez (2020)
Myrtaceae													
Eugenia	1.80	0.18	0.76	0.39		1.08	2.13		0.18	T/S	WA	N/P	Velandia *et al.* (2012)
Myrcia splendens	1.40	2.00	2.08	3.50	5.37	1.08	0.97	6.34	1.41	S	An/FF/WA	N/P	Ramírez-Arriaga *et al.* (2011)
Myrtaceae 1	1.80	2.90	0.19	0.58		3.23	2.33		5.48	T	An/FF	N/P	Velandia *et al.* (2012)
Phyllanthaceae													
Phyllanthus		0.54		0.19		0.72		1.41	0.18	S	An/FF	N/R	Matos & Santos (2017)
Rhizophoraceae													
Rhizophora mangle	4.21	9.07	5.10	3.11	7.95	3.23	12.02	5.81	3.36	T	MG	N/P	Sucerquia *et al.* (2019)
Rubiaceae													
Psychotria carthagenensis	1.40	1.63	0.19	1.94		0.72			0.88	T/S	FF/Ps	N/P	González-Avilés *et al.* (2023)
Randia	0.60	0.18	0.57	1.17		0.18	0.97	0.53	1.24	T/S	FF/Ps	N	Matos & Santos (2017)
Spermacoce verticillata	0.20			0.19		0.18		0.18	0.18	S	An/FF	N/P	Giraldo *et al.* (2011); Reis *et al.* (2023)
Rutaceae													
Zanthoxylum		0.18	0.57	0.19					1.41	T/S	FF	N	Flores *et al.* (2021)

Table 1. Cont.

Pollen type	Sample									GH	Vegetation type	Plant resource	
	LL1	LL2	LL3	LL4	FH1	FH2	FH3	FH4	FH5			Type	Reference
Sapindaceae													
Melicoccus bijugatus	10.22	8.17	4.77	1.80	0.97		1.41	6.99	7.38	T	FF	N	Wilson *et al.* (2019)
Paullinia cururu		1.27	0.38	1.17	0.80	0.36	0.78	0.70	0.18	S	FF	N/P	Giraldo *et al.* (2011)
Solanaceae													
Cestrum			0.19	0.39						S	An/FF	P	Nascimento *et al.* (2014)
Urticaceae													
Cecropia peltata	16.63	9.62	7.18	10.29	8.95	15.26	9.50	8.10	6.18	T	FF	P	Giraldo *et al.* (2011)
Verbenaceae													
Lippia alba	8.42	5.63	5.29	2.52		5.21		4.23		Sb	FF	N/P	Reis *et al.* (2023)
Petrea			0.38	0.58	0.40			0.18	0.18	S/L/C		N	González-Suárez *et al.* (2020)
Phyla nodiflora		0.18	0.95	2.91	0.40	1.26	0.39	1.41	1.77	H	An/FF	N/P	Solomon *et al.* (2021)
Vitaceae													
Vitis	1.20	1.45	2.84	4.08		2.15	1.74		3.53	C	FF	N	Montoya-Pfeiffer *et al.* (2014)
Indeterminate types (%)	0.40 (1 t)	1.09 (3 t)	2.65 (7 t)	1.36 (4 t)	4.37 (3 t)	2.15 (3 t)	1.74 (3 t)	2.46 (3 t)	6.01 (4 t)				

FH: Flower Hill; **LL:** La Loma; **GH:** growth habit; **T:** tree; **S:** shrub; **Sb:** subshrub; **H:** herbaceous; **L:** liana; **C:** climbing; **Ep:** epiphyte. **Plant resource: N:** nectar; **O:** oil; **P:** pollen; **R:** resin. **Vegetation type: An:** anthropic; **FF:** forest formations; **WA:** wet areas; **MG:** mangrove; **Ps:** psammophilous vegetation.

To quantify the use and preference of resources in the study area, the importance index (IE_j) for each pollen type was calculated based on the relative abundance and frequency of the respective pollen types among the apiaries (Nates-Parra *et al.*, 2013) using the following formula:

$$IE_j = \textit{Mean relative abundance of type J x (Number of samples containing pollen type J/ Total number of samples)}$$

Results

The pollen spectrum of the analyzed red propolis samples showed 76 pollen types, of which 68 (89.47%) were identified as related to 32 plant families, 61 genera, and 42 species (see Table 1). The most representative families were Leguminosae and Asteraceae (each with seven pollen types), Malvaceae (five), Acanthaceae, Anacardiaceae, and Combretaceae (each with four types). These families were followed by Myrtaceae, Rubiaceae, and Verbenaceae (each with three types), Arecaceae, Convolvulaceae, Lamiaceae, Malpighiaceae, and Sapindaceae (each with two types), and the remaining 18 families contribute one pollen type.

The pollen types per sample ranged from 34 (sample LL1) to 58 (samples LL3 and LL4; Table 1). The samples from the La Loma apiary presented 71 pollen types, while those from Flowers Hill presented 63 pollen types. The unidentified pollen types represented 10.53% (eight types) of the pollen spectrum. The sample FH5 exhibited the highest rate of unidentified pollen types (6.01%; four pollen types), while sample LL1 had the lowest rate (0.40%; one pollen type).

Fifteen pollen types were recorded in all analyzed samples: *Aeschynomene americana*, Anacardiaceae 1, *Avicennia germinans*, *Cecropia peltata* (Fig. 2, P), *Cocos nucifera* (Fig. 2, F), *Cyanthillium cinereum*, *Dalbergia brownei* (Fig. 2, J), *Laguncularia racemosa*, *Leucaena leucocephala*, *Metopium brownei*, *Mimosa pudica* (Fig. 2, L), *Myrcia splendens*, *Rhizophora mangle*, *Rourea glabra*, and *Spondias mombin*. In addition to these pollen types, another 34, including three unidentified, were also registered in more than 50% of the samples, although the abundance of the unidentified types was low. Moreover, 18 pollen types were found in 20 to 50% of the samples, and six were found in less than 20% (Tab. 1). The highest frequency for a pollen type was recorded in the sample LL3, in which 25.14% of the identified pollen grains corresponded to the *Mimosa pudica* type (Tab. 1).

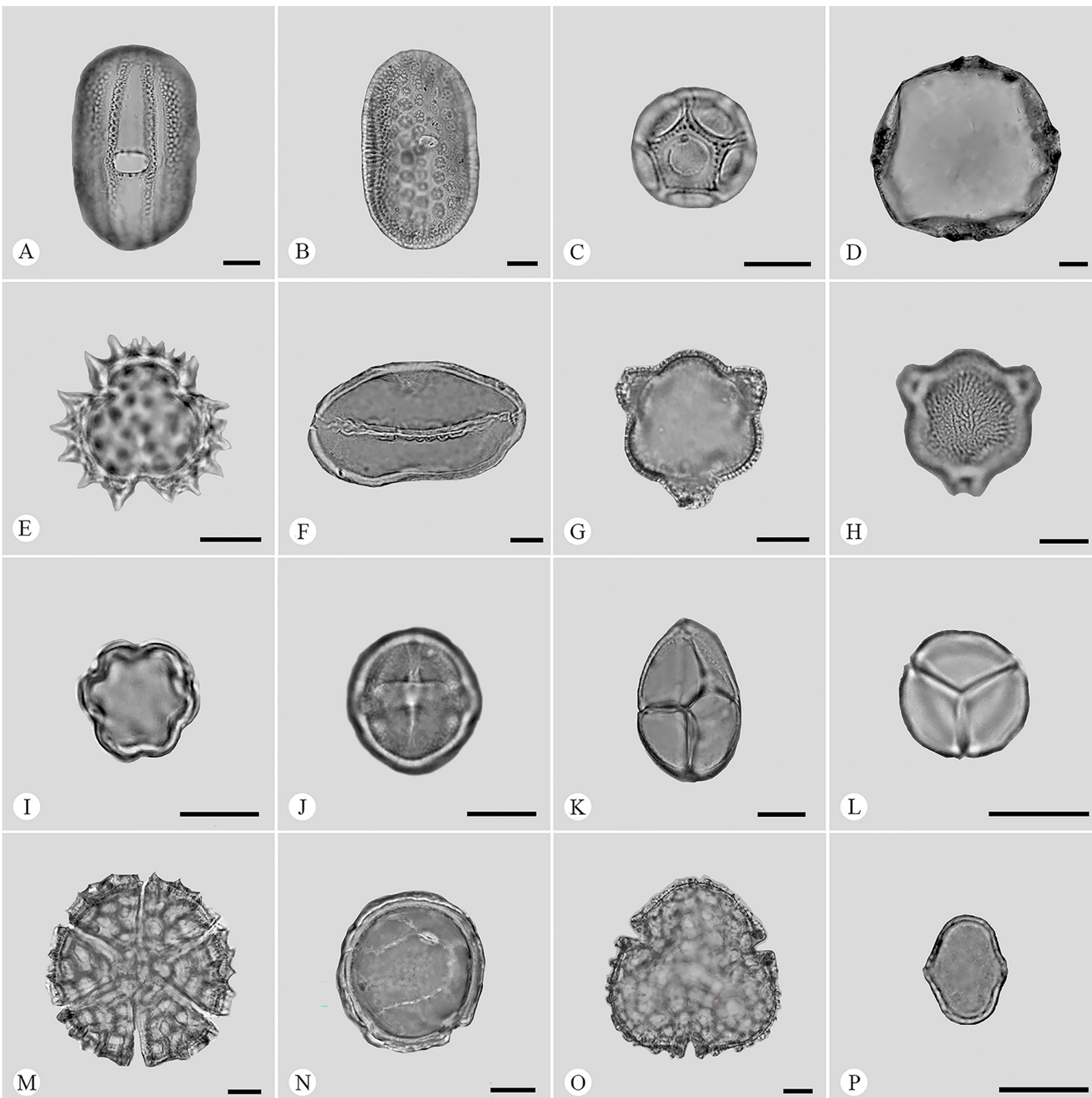

Figure 2. Pollen types registered in red propolis samples from San Andrés Island. **A-B. Acanthaceae: A.** *Dicliptera sexangularis*. **B.** *Justicia carthagenensis*. **C. Amaranthaceae:** *Alternanthera sessilis*. **D. Apocynaceae:** *Mandevilla subcarnosa*. **E. Asteraceae:** *Erigeron canadensis*. **F. Arecaceae:** *Cocos nucifera*. **G-H. Burseraceae:** *Bursera simaruba*. **I. Combretaceae:** *Conocarpus erectus*. **J-L. Fabaceae: J.** *Dalbergia brownie*. **K.** *Mimosa 1*. **L.** *Mimosa pudica*. **M. Lamiaceae:** *Ocimum*. **N. Malpighiaceae:** *Stigmaphyllon*. **O. Malvaceae:** *Ceiba pentandra*. **P. Urticaceae:** *Cecropia peltata*. Scale: 10 µm.

The highest importance values (IE_j) were registered for the following pollen types: for Flowers Hill, *M. pudica* (11.30), *C. peltata* (9.60), *B. crassifolia* (7.73) *R. mangle* (6.47), *C. nucifera* (6.02), and for La Loma, *M. pudica* (13.70), *C. peltata* (10.93), *M. bijugatus* (6.24), *D. brownie* (6.17), *L. alba* (5.47) (Figs. 3 A, B).

Among the identified pollen types, some of them are related to resin-producing taxa [Anacardiaceae 1, *Bursera simaruba* (L.) Sarg. (Fig. 2, G-H), and *Phyllanthus* L.], while others are related to taxa that produce floral oils [Anacardiaceae 1, *Byrsonima crassifolia* (L.) Kunth, and *Stigmaphyllon* A.Juss. (Fig. 2, N); Tab. 1]. These two groups represented 7.35% of the total types. Moreover, 56 pollen types showed an affinity with nectariferous and/or polliniferous taxa, totaling 82.35% of the pollen spectrum. Seven pollen types had no information regarding the availability of any resource that honeybees could use (Tab. 1).

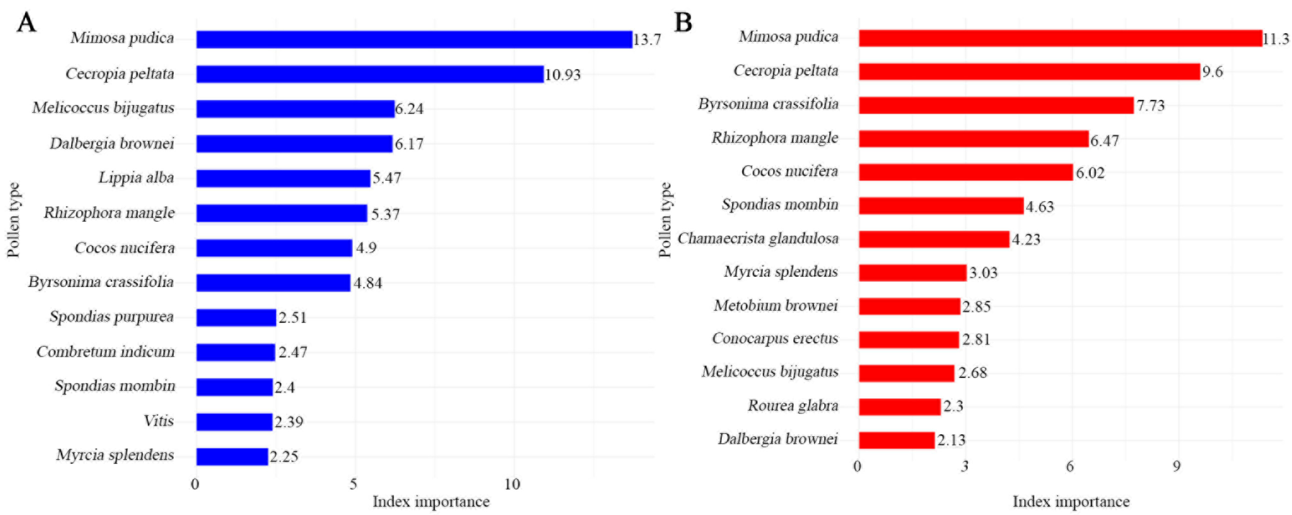

Figure 3. Importance index of pollen types (IE_j) used by *Apis mellifera* in the production of red propolis from San Andrés Island (Colombia) that exhibited a value higher than 2.00. **A.** La Loma, **B.** Flowers Hill.

The most frequent growth habits of plants related to the pollen types were as follows: shrubby (28 pollen types; 41.17%), arboreal (24; 35.29%), herbaceous (18; 26.47%), subshrubby (9; 13.23%), climbing (6; 8.82%), lianas (4; 5.88%), and epiphyte (1; 1.47%). Some pollen types were associated with taxa presenting more than one growth habit. However, among the taxa with only one growth habit, the arboreal habit contributed the most to the pollen spectrum (49.88%), followed by the herbaceous (20.94%) and shrubby (13.71%) habits (Fig. 4, A).

Some pollen types associated with a specific vegetation type were found (Tab. 2). The vegetation from forest formations contributed the most to the pollen spectrum (48.40%), followed by vegetation associated with anthropic areas (26.50%), mangroves (17.21%), and wetland areas (7.89%) (Fig. 4, B). Some taxa were associated with more than one type of vegetation.

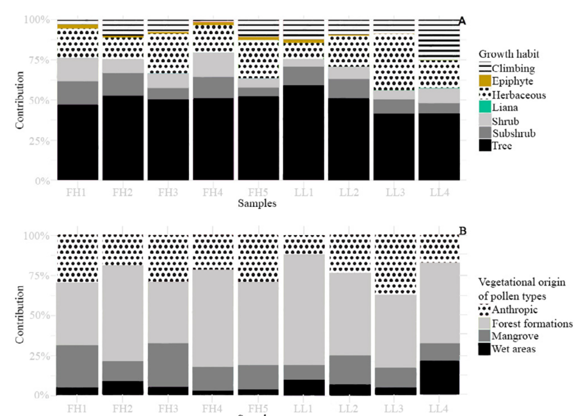

Figure 4. Contribution of growth habit of taxa related to pollen types (A) and vegetational origin of pollen types (B) present in red propolis samples from San Andrés Island, Colombia. **FH:** Flowers Hill, **LL:** La Loma.

Table 2. Pollen-type marker of vegetation in red propolis samples collected by *Apis mellifera* on San Andrés Island, Colombia.

Mangrove	Wet areas	Forest formations			Anthropic areas	
Avicennia germinans	*Justicia carthagenensis*	*Metopium brownei*	*Sabal mauritiiformis*	*Ocimum* 1	*Bidens pilosa*	*Tradescantia*
Conocarpus erectus	*Alternanthera sessilis*	*Spondias mombin*	*Tillandsia dasyliriifolia*	*Ocimum* 2	*Cyanthillium cinereum*	*Cycas*
Laguncularia racemosa	*Randia*	*S. purpurea*	*Rourea glabra*	*Gossypium*	*Eclipta alba*	*Cnidoscolus aconitifolius*
Rhizophora mangle	*Dicliptera sexangularis*	*Ceiba pentandra*	*Cecropia peltata*		*Emilia sonchifolia*	
	Dalbergia brownei	*Trema micrantha*	*Lippia alba*		*Erigeron canadensis*	
	Stigmaphyllon	*Vitis*	*Melicoccus bijugatus*		*Mimosa pudica*	

The pollen composition significantly differed between the apiaries, as evidenced by the multivariate analysis. Their location explained approximately 31% of the variance (r^2=0.31, p<0.01). The pollen similarity dendrogram between the samples revealed that they clustered into three clades (Fig. 5). In clades A and B, the samples from Flowers Hill grouped with one sample from La Loma (LL2), with a similarity of over 65%. Samples LL3 and LL4 grouped in clade C, which showed a similarity of approximately 55% with clades A and B, and sample LL1 did not group with any. Samples FH2 and FH5 had the highest similarity, sharing nine pollen types.

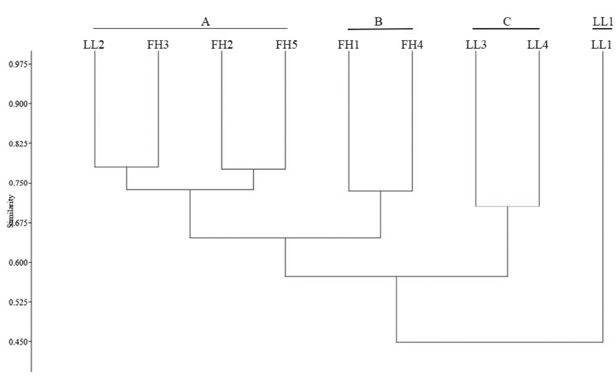

Figure 5. Similarity (Jaccard coefficient) between red propolis samples from San Andres Island, Colombia. Formation of three clades (A-C) according to pollen similarity. **FH:** Flowers Hill, **LL:** La Loma.

The NMDS (Fig. 6) also showed the difference in the distribution of pollen types, indicating the formation of two polygons corresponding to the different apiaries (FH and LL) based on the presence of pollen types. The La Loma apiary (LL) had more exclusive pollen types (*e.g. B. pilosa, Cestrum, D. sexangularis, Gossypium, M. subcarnosa, Ocimum* 2, *Stigmaphyllon, S. gaertneri, S. purpurea, T. micrantha, Verbesina*). In contrast, the Flowers Hill apiary (FH) had only a few exclusive pollen types: *C. aconitifolius, Ipomoea* 1, *J. carthagenensis*, and *Ocimum* 1.

Discussion

Pollen analysis of red propolis samples from San Andrés Island revealed 51 pollen types and 15 families that had not been previously described by Salamanca-Grosso & Osorio-Tangarife (2019), who conducted the first study on propolis collected on the island. This is mainly due to the location of the apiaries, as the vegetation can vary between them. In this study, the frequency and presence of certain pollen types (*Mimosa pudica*, *Cecropia peltata*, *Byrsonima crassifolia*, *Rhizophora mangle*, and *Cocos nucifera*) within the area are highly consistent. Therefore, it is possible to infer that these species contribute significantly to the island's apicultural production.

The Leguminosae family has the highest number of species reported on the island, as evidenced by being the family with the most pollen types (Tobar-Vargas & Kondo, 2015). This family has also been reported as significantly crucial in the diet of bees by Avelino & Santos (2018) and Matos *et al*. (2014) and Matos and Santos (2017) who analyzed different types of propolis from Northeastern Brazil, and from the state of Bahia, Brazil, respectively, and Salamanca-Grosso & Osorio-Tangarife (2019).

Among the pollen types of the Leguminosae family, the *Dalbergia brownei* pollen type stands out, as it may be related to the red coloration of the propolis, similar to the *D. ecastaphyllum* type from which Brazilian red propolis is produced (Rufatto *et al*., 2017). The genus *Dalbergia* L.f. (Leguminosae: Papilionoideae) comprises nearly 300 species with significant pharmacological potential and represents an important source to produce red propolis (Lotti *et al*., 2010; Saha *et al*., 2013).

It is worth noting that after Hurricane Iota in 2020, the frequency of *D. brownei* (Jacq.) Schinz plants increased significantly. Although initially reported as exclusive to swampy areas (Tobar-Vargas & Kondo, 2015), it has now occupied large mangrove areas. It is even considered an invasive species according to Corporación Ambiental CORALINA (unpublished data). According to Salamanca-Grosso & Osorio-Tangarife (2019), its pollen type was recorded in low frequencies.

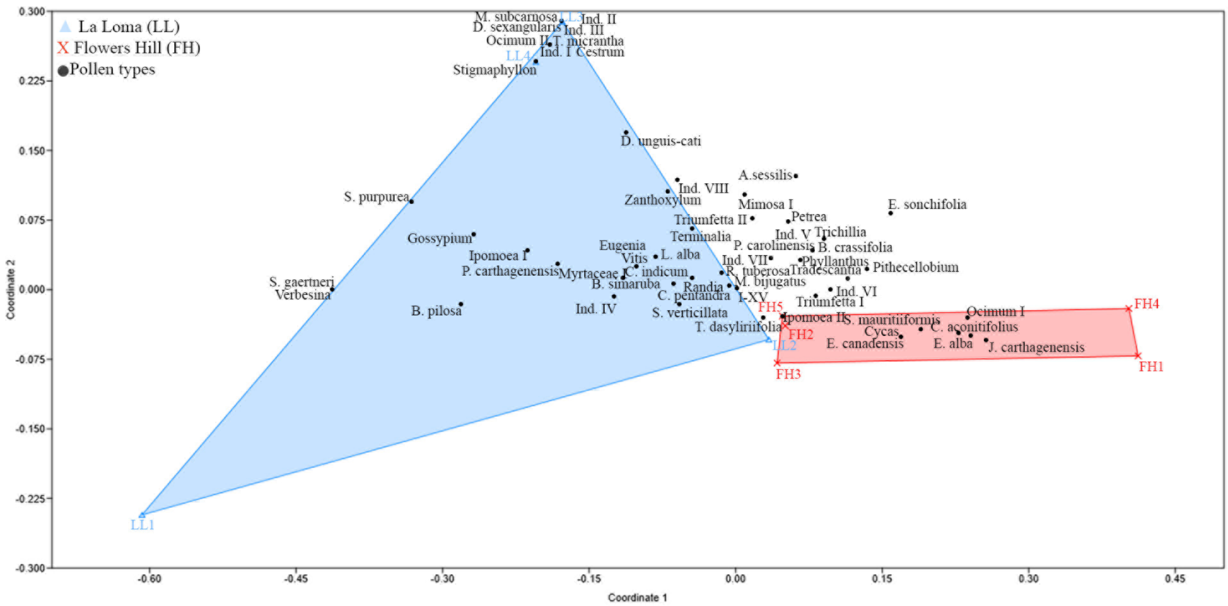

Figure 6. Non-Metric Multidimensional Scale (NMDS) showing the composition of pollen types found in red propolis samples from San Andres Island, Colombia. I-XV: pollen types present in all samples. **FH:** Flowers Hill, **LL:** La Loma.

Furthermore, Asteraceae is another important family for honeybees, as they provide abundant nectar and pollen per floral unit and are easily accessible and manipulable by honeybees. Therefore, it is common to find a representative number of species from the Asteraceae family in propolis samples (Silva *et al*., 2013; Casas-Restrepo *et al*., 2021).

The main pollen types in the spectrum were *Mimosa pudica* (Leguminosae) and *Cecropia peltata* (Urticaceae), which were present in all samples with the highest importance index for both apiaries. *Mimosa pudica* L. is valued for its high pollen production and widespread distribution (Matos *et al*., 2014), while *C. peltata* L., an anemophilous species, is common in secondary forests, aligning with the study area (Lowy, 2000; Tobar-Vargas & Kondo, 2015). These results are consistent with previous studies (Barth & Luz, 2009; Freitas *et al*., 2010), but differ from the findings by Salamanca-Grosso & Osorio-Tangarife (2019). An unusual pollen type, *Mimosa* 1, was also detected, which is uncommon as *M. pudica* L. is the unique species of the genus *Mimosa* L. recorded in the study area (Tobar-Vargas & Kondo, 2015).

Rhizophora mangle L., the dominant mangrove species on the island (Murcia *et al*., 2012), is widespread near the apiaries and its pollen type showed the highest importance values among mangrove species in the samples (6.47 in Flowers Hill and 5.37 in La Loma). *Rhizophora mangle* L. produces abundant pollen and secretes a sugary liquid that attracts honeybees (Sucerquia *et al*., 2019). Salamanca-Grosso & Osorio-Tangarife (2019) identified it as the source of red propolis on San Andrés Island, consistent with previous reports linking it to red propolis production in Cuba (Ledon *et al*., 2002) and Northeast Brazil (Barth & Luz, 2009).

Furthermore, *Cocos nucifera* L. is a species that has a wide distribution on the island, as there are extensive areas dedicated to its cultivation, and it is also distributed as part of the beach vegetation and forested areas. Its pollen type was observed in all red propolis samples and has been reported by other authors. Notably, Avelino & Santos (2018) identified this pollen type in samples of red, brown, and green propolis from Northeastern Brazil. It is a very common pollen type in beekeeping products, especially in coastal areas, as highlighted by Alves & Santos (2014).

Five pollen types associated with taxa that produce resin and floral oils were identified (Anacardiaceae 1, *Bursera simaruba*, *Byrsonima crassifolia*, *Phyllanthus*, and *Stigmaphyllon*). Regarding the Anacardiaceae family, species such as *Anacardium occidentale* L. are found on the island and represent a source of resin and floral oils, as reported by Barth & Luz (2009). Moreover, *B. simaruba* (L.) Sarg exhibits an aromatic resin mainly found under the bark, which is used by eusocial bees in propolis production (Missouri Botanical Garden [unpublished data]). Furthermore, *B. crassifolia* (L.) Kunth and *Stigmaphyllon* A.Juss., belonging to the Malpighiaceae family, are highlighted by Vogel (1990) as important sources of floral oils, emphasizing their relevance as food sources for honeybees.

Additionally, species considered as nectariferous and polliniferous were found. *Spermacoce* L. (Rubiaceae) is an example of a plant group classified as nectariferous, and its percentage of pollen types is generally low in analyses (Matos *et al*., 2014). In this case, *S. verticillata* pollen type was frequent in the analyzed samples, but its abundance was low. In the studies carried out by Freitas *et al.* (2010) and Avelino & Santos (2018), its pollen contribution was also low. Therefore, it was considered as a species related to hive-feeding activities. On the other hand, the previously mentioned genera *Mimosa* L. and *Cecropia* L. are polliniferous taxa (Matos & Santos, 2017).

Regarding the growth habit, shrubs and trees represented a large portion of the pollen types, suggesting a dominance of these growth forms around the apiaries. Pereira *et al.* (2004) noted that the arboreal stratum is the primary resource supplier for most bee species (providing nectar, pollen, oil, and resin), and along with the shrub layer, contributes significantly to propolis production (Matos & Santos, 2017). However, both arboreal and herbaceous strata were abundant in pollen contributions, as expected, given the presence of species known for high pollen production, such as *C. nucifera* L., *C. peltata* L., *R. mangle* L., and *M. pudica* L.

Some authors have reported the generalist habit of *Apis mellifera* in searching for food (Barth & Luz, 2009; Matos *et al*., 2014; Matos & Santos, 2017; Avelino & Santos, 2018), which is evident in this study considering the diversity of pollen types registered. However, there is a certain dominance of one vegetation type since pollen types of forest species such as *C. peltata, M. brownie, M. bijugatus, P. cururu*, *R. glabra,* and *S. mombin* were the most frequent, possibly due to their proximity to the apiaries, thus being the immediate food source.

However, species related to anthropic activities were also very present in the pollen spectrum of the analyzed red propolis samples, which evidences anthropization on the island. Some taxa associated with pollen types that act as indicators of crops were found, such as *Gossypium* L. (cotton), as well as some ornamental species such as *Tradescantia* L. (canoa), and *Cycas* L. (Palma funeral). There were also pollen types indicative of wet areas found in most samples (*e.g. A. sessilis, D. brownei, Randia*, and *Stigmaphyllon*), indicating the presence of some small wetlands in the area. The identification of pollen markers is important because it facilitates a diagnosis of the interactions that honeybees have with the local vegetation and provides insights into the state of the local flora. In this case, it is observed how the vegetation is mainly affected by anthropization.

The significant difference between the apiaries may be linked to the pollen types unique to each apiary. In the La Loma (LL) apiary, pollen types were associated with taxa from more anthropized areas, including cultivated species (*Ocimum* L. 2), ruderal species (*e.g.*, *Bidens pilosa* L., *Cestrum* L., *Scleria gaertneri* Raddi, *Verbesina* L.), and ornamental plants (*Mandevilla subcarnosa* (Benth.) Benth. & Hook.f.). This is due to the presence of grasslands and cultivated areas in the island's southeast, explaining the abundance of herbaceous plant pollen. In contrast, the Flowers Hill (FH) apiary had fewer pollen types linked to anthropized areas, as it is more influenced by less disturbed natural mosaics. Common pollen types across all samples are related to taxa widely distributed on the island *e.g. Aeschynomene americana* L., *Cyanthillium cinereum* (L.) H.Rob., *C. nucifera* L., *C. peltata* L., *D. brownie* (Jacq.) Schinz*, Laguncularia racemosa* C.F.Gaertn.*, M. pudica* L.*, R. mangle* L.).

The identification of plants that provide resources for beekeeping is important for this industry since this information indicates the vegetal sources used for collecting nectar, pollen, resins, and floral oils, aiming to maximize the use of these trophic resources, both in the establishment of the apiaries, as well as in the preservation of the natural vegetation. The Leguminosae and Asteraceae families were most important for honeybees since they help in their maintenance (nectar and pollen availability). Species such as *B. crassifolia* (L.) Kunth*, B. simaruba* (L.) Sarg*, Phyllanthus* L., and *Stigmaphyllon* A.Juss. are important resources for *Apis*, since they provide resins and/or oils. *Dalbergia brownie* (Jacq.) Schinz and *R. mangle* L. could be important sources responsible for the coloration of the red propolis of San Andres Island.

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Authors' Contributions

Vanegas-Florez OJ.: Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing-original draft, Writing-review and editing; **Villa PM.:** Formal analysis, Visualization, Writing-original draft, Writing-review, and editing; **Salamanca-Grosso G:** Resources, Visualization, Writing-original draft, Writing-review and editing; **Santos FAR.:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Visualization, Writing-original draft, Writing-review and editing.

Declarations of Interest

The authors declare no conflicts of interest.

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