Pollen Profile of Geopropolis Samples Collected by Native Bees (Meliponinae) in South American Countries

by

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ABSTRACT

A total of 16 geopropolis samples from Brazil, Bolivia and Venezuela were analysed in the present study. Samples were submitted to the standard procedure used to obtain pollen grains and additional structured elements. At least 300 pollen grains per sample were counted; five samples did not reach 300 grains. The pollen types of Brazilian samples displayed Melastomataceae as the predominant pollen; Arecaceae, *Cecropia* and one as yet unidentified pollen type were established as accessory pollen. The Bolivian samples presented *Cecropia* and *Solanum* as the dominant pollen and Eucalyptus, Inga and Rubiaceae as accessory pollen. Venezuelan samples presented *Melochia* as the dominant pollen type and *Cassia*, *Crotalaria*, *Didymopanax*, Fabaceae, Melastomataceae, *Myrcia*, Rubiaceae, *Tabebuia* and one as yet unidentified pollen type as accessory pollen. The study showed a high diversity of vegetation surrounding the collection sites, indicative of the resources available for geopropolis production, and a rare superposition of plant *taxa* even by the same bee species.

Keywords: geopropolis, Meliponinae, palynology, vegetation, Brazil, Bolivia, Venezuela

INTRODUCTION

Meliponini are social bees that are considered perennial in the floristic structure of communities. They are considered generalist pollinators, which are bees that visit and in many cases pollinate plant species. Biesmeijer *et al.*

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(2005) noted that the generalist species of the Apidae Family have easy access to the pollen and nectar of different flowers and that their productivity depends on the skill with which they handle the floral parts. These bees are pre-adapted to foraging and cross-pollinating trees that are flowering en masse. Although usually docile, stingless bees may respond aggressively to *Apis mellifera* L. when in a race to obtain food. This competition greatly reduces the trophic resources available (Roubik, 1978; Schaffer *et al.* 1983).

Apis mellifera bees use propolis to prevent mechanical damages to their hives and to seal them. Propolis acts as a thermoregulatory agent for the hive, preventing its potential exposure to air currents and colony infestation (Manrique & Soares 2002, Teixeira *et al.* 2003). The biological and pharmacological activities of propolis have been widely studied (Marcucci 1995, Kujumgiev *et al.* 1999, Banskota *et al.* 2001). Propolis that has been elaborated by stingless bees is named geopropolis because the bees mix soil with wax (Nogueira-Neto, 1953). In comparison to propolis, geopropolis has been poorly characterised. Pharmacological studies, however, have shown that it harbours antibacterial and antioxidant activities and contains a high concentration of flavonoids (Bankova &Popova 2007, Dutra *et al.* 2008, Manrique & Santana 2008).

Geopropolis, or bee glue, is characterised as a mixture of resin exudates, from several plant sources that have been mixed, with waxes, silt and sandy fragments (Nogueira-Neto 1997). Pollen grains appear as contaminants (Barth & Luz 2003). The resulting pollen spectrum obtained from the insoluble residue of geopropolis is composed of nectariferous, polliniferous and anemophilous pollen grains. Thus, pollen analysis is a valuable tool in determining the phytogeographical origin of geopropolis, which enables the efficient detection of the different productive regions (Ricciardelli D'Albore 1979, Barth *et al.* 1999).

The propolis *Apis* may vary in its consistency, ranging from firm, fibrous or elastic in some cases to soft and sticky with small wood particles in others. Propolis may vary in colour from light yellow, red and greenish-brown to black, depending upon the origin of the vegetation and other environmental factors of the producing locality (Marcucci *et al.* 2001, Salatino *et al.* 2005). Geopropolis may have the same aforementioned characteristics described above but additionally contain clay particles. However, geopropolis never contains plant trichomes (Barth & Luz, 2003).

Pollen analyses of propolis samples were carried out primarily by Ricciardelli D'Albore (1979), who analyzed 56 samples from several countries, and Warakomska & Maciejewicz (1992), who analysed propolis from Polish regions. Some studies, in particular the analyses carried out in Brazil by Barth (1998), Barth *et al.* (1999), Bastos (2001), Barth & Luz (2009), Luz & Barth (2009), Freitas *et al.* (2010) and Freitas *et al.* (2011), focus on the palynology of propolis samples from South America

Studies concerning the palynological analysis of geopropolis samples are scarce, though notable examples include Barth & Luz (2003), Barth (2006) and Barth *et al.* (2009). The first paper focused primarily on the 10 geopropolis samples obtained from the Brazilian states of Espirito Santo, Minas Gerais and São Paulo, where the dominant pollen was from either *Eucalyptus* (*Melipona quadrifasciata*, São Paulo, Myrtaceae) or *Schinus* (*Tetragonisca angustula*, Minas Gerais, Anacardiaceae); the accessory pollen were the *Myrcia* and Melastomataceae/*Combretum* types.

Barth (2006) analysed six geopropolis samples obtained by different bee species inside Ribeirão Preto, São Paulo. The dominant pollen detected in the geopropolis of each species was exclusively *Trigona recursa*. However, the accessory pollens differed: Lestrimellita limao geopropolis showed accessory Eucalyptus pollen; *Melipona quadrifasciata* displayed the *Mimosa scabrella* accessory pollen; and *Trigona angustula* exhibited the *Cecropia* accessory pollen. Pollen analysis reflected the environmental conditions around the apiary and the plants of trophic preference for the bees.

Barth *et al.* (2009) studied four archaeological geopropolis samples. These samples were obtained from the Januária, Minas Gerais region. Evidently, no bee species were known. The pollen spectra reflected the types of vegetation proximal to the location where the natives collected these geopropolis samples. The dominant pollen belonged to the Arecaceae (palms) and Anacardiaceae (*Anacardium*, *Spondias*) families. Gallery forests, open-humid land and open-dry vegetation were identified in the pollen analysis.

The present study aims to analyse and characterise South American geopropolis samples to determine the phytogeographical regions from which the samples were obtained. This study also aims to provide a physico-chemical analysis of the geopropolis for both quality control and for the beekeepers inventory.

MATERIALS AND METHODS

Sixteen geopropolis samples produced by seven different bee species were analysed. Four samples were obtained in the state of Paraná (Brazil) and in the state of Santa Cruz (Bolivia). Eight samples were obtained from the Amazonas and Falcón states (Venezuela) (Table 1). Of the nine identified and one unidentified bee species, each one occurred in its specific country.

The palynological processing of the samples followed the standard methodology (Barth, 1998), whereby pollen were extracted from 0.5 g of scraped propolis overnight with ethanol. Next, the sediment was treated with KOH,

Table1. Bee species and origin of geopropolis samples from Brazil, Bolivia and Venezuelan municipality

Bee species	Common name	Country	Origin/ Municipality
Tetragonisca angustula (Ta1)	Jataí	Brazil	Antonina, Paraná state
Tetragonisca angustula (Ta2)	Jataí	Brazil	Tragaçaba, Guaraqueçaba, Paraná state
Melipona quadrifasciata (Mq)	Mandaçaia	Brazil	Tragaçaba, Guaraqueçaba, Paraná state
Melipona mondury (Mm)	Uruçu-amarela	Brazil	Potinga, Guaraqueçaba, Paraná state
Scaptotrigona depilis (Sd1)	Obobosí	Bolivia	Parque Nacional Amboró, Carmen Surutú, Santa Cruz state
Scaptotrigona depilis (Sd2)	Obobosí	Bolivia	Parque Nacional Amboró, Carmen Surutú, Santa Cruz state
Scaptotrigona polysticta (Sp)	Suro negro	Bolivia	Parque Nacional Amboró, Carmen Surutú, Santa Cruz state
Melipona grandis(Mg)	Erereú barcina	Bolivia	Parque Nacional Amboró, Carmen Surutú, Santa Cruz state
Tetragona clavipes (Tc1)	Ajavitte	Venezuela	Caño Tabika, Paria Grande, Amazonas state
Tetragona clavipes (Tc2)	Ajavitte	Venezuela	Caño Tabika, Paria Grande, Amazonas state
Tetragona clavipes (Tc3)	Ajavitte	Venezuela	Caño Tabika, Paria Grande, Amazonas state
Scaptotrigona sp.(S)	Sonquette	Venezuela	Pijiguao, Paria Grande, Amazonas state
Lestrimelitta limao (Ll)	Limoncita	Venezuela	Pijiguao, Paria Grande, Amazonas state
Melipona favosa (Mf1)	Erica	Venezuela	Península de Paraguaná, Falcón state
Melipona favosa (Mf2)	Erica	Venezuela	Península de Paraguaná, Falcón state
Melipona favosa(Mf3)	Erica	Venezuela	Península de Paraguaná, Falcón state

subjected to ultrasound and sieved to eliminate the large fragments. Samples from this stage of the procedure were mounted on two microscope slides to observe the organic residues that may be destroyed in this sequence of events.

The acetolysis method (Erdtman, 1952) was then applied, and samples were mounted on two additional microscope slides using glycerin jelly, one stained with basic fuchsin and the other non-stained. The target sum was 300 pollen grains or more per sample. The definition of pollen classes presented by Zander (Louveaux *et al.* 1978) was used for qualitative and quantitative analyses. Samples were observed using light and polarised light microscopy. Pollen type identification followed Barth (1989), Roubik & Moreno (1991) and Vit (2005).

RESULTS

Geopropolis samples obtained in several counties of Brazil, Bolivia and Venezuela harbour a great variety of pollen grains. The pollen analysis is presented in Table 2, Figure 1. It was possible to identify 35 pollen types with frequencies higher than 3% of the total pollen count (pollen sum). Most of the samples showed a high quantity of pollen grains. All propolis samples obtained by *Melipona* bees, except the sample of *Melipona quadrifasciata* (Mq, Brazil), showed a low frequency of pollen grains and did not achieved the target limit of 300 pollen grains counted.

Sand, clay particles and plant tissue fragments were the most commonly observed structured elements in the propolis sediments after chemical treatment (Table 3). Hyphae and fungal spores were less common. Amorphous organic material and the remainders of resin could be observed in only some of the samples. The *Melipona mondury* sample (Mm, Brazil) presented pollen grains that were damaged by oxidation (air exposition).

Brazil/Paraná state – The geopropolis samples of both species of *Melipona* contained the dominant pollen from Melastomataceae. No accessory pollen from either Matayba or Myrcia pollen was isolated. The *Tetragonisca* samples displayed the *Cecropia* accessory pollen. The Ta2 sample also contained the Arecaceae pollen and both isolated pollen from *Sapium*.

Bolivia/National Park Amboró – The *Scaptotrigona depilis* (Sd1) sample presented *Cecropia* pollen as the dominant pollen and *Eucalyptus* pollen as

Table2a. Pollen types frequencies of gepropolis samples. DP = dominant pollen (> 45%), AP = accessory pollen (15% to 45%), IP = isolate pollen (3% to15%); Mf= *Melipona favosa*, Mg= *Melipona grandis*, Mq= *Melipona quadrifasciata*, Mm= *Melipona mondury*, Sd = *Scaptotrigona depilis*, Sp= *Scaptotrigona polystica*, Ta= *Tetragonisca angustula*.

Pollen types	Paraná/Brazil				Santa Cruz/Bolívia			
	Antonina	Tagaçaba/ Guaraqueçaba		Potinga/ Guaraqueçaba	National Park Amboró			
	Ta1	Ta2	Mq	Mm	Sd1	Sd2	Sp	Mg
Alchornea (Euphorbiaceae)	IP (13.2%)	IP (7.7%)	IP (3.4%)	IP (9.6%)				
Anadenanthera (Mimosaceae)								
Arrabidaea (Bignoniaceae)								
Arecaceae		**AP** (25.8%)						IP (4.0%)
Asteraceae				IP (4.8%)		IP (7.1%)		
Bignoniaceae							IP (5.1%)	
Borreria (Rubiaceae)								
Cassia (Caesalpiniaceae)								
Cecropia (Cecropiaceae)	**AP** (26.0%)	**AP** (32.0%)			**DP** (63.7%)			**AP** (28.6%)
Celtis (Ulmaceae)								
Cestrum (Solanaceae)						IP (5.2%)		
Combretaceae						IP (8.1%)		
Crotalaria (Fabaceae)								
Cupania (Sapindaceae)							IP (8.0%)	
Didymopanax (Araliaceae)								
Eucalyptus (Myrtaceae)					**AP** (18.7%)	IP (7.1%)		
Euphorbiaceae					PI (3.6%)	IP (5.2%)		

the accessory pollen; the *Melipona grandis* sample presented the pollen of Solanaceae as the dominant pollen and the pollen of *Cecropia* as the accessory pollen. The *S. depilis* (Sd2) and *S. polysticta* samples did not show any pollen dominance but did display an accessory pollen from Rubiaceae in the first and last samples of *Inga*.

Venezuela/Amazonas state – *Melochia* provided the unique, dominant pollen in the *Tetragona clavipes* (Tc1) sample. The *Didymopanax* and Fabaceae-Faboideae accessory pollen type was abundant in the Tc2 sample;

Table2a (cont). Pollen types frequencies of gepropolis samples from Brazil and Bolivia. DP = dominant pollen (> 45%), AP = accessory pollen (15% to 45%), IP = isolate pollen (3% to15%); Mf= *Melipona favosa*, Mg= *Melipona grandis*, Mq= *Melipona quadrifasciata*, Mm= *Melipona mondury*, Sd = *Scaptotrigona depilis*, Sp= *Scaptotrigona polystica*, Ta= *Tetragonisca angustula*.

Pollen types	Paraná/Brazil				Santa Cruz/Bolívia			
	Antonina	Tagaçaba/ Guaraqueçaba		Potinga/ Guaraqueçaba	National Park Amboró			
	Ta1	Ta2	Mq	Mm	Sd1	Sd2	Sp	Mg
Fabaceae-Faboidea							IP (7.3%)	
Inga (Mimosaceae)							**AP** (35.3%)	
Loranthaceae	IP (3.8%)							
Matayba (Sapindaceae)			IP (6.8%)	IP (13.2%)				
Melastomataceae		IP (3.5%)	**DP** (75.5%)	**DP** (50.6%)	IP (4.5%)	IP (9.1%)		
Melochia (Sterculiaceae)								
Mimosa verrucosa (Mimosaceae)								
Mimosa scabrella (Mimosaceae)			IP (3.7%)		IP (3.6%)			
Moraceae								
Myrcia (Myrtaceae)			IP (4.0%)	IP (6.0%)				
Piper (Piperaceae)						IP (3.6%)		
Poaceae								IP (4.0%)
Rubiaceae						**AP** (32.4%)		
Schinus (Anacardiaceae)							IP (4.8%)	
Sapium (Euphorbiaceae)	IP (11.9%)	IP (3.9%)						
Serjania (Sapindaceae)							IP (8.0%)	
Solanaceae								**DP** (50.8%)
Tabebuia (Bignoniaceae)								
∑unidentified	**AP** (17.0%)							
Number of pollen grains counted	311	310	322	83	336	308	314	126

Melastomataceae stood out in the Tc3 sample; Melastomataceae, *Melochia* and Rubiaceae stood out in the *Scaptorigona* sp. sample; and Crotalaria stood out in the *Lestrimelitta limao* sample. The three *Tetragona* geopropolis samples

Table2b. Pollen types frequencies of gepropolis samples from Venezuela. DP = dominant pollen (> 45%), AP = accessory pollen (15% to 45%), IP = isolate pollen (3% to15%); Ll= *Lestrimelitta limao*, Mf= *Melipona favosa*, Mm= *Melipona mondury*, S= *Scaptotrigona sp.*, Sp= *Scaptotrigona polystica*, Tc= *Tetragona clavipes*.

Pollen types	Amazonas state/Venezuela							Falcón state/Venezuela
	Caño Tabika/ Paria Grande			Pijiguao/ Paria Grande				Península Paranaguá
	Tc1	Tc2	Tc3	S	Ll	Mf1	Mf2	Mf3
Alchornea (Euphorbiaceae)		IP (4.2%)			IP (3.8%)			IP (9.0%)
Anadenanthera (Mimosaceae)				PI (7.2%)				
Arrabidaea (Bignoniaceae)						IP (4.4%)		
Arecaceae	IP (3.5%)	IP (4.6%)						IP (4.5%)
Asteraceae								
Bignoniaceae			IP (5.3%)			IP (6.1%)		
Borreria (Rubiaceae)			IP (4.3%)					
Cassia (Caesalpiniaceae)						**AP** (26,7 %)	**AP** (39.0%)	
Cecropia (Cecropiaceae)								
Celtis (Ulmaceae)					IP (4.8%)			
Cestrum (Solanaceae)								
Combretaceae				IP (10.1%)				
Crotalaria (Fabaceae)					**AP** (42.3%)			
Cupania (Sapindaceae)								
Didymopanax (Araliaceae)		**AP** (31.3%)		IP (3.9%)				
Eucalyptus (Myrtaceae)			IP (6,3%)	IP (4.9%)	IP (7.7%)			IP (6.8%)
Euphorbiaceae						IP (6.6%)		
Fabaceae-Faboidea		**AP** (15.3%)						IP (9.0%)
Inga (Mimosaceae)								
Loranthaceae								
Matayba (Sapindaceae)								
Melastomataceae			**AP** (17.5%)	**AP** (15.3%)		IP (3.1%)		

each presented isolated pollen from *Myrcia* and Poaceae.

Venezuela/ Falcón state – No dominant pollen was detected in the three geopropolis samples of *Melipona favosa*. The accessory pollen from *Cassia* was detected in two samples (Mf1 and Mf2). The *Tabebuia* pollen type was

Table2b. (cont). Pollen types frequencies of gepropolis samples from Venezuela. DP = dominant pollen (> 45%), AP = accessory pollen (15% to 45%), IP = isolate pollen (3% to15%); Ll= *Lestrimelitta limao*, Mf= *Melipona favosa*, Mm= *Melipona mondury*, S= *Scaptotrigona sp.*, Sp= *Scaptotrigona polystica*, Tc= *Tetragona clavipes*.

Pollen types	Amazonas state/Venezuela					Falcón state/Venezuela		
	Caño Tabika/ Paria Grande			Pijiguao/ Paria Grande		Península Paranaguá		
	Tc1	Tc2	Tc3	S	Ll	Mf1	Mf2	Mf3
Melochia (Sterculiaceae)	**DP** (49.2%)		IP (10.9%)	**AP** (18.9%)		IP (8.8%)		
Mimosa verrucosa (Mimosaceae)								IP (6.8%)
Mimosa scabrella (Mimosaceae)								
Moraceae			IP (12.9%)		IP (11.5%)	IP (4.8%)		
Myrcia (Myrtaceae)	IP (6.1%)	IP (9.4%)	IP (3.6%)					**AP** (15.9%)
Piper (Piperaceae)								
Poaceae	IP (11.8%)	IP (14.3%)	IP (8.6%)		IP (5.1%)	IP (12.7%)	IP (9.3%)	IP (11.4%)
Rubiaceae			IP (6.6%)	**AP** (17.6%)				
Schinus (Anacardiaceae)								
Sapium (Euphorbiaceae)								
Serjania (Sapindaceae)								
Solanaceae								
Tabebuia (Bignoniaceae)							**AP** (24.6%)	
∑unidentified						IP (4.8%)	IP (11.0%)	**AP** (25.0%)
Number of pollen grains counted	313	307	302	307	312	228	118	44

detected in the Mf2 sample, and the *Myrcia* pollen type was detected in the Mf3 sample. Poaceae pollen grains were found in all samples.

DISCUSSION

The geopropolis samples analysed in the present study were from Brazil, Bolivia and Venezuela and were characterised by pollen assemblages, which provided evidence for the presence of different plants and vegetation.

Brazil/Paraná – The samples from Paraná state were produced by three different bee species. Both the *T. angustula* (Ta1, Ta2) samples contained *Cecropia* and *Sapium* pollen, even though they were obtained from different locations (Antonina and Traçaba). Each of the two samples from *Melipona* (*M. quadrifasciata* and *M. mondury*) contained the *Matayba*, Melastomataceae

Table 3. Origin of the geopropolis samples analysed and evaluation of additional structured elements except pollen grains. (-) = without, (+) =few, (++) = medium (+++) = frequent structured elements. Ll= *Lestrimelitta limao*, Mf= *Melipona favosa*, Mg= *Melipona grandis*, Mq= *Melipona quadrifasciata*, Mm= *Melipona mondury*, S= *Scaptotrigona* sp., Sd = *Scaptotrigona depilis*, Sp= *Scaptotrigona polystica*, Ta= *Tetragonisca angustula*, Tc= *Tetragona clavipes*.

Origin/ Municipality/ Bee species	**Hyphae and fungal spores**	**Sandy/clay fragments**	**Plant tissue fragments**	**Amorph organic material**	**Resin**
Antonina, Paraná state (Ta1)	+++	+	++	+	+
Tragaçaba, Guaraqueçaba, Paraná state (Ta2)	+	+	++++	++++	+
Tragaçaba, Guaraqueçaba, Paraná state (Mq)	+	+	++++	++++	+
Potinga, Guaraqueçaba, Paraná state (Mm)	+	+++	+	+++	-
Parque Nacional Amboró, Carmen Surutú, Santa Cruz state (Sd1)	+	+++	+	-	-
Parque Nacional Amboró, Carmen Surutú, Santa Cruz state (Sd2)	+++	+	++	-	-
Parque Nacional Amboró, Carmen Surutú, Santa Cruz state (Sp)	++	++	-	-	+
Parque Nacional Amboró, Carmen Surutú, Santa Cruz state (Mg)	++	++	+++	+++	-
Caño Tabika, Paria Grande, Amazonas state (Tc1)	+	++	+	-	+
Caño Tabika, Paria Grande, Amazonas state (Tc2)	-	+++	-	-	-
Caño Tabika, Paria Grande, Amazonas state (Tc3)	+	+++	+++	-	-
Pijiguao, Paria Grande, Amazonas state (S)	+	++	+++	-	++
Pijiguao, Paria Grande, Amazonas state (Ll)	+	+++	++	-	+
Península de Paraguaná, Falcón state (Mf1)	+	+++	+	-	+
Península de Paraguaná, Falcón state (Mf2)	-	+++	-	+	-
Península de Paraguaná, Falcón state (Mf3)	-	+++	-	+	+

and *Myrcia* pollen types. All geopropolis samples from Paraná state contained isolated pollen grains from *Alchornea*.

Cecropia is widely distributed from the Bahia state in the northeast region to the Paraná state in southern Brazil. This anemophilous pollen was also found in geopropolis samples from the São Paulo state (Barth & Luz 2003, Barth 2006). *Matayba* genus is characteristic of the mountain rainforest of the Paraná state (Reginato & Goldenberg, 2007), being opportunist in cleared and abandoned areas in the forest and may cause competition for shadding (Lorenzi 2008, Cheung *et al.* 2009, Liebsch *et al.* 2007). This pollen type has never been previously detected in Brazilian geopropolis samples.

Plant species of Melastomataceae occur mainly in the vegetation of the Atlantic Forest. Its pollen, collected by *M. quadrifasciata* and *T. angus*tula bees, was detected in geopropolis samples from the São Paulo, Minas Gerais and Espírito Santo states (Barth & Luz, 2003). Barth & Luz (2003) described the *Myrcia* pollen type as an accessory pollen in *M. quadrifasciata* samples and as an isolated pollen in *T. angustula* samples from the São Paulo, Espírito Santo and Minas Gerais states. Barth (2006) described this pollen type as an isolated pollen in geopropolis samples from *M. quadrifasciata*, which were collected in Ribeirão Preto, São Paulo.

Alchornea pollen was found in all the analysed samples as an isolated pollen type. This pollen is not frequently found in propolis and geopropolis samples of Brazil. Barth & Luz (2003) detected this pollen in one sample from the Bahia state. Nevertheless, these plants are characteristic of Paraná state vegetation (Borgo *et al.* 2011, Cheung *et al.* 2009, Liebsch *et al.* 2007). Imperatriz-Fonseca *et al.* (1993) described the constant visit of stingless bees to *Alchornea sidaefolia* flowers in São Paulo.

Arecaceae pollen was characterised as an accessory pollen in a *T. angustula* (Ta2) sample. Barth & Luz (2003) and Barth (2006) described Arecaceae as an isolated pollen in geopropolis from São Paulo. *Sapium* and Arecaceae are commonly found in the Paraná state (Borgo *et al.* 2011, Cheung *et al.* 2009, Liebsch *et al.* 2007). The majority of the plant species identified by pollen assemblage in the geopropolis samples of the Paraná state were pioneer trees; some lianas were also identified. Pollen grains of herbs were not represented (<3%), indicating the preferential location of the bee nests on the forest borders.

Bolivia/National Park Amboró – Geopropolis samples of three bee species were obtained from the same locality. The *Scaptotrigona depilis* samples showed *Cecropia*, Eucalyptus and Rubiaceae pollen grains in the highest percentages. These pollen grains were also found in the geopropolis samples from Brazil, which were analysed by Barth & Luz (2003) and Barth (2006).

The *Scaptotrigona polystica* sample contained a high percentage of the *Inga* pollen type. Barth & Luz (2003) found this pollen type in one geopropolis sample from the Paraíba region in the Northeast of Brazil. Smith & Killeen (1995) noted that *Inga* was a large genus and that 11 species were found in the tropical forest of Bolivia. Lorenzi (2002) noted that the *Inga cylindrica* species grows in open areas and on riverbanks.

All pollen types detected in the *Melipona grandis* sample (>3%) were never found in *Scaptotrigona* samples, in which Solanaceae pollen was dominant. It is known that flowers of the Solanaceae family have poricidal anthers and that bees extract pollen through the vibrations caused by the flapping of their wings (Bezerra & Machado, 2003). This acts removes large amounts of pollen from the anthers. Absy & Kerr (1977) and Absy *et al.* (1980) noted that the Solanaceae family is one of the most important nectar plants that the *Melipona seminigra merrilae* visit for honey production in the Brazilian Amazonas state. It is also found in geopropolis samples from Brazil (Barth & Luz, 2003).

The pollen spectra of geopropolis samples obtained in the Bolivian localities are bee dependent. Pollen grains of herbs were rare in these geopropolis samples. *Cecropia* was the most frequent pollen, and together with *Eucalyptus*, *Inga* Rubiaceae and Solanaceae pollen, it reflects the vegetation of a semi-open and humid forest that has been devastated by human influence.

Venezuela/Amazon state - The geopropolis samples elaborated by three bee species in the two localities of the Paria Grande region showed different pollen spectra. The pollen spectrum of *Tetragona clavipes* (Tc3) was the richest of all samples analysed and was similar to that of *Scaptotrigona* sp., considering the accessory pollen of Melastomataceae and *Melochia*. Fernandez & Grande (2007) noted that the *Melochia* genus is represented in Venezuela for 16 species present in low to intermediate altitudes along the savanna and grassland areas. Its pollen grains were never previously detected in geopropolis samples. Ramirez & Navarro (2010) reported that *Melochia tomentosa* was largely pollinated by bees.

Crotalaria, Didymopanax, Fabaceae-Faboidea, Melastomataceae and Rubiaceae were characterised as accessory pollen types, which is characteristic of the forest region. Hernández-Rosas (2001) reported that the Amazonas state has high humidity throughout the year and that its vegetation has complex structures with great diversity. The large, dense forests have gone largely undisturbed and have suffered little anthropogenic damage.

Vit & Ricciardelli D'Albore (1994) observed the *Crotalaria* pollen type in eight samples of *Melipona* honey from Venezuela, which may indicate open or disturbed vegetation in a forest area (Flores & Miotto, 2005). Freitas *et al.* (2010) also observed this pollen type in one brownish propolis sample collected next to a coastal area in Southeast Brazil.

Aside from the isolated pollen frequency of *Eucalyptus* and *Myrcia*, the geopropolis sample produced by *Liestrimellita limao* was the most distant from all other remaining samples because of the high percentage of the *Crotalaria* pollen type. This pollen type is characteristic of the open areas or forest edges (Flores & Miotto, 2005) that distance this sample from those obtained in dense forests. The difference in the pollen spectra of this bee species compared to the others in the Amazon state can be explained by the preference of food resources during the geopropolis production.

Venezuela/Falcón state – Two of the three geopropolis samples of *Melipona favosa* from Peninsula Paranaguá area showed *Cassia* as the secondary pollen. Vit & Ricciardelli D'Albore (1994) noted the presence of *Cassia* pollen grains in 34 Venezuelan honey samples; these grains predominated in one sample. Barth & Luz (2003) and Barth (2006) also found the *Cassia* pollen type in the geopropolis samples from Brazil in the samples of red propolis produced by *A. mellifera*, although always at low concentrations (Barth & Luz, 2009). Cassia species may occur in drier areas or drained soils (Borchert, 1994; Marchant *et al.* 2002).

Tabebuia and Myrcia pollen grains were recognised as accessory pollen in different samples. These pollen types were constant in Brazilian propolis samples (Barth & Luz, 2009; Freitas *et al.* 2010; Freitas *et al.* 2011) and in geopropolis samples (Barth & Luz, 2003; Barth 2006).

Pollen grains of Poaceae, which are of anemophilous dispersion, were identified by significant quantity (>3%) in all samples of Venezuela, except that of *Scaptorigona* sp. It reflects open areas adjacent to forests. It is common to identify this pollen in geopropolis samples. Barth (2006) highlights the importance of anemophilous pollen types, such as the *Cecropia* and Poaceae types, indicating that these pollen grains stick to the resin during geopropolis manufacture and provide information about the phytogeographical region of production.

The resin-producing plants visited by Meliponinae are difficult to identify in geopropolis and propolis samples. The flowering period does not necessarily correlate with the resin exudation. In red propolis samples of *Apis*, Barth & Luz (2009) frequently identified the pollen grains of *Schinus*, which is a resiniferous plant species from the Brazilian northeast region. Field observations are indispensable (Barth, 2004).

The pollen analysis of the geopropolis samples showed the wide diversity of vegetation surrounding the collection sites and a rare superposition of plant *taxa*, even by the same bee species. Analyses reflect the local and not the regional environment.

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