



Native and ornamental exotic resources in pollen loads and garbage pellets of four stingless bees (Apidae, Meliponini) in an urban environment with riparian native forest

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Abstract: An entomopalynological study to assess pollen use by wild bees was performed in an urban environment with riparian native forest in the Chaco region of Argentina. A combined method for sampling pollen loads and garbage pellets at nest entrances in four stingless bees with different entrance size and forager traffic was here successfully used. Sampling was carried out in a short period of time (late winter) and located within a distance of 50 m. Pollen analysis using acetolysis showed that most identified pollen types (31) were from native species, 12 from ornamental exotic species, 5 included native and ornamental exotic species and 18 of uncertain status. A much broader pollen spectrum was present in garbage pellets (62 types) than loads (14 types). A few resources from previous blooming (late spring-summer) were identified in garbage pellets, which were mainly composed of current flowerings. From the 66 pollen types found in all samples, many (18) remained unidentified due to the complex nature of the vegetation surrounding the nests and the generalist behaviour of these bees. The versatile foraging behaviour on spontaneous (mostly native) and cultivated (mostly ornamental exotic) pollen resources is an important issue for the conservation and rearing of stingless bee colonies in urban habitats where native flora is preserved, particularly the riparians, where a high richness of blooming plants is available.

Key words: entomopalynology, *Geotrigona argentina*, *Plebeia catamarcensis*, polylectic bee, *Scaptotrigona jujuyensis*, *Tetragonisca angustula fiebrigi*.

INTRODUCTION

Palynological study is a powerful tool when researching plant-bee interactions, as they are often difficult to observe by means of direct flower visiting (Cane and Sipes 2006). This is even more important in the special case of generalist bees such as the Meliponini, which forage on a great diversity of pollen and nectar resources (Imperatriz-Fonseca

et al. 1984, Ramalho et al. 1985, 1990, Roubik 1989, Roubik and Moreno Patiño 2009, Rech and Absy 2011, Vossler et al. 2010, Vossler 2015, 2018, 2019a, b) and frequently at the upper stratum of the forest (Ramalho 2004). Observations and sampling of bees visiting flowers is particularly difficult in urban environments, due to impediments such as restricted access to some areas that are being foraged. Research on bee diet in urban habitats will become increasingly important for native bee management and conservation due to human population growth.

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Therefore, palynological studies of bee provisions will be of utmost importance.

To pollen transport from flowers to nest, a great variety of body structures exists in bees (group Anthophila), being the corbicula the most specialized one (Michener 1999). The corbiculate bees of the tribes Meliponini transport a bulk of pollen, commonly named pollen load or pollen basket, on their hind tibia. Although this pollen load is predominantly composed of pollen grains, it is moistened with fluids such as nectar which allows the transport of highly diverse size grains (Thorp 1979, 2000). Moreover, highly concentrated nectar has been detected in the crop of pollen workers in six *Trigona* (Meliponini) when leaving the nest for pollen foraging, presumably for adhesion of loads to the corbicula and agglutination (Leonhardt et al. 2007). The pollen analysis of corbicular loads from bees incoming to the nest allows the identification of the currently foraged botanical resources predominantly the pollen ones. On the contrary, the analysis of garbage pellets carried by leaving workers shows the resources consumed by brood the previous weeks after larval development, but also those currently consumed by workers (Eltz et al. 2001). Garbage pellets are nest residuals of cerumen of brood cells, pollen grains from feces, cocoons, brood and adults (Roubik 1989, Eltz et al. 2001). These residues are accumulated at certain areas in the nest and later molded into pellet by specific workers, named 'waste dump workers', who carry them in their mandibles outside the nest or transfer them to another worker (Medina et al. 2014). In most stingless bees, pellets are dropped at variable distances from the nest, from 1 to 35 (mean = 18 m) or to 45 m (mean = 32.1 m) in the case of *Melipona compressipes* and *M. scutellaris*, respectively (Kerr and Kerr 1999). Pellets are released far from the nest entrances to avoid nest detection by natural enemies of stingless bees such as phorids and other parasites and predators (Kerr and Kerr 1999).

The aims of this study were to test a method to sample pollen loads and garbage pellets from stingless bees having diverse nest entrance size and forager traffic, to identify their botanical origin in an urban environment with riparian native forest by means of pollen analysis and to compare the information provided by both kinds of samples.

MATERIALS AND METHODS

STUDY AREA: THE RIPARIAN CHACO FOREST

The Chaco region is a large sedimentary plain of about 1,000,000 km², extending to the north and south of the Tropic of Capricorn over northern Argentina, western Paraguay, eastern Bolivia and part of southeastern Brazil (Prado 1993). The original vegetation consisted of a mosaic formed by xerophytic forests, gallery forests, and soil-determined or fire-generated savannas (Adámoli et al. 1990). The present study was carried out in a riparian medium-tall forest at the locality Villa Río Bermejito (25° 37' S, 60° 15' W), province of Chaco, Argentina. This site characterizes by the presence of riparian forest species typical of the Chaco region and ornamental cultivated species due to urbanization. The climate at the study area is strongly seasonal with very hot summers (December to March) and low temperatures and frost during winter (July to September) (Prado, 1993); there is a great yearly variation in rainfall, with a marked dry season in winter-spring and a rainy season from October to April (Papadakis 1973).

SAMPLING OF POLLEN LOADS AND GARBAGE PELLETS

Pollen loads and garbage pellets from four stingless bee genera were synchronously sampled to test the applicability of the method here used: *Scaptotrigona jujuyensis* (Schrottky) (10 pollen loads and 1 pellet), *Tetragonisca angustula fiebrigi* Schwarz (4 and 7), *Geotrigona argentina* Camargo and Moure (4 and

0) and *Plebeia catamarcensis* (Holmberg) (4 and 3). Loads and garbage were separately sampled and pooled for further pollen analysis. They were taken from nest entrances during a very short period (two consecutive days at 8 and 9 September 2018) in late winter when many woody elements of the Chaco forest start their flowering. These wild nests (one per species) were located within a distance of 50 m, *S. jujuyensis* and *T. angustula fiebrigi* were found in a same living tree trunk of *Geoffraea decorticans* in full blooming, *G. argentina* in the ground at 5 m away from the others and *P. catamarcensis* in a brick wall at 50 m away from the others.

To avoid pollen cross-contamination between loads and garbage pellets, as can occur when using entomological net or aspirator, a method quite similar to that proposed by Eltz et al. (2001) was here used.

The trap used consisted of a semi-rigid transparent nylon bag placed in front of the nest entrance and separated from it by a space of about 2-20 cm wide to allow the access of the incoming and outgoing bees to the bag. A plastic cup functioning as a funnel was located under this bag to collect the samples. The bag was fixed to the substrate by means of sellotape and wooden sticks (Fig. 1a, c, e).

Some of the incoming foragers carrying pollen loads and some outgoing bees carrying garbage pellets were trapped in the bag. The loads in the hind legs and the garbage ball in their mandibles were not easily released in most cases, and when they were it was not possible to collect them from the plastic cup due to their moist nature (Fig. 1b, d). To secure both kinds of samples, individual bees had to be gently pressed with the fingers and then collected with the help of a cotton bud and stored in a plastic tube for further processing in the laboratory. Only in very few cases, the bees released the loads and pellets themselves. Special care was taken to avoid pollen cross-contamination between loads and garbage, and by using this method (bag

and hand) it was successfully avoided. The time required for successfully collecting samples was between 15 minutes to more than one hour.

It is also worth mentioning that attaching the bag to the nest substrate is not an easy task due to the effect of the wind and the position of nest entrances. Concerning the use of plastic cup as funnels to collect garbage pellets, it was successfully applied from horizontal nest entrances of *S. jujuyensis* colonies on vertical trunks (Vossler F.G., unpublished data). It is possible that its ineffective implementation on the nest of *S. jujuyensis* here studied was due to the vertical position of the entrance tube as it was located on a leaning tree. As these four stingless bees are not tremendously aggressive, this method was complemented by catching bees by hand, the incoming ones carrying pollen loads as well as the outgoing with garbage pellets (Fig. 1f). This was possible due to the presence of the bag in the position already mentioned as it slows the transit and allows the catching. In the special case of *G. argentina*, both methods were needed simultaneously due to its very timid behaviour towards an observer when it is near the nest.

POLLEN PROCESSING AND IDENTIFICATION

Pollen samples were dissolved in distilled water at 80-90 °C for 10-15 minutes, stirred by hand for one minute, and filtered. Finally, to obtain pollen sediment, samples were centrifuged at 472 x g for 5 minutes, and acetolyzed (Erdtman 1960). Pollen types were identified using a light microscope Leitz Laborlux at 400 and 1,000 x magnification. Pollen grain identification was carried out comparing grains from loads and pellets with acetolyzed reference pollen from flowers of native and exotic species (ornamental and spontaneous) grown in the site studied. Plant systematics was updated following APG IV (2016) classification.

RESULTS

A total of 66 pollen types were found in all samples, most of them were identified (48 types in 27



Figure 1 - Nylon bag used for sampling load and garbage pellets: a_ from *Plebeia catamarcensis* (nesting in brick wall, horizontal tube); c_ from *Tetragonisca angustula fiebrigi* (leaning tree trunk, horizontal tube); e_ from *Geotrigona argentina* (nesting in the ground, horizontal soil surface, bees departing upwards). b_ Pollen loads (red arrow) of *P. catamarcensis* trapped in the nylon bag; d_ Pollen loads from incoming workers (red arrow) and garbage pellet from outgoing workers (yellow arrow) of *T. angustula fiebrigi* trapped in the nylon bag; f_ Nest entrance from *Scaptotrigona jujuyensis* in leaning tree trunk. A garbage pellet carried in the mandible of a leaving worker can be seen (yellow arrow).

families). Only 18 types remained unidentified due to the complex nature of the vegetation surrounding the nests and the generalist behaviour of these bees (Table I).

A total of 13 pollen types from 10 families and 1 unidentified type were present in the load samples of the four stingless bee species (Table I; Fig. 2a-c). Five pollen types (three of them abundantly represented: those $\geq 5\%$) were identified in the

pollen loads of *S. jujuyensis*, 3 abundant types in *T. angustula fiebrigi*, 10 types (four abundant) in *P. catamarcensis*, and two abundant types in *G. argentina* (Table I). As expected, loads of the four stingless bees were composed of pollen types from current flowerings of ornamental and native plant species of the riparian Chaco forest (as it was detected when comparing them with floral phenology of the site studied).

A total of 45 pollen types from 26 families and 17 unidentified types were present in the garbage pellet samples of three stingless bee species (for *G. argentina*, no garbage pellets were sampled) (Table I; Fig. 2d; Fig. 3). The only garbage pellet from *S. jujuyensis* was composed of 30 types (5 of them abundant), those of *T. angustula fiebrigi* of 48 types (7 abundant), and of *P. catamarcensis* of 2 abundant types (Table I; Fig. 2d; Fig. 3).

A total of 31 pollen types from taxa native to the riparian Chaco forest (two of them cultivated), 12 from ornamental exotic taxa (one of them introduced), 5 types including native and ornamental exotic species and 18 from unidentified taxa were present in all samples together (Table I). From them, 7 native, 5 ornamental exotic, 1 type including native and ornamental exotic species and 1 unidentified type were present in loads and 29 native, 11 ornamental exotic, 2 types including native and ornamental exotic species and 20 unidentified types in garbage pellets (Table I). One invasive ornamental exotic tree (*Melia azedarach*) was poorly represented in loads of *P. catamarcensis* and garbage pellets of *T. angustula fiebrigi* (Table I).

DISCUSSION

In stingless bees, pollen loads are frequently totally or predominantly composed of one resource, but mixed loads can more often be found during unfavourable climate periods such as droughts (Ramalho et al. 1994, Freitas et al. 2017, Barth et al. 2018). Threshold values of 99% and 90% (or 60% if no accessory pollen was present) were used to identify single or monofloral versus mixed or bifloral/ heterofloral pollen loads by Ramalho et al. (1994) and Barth et al. (2018), respectively. In the present study, the number of pollen types identified was less than the number of load pairs analyzed except in the 4 load pairs of *P. catamarcensis* where 10 pollen types were identified. As this study was performed in a period of high diversity of floral

resources and favourable climate conditions it is assumed that loads were monofloral, except in *Plebeia*. Mixed loads in *P. catamarcensis* were not due to unfavourable climate conditions neither to the foraging on plants with similar flower and/ or inflorescence morphology (as reported for *Scaptotrigona bipunctata* by Ramalho et al. (1994)) as shape, clustering, colour and other flower characters of foraged types were very different.

Only a few mixed loads were reported for *Plebeia remota* (1 out of 54) and *P. droryana* (2 out of 41) in Brazil (Ramalho et al. 1994). Further studies on individual pollen load composition are necessary to test whether mixed pollen loads are a common pattern in *P. catamarcensis* even in periods of abundantly available floral resources.

In the present study, the majority of pollen types composing garbage pellets was from current blooming (late winter in September), contrary to the expected composition. The dominance of pollen grains from *Citrus* and type Areaceae-Asparagaceae-Asphodelaceae in the garbage of *Plebeia catamarcensis* is possibly due to foraging occurring some weeks before sampling and residuals were quickly processed, the same was observed for the other stingless bees as many types were shared among loads and garbage. However, in the cases of *T. angustula fiebrigi* and *S. jujuyensis*, the presence of pollen from late spring-summer flowerings could suggest that more time was required for processing the pollen stored or for removing the high number of storage pots found in these larger nests. Among the few pollen types from species blooming exclusively during warm months likely from November to February (late spring and summer) found in the garbage were the native trees *Pterogyne nitens* (abundant in *S. jujuyensis*: 34.21%) and *Sapindus saponaria* (abundant in *S. jujuyensis*: 5.11%), and the ornamental exotic trees *Largestroemia indica* (poorly represented in *T. angustula fiebrigi*: 1.19%), *Delonix regia* (poorly represented in *T. angustula fiebrigi*:

TABLE I
Relative abundance (%) of pollen types in pollen loads and garbage pellets (faeces) from *Scaptotrigona jujuyensis*, *Tetragonisca angustula fiebrigi*, *Plebeia catamarcensis* and *Geotrigona argentina*, in alphabetic order of their families. Pollen types abundantly represented (>5%) are shown in bold. References of species status: exotic ornamental species (*); native cultivated species (cultivated native); native spontaneous species (W); exotic spontaneous species (!); types including native and ornamental exotic species (W/***); uncertain status, from unidentified types (?).**

plant family	pollen type	species status	<i>Scaptotrigona jujuyensis</i>	<i>Tetragonisca fiebrigi</i>	<i>Plebeia catamarcensis</i>	<i>Geotrigona argentina</i>
			loads	loads	loads	loads
			faeces	faeces	faeces	faeces
Anacardiaceae	type <i>Schinopsis</i>	W/***	7.94	3.56		
Apiaceae	Apiaceae	W		0.26		
	Eryngium	W		0.39		
Arecaceae	Arecaceae	***	0.18			
	<i>Phoenix</i>	***		26.78		
Arecaceae affinity	type Arecaceae-Asparagaceae-Asphodelaceae	***	+	36.42	18.49	56.17
Asteraceae, Astereae-Eupatoriaceae	type <i>Baccharis-Mikania</i>	W	+	2.9		0.33
Asteraceae, Cichorieae	<i>Hypochoeris</i>	W				
Asteraceae, Eupatoriaceae	<i>Eupatorium</i> type 1 (<i>Critonia</i> affinity)	W		2.11		
Asteraceae, Eupatoriaceae	<i>Eupatorium</i> type 2	W	1.06			
Asteraceae, Heliantheae	<i>Parthenium hysterophorus</i>	W		1.85		
Asteraceae, Heliantheae	type Heliantheae (type <i>Bidens</i>)	presumably W	+	0.26		
Asteraceae, Inuleae	type <i>Tessaria</i>	W	0.18			
Asteraceae, Mutisicaceae	<i>Holocheilus hieracioides</i>	W	0.18	0.26		
Basellaceae	<i>Anredera cordifolia</i>	W	+			
Bignoniaceae	<i>Handroanthus impetiginosus</i>	cultivated native-W		50.14	9.33	
Cactaceae	<i>Rhipsalis lumbricoides</i>	W			1.14	
Calyceraceae	<i>Acicarpa tribuloides</i>	W		1.32		
Cannabaceae	<i>Celtis</i>	W		1.85		
Casuarinaceae	<i>Casuarina cunninghamiana</i>	***		5.94		
Euphorbiaceae	<i>Sapium haematospermum</i>	W		+		
Fabaceae, Caesalpinoideae	<i>Delonix regia</i>	***		1.05		
	<i>Pterogyne nitens</i>	W	34.21	+		
Fabaceae, Caesalp, mimosoid clade	<i>Prosopis</i>	W	21.73	9.1	31.42	35.66
			7.76			

TABLE I (continuation)

plant family	pollen type	species status	Scaptotrigona jujuyensis		Tetragonisca febrigi		Plebeia catamarcensis		Geotrigona argentina	
			loads	faeces	loads	faeces	loads	faeces	loads	faeces
	type <i>Enterobium contortisiliquum</i> (monads from polyad)	W		3						
	type <i>Senegalia praecox</i>	W	0.22	0.18						
	type <i>Vachellia aroma</i>	W		1.06						
Fabaceae, Cercidoideae	<i>Bauhinia</i>	***						0.16		
Fabaceae, Papilionoideae	<i>Geoffraea decorticans</i>	W	0.44	3.17		1.71				
Iridaceae	<i>Sysirinchium</i>	W		+		+				
Lamiaceae	Lamiaceae	W/***				0.13				
	<i>Salvia</i>	W/***				0.13				
Lythraceae	<i>Heimia salicifolia</i>	W		0.07						
	<i>Lagerstroemia indica</i>	***		+		1.19				
Meliaceae	<i>Melia azedarach</i>	***-!				0.13			1.8	
Menispermaceae	<i>Cissampelos pareira</i>	W				0.13				
Moraceae-Urticaceae	type Moraceae-Urticaceae	***/W	50.11	0.07		8.57		0.33		
Myrtaceae	Myrtaceae	***				0.13				
Nyctaginaceae	<i>Bougainvillea</i>	***	27.49	+		0.26				
	Pisonia zapallo	W		4.76		+				64.34
Oxalidaceae	<i>Oxalis</i> type 1	W				+				
	<i>Oxalis</i> type 2	W				+				
Proteaceae	<i>Grevillea robusta</i>	***		+		0.26				
Rutaceae	<i>Citrus</i>	***				13.44		36.66		43.82
Salicaceae	<i>Xylosma venosa</i>	cultivated native-W		28.4		6.6				
Sapindaceae	<i>Sapindus saponaria</i>	W		5.11		3.17				
Sapotaceae	<i>Sideroxylon obtusifolium</i>	W				1.58				
Verbenaceae	Verbenaceae	W/***		+						
Unidentified A	grains small size, porate	?				0.26				
Unidentified B (Eudicot)	grains medium size, 3-colporate, subrhombic	?				+				
Unidentified C (Eudicot)	grains small size, 3-colporate suboblate	?				0.13				

TABLE I (continuation)

plant family	pollen type	species status	<i>Scaptotrigona jujuyensis</i> loads	<i>Scaptotrigona jujuyensis</i> faeces	<i>Tetragonisca fiebrigi</i> loads	<i>Tetragonisca fiebrigi</i> faeces	<i>Plebeia catamarcensis</i> loads	<i>Plebeia catamarcensis</i> faeces	<i>Geotrigona argentina</i> loads
Unidentified D (Eudicot)	grains small size, 3-colporate suboblate	?			0.26				
Unidentified E (Monocot)	grains medium size, monosulcate, boat-shaped, microreticulate	?			0.13				
Unidentified F (Monocot)	grains small size, monosulcate, boat-shaped, psilate	?			0.13				
Unidentified G (Eudicot)	grains small size, 3-colpate, microechinate	?			+				
Unidentified H (Eudicot)	grains small size, 3-colporate lalongate	?			0.26				
Unidentified I (Eudicot)	grains medium size, 3-colporate, subtriangular, verrucate	?			+				
Unidentified J (Eudicot)	grains small size, 3-colporate, oblate, folded wall	?			0.13				
Unidentified K	grains small size, folded thin wall	?			0.13				
Unidentified L (Monocot)	grains small size, monosulcate, boat-shaped	?			0.39				
Unidentified M (Eudicot)	grains large size, 3-colporate, prolate	?		0.53	0.13				
Unidentified N (Eudicot)	grains small size, 3-colporate, microreticulate	?		0.88					
Unidentified Ñ (Eudicot)	grains medium size, 3-colporate, prolate	?		+					
Unidentified O (Eudicot)	grains medium size, 3-colporate, subrectangular	?		+					
Unidentified P (Eudicot)	grains medium size, 3-colpate, echinate	?		+					
Unidentified Q	grains medium size, inaperturate, echinate	?							0.33

1.05%) and *Grevillea robusta* (poorly represented in *T. angustula fiebrigi*: 0.26%). Eltz et al. (2001) assumed that pollen grains from garbage were from brood and worker feces. They also believed that defecation by workers in the nest explained the fact that some resources were abundantly shared among garbage and loads, as cross-contamination between loads and garbage was assumed to occur in low proportion (less than 0.5% representation of the pollen volume).

The study on *Trigona (Tetragonula) collina* in Malaysia showed that single garbage pellets contained from 7 to 11 pollen types (Eltz et al. 2001), being a third the amount found here for a single pellet of *Scaptotrigona* (30 types). Furthermore, the 7 pellets here studied from *T. angustula fiebrigi* were composed of 48 types. Compared to pollen loads, the higher richness of pollen types in the garbage could be the result of floral resources being exploited by many foragers for long and the removal of different storage pots. The values here obtained for a reduced sampling are high in comparison to the recorded in the Chaco dry native forest for many nests, seasons and years (Vossler et al. 2010, 2014, Vossler 2019a, Vossler F.G., unpublished data). The urban landscape here studied has both ornamental exotic and native species from riparian Chaco forest providing a greater plant richness than the native forest alone.

Comparing pooled samples of loads and garbage per species, the number of pollen types was 5 and 30 for *S. jujuyensis*, 3 and 48 for *T. angustula fiebrigi* and 10 and 2 for *P. catamarcensis*. This number was twice as big in garbage than loads for *T. collina* in Sabah, Malaysia (Eltz et al. 2001). On the other hand, these authors observed that almost all (94%) of pollen types in loads (total: 32) did also appear in the garbage samples but only 65% of the ones found in garbage samples (total: 46) were also detected in pollen loads. In the present study, most pollen types from loads (except 4 from *Plebeia*) were represented in garbage (8 cases considering

the same bee species and 2 among different species) (Table I). Contrary to the finding of Eltz et al. (2001), most pollen types were exclusive from garbage (Table I), likely due to the short time of sampling in the present study.

Similar to the method used by Eltz et al. (2001), the combined method here used was a successful tool to assess pollen diet in Meliponini bees and it could be used to make comparisons among colonies from the same and different species, at different times of the day, in different months and seasons, and at different sites without the need of opening the nests. By means of this method, it would be also interesting to reveal the dynamics of pollen provisioning by these subtropical stingless bees, both in native forest and anthropized landscapes.

This sampling method helped to obtain two kinds of information on pollen resources foraged by native bees analyzed by entomopalynological techniques at the same time, and it was successfully applied to stingless bees of different nest entrance size and forager traffic, and to wild colonies nesting in trunks, ground and brick substrates, of the genera *Plebeia*, *Geotrigona*, *Tetragonisca* and *Scaptotrigona*. In stingless bees, traffic is known to be strongly correlated with entrance/bee ratio (Couvillon et al. 2008) as easy and quick entry of individuals carrying resources inside the nest must be guaranteed by wide nest entrance holes. In the case of the Chaqueni species here studied, nest entrance area is greater in *Scaptotrigona*, followed by *Tetragonisca*, *Geotrigona* and *Plebeia* (Vossler 2012) but forager traffic (and entrance/bee ratio) was more intensive in *Scaptotrigona*, followed by *Tetragonisca*, *Plebeia* and *Geotrigona*.

Versatile digestive physiologies characterize bees capable to forage on a broad spectrum of pollen plants such as the stingless bees (Cane and Sipes 2006), even from previously unknown resources such as ornamental exotic species. This fact has implications in the conservation of colonies naturally established in urban and semi-

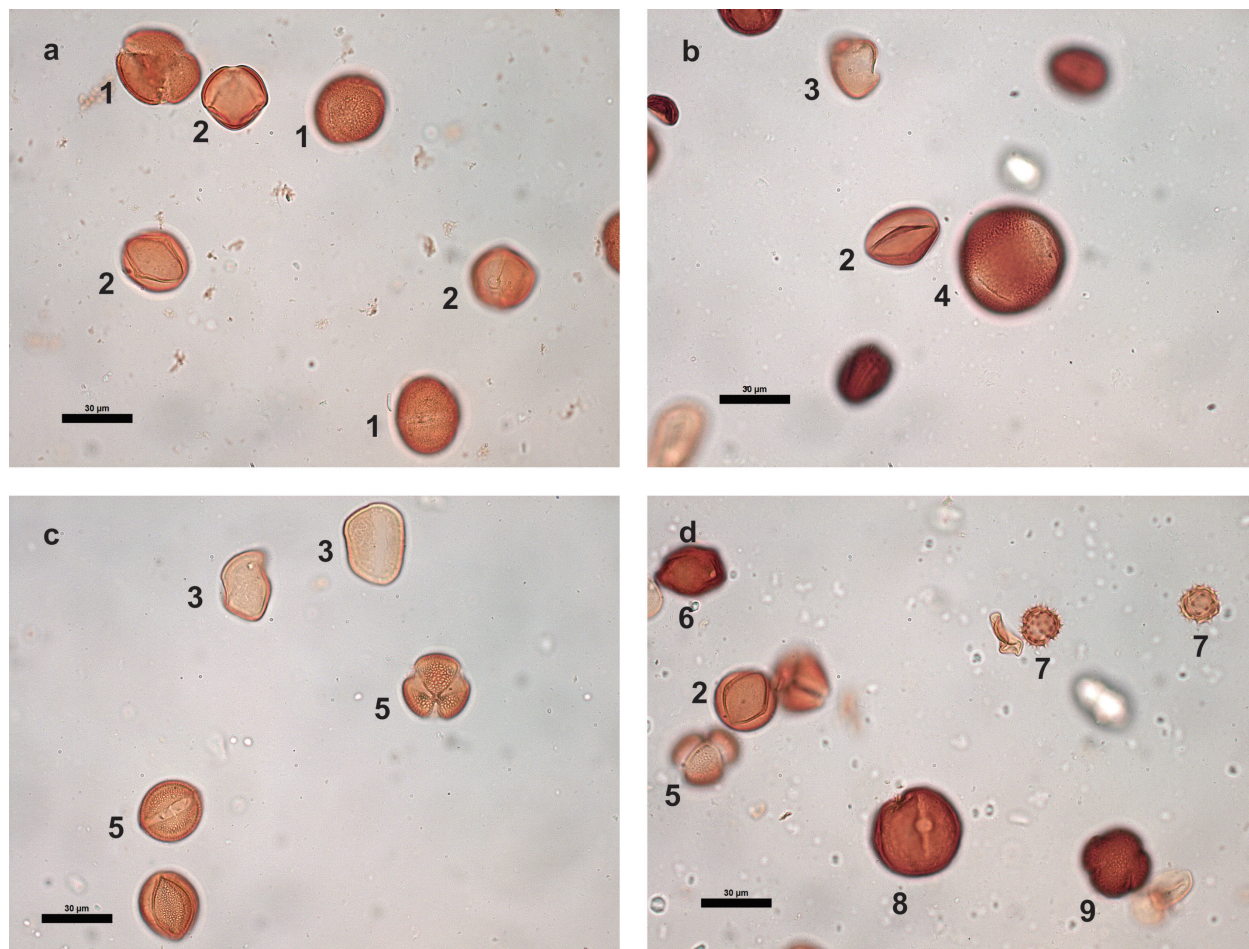


Figure 2 - Pollen types in pollen loads of *G. argentina* (a), *P. catamarcensis* (b) and *T. angustula fiebrigi* (c), and in garbage pellets of *T. angustula fiebrigi* (d): 1_ *Pisonia zapallo*; 2_ *Prosopis*; 3_ type Arecaceae-Asparagaceae-Asphodelaceae; 4_ *Rhipsalis lumbricoides*; 5_ *Handroanthus impetiginosus*; 6_ *Casuarina cunninghamiana*; 7_ type *Baccharis-Mikania*; 8_ *Melia azedarach*; 9_ *Citrus*. Bar = 30 µm.

urban areas where many bloomings are available for foraging. Moreover, the versatile foraging behaviour on native and exotic pollen resources is important for meliponiculture practices in urban habitats where native flora is preserved (such as the observed in the site of the present study), as the production of bee products can be increased. Furthermore, pollination of native plants by wild or reared stingless bees could be favoured promoting their conservation in urban environments.

Predictable ecological patterns that emerge from studies on native bees in urban environments can provide guidance on how future urban

constructs can be designed to provide habitat for conserving and protecting native bees (Frankie et al. 2013). The rearing of Meliponini in urbanized landscapes such as house gardens is possible due to the stingless nature of these bees, although tremendously aggressive species exist (Roubik 2006), they are not present in the Chaco region (Vossler 2012).

The importance of ornamental exotic plants in the pollen diet of the Chaquenan stingless bees has not previously been addressed. This information is useful for stingless bee beekeepers as many times colonies reared in the native forest

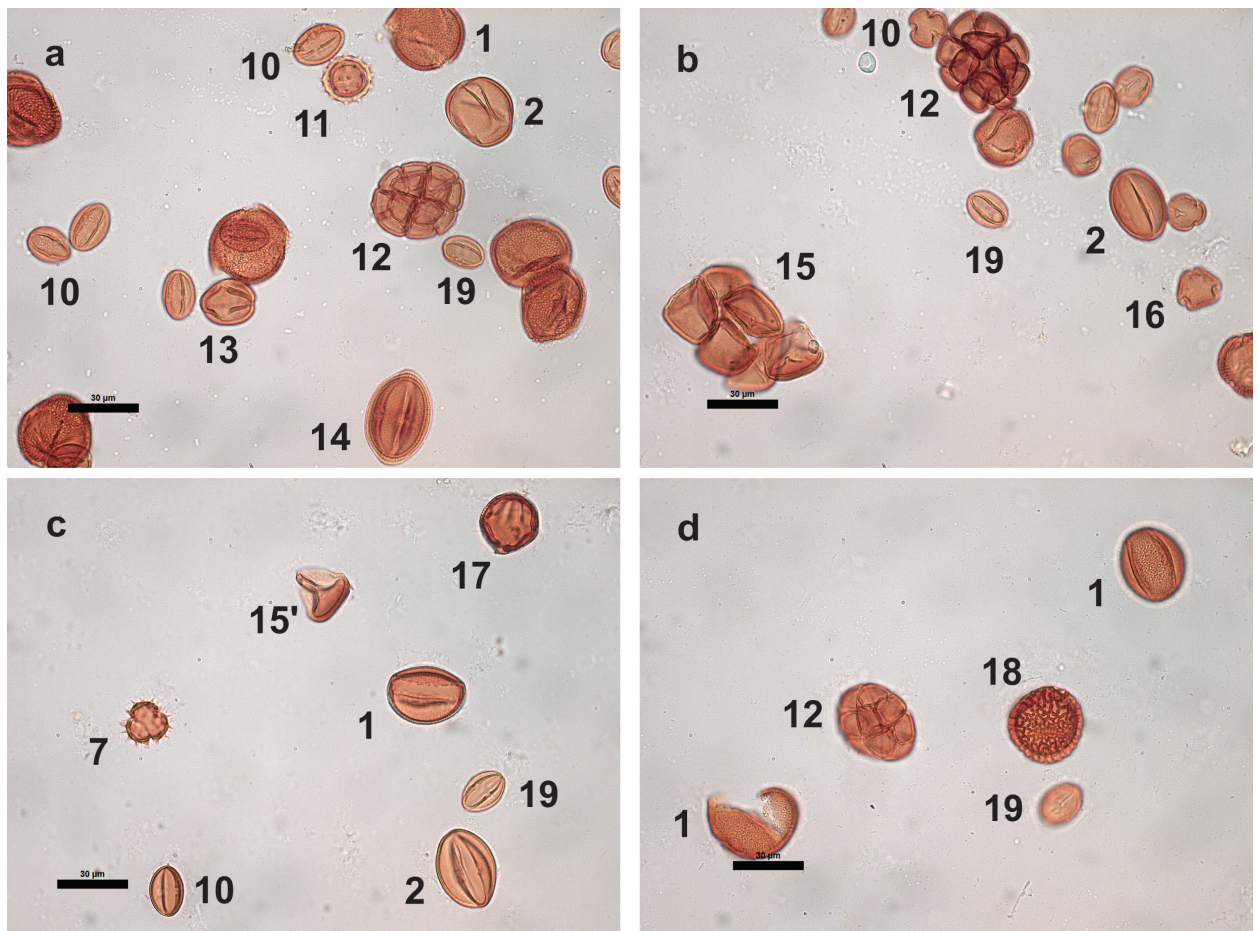


Figure 3 - Pollen types in garbage pellets of *S. jujuyensis* (a–d): 10_ *Xylosma venosa*; 11_ *Parthenium hysterophorus*; 12_ type *Senegalia praecox*; 13_ *Geoffraea decorticans*; 14_ *Holocheilus hieracioides*; 15_ type *Enterolobium contortisiliquum* (15_a fragment of polyad and 15'_ an isolated monad); 16_ *Sapindus saponaria*; 17_ type *Schinopsis*; 18_ *Bougainvillea*; 19_ *Pterogyne nitens*.

are temporally maintained in urban environments. Most entomopalynological studies in Chaquenan stingless bees were performed in the native dry forest (Vossler et al. 2010, 2014, Vossler 2015, 2018, 2019a, 2019b), where pollen resources are obtained largely from trees and shrubs from diverse plant families rarely conserved or cultivated in urban habitats of most localities in the Chaco region. In most cases, urban plants are ornamental exotic species and many times they are not adapted to the dry climate conditions and must be periodically replaced. Urban greening using native species of the Chaco forest is possible but it is not done,

mainly due to its thorns, size and wild nature. It is necessary to create awareness of the importance of native species conservation in urban areas.

Pollen foraging on exotic species could also have a negative effect in conservation of natural environments, as it could increase the reproductive success of potential invader plants. In the case of *Melia azedarach*, an invasive tree established in this site a long time ago, although it was foraged by these bees, its pollination syndrome is associated to moths. Further studies are necessary. Other ornamental plants with incipient invasive status have been reported in vegetation studies in

this site (Vossler and Delucchi 2019) but pollen usage has not yet been reported in the Chaquenan stingless bees although it has been mentioned for other regions. Forestry in urban environments should consider the potential invasive behaviour of ornamental species before they are cultivated. Although it is many times difficult to predict it, plants of evident invasive behaviour should be removed from urban environments before they are established. This is particularly important in riparian habitats as water streams transport seeds, fruits and vegetative organs.

Further palynological studies of garbage pellets in a long-term sampling period would be interesting to record a greater number of pollen resources throughout the year in urban habitats, which would likely includes other exotic plants.

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REFERENCES

- ADÁMOLI J, SENNHAUSER E, ACERO JM AND RESCIA A. 1990. Stress and disturbance: vegetation dynamics in the dry Chaco region of Argentina. *J Biogeogr* 17: 491-500.
- APG IV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Bot J Linn Soc* 181: 1-20.
- BARTH OM, FREITAS AS AND VANDERBORGTH B. 2018. Pollen Storage by *Melipona quadrifasciata anthidioides* in a Protected Urban Atlantic Forest Area of Rio de Janeiro, Brazil. In: Vit P, Pedro SRM and Roubik DW (Eds), *Pot-pollen in stingless bee mellitology*, Cham: Springer, Cham, Switzerland, p. 103-109.
- CANE JH AND SIPES S. 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. In: Waser NM and Ollerton J (Eds), *Plant-pollinator interactions. From specialization to generalization*, Chicago: The University of Chicago Press, Chicago, USA, p. 99-122.
- COUVILLON MJ, WENSELEERS T, IMPERATRIZ-FONSECA VL, NOGUEIRA-NETO P AND RATNIEKS FLW. 2008. Comparative study in stingless bees (*Meliponini*) demonstrates that nest entrance size predicts traffic and defensivity. *J Evol Biol* 21: 194-201.
- ELTZ T, BRÜHL CA, VAN DER KAARS S AND LINSENMAIR KE. 2001. Assessing stingless bee pollen diet by analysis of garbage pellets: a new method. *Apidologie* 32: 341-353.
- ERDTMAN G. 1960. The acetolysis method, a revised description. *Sven Bot Tidskr* 54: 561-564.
- FRANKIE GW, VINSON SB, RIZZARDI MA, GRISWOLD TL, COVILLE RE, GRAYUM MH, MARTINEZ LES, FOLTZ-SWEAT J AND PAWELEK JC. 2013. Relationships of bees to host ornamental and weedy flowers in urban Northwest Guanacaste province, Costa Rica. *J Kansas Entomol Soc* 86: 325-351.
- FREITAS AS, VANDERBORGTH B AND BARTH OM. 2017. Pollen resources used by *Melipona quadrifasciata anthidioides* Lepeletier in an urban forest in Rio de Janeiro city, Brazil. *Palynology* 42: 392-399.
- IMPERATRIZ-FONSECA VL, KLEINERT-GIOVANNINI A, CORTOPASSI-LAURINO M AND RAMALHO M. 1984. Hábitos de coleta de *Tetragonisca angustula angustula* Latreille (Apidae, Meliponinae). *Bol Zool Univ São Paulo* 8: 115-131.
- KERR AS AND KERR WE. 1999. *Melipona* garbage bees release their cargo according to a Gaussian distribution. *Rev Bras Biol* 59: 119-123.
- LEONHARDT SD, DWORSCHAK K, ELTZ T AND BLÜTHGEN N. 2007. Foraging loads of stingless bees and utilisation of stored nectar for pollen harvesting. *Apidologie* 38: 125-135.
- MEDINA LAM, HART AG AND RATNIEKS FLW. 2014. Waste management in the stingless bee *Melipona beecheii* Bennett (Hymenoptera: Apidae). *Sociobiology* 61: 435-440.
- MICHENER CD. 1999. The corbiculae of bees. *Apidologie* 30: 67-74.
- PAPADAKIS J. 1973. La región chaqueña. *Ecología, suelos, posibilidades agrícolas*. *Ciencia e Investigación* 29: 182-201.
- PRADO DE. 1993. What is the Gran Chaco vegetation in South America? I. a review. *Contribution to the study of flora and vegetation of the Chaco*. *V. Candollea* 48: 145-172.
- RAMALHO M. 2004. Stingless bees and mass flowering trees in the canopy of Atlantic forest: a tight relationship. *Acta Bot Bras* 18: 37-47.
- RAMALHO M, IMPERATRIZ-FONSECA VL, KLEINERT-GIOVANNINI A AND CORTOPASSI-LAURINO M. 1985. Exploitation of floral resources by *Plebeia remota*

- Holmberg (Apidae, Meliponinae). *Apidologie* 16: 307-330.
- RAMALHO M, KLEINERT-GIOVANNINI A AND IMPERATRIZ-FONSECA VL. 1990. Important bee plants for stingless bees (*Melipona* and *Trigona*) and Africanized honeybees (*Apis mellifera*) in neotropical habitats: a review. *Apidologie* 21: 469-488.
- RAMALHO M, GIANNINI TC, MALAGODI-BRAGASKS AND IMPERATRIZ-FONSECA VL. 1994. Pollen harvest by stingless bee foragers (Hymenoptera, Apidae, Meliponinae). *Grana* 33: 239-244.
- RECH AR AND ABSY ML. 2011. Pollen sources used by species of Meliponini (Hymenoptera: Apidae) along the Rio Negro channel in Amazonas, Brazil. *Grana* 50: 150-161.
- ROUBIK DW. 1989. Ecology and natural history of tropical bees. New York: Cambridge University Press, 526 p.
- ROUBIK DW. 2006. Stingless bee nesting biology. *Apidologie* 37: 124-143.
- ROUBIK DW AND MORENO PATIÑO JE. 2009. *Trigona corvina*: An ecological study based on unusual nest structure and pollen analysis. *Psyche*, Article ID 268756, 7 pages DOI:10.1155/2009/268756.
- THORP RW. 1979. Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. *Ann Missouri Bot Gard* 66: 788-812.
- THORP RW. 2000. The collection of pollen by bees. *Plant Syst Evol* 222: 211-223.
- VOSSLER FG. 2012. Flower visits, nesting and nest defence behaviour of stingless bees (Apidae: Meliponini): suitability of the bee species for meliponiculture in the Argentinean Chaco region. *Apidologie* 43: 139-161.
- VOSSLER FG. 2015. Small pollen grain volumes and sizes dominate the diet composition of three South American subtropical stingless bees. *Grana* 54: 68-81.
- VOSSLER FG. 2018. Are stingless bees a broadly polylectic group? An empirical study of the adjustments required for an improved assessment of pollen diet in bees. In: Vit P, Pedro SRM and Roubik DW (Eds), *Pot-pollen in stingless bee melittology*, Cham: Springer, Cham, Switzerland, p. 17-28. DOI: 10.1007/978-3-319-61839-5_2.
- VOSSLER FG. 2019a. Pollen diet assessment and flower association in *Melipona orbignyi* and recommendations on management and conservation of stingless bees in the Chaco dry forest of South America. *Apidologie* 50: 391-413.
- VOSSLER FG. 2019b. Foraging behaviour of colonies of the stingless bee *Melipona orbignyi* (Hymenoptera: Apidae: Meliponini) in a dry forest assessed by multivariate analysis from palynological data. *Grana* 58: 383-392.
- VOSSLER FG, FAGÚNDEZ GA AND BLETTLER DG. 2014. Variability of food stores of *Tetragonisca fiebrigi* (Schwarz) (Hymenoptera: Apidae: Meliponini) from the Argentine Chaco based on pollen analysis. *Sociobiology* 61: 449-460.
- VOSSLER FG, TELLERÍA MC AND CUNNINGHAM M. 2010. Floral resources foraged by *Geotrigona argentina* (Apidae, Meliponini) in the Argentine Dry Chaco forest. *Grana* 49: 142-153.
- VOSSLER FG AND DELUCCHIG. 2019. Leñosas adventicias en el bosque chaqueño y concientización para su control: *Tecoma stans* (Bignoniaceae), *Leucaena leucocephala* (Fabaceae) y *Melia azedarach* (Meliaceae) en la provincia del Chaco, Argentina. In: XXXVII Jornadas Argentinas de Botánica, San Miguel de Tucumán. *Bol Soc Arg Bot* 54(Supl.): 161-162.