



Original Papers

Main pollen characters in the diet of four stingless bees (Apidae: Meliponini) in the Chaco dry forest

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Abstract

The pollen morphology of 59 floral resources found in 30 nests of four stingless bees was analyzed using nine characters. For identification and description, the pollen was acetolyzed and for cytoplasm measurements, the non-acetolyzed grains were fuchsin stained. Ten pollen types were chosen at random to be described in detail and microphotographed using light and scanning microscopes: *Achatocarpus praecox*, *Trithrinax schizophylla*, *Parthenium hysterophorus*, *Anisocapparis speciosa*, *Capparicordis tweediana*, *Cynophalla retusa*, *Sarcotoxium salicifolium*, *Caesalpinia paraguariensis*, *Parkinsonia praecox*, and *Pisonia zapallo* var. *guaranitica*. Among the plant species foraged, the main pollen classes were monads, spheroidal cytoplasm shapes (P/E ratio), spheroidal and ellipsoidal cytoplasm shapes (geometric), small to medium cytoplasm sizes and volumes, tri-zono-colporate apertures, and meshed and psilate-softly ornamented. The main pollen morphological characters were associated with: a_ extreme versatility in foraging behaviour of stingless bees (polylecty, wet pollen load transportation, illegitimate collection of floral pollen, pollen stealing, recruitment of foragers); b_ some morphological attributes (low hairiness on legs, small bodies); and c_ the predominant floral morphology (small, pale, generalist) of the woody flora of the Chaco dry forest. To better understand pollen selection by bees, more pollen morpho-anatomical characters should be studied under different techniques and preparation variants of the same samples.

Key words: bee pollen, *Geotrigona argentina*, *Melipona orbignyi*, *Scaptotrigona jujuyensis*, *Tetragonisca fiebrigi*.

Resumen

La morfología del polen de 59 recursos florales hallados en 30 nidos de abejas sin aguijón fue analizada utilizando nueve caracteres. Para la identificación y descripción el polen fue acetolizado, y para realizar las mediciones los granos no acetolizados fueron teñidos. Nueve tipos polínicos fueron elegidos al azar para ser descritos en detalle y microfotografiados utilizando microscopio óptico y electrónico de barrido: *Achatocarpus praecox*, *Trithrinax schizophylla*, *Parthenium hysterophorus*, *Anisocapparis speciosa*, *Capparicordis tweediana*, *Cynophalla retusa*, *Sarcotoxium salicifolium*, *Caesalpinia paraguariensis*, *Parkinsonia praecox*, and *Pisonia zapallo* var. *guaranitica*. Entre las especies de plantas recolectadas, las principales clases de polen fueron monadas, formas citoplasmáticas esferoidales (relación P/E), formas citoplasmáticas esferoidales y elipsoidales (geométricas), tamaños y volúmenes citoplasmáticos pequeños y medianos, aperturas tri-zono-colporadas, y mallados y lisos-suavemente ornamentados. Los principales caracteres morfológicos del polen se asociaron con: a_ versatilidad extrema en el comportamiento recolector de las abejas sin aguijón (polilectia, transporte de cargas polínicas húmedas, recolección ilegítima de polen floral, robo de polen, reclutamiento de obreras); b_ algunos atributos morfológicos (pilosidad reducida en patas, cuerpos pequeños); y c_ la morfología floral predominante (pequeña, pálida, generalista) de la flora leñosa del bosque seco chaqueño. Para comprender mejor la selección del polen por abejas, deberían estudiarse más caracteres morfo-anatómicos del polen con diferentes técnicas y variantes de preparación en las mismas muestras.

Palabras clave: polen de abejas, *Geotrigona argentina*, *Melipona orbignyi*, *Scaptotrigona jujuyensis*, *Tetragonisca fiebrigi*.

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Introduction

Morphological characters of pollen grains associated with pollination agents have been approached in several ways (Hesse 1981, 2000; Pacini 2000; Furness & Rudall 2004; Pacini & Hesse 2005; Halbritter *et al.* 2018). Specific traits of pollen grains have been related to particular pollinator agents, such as verrucate ornamentation in pollen of Papilionoideae (Fabaceae) associated with bird and bat pollination (Ferguson & Skvarla 1982). In the same way, the differences in pollen ornamentation and stickiness among *Erythrina* species have also been associated with different pollinator agents (Hemsley & Ferguson 1985). These authors suggested that the acquisition of some pollen characters was due to adaptive selection (Ferguson 1985; Furness & Rudall 2004). However, other authors indicated that pollen morphological features specific to pollination vectors are rare in nature and difficult to demonstrate (Hesse 2000; Stroo 2000). It has also been discussed that pollen treatments such as acetolysis difficult the finding of associations with pollinators and therefore recommended studying pollen in its natural state (Ferguson 1985). Although most pollen studies include acetolyzed grains to achieve a good identification, many important pollen characters selected by animal pollinators, such as colour, odour, coatings, etc, are lost (Hesse 1981, 2000; Ferguson 1985; Dobson & Bergström 2000; Lunau 2000; Pacini 2000). Although the protein content has been proven as a factor of selection by bees, no direct association was found (Roulston *et al.* 2000). Selection of most pollen characters by pollinators remains unclear and insufficiently studied (Hesse 2000) including that performed by bees (Hymenoptera: Anthophila). For instance, the selection of pollen odour, colour and coating has never been evaluated in bees, even though they are attractive characters to floral visitors.

Stingless bees or meliponines (Hymenoptera: Apidae: Meliponini) are a mainly tropical and subtropical highly eusocial clade of corbiculate bees with more than 600 species in approximately 60 supraspecific groups considered as genera or subgenera (Michener 2007, 2013). They are the most abundant group of bees in the tropical forest canopies, due to their perennial and populous nests from a few dozen to hundreds of foragers of generalist habit that interact with the flowers of most flowering plant species to obtain food resources (pollen and nectar) (Roubik 1989, 2006; Ramalho 2004; Michener 2007; Monteiro

& Ramalho 2010). The pollen generalist habit (polylecty) is a need and a condition for the survivorship of such bees with large and perennial nests with high reproductive rate (Michener 2007). Moreover, some of these floral visiting species are able to facultatively forage on non-floral resources, and even steal from stores of other nests from the same or different species (Roubik 1989; Sakagami *et al.* 1993).

In the Neotropics, the interaction between stingless bees and flowers has been deeply studied (Roubik 1989; Ramalho *et al.* 1990; Wilms *et al.* 1996; Ramalho 2004; Monteiro & Ramalho 2010; Vossler *et al.* 2010; Ferreira & Absy 2015, 2017; Vossler 2015, 2019a,b, 2021; Rezende *et al.* 2019; Flores *et al.* 2021). Most species have strong recruitment behaviour on floral resources, sometimes monopolizing specific flowerings (Ramalho 1990). In some communities studied, niche partitioning and overlapping have also been displayed (Ramalho 1990; Wilms *et al.* 1996; Ramalho *et al.* 2007; Ferreira & Absy 2015, 2017; Rezende *et al.* 2019). Some studies have also evidenced preferences for flowering strata and the morphology, colour, size and clustering of flowers, and even for small to medium pollen sizes and volume (Ramalho *et al.* 1989; Ramalho 2004; Vossler 2015). To better understand the interactions between bees and plants, it is necessary to study more pollen characters, mainly those of natural and non-acetolyzed pollen grains.

The aim of this study was to identify the ecological/functional morphology in pollen characters selected by stingless bees. For this, information about the main pollen characters was provided from 59 pollen species / pollen types identified in nest storages of four species of stingless bees in the Chaco dry forest. A total of nine qualitative and quantitative characters from acetolyzed and stained non-acetolyzed grains, respectively, were described and measured. Furthermore, to show diversity of fine structure of exine characters, pollen grains of ten floral resources predominant in the stingless bee diets were selected at random to be described and illustrated in detail. The link between the main morphological characters of pollen grains and stingless bees is discussed.

Material and Methods

Study area

The climate in the study area is strongly seasonal with very hot summer (December to

March) and low temperatures and frost during winter (July to September) (Prado 1993); there is a manifest yearly variation in rainfall, with a marked dry season in winter-spring and a rainy season from October to April (Papadakis 1973). In the “palosantal” area of the Dry Chaco and the “quebrachal” of the Transitional Chaco, the vegetation consists of diverse plant lineages with dominance of legumes (Fabaceae) among the woody flora of the forests but also in open areas and Asteraceae and Poaceae among herbs and shrubs often in open areas (Prado 1993). Many other plant lineages and diverse habits of Angiosperms were also important both in richness and abundance among the vegetation where stingless bee nest samples were taken, they were Achatocarpaceae, Amaranthaceae, Anacardiaceae, Apocynaceae, Arecaceae, Bignoniaceae, Boraginaceae, Bromeliaceae, Cactaceae, Cannabaceae, Capparaceae, Celastraceae, Cervantesiaceae, Convolvulaceae, Cucurbitaceae, Cyperaceae, Euphorbiaceae, Lamiaceae, Loranthaceae, Malpighiaceae, Malvaceae Bombacoideae, Malvaceae Malvoideae, Nyctaginaceae, Passifloraceae, Polygonaceae, Portulacaceae, Rhamnaceae, Rubiaceae, Rutaceae, Santalaceae, Sapindaceae, Sapotaceae, Simaroubaceae, Solanaceae, Verbenaceae, Ximeniaceae, Zygophyllaceae, among others less represented in richness or abundance (Vossler 2013).

Samples studied

A total of 59 pollen grains from 34 families of Angiosperms found in the pollen and honey storage pots from 30 nests of the stingless bees *Geotrigona argentina* (6 nests), *Tetragonisca fiebrigi* (11 nests), *Scaptotrigona jujuyensis* (4 nests) and *Melipona orbignyi* (9 nests) from the Argentine Dry and Transitional Chaco subtropical forests (Vossler *et al.* 2010; Vossler 2015, 2019a,b, 2021) were studied considering nine pollen morphological characters: cytoplasm volume, cytoplasm size, cytoplasm P/E ratio shape, dispersal unit, cytoplasm geometrical shape, aperture number, aperture position, aperture form, and ornamentation (Tabs. S1-S2, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>). Pollen grains of the 59 floral resources were measured from 22 pollen species from herbarium specimens and 37 pollen types from bee nests. For identification, description and characterization of qualitative characters, the pollen was acetolyzed (Erdtman 1960) and for

cytoplasm measurements (quantitative characters), the non-acetolyzed grains were fuchsin stained (Wodehouse 1935). The cytoplasm of the pollen grain was here considered as the cellular content of the vegetative and generative cells as a whole and enclosed by the pollen wall. This concept of pollen cytoplasm as a whole is often used in the palynological lexicon (Punt *et al.* 2007; Hesse *et al.* 2009; Halbritter *et al.* 2018).

Main pollen morphological characters in stingless bee diets

Pollen grain morphology of this pollen community was characterized considering the following nine characters from non-acetolyzed (cytoplasm volume, cytoplasm size, cytoplasm geometric shape and cytoplasm P/E ratio shape) and acetolyzed samples (dispersal unit, three aperture features and ornamentation) (Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>). As most nutrient requirements for bees are found in pollen cytoplasm, measurements were only from the cytoplasmic limits from non-acetolyzed grains and thus pollen wall was excluded from volume, size and shape calculations. It is necessary to clarify that cytoplasm size is a different character from total pollen size, whose values are quite similar in grains with thin walls but different in grains with thick walls or noticeably ornamented such as the echinate. Consequently, cytoplasm volume and total pollen volume are also different characters. Total pollen size and volume were not measured here. The quantitative characters of the pollen selected by bees (cytoplasmic size, cytoplasmic volume, shape based on the P/E ratio and geometric shape) were classified in classes following Erdtman (1943, 1952), Punt *et al.* (2007), Hesse *et al.* (2009) and Vossler (2015). For instance, categories for cytoplasm sizes are very small (< 10 µm), small (10–25 µm), medium (26–50 µm), large (51–100 µm) and very large (> 100 µm). Categories for cytoplasm volume are very small (< 524 µm³), small (524–8,181 µm³), medium (8,182–65,448 µm³), large (65,449–523,583 µm³) and very large (> 523,583 µm³). The classes of cytoplasm shape based on P/E ratio values are seven: prolate (< 0.5), oblate (0.5–0.75), suboblate (0.75–0.88), spheroidal (0.88–1.14), subprolate (1.14–1.33), prolate (1.33–2), and perprolate (> 2). Categories for cytoplasm shapes based on geometrical shapes were three: sphere, ellipsoid, and triangular base prism. For details see Vossler (2015).

Among the qualitative characters (dispersal unit, three aperture features and ornamentation), they were classified in slightly different way to that of palynological literature, as the aim of this study was to identify the ecological/functional morphology in pollen characters selected by bees. Thus, monads and individual grains from broken (acalymmate and pseudocalymmate) polyads were considered as monads. Only whole polyads (calymmate) were considered as a dispersal unit different to monads. Number of apertures was classified as polyaperturate when more than 5 apertures were present, and the predominant number of apertures of the population of grains was used when it was 1 to 5. Position of apertures was classified in panto- (globally distributed), zono- (on the equator) and polar-aperturate (either distal or proximal). Polyads were treated as a whole dispersal unit, therefore the number and position of apertures were considered to be many and globally distributed (poly- and panto-aperturate). Forms of apertures were classified as elongated (colpus, colporus and sulcus were unified as they were considered to have similar ecological functionality) and spheroidal (porate and poroids). The ornamentation of pollen wall was classified under LM (light microscope), and fine sculpture was further described and corroborated in the grains microphotographed at SEM (scanning electron microscope). Thus, ornamentation was classified as psilate-softly ornamented (mainly psilate and scabrate), meshed (microreticulate, reticulate, striato-reticulate, foveolate, and perforate ornamentations), microechinate, echinate, and unspecified (rugulate, verrucate). Although mesh ornamentation is not a palynological term, it was here used to include many wall sculptures consisting of a pattern with deeper or perforated areas (typically “lumina” and holes) surrounded by higher walls (typically “muri”). The diverse morphological ornamentations included are here interpreted to have similar ecological functionality concerning to the storage of pollenkitt or other coatings primarily in these deeper areas, important for attraction and adherence to pollinators [see Hesse (1981); Dickinson *et al.* (2000); and Edlund *et al.* (2004)].

Description of selected pollen grains

Pollen types with more than 5% representativeness were considered as the abundant resources, following Vossler (2018). Ten pollen types abundant in stingless bee diets (Vossler

et al. 2010; Vossler 2015, 2019a,b, 2021) were chosen at random to be described in detail and microphotographed using a light microscope Nikon Eclipse E200 at 400 and 1,000 x magnification and a scanning microscope JEOL JSM-T-100: *Achatocarpus praecox* Griseb. (Achatocarpaceae), *Trithrinax schizophylla* Drude (Arecaceae), *Parthenium hysterophorus* L. (Asteraceae), *Cynophalla retusa* (Griseb.) Cornejo & Iltis, *Sarcotoxium salicifolium* (Griseb.) Cornejo & Iltis, *Anisocapparis speciosa* (Griseb.) Cornejo & Iltis, *Capparicordis tweediana* (Eichler) Iltis & Cornejo (all Capparaceae), *Caesalpinia paraguariensis* (D. Parodi) Burkart, *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins (both Fabaceae), and *Pisonia zapallo* Griseb. var. *guaranitica* Toursark. (Nyctaginaceae) (Tab. S2, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>). For description, pollen terminology used was from Punt *et al.* (2007), Hesse *et al.* (2009) and Halbritter *et al.* (2018). Pollen characters included: dispersal unit, number, position and type of apertures, polarity, symmetry, shape based on P/E ratio, size and wall sculpture and structure. A total of 25 measurements were taken for polar and equatorial diameters, while 15 for exine characters. Pollen grain description was performed mainly from reference plant specimens. Abbreviations: PD (polar diameter), ED (equatorial diameter). Ecological data of these ten described pollen grains as well as others important in the stingless bee diets are in Table S2 (available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>).

Results

Main pollen morphological characters in stingless bee diets

Considering the volume of pollen cytoplasm, four out of the five volume classes were represented: very small, small, medium, and large, predominating small and medium ones (Fig. 1a-b). In the same way, considering the size (largest diameter either polar or equatorial) of pollen cytoplasm, four out of the five size classes were represented: very small, small, medium and large, predominating small and medium ones (Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>). The cytoplasm shape classes based on P/E ratio values showed that all seven classes were represented, predominating the spheroidal one (0.88–1.14 μ m),

while based on cytoplasm geometrical shapes, spheroidal, ellipsoidal and prismatic shapes were represented, predominating spheroidal and ellipsoidal (Fig. 1a-b; Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>).

Monads largely predominated among dispersal units. Triaperturate was followed

by polyaperturate among aperture numbers, zonoaperturate followed by pantoaperturate among aperture positions, elongate (predominantly colpate) followed by spheroidal (porate) among aperture forms. The psilate-softly ornamentation predominated followed by microreticulate ornamentation, but when all meshed ornamented grains were unified (reticulate, microreticulate,

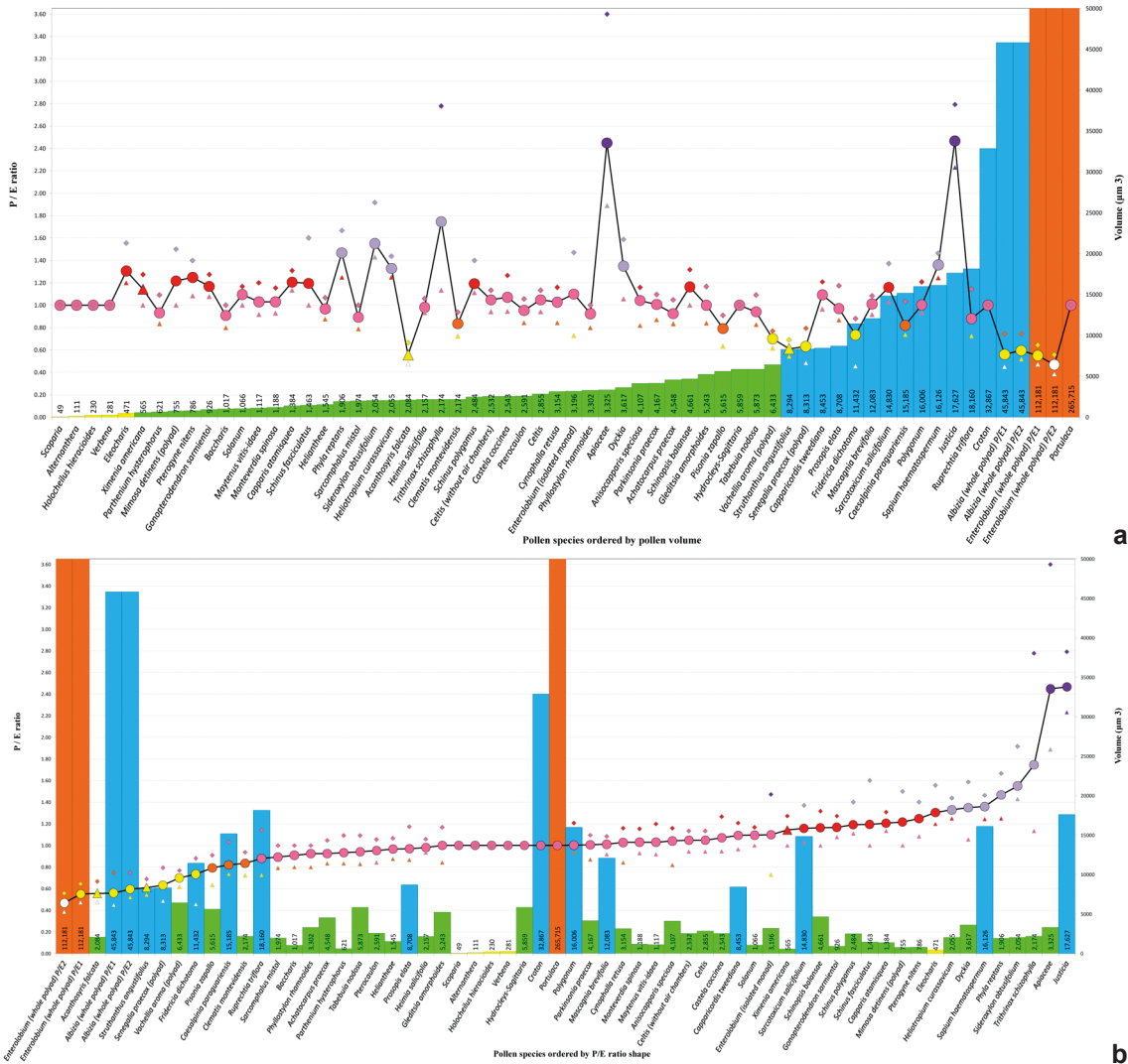


Figure 1 – a-b. Relationship between pollen volume and P/E ratio shape, from cytoplasm measurements of 59 pollen grains (plus three variants) from plant species/pollen types foraged by the four stingless bees in the subtropical Chaco dry forest – a. pollen species in increasing order according to pollen cytoplasmic volume (µm³) mean values; b. pollen species in increasing order according to pollen shapes given by P/E ratio mean values (circle). Minimum (triangle) and maximum (diamond) values are also shown. Volume classes (bar colour): very small (yellow), small (green), medium (sky blue), large (orange). P/E ratio shape classes (circle/triangle colour): peroblate (white), oblate (yellow), suboblate (orange), spheroidal (rose), subprolate (red), prolate (lilac), perprolate (violet). Geometrical shapes: ellipsoids and spheres (circles); prisms (triangles).

striato-reticulate, foveolate, perforated), mesh ornamentation predominated (Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>).

Description of acetolyzed pollen grains

To show diversity of fine structure of exine characters of the pollen foraged by stingless bees, ten pollen types abundant in their diet were selected at random for description, in alphabetic order of their families (Figs. 2–6). Furthermore, the ornamentation details of these and other five pollen types abundant in pollen samples (Tab. S2, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>) were corroborated using LM and SEM microphotographs (Fig. 6e–h). Some new surface characters were observed on the exine wall only under SEM microscope, such as aperture membrane granulate in *Achatocarpus praecox*, microreticulate in *Caesalpinia paraguariensis*, and microechinate in *Pisonia zapallo* var. *guaranitica*, wall surface with perforations in *Trithrinax schizophylla*, *Parthenium hysterophorus* and *Pisonia zapallo* var. *guaranitica*, and nanoechini in *Solanum*. Such observations indicate the need for these complementary techniques to further understand the surface characteristics of pollen grains that may be associated with the collection and/or transport of pollen by bees.

Achatocarpaceae.

***Achatocarpus praecox*.** Figs. 2a–b; 5a
Monad, pantoporoidate, spheroidal, apolar. Diameter 24–30 µm. Poroids 5–7 often 6, with ornamented aperture membrane. Exine 1–2 µm thickness, tectate, scabrate. SEM: Ornamentation scabrate on the surface, with granulate aperture membrane. Herbarium reference: Vossler s.n. (LP; BA).

Arecaceae.

***Trithrinax schizophylla*.** Figs. 2c–f; 5b,d
Monad, monosulcate, heteropolar, bilaterally symmetric. Shapes with significant variation, from biconvex to plane-convex (boat-shaped, rarely pear-shaped, others) in polar view; spheroidal to ellipsoidal in equatorial view. PD: 19–26 µm, major ED: 33–42 µm, minor ED: 25–36 µm. Sulcus long with psilate aperture membrane. Exine 1–2 µm thickness, semitectate, microreticulate. Sexine/nexine ratio 1:1. SEM: Ornamentation perforate-

microreticulate on the surface. Observations: pollen grains studied were from nest storages.

Asteraceae.

***Parthenium hysterophorus*.** Figs. 2g–i; 5c
Monad, tricolporate, spheroidal, radially symmetric. Amb circular in polar and equatorial views. PD = ED: 20–21 µm. Colpus long, porus lalongate. Exine 5 µm thickness, tectate, columellate, echinate, caveate, cavea 1 µm thickness. Echinus 3 µm length, wide, perforated from the base to the middle of its length, exine wall 1 µm thickness between neighbouring echini. SEM: Ornamentation perforate on the wall surface between echini. Herbarium reference: Vossler s.n. (LP; BA).

Capparaceae.

***Anisocapparis speciosa*.** Figs. 3g,j–l; 6a–b
Monad, tricolporate, suboblate to prolate, radially symmetric. Amb elliptic-subrhombic in equatorial view, subcircular in polar view. PD: 22–30 µm, ED: 19–24 µm. Colpus long with psilate aperture membrane, porus lalongate with granulate aperture membrane. Exine 1 µm thickness, semitectate, microreticulate, with polar thickenings. Sexine/nexine ratio 1:1. SEM: Observations coincide with those made with LM. Herbarium reference: Vossler s.n. (LP; BA).

***Capparicordis tweediana*.** Figs. 2j–l; 5g
Monad, tricolporate, suboblate-spheroidal to perprolate, radially symmetric. Amb elliptic-subrhombic in equatorial view, circular in polar view. PD: 25–40 µm, ED: 18–27 µm. Colpus long with psilate aperture membrane, porus lalongate with granulate aperture membrane. Exine 2 µm thickness, semitectate, microreticulate. Sexine/nexine ratio 1:1. SEM: Observations coincide with those made with LM. Herbarium reference: Vossler s.n. (LP; BA).

***Cynophalla retusa*.** Figs. 3a–d; 5e
Monad, tricolporate, spheroidal to subprolate, radially symmetric. Amb elliptic-subrhombic in equatorial view, circular in polar view. PD: 23–29 µm, ED: 20–24 µm. Colpus long with granulate aperture membrane, porus lalongate with granulate aperture membrane. Exine 1 µm thickness, tectate, microechinate. Sexine/nexine ratio 1:1. SEM: Microechini are irregularly distributed on the surface, aperture membrane densely ornamented with granula of variable size. Herbarium reference: Vossler s.n. (LP; BA).

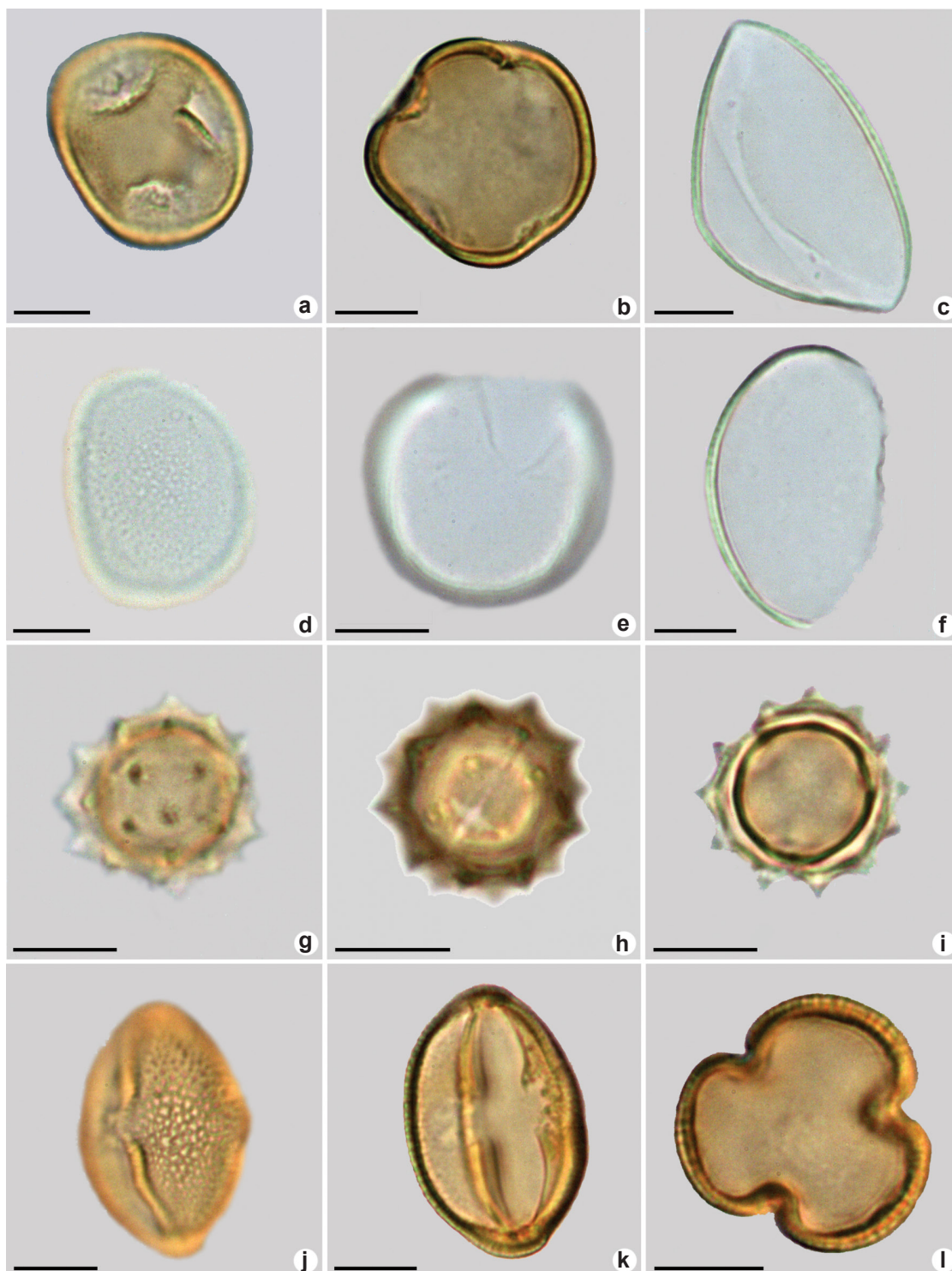


Figure 2 – a-l. LM micrographs of pollen grains from herbarium specimens (except c-f from bee nests) – a-b. *Achatocarpus praecox* (Achatocarpaceae) – a. pollen surface, upper focus; b. optical section; c-f. *Trithrinax schizophylla* (Arecaceae) – c. distal polar view, optical section; d. proximal polar view, surface; e. equatorial view, surface; f. equatorial view, optical section; g-i. *Parthenium hysterophorus* (Asteraceae) – g. polar view, surface; h. equatorial view, surface; i. polar view, optical section; j-l. *Capparicordis tweediana* (Capparaceae) – j. equatorial view, surface; k. equatorial view, optical section; l. polar view, optical section. Scale bar = 10 μ m.

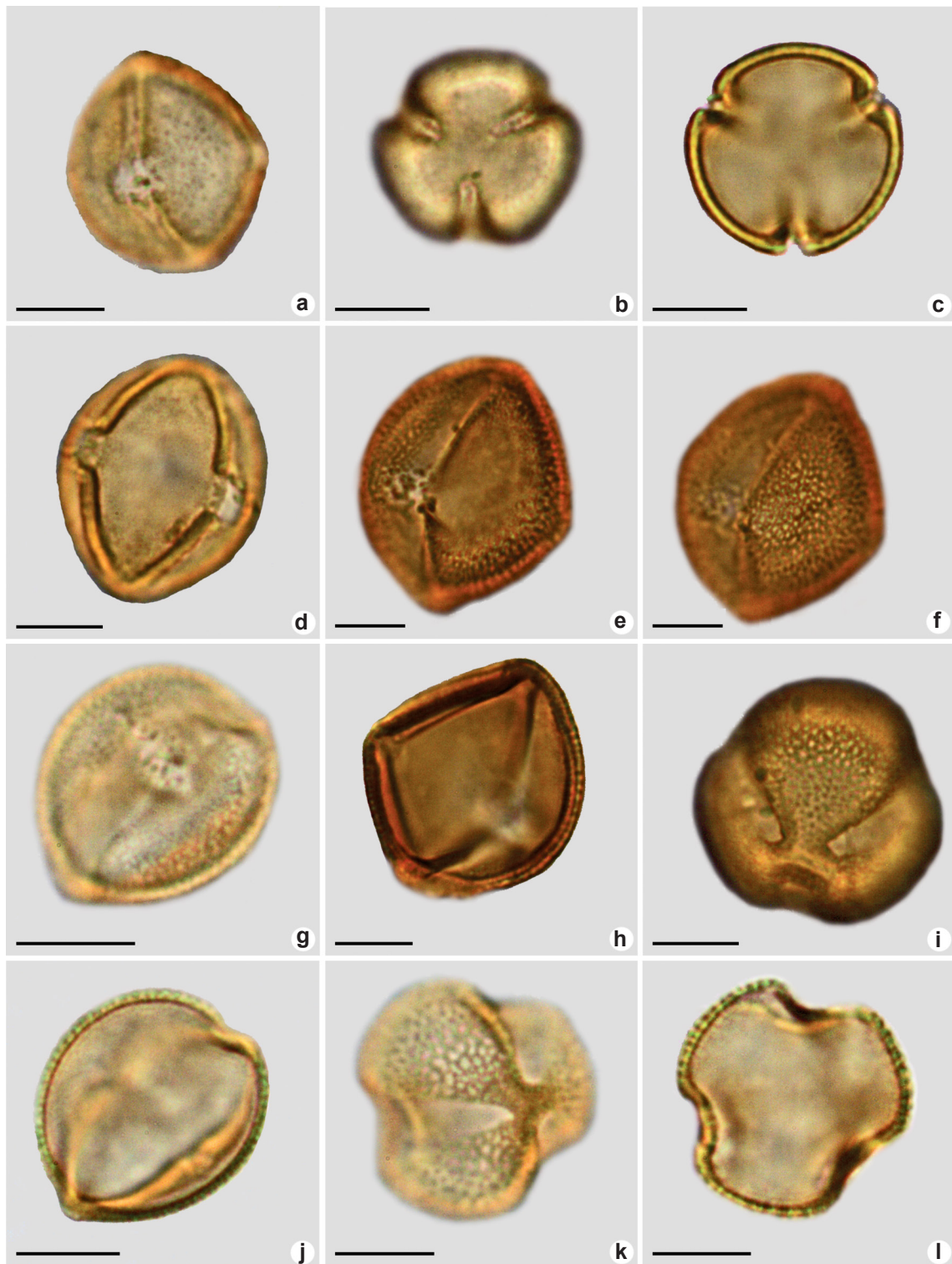


Figure 3 – a–l. LM micrographs of pollen grains from herbarium specimens – a–d. *Cynophalla retusa* (Capparaceae) – a,d. equatorial view, surface; b. polar view, surface; c. polar view, optical section; e–f, h–i. *Sarcotoxium salicifolium* (Capparaceae) – e–f. equatorial view, surface; h. equatorial view, optical section; i. polar view, surface; g,j–l. *Anisocapparis speciosa* (Capparaceae) – g. equatorial view, surface; j. equatorial view, optical section; k. equatorial view, surface; l. polar view, optical section. Scale bar = 10 μ m.

***Sarcotoximum salicifolium*.** Figs. 3e-f,h,i; 5f,h

Monad, tricolporate, spheroidal to perprolate, radially symmetric. Amb elliptic-subrhombic in equatorial view, circular in polar view. PD: 30–47 μm , ED: 21–37 μm . Colpus long with psilate aperture membrane, porus lalongate with granulate aperture membrane. Exine 2 μm thickness, semitectate, microreticulate. Sexine/nexine ratio 1:1. SEM: Observations coincide with those made with LM. Herbarium reference: Vossler s.n. (LP; BA).

Fabaceae, subfamily Caesalpinioideae.***Caesalpinia paraguariensis*.** Figs. 4a-c; 6c

Monad, tricolporate, suboblate to spheroidal, radially symmetric. Amb elliptic in equatorial view, circular in polar view. PD: 33–43 μm , ED: 38–48 μm . Colpus short up to 10 μm length, porus lolongate. Margo broad of up to 20 μm width, microreticulate. Exine 3 μm thickness, semitectate, reticulate. Columellae capitate, muri simplicolumellate. Sexine/nexine ratio 2:1. SEM: Microreticulate aperture membrane. Herbarium reference: Vossler s.n. (LP; BA).

***Parkinsonia praecox*.** Fig. 4d-h

Monad, tricolporate, spheroidal to subprolate, radially symmetric. Amb elliptic in equatorial view, subcircular in polar view. PD: 24–27 μm , ED: 20–22 μm . Colpus long with bridge, colpus diffuse. Margo broad of up to 8–10 μm width, scabrate. Exine 1.5–2 μm thickness, semitectate, reticulate. Columellae capitate, muri simplicolumellate, lumina irregular, smaller in diameter towards the aperture, lumina with bacula. Sexine/nexine ratio 2:1. Herbarium reference: Vossler s.n. (LP; BA).

Nyctaginaceae.***Pisonia zapallo* var. *guaranitica*.** Figs. 4i-l; 6d

Monad, tricolpate, suboblate to prolate, radially symmetric. Amb circular to elliptic in equatorial view, subcircular to circular in polar view. PD: 30–37 μm , ED: 23–34 μm . Colpus long with microgranulate aperture membrane. Exine 2 μm thickness, tectate, microechinate-microgranulate. Sexine/nexine ratio 1:1. SEM: Microechini and perforations irregularly distributed on the surface, with microechinate and microgranulate aperture membrane. Herbarium reference: Vossler s.n. (LP; BA).

Discussion

The present study shows the main characters found in the pollen foraged by stingless bees,

a group of bees versatile in pollen collecting behaviour, and a link between them is analyzed here.

Some morphological traits from a dataset of 37 pollen grains foraged by stingless bees in this forest have been assessed in a previous study (Vossler 2015). This study detected a positive correlation between volume and size of pollen cytoplasm and flower size, and similar findings were obtained for pollen associated with bat pollinated flowers (Stroo 2000). In the present study, the main pollen classes found in the nest storages of four stingless bees were monads, spheroidal cytoplasm shapes (P/E ratio), spheroidal and ellipsoidal cytoplasm shapes (geometric), small to medium cytoplasm sizes and volumes, tri-zono-colporate aperturate and meshed and psilate-softly ornamented. Although pollen shapes (P/E ratio and geometrics), sizes as well as number, position and form of apertures are primarily linked to harmomegathic effect whose main purpose is to protect the male gametophyte against desiccation during pollen presentation and dispersal, they are also often related to pollination biology (Pacini 2000; Edlund *et al.* 2004; Hesse *et al.* 2009). For instance, larger grains provide larger cytoplasm volume and consequently more nutrients for pollinators than smaller grains. And, the apertures are sites for germination of pollen tube that also function as sites for the entry of digestive enzymes and nutrient absorption for bee larvae (Danforth *et al.* 2019).

The main characters of pollen found in nest storages were here linked to a combination of intrinsic factors of stingless bees [small to medium cytoplasm pollen sizes and volumes, found usually in small flowers with which they are commonly associated (Ramalho 2004; Vossler 2015)] and the dominant floral morphology from different plant lineages of the woody flora of the Chaco dry forest (all pollen characters are possible, taxonomically related to plant origin). In the sites studied in the Chaco region (the “palosantal” area of the Dry Chaco and the “quebrachal” of the Transitional Chaco), most of the woody flora (trees and shrubs) have small, actinomorphic, pale coloured, open flowers with short or reduced perianth and easy access to pollen and nectar resources (generalised flowers), and produce a large number of flowers in a relatively short to moderate period of the year [see Appendix I in Vossler (2013)]. Among them are the Fabaceae (*Prosopis*, *Senegalia*, *Vachellia*, *Mimosa*, *Pterogyne*, *Gleditsia*, *Albizia*, *Enterolobium*, *Chloroleucon*), Celastraceae (*Maytenus*,

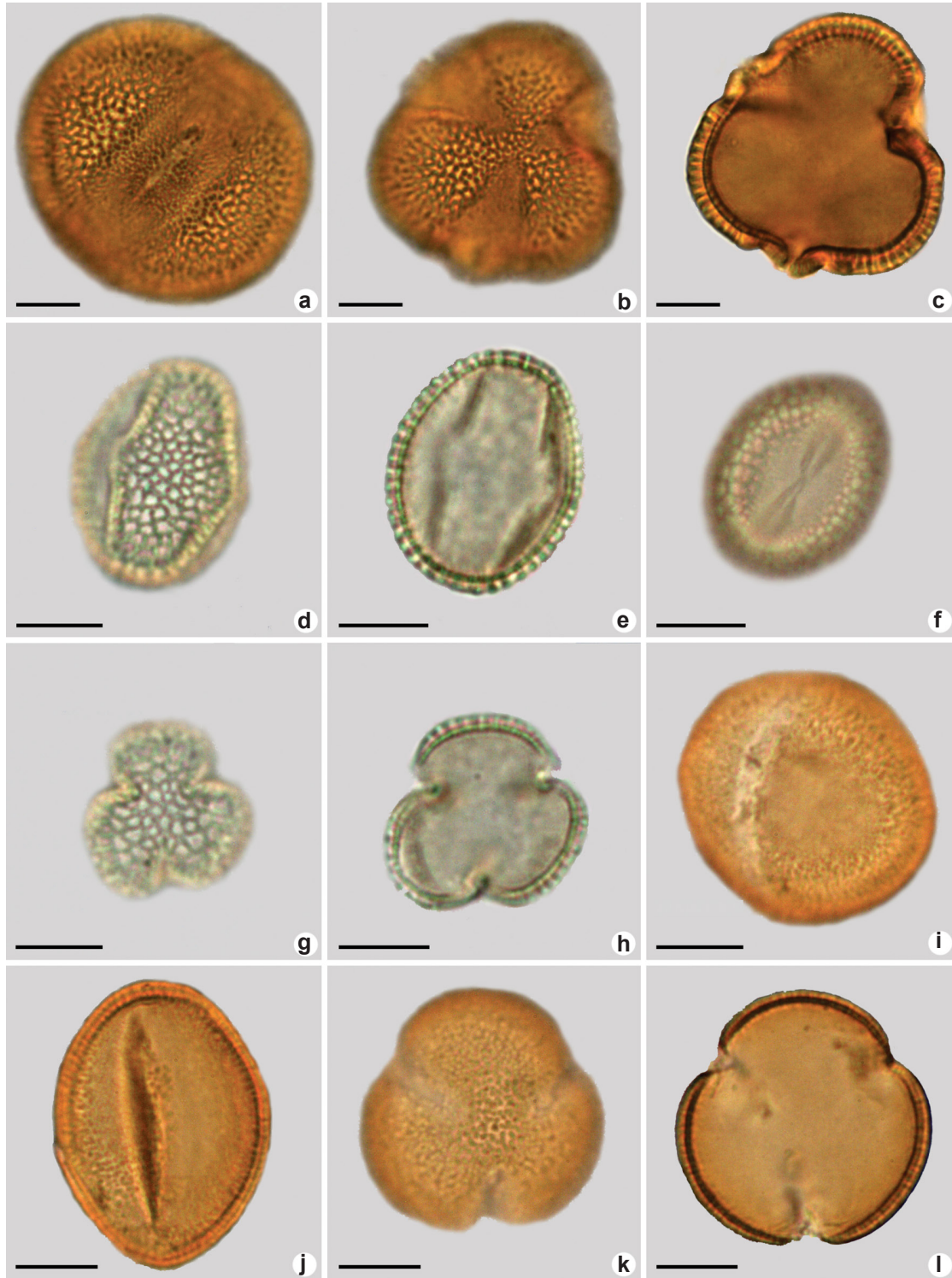


Figure 4 – a–l. LM micrographs of pollen grains from herbarium specimens – a–c. *Caesalpinia paraguariensis* (Fabaceae) – a. equatorial view, surface; b. polar view, surface; c. polar view, optical section; d–h. *Parkinsonia praecox* (Fabaceae) – d, f. equatorial view, surface; e. equatorial view, optical section; g. polar view, surface; h. polar view, optical section; i–l. *Pisonia zapallo* var. *guaranitica* (Nyctaginaceae) – i. equatorial view, surface; j. equatorial view, optical section; k. polar view, surface; l. polar view, optical section. Scale bar = 10 μ m.

Monteverdia), Rhamnaceae (*Sarcomphalus*), Simaroubaceae (*Castela*), Cannabaceae (*Celtis*), Solanaceae (*Lycium*), Anacardiaceae (*Schinopsis*, *Schinus*), Zygophyllaceae (*Porlieria*), Santalaceae (*Phoradendron*, *Jodina*), Loranthaceae (*Struthanthus*, *Tripodanthus*), Boraginaceae (*Cordia*), Arecaceae (*Trithrinax*), Euphorbiaceae (*Sapium*, *Jatropha*, *Croton*), Sapotaceae (*Sideroxylon*), Nyctaginaceae (*Pisonia*), Polygonaceae (*Salta*, *Coccoloba*), Achatocarpaceae (*Achatocarpus*), Cervantesiaceae (*Acanthosyris*), Sapindaceae (*Sapindus*, *Diplokeleba*), and Rutaceae (*Fagara*) [see Appendix I in Vossler (2013)]. The convergence of size, colouration and general configuration in flowers of diverse lineages in the Neotropics has been interpreted as diffuse coevolution (Wilms *et al.* 1997; Ramalho 2004) and the same seems to occur in the Chaco region (Vossler 2015). The small to medium size and volume of pollen grains have been related to small flower size (Vossler 2015), but the diversity of shape, some types of ornamentation and the apertures of pollen would be conservative traits (related to plant phylogeny). The small bodies of the stingless bees help to establish a tight association with these smallest flowers (Ramalho 2004; Vossler 2015). These generalised flowers are important for bees that store large amounts of pollen and honey in their perennial and highly populous colonies such as the stingless bees. For these reasons, a high diversity of pollen characters are possible among the resources foraged by stingless bees (mainly in relation to dispersal unit, P/E ratio and geometrical shapes, and number, position and form of apertures).

Among the behavioural aspects of stingless bees related to pollen morphology is their extreme versatility in the foraging process. Therefore, the high diversity of pollen characters found in the pollen diet of the four Chaquenan stingless bees is given by polylecty (innate behaviour of pollen collection from diverse plant families), in which is expected to found diverse general pollen morphology (Cane & Sipes 2006; Müller & Kuhlmann 2008). Furthermore, wet pollen load transportation (the usage of nectar to form humid packed pollen loads) enables transport on the corbicula a high diversity of sizes and sculptures of pollen grains (Thorp 1979, 2000). The illegitimate pollen collection from flowers allows to collect pollen directly from anthers with long filaments in flowers disproportionately large when comparing to stingless bee sizes (these flowers

commonly have medium to large pollen sizes and volumes) (Roubik 1989). Pollen stealing from other stingless bees (under stress conditions, intra- and interspecific stealing of pollen and honey from other bee nests might occur, collecting thus diverse general pollen morphology stored in nests of other stingless and even from *Apis* bees) (Sakagami *et al.* 1993). The recruitment of foragers results in pollen collection from a few floral resources from massive flowerings and consequently a few pollen types (and therefore a particular pollen morphology) predominates in nest stores (Lindauer & Kerr 1960). All these behavioural mechanisms together could help explain the high morphological diversity of pollen types found in nest stores, even from non-generalised and non-small flowers of the Chaco forest, such as those from Capparaceae (*Anissocapparis*, *Cynophalla*, *Sarcotoxium*, *Capparicordis*), some Fabaceae (*Caesalpinia*, *Parkinsonia*), and Zygophyllaceae (*Gonopterodendron*). Pollen collection from other non-small flowers of Solanaceae (*Solanum*) and Ximeniaceae (*Ximenia*) can be explained by the buzzing behaviour highly developed in *Melipona* [see Vossler (2019a)].

Among the stingless bee morphological attributes are the low hairiness of the legs which hinders the collection and/or transporting of spiny large to very large pollen grains which were rare among the samples here studied. However, it allows such processes in sticky pollens usually coated with pollenkitt, which is abundant in exines with ornamentation having mesh or holes (microreticulate, reticulate, striato-reticulate, foveolate, perforated) (Hesse 1981; Dickinson *et al.* 2000; Edlund *et al.* 2004) abundant among the pollen types foraged (Figs. 2-6). For instance, Hesse (1981) found that sticky pollen is frequent among entomophilous versus powdery pollen always in anemophilous plants, due to different distribution and consistency of the pollenkitt on the pollen surface and in relation to the fine structure of the exine. For this reason, exine structure and sculpture plays an important role in pollenkitt presentation for pollinators. However, other studies showed that also psilate grains with negligible amounts of pollenkitt have acquired stickiness by means of a different unique mechanism also related to entomophily (Weber & Halbritter 2007). Pollen without pollenkitt related to bee pollination was also documented in buzz pollinated flowers (Thorp 1979). Besides, these grains are usually small and unsculptured (*i.e.*, *Solanum*, Fig. 6e) and collecting bees have densely

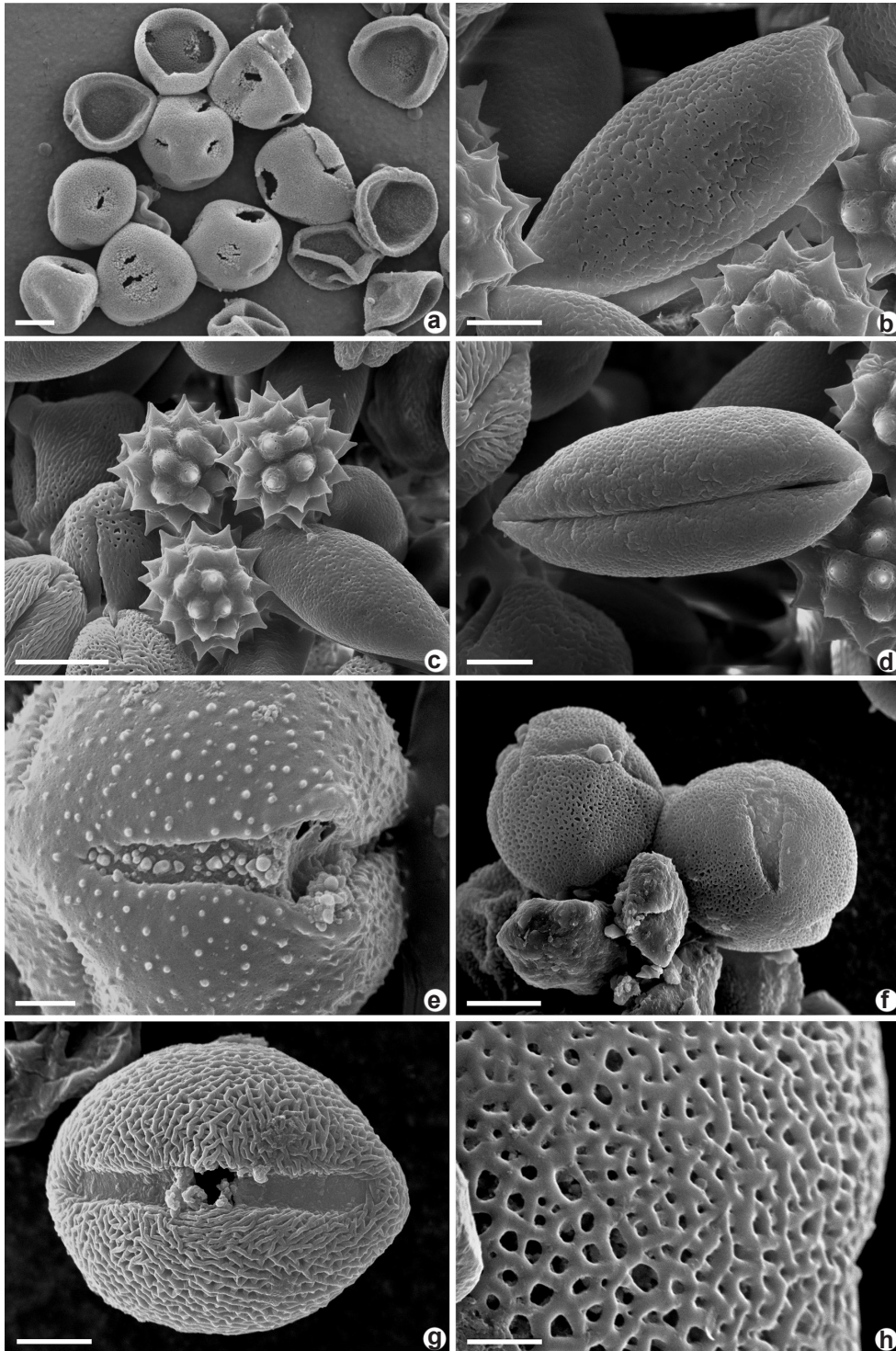


Figure 5 – a–h. SEM micrographs of pollen grains from herbarium specimens (except b, c and d from bee nests) – a. *Achatocarpus praecox* (Achatocarpaceae), general aspect of turgescient and infolded grains; b,d. *Trithrinax schizophylla* (Arecaceae) – b. equatorial view; d. distal polar view; c. *Parthenium hysterophorus* (Asteraceae), grains in different views; e. *Cynophalla retusa* (Capparaceae), oblique equatorial view; f, h. *Sarcotoximum salicifolium* (Capparaceae) – f. polar and equatorial views; h. surface detail; g. *Capparicordis tweediana* (Capparaceae), equatorial view. Scale bar: a, c, f = 10 μ m; b, d, g = 5 μ m; e, h = 2 μ m.

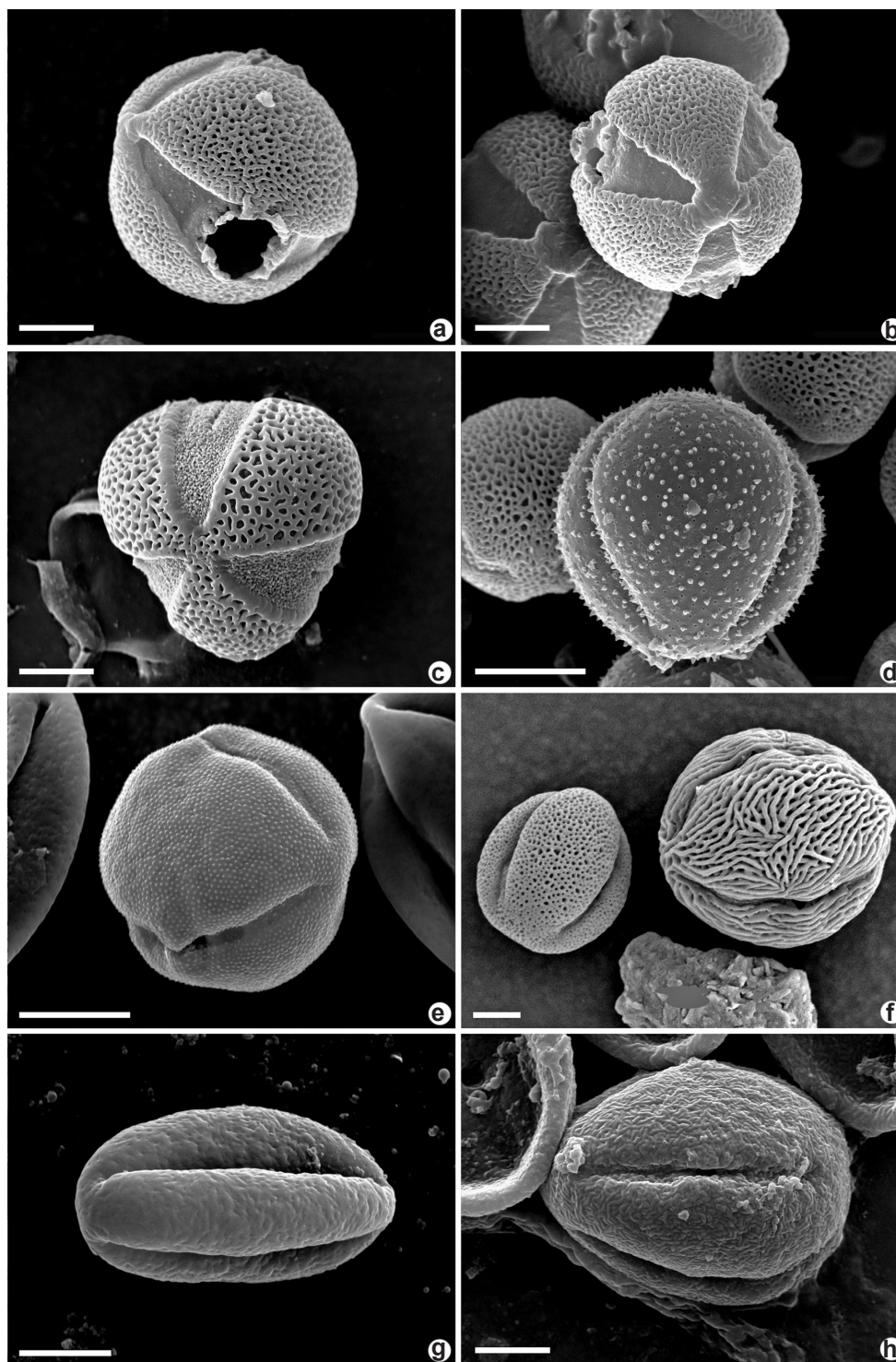


Figure 6 – a-h. SEM micrographs of pollen grains from herbarium specimens (except d, e, f and h from bee nests) – a-b. *Anisocapparis speciosa* (Capparaceae) – a. oblique equatorial view; b. polar view; c. *Caesalpinia paraguariensis* (Fabaceae) polar view; d. *Pisonia zapallo* var. *guaranitica* (Nyctaginaceae), equatorial view; e. *Solanum* (Solanaceae), oblique equatorial view; f. type *Maytenus* (Celastraceae) (1) and type *Schinopsis* (Anacardiaceae) (2), both in oblique equatorial view; g. *Pterogyne nitens* (Fabaceae), oblique equatorial view; h. *Sideroxylon obtusifolium* (Sapotaceae), equatorial view. Scale bar: a, b, f, g, h = 5 μ m; c, d, e = 10 μ m.

haired scopae or add oil or nectar to pollen loads (Thorp 1979, 2000). *Solanum* grains are heavily foraged by *Melipona*, a buzz pollinating stingless bee (Tab. S2, available on supplementary material <<https://doi.org/10.6084/m9.figshare.26956084.v1>>) (Ramalho *et al.* 1990; Vossler 2019a,b). Large and very large, pantoaperturate and spiny grains such as those of *Portulaca*, *Ipomoea*, Malvaceae *s.str.*, Cactaceae, Cucurbitaceae as well as the large grains with viscin threads from Onagraceae were avoided or rare components in the diet of these bees and seems to be atypically collected (Vossler *et al.* 2010). These grains are linked to particular configuration of pollen collecting hairs in disparate lineages of bees but absent in stingless bees (Thorp 1979, 2000; Michener 1999).

Some of the morphological characters here considered are among the commonly used for pollen description, but there were others which could not be observed as they are often greatly modified or disappear after acetolysis in laboratory such as wall coatings and pollen clumping. It is known that pollen treatments such as acetolysis difficult the finding of associations with pollinators and therefore is recommended studying pollen in its natural state (Ferguson 1985; Hesse 2000). Among the features that are lost after acetolysis are pollen coatings with viscous fluids such as pollenkitt, tryphine, elastoviscin, as they make it difficult to observe the sculpture and therefore taxonomic identification (Pacini 2000). Mechanisms of pollen clumping important for pollination are also lost, such as common walls in tetrads and polyads (Pacini 2000). Conversely, other features are resistant to acetolysis such as the viscin threads of Onagraceae, important for entangling pollen grains and adhering to pollinators (Hesse *et al.* 2000; Pacini 2000).

In an attempt to analyze some characters from non-acetolyzed and others from acetolyzed pollen, the main characters of pollen foraged were identified. They could either be due to selection by the bees, to the abundant availability of floral resources in the forest or both. To further the studies on pollen morphology associated with animal pollination, different combined laboratory treatments in the same set of pollen samples are needed, including different techniques such as fluorescence, staining and acetolysis as well as variants in samples preparation. These suggested palynological analyzes will deepen the knowledge of the interactions between plants and the varied clades of bees or other pollinators, and will allow

expanding the characteristics of the various kinds of specializations of bees for pollen plant-hosts.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that there is no data sharing of this manuscript.

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