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Full Paper

Structural implications for nectar secretion by nectaries of three columnar cacti

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Abstract: Floral nectaries are essential for plant reproduction. Their position and shape are important because these factors determine the amount of nectar secreted and therefore the pollinators that are attracted. The main objective of this study is to determine the position, shape and macromorphology of floral nectaries for three columnar cacti, namely *Polaskia chende*, *P. chichipe* and *Stenocereus quevedonis*. By means of light microscopy, scanning electron microscopy and histochemical tests, the floral nectar-secreting structures were investigated. Different secreting structures were found for the three species, with *S. quevedonis* having the largest nectar secreting area consisting of stomates located at the base of the stem filaments (1.9 mm^2). The nectar secretory pores of *P. chichipe* measured 0.93 mm^2, while the cuticular fissures of *P. chende* measured 0.8 mm^2. For the case of these three species, the surface available for nectar secretion appears to determine the volume of nectar secretion. The relationships between nectarial chamber size, total nectar volume secreted and size of secreting structure found for the three species suggest that the surface area available is the central influential variable that determines the volume of secreted nectar.

Keywords: floral morphology, Mexico, nectar, Pachycereae, reproductive ecophysiology, columnar cacti, *Polaskia chende*, *Polaskia chichipe*, *Stenocereus quevedonis*

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INTRODUCTION

The floral nectary is an anatomical feature of angiosperms with great evolutionary and ecological relevance because nectar, an aqueous solution primarily containing sugars, can mediate pollination [1-3]. The structure is characterised by a specialised epidermis and parenchyma cells that synthesise, accumulate and release the nectar solution [4]. Anatomical studies have shed some light on the structural and functional traits of floral nectaries, but the actual mechanisms of nectar unloading remains unclear [5,6]. In addition, their position in a flower can determine the nature of plant-pollinator interactions [7]. For instance, the flowers of *Dolichandra cynanchoides* and *Parabignonia chodatii* present nectaries on the outer or inner surface of the sepals that have no relation to pollination [8] while the positions of the nectaries of *Echinacea purpurea* and *Hymeneaea stigonocarpa* do promote pollination [9, 10].

The nectar of the flowers of columnar cacti (Family: Cactaceae) can be the main source of water and nutrients for pollinator communities in arid and semi-arid environments of the New World [11]. This is especially important in Mexico, where the ensuing fruits of most species of the mentioned columnar cacti are consumed by humans [12]. Thus, an understanding of the reproductive biology of these plants can have economical implications, especially when considering that the impending climate change could result in phenological decouplings of pollinators and flowers [3] or a decline of pollinators as it appears to be occurring at present [13].

To advance our understanding of the mechanism of nectar secretion in the columnar cacti, we study the floral anatomy of three columnar cacti whose fruits are edible. In particular, we determine the position, shape and macromorphology for the floral nectaries of the sympatric and congeneric *Polaskia chende* and *P. chichipe* as well as of *Stenocereus quevedonis* in order to gain insight into the mechanism of nectar secretion in these species. To our knowledge, this research represents the first contribution towards the understanding of nectary morphology in the Pachycereae subfamily.

MATERIALS AND METHODS

Plant Material

Flowers of *Polaskia chende* (Rol.-Goss.) A.C. Gibson & K.E. Horak and *P. chichipe* (Rol.-Goss.) Backeb. were collected on 5 May 2010 from a natural population located in San Luis Atolotitlán, Puebla, in the Tehuacán-Cuicatlán Biosphere Reserve (18°10'43" N, 97°26'38" W). This is a semi-arid region spanning 10,000 km^2, with an average annual rainfall of 400 mm and an annual mean temperature of 21°C [14]. The flowering for these species occurs during late winter and spring [15]. From a similar latitude, flowers of *Stenocereus quevedonis* (J. G. Ortega) Buxb. were collected on 15 May 2010 from a natural population located in La Pitirera, Michoacán, in the Zicuirán-Infiernillo Biosphere Reserve (18°18'45.34" N, 101°52'37.76" W). Also a semi-arid region, Infiernillo spans 2,651 km^2, with an average annual rainfall of 500 mm and an annual mean temperature of 28°C [16]. The flowering for *S. quevedonis* occurs from January to May [17]. In all cases, the plants were identified following Anderson [18].

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Anatomical Studies

The flowers, which were collected during anthesis, were fixed in formaldehyde: acetic acid: 96% ethanol: water (2:1:10:7) [19] and later examined with a stereo-microscope (Olympus SZX12, Japan) in order to locate the position of the nectaries.

Nectaries were dissected to conduct the anatomical studies described below. They were dehydrated in a graded series of ethanol (30, 50, 70, 85, 96 and 100%) for 2 hr in each step and finally in xylol for 40 min. before being embedded in paraplast paraffin. Longitudinal and transverse serial sections (9 µm thick) were obtained with a microtome (Spencer 820, American Optical, USA). The sections were stained with either safranine - Fast Green, periodic acid Schiff's reagent (PAS reaction) or Lugol's solution to induce a histochemical reaction. The tissue samples were observed under a microscope (Micron 1.07, Westover Scientific, USA).

Structural Studies

Flower tissue samples were dehydrated in the ethanol series as described above but with repeating the absolute ethanol step three times to complete micromorphological observations of the nectar-secreting structures. The dehydrated samples were then placed in a critical-point drier (AutoSamDri 815, Tousimis, USA), coated with gold and examined under high-vacuum with a scanning electron microscope (JSM-6360-LV, Jeol, Japan). Magnifications utilised were ×40, ×2,000 and ×15,000 for *P. chende*, ×50, ×200 and ×2,700 for *P. chichipe*, and ×40, ×500, ×3,500 for *S. quevedonis*.

Data Analyses

Statistical analyses were performed with SigmaStat (SPSS Science, Chicago, USA). Values of p are from pairwise Tukey's tests following one-way ANOVAs. Data are presented as mean ± 1 S.E. (n = sample size).

RESULTS

The flowers of *Polaskia chende*, *P. chichipe* and *Stenocereus quevedonis* were bisexual with an inferior ovary (Figure 1). The secretory tissue was located in the area where the receptacle differentiates from the ovary. The receptacle and the base of the anther filaments fuse to form a nectarial chamber, which makes up a secretory ring around the style.

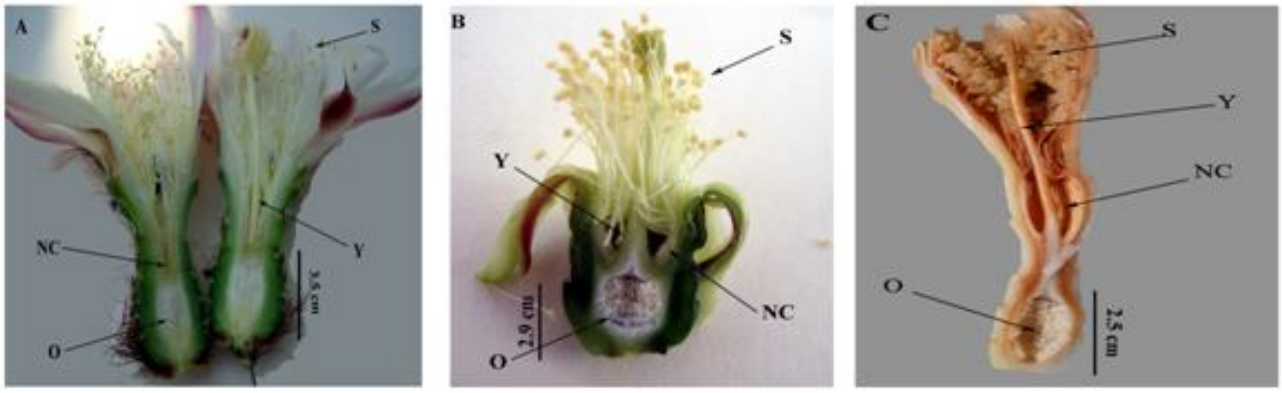

Figure 1. Floral anatomy for *Polaskia chende* (A), *Polaskia chichipe* (B) and *Stenocereus quevedonis* (C). For these three bisexual flowers, reproductive structures are labelled, i.e. nectariferous chamber (NC), ovary (O), stamens (S) and style (Y)

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The secretory tissue is composed of cells that have a large nucleus, a distinguishable nucleolus and dense cytoplasm (Figures 2A, D, G). This tissue also presents vascular bundles with both xylem and phloem branches (Figures 2A-I). In turn, the epithelial cells possess fewer cellular components (Figures 2B, C, E, F, H, I). The epidermis is composed of a layer of cubical cells while the sub-epidermal tissue consists of a variable number of layers of parenchyma cells without a specific arrangement.

The Lugol's stain reveals a greater accumulation of starch within the cells of the secretory tissue than in the rest of the floral tissues for all three species (Figures 2B, E, H). However, the frequency of the starch-containing cells varies among species, with *S. quevedonis* having the highest content and *P. chende* the lowest (Table 1). A similar pattern was found with the PAS reaction that stains the insoluble polysacharides of cell walls (Figures 2C, F, I).

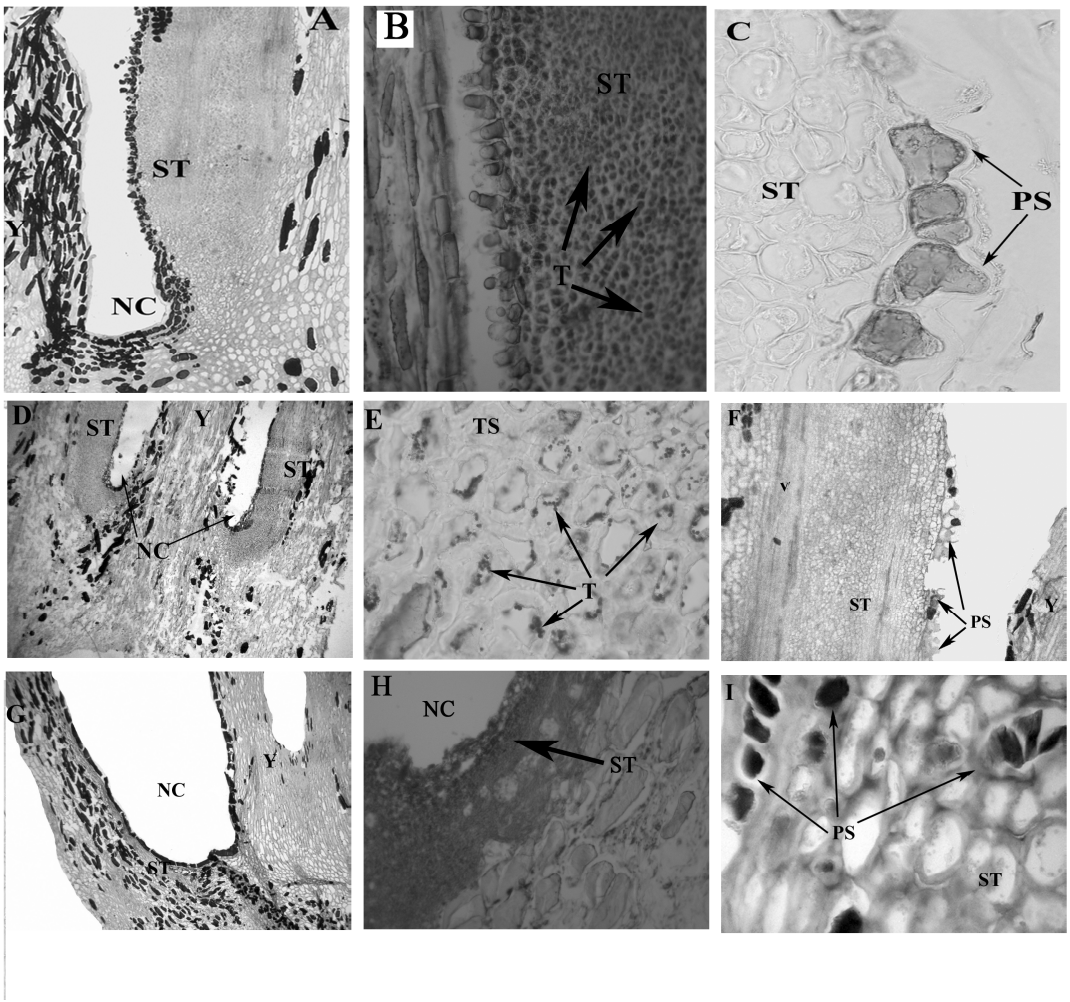

Figure 2. Nectary sections of *P. chende* (A, B, C), *P. chichipe* (D, E, F) and *S. quevedonis* (G, H, I). The structural features of the nectaries were identified with a safranine - Fast Green stain (A, D, G). Stored carbohydrates in the secretory tissue were identified with Lugol's solution (for starch: B, E, H) and with PAS reaction (for other insoluble polysaccharides: C, F, I). Structural features are nectariferous chamber (NC), insoluble polysaccharides (PS), stamens (S), nectar secretory tissue (ST), starch (T), style (Y) and vascular elements (V).

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The cuticular fissures of *P. chende* (Figures 3A-C) have the smallest area for nectar secretion among the cacti considered. For this species the cuticle fissures contain a total nectar secretory surface of 0.0836 mm^2 (Table 2). The pores of *P. chichipe* (Figures 3D-F) have the second largest secreting area (0.0936 mm^2). For this species, the pores have a 12% greater area than the fissures of *P. chende*. The flowers of *S. quevedonis* have stomates connecting the nectaries to the nectariferous chamber (Figures 3G-I). For this species, the total secreting area is 1.976 mm^2 (Table 2), the largest among the species considered.

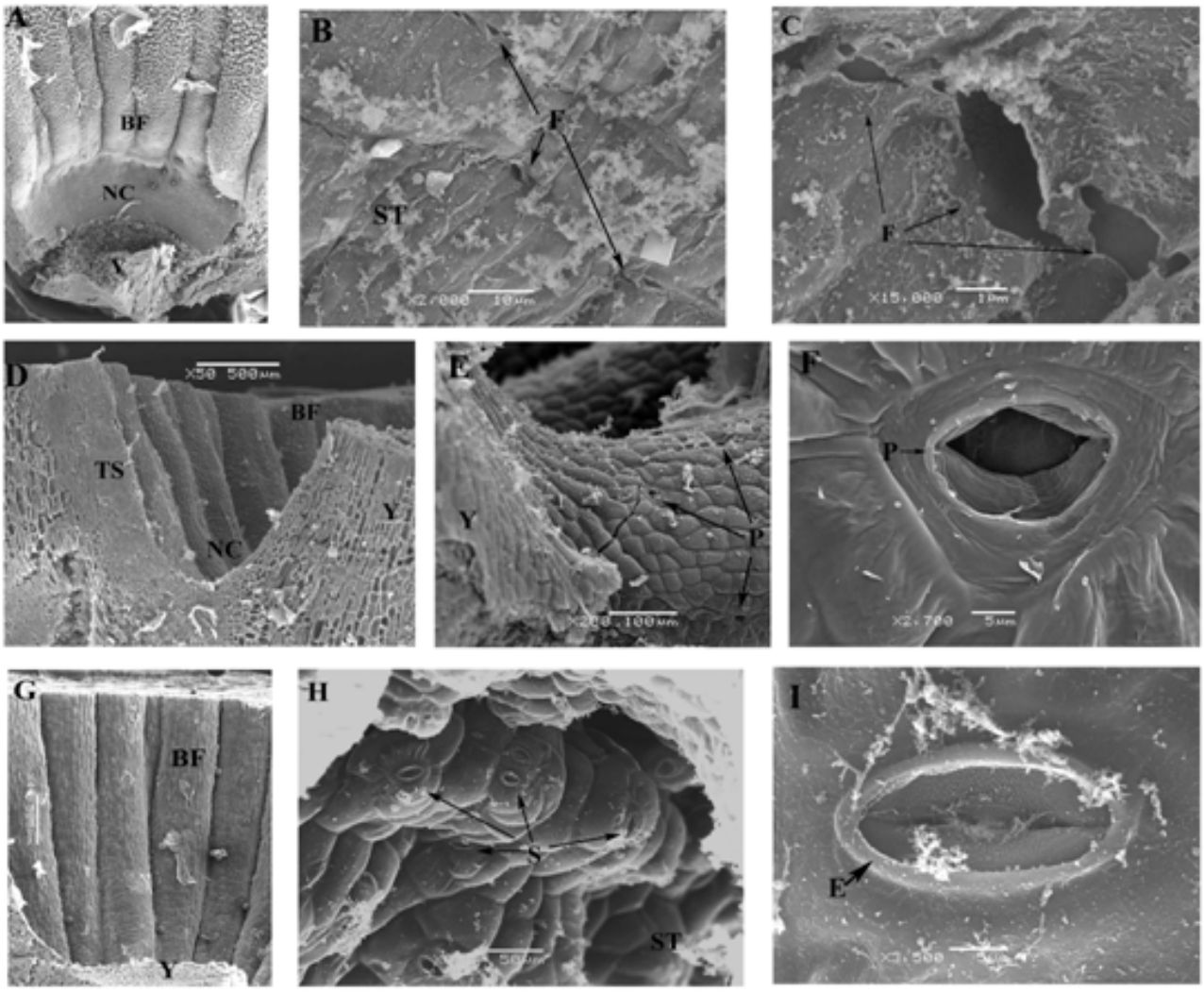

Figure 3. Micromorphological characteristics of the nectary secretory tissue in flowers of three columnar cacti. For *P. chende* (A, B, C), nectar is released through cuticle fractures (F); *P. chichipe* (D, E, F) presents secretory pores (P); and *S. quevedonis* (G, H, I) has stomates (S). Light-coloured debris observed in the micrographs is solidified nectar. Other structural features identified for reference are: base of filaments (BF), nectariferous chamber (NC), nectar secretory tissue (ST) and style (Y).

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Table 1. Anatomical characteristics of secretory tissue of three columnar cacti. Data are presented as mean ± 1 S.E. (n = 5 flowers from different individuals). Different letters in each column indicate statistical differences ($p< 0.05$) from Tukey test following one way ANOVA.

Species	Number of cell layers	Cell wall thickness (μm)	Nucleus diameter (μm)	Percentage of cells with starch	Percentage of cells with polysaccharides
Polaskia chende	7 ± 0.19^{a}	1.04 ± 0.02^{a}	3.1 ± 0.08^{a}	49 ± 0.43^{a}	11.5 ± 0.84^{a}
Polaskia chichipe	11 ± 0.17^{ab}	0.94 ± 0.03^{b}	1.9 ± 0.08^{b}	74 ± 1.07^{ab}	10.8 ± 0.53^{a}
Stenocereus quevedonis	18 ± 0.34^{b}	1.2 ± 0.03^{ab}	2.36 ± 0.13^{c}	91 ± 0.48^{b}	12.6 ± 0.94^{a}

Table 2. Frequency and size of secreting structures of three columnar cacti. Data are shown as mean ± 1 S.E. (n = 2 fields of view for each one of 5 flowers or 0.1 mm^2 per flower). Different letters in each column indicate statistical differences ($p< 0.05$) from Tukey test following one way ANOVA.

Species	Secretory structure	Structure frequency	Maximum length (10^{-3} mm)	Minimum length (10^{-3} mm)	Structure area (10^{-5} mm^2)	Total secreting area (mm^2)
Polaskia chende	Fissures	$37.7mm^{-2}$	0.0025 ± 0.15^{a}	0.00089 ± 0.04^{a}	0.0022 ± 0.19^{a}	0.0836^{a}
Polaskia chichipe	Pores	$7.5\ mm^{-2}$	0.0112 ± 0.22^{b}	0.0111 ± 0.30^{b}	0.0117 ± 0.48^{b}	0.0936^{a}
Stenocereus quevedonis	Stomates	$10.4mm^{-2}$	0.024 ± 0.61^{c}	0.0096 ± 0.13^{b}	0.1796 ± 4.54^{c}	1.9756^{b}

DISCUSSION

The position of nectaries for the cacti considered here, along with field observations of pollinator activity, confirms that these nectaries are involved in plant reproduction [15]. For the case of columnar cacti in Mexico, the importance of the interaction with pollinators has been widely documented [11-17]. This is important considering that not all floral nectaries are involved in pollination, as is the case for some species in the Bignoniaceae from Argentina whose floral nectaries play a protective role against herbivores [9].

For the three species in this study, the nectar secretory tissue was found at the base of the anther filaments of their bisexual flowers. Such a pattern, known as a disk, is most prevalent among the middle and upper levels in the evolution of dicots [4, 8, 9, 20]. Solutes are delivered to the nectary tissue where they are stored as different polysaccharides, as observed here for the cells containing starch and other insoluble polysaccharides. The calcium oxalate crystals found near the vascular bundles in the nectaries of *S. quevedonis* are quite common in nectaries vascularised by phloem [11, 21].

The nectaries are easily distinguishable from the surrounding parenchyma for the three cacti. In particular, multiple cell layers of thickened cell wall and a conspicuous nucleus were observed. These anatomical characteristics of nectary cells have also been found for other species whose nectary cell walls are thicker than those of other floral tissues [4, 8, 21-25].

Whether the presence of thickened cell walls such as those found here for cactus nectaries enables the floral tissues to withstand the very high hydrostatic pressures resulting from water displacement in response to solute accumulation requires further investigation [1]. However, hydrostatic pressures measured inside of storage cells can exceed 2 MPa while the normal turgor

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pressure for a cactus cell is in the order of 0.15 MPa [26, 27]. Concerning the conspicuously enlarged nuclei of the nectary tissue, a basic observation in cell biology is that a cell nucleus is only visible when it is undergoing intense metabolic activity, generally related to mitosis [28].

Based on the anatomical and macromorphological evidence gathered in the present work, the secreting of nectar to the nectariferous chamber occurs directly through fissures for *P. chende* while for *P. chichipe* and *S. quevedonis*, this occurs through pores and stomates respectively. This study documents nectar secretion through fissures and pores in the Cactaceae for the first time. For *Mammillaria san-angelensis*, *Opuntia tomentosa* and *Pereskia lychnidiflora*, the few cacti whose nectary structure has been studied, stomates release the nectar solution into the nectariferous chamber [24]. For female and male flowers of *Cucurbita pepo*, nectar secretion also occurs through stomates, although nectar release through cuticle fissures has also been observed [29]. For the three species in this study, nectaries are highly vascularised, similar to the case of *Mammillaria san-angelensis* and *Opuntia tomentosa* [24].

With respect to nectar secretion, a positive correlation between floral length and volume of nectar solution produced has been documented [23]. The species considered in this study appear to follow a similar pattern with nectar secretion responding to the total area of the secretory tissue. Thus, the nectarial chamber of *P. chichipe* yields 13 µL/day and that of *P. chende* yields 9 µL/day, while the much larger nectarial chamber of *S. quevedonis* yields 2 mL day^{-1} [30].

The relationships found between nectarial chamber size, total nectar volume secreted, and size of secretory structures in *P. chende*, *P. chichipe* and *S. quevedonis* suggests that structural constraints contribute to the volume of nectar secretion. However, information about the structure and micromorphology of cactus nectaries remains scant.

CONCLUSIONS

The nectary location and secretory structure have been studied for three columnar cacti, namely *Polaskia chende*, *P. chichipe* and *Stenocereus quevedonis* by identifying a relationship between the nectary structure, the area of nectary chamber and the volume of nectar solution produced.

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