



Pollen release mechanisms and androecium structure in *Solanum* (Solanaceae): Does anther morphology predict pollination strategy?



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ABSTRACT

Most species of *Solanum* L. (Solanaceae) exhibit a floral morphology typical of the genus: Yellow poricidal anthers with rigid walls contrasting in color with the corolla. However, some species of *Solanum* sect. *Cyphomandropsis* differ from most of the other species of *Solanum* by having flowers without contrasting colors and large anthers with flexible walls. These features resemble those of some closely related species belonging to *Solanum* sect. *Pachyphylla* that exhibit a bellows pollination mechanism whereby male euglossine bees cause the compression of thin anther walls and trigger pneumatic pollen release without vibration. Herein we studied the reproductive and pollination biology of a population of *Solanum luridifuscens* (sect. *Cyphomandropsis*), a species with purple corolla and anthers, expecting to find a bellows mechanism of pollination. Both artificial mechanical stimuli applied with forceps and vibrations transmitted with an electric toothbrush resulted in the release of pollen from the anthers. Females of six species of bees visited the flowers and vibrated the anthers to collect pollen. Only one male euglossine bee visited the flowers, without causing pollen release. *Solanum luridifuscens* exhibits pollen flower characteristics that are common among species of *Solanum*, such as the absence of nectar and the presence of poricidal anthers that release dry pollen by vibration. However, it also exhibits features that resemble mature anthers of perfume flowers typical of *Solanum* sect. *Pachyphylla*, such as a dorsally developed connective covered with purple papillae that do not contrast with the corolla plus flexible anther walls that are two or three cell layers thick on the lateral and ventral surfaces. Despite this distinct anther morphology, pollen of the flowers of *S. luridifuscens* was removed exclusively by female bees using a typical vibratory mechanism. Morphological traits alone, thus, are not sufficient to predict the pollination mechanism of species of *Solanum*.

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1. Introduction

Adaptive radiation involving different pollinators has been one of the most important processes in speciation, diversification and evolution of angiosperms (Friis et al., 1987; Proctor et al., 1996; Hodges and Derieg, 2009). This is particularly true for Neotropical Solanaceae (Cocucci, 1999). In the genera *Calibrachoa* Cerv., *Lochroma* Benth., *Nicotiana* L. and *Petunia* Juss., for example, there are species that are pollinated by bees, hawkmoths and hummingbirds (Cocucci, 1999; Smith et al., 2008; Stehmann et al., 2009; Gübitz et al., 2009; Knapp, 2010). In an evolutionary context, morphological adaptations associated with different pollinators

have been considered homoplastic in Solanaceae (Knapp, 2010), although members of the subfamily Solanoideae, which includes the speciose genus *Solanum* L., are specialized for bee pollination.

Characterized by the absence of nectaries, the flowers of *Solanum* offer only dry pollen stored in rigid, thick poricidal anthers, which are usually bright yellow and contrast with the color of the petals (Vogel, 1978; Endress, 1994). Female bees of certain species (Augochlorini, Bombini, Centridini, Euglossini, Xylocopini, among others) vibrate anthers to collect pollen grains. In this type of pollen removal, bees bend their body over the apex of the anther cone and, grasping it firmly, vibrate the flowers by contraction and relaxation of alar muscles at a frequency of about 50–2000 Hz (Buchmann, 1983; De Luca and Vallejo-Marín, 2013). An audible sound is emitted while a cloud of pollen is released from the apical pores of the anthers. Pollination resulting from this process has been termed “buzz-pollination” (Buchmann, 1983).

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An alternative pollen release mechanism evolved in *Solanum* sect. *Pachyphylla* (Dunal) Dunal, which encompasses the former genus *Cyphomandra* L. (Bohs, 1995). In the so-called bellows pollination mechanism, pollen grains are pneumatically released due to the collapse of placentoid tissue and thin walls of the anthers. Just before anthesis the placentoid tissue collapses and the anthers are filled with air. By slight mechanical pressure on anther walls, which act as elastic membranes, male euglossine bees cause the release of pollen since part of the air contained in the anthers is expelled and carries pollen with it (Sazima et al., 1993).

The flowers of sect. *Pachyphylla*, which exhibit this bellows mechanism, possess anthers that do not contrast in color with the perianth. The connective is broadened (Sendtner, 1845), has a papillose surface and functions as an osmophore, producing volatile substances that attract male bees of the tribe Euglossini (Apidae) (Soares et al., 1989; Gracie, 1993; Sazima et al., 1993). While scraping the glandular surface to collect perfumes, the male bees cause the compression of the anthers and the expulsion of pollen, which adheres to their body and is, thus, available for pollination. Although male perfume collectors are the primary pollinators, females that collect pollen by vibration are also found on flowers, albeit sporadically (Sazima et al., 1993). In addition to acting as the predominant attractant, floral perfumes are the only resource pursued for male euglossine bees in these nectarless flowers.

Bellows-like mechanisms also have been described in some genera of Melastomataceae. The inconspicuous flowers of *Blakea* release clouds of pollen after being pressed by rodents searching for its copious nectar (Lumer, 1980). In *Axinaea* a bulbous aerenchymatous appendage, derived from connective tissue, causes flushes of air to release pollen onto pollinating birds. The appendage also functions as a hexose-rich, highly nutritious food body for the pollinating passerines (Dellinger et al., 2014). As observed in Solanaceae, these bellows-like mechanisms evolved in poricidal anthers in a family where buzz-pollination is the typical pollen removal strategy.

The sect. *Pachyphylla* is phylogenetically related with the sect. *Cyphomandropsis*, both placed within the clade *Cyphomandra*, which contains about 50 species of exclusively Neotropical shrubs or small trees, with its most consistent morphological synapomorphy being the presence of large chromosomes and large amounts of nuclear DNA (Bohs, 1994, 2001, 2007; Weese and Bohs, 2007). These two sections are distinguished morphologically mainly in characters of the connective of the anthers. Unlike *Solanum* sect. *Pachyphylla*, species of *Solanum* sect. *Cyphomandropsis* do not have such well-developed connectives, however, some of them exhibit characteristics similar to members of sect. *Pachyphylla*. Passareli and Cocucci (2006b) studied the anther ontogeny of three species of *Cyphomandropsis*, two with thin, flexible walls at maturity (*Solanum confusum* C.V. Morton and *Solanum stuckertii* Bitter) and one with thick, rigid walls (*Solanum glaucophyllum* Desf.). *Solanum confusum* and *S. stuckertii* have developed connectives with glandular characters, but without papillate cells (Passareli and Bruzzone, 2004; Passareli and Cocucci, 2006b). In the three species, the placentoid does not stay expanded during anthesis, as usual in thicker anthers (four or more cell layers), but presents collapsed at anthesis time (Passareli and Cocucci, 2006b), similar to the species of *Solanum* sect. *Pachyphylla* that generally exhibit the bellows pollination mechanism (Sazima et al., 1993). Passareli and Cocucci (2006a) reported that anthers with single-layered thecae become temporarily clogged and that strokes with a forceps on the anthers are needed to allow the extractions to continue, suggesting that the pneumatic mechanism may play a simultaneous role with the vibratory mechanism by un-obstructing the pores of the anthers and thereby allowing the division of pollen among pollinators with different abilities. Despite these similarities, the pneumatic pollination mechanism has yet to be documented operating in nature in

species of sect. *Cyphomandropsis*. To date, floral visitors have been recorded for only two Argentine species of this section, *S. stuckertii* and *S. glaucophyllum*, both of them pollinated by bees that vibrate flowers to collect pollen (Passareli and Bruzzone, 2004).

In this study, we investigated the floral biology, reproductive system, pollen release mechanism, anther wall structure and pollination of *Solanum luridifuscescens* Bitter, a species belonging to sect. *Cyphomandropsis* (Bohs, 2001). The flowers of the studied population possess anthers with flexible walls covered by purple papillae that do not contrast with the corolla, resembling those of sect. *Pachyphylla*. Thus, we tested the hypothesis that the flowers of *S. luridifuscescens* are mainly pollinated by perfume collecting male euglossine bees and that the pollen dispersal mechanism functions via pneumatic anthers and resembles the bellows-like mechanism found in *Solanum* sect. *Pachyphylla*.

2. Methodology

2.1. Study area and population

The study was conducted in the Reserva Particular do Patrimônio Natural (RPPN) Mata do Sossego, a private nature reserve located in the “Zona da Mata Mineira”, in the municipalities of Simonésia and Manhuaçu in the state of Minas Gerais, Brazil, situated 324 km from the state capital Belo Horizonte. Being part of the Atlantic Rainforest, the vegetation is characterized as a Seasonal Semideciduous Tropical Montane Forest (Veloso et al., 1991). Monthly mean temperature and rainfall vary from 13 to 17 °C and 10 to 17 mm, respectively, during the cooler, dry season (April to September), and from 22 to 26 °C, and 103 to 283 mm, respectively, during the warmer, humid season (October to March) (Fundação Biodiversitas, 2013).

Plants of *Solanum luridifuscescens* grow in natural forest clearings, which are difficult to access because of the steep terrain. The study population is close to the scenic overlook of the reserve, and is scattered around the “Trilha das Hortências”. It consists of 12 shrubs up to 2.5 m in height, at an elevation of approximately 1540 m (S 20° 04' 05.2"/W 42° 05' 18.6"). The plants were studied through two flowering seasons between May 2013 and December 2014.

2.2. Floral biology, reproductive system and pollen release

Flower opening was determined for 40 marked flower buds at intervals of one hour, and duration of anthesis was determined by monitoring the flowers until senescence. The reproductive system was assessed through controlled pollination experiments with four treatments on 12 individuals each (48 flowers): 1-hand cross-pollination; 2-hand self-pollination; 3-spontaneous self-pollination; and 4-control. For hand cross-pollination and hand self-pollination, flower buds were bagged until the day of opening, at which time the bags were removed and the flowers pollinated between 1500 h and 1700 h and then re-bagged. For spontaneous self-pollination, flower buds were maintained bagged on each individual throughout anthesis. The un-bagged control flowers, which were accessible to visitors, were marked on each individual plant.

To remove pollen from the anthers, flowers of six individuals on the first day of anthesis were held parallel to a flat surface and the anthers were compressed with forceps to test for the pneumatic mechanism of pollen release (N = 17). In another 17 flowers pollen was extracted using an electric toothbrush (Dontodent, mod. 4695, 2.4–3 v, manufactured in Switzerland, frequency 50–60 Hz) with a pin attached to the end such that the vibrating pin simulates the visit of a vibrating bee.

Pollen was transferred to the stigma until the entire stigmatic region remained covered. For hand cross-pollination, five

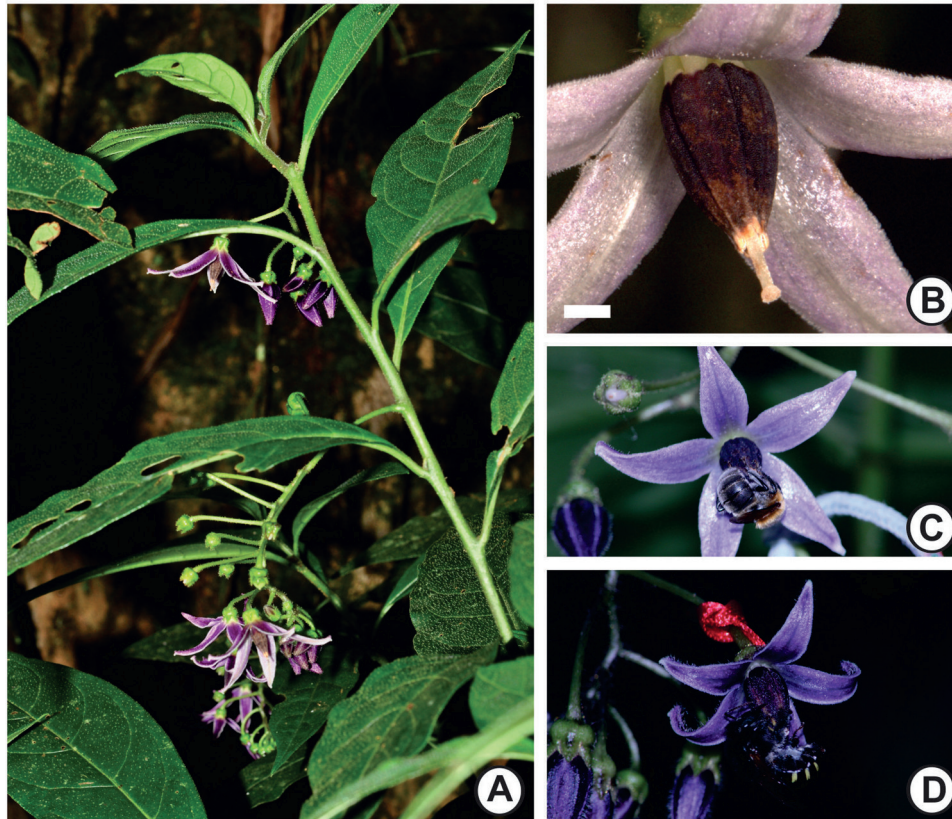


Fig. 1. *Solanum luridifuscescens* Bitter in RPPN Mata do Sossego, Simonésia, Minas Gerais, Brazil. (A) Fertile branch. (B) Details of a mature flower (scale bar 2 mm). Purple papillae on the abaxial surface of the anthers. Injury caused by the visitors can be seen as necrotic marks mainly in the apex of the anther cone. Glabrous and cylindrical style and truncate stigma. (C) *Melipona bicolor*. (D) *Melipona quadrifasciata*.

individual pollen donors were used for each recipient. The quantity of fruits produced under natural conditions (control flowers) was compared to that of the bagged flowers.

Stigmatic receptivity was tested in flowers of nine individuals from the 1st through the 4th day of anthesis (10 flowers each day, totaling 40 flowers) by their reaction with 0.25% potassium permanganate solution (Robinson, 1924). The flowers were submerged in the solution for 10 min, washed with distilled water, dried on filter paper and the stigma observed with a magnifying glass. Stigmas stained brown were considered receptive.

2.3. Anther wall structure

For the study of the anatomical characteristics of anther walls we used light microscopy. Samples collected at mature stage (pre-anthesis flower buds) were fixed in Karnovsky solution (Karnovsky, 1965), dehydrated in an ethanol series, and embedded in hydroxy-ethyl-methacrylate resin (Leica). Transverse and longitudinal sections (5 μ m) were obtained using a microtome and stained with 0.05% Toluidine Blue, pH 4.3 (O'Brien et al., 1964).

2.4. Floral visitors

The observation and collection of bees was performed during five four-day periods (November/2013; January, September, November and December/2014), between 0700 h and 1500 h, for a total of 160 h over 20 days of sampling. To determine the relative frequency of visits by bee species, we counted flower visits and calculated the percentage from the total number of visits of all bees (N = 1095 visits) during the whole sampling period.

The attractiveness of flowers was evaluated in a single plant throughout anthesis by determining the average number of visits of the most common bee species (*Melipona bicolor*) at one-hour intervals on marked flowers from the 1st to through the 4th day of anthesis. Two flowers at each day of anthesis (total of eight flowers) were marked per observation day. The test was repeated using different flowers for four more days for a total of 40 flowers. Unmarked flowers were not removed in order not to interfere with the attractiveness of the plant as a whole. To determine the effect of floral longevity (i.e. days of anthesis) on the average frequency of visits, we analyzed a one-way ANOVA. We tested normality and homogeneity of data by means of Shapiro–Wilk's and Levene's tests, respectively. ANOVA was conducted using Software PAST for Windows, version 3.12.

The behavior of bees during flower visits was analyzed through direct-observation, videos and photographs taken with a digital camera. Collected bees were deposited in the entomology collection of Universidade Federal de Minas Gerais (UFMG). Voucher specimens of *Solanum luridifuscescens* were deposited in the herbarium of UFMG (BHCN 175529 and 175534).

In order to confirm the presence of euglossine males in the study area, scent traps were used during one of the sampling periods following an adaptation of the methodology of Campos et al. (1989). The traps were made from two-liter PET bottles with two funnel-shaped side openings. The scent-baits, cotton wads impregnated with different compounds (eucalyptol, eugenol, linalool and vanillin), were placed inside bottle-traps, which were hung in the surroundings of a plant. The plants were also observed during three nights to assess the presence of nocturnal flower visitors.

Table 1
Pollination experiments conducted with *Solanum luridifuscescens*.

Treatments	No flowers	No fruits	%
Spontaneous auto-pollination	12	0	0
Manual auto-pollination	12	0	0
Control (accessible flowers)	12	1	8.3
Manual cross-pollination	12	4	33.3

3. Results

3.1. Floral biology and reproductive system

The inflorescences of *Solanum luridifuscescens*, branching systems with scorpioid monochasia, produced a mean of 15 ± 4.4 hermaphroditic flowers ($N=36$ inflorescences, three per individual) (Fig. 1a). Up to four flowers opened simultaneously in an inflorescence. After the opening of the first flower, there was an interval of at least two days until the opening of the next flower of the same inflorescence. The flowers are actinomorphic and pentamerous, and the petals have an acute apex and a small ventral subapical projection. The corolla is star-shaped, subcoriaceous, and purple, with petals having whitish margins and a whitish midrib. The five poricidal anthers are connivent and lanceolate, and the connective is well developed and covers the entire dorsal surface of the theca. The epidermis of the connective shows purple papillae, giving the abaxial surface of the anther a warty appearance (Fig. 1b). The flowers have a very weak sweetish scent to the human nose.

Flowering occurred between September and February, and most of the flowers (29 flowers, 73%) started anthesis between 0600 h and 0800 h, with the other buds opening throughout the day. The flowers closed every night and lasted on average 5 ± 0.5 days. Flower closing and reopening lasted two to three hours between 1600 h and 1900 h, and 0400 h and 0700 h, respectively. At the beginning of anthesis, the petals were short and reflexed, becoming elongate and less arched as anthesis proceeded. All anther pores opened during the first three hours of anthesis, but the opening process lasted only until 1500 h on cold days.

Flowers exhibited style growth throughout anthesis. The distance between the stigma surface and the anther pores was short (about 2 mm) in fully developed styles. On the 5th day, the petals withered and fell off, along with the androecium, whereas the style remained attached to the ovary for a day or two before falling off. From the 3rd day of anthesis, the corolla, which had at first been purple, became whitish. Except for the first three hours of anthesis (3 flowers, 8%), the stigmas stained intensely brown with potassium permanganate solution, indicating that the stigmatic surface became receptive already during the first day of anthesis and maintained viability throughout the rest of anthesis. The pollination experiments demonstrated that the species is self-incompatible, and only set fruits after hand cross-pollination and bee visits (Table 1).

3.2. Floral visitors

Females of six bee species visited the flowers of *S. luridifuscescens* and vibrated the anthers to remove pollen (Fig. 1c and d): *Augochlora* sp., *Augochloropsis* sp. (Halictidae, Augochlorini), *Bombus brasiliensis* Lepeletier (Apidae, Bombini), *Melipona bicolor* Lepeletier and *Melipona quadrifasciata* Lepeletier (Apidae, Meliponini), and *Thygater paranaensis* Urban (Apidae, Eucerini). Worker bees of *M. bicolor* were the most frequent flower visitors (58.5% of the visits), followed by *M. quadrifasciata* (20.0%) and *Augochloropsis* sp. (14.0%) (Fig. 2). Moreover, one male of *Eulaema nigrita* (Apidae, Euglossini) visited five flowers of a single plant. The

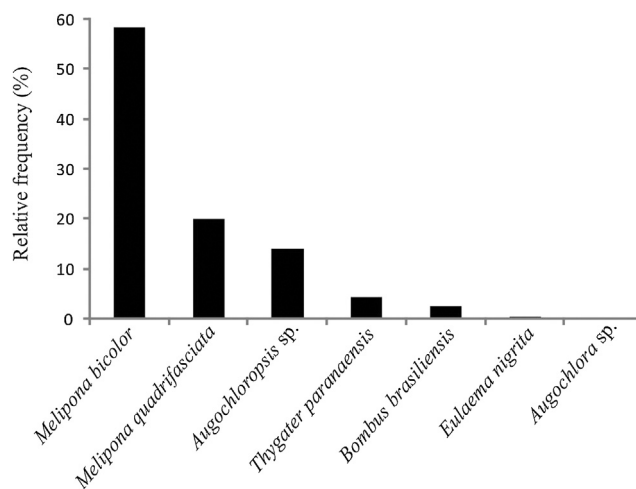


Fig. 2. Relative frequency of flower visiting bees of *Solanum luridifuscescens* based on a total of 1095 visits.

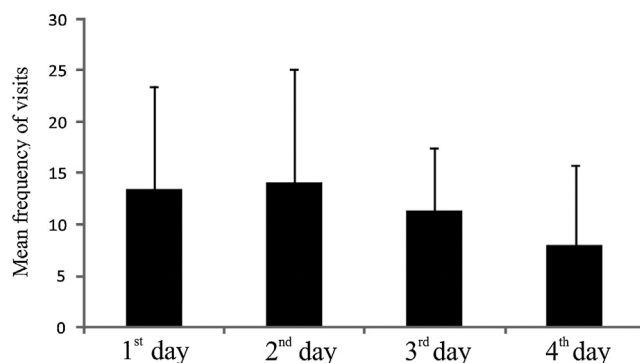


Fig. 3. Attractiveness of flowers of *Solanum luridifuscescens* to visitors from the first to the fourth day of anthesis. Bars represent mean frequency (with standard deviation) of visits of *Melipona bicolor* based on a total of 471 visits to 40 flowers.

bee landed on the flowers and inserted its head into the short floral tube, but no pollen was released during these visits.

Floral visits began at 0700 h, coinciding with the beginning of anthesis and the reopening of flowers on day two through four of anthesis. Females of all vibrating species clung to the five anthers with their legs and vibrated them simultaneously. They landed upside down, perpendicular to the flower axis, curved their body, clung to the apex of the cone using their three pairs of legs, curled-up on the stigmatic surface, and vibrated the flowers while at the same time the ventral part of their body touched the stigma. All species commonly rotated their body on the anther cone in-between vibrations.

During these movements the stigma touched the ventral surface of the foragers, as well as the internal parts of their legs, where pollen adhered after vibrations. The average number of flower visits per plant was: *Melipona bicolor* 16.3 ± 8.4 ($N=10$); *Melipona quadrifasciata* 16.4 ± 8.3 ($N=10$); *Thygater paranaensis* 12.5 ± 6.7 ($N=10$) and *Augochloropsis* sp. 2.6 ± 1.3 ($N=10$). Females of *Augochlora* sp. and *Bombus brasiliensis*, as well as the single euglossine male, were only sporadic visitors of the focal plants.

We did not detect any significant difference in the frequency of bees visiting flowers on the 1st, 2nd, 3rd and 4th day of anthesis ($F_{3,16} = 0.46$, $p = 0.714$; Fig. 3). However, we did detect a decrease in the frequency of visits over time (mean \pm SD: 13.5 ± 10.0 and 8.1 ± 7.8 for 1st and 4th day of anthesis, respectively). The flower visits were rather uniformly distributed during the day until 1500 h (Fig. 4). Males of three euglossine bees were captured in traps baited

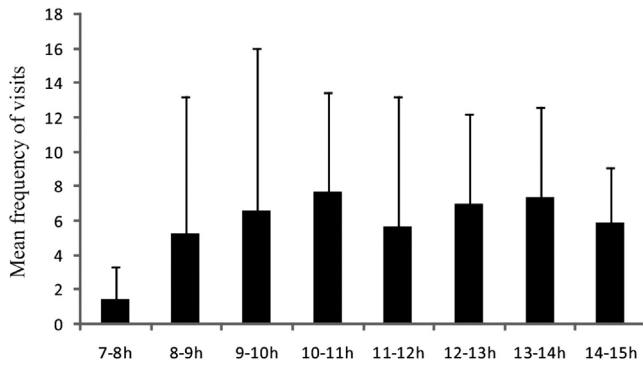


Fig. 4. Mean frequency (with standard deviation) of visits by workers of *Melipona bicolor* to flowers of *Solanum luridifuscescens*, per hour throughout the day (N = 5 days; 40 flowers, 10 each stage: 1st to the 4th day of anthesis).

with eucalyptol, eugenol and vanillin: *Eufriesea violacea* Blanchard (3), *Eulaema nigrita* Lepageletier (6) and *Eulaema cingulata* Fabricius (2). No floral visitors were found at night.

3.3. Anther wall structure

The connective is more strongly developed on the dorsal side of the anthers and the epidermis covering the connective tissue is distinctly papillate (Fig. 5a). Neighboring anthers are tightly connected to each other by interlocking epidermal cells (arrowheads in Fig. 5a). The anther walls are thin and delicate (Fig. 5a). They consist of the epidermis and one or two thin, subepidermal cell layers of small, juxtaposed cells (Fig. 5b and c). No thickened cell walls are present in any of the cell layers of the anther walls.

3.4. Pollen release

Gentle experimental compressions made with forceps to the middle region of the entire cone of anthers caused explosive expulsion of pollen jets for a distance of up to 20 mm from the pores. This artificial stimulation released pollen from all five anthers simultaneously. Vibrations applied to the anthers by contact with the pin tip of the electric toothbrush also caused the release of pollen from the pores. The vibrations of the toothbrush, however, caused the pollen to be released continuously, and for about half of the distance effected by anther compressions.

4. Discussion

In the flowers of *Solanum luridifuscescens*, jets of pollen are released after light mechanical pressure to the anther cone, similar to that described for two other species of *Solanum* sect. *Cyphomandropsis*, *S. glaucophyllum* and *S. stuckertii* (Passarelli and Cocucci, 2006a), and for the species of *Solanum* section *Pachyphylla* pollinated by Euglossini males (Soares et al., 1989; Gracie, 1993; Sazima et al., 1993). Although the physical features of the anthers of the studied population allow the artificial stimulus of the bellows mechanism, in nature we exclusively recorded pollen release through vibration by female bees, as is usual for flowers of the genus (Buchmann, 1983; De Luca and Vallejo-Marín, 2013). The only euglossine male, *Eulaema nigrita*, visiting the flowers did not release pollen during flower visits since the animal did not press on the anthers. Thus, at our study site, we found no pollination of flowers by euglossine males in search of floral perfumes, and it seems that effective pollination by the bellows mechanism remains restricted to *Solanum* sect. *Pachyphylla*.

At the study site, workers of *Melipona bicolor* and *M. quadrifasciata* were the most frequent visitors, while *Bombus brasiliensis* was

rare, differing from what has been frequently observed in species of *Solanum* with flowers of comparable size, where medium to large vibrating bees (Bombini and Xylocopini), in general, were the most frequent visitors (Buchmann, 1983; Buchmann and Cane, 1989; Bezerra and Machado, 2003; Passarelli and Bruzzone, 2004; Dupont and Olensen, 2006; Quesada-Aguilar et al., 2008; Sekara and Bieniasz, 2008; Burkart et al., 2011, 2014).

It is interesting that older flowers, from the second day onward, that showed visual marks from previous flower visits were visited at frequencies similar to that for younger flowers. Such visual changes show old flowers with fewer resources, which have often been found to be visited less frequently by bees (Pellmyr, 1988; Shelly et al., 2000). Some bees are able to assess the availability of pollen while vibrating *Solanum* flowers, and adjust their behavior during flower visits accordingly (Buchmann and Cane, 1989; Burkart et al., 2014). Visitors (*Augochloropsis* sp. and *Bombus atratus* Franklin) avoided old flowers of *S. glaucophyllum* whose anthers became brown after damage caused by the jaws and legs of visiting insects (Passarelli and Bruzzone, 2004). Decrease in resource availability causes an alteration to pollen harvest behavior such that bees begin to spin over the same flower repeatedly, and induces a greater number of visits (Buchmann, 1983; Bezerra and Machado, 2003). Although we did not detect any significant difference in frequency of bee visits over time, visitation decreased about 40% from 1st to 4th day of anthesis.

The studied population of *S. luridifuscescens* is self-incompatible and, therefore, depends on pollinators to set fruits. Of five other species of *Solanum* section *Cyphomandropsis*, three showed gametophytic self-incompatibility whereas the other two were self-compatible (Bohs, 2001). The higher fruit and seed set resulting from hand cross-pollination when compared to that recorded under natural conditions indicate pollen limitation. Ashman et al. (2004) supposed that the quality of pollen may differ between manual and natural cross-pollination. These differences in the origin of pollen between the pollen loads of different pollinators may have contributed to the lower fruit set in control flowers in comparison to hand cross-pollinated flowers, or there just was a smaller amount of pollen deposited by the bees on stigmas in control flowers. The most frequent pollinators (*Melipona bicolor* and *M. quadrifasciata*) visited many flowers on the same individual plant before leaving, thereby increasing the amount of geitonogamous pollen on the stigmatic surface. In other species, from the fourth consecutive visit of an animal to the same plant onward, no further fruit set was recorded (Pinto et al., 2008). In the present study, the climatic conditions at the study site might also have diminished fruit set. The studied population is situated near the top of a mountain in montane Atlantic Forest, where fog and rain showers are frequent during the flowering period of *S. luridifuscescens*. On foggy days, flower visitors were absent, or almost so, resulting in little to no pollen flow.

As with other species of sect. *Cyphomandropsis*, *S. luridifuscescens* has hermaphroditic and monomorphic flowers (Bohs, 2001). However, due to the elongation of the style, flowers show temporal heteromorphism. In andromonoecious flowers of *Solanum carolinense* L., long styles reduced the removal of pollen because they make it difficult for the anthers to be firmly gripped by pollinating bees (Quesada-Aguilar et al., 2008). This optimizes cross-pollination by permitting the contact of the stigma with the pollen on the animal's body. On the other hand, short styles did not remove pollen from the bee pollinators and did not interfere with the bee's removal of pollen from the anthers. In contrast, Nunes-Silva (2011) demonstrated that foragers of *Melipona fasciculata* Smith removed a greater quantity of pollen from flowers of *Solanum melongena* L. (eggplant) with long styles than in those with short styles, although Sekara and Bieniasz (2008) observed that cross-pollination by *Bombus terrestris* L. is favored in flowers with

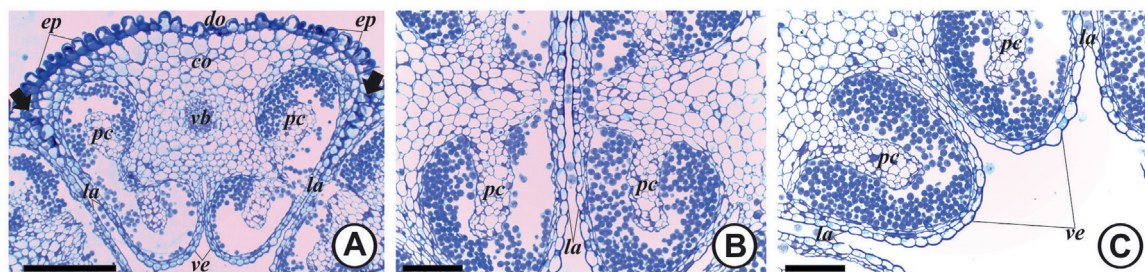


Fig. 5. Transverse sections of mature anthers of *Solanum luridifuscescens* (light microscopy). (A) Cone of anthers (scale bar 500 μm). (B) Detail of lateral walls (scale bar 200 μm). (C) Detail of ventral wall (scale bar 200 μm). [arrowheads interlocking epidermal cells connecting neighboring anthers, *co* connective, *do* dorsal wall, *ep* epidermis, *la* lateral wall, *pc* pollen sac placentoid, *vb* vascular bundle, *ve* ventral wall].

long styles. Thus, continuous style growth in *S. luridifuscescens* may be a strategy for reducing the rate of geitonogamy, with the younger flowers with short styles serving mainly as pollen donors, and older flowers, with long styles, preferentially serving as pollen receptors. This hypothesis is supported by the fact that during the first hours of anthesis the stigmatic surface is not yet ready to receive pollen grains.

Solanum luridifuscescens shows characteristics typical of most *Solanum* species (pollen flower syndrome): its flowers lack nectar and the poricidal anthers release dry pollen by vibration. It also exhibits features similar to those found in mature anthers of species with the perfume flower syndrome, which belong to *Solanum* sect. *Pachyphylla*: dorsally a developed connective covered with purple papillae that does not contrast with the corolla and laterally and ventrally flexible anther walls of two or three cells in thickness. The anatomy of the mature anther wall is identical in all *Pachyphylla* species studied so far, all of which, with the exception of *Solanum pinetorum* (L.B. Sm. & Downs) Bohs, have the epidermis as the only remaining cell layer of the pollen sacs (Sazima et al., 1993). There are species of sect. *Cyphomandropsis* with single-layered anthers, as is the case with *S. stuckertii* and *S. confusum*, and species with 3–4 layers, as is found in *S. glaucophyllum* (Passarelli and Cocucci, 2006a,b).

Internal relationships of the *Cyphomandra* clade are not well known, and *S. luridifuscescens* was not yet included in any phylogeny. However, phylogenetics studies using ITS data revealed four subclades well supported within this clade (Bohs, 2007). These subclades largely conform with informal groups of species in *Pachyphylla* and *Cyphomandropsis* sections inferred from morphological characters (Bohs 1994, 2001). Although many species of each section to be grouped together, the sections do not form monophyletic groups in the most parsimonious trees (Bohs, 2007). Therefore, the acquisition of anthers with thin walls seems to have occurred more than once in the clade *Cyphomandra*, given the non-monophyletic groups that comprise it (Bohs, 2007).

In the unique pollination mechanism found in the *Axinaea* (Melastomataceae), mechanical compression in the aerenchymatous tissue of the appendage results in air current through pollen chamber that, due displacement of the air contained in the intercellular spaces, flushes the pollen grains through apical anther pores. The connective appendages act as structures detached from the pollen chambers. In this case, bellows work only once per stamen, whereas the other studied species of Solanaceae and Melastomataceae have the pollen chambers functioning as bellows-like structures which may be activated repeatedly. The bellows-like mechanism seems to be linked with the evolution of additional rewards (besides pollen), such as food bodies (*Axinaea*), nectar (*Blakea*) or perfumes (*Solanum*) (Dellinger et al., 2014).

It would be interesting to document in more detail the structure of the anther and the floral scent of *S. luridifuscescens*, as well that in other species of *Solanum* sect. *Cyphomandropsis*,

particularly *S. confusum*, which exhibits the perfume syndrome (Passarelli and Bruzzone, 2004), and *Solanum fusiforme* L. B. Sm. and Downs, which may be the sister group to all other species of the clade *Cyphomandra* (Bohs, 2007). Our results indicate that morphological traits alone are not sufficient to understand the evolution of one pollination mechanism from another in the genus *Solanum*. The key to understanding the evolution of the bellows mechanism in Neotropical *Solanum* may be related to the presence of osmophores and chemical signaling.

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