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MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF ANTHERS FROM SPECIES OF *SOLANUM* SECT. *CYPHOMANDROPSIS*[1]

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Abstract

The study of anther development of *Solanum* sect. *Cyphomandropsis* species (*Solanum confusum* Morton, *Solanum glaucophyllum* Desf. and *Solanum stuckertii* Bitter) provide information about differences between the anther wall layers and placentoid behaviour at maturity and according with the floral syndrome. It is known that in *Solanum* species with vibratory mode of pollen release, anther wall is thick with four or more layers and the placentoid staid expanded during anthesis. In *Solanum* species with a bellow like mechanism of release (e.g. section *Pachyphylla*) pollen wall is thin (one or two layered) and the palcentoid presents a double expansion at maturity being collapsed at anthesis time. The anther locule stays empty allowing the pollen to be bellowed out. This feature was known exclusively for sect. *Pachyphylla* but in this work it is observed that it is extended in the genus. In section *Cyphomandropsis* with both pollen release modes working together intermediate anther morphology is found and anther behavior at anthesis time is similar to that of section *Pachyphylla*.

Keywords : Anther development, morphology

Introduction

The genus *Solanum* is extremely large with 1500 species, of which 1000 occur in South America (Hunziker 1979). Despite being a large taxon, floral morphology is fairly uniform in the genus *Solanum* (Buchmann 1983, Cocucci 1988, Gracie 1993). Symon (1979) considers this floral uniformity as a plant-insect mechanism of specialisation. Studies on androecium, and especially on the internal structure of the anthers in this genus were very scarce and due to the large size of the genus they are not sufficiently representative (Bhandari & Sharma 1988). Lately new studies about anther structure, anatomical and morphological investigations on selected species of *Solanum* have made interesting contributions, specially on the anther dehiscence mechanisms in the genus (Child & Lester 2001).

Solanum sections show different pollen release modes, related with anther structure and floral syndrome (Sazima et al. 1993). *Solanum* (sensu lato) flowers are buzz-pollinated by female bees, contrary to species of sect. *Pachyphylla* present a bellow-like mechanism worked by male bees (Sazima et al. 1993).

Section *Cyphomandropsis* is considered close to sect. *Pachyphylla*. This section was earlier included in genus *Cyphomandra* but lately Bohs (1995) and Bohs et al. (1999) place this genus in *Solanum*. Nee (1999) has included its species in sect. *Pachyphylla*. It was observed that sect. *Cyphomandropsis* includes intermediates between typical *Solanum* and sect. *Pachyphylla* regarding both, anatomy and function (*S. stuckertii*). Other members of this section, exhibit *Pachyphylla* like features at anther level and may release pollen by a pneumatic mechanism (*Solanum confusum*) or present buzz-pollinated flowers (*Solanum glaucophyllum*, Passarelli 1998).

The aim of this paper is to report structural changes during anther maturation in species of

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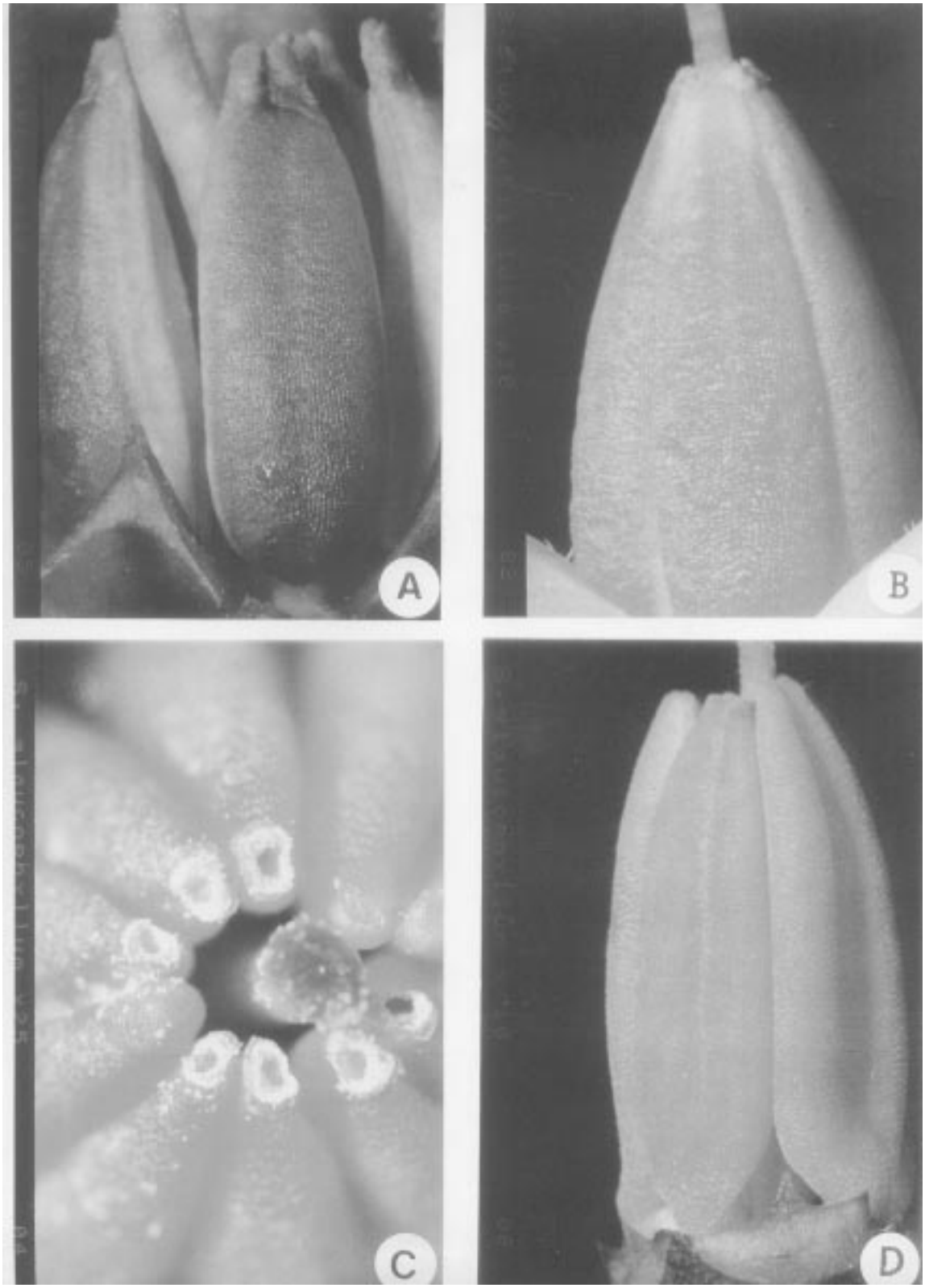

Fig. 1A-D — *Solanum* sect. *Cyphomandropsis* anthers. **A.** *S. confusum.* Aspects of the connective. Dark purple anthers are not contrasting with corollas colour. **B.** *S. stuckertii*, yellow and bright anthers that remain "stuck" to each other during anthesis. **C-D.** *S. glaucophyllum*. **C.** Anthers at anthesis time. Some of the pores are open, others are still shut. **D.** Yellow and bright anthers without an external notorious connective.

Solanum sect. *Cyphomandropsis* with modifications at anthesis stage. It attemps to correlate the findings with the function during pollen release.

Materials and Methods

Voucher specimens from the studied material are:

Solanum confusum Morton, Argentina. Prov. Tucumán. Quebrada de Los Sosa, 22 de Enero de 1995, Km 37. Passarelli 112 (LP).

Solanum glaucophyllum Desf., Argentina. Prov. Buenos Aires. Ensenada, camino Rivadavia, 4 de Diciembre de 1991, Passarelli 4(LP); idem Passarlli 5 (LP).

Solanum stuckertii Bitter, Argentina. Prov. Córdoba. Depto. Calamuchita, Falda de Reartes, ruta 5, Km 10. 2 de Diciembre de 1992. Passarelli 8 (LP); idem Passarelli 9 (LP).

Anthers anatomy was studied on sections of fresh material and on fixed material in a mixture 3:1 of ethanol and glacial acetic acid. Material was dehydrated by ethanol-xylene and embedded in Paraplast. Sections caps were cut with a Minot microtome, and stained with safranine and fast green (Johansen 1940). The different stages of floral development were studied. External features of the androecium were observed with stereoscopic microscope and with scanning microscope Jeol JSMT 100, previously dehydrated for critical point.

Results

EXTERNAL MORPHOLOGY — Five bithecous and poricidal stamens constitute the androecium, corresponding to the basic type of the family Solanaceae. The top of the anthers is blunt and bilobed with a subapical constriction, (Fig. 1A, B) being absent in *Solanum glaucophyllum* (Fig. 1D). This area is

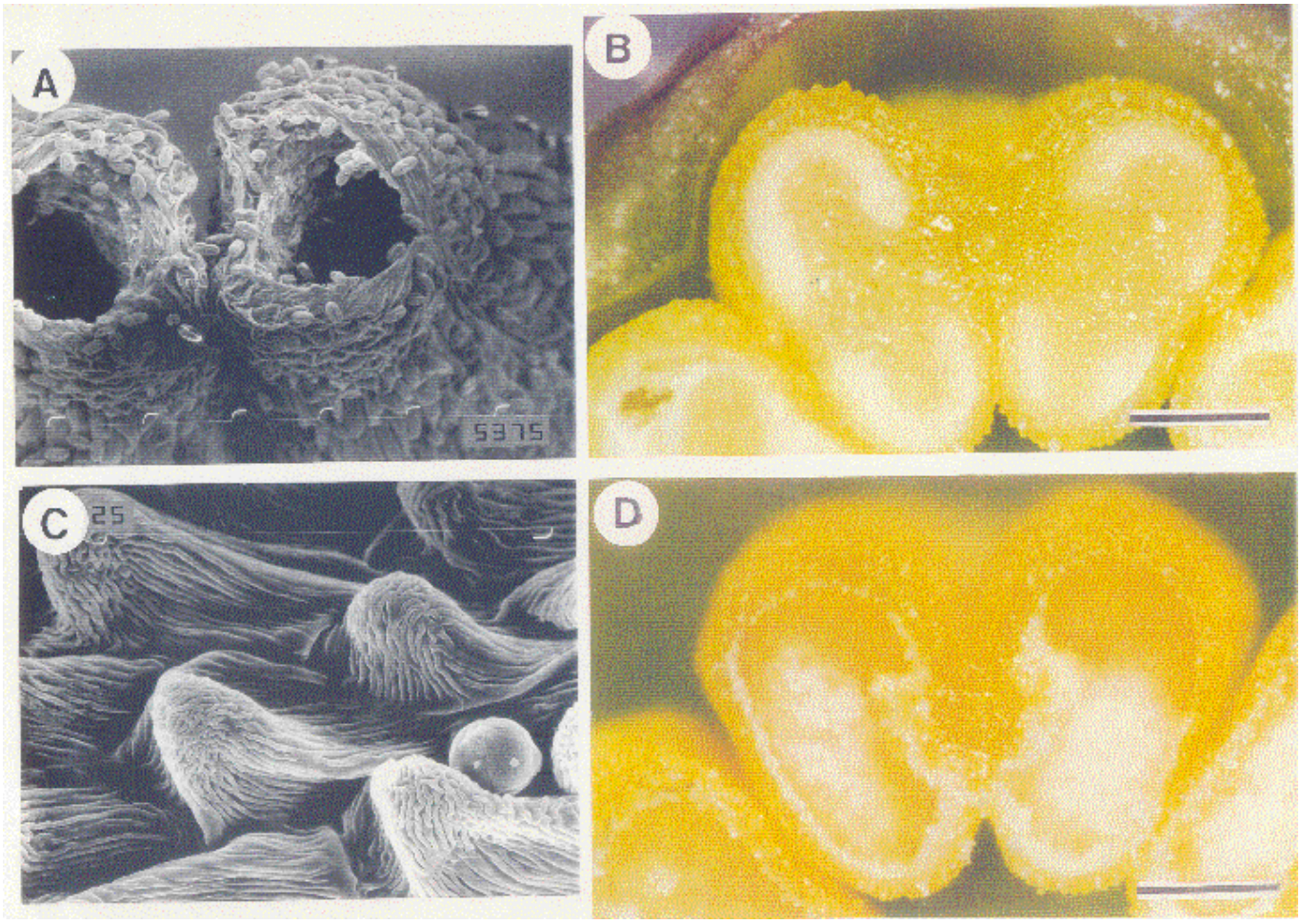

Fig. 2A-D — *Solanum glaucophyllum*. **A.** SEM. Anthers pores at anthesis. **B.** Young anther in the first stages of development. The palcentoid gives the characteristic horseshoe shape. **C.** SEM. Anther papillae. **D.** Flower at anthesis. Anther sacs become confluent. Scale bars : A-C, 24mm; B-D, 10mm.

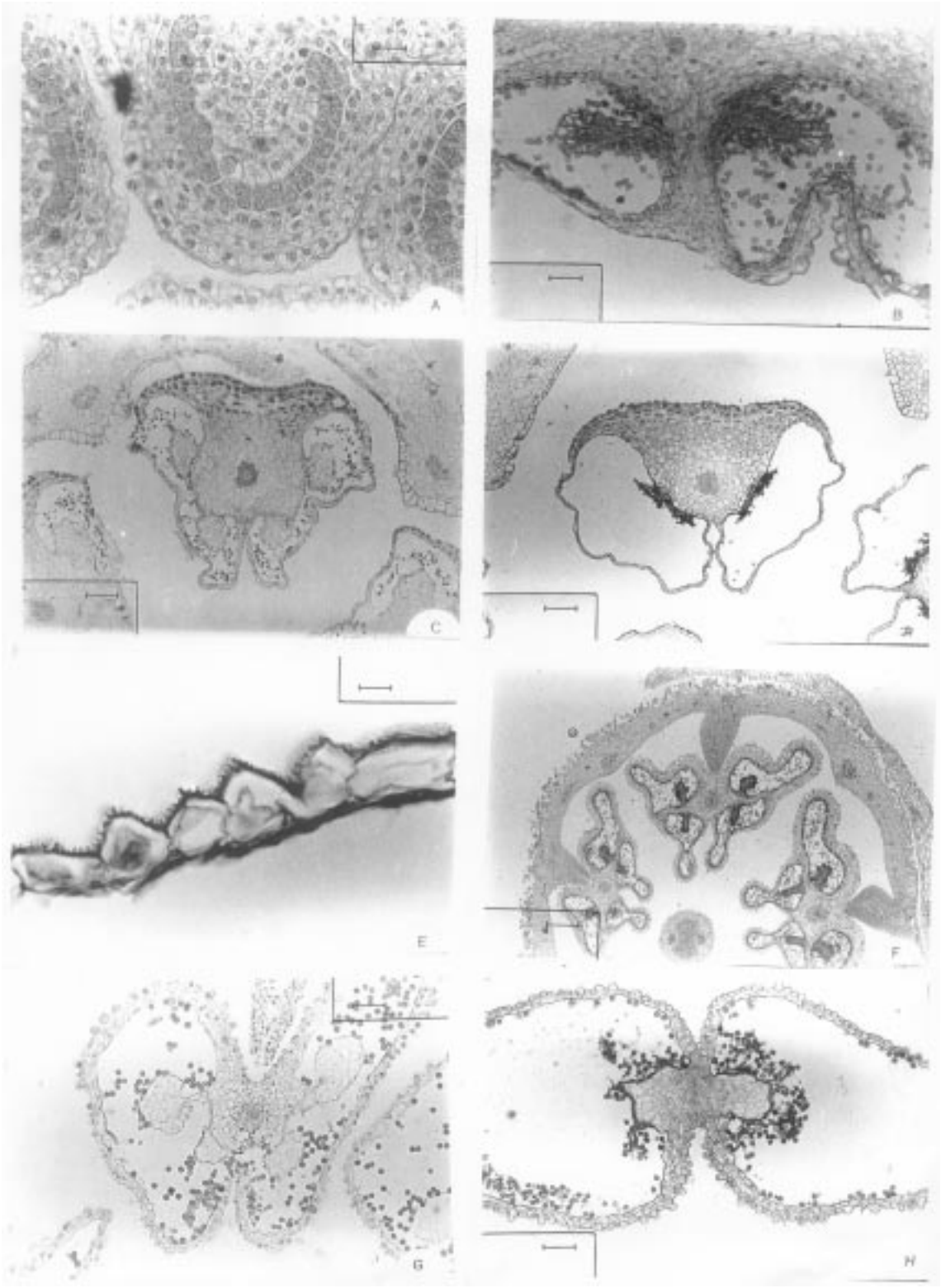

Fig. 3A-H — Cross-sections from *S. confusum* anthers. **A.** Initial stage. **B.** Stage after meiosis. The tapetum is in the most active stage. The cells present abundant content. **C.** Tapetum degeneration begins. The polinics sacs cavities bring together. The connective is well developed. Microspores are pushed against the anther wall by the placentoid. **D.** Whole anther at anthesis. **E.** The wall is only constituted by the epidermis. **F.** *Solanum glaucophyllum.* Differentiation during meiosis. **G.** Anther during anthesis. **H.** Whole anther with the placentoid collapsed. Anther wall is thick.

sterile and it is specialised for dehiscence. The cells possess thickened walls, this is the only zone with endothecium. The anthers are indehiscent in the longitudinal line (Fig. 1C, 2A).

In *S. confusum* and *S. stuckertii* the connective is externally distinguished (Fig. 1A, B), and possess a thicker tissue than in *S. glaucophyllum* (Fig. 1D). The gap among the connective and the anther wall shows a gradual transition. In *S. stuckertii* the anthers remain "stuck" to each other during anthesis through the cuticle (Fig. 1B).

In *Solanum glaucophyllum*, the thecas occupy the largest extension in the anther, the connective is not visible externally and it is only developed in the interior, in the central region of the anther (Fig. 3H). It doesn't present the voluminous shape found in *S. confusum* and *S. stuckertii*. Yellow and bright anthers of *Solanum glaucophyllum* and *S. stuckertiit* contrasting with the corolla, are attractive to insects, (Fig. 1B, D). In *S. adelphum*, on the other hand, anthers are dark purple, with yellowish areas in the dorsal part and in the area near the pore. This coloration is not contrasting with the corolla one (Fig. 1A).

ANTHERS ANATOMY — The anther was 4 chambered with the characteristic horseshoe shape, originated by the presence of the placentoids (Fig. 2B, 3A). During the anthers development very important changes take place in the tissues disposition in relation to the pollen release mode. Three stages can be distinguished:

1. EARLY DIFFERENTIATION — The sporogenous tissue is divided by mitosis forming archeosporium. Two cells layers with dense cytoplasm and very visible nuclei constitute this tissue. (Fig. 3A). Primary parietal layer originates for successive divisions in the different strata of the anther wall. Before meiosis five well-differentiated layers are observed. The epidermis is one layered with isodiametric cells, subtended by a layer of bigger cells, two intermediate layers and the external tapetum with cells facing the sporogenous tissue. The internal tapete abutting the connective, forms part of the placentoid. It is very different from the external one because it presents longer cells with intensely coloured content. A projection of parenchymatic tissue located below the internal tapete is a continuation of the placentoid (Fig. 4A, B).

2. DIFFERENTIATION DURING THE MEIOSIS — By means of post-meiotically division tetrahedral tetrads are formed (fig. 4B). Several changes are observed in the anther structure. The epidermis continues conformed by isodiametric cells, below it a layer of cells with thin walls is observed (no endothecium is found) and toward the interior two intermediate, collapsed layers are observed. The tapeta is in the most active stage and its cells present abundant content. At this moment the difference between the internal and external tapeta is notable. The external one presents elongated cells of smaller size that the epidermic cells, with very prominent nuclei. The internal tapetum, on the contrary, is well developed, its cells are very prominent, some of them binucleated, with strong coloured nuclei. parenchymatic strata of the placentoid decreased completely (Fig. 3F, 4C, 5A).

3. POST-MEIOTIC DIFFERENTIATION- After meiosis tapetum degeneration begins. In the stomium area the anther sacs become confluent (Fig. 3G).When the microspores are formed, the placentoid expands again, having their cells the vital aspect of the first stage. This fact pushes marginally the pollen grains against the theca wall (Fig. 3C, 4D, 5D).

ANTHESIS — In *Solanum confusum* and *S. glaucophyllum*, in the previous hours before anthesis placentoid collapses for second time (Fig. 3G), and anther shows an empty cavity again (Fig. 3D). In *S. stuckertii*, on the contrary, the placentoid is still expanded, during the first hours of the anthesis, when the pores are open (Fig. 4D). Retraction takes place in the afternoon of the first day of anthesis (Fig. 5F). This change in the structure agree with differences in microspores viability (Passarelli 1999) because pollen grains can germinate since the second day of anthesis.

The wall of the mature anther is very thin in two of the studied species; in *Solanum confusum* it is only formed by the outer epidermis whereas in *S. stuckertii*, by the epidermis and a subepidermical layer. On the other hand, in *S. glaucophyllum* the wall is remarkably thick on account of 3 or 4 layers under the papillous epidermis (Fig. 2C, 3H).

Discussion

One of the main differences between several sections of *Solanum* (s. lat.) and species of sect. *Pachyphylla*, is androecium coloration, which has great importance in the biology of both floral types (Passarelli 1998, Sazima et al. 1993). In sect. *Pachyphylla* species, a swollen and thick connective extends widely over the theca and anthers colours are not contrasting with the corolla so attraction in this genus is not pollen but the odour. Therefore it is not

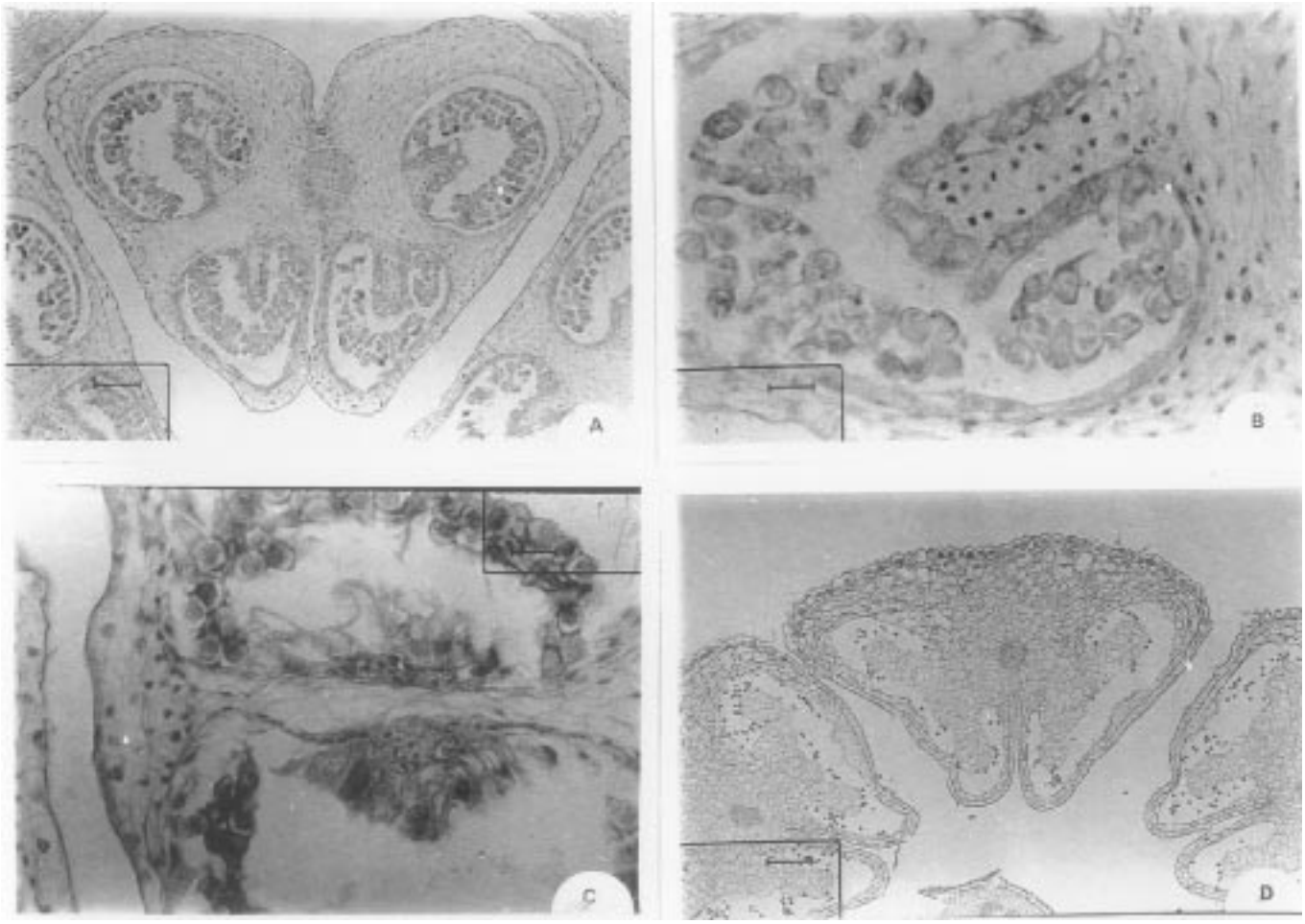

Fig. 4A-D — *Solanum stuckertii.* **A.** Anther during meiosis. Internal tapetum begins differentiation. **B.** Tetrahedral tetrads are just formed. **C.** Stage after meiosis. Internal placentoid at the maximum differentiation. **D.** Previous stage to the anthesis.

necessary pollen imitation presented by the anthers of others *Solanum* species (Grace 1993). Bohs (1986) affirms that sect. *Pachyphylla* flowers, contrary to those of other *Solanum* sections present anthers of diverse tones: white, yellowish, violet, but none yellow brilliant, contrasting with the corolla. In this case, two of the studied species (*Solanum glaucophyllum* and *S. stuckertii*) possess showy flowers with yellow androecium which attracts the pollinators, conserving the characteristics of the genus. On the contrary, in *S. confusum* the androecium has strong similitude with that observed in *Solanum pinetorum* (= *Cyphomandra pinetorum*), sect. *Pachyphylla*. Morphological similarity is also noticed in the presence of an enlarged connective, in the dorsal area of each teak. This feature is also present in *S. stuckertii* species with intermediate morphology between *Solanum* (*s. lat.*) and sect. *Pachyphylla.*

In *Solanum confusum* and *S. stuckertii*, the connective has glandular characters, but the epidermal cells are not papillous (Cocucci 1996). In the present work these features are confirmed and epidermal papillae are observed in *S. glaucophyllum.*

ANATOMY — The stamen usually presents different modifications, generally related with the floral mechanisms. In *Solanum* section *Cyphomandropsis* the structure of the wall of the anther has an important participation in the way of liberation of the grains of pollen (Passarelli & Cocucci, personal communication).

The structure of the anthers in the early development stages, corresponds to the basic type of the family *Solanaceae* (Mascré 1921, Davis 1966). When anthers are mature, a series of changes happen. The placentoid, always present in subclass *Asteridae* (Eames 1961, Hartl 1964, Dharamadhaj et al. 1978) can increase its volume or to be collapsed. “In *Cyphomandra*, (now sect. *Pachyphylla*) it contracts

during the meiosis. When the pollen grains are mature, the placentoid enlarges notably and it almost occupies the whole interior of the teak. Hours before the anthesis, it contracts abruptly for a second time" (Sazima et al. 1993). This mechanism is supplemented with a flexible anther wall. In species of *Solanum* (*s.lat.;* e.g. *S. granulosum-leprosum* Dunal, of sect. *Brevantherum* and *S. jasminoides* Paxton, sect. *Jasminosolanum*) the placentoid stays expanded during the anthesis (unpublished). Double expansion takes place in the species of *Solanum* sect. *Cyphomandropsis* studied here, being similar to that of sect. *Pachyphylla* (Sazima et al. 1993). These are the only data about this fact; in the consulted bibliography the second increase of the placentoid is not mentioned (Bernardello 1987, Prasad & Singh 1978, Sharma et al. 1987). Rodrigues (1998) who studied the species of sect. *Cyphomandropsis* in her interesting Doctoral Thesis considered the placentoid like a collapsed tissue, because she makes the histologic observation,

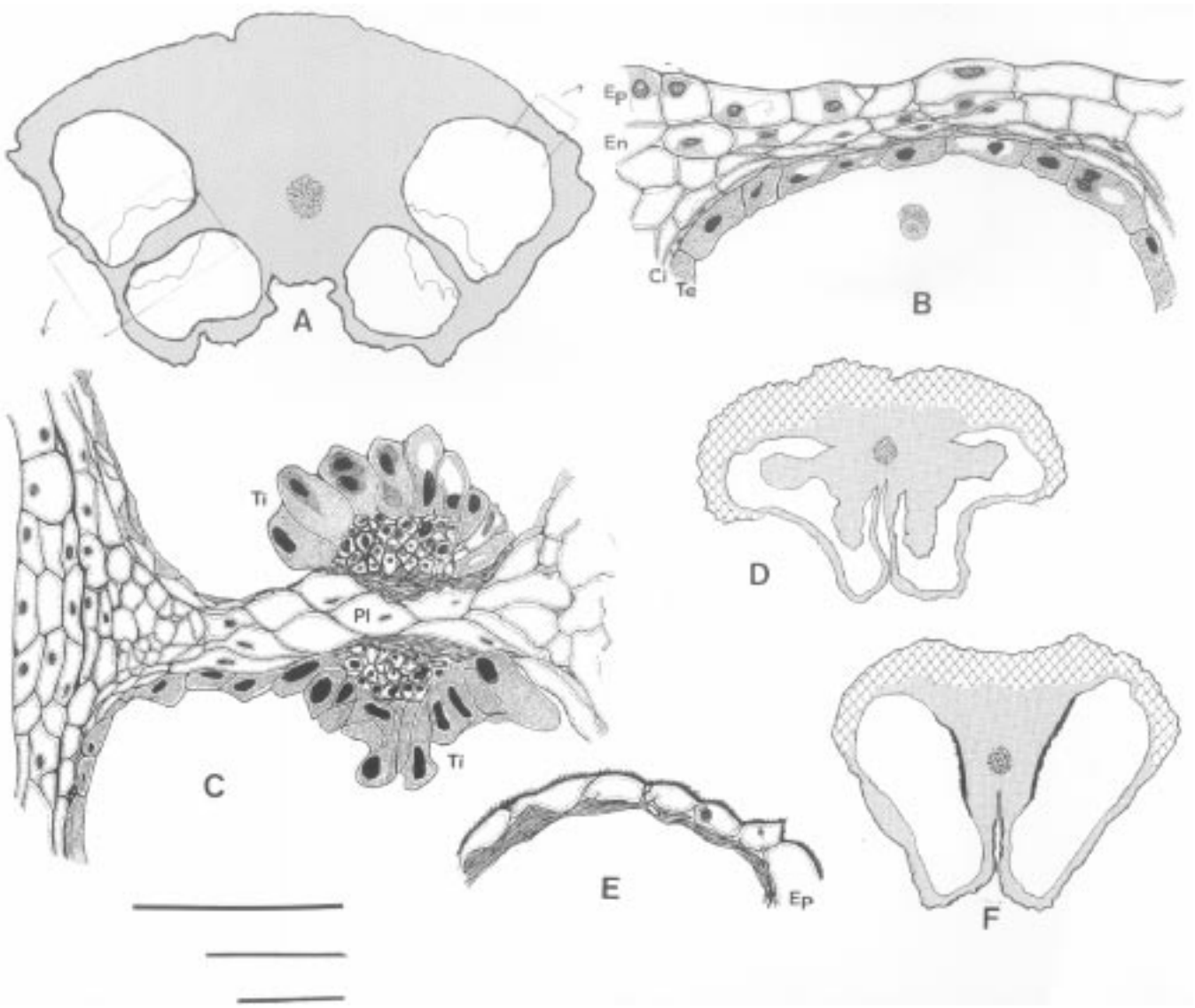

Fig. 5A-F — *Solanum stuckertii*. **A,B,C.** Differentiation during meiosis. **A.** General view from the anther. **B.** Anther wall detail from pointed compartment in A. **C.** Stage of great differentiation among internal and external tapetum. **D, E.** Anther wall with only the epidermis. **F.** Anther at anthesis. Marked zone points osmophore.
Ep : epidermis; En : layer corresponding to the endothecium; Ci : intermediate layers, colapsed; Te : external tapetum; Ti : internal tapetum; Pl : planetoid.
Scale bars : B,C,E 200 μm, F 300 μm, D 60 μm

not the histogenesis one.

The prominent connective of the anthers of *Solanum c onfusum* and *S. stuckertii*, contribute to maintain the volume of the teak. In *Solanum glaucophyllum*, species without the voluminous external connective, the swelling of the epidermic papilla absent in the other two species can contribute to maintain the teaks shape when the placentoid is retired. In sect. *Pachyphylla* it is first plurilayered, being only reduced in the mature state to the epidermis. In *Solanum* (s. lat.) where the anthers are emptied by means of vibrations, the wall is still plurilayered in the maturity" (Sazima et al. 1993, pers. obs. *Solanum granulosum-leprosum* Dun.). This character would grant so the necessary rigidity to facilitate the vibratory extraction of the pollen.

In the species of *Solanum* sect. *Cyphomandropsis*, intermediate conditions are observed between both. In *Solanum adelphum* and *S. stuckertii* the anther wall at anthesis is bilayered (as in sect. *Pachyphylla*). In *Solanum glaucophyllum* it is wider, because the epidermis and two intermediate layers constitute it. These structures participate in the vibratile way of pollen release (Passarelli 1998).

Among the adaptations in this genus for pollen distribution the restriction of cells with augmented walls (endothecium) to the apex of the anther is related with dehiscence (Cocucci 1988). Several authors (Buchmann 1983, Hossain 1973) mention the presence of membranes in this area. However, as it was observed, it is parenchima related with internal tissuess. The stomium or the open slit is situated beyond the fertile zone upward into the sterile parts tills the tip (Passarelli 1998, Child 2001).

According with the classification of (Child et al. 2001), in the three studied species the stamens open by pores. The gradual opening of the pores along the day, contributes to the dosage of pollen.

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