

Features related to anther opening in *Solanum* species (Solanaceae)

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Received 27 October 2005; accepted for publication 4 June 2008

The mode of anther opening and the morphological and histological variability of the stomium are described in 30 *Solanum* species. Poricidal, poricidal-longitudinally dehiscent and longitudinally dehiscent anthers are observed. In the three types, the stomium may be diverse with regard to shape and histological characteristics before opening, but is always composed of small epidermal cells as the sole anther wall layer; the stomial cells may be differentiated only in part of the anther length. Particular crescent-shaped structures in the epidermis, called 'ridges', are observed to line the stomium in most species. These ridges may be related to the stomium opening, working together with the cells with thickened walls of the anther. Cells with thickened walls are developed in the endothecium, middle layers and/or connective tissue at the apical end of the anther, surrounding the pore; only in the longitudinally dehiscent anthers of *S. nitidum* does an endothecium with thickened cell walls develop along its entire length. At least two histological features (the differentiation of small stomial epidermal cells as a unique layer, and the distribution of cells with thickened walls) seem to constrain the form of the open stomium. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, 158, 344–354.

ADDITIONAL KEYWORDS: cells with thickened walls – endothecium – poricidal anthers – stomial epidermal cells – stomium.

INTRODUCTION

Longitudinally opening anthers are the most common form in angiosperms (Endress, 1994, 1996). Nevertheless, other mechanisms of anther opening exist, which may respond to different stomium shapes (for example, valvate anthers in Hamamelididae; Hufford & Endress, 1989) and/or to different histological structures (for example, poricidal anthers in species of *Senna*; Venkatesh, 1957). With regard to histology, particular features (for example, distribution of cells with thickened walls) have been found to determine the opening of pores or partial slits in several cases (Venkatesh, 1955, 1956a, b, 1957; Keijzer, Leferinkten Klooster & Reinders, 1996; Matsui, Omasa & Horie, 1999, 2000), and other features (for example, endothelial-like connective hypodermis) seem to be related to the development of valves in Hamamelididae species (Hufford & Endress, 1989).

According to Bernhardt (1996), the mode of anther opening is one of the factors which may influence the pollination syndrome of a flower. The flowers of the large genus *Solanum* (c. 1000 species; Hunziker, 2001) have been referred to the *Solanum* type (Vogel, 1978; Buchmann, 1983; Endress, 1994, 1996), and Bernhardt (1996) considered the bellows-like mechanism developed in some of the former *Cyphomandra* species [for example, *Solanum sciadostylis* (Sendtn.) Bohs] to be derived from the standard *Solanum*-type flowers. Although the presence of poricidal anthers is one of the features that characterizes *Solanum*-type flowers as a whole (Buchmann, 1983; Endress, 1994), including those with a bellows-like mechanism (Sazima *et al.*, 1993), other forms of anther opening have been observed in species that follow the same syndrome (Endress, 1996). Indeed, within *Solanum*, there are species that develop different modes of anther opening. The most common cases in *Solanum* are poricidal anthers, which open only through apical pores, and poricidal-longitudinally dehiscent anthers,

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in which each theca opens apically through a pore that continues downwards with a longitudinal slit (Barboza & Hunziker, 1991; Hunziker, 2001; Knapp, 2002). In addition, a few species with longitudinal anthers have also been mentioned (Siddiqui & Khan, 1988), including the former *Lycopersicon* species (Carrizo García, 2003; Carrizo García & Barboza, 2006).

Based on this background, an integral study of the features related to anther opening at the morphological and histological levels of organization was attempted in a group of *Solanum* species, in order to analyse the possible interaction between them.

MATERIAL AND METHODS

A sample of 30 *Solanum* species belonging to several infrageneric taxa was studied (Table 1). The observations were made on materials preserved in 5% formalin, 10% acetic acid and 50% ethanol (FAA), which are deposited at the CORD spirit collection.

MACROSCOPIC OBSERVATIONS

The shape of the stomium before opening and its progress from opening to anther senescence were observed. These observations were made in buds and flowers at different flowering stages (different positions along the inflorescence) with a stereoscopic microscope. Closed and opened anthers were selected for scanning electron microscopy (SEM) analysis to illustrate the stomium shape before and after opening. The preserved anthers were dehydrated in ethanol and acetone, critical point dried, coated with gold-palladium, and finally analysed in a JEOL JSM T-100 scanning electron microscope (Scanning Electron Microscopy Service, University of La Plata, Argentina).

HISTOLOGY

The concept of the stomium used by Hufford & Endress (1989: 303) was followed in this work. The histology of the anther wall facing the septum and neighbouring tissues was studied in closed anthers from the largest flower bud before anthesis. The materials were dehydrated, embedded in Paraplast and serially cut in cross-sections (12 µm thick). The sections were stained with 0.05% cresyl violet in water (Carrizo García, 2002a) and analysed with a light microscope. The distribution of cells with thickened walls along the anther was outlined in entire anthers cleared with 50% sodium hypochlorite in water, stained with 0.05% safranin in water and observed with a light microscope. In addition, the cross-sections were analysed under polarized light to

illustrate the thickened cell walls across the anther. All descriptions follow a basipetal direction.

RESULTS

GENERAL FEATURES

The anthers are more or less longitudinally elongate and straight in most species, laterally bent in *S. pennellii* (Carrizo García, 2003) and dorsiventrally bent (sinuate) in *S. trisectum* (Carrizo García, 2001). Although *S. consimile*, *S. pennellii*, *S. wendlandii* and *S. trisectum* have anthers of different lengths, the features observed are the same in all anthers. The anthers are dithecal, and each theca has two pollen sacs. The anthers are slightly dorsiventrally asymmetrical, because the ventral pollen sacs (those facing the gynoecium) are smaller than the dorsal sacs, except in *S. trisectum*, whose anthers are dorsiventrally symmetrical.

STOMIUM MORPHOLOGY

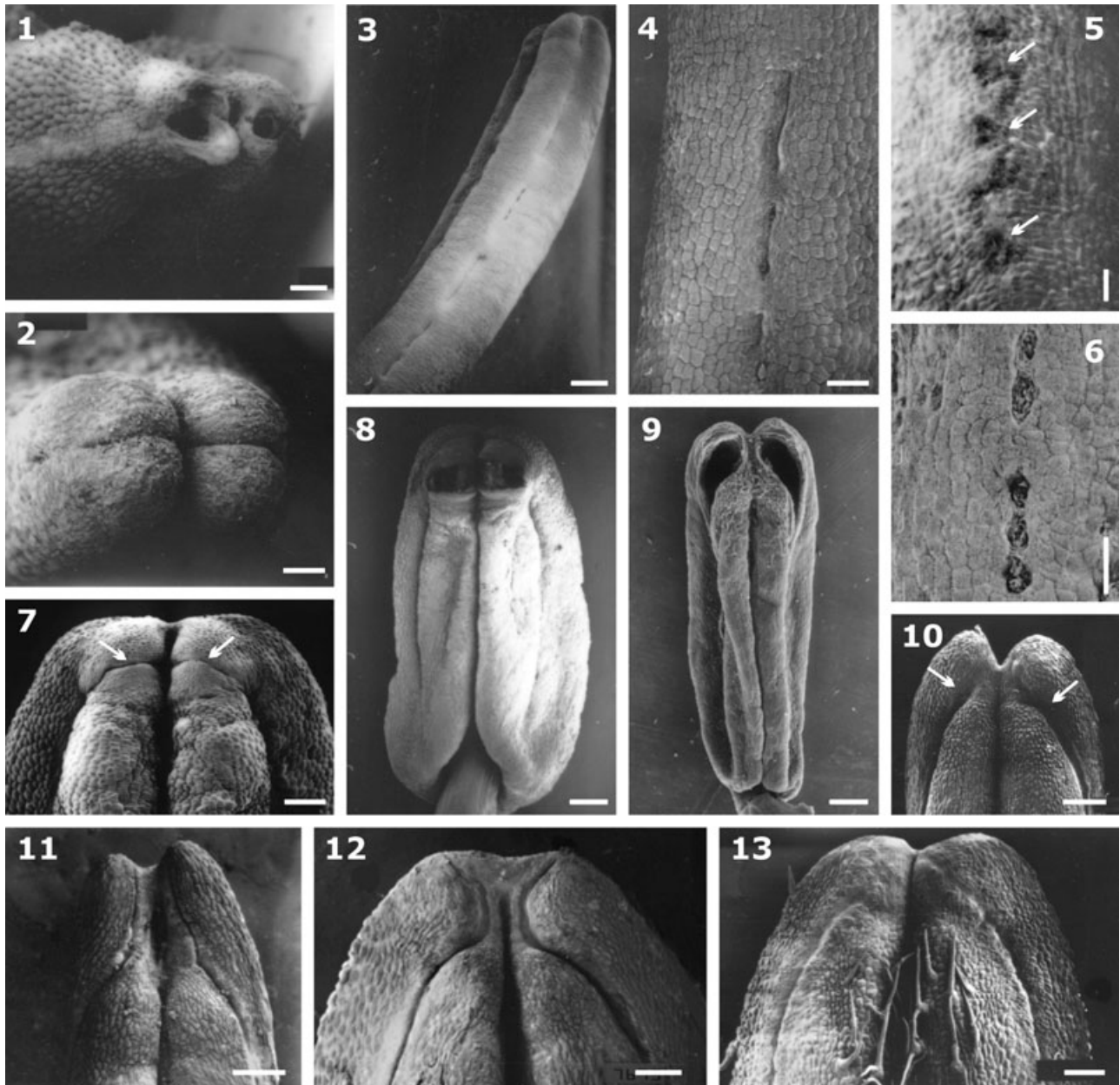
The stomium is placed between the two pollen sacs of each theca, and is recognized superficially before it opens as a marked depression in the anther wall. The stomium shape is variable both before and after opening.

Poricidal stomia

In *S. betaceum*, *S. corymbiflorum*, *S. fusiforme*, *S. glaucophyllum* and *S. stuckertii*, although a straight longitudinal furrow is observed along the lateral surface of each theca, only the end that extends over the theca apex opens. This aperture widens to form a rounded pore (Fig. 1), with the rest of the furrow remaining intact. In *S. toldense*, each theca has a slit extending over the apex (Fig. 2), and several short lateral slits aligned in the lateral surface up to the base (Figs 3, 4). All of these slits open, and neighbouring lateral slits can merge whilst enlarging longitudinally to form longer slits. Only the apical aperture widens to form a rounded pore; the other apertures remain inconspicuous. In *S. consimile*, each theca has an apical slit extending over the apex, and a number of smooth lateral depressions (Fig. 5). The apical slit opens and widens to form a pore, and scattered minute pores open along the lateral surface (Fig. 6). In *S. endoadenium*, *S. seaforthianum* and *S. tucumanense*, each theca has a longitudinal furrow with a curve towards the connective tissue at its apical end (Fig. 7); this curve begins on the ventral side of the theca, slightly below its apex (Fig. 7). The stomium opens only in the apical curve, where a pore is formed (Fig. 8). The anthers of *S. endoadenium* have small oblique pores, whereas, in *S. tucumanense*, the pores are larger. In *S. seaforthianum*, the apical curve of

Table 1. *Solanum* species studied and collection data. Infrageneric placement of the species based mainly on Nee (1999)

Species	Infrageneric taxon	Voucher	Source
<i>Solanum fusiforme</i> L.B.Sm. & Downs	Subgen. <i>Bassovia</i> , sect. <i>Cyphomandropsis</i>	Barboza <i>et al.</i> 471 and 479	Argentina, Misiones
<i>Solanum glaucophyllum</i> Desf.	Subgen. <i>Bassovia</i> , sect. <i>Cyphomandropsis</i>	CORD 588, Barboza <i>et al.</i> 357	Argentina, Corrientes
<i>Solanum stuckertii</i> Bitter	Subgen. <i>Bassovia</i> , sect. <i>Cyphomandropsis</i>	Hunziker 25325	Argentina, Córdoba
<i>Solanum betaceum</i> Cav.	Subgen. <i>Bassovia</i> , sect. <i>Pachyphylla</i>	CORD, f. 4025	Argentina, Buenos Aires, cult.
<i>Solanum corymbiflorum</i> (Sendtn.) Bohs	Subgen. <i>Bassovia</i> , sect. <i>Pachyphylla</i>	Mentz 270	Brazil, Rio Grande do Sul
<i>Solanum trizygum</i> Bitter	Subgen. <i>Bassovia</i> , sect. <i>Pteroidea</i>	Benítez de Rojas 5939	Venezuela
<i>Solanum wendlandii</i> Hook.f.	Subgen. <i>Leptostemonum</i> , sect. <i>Herposolanum</i>	CORD 291	Argentina, Córdoba, cult.
<i>Solanum consimile</i> C.V.Morton	Subgen. <i>Leptostemonum</i> , sect. <i>Torva</i>	Barboza <i>et al.</i> 279	Bolivia
<i>Solanum toldense</i> Matesevach & Barboza	Subgen. <i>Leptostemonum</i> , sect. <i>Torva</i>	Barboza <i>et al.</i> 281	Argentina, Salta
<i>Solanum argenteum</i> Dunal	Subgen. <i>Solanum</i> , sect. <i>Brevantherum</i>	Barboza <i>et al.</i> 804	Brazil, Rio de Janeiro, cult.
<i>Solanum concinnum</i> Sendtn.	Subgen. <i>Solanum</i> , sect. <i>Brevantherum</i>	Barboza <i>et al.</i> 793	Brazil, Sao Paulo
<i>Solanum granuloso-leprosum</i> Dunal	Subgen. <i>Solanum</i> , sect. <i>Brevantherum</i>	Barboza <i>et al.</i> 365	Argentina, Corrientes
<i>Solanum ramulosum</i> Sendtn.	Subgen. <i>Solanum</i> , sect. <i>Brevantherum</i>	Barboza <i>et al.</i> 408	Argentina, Misiones
<i>Solanum endoadenium</i> Bitter	Subgen. <i>Solanum</i> , sect. <i>Dulcamara</i>	Barboza <i>et al.</i> 578	Argentina, La Rioja
<i>Solanum seaforthianum</i> Anders.	Subgen. <i>Solanum</i> , sect. <i>Dulcamara</i>	Barboza <i>et al.</i> 799	Brazil, Rio de Janeiro, cult.
<i>Solanum tucumanense</i> Griseb.	Subgen. <i>Solanum</i> , sect. <i>Geminata</i>	Barboza <i>et al.</i> 1068 Morero 76	Argentina, Jujuy Argentina, Córdoba
<i>Solanum argentinum</i> Bitter	Subgen. <i>Solanum</i> , sect. <i>Holophylla</i>	CORD 581	Argentina, Córdoba
<i>Solanum caavurana</i> Vell.	Subgen. <i>Solanum</i> , sect. <i>Holophylla</i>	Barboza <i>et al.</i> 390	Argentina, Corrientes
<i>Solanum nitidum</i> Ruiz & Pav.	Subgen. <i>Solanum</i> , sect. <i>Holophylla</i>	CORD, f. 4022	Bolivia
<i>Solanum lycopersicoides</i> Dunal	Subgen. <i>Solanum</i> , sect. <i>Lycopersicon</i>	CORD, f. 4023	Netherlands, Nijmegen, cult.
<i>Solanum pennellii</i> Correll	Subgen. <i>Solanum</i> , sect. <i>Lycopersicon</i>	CORD, f. 4024	Netherlands, Nijmegen, cult.
<i>Solanum acaule</i> Bitter	Subgen. <i>Solanum</i> , sect. <i>Petota</i>	Negritto <i>et al.</i> 425	Argentina, Salta
<i>Solanum commersonii</i> Dunal	Subgen. <i>Solanum</i> , sect. <i>Petota</i>	Barboza <i>et al.</i> 1002	Argentina, Corrientes
<i>Solanum spagazzinii</i> Bitter	Subgen. <i>Solanum</i> , sect. <i>Petota</i>	Barboza 323	Argentina: Salta
<i>Solanum americanum</i> Mill.	Subgen. <i>Solanum</i> , sect. <i>Solanum</i>	CORD 585	Argentina, Santa Fe
<i>Solanum caesium</i> Griseb.	Subgen. <i>Solanum</i> , sect. <i>Solanum</i>	Barboza <i>et al.</i> 1055	Argentina, Jujuy
<i>Solanum huayavillense</i> Del Vitto & Petenatti	Subgen. <i>Solanum</i> , sect. <i>Solanum</i>	Barboza <i>et al.</i> 308	Bolivia, Tarija
<i>Solanum incisum</i> Griseb.	Subgen. <i>Solanum</i> , sect. <i>Solanum</i>	Hunziker 10764	Argentina, Córdoba
<i>Solanum pilcomayense</i> Morong	Subgen. <i>Solanum</i> , sect. <i>Solanum</i>	Barboza <i>et al.</i> 360	Argentina, Corrientes
<i>Solanum trisectum</i> Dunal	Uncertain	Hunziker 25650	Netherlands, Nijmegen, cult.



Figures 1–13. Morphological features of the stomium before and after opening in poricidal and poricidal-longitudinally dehiscent anthers of *Solanum* species. Figs 1–8. Poricidal anthers. Figs 9–13. Poricidal-longitudinally dehiscent anthers. Fig. 1. Apical pores in an open anther of *S. glaucophyllum*. Fig. 2. Short apical slits that form the pores in *S. toldense*. Fig. 3. Lateral surface of an anther of *S. toldense* before opening, showing a pattern of short lateral longitudinal slits. Fig. 4. Detail of several lateral longitudinal slits of an anther of *S. toldense*. Figs 5, 6. Pattern of lateral pores before (Fig. 5; arrows) and after (Fig. 6) opening in *S. consimile*. Fig. 7. Apical pores (arrows) before opening in *S. seaforthianum*. Fig. 8. Ventral side of an open anther of *S. seaforthianum*. Note the close placement of the two apical pores and the lateral furrows continuous with the pores. Fig. 9. Ventral surface of an open anther of *S. argentinum*. Fig. 10. Apical end of the stomium before opening in an anther of *S. argentinum*. The wider apical end of the stomium (arrows) forms the pore. Figs 11–13. Apical end of the stomium before opening in *S. incisum* (Fig. 11), *S. concinnum* (Fig. 12) and *S. ramulosum* (Fig. 13), showing the different patterns of apical curvature in the stomium, where pores will be formed. Scanning electron micrographs. Scale bars: Figs 1, 4–6, 13, 100 μm ; Figs 7, 10–12, 150 μm ; Fig. 9, 200 μm ; Figs 2, 3, 8, 250 μm .

the stomium is pronounced, and therefore the large pores formed are transversally elongate (Fig. 8).

Poricido-longitudinal stomia

A group of species exists in which the opened stomium is formed by an apical pore and a longitudinal slit (Fig. 9). The pore is usually placed on the ventral side of the theca. The pore is continuous with a longitudinal slit running down to the base on the lateral surface, where the anther walls separate slightly from each other. Nevertheless, before anther opening, the apical end of the stomium (that is, where the pore will be formed) is morphologically diverse. In *S. argentinum*, *S. caesium*, *S. granuloso-leprosum*, *S. pennellii* and *S. trizygum*, the stomium shows an apical sigmoid curve in its wider part (Fig. 10). An elongate pore is formed afterwards in this wider part (Fig. 9). In *S. americanum*, *S. argenteum*, *S. incisum* and *S. pilcomayense*, the stomium also shows a sigmoid curve at its apical end, but the width of the stomium is uniform along the theca (Fig. 11). A pore is formed in the apical curve of the stomium. In *S. concinnum* and *S. huayavillense*, the stomium presents a strongly pronounced sigmoid curve at its apical end (Fig. 12), where a pore almost transversally placed is then formed. In *S. acaule*, *S. caavurana*, *S. commersonii*, *S. ramulosum* and *S. spagazzinii*, the apical end of the stomium exhibits only a single slight curve towards the connective on the ventral surface of the theca (Fig. 13), where a wide elliptical pore is formed. In *S. wendlandii*, the stomium forms a slit along the theca that surrounds the apical end, and a rounded pore is formed in the upper curve of the slit.

Longitudinal stomia

In *S. lycopersicoides*, *S. nitidum* and *S. trisectum*, the stomium opens as a slit along the lateral side of the theca (in the latter species, the slit curves following the sinuous outline of the anthers). In *S. trisectum* and *S. nitidum*, the aperture is uniform along the entire length, although it is wider in the latter (Fig. 14). In *S. lycopersicoides*, the stomium aperture can be even wider, particularly in its apical quarter, where the borders of the anther walls may be bent slightly outwards (Fig. 15).

HISTOLOGICAL FEATURES

Stomium

Before anthesis, each theca is unilocular (Figs 16, 26, 27), because the septum that separates the two pollen sacs is already broken. The stomium differentiates in the anther wall facing the septum. In longitudinally dehiscing and poricidal-longitudinally dehiscing anthers, the septum breaks immediately below the



Figures 14, 15. Morphology of the open stomium in longitudinally dehiscing anthers of *Solanum* species. Fig. 14. Ventral side of anther of *S. nitidum*. Fig. 15. Ventral side of anther of *S. lycopersicoides*. Note the regular aperture in *S. nitidum* (Fig. 14) and the wider aperture in *S. lycopersicoides* (Fig. 15), where the walls are bent slightly outwards in the apical third (arrows). Scanning electron micrographs. Scale bars, 250 μm .

epidermis all along the stomium. In contrast, in poricidal anthers, septum breakage immediately below the epidermis occurs only in the parts of the anther wall that will open (Figs 16, 17), whereas it occurs several layers below the epidermis in the rest of the theca (Fig. 18) where it does not open. In addition, the septum usually remains intact in a small portion at the basal end of these anthers (Fig. 19), and the two pollen sacs are still present in each theca. As a consequence, the part of the anther wall that opens effectively, and that can be properly called a stomium, is recognized in cross-section as being formed only by small epidermal cells, which are the only cells of the wall in that part.

Poricidal stomia: Two to three small epidermal cells form the stomium at the apical end of the anther in *S. betaceum*, *S. corymbiflorum*, *S. endoadenium*, *S. fusiforme*, *S. glaucophyllum* (Fig. 16), *S. stuckertii* and *S. tucumanense*. Further down the anther, the epidermis becomes homogeneous and one to three layers of small cells are present below the epidermis, where the anther does not open (Fig. 18).

All the sites (apical and lateral) recognized as stomia in *S. consimile* and *S. toldense* are histologically equivalent to the unique pore described in the preceding paragraph (Fig. 17). In these two species,

the number of anther wall layers below the epidermis increases and decreases, defining the sites of lateral opening along the theca.

In *S. seaforthianum*, the pores are formed by eight to ten tiny epidermal cells in a cross-section (the apical curve observed superficially; Fig. 7).

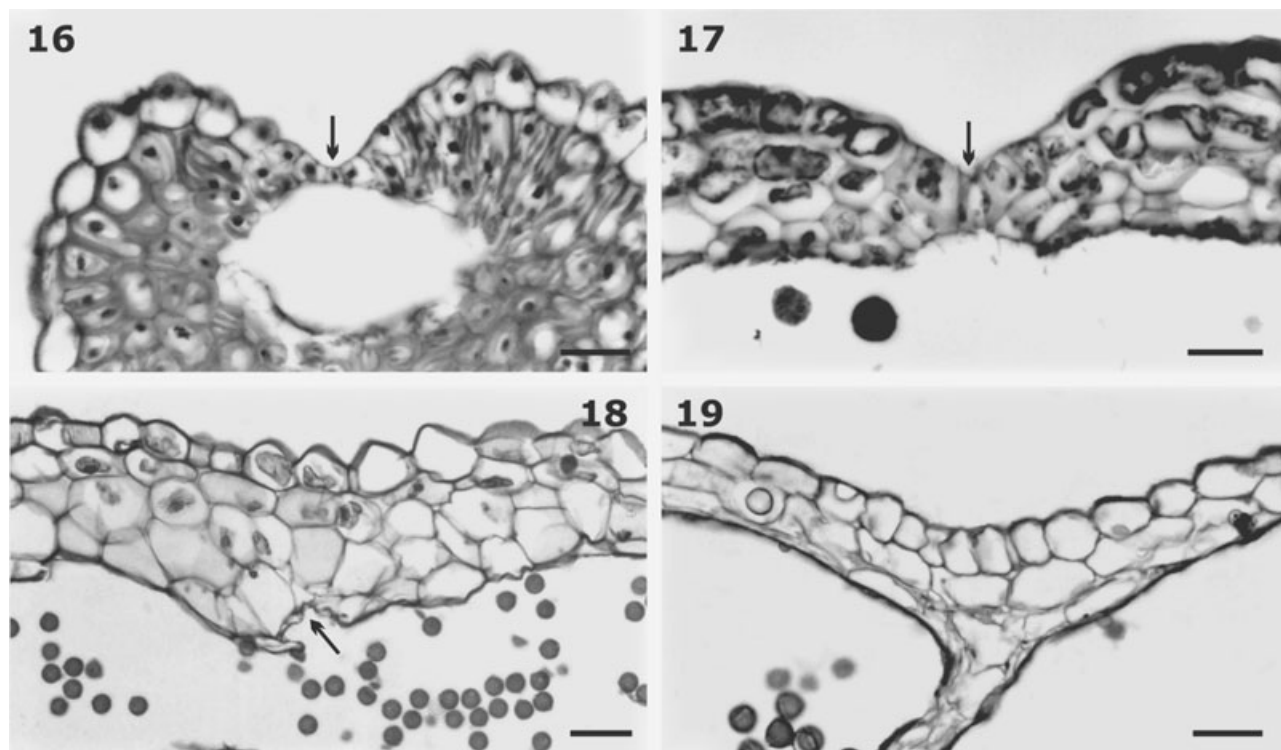
Poricido-longitudinally dehiscing stomia: The apical pore and the longitudinal slit are clearly distinguishable from each other in *S. argentinum*, *S. granuloso-leprosum*, *S. pennellii* and *S. trizygum*, as eight to ten cells form the wider apical part (Fig. 20) and only two cells form the slit (Fig. 21). In *S. trizygum* and *S. pennellii*, the epidermis on each side of the slit forms a particular crescent-shaped structure, in which approximately three cells are radially enlarged to form a 'ridge' (Figs 20, 21). In *S. granuloso-leprosum*, ridges are formed by six to eight cells lining the wider apical end of the stomium, and by three to five cells along the slit.

In *S. americanum*, *S. caesium* and *S. huayavillense*, the pore is slightly different from the slit, as four to five cells form the pore and approximately two cells

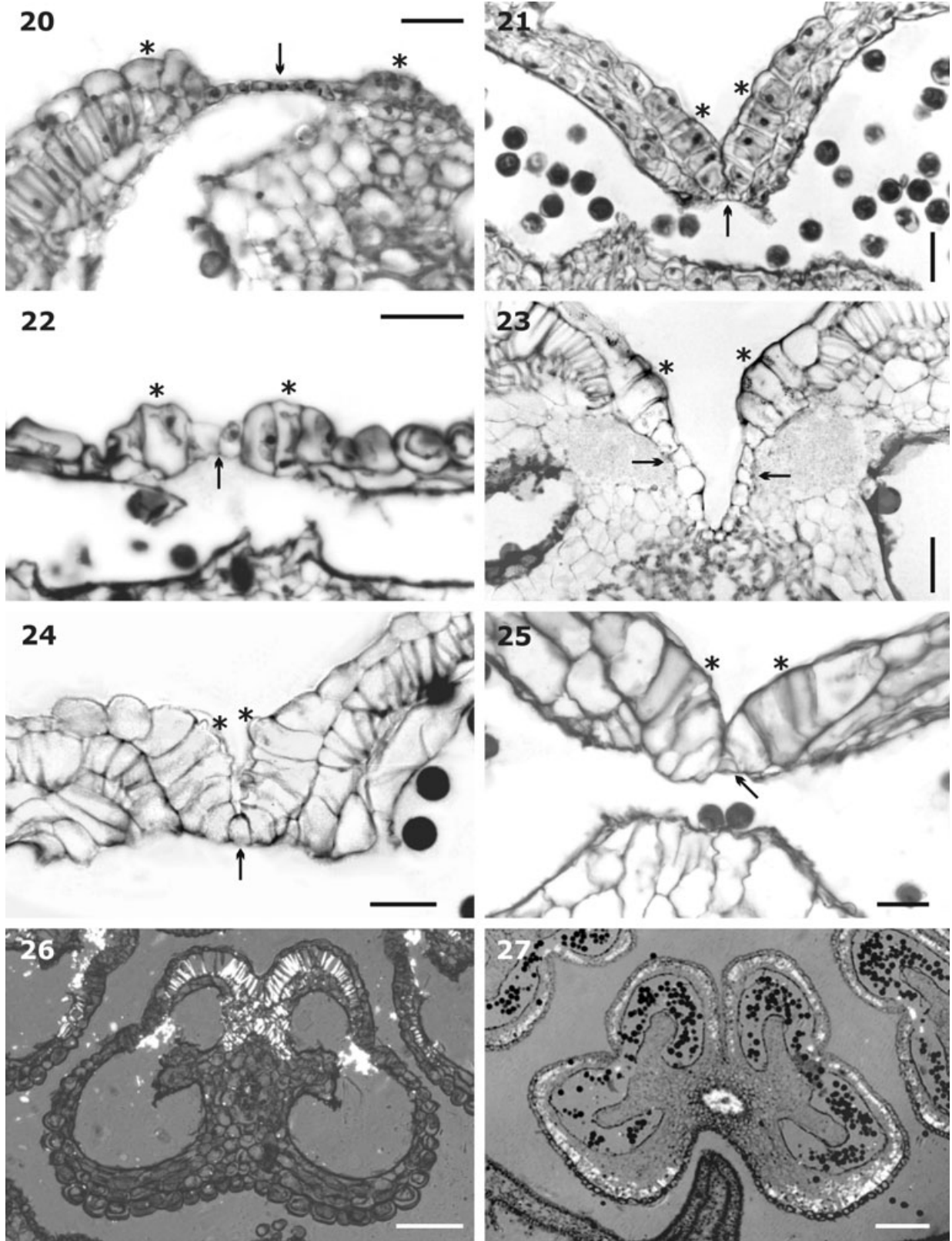
form the slit (Fig. 22). There are ridges along the whole stomium, formed by two to three cells in *S. americanum* (Fig. 22) and by approximately five cells in *S. caesium* and *S. huayavillense*.

In *S. acaule*, *S. argenteum*, *S. caavurana*, *S. commersonii*, *S. concinnum*, *S. incisum*, *S. pilcomayense*, *S. ramulosum*, *S. spegazzinii* and *S. wendlandii*, the stomium is formed by (1)2–3(4) small epidermal cells along its entire length (Figs 23–25); therefore, despite the possible variations, the pore and slit are not clearly distinguishable histologically. In particular, in *S. incisum* and *S. ramulosum*, the stomial cells are only slightly smaller than the rest of the layer. These ten species present ridges formed by 3–5(6) cells along the entire stomium (Figs 24, 25), although the ridges are smaller towards the apical end (Fig. 23).

Longitudinal stomia: Two to four cells form the stomium along its entire length in *S. lycopersicoides*, *S. nitidum* and *S. trisectum*. There are ridges formed by three to five cells in the four species; these line the whole stomium in *S. nitidum* and *S. trisectum*, but, in



Figures 16–19. Histological features of the stomium and surrounding tissues in poricidal anthers of *Solanum* species. Cross-sections. Fig. 16. Apical pore (arrow) before opening in *S. glaucophyllum*. Fig. 17. Lateral slit (arrow) before opening in *S. toldense*. Fig. 18. Anther wall facing the septum in the middle part of the anther in *S. tucumanense*. The arrow points to the zone in which the septum was attached. Note the number of layers below the epidermis and the absence of a stomium. Fig. 19. Basal part of the anther of *S. seaforthianum*. Note the complete septum and the absence of a stomium. Scale bars, 40 μ m.



Figures 20–27. Histological features of the stomium and surrounding tissues in poricidal-longitudinally dehiscent and longitudinally dehiscent anthers of *Solanum* species. Figs 20–26. Poricidal-longitudinally dehiscent anthers. Fig. 27. Longitudinally dehiscent anther. Cross-sections. Figs 20–25. Bright field. Figs 26, 27. Polarized light. Figs 20, 23. Apical end of the stomium (arrow) in which the pore will be formed. Note the small epidermal ridges (asterisks) lining the stomium. Fig. 20. Stomium formed by a zone of eight cells in *S. trizygum*. Fig. 23. Apical part of the stomium formed by three to four cells in the two thecae of an anther of *S. concinnum*. Figs 21, 22, 24, 25. Lateral slit of *S. pennellii* (Fig. 21), *S. americanum* (Fig. 22), *S. caavurana* (Fig. 24) and *S. wendlandii* (Fig. 25). Note the epidermal ridges (asterisks) lining the stomium (arrow). Figs 26, 27. Distribution of cells with thickened walls (bright cells). Fig. 26. Cells with thickened walls on the ventral side at the apical end of the anther of *S. americanum*. Fig. 27. Basal third of an anther of *S. nitidum* showing endothecial cells across the entire anther wall. Scale bars: Figs 20–25, 40 µm; Fig. 26, 100 µm; Fig. 27, 300 µm.

S. lycopersicoides, are absent in the apical part where cells with thickened walls are present (see below).

Distribution of cells with thickened walls

Because cells from several subepidermal layers and the connective tissue develop thickened walls, they are treated as a whole, including the endothecium. The thickenings are reticulate ribs in all cases (Carrizo García, 2002b), although they can follow different patterns in the connective tissue and anther wall. In general, in all species studied, cells with thickened walls are present at the apical end of the theca, mainly on the ventral side (Fig. 26); these cells gradually decrease in number downwards, until they disappear. There is a minor variation in this pattern amongst the species. Cells with thickened walls surround the pores in poricidal anthers, but do not surround the lateral apertures of *S. consimile* and *S. toldense*. In poricidal-longitudinally dehiscent anthers, cells with thickened walls show a similar distribution to that in poricidal anthers, extending around the apical pore and sometimes also on the sides of the upper part of the slit.

In *S. trisectum*, cells with thickened walls are restricted to a small zone at the uppermost end of the thecae. *Solanum lycopersicoides* has cells with thickened walls in approximately the apical quarter of the anther, which coincides with the zone in which the stomium aperture is wider. In *S. nitidum*, although a greater density of cells with thickened walls is found at the apical end of the thecae (that is, all the subepidermal layers and part of the connective tissue), an endothecium with thickened cell walls is present down to the basal end of the thecae (Fig. 27).

DISCUSSION

STOMIUM MORPHOLOGY

The mode of anther opening in *Solanum* is not uniform. Siddiqui & Khan (1988) studied ten *Solanum* species and observed different mechanisms of anther opening. A similar variability was found in the sample of *Solanum* species analysed here. Anther

opening in *Solanum* can occur via: (1) a simple pore at the apical end of each theca, in some cases accompanied by other lateral apertures; (2) an apical pore that continues downwards with a longitudinal slit; or (3) a longitudinal slit that runs along each theca. It is possible that additional variations may be found if the sampling of the species is enlarged. Although the genus *Solanum* is identified as having *Solanum*-type flowers (including the cases with a bellows-like mechanism), characterized by poricidal anthers that keep the pollen enclosed until the pollinator vibrates the anther (Buchmann, 1983; Endress, 1994, 1996), the anthers may also open via other modes. The presence of other forms of anther opening, different from the strict poricidal mechanism, is not an exclusive feature of *Solanum* amongst the taxa with the same pollination syndrome, as other cases have been reported in Rubiaceae (Puff *et al.*, 1995). However, it must be emphasized that, although the mode of anther opening is diverse in *Solanum*, because the anther walls do not bend outwards completely in the species observed, the pollen remains more or less hidden in the anther.

Before opening, the stomium shape is also diverse, even amongst species with the same mechanism of opening. Accordingly, the part of the stomium that corresponds to the pore is also diverse. In agreement with Buchmann's (1983) criteria, the so-called pore can represent morphologically not only a pore, but also a short slit, as in *S. glaucophyllum* (longitudinal slit) or *S. seaforthianum* (transverse slit). An interesting example is *S. consimile*, in which the apical pore of the theca corresponds to a short slit, whereas the lateral apertures may be considered as true pores, although the latter may not be involved in pollen shedding. In addition, amongst the species with poricidal-longitudinally dehiscent stomia, the pore is formed in a distinct part of the stomium, as a slightly widened portion (for example, *S. argentinum*) and/or the curved apical end (for example, *S. concinnum* and *S. ramulosum*). These observations suggest that the formation of a wide apical pore does not depend exclusively on the stomium shape.

HISTOLOGY OF THE STOMIUM AND NEIGHBOURING CELLS

After septum breakage, the parts in which the anther will open can be clearly distinguished because, in these parts, the wall is formed only by the epidermis, and the epidermal cells are smaller than in the non-dehiscing area. Thus, it is easy to predict which parts of the anther wall will not open: where cell layers appear below the epidermis or where the septum is not broken, as in the basal end of some poricidal anthers (for example, *S. glaucophyllum*, *S. tucumanense* and *S. corymbiflorum*). Although a superficial furrow or different markings that seem to represent the stomium are present, the differentiation of the stomium at the histological level is restricted to the sites at which the anther wall will open effectively. By the ablation of specific cells in tobacco, Beals & Goldberg (1997) have found that the formation of a definite stomium is required to achieve anther opening.

The ridges observed lining the stomium have been defined here as a group of radially elongate epidermal cells, which seem to exert pressure over the cells of the stomium. This type of ridge has also been observed in other species of Solanaceae, such as *Physochlaina orientalis* G. Don, *Deprea paneroi* Benitez & Martínez and *Nicotiana glauca* Graham (Carrizo García, 2002a), *Solanum lycopersicum* L. (Bonner & Dickinson, 1989) and wild tomatoes (Carrizo García & Barboza, 2006). In addition, the formation of ridges is obvious in *Grabowskia boerhaviaefolia* (L.) Schltdl. and *Exodeconus miersii* (Hook.) D'Arcy (D'Arcy, Keating & Buchmann, 1996: 162). The formation of these structures is not exclusive to Solanaceae, as ridges formed by larger cells have been described, for instance, in *Cassia* (called 'lips'; Venkatesh, 1956b), where they have been associated with the widening of the stomium aperture. Bonner & Dickinson (1989) related the ridges directly with the stomium opening in *S. lycopersicum*, possibly working together with the endothecium by generating and transmitting a mechanical force to the cells of the stomium. Although the ridges are smaller in the *Solanum* species studied here, they may function in a similar manner. The ridges may contribute to stomium opening, working together with the layers of cells with thickened walls, or alone where cells with thickened walls are absent, as proposed for wild tomatoes (Carrizo García & Barboza, 2006).

CELLS WITH THICKENED WALLS

Eames (1977) stated that: 'the details of opening vary greatly, especially the extent of retraction of the walls, ... the variations depend, at least in part, upon the extent of the fibrous layer'. The participation of layers

with thickened cell walls in anther wall shrinkage and the consequent widening of the anther aperture has been demonstrated in particular mutants (Dawson *et al.*, 1999; Steiner-Lange *et al.*, 2003). Endress (1996) provided several examples of species in which the relationship between the form of anther opening and the presence of layers with thickened cell walls does not follow a definite pattern, including cases of *Solanum*-type flowers. However, in the *Solanum* species studied here, it is possible to relate the width of the anther aperture with the presence and distribution of cell layers with thickened cell walls, which have a distinctive influence on the shape of the open stomium. In poricidal and poricidal-longitudinally dehiscing anthers, cells with thickened walls are developed only around the apical pores, where they are possibly involved in the widening of the pores by wall shrinkage. Furthermore, the presence of cells with thickened walls in the connective tissue, working together with those of the anther wall, may enhance the aperture, as suggested by Hufford & Endress (1989). Although, in some poricidal-longitudinally dehiscing stomia, there is a different part of the stomium in which the pore will be formed, it seems clear that the wider opening is reached where cells with thickened walls surround the stomium. In species of other families, the formation of the pore has also been related to the presence of thickened cells in the surrounding tissues (Venkatesh, 1956a, b, 1957; Keijzer *et al.*, 1996; Manning, 1996; Matsui *et al.*, 1999, 2000). The inconspicuous lateral apertures in the anthers of *S. toldense* and *S. consimile*, and the lateral slit in the anthers of poricidal-longitudinally dehiscing species of *Solanum*, may be explained by the absence of cells with thickened walls in these parts of the theca. With regard to the case of longitudinally dehiscing anthers, the relationship proposed between the presence of cells with thickened walls and the width of the opening may explain why, in *S. lycopersicoides* anthers, the opening is wider in the apical part. Cells with thickened walls differentiate only near the apical quarter of the anther, where they may cause the broader aperture of the stomium and even a partial outward bending of the anther wall. A similar pattern has been observed in *S. lycopersicum* (Bonner & Dickinson, 1989) and in wild tomatoes (Carrizo García & Barboza, 2006), species closely related to *S. lycopersicoides*, in which the anther opening is longitudinal, but the aperture is wider in the apical part where cells with thickened walls develop. Conversely, the absence of cells with thickened walls along almost the entire length of the anthers in *S. trisectum* may explain the narrow stomium aperture in this species. The anther aperture in *S. nitidum* is more or less regular along its entire length, and this may be a result of the presence of cells with thickened walls along the entire anther. This case

closely resembles a typical longitudinally dehiscing anther, as in several other species of Solanaceae that have a fibrous endothecium along the entire anther (Carrizo García, 2002a, b).

CONCLUSIONS

Anther opening is diverse within *Solanum*, but the shape of the closed stomium does not, by itself, explain the variation observed. There are at least two main histological features that seem to constrain the form of the opened stomium: (1) the differentiation of small stomial epidermal cells as the sole anther layer in which the anther will open (a feature connected to septum breakage and the disappearance of the sub-epidermal layers); and (2) the distribution of cells with thickened walls, which define the width of the opening (wide or inconspicuous pores or slits). A final question arises with regard to the relevance of the form of anther opening as a diagnostic feature in *Solanum* systematics. Because the form of anther opening is a consequence of a particular inner structure, it may possibly provide information about the relationships between species, or may only represent minor adjustments in relation to the pollination mechanism of each species.

ACKNOWLEDGEMENTS

We thank Patricia Sarmiento (Scanning Electron Microscopy Service, University of La Plata, Argentina) for her valuable help in processing the material studied, and Juan Rueda for technical assistance with the scanning electron micrographs. We are also grateful to Consejo Nacional de Investigaciones Científicas y Técnicas, Ministerio de Ciencia y Tecnología (Córdoba), Secretaría de Ciencia y Tecnología (Universidad Nacional de Córdoba) and the Myndel Botanical Foundation for financial support.

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