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Pararchidendron pruinosum (*Mimosaceae-Ingeae*): ESEM Investigations on Anther Opening, Polyad Presentation and Stigma

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With 52 Figures

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Summary

TEPPNER H. & STABENTHEINER E. 2010. *Pararchidendron pruinosum* (*Mimosaceae-Ingeae*): ESEM investigations on anther opening, polyad presentation and stigma. – Phyton (Horn, Austria) 50 (1): 109–126, with 52 figures.

Anthers and anther opening of *Pararchidendron pruinosum* (BENTH.) NIELSEN var. *junghuhnianum* (BENTH.) NIELSEN were investigated using environmental scanning electron microscopy (ESEM). In general, observations were in good accordance with other *Mimosaceae*, but in the details of many characteristics there are deviations. The thecae sit parallel on a very wide and thick connective. The stomium is distinctly subsided. Opening of the thecae begins in the centre. The margin of the valves tends to be bent inwards and finally overtops the plane of the main part of the valves. As usual in *Mimosaceae*, the remnants of the parenchymatic transversal septum are distinct on the valves. In the open anther the two longitudinal bulges of the inward folded valves of a theca abut on each other usually. In the completely open anther the position of the 16-grained, relatively thick polyads presented on the flat parts of the valves usually, is often a little distorted. A large amount of uneven distributed pollenkitt is present. The rim-surrounded stigmatic cup with stigmatic fluid

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is of the wet, non-papillate type. Many of the presented structures or processes seem to be less specialized and thus, these features may be plesiomorphous at least within *Ingeae*.

Zusammenfassung

TEPPNER H. & STABENTHEINER E. 2010. *Pararchidendron pruinosum* (*Mimosaceae-Ingeae*): ESEM investigations on anther opening, polyad presentation and stigma. [*Pararchidendron pruinosum* (*Mimosaceae-Ingeae*): ESEM-Studien über Antheren-Öffnen, Polyaden-Präsentation und Narbe]. – Phyton (Horn, Austria) 50(1): 109–126, mit 52 Abbildungen.

Der Antherenbau und das Antherenöffnen von *Pararchidendron pruinosum* (BENTH.) NIELSEN var. *junghuhnianum* (BENTH.) NIELSEN wurden mittels ESEM (environmental scanning electron microscopy) untersucht. Die Merkmale stimmen grundsätzlich gut mit anderen bisher untersuchten *Mimosaceae* überein, aber in den Details gibt es viele Abweichungen. Die Theken liegen parallel auf dem sehr breiten und dicken Konnektiv. Das Stomium ist in eine Rinne eingesenkt. Das Öffnen der Theka beginnt im Zentrum. Der Rand der Valven rollt sich ein und liegt schließlich höher als der ebene Hauptteil der Valven. Die Reste des parenchymatischen, transversalen Septums sind wie üblich auf den Valven deutlich. In der offenen Anthere stoßen die beiden Längswülste der einwärts gefalteten Valven einer Theka üblicherweise aneinander. In der komplett geöffneten Anthere liegen die 16-körnigen, relativ dicken Polyaden nicht immer am Ort ihrer Entstehung; vielmehr ist die Anordnung oft etwas gestört. Die große Menge von Pollenkitt ist ungleichmäßig verteilt. Das von einem Randsaum umgebene, flach schüsselförmige Stigma mit Narbensekret gehört dem feuchten, nicht-papillösem Typ an. Viele der genannten Strukturen und Vorgänge erscheinen weniger spezialisiert, weshalb diese Merkmale, zumindest innerhalb der *Ingeae*, plesiomorph sein dürften.

1. Introduction

Continuing our studies on the variability in anther opening and its visual presentation in *Mimosaceae*, especially in *Ingeae* (TEPPNER & STABENTHEINER 2006, 2007) we investigated the Malesian *Pararchidendron pruinosum* (BENTHAM) NIELSEN var. *junghuhnianum* (BENTHAM) NIELSEN which is grown in the Botanic Garden of the Institute of Plant Sciences of the University of Graz. One of the two plants flowered in 2008 for the first time, at a size of c. 2.6 m. The treelet produced single or paired pseudoumbels on minute brachyblasts in the leaf axils. One partial inflorescence consisted of c. 15–50 flowers with 22–28 stamens each. The colour of the filaments turned from white to cream or ochre-brown during anthesis. Details of flower morphology and anthesis are described in TEPPNER 2010.

2. Material and Methods

Pararchidendron pruinosum (BENTH.) NIELSEN var. *junghuhnianum* (BENTH.) NIELSEN [det. H. TEPPNER May 27, 2009, using NIELSEN 1992 and COWAN 1998]. Grown in the temperate greenhouse (May-September outdoor) of the Botanic Garden of the Institute of Plant Sciences, University of Graz, Austria, Europe (see TEPPNER 2010: 92).

Whole partial inflorescences (pseudo-umbels) were brought to the laboratory for observation by stereomicroscope (dissecting microscope) Wild M 3B and Nikon SMZ645 or ESEM (environmental scanning electron microscope).

For ESEM investigations (see STABENTHEINER & al. 2010) fresh anthers or style tips were mounted on aluminium stubs using C-impregnated double sided tape. For the view into the stigmatic cup the style tips were mounted on the sides of a roof-like folded tape. The samples were investigated without any further preparation using a Philips XL 30 ESEM (FEI), using the following conditions: 0.8–0.9 torr chamber pressure, 9 mm working distance, 20 kv acceleration voltage, LFGSED (large field gaseous secondary electron detector). For studying the opening process, ripe but still closed anthers from flowers at the begin of the opening phase were transferred to the chamber of the ESEM, where opening of the anthers began to start after c. 5–20 minutes. For unknown reasons a number of anthers remained closed in all preparations. The results were compared with anthers which opened under greenhouse conditions or outdoor under a roof (during a rain period), respectively.

Additionally to the description of essential morphological characteristics (Fig. 1–20) we had the intention to illustrate the opening process by series of images from a number of anthers (fife) to show different views and some variability of the process (Fig. 21–48).

The figures were edited using Corel Photo Paint X4 (2008) and figure plates were composed using Corel Draw X4 (2008).

3. Results

3.1. Anther

The long filaments are coiled up in the flower buds and unroll and stretch during the first phases of anthesis (Fig. 1; TEPPNER 2010). The epidermal cells are slightly elongated, bulged and ridged. At the apical end of the filament a narrow isthmus of c. 0.1 mm length is developed; its epidermal cells are much elongated, narrow and smooth (Fig. 3, 8). Dehydration of the isthmuses' epidermal cells (Fig. 9) is the first sign of full ripeness of the anther and the impending opening phase.

Anthers are c. 0.25–3.0 mm long, c. 0.35–0.45 mm wide and c. 0.3 mm thick (Fig. 2–9). The connective is very thick and takes nearly the whole width of the anther (e. g., Fig. 5, 6, 8). At the apical end the connective forms an apparent bulge (Fig. 2, 4, 6, 7). In the sinus of this bulge the filament inserts (Fig. 3, 8, 9). A distinct attachment zone of c. 0.1×0.05 mm is developed, the epidermal cells showed a bulged, smooth surface. In the optical section of an anther in the light microscope this attachment zone appears as a part of the dorsal apex of the filament (TEPPNER 2010: 98–99, Fig. 7, 8). Between the two thecae inserted parallel on the connective a small but distinct interstice is developed (Fig. 2, 4, 6, 27, 32).

The stomium extends over the distal and proximal shoulders of the thecae up to the base of the valves at the connective. The margins of the valves tend to be bent inward along the stomium, thus the latter is subsided into a groove.

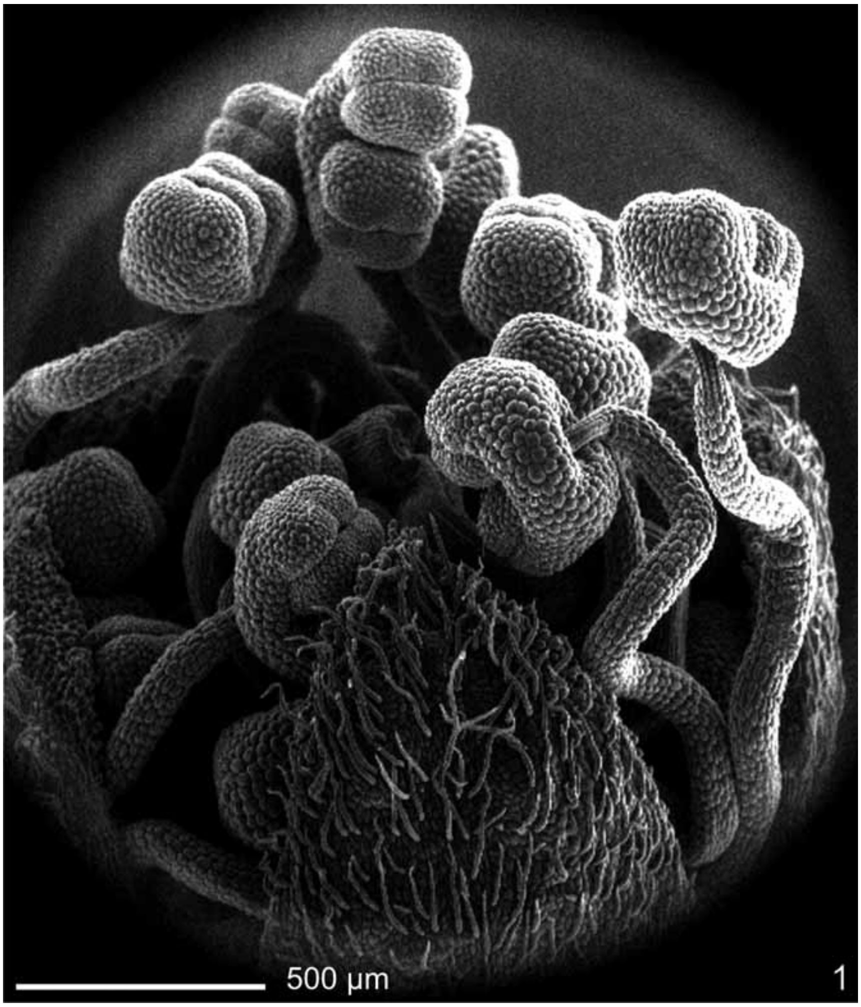


Fig. 1. *Pararchidendron pruinosum*, flower bud at the beginning of opening: the corolla lobes dehisced and the coiled filaments begin to stretch. Distal part of style and stigma in the centre of the figure.

The outer surface of the epidermal cells is bulged to papillose (with the exception of the rows along the stomium), most prominent on the connective (e. g., Fig. 2–8), the surface is ridged.

The endothecium, responsible for the anther opening, can be studied in the light microscope only (TEPPNER 2010).

The tapetal membrane with the orbicules can clearly be seen in the stages after meiosis in the light microscope. In the ESEM, however, this

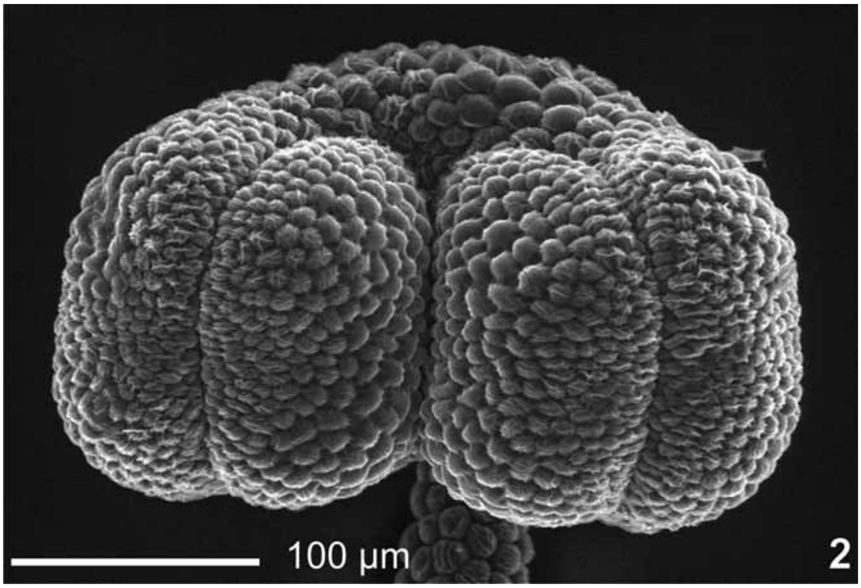


Fig. 2. *Pararchidendron pruinosum*, anther, ventral side.

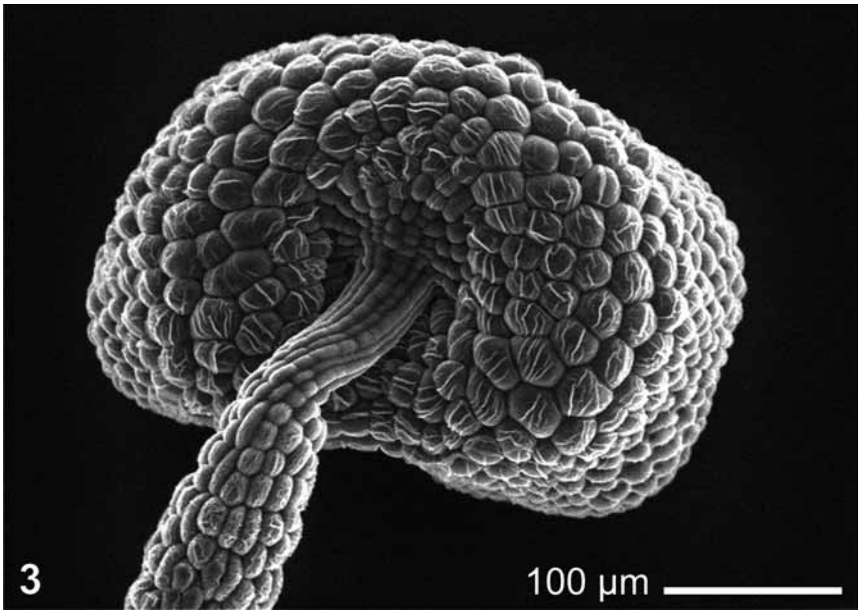


Fig. 3. *Pararchidendron pruinosum*, anther, dorsal side. The attachment zone with small, smooth, bulged cells above the filament insertion and the depression below. Note the elongated, smooth cells of the filament isthmus and the bulged and ridged cells of the remaining filament.

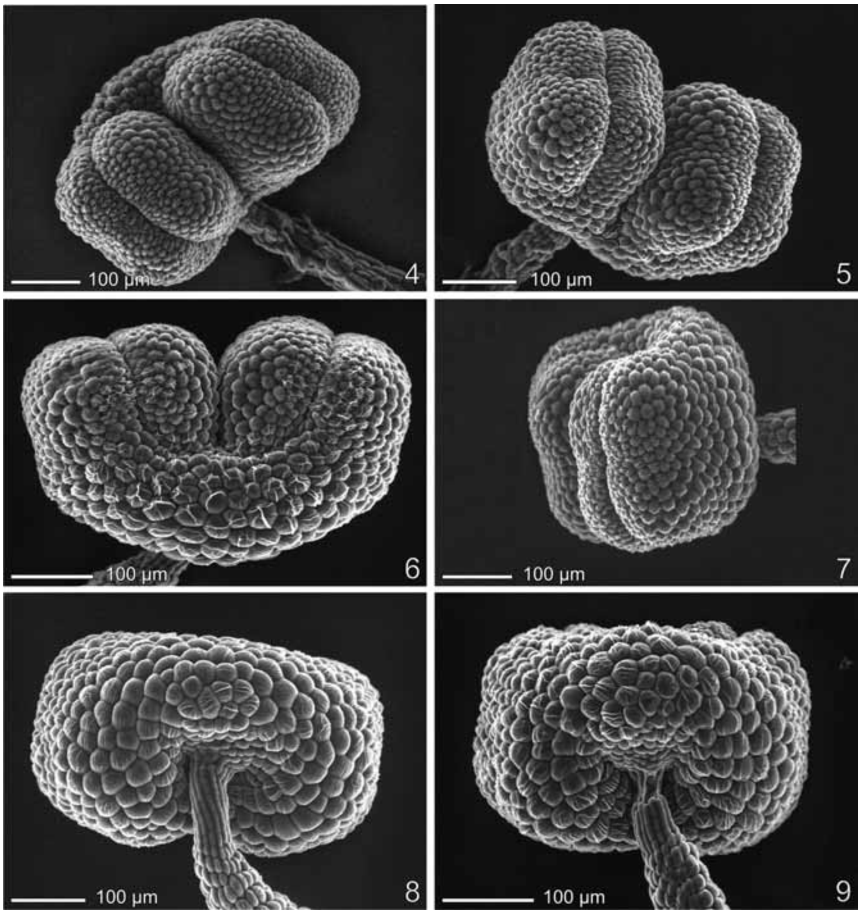


Fig. 4–9. *Pararchidendron pruinosum*, different views of ripe but still closed anthers. – Fig. 4. Ventral side. – Fig. 5. Ventral view, slanted from below. – Fig. 6. View of the distal (apical) end. – Fig. 7. Side view, the apical end above. – Fig. 8. Dorsal side with intact isthmus cells. – Fig. 9. Dorsal side, a slightly later stage, isthmus cells partly dried up.

membrane, appressed to the valve, is difficult to be observed (probably by superficial contamination with pollenkitt) (Fig. 13, 14, 16).

The anthers persist on the withered and dried filaments.

3.2. Polyads

The polyads are 16-grained with 8 pollen grains in two planes in the centre and 8 grains in one plane on the periphery. The diameter measures c. 50–75 µm, thickness is c. 40–45 µm. Side views of polyads suggest, that

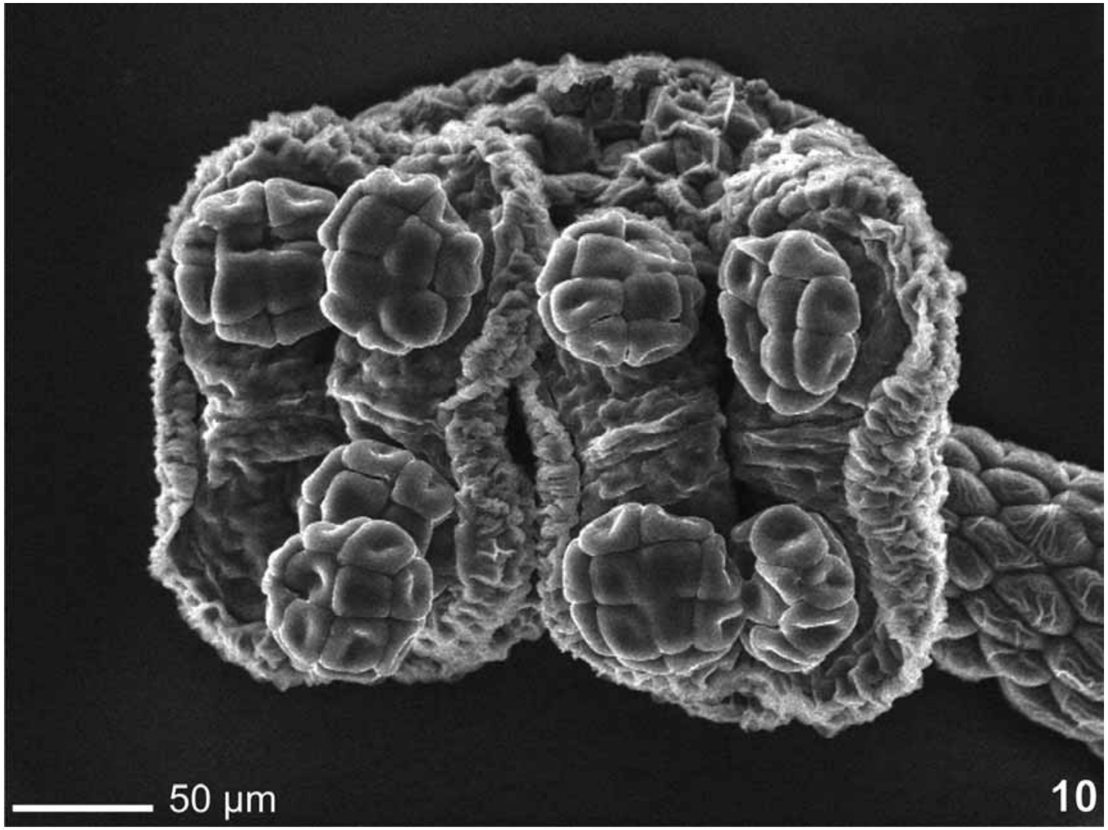


Fig. 10. *Pararchidendron pruinosum*, anther fully opened under natural conditions.

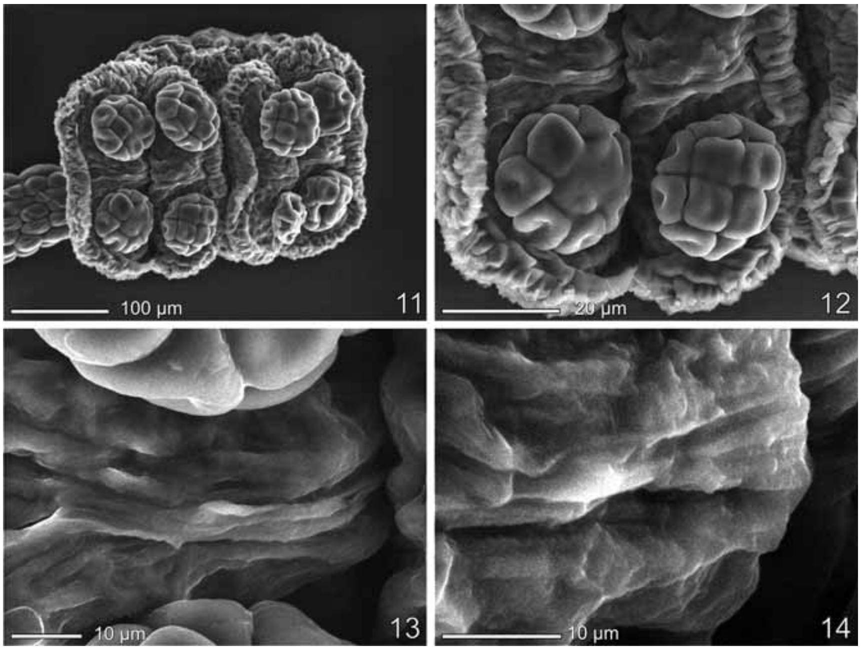


Fig. 11–14. *Pararchidendron pruinosum*, anther fully opened under natural conditions under a roof. Whole anther and details from the lower half of the anther. – Fig. 11. Open anther, apex above, margins of the valves bent inward, remnants of the transversal septa as ridges on the valves. – Fig. 12. The lower two locule halves of the left theca with the remnants of the transversal septum and two polyads, the left one with a large patch of pollenkitt. – Fig. 13. Remnants of the transversal septum on the left valve of the left theca. – Fig. 14. Detail of the left valve of the right theca, granulate structures: the tapetal membrane.

both faces of the polyad are similar but not identical [Fig. 31, 44 and comparison of Fig. 19 and 20 (lower or outer side) with the other figures (upper or inner side of the polyads)]. Most probably the bulges at the lower (outer) side of the polyads are more even (Fig. 20) and the ESEM investigations suggest, that the layer of pollenkitt is thin at this side.

The polyads are covered by pollenkitt. Thin layers cannot be proven with the ESEM. Thick irregular patches however, can be observed (e. g. Fig. 12, 18) as well as pollenkitt strings between polyads (Fig. 16, 44) or between polyad and valve (Fig. 18).

3.3. Opening Process and Polyad Presentation

Comparison of Fig. 1, 3, 5, 6-9 and 32 on the one hand with Fig. 27, 28, 33 and 45 on the other, shows clearly that the first sign of anther opening is

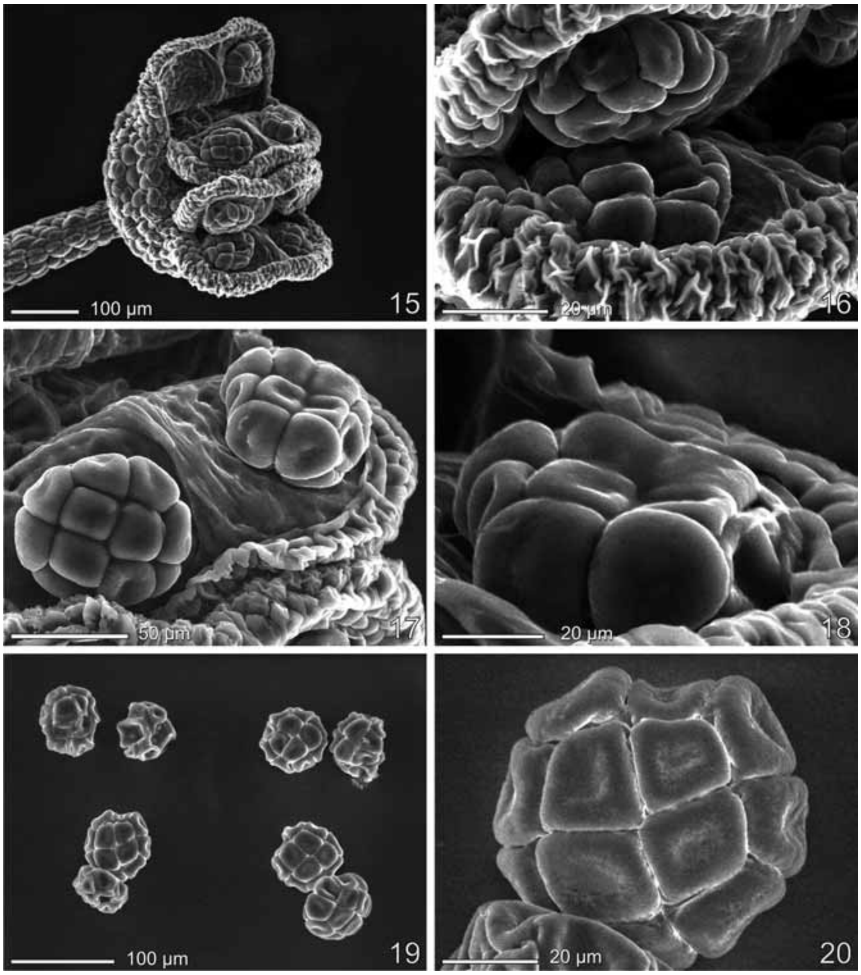


Fig. 15–18. *Pararchidendron pruinosum*, anther partly opened in the ESEM, distal end left. – Fig. 15. Remnants of the longitudinal septum visible at the bottom of the upper theca. – Fig. 16. Two opposite polyads in the opening theca, stuck with pollenkitt. – Fig. 17. One valve of an open locule of the upper theca with two polyads and the remnants of the transversal septum. – Fig. 18. A polyad with a large patch of pollenkitt in its locule-half.

Fig. 19–20. Under side (= outer or adherent side) of the polyads from a naturally opened anther. – Fig. 19. All eight polyads of an anther "stamped" onto the viscid tape of the stub. – Fig. 20. Detail of Fig. 19.

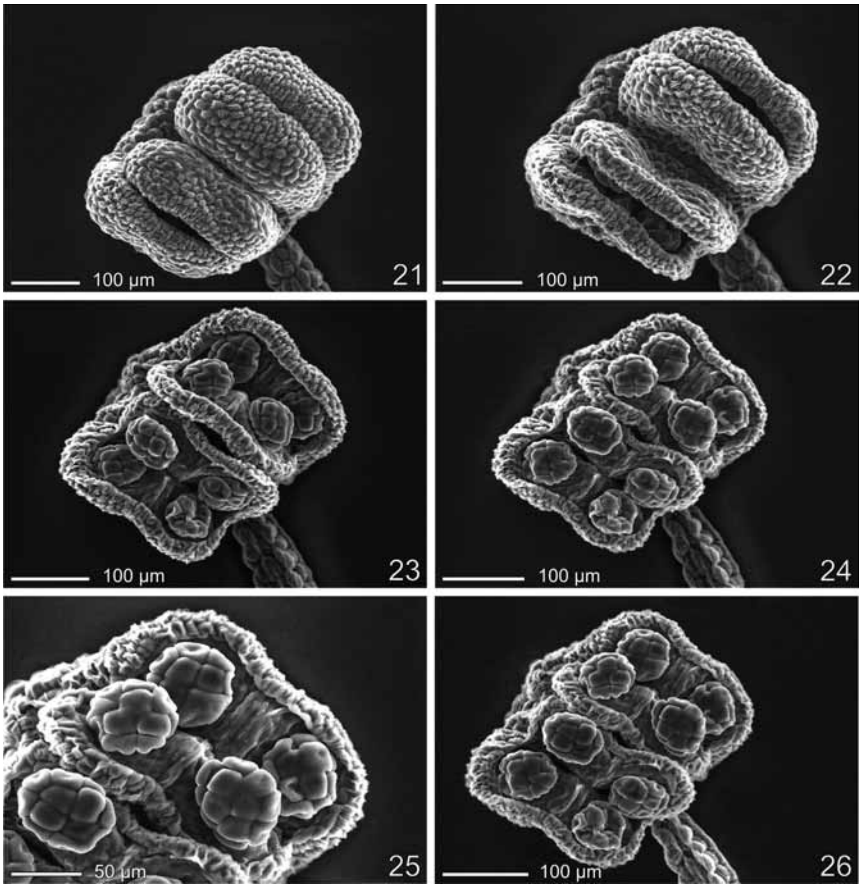
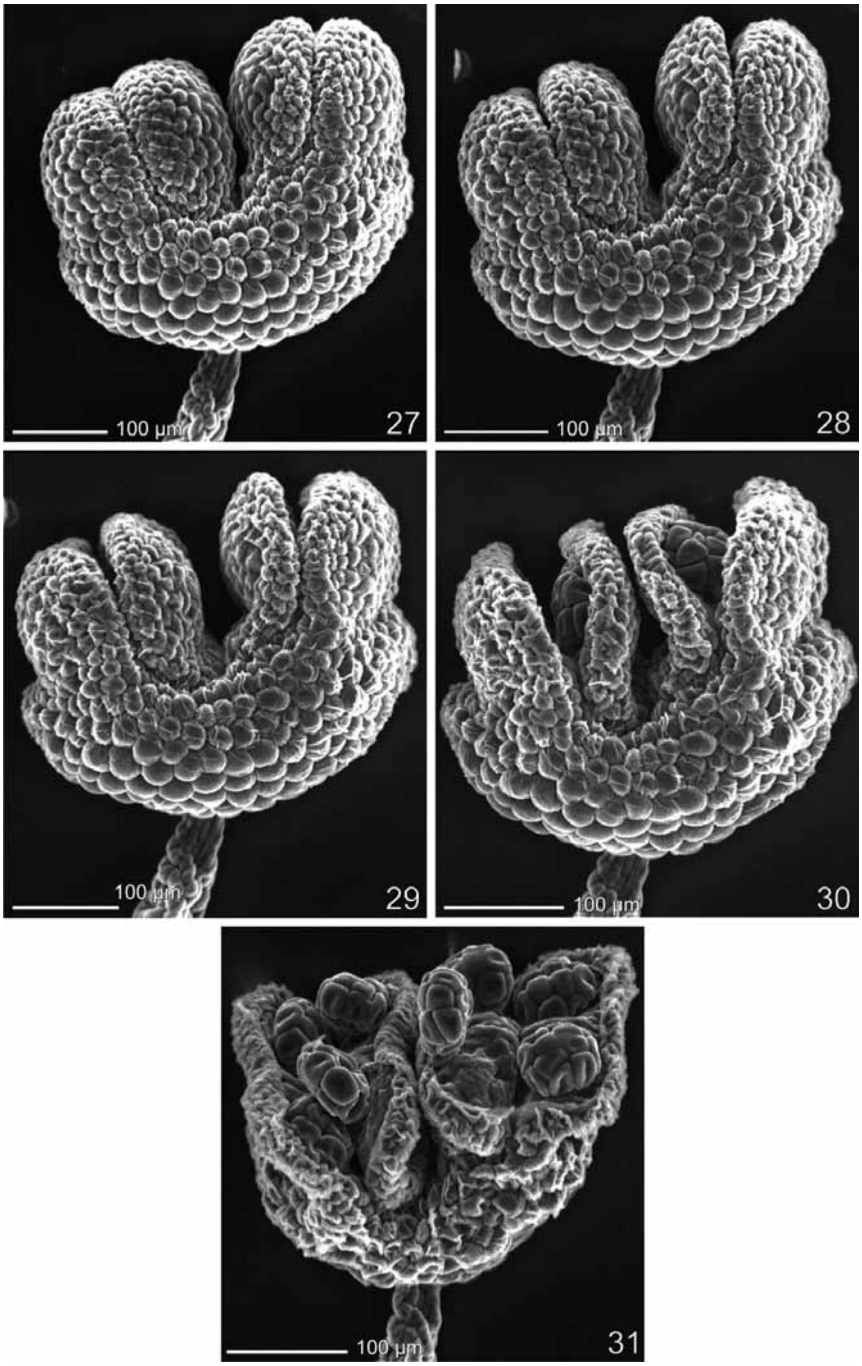


Fig. 21–26. *Pararchidendron pruinosum*, opening process of an anther in the ESEM. – Fig. 21. Stomium fully opened in both thecae, only a little later in the left one. – Fig. 22. Opening of the valves a little progressed. – Fig. 23 and 24. Wide open thecae, inner valves overlap alternating. – Fig. 25. Detail of Fig. 24, right theca. – Fig. 26. Fully opened anther. – Time span between Fig. 21 and 24 c. 40 minutes, between Fig. 21 and 26: c. 2 hours 18 minutes.

Fig. 27–31. *Pararchidendron pruinosum*, opening process of an anther in the ESEM, view of the distal end. The indentation at the base of the valve, caused by the longitudinally inward folding, in all figures. – Fig. 27–30 the first stages. – Fig. 31. The fully opened anther, in the left theca the valves not flat, exceptionally. – Time span between Fig. 27 and 30. c. 15 minutes, between Fig. 30 and 31. c. 1 hour 21 minutes.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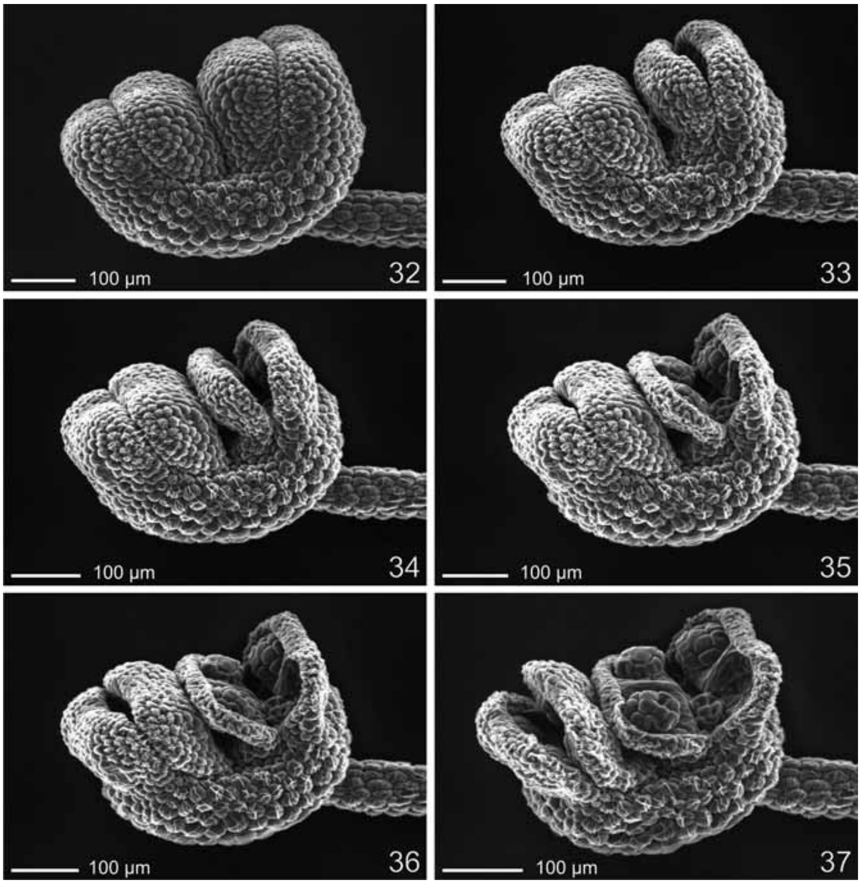

Fig. 32–37. *Pararchidendron pruinosum*, opening process of an anther in the ESEM, view slanted to the distal end. – Fig. 32. Anther before opening. – Fig. 33. Start of opening in the right theca. – Fig. 35. Start of opening in the two thecae distinctly asynchronous, start in the left theca. – Fig. 37. Opening progressed, one probably degenerated polyad squeezed between the longitudinal bulges of the valves. – Continuation in Fig. 38 and 39.

the begin of a longitudinal indentation at the very base of the valves.This inward folding of the valve-base is quickly followed by the opening of the thecae at the stomium. Opening starts more or less in the centre (middle) (Fig. 35–36, 41) and proceeds very quickly to the ends at the connective (Fig. 21, 22, 28, 33–36, 45–46). With the further opening of the valves the longitudinal inward folding of the valves progresses (Fig. 21, 22, 28–30, 33–38, 40–43, 45–47). Finally the longitudinal bulges of the two valves touch one another along the middle (boundary between the two locules). At the

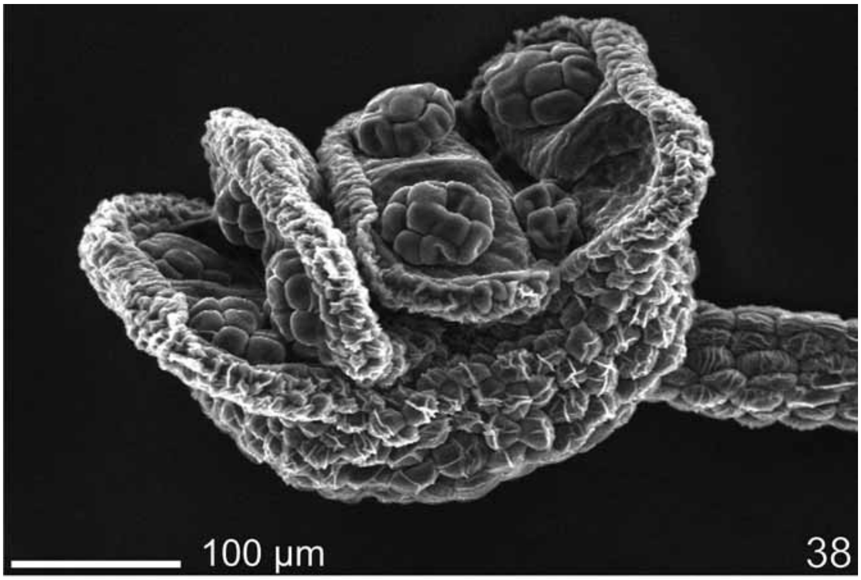

Fig. 38. *Pararchidendron pruinosum*, opening process of an anther (continuation from Fig. 32–37), different stage in the two thecae. – Time span between Fig. 32 and 38 c. 1 hour 4 minutes. – Continuation and end in Fig. 39.

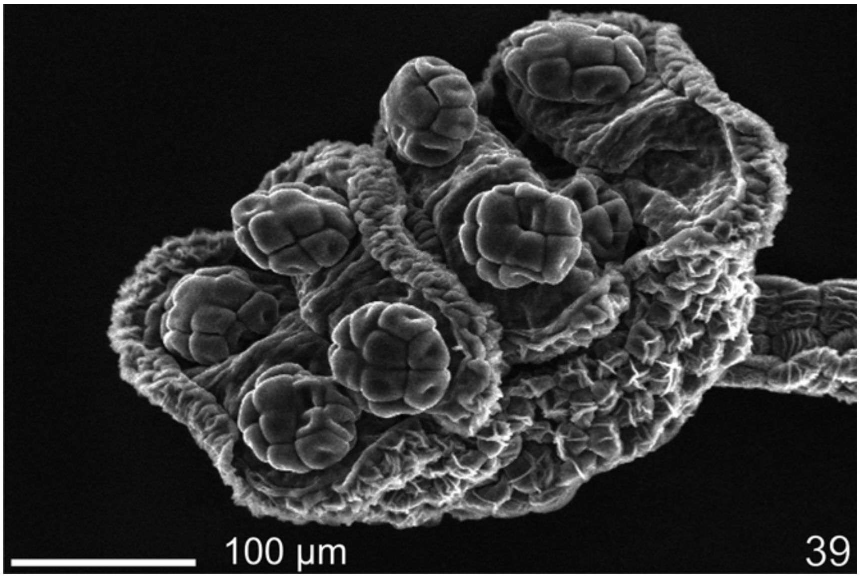

Fig. 39. *Pararchidendron pruinosum*, opening process of an anther (continuation from Fig. 32–38), both thecae fully open. – Time span between Fig. 38 and 39 c. 1 hour 13 minutes.

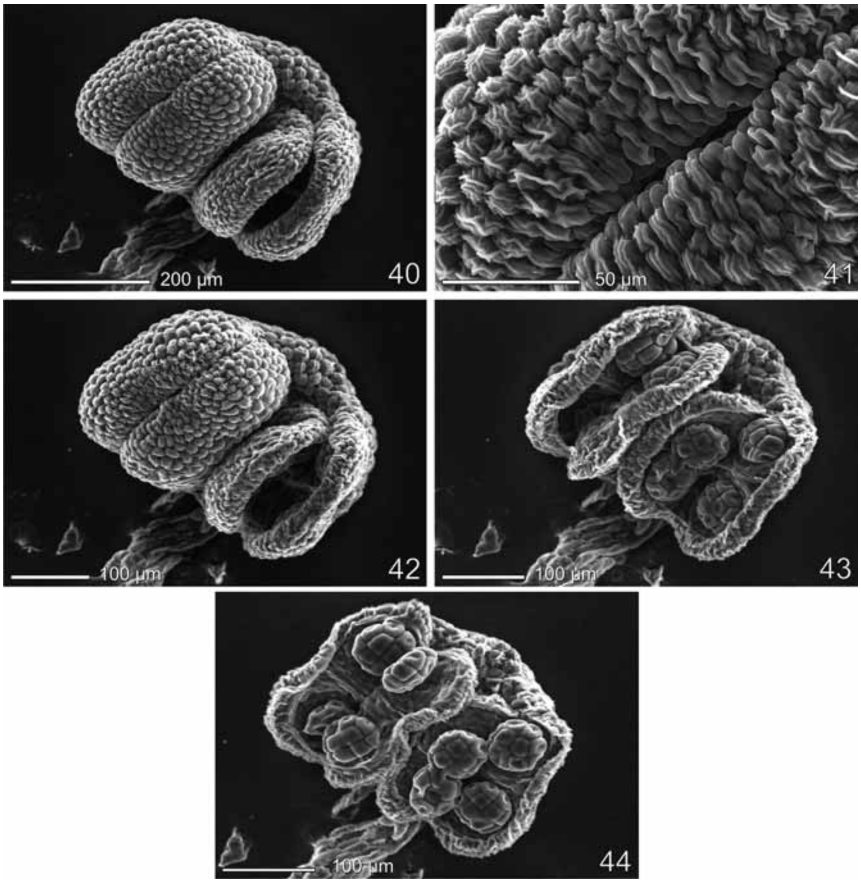

Fig. 40–44. *Pararchidendron pruinosum*, opening process of an anther in the ESEM, view of the ventral side, distal end above. – Fig. 40. Start of the splitting between the valves in the centre of the left theca. – Fig. 41. Detail of the left theca in Fig. 40. – Fig. 42 and 43. Progress of the opening. – Fig. 44. Fully opened anther, in the left locule-half a degenerated polyad. – Time span between Fig. 40 and 43 c. 40 minutes, between Fig. 40 and 44 c. 2 hours 10 minutes.

end of this process the four valves (their parts distal of the longitudinal bulges) of the open anther form a more or less flat surface, presenting the polyads (Fig. 10, 11, 26, 39 and 48).

Usually, the inward folding of the valves proceeds so quickly, that the remains of the longitudinal septum between the two locules of a theca are rarely to be seen (Fig. 15, 17, 43) and are hidden under the longitudinal bulges, usually. In contrast, the remnants of the dissolved transversal septum form distinct transveral ridges on the open valves (Fig. 10-17, 23-26

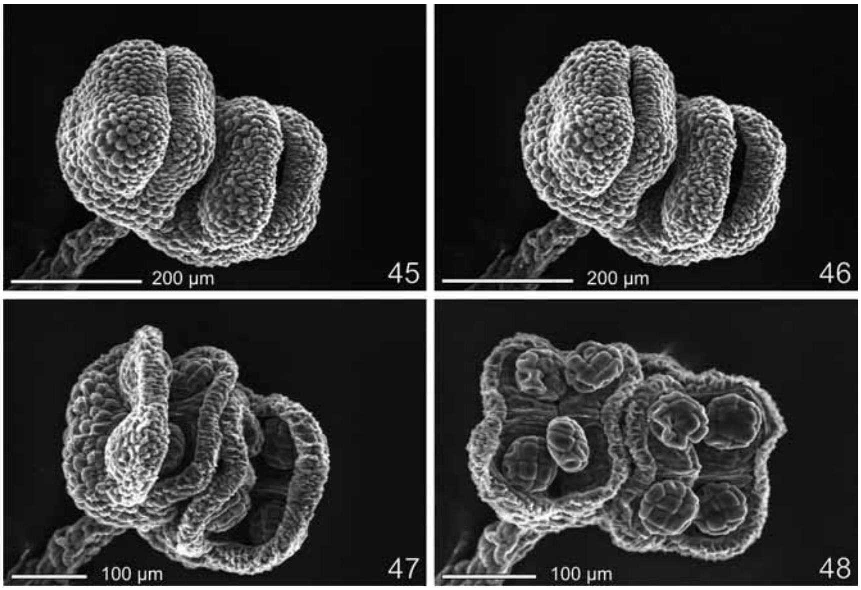

Fig. 45–48. *Pararchidendron pruinosum*, opening process of an anther in the ESEM, slanted to the proximal end. – Fig. 45. Start of opening at different time in the two thecae. – Fig. 46 and 47. Progress of the opening. – Fig. 48. Fully opened anther. – Time span between Fig. 45 and 47 c. 48 minutes, between Fig. 45 und 48 c. 1 hour 53 minutes.

etc.). Theoretically, the border between the remains of the transversal septum and the surface of the cavity of the locule-halves should be distinct by the absence of the tapetal membrane on the former. In *Pararchidendron* this is not true because of the indistinctness of the tapetal membrane (Fig. 13,14).

The inner surface of the locule-halves is irregularly and unevenly bulged (Fig. 10, 11, 15, 31, 37–39). The slight inward bending of the margin of the closed valves (e. g., Fig. 4–7) is maintained. Finally, they form a slightly rolled, elevated margin on the outer side (e. g., Fig. 10, 11, 15–18, 35–39, 47–48). At the inner contact zone of the two thecae the valves lie side by side (Fig. 11, 31, 48) or they overlap in various ways (Fig. 10, 23–26, 39, 44).

Finally, the polyads are presented more or less like on a table. They often adhere at the inner side of the valves at the place of their origin (Fig. 10 right theca, Fig. 11 left theca, Fig. 48 right theca). However, position and orientation on the open valves can vary frequently. They can adhere, e. g. with the narrow side (Fig. 31 left theca, Fig. 39 above, Fig. 44 above) or even at the margin of the valve (Fig. 11 left theca, Fig. 31 right

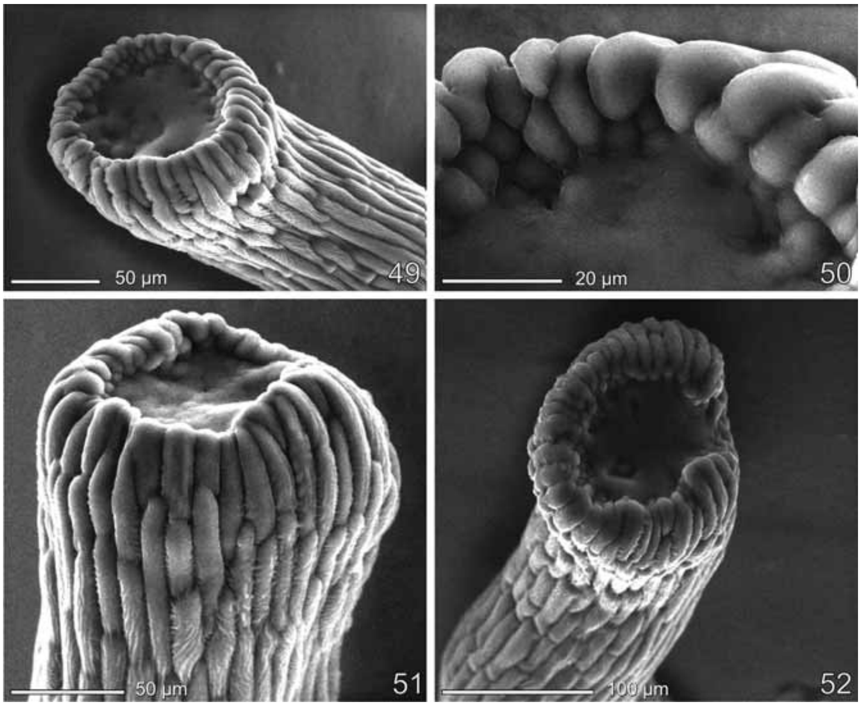

Fig. 49–52. *Pararchidendron pruinosum*, style tip with the cup-like stigma with stigmatic fluid, surrounded by a rim (wide in Fig. 49, narrow in Fig. 51). Detail of rim and stigmatic fluid in Fig. 50.

theca); furthermore a polyad can adhere to the polyad of a neighbour-locule (Fig. 10 left theca, 48 left theca) or polyads can be connected by pollenkitt at their edge (Fig. 44 right theca).

In Fig. 10 and 11 anthers are shown that opened under natural conditions. The final stages of anther opening in the ESEM are the same (Fig. 26, 39, 44, 48).

The o p e n i n g time of the anthers in the ESEM can be calculated as c. 1–1 $^{1}/_{2}$ hours. The time span between the first and last figure of a series given in the figure legends may be longer when the last photo is not taken immediately at the end of movements but somewhat later. In *Inga* with larger anthers natural opening was easier to observe exactly and it was found that approximately the same time is needed as in the ESEM (TEPPNER & STABENTHEINER 2006: 144). So, this can also be supposed for *Pararchidendron*.

3.4. Stigma

The style is a little bit thicker and stiffer than the filaments. The epidermal cells of the style are elongated and finely longitudinally to transversally ridged (Fig. 49–52). The cells of the cup rim (Fig. 50) at the tip of the style are more or less smooth and surround a flat stigmatic cavity (cup) which is filled with variable amounts of stigmatic fluid (Fig. 49–52). Therefore, the stigma is of the wet, non-papillate type. The diameter of the cup opening measures c. 75–100 µm, and is thus a little bit larger than the diameter of the polyads (c. 50–75 µm).

4. Discussion

The anthers of *Pararchidendron* fit well with the profile of many *Mimosaceae* as described, for e. g., by Endress & Stumpf 1991 and Tucker 1996: small anthers, well developed connective, outward bulging epidermal cells, stomium extends over both shoulders of the thecae up to the base of valves, glabrous filaments coiled in the bud, distinct narrow isthmus at the distal end of the filament.

In *Pararchidendron* the thick connective encompasses the whole width of the anther, similar as, e. g., in *Inga* (Teppner & Stabentheiner 2006) and is characterized by a bulge at the distal end. Attachment zone above and depression below the insertion of the filament also shows similarities with *Inga, Pithecellobium* and others. As compared to other *Ingeae* the stomium is more distinctly subsided. The dehydration of the isthmus cells occurs in a similar way as described for *Inga* (Teppner & Stabentheiner 2006).

The longitudinally inward folding of the valves (indentation) between connective and base of the valves is very distinct and seems to be a characteristic of *Ingeae*. Opening, the separation of the valves, starts in the centre of the theca and progresses quickly over the whole stomium. Outstanding in *Pararchidendron* is the quick inward-folding of the valves to form longitudinal bulges. Usually, they touch so that the remnants of the longitudinal septum (separating the two locules) are masked. Contrary to *Inga*, the margins of the valves roll inward and lie above the plane of the valve. The tapetal membrane cannot be distinctly observed in this investigation made, probably because it is too thin.

Whereas in *Inga* the flat polyads lie very regularly on the exposed flat parts of the valves, there is a remarkable irregularity in the position of the polyads in the open anther in *Pararchidendron*. Possible reasons are 1) the uneven surface of the valves, 2) the inward rolled margins of the valves, which can hinder or hold the polyads, 3) the thick and relatively round polyads, 4) the not very flat outer side of the polyads and no exactly corresponding mould in the fully opened valves and 5) irregular distribution of larger patches of pollenkitt.

All these are evidences for less specialization, so that the pollen presentation in *Pararchidendron* is supposed to be more primitive as compared to, for e. g., *Inga*.

The stigma structure is practically identical with that of *Pithecellobium dulce* and *Albizia julibrissin* and very similar to *Zapoteca tetragona* (*Ingeae*; from the examples we investigated). These stigmata of the wet, non-papillate type (term: HESLOP-HARRISON 1981) show similarities with some *Caesalpinieae* (OWENS 1990), so that this may be a plesiomorph character within *Mimosaceae*, or at least *Ingeae*, too.

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