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Anther Opening, Polyad Presentation, Pollenkitt and Pollen Adhesive in Four *Calliandra* Species *(Mimosaceae-Ingeae)*

By

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With 97 Figures

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Summary

TEPPNER H. & STABENTHEINER E. 2007. Anther opening, polyad presentation, pollenkitt and pollen adhesive in four *Calliandra* species (*Mimosaceae-Ingeae*). – Phyton (Horn, Austria) 47 (1-2): 291–320, with 97 figures. – English with German summary.

The unique mode of anther opening and pollen presentation of *Calliandra* was investigated in *C. angustifolia* BENTHAM, *C. haematocephala* HASSKARL, *C. tergemina* (L.) BENTHAM and *C. tweedii* BENTHAM. Each theca opens by a longitudinal slit, usually beginning from the ends of the theca. Sporadically the opening starts at the centre (eye). Then both theca walls (valves) bend back with two actions occurring simultaneously: 1) the main part of the theca walls bends back to the dorsal side and the middle of the anther, respectively; thereby the wall is infolded longitudinally and thus an inward-looking longitudinal bulge originates from the yellow part of the theca walls. 2) the slightly spoon-like ends of the longitudinal bulge zone are bent

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into a more or less perpendicular position. Before opening, the pollen adhesive (it is originating from the mucilage chambers in the narrowed, proximal end of the lumen of the locule-halves) is attached to the polyad tips. Immediately with the opening and the connected dislocation of the polyads the mucilage is stretched initially, then it loosens from the chambers and finally forms the drops on the polyads tip. The polyad is covered by a thin layer of pollenkitt, therefore the rounded basal part and the two faces of the polyad are sticky and adhere to the spoon-like ends of the longitudinal bulges of the theca walls. So affixed, the polyads must follow the movements of the wall and finally become turned and bent upright with the adhesive drop on top. Thus the complicated movements of the anther wall as well as two kinds of pollen sticker (pollenkitt and pollen adhesive) are responsible for polyad presentation and attachment.

Zusammenfassung

TEPPNER H. & STABENTHEINER E. 2007. Antheren-Öffnen, Polyaden-Präsentation, Pollenkitt und Pollenklebstoff bei vier *Calliandra*-Arten (*Mimosaceae-Ingeae*). – Phyton (Horn, Austria) 47 (1–2): 291–320, mit 97 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die einzigartige Öffnungsweise der Antheren und Pollenpräsentation von *Calliandra* wurde an *C. angustifolia* BENTHAM, *C. haematocephala* HASSKARL, *C. tergemina* (L.) BENTHAM und *C. tweedii* BENTHAM untersucht. Jede Theka öffnet sich mit einem Längsspalt, meist von den Enden der Theka beginnend. Sporadisch kann das Öffnen auch im Zentrum (Auge) der Theka beginnen. Dann schlagen sich die beiden Thekenwände (Valven) zurück, wobei zwei Bewegungsvorgänge gleichzeitig ablaufen: 1.) Der Hauptteil der Thekenwände krümmt sich zur Dorsalseite der Anthere bzw. zur Mitte der Anthere zurück, wobei sich die Wand der Länge nach einfaltet: dadurch entsteht ein nach innen gerichteter Längswulst aus dem gelben Teil der Thekenwände. 2.) Die leicht löffelförmigen Enden am Längswulst knicken in eine mehr oder weniger senkrechte Position abwärts. Vor dem Öffnen wurde der Pollenklebstoff aus den Schleimkammern in den verschmälerten, proximalen Enden des Lumens der Lokulamenthälften der Polyadenspitze angeheftet. Unmittelbar mit dem Öffnen und der damit verbundenen Verlagerung der Polyaden wird der Schleim zunächst gedehnt, dann von der Schleimkammer gelöst und rundet sich nun zum Tropfen auf der Polyadenspitze ab. Die Polyaden sind rundum von einer dünnen Schicht Pollenkitt überzogen und somit auch auf der gerundeten Basis und den beiden Flanken klebrig; sie sind durch den Pollenkitt den Löffeln an den Enden der Längswülste angeklebt und müssen daher jeder Bewegung der Thekenwand folgen, bis sie mit dem Pollenklebstofftropfen nach oben mehr oder weniger senkrecht stehen. Es sind also die komplizierten Bewegungen der Antherenwand ebenso wie die beiden Typen von Pollen-Haftmittel (Pollenkitt und Pollenklebstoff) für Polyaden-Präsentation und -Anheftung verantwortlich.

1. Introduction

Since 1979 one of the authors (H. T.) is interested in and concerned with *Calliandra*, especially in the fascinating anther mechanisms. Anther development and pollen presentation in *Calliandra* were investigated in a diploma thesis (PRENNER 1998) and the results presented to the scientific

community (PRENNER 2004, PRENNER & TEPPNER 2005). However, more work was necessary to more completely describe the mechanism of anther opening and polyad presentation.

Thus, a careful reinvestigation of the opening process with the help of a stereomicroscope (H. T.) resulted in a better understanding of this problem. The ESEM technique (environmental scanning electron microscopy, KOLB & STABENTHEINER 2003; E. S.) enabled the investigation at higher magnification, the observation of the dynamic opening process, without destructive preparations and offered an ideal possibility for an impressive presentation of the results.

2. Material and Methods

Plants from the following species were grown in the greenhouse of the Botanic Garden of the Institute of Plant Sciences of the University of Graz, Austria, Europe. The infrageneric grouping follows BARNEBY 1998.

Sectio *Androcallis* BARNEBY

Series *Androcallis*

Calliandra tweedii BENTHAM. – Origin: Purchase from R. & K. BAUM, D-71229 Leonberg, received 7.6.2000, one live plant.

Series *Ambivalentes* BARNEBY

Calliandra haematocephala HASSKARL var. *haematocephala*. – Origin: Purchase from the Emil KUR collection, Czech Republic, received July 1998, as *C. emarginata* and *C. inaequilatera*, respectively, three live plants.

Series *Macrophyllae* BENTHAM

Calliandra angustifolia BENTHAM. – Origin: Peru, Dpt. Pasco, Pozuzo, ca. 820 m, August 30, 1981, H. TEPPNER 81/518 & K. KEPLINGER, live plant.

Calliandra tergemina (L.) BENTHAM var. *emarginata* (WILLD.) BARNEBY. – Origin: Purchase from the Emil KUR collection, Czech Republic, received July 1998, as *C. haematocephala*, one live plant.

All four species grow in the division for tropical plants.

Single flowers or whole inflorescences were brought in the laboratory for observation by stereomicroscope or ESEM.

The stereomicroscopes Wild M 3B and Nikon SMZ645 were used.

For ESEM investigations fresh anthers were mounted on aluminium stubs using C-impregnated double sided tape and investigated without any further preparation using a Philips XL 30 ESEM scanning electron microscope (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 the study of the opening process, ripe but still closed anthers from open flowers were transferred into the chamber of the ESEM, where the opening process of the anthers started after 5–30 minutes.

Additionally, in *C. angustifolia*, also fixation with 2.5% glutaraldehyde, dehydration using acetone series, critical point drying, sputtercoating with gold and observation in the high vacuum were used (Fig. 77–78).

The stickiness of the pollenkitt was tested by touching the polyads with the tip of a needle.

3. Results

3.1. Notes on Anthesis and Morphology

3.1.1. *Calliandra tweedii* BENTHAM

The flowering period of *C. tweedii* in our greenhouse lasts from November to beginning of March with single inflorescences sporadically occurring outside of this period. The sequence of flower opening starts from the base of the head or is irregular (Fig. 3). Full anthesis of a head takes more or less two days where some irregularities in flower opening within a head are possible (some flowers precursory, some delayed). Tighten (bundle up) of the anthers within a flower (usually third day; VOGEL 1954: 215, Fig. 115 III) is regarded as the end of anthesis; wilting follows. One head in anthesis (Fig. 3) has a diameter of c. 8-9 cm and contains c. 20–23 flowers and often an additional one in the axil of the scale below on the peduncle. A single flower is c. 4.0–4.5 cm long, corolla ca. 6.3–9.0 mm long, c. 30–59 stamens, filament tube c. 3.5–4.5 mm, all flowers with gynoeceum, but nectary only in the central flowers (up to 10), nectary a thick, hollow cylinder, c. 0.3–0.8 mm high (Fig. 4), attached to the stemonozone for c. 1/2. The nectar fills the basal c. 10 mm within the filaments. Habitual differences between nectariferous central flowers (e. g., larger buds, pedicels 0–1 mm, diameter of the corolla c. 3.0–4.5 mm, up to c. 60 stamens) and nectarless peripheral ones (e. g., pedicels 2-3,4 mm, diameter of corolla c. 2 mm, up to c. 40 stamens) are not very sharp. No odour is discernible. Attempts for fruit-set by manual selfing failed. Nevertheless, only once, one fruit with two seeds originated without artificial pollination.

For further details of the flowers see BARNEBY 1998: 57-58 and LOEW 1904: 350–351.

3.1.2. *Calliandra haematocephala* HASSKARL

C. haematocephala usually blooms in our greenhouse in February and March usually. The sequence of opening of flowers within a head is variable and can either begin on the top or on the base. Full anthesis of a head (all flowers open) with fully extended filaments, open anthers and nectar

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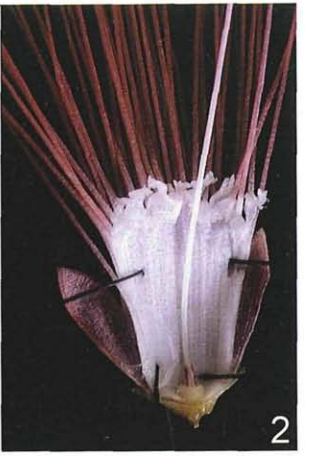
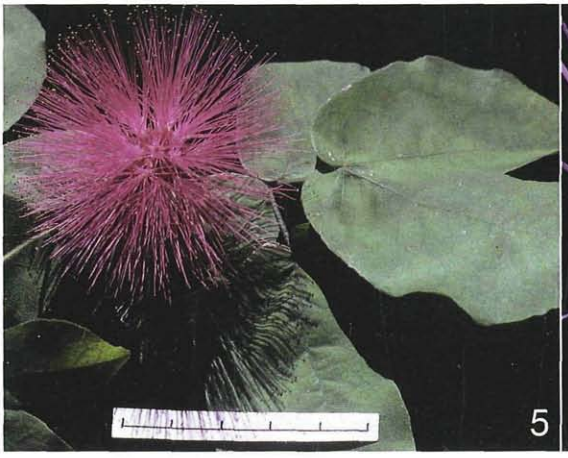
Fig. 1–2. *Calliandra haematocephala*. – Fig. 1. Flowering head, c. 10 cm in diameter. – Fig. 2. Flower slit longitudinally, the filament tube (c. 1.1 cm high) extended, with gynoeceum and nectary.

Fig. 3–4. *Calliandra tweedii*. – Fig. 3. Flowering head, c. 8.5 cm in diameter, 10 flowers and 13 buds. – Fig. 4. Central flower, filament tube extended for showing gynoeceum and nectary (0.7 mm in diameter). Filament tube c. 4.0 mm high.

Fig. 5–6. *Calliandra tergemina*. – Fig. 5. Flowering head. Scale bar equals 5 cm. – Fig. 6. Flower slit longitudinally, filament tube extended. Filament tube c. 8.0 mm high, nectary 0.75 mm in diameter.





in the filament tube, lasts usually two days. Often anthesis of a smaller part of the flowers of a head can start at noon or afternoon of the foregoing day, however anther opening and nectar production not necessarily occurs on this day. One head in anthesis (Fig. 1) has a diameter of c. 7–10 cm and contains in our plants c. 40–60 flowers. A single flower is c. 4.5 cm long, the stigma from 1 cm below up to 0.5 cm above the anthers, corolla ca. 1 cm long, c. 33–44 stamens, filament tube c. 1.1–1.5 cm, rim of the tube inward with c. 15–20 white lobes (Fig. 2, with papillose and partly slashed margins), curved inward so that the access to the nectar is completely closed. All flowers show a nectary (a hollow cylinder 1.3–1.5 mm in diameter and 1 mm high) (Fig. 2), a functional gynoeceum can be found in all flowers, only in a few central flowers, or they are lacking at all in a head. The male flowers have a small carpellodium. The nectar fills c. half of the filament tube. No odour is discernible. Sporadically (self ?) pollination occurs in the greenhouse, so that few fruits are produced.

Further details on the flowers in NEVLING & ELIAS 1971: 77–82 and BARNEBY 1998: 108.

3.1.3. *Calliandra angustifolia* BENTHAM

In the greenhouse the main flowering period usually lasts from September to November, in some years flowering began in June or July or continued till December or January. Anthesis of the single heads begin in the afternoon, opening of the anthers occurs at onset of dusk approximately and wilting of the flowers takes place on the next morning (PRENNER 1998, TEPPNER 2007a). A head in anthesis is up to 10 cm in diameter, and contains c. 20–35 flowers. Few central flowers of a head can be provided with a wide, petaloid filament tube and a distinct disc-nectary, whereas the peripheral flowers show a normal, narrow filament tube and no nectary. Both types can be either hermaphrodite or male: the four flower types occur in variable numbers and combinations in the heads. The single flowers are c. 4.0–6.0 cm long. For *C. angustifolia* a separate paper is planned which will contain more details and images (cf. also BARNEBY 1998: 125).

3.1.4. *Calliandra tergemina* (L.) BENTHAM

In our greenhouse flowering occurred in February and from June to November. Anthesis of a head begins in the morning and lasts two days. A head in anthesis (Fig. 5) has a diameter of c. 5.5–8.3 cm and contains (10-) 24–29 flowers. A single flower is c. 2.5–3.2 cm long, styles as long or little shorter than filaments, calyx c. 1.5–2.8 mm, corolla c. 5.6–6.7 mm long, campanulate distally, c. 23–31 stamens, filament tube c. 6.0–8.0 mm, stemonozone 0.5–0.7 mm high, rim of the tube outward with c. 12–15 white and glabrous teeth, ca. 0.2–0.7 mm long, between the filaments. All flowers of a head have a gynoeceum and nectary but sporadically up to 1/2 of the

flowers (peripheral) show a reduced style (c. 1/2 of the normal length, rarely shorter) and a dry stigma. The nectary is a hollow cylinder of 0.5–0.7 mm diameter and 0.5–0.8 mm hight (Fig. 6), attached to the stemonozone for 0.15–0.3 mm. The nectar stands up to 1–2 mm above the rim of the filament tube. No odour is discernible.

Further details on the flowers in BARNEBY 1998: 127–130.

3.2. Anther Structure

The body plan of the *Calliandra*-anther is described and figured in detail in PRENNER & TEPPNER 2005.

The anthers measure c. (0.2-)0.3–0.4 × (0.25-)0.35–0.5 mm (e. g., Fig. 7, 41, 71 and 86). On the dorsal side of the connective and along the stomium, the epidermal cells build conical, longitudinally ribbed papillae. Those in few rows along the stomium are elongated to short hairs and interlock over the stomium (e. g., Fig. 14, 26, 42, 67, 72, 88). The inner row of these hairs can be slender and smooth (especially in *C. haematocephala*, e. g., Fig. 38, 44, 46, 60). The central part of the stomium is a little dilated. This can be seen distinctly, when the papillate hairs let this area more or less free (uncovered) so that an 'eye' or 'window' is formed (*C. angustifolia* , *C. haematocephala*; Fig. 65, 71, 72; 41, 42, 59). This dilatation is less distinct, when the center is also more or less covered by these hairs (e. g., Fig. 7, 85). Furthermore, in *C. haematocephala* on each side of the eye a tuft of somewhat elongated, erect papillae is formed (Fig. 42) (less expressed in *C. angustifolia*, Fig. 72).

The two thecae are widely separated by the massive connective and lie parallel, in an angle of c. 65 degrees (Fig. 8, 82 and 83). The attachment point and the apical end of the filament (isthmus) are ramparted from the connective. Thus the filament inserts in the base of a short tube (c. 0.15 mm deep) (Fig. 9 and 11–13). So, the position of the anther perpendicular to the filament is somewhat stabilized. Because of the deep insertion of the filament, the attachment zone around it is very small (Fig. 12–13). The smooth isthmus cells (Fig. 9 and 10) form c. 20 longitudinal rows and turn over into normal, transversely ribbed filament epidermis cells (Fig. 10). The desiccation of the isthmus during anthesis was not studied in detail, but the first steps of dehydration (Fig. 11, 12) indicate a similar process as described for *Inga* (TEPPNER & STABENTHEINER 2006: 145, 150–151). Each theca is partite by a small parenchymatous, transversal septum and by a massive, inward protruding part of the connective (TEPPNER 2007c). So each theca consists of two locules and four locule-halves (scheme of an anther in PRENNER & TEPPNER 2005: 272). The lumen of the locule-halves of a ripe anther is lined by the orbicule-bearing tapetal membrane, which is strongly appressed to the valves (Fig. 17, 19–22, 34, 38, 78) (TEPPNER 2007c: 24, 31) and, thus, opens in general with the valves. In the ESEM mode (wet samples) the orbicules can not be seen very clearly.

The anthers are red on the dorsal side and on a broad stripe along the dehiscence sutures of the thecae, whereas the sides of the theca walls are more or less (less expressed in *C. tweedii*) bright yellow (in part the stripe above the transversal septum is also red, at least on the outer side). In this yellow zone of the locule-halves the endothecial cells with their characteristic thickenings of the walls are mainly located and are connected over the transversal septum by bridges of thickened endothecial cells, a broad one on the inner, a narrower one on the outer theca wall (TEPPNER 2007c: 18, 22, 33).

Pollen adhesive is formed by disintegration of approximately globose groups of parenchymatic cells of the middle layer. They lie in the narrowed proximal end of the lumen of the locule-half, adjacent to the anther wall and the transversal septum (e. g., Fig. 39, 40) (PRENNER & TEPPNER 2005, TEPPNER 2007c). Thus, finally four slime drops lie in the four mucilage chambers opposite to the polyad tips of a theca.

The whole polyad is covered by a thin layer of pollenkitt which is visible as a bright line in the optical section of the polyad in the light microscope, maybe locally a little thickened (flat patches). Because in *Calliandra* the pollenkitt is not forming droplets in water (but in diluted acetic acid), the easiest way to proof the presence of pollenkitt is in air, where it leaps to the cover slip forming characteristic contact zones (Fig. 79; as described in TEPPNER 2007c: 4-5). The viscosity of the pollenkitt is easy to demonstrate. If the basal end of a polyad is touched by a tip of a needle or on the faces (both faces behave similar) with the side of a needle tip, the polyads adhere to the needle. If, accidentally, polyads are attached by the pollen adhesive to a filament or an anther, and a needle touches the polyad, then the filament follows the movement of the needle a short time till the connection by pollenkitt is released and the filament flings back. Thus the adherence by pollenkitt is weaker than that by pollen adhesive.

3.3. Normal Opening and Polyad Presentation

The first sign of the impending anther opening is the longitudinal inward-folding at the very base of the theca walls, so that, outside, deep furrows can be discerned (Fig. 86 as compared to Fig. 85). Probably this enlarges the tension in the wall, leading to the opening of a slit in the stomium and the overcoming of the coherence of the interlocking hairs along the stomium. Usually, the opening of the stomium begins at both ends of the thecae, often a little asynchronous in the two theca-halves (e. g., Fig. 27, 59 and 87), and proceeds along the stomium towards the eye (e. g., Fig. 23, 45, 47, 66, 87). Before the rupture, the cells of the eye appear blurred (Fig. 48, 74), most probably by trickling of the mucilage to the inner side of the eye cell walls. Finally, the centre of the stomium at the eye ruptures and at this moment the valves separate often jerkily, and then evenly bend back

(outwards to the dorsal side of the anther and the space between the thecae, respectively) (e. g., Fig. 25, 49, 53, 69, 90, 95).

Shortly after the start of opening the two opposing mucilage chambers attached to the theca walls can be seen. Between the two a small septum is to be seen, which is regarded as the true transversal septum (corresponding to the transversal, parenchymatous septum in *Inga*); this septum appears collapsed and no cellular structure can be discerned: thus the septum seems to be partly dissolved before opening of the anther. Contrary, the below adjacent cell remnants of the thick part of the locule-separating tissue (interpreted as connective protrusion in TEPPNER 2007c: 22) appear relatively sharp (Fig. 17, 34, 38, 54); thus it seems possible, that the theca walls bending-back break from this transversal tissue just during the opening process. A further proof for this assumption is the circumstance, that these residual cells are usually free from tapetal membrane (Fig. 20, 34, 38, 78). The occasional occurrence of tapetal membrane fragments (Fig. 21) on this cell remnants can not be explained at present. As soon as a distinct cleft originated, it can be seen that the tip of a polyad is connected with the mucilage chamber in the back-bending theca wall of the relevant locule-half by a thread of mucilage (e. g., Fig. 25, 48, 49, 53, 90, 93). Originally the two polyads of one theca-half lie parallel, face by face (e. g., Fig. 29 below, 53, 75), by the beginning opening and the dislocation of the polyads the mucilage is stretched (e. g., Fig. 50, 51, 54, 55, 93, 97). These threads persist relatively long in the further opening anther, detach finally resulting in the rounded mucilage drop on the apical end of the polyads (e. g., Fig. 33–37, 56, 80–83). Because the mucilage chambers on the open theca walls seem to be free of mucilage essentially, it is more probable that the mucilage threads loose from the chamber, rather than the threads rupture.

The mucilage threads in the opening anthers can always be observed under the stereomicroscope. As observed with the stereomicroscope in cut heads c. 7 – 15 minutes are needed from the start of opening up to forming the drops. For the full opening it takes c. 1/2 – 3/4 hour, under cooler conditions also up to 1 hour 15 minutes. The time span for opening lies approximately in the same dimension when they were observed in the ESEM.

The following stages of opening, the further bending back of the theca walls, is characterized by a forced inward protruding of the yellow parts of the theca walls forming a longitudinal bulge (beginning in Fig. 62 and 64, further development, e.g., in Fig. 69, 55 and 39). This bulge is narrowest at the transversal septum and widest c. a little distal from the middle of the theca half (e. g., Fig. 16, 33–37 and 80). The ends of the longitudinal bulge show a somewhat spoon-shaped form and are bended down during the anther opening (simultaneously to the infolding of the bulge) up to a steeply oblique or nearly perpendicular position (e.g., Fig. 16–19, 36, 57, 80). This complicated mode of bend is mainly due to the different thickenings of the endothecium, because all other layers are largely degenerated in the

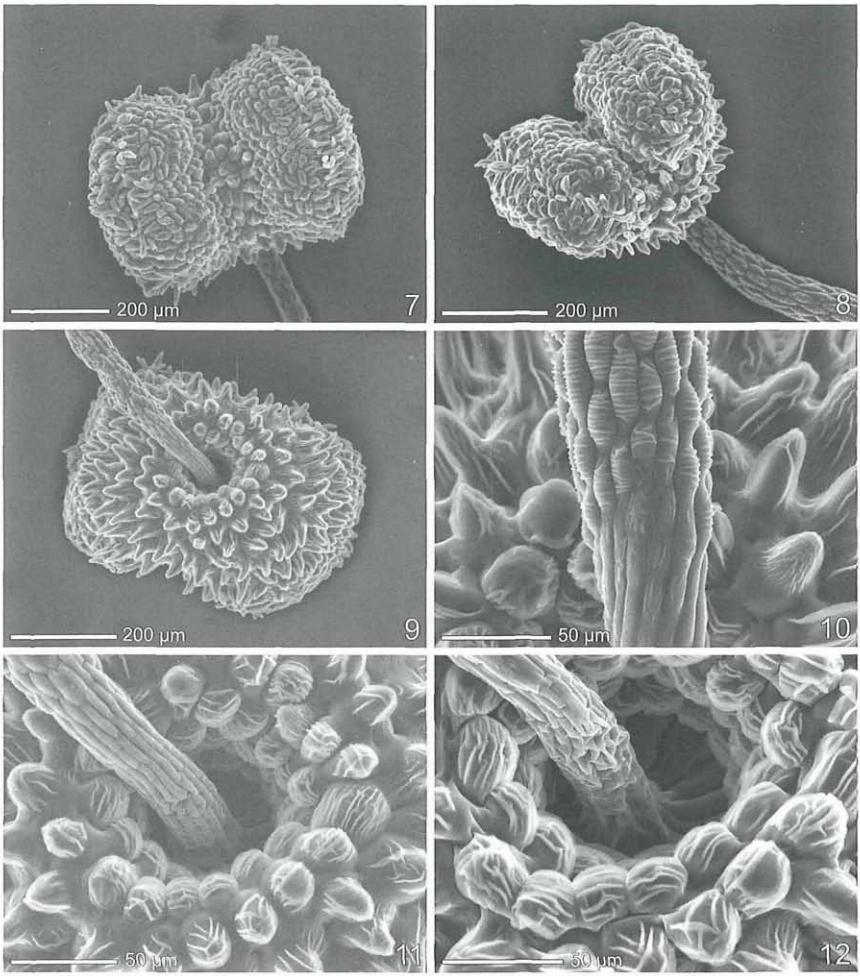


Fig. 7–12. *Calliandra tweedii,* ripe, still closed anthers. – Fig. 7. Ventral side. – Fig. 8. Apical end, view a little oblique. – Fig. 9. Dorsal side, filament bent sidewards artificially, inserting in a small cylindrical tube. – Fig. 10. Filament, transition between the epidermal cells of the isthmus and the normal cells of the filament. – Fig. 11. Detail of Fig. 9. Filament isthmus and attachment zone, first signs of shrivelling of the isthmus cells by drying up. – Fig. 12. Ditto, 35 minutes later, drying up progressed. The very small attachment zone on the base of the tube.

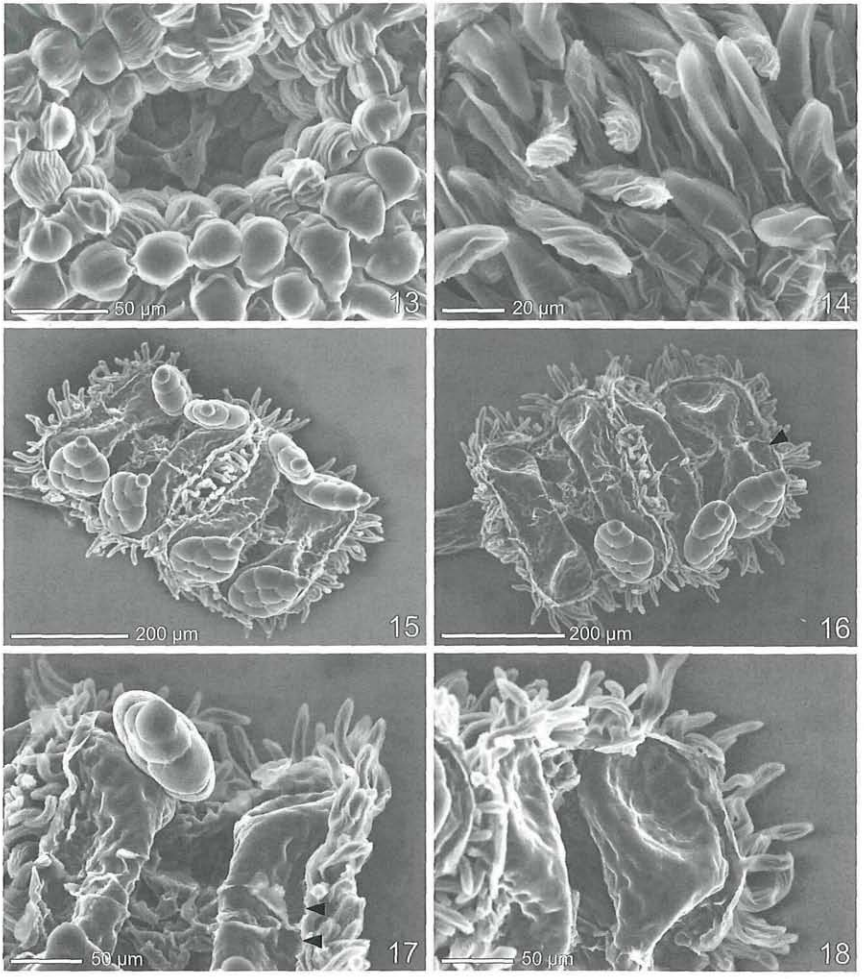


Fig. 13–14. *Calliandra tweedii*, ripe, still closed anthers. – Fig. 13. The small filament attachment zone of the base of the tube, after removing of the filament. – Fig. 14. The hairs (epidermal papillae) along the stomium interlock.
Fig. 15–18. Naturally opened anthers from the greenhouse. – Fig. 15. Fully open anther with eight oblique-erect polyads. – Fig. 16. Fully open anther, five polyads removed for showing the longitudinal bulges with their spoon-like ends. The arrowhead marks one of the two adjacent mucilage chambers. – Fig. 17. Half of a fully open theca, one polyad adhering to the downward bent bulge-end (spoon), other polyad removed. Below mucilage chambers (with arrowheads), remains of transversal septum and connective protrusion, and the tapetal membrane. – Fig. 18. Half of the inner side of a theca valve with the spoon-shaped end of the longitudinal bulge.

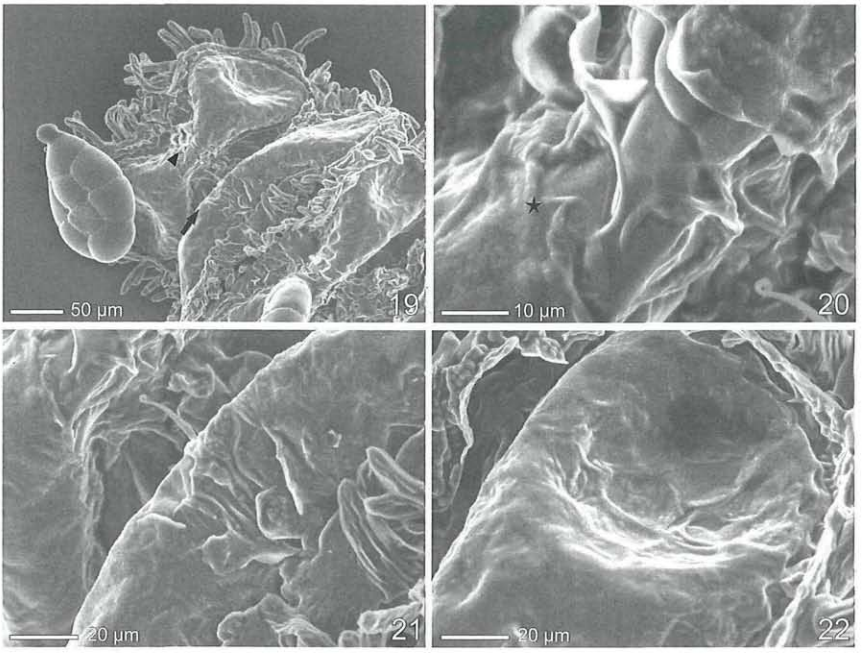


Fig. 19–22. *Calliandra tweedii*, tapetal membrane. – Fig. 19. One theca of a naturally opened anther. Centres of the bulges in the following images. – Fig. 20. Detail from Fig. 19, left bulge (arrowhead), remains of the connective protrusion bordered by the margin of the orbicule-bearing tapetal membrane (asterisk). – Fig. 21. Detail from Fig. 19, right bulge (arrow), remains of the connective protrusion covered with loosen fragments of the tapetal membrane. – Fig. 22. The spoon-like end of a longitudinal bulge is covered by the strongly appressed, orbicule-bearing tapetal membrane.

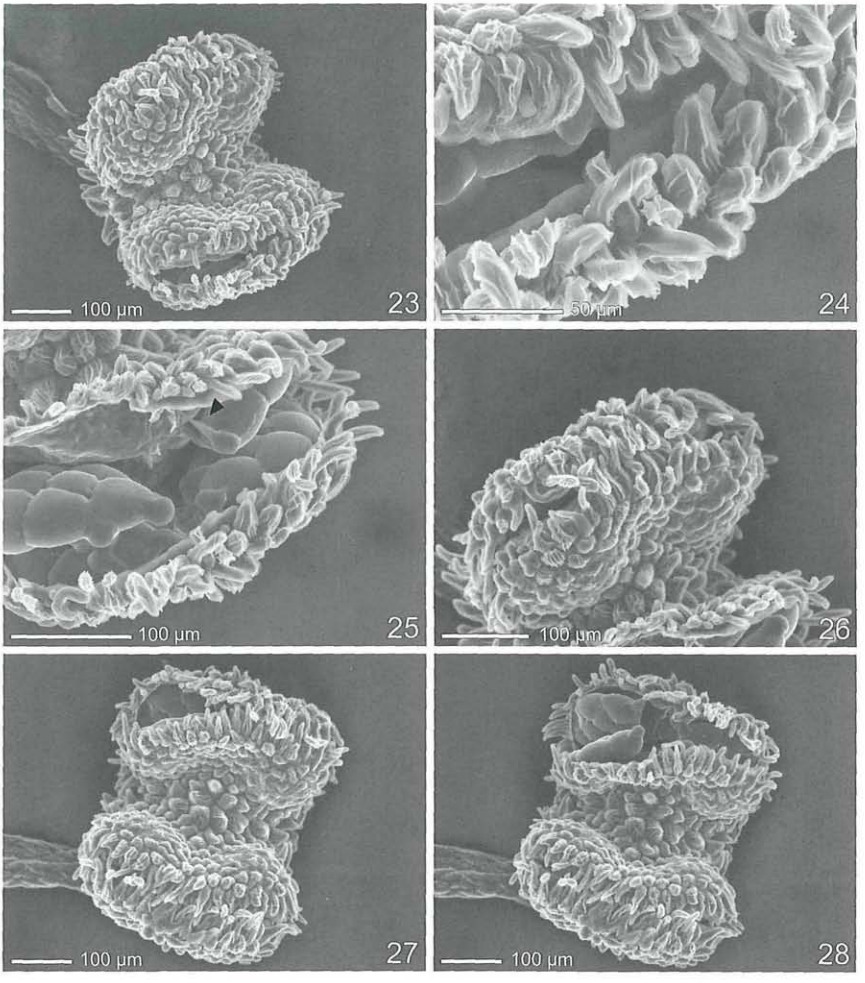


Fig. 23–28. *Calliandra tweedii*, opening process in the ESEM. – Fig. 23. Slit in the stomium, beginning from one end in the below theca. – Fig. 24. The slit reaches the margin of the eye (right). – Fig. 25. Theca shortly after rupture of the eye. Mucilage in part in connection (stretched) with the mucilage chamber (arrowhead), in part liberated and forming the rounded drop. – Fig. 26. The second theca of this anther begins to open from both ends simultaneously. – Time span between Fig. 23 and 26: c. 4 minutes. – Bubbles on the pores are artefacts.
Fig. 27–28. Begin of opening from the ends of the thecae in another anther; continuation in Fig. 29–34.

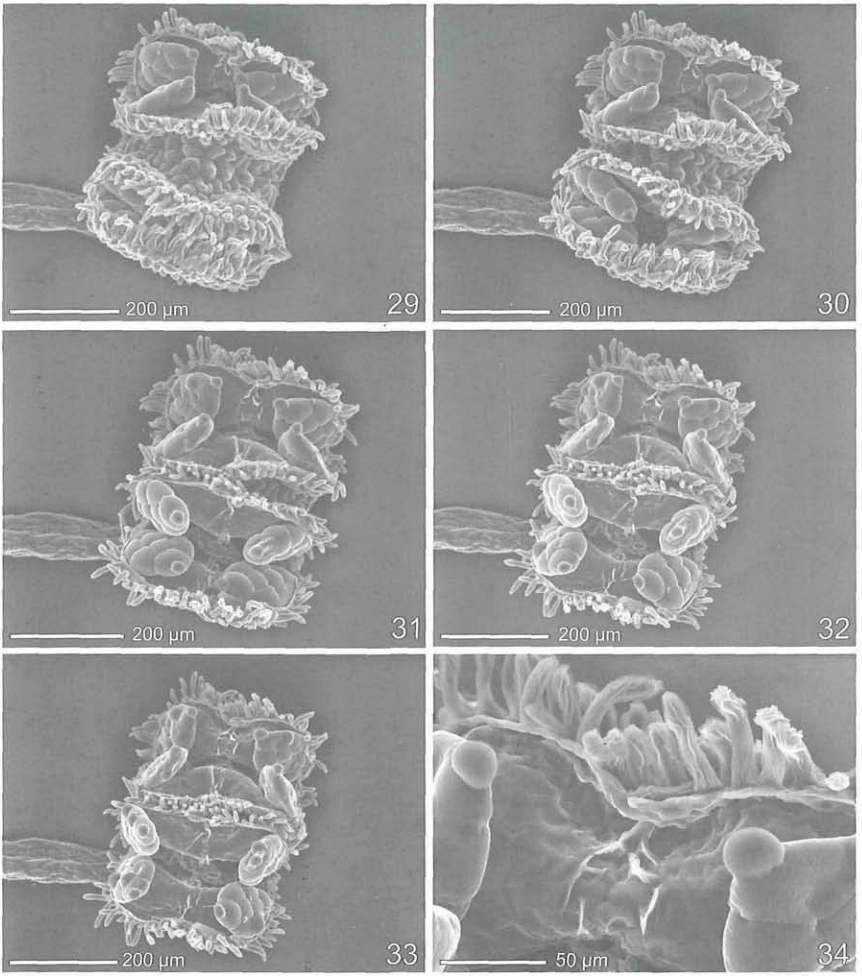


Fig. 29–34. *Calliandra tweedii*, opening of an anther in the ESEM (continuation from Fig. 27–28). – Fig. 29. Start of opening on both ends in the lower theca, progressed opening in the upper one, drop of the polyad right above still connected with the mucilage chamber. – Fig. 30. Thecae further opened, below right, the drops on the neighbour-polyads confluent. – Fig. 31. Opening process largely progressed, neighbour-polyads still in V-position. – Fig. 32 and 33. Anther nearly fully opened. – Fig. 34. Detail from Fig. 33. The central part of the uppermost valve. Mucilage chambers and septum in the centre. Above the longitudinally broken epidermis of the stomium. The septum between the two mucilage chambers. Below the remains of the connective protrusion bordered by the tapetal membrane. – Time span between Fig. 27 and 34: c. 26 minutes.

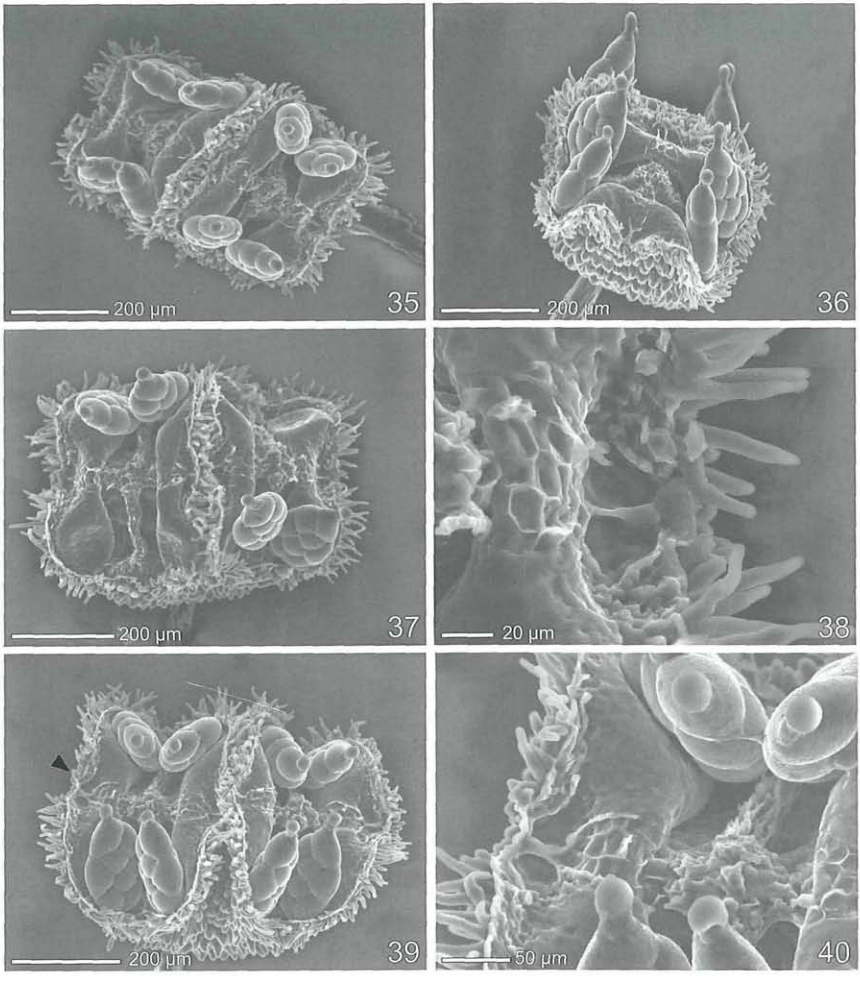


Fig. 35–38. *Calliandra haematocephala,* naturally opened anthers from the greenhouse. – Fig. 35. View from above. – Fig. 36. Lateral view from a little above. – Fig. 37. View from above, four polyads removed for showing the longitudinal bulges. – Fig. 38. Detail from Fig. 37, right valve. In the centre the two mucilage chambers and the septum, left the remain of the connective protrusion, bordered by the margins of the tapetal membrane.

Fig. 39–40. Approximately half-opened anther, valves not fully bent backwards, thus the form of the lumen of the locule-halves (narrowed end with mucilage chamber) discernible, especially in the left valve (arrowhead). – The detail in Fig. 40.

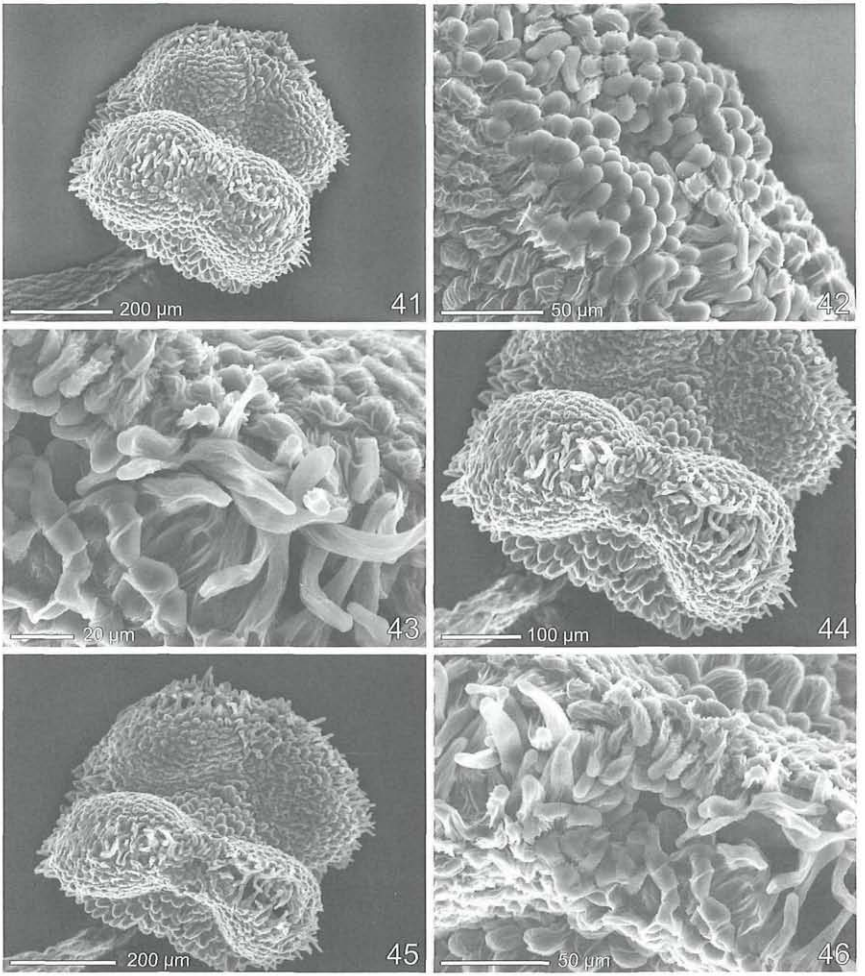


Fig. 41–46. *Calliandra haematocephala*, first part of the opening process of an anther in the ESEM. – Fig. 41. Closed anther. – Fig. 42. Eye and adjoining tufts of papillae from another anther. -Fig. 43. Opening of a theca beginning at the ends, eye (left) still intact, slit in the stomium still covered by interlocking papillae (below right). – Fig. 44. Opening of a theca beginning at the ends, a little later than Fig. 43. – Fig. 45. Opening in both thecae a little progressed. – Fig. 46. The slit in the stomium (right) reaches the eye. – Continuation in Fig. 47-52.

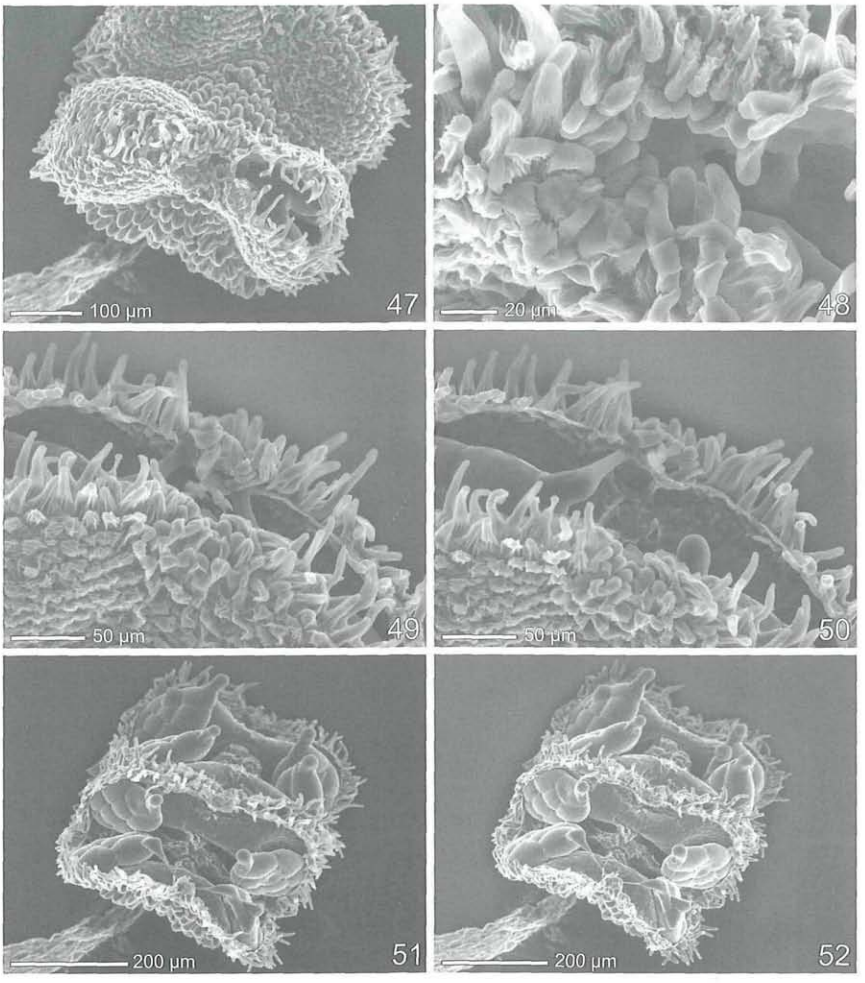


Fig. 47–52. *Calliandra haematocephala,* opening process of an anther in the ESEM (continuation from Fig. 41–46). – Fig. 47. Progressed opening in the lower theca. – Fig. 48. In the lower theca the eye (with diffuse appearance) begins to split. The mucilage between a polyad and the mucilage chamber begins to stretch. – Fig. 49. Upper theca immediately after rupture of the eye. Mucilage threads between polyads and mucilage chambers. – Fig. 50. Opening in the upper theca progressed. In one polyad mucilage stretched between polyad tip and eye, the other polyad with a rounded drop of pollen adhesive, already. Mucilage chambers, septum and connective protrusion visible. – Fig. 51 and 52. Nearly completed opening, a little lesser in the theca below, where two adhesive drops are still connected by threads with the mucilage chambers. – Time span between Fig. 41 and Fig. 52: c. 113 minutes, between Fig. 43 and Fig. 51: c. 37 minutes. Bubbles on the polyad pores are artefacts.

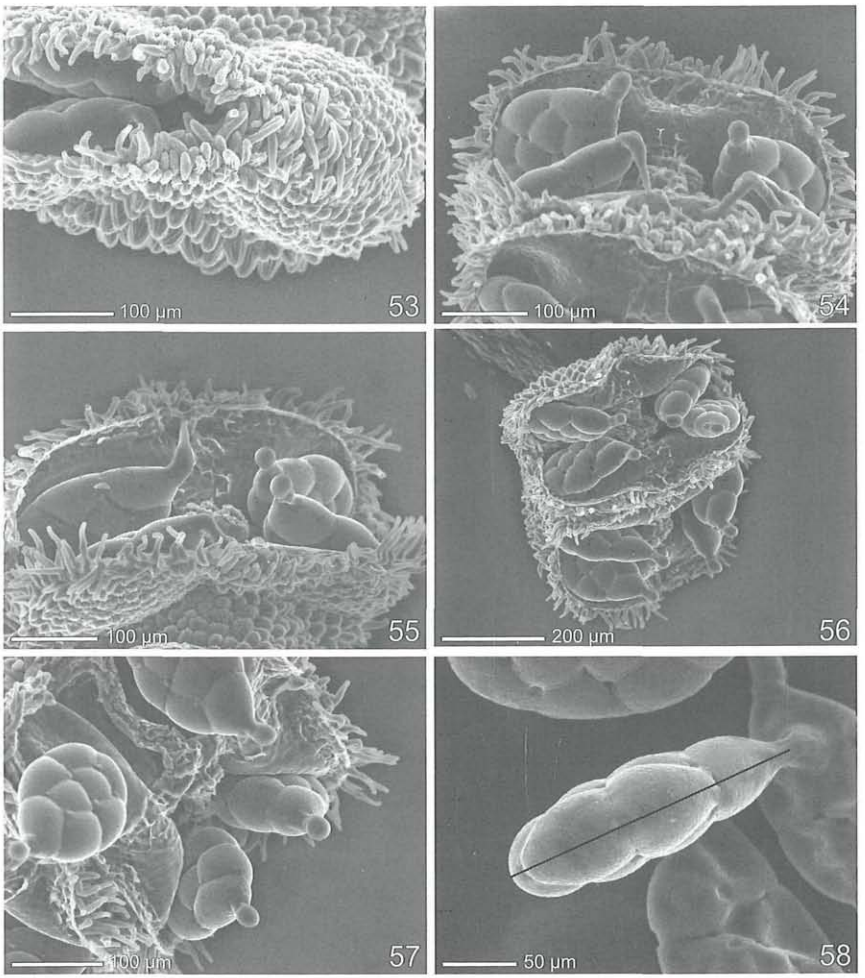


Fig. 53–58. *Calliandra haematocephala*, stages of opening from different anthers. – Fig. 53. A theca after splitting of the eye. Mucilage stretched between mucilage chambers and polyad tips. – Fig. 54–57. All four mucilage drops of a theca free from each other from the beginning, but in part mucilage threads remain connected some time with their mucilage chambers. In Fig. 57 adherence of the two right polyads on the perpendicular spoons is shown. – Fig. 58. Polyad taken from an anther in view of the narrow side, sticks on another polyad. The line through the polyad ends shows its asymmetry.

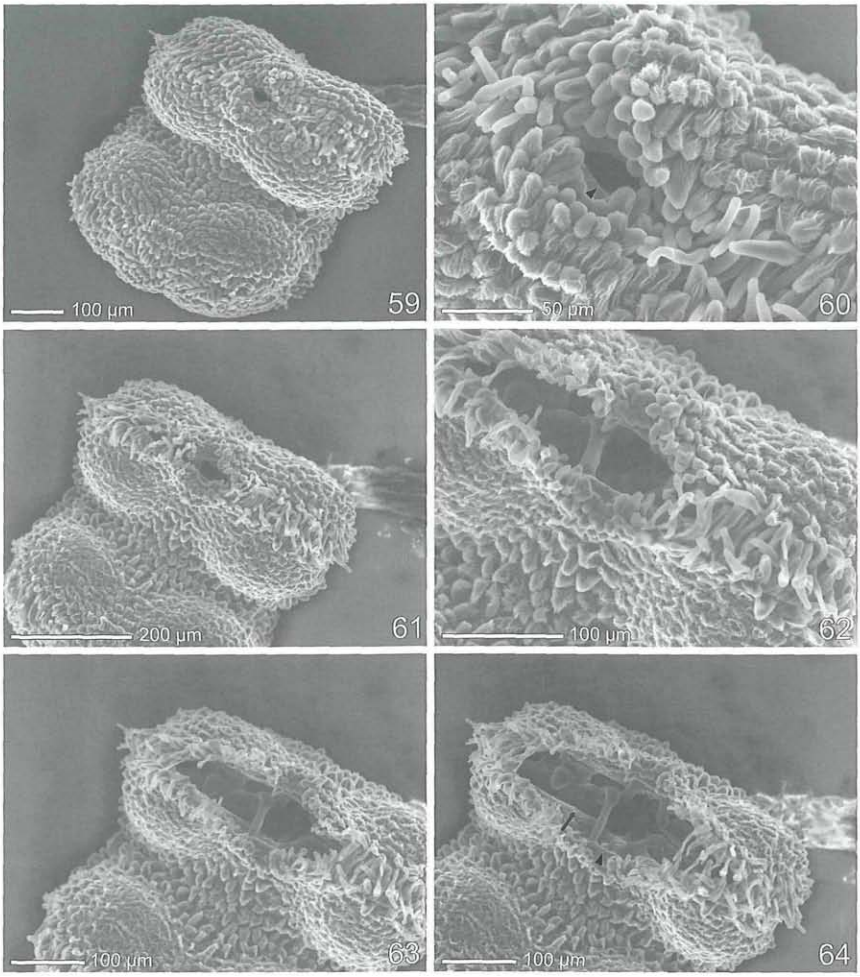


Fig. 59–64. *Calliandra haematocephala*, start of opening at the eye, exceptionally. The first phases of the opening process. Arrowheads: epidermis of the eye, arrow: inward folded longitudinal bulge. In Fig. 63 and 64, the four mucilage drops still in contact with their mucilage chambers. – Time span between Fig. 59 and 64: c. 42 minutes.

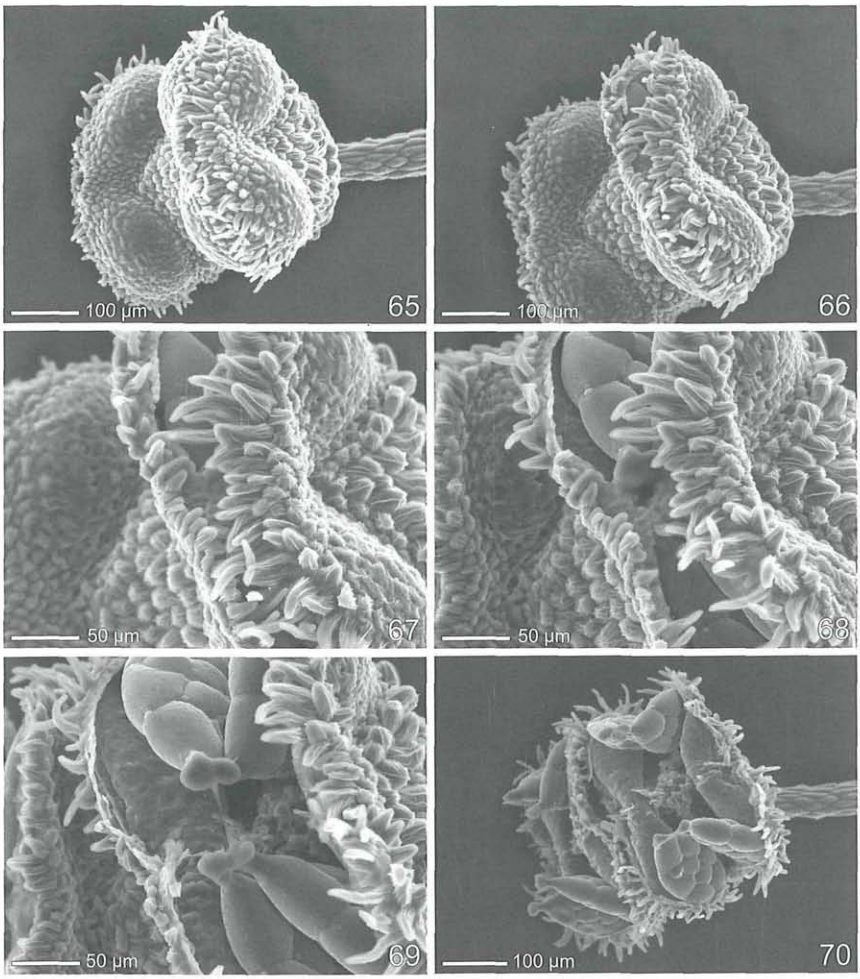


Fig. 65–70. *Calliandra angustifolia*, some stages of opening of one anther in the ESEM. – Fig. 65. Inwards oriented fold at the very base of the valves and begin of opening at the ends of the thecae. – Fig. 66. Splitting along the stomium progressed, eye still intact. – Fig. 67. The split reaches the eye. – Fig. 68. Rupture of the eye begins, above the eye the fused mucilage drops of the two neighbouring polyads visible. – Fig. 69. Shortly after rupture of the eye, opening of the valves progressed, mucilage drops of neighbour-polyads fused as in Fig. 68. A thin mucilage thread between the opposite drop-pairs. – Fig. 70. Bending-back of the valves largely progressed (but not finished), longitudinal bulges developed and polyads erected, drops of the neighbour-polyads still connected in the right theca (shown also in Fig. 68–69), completely separated in the left theca, already. – Time span between Fig. 65 and 70: c. 21 minutes.

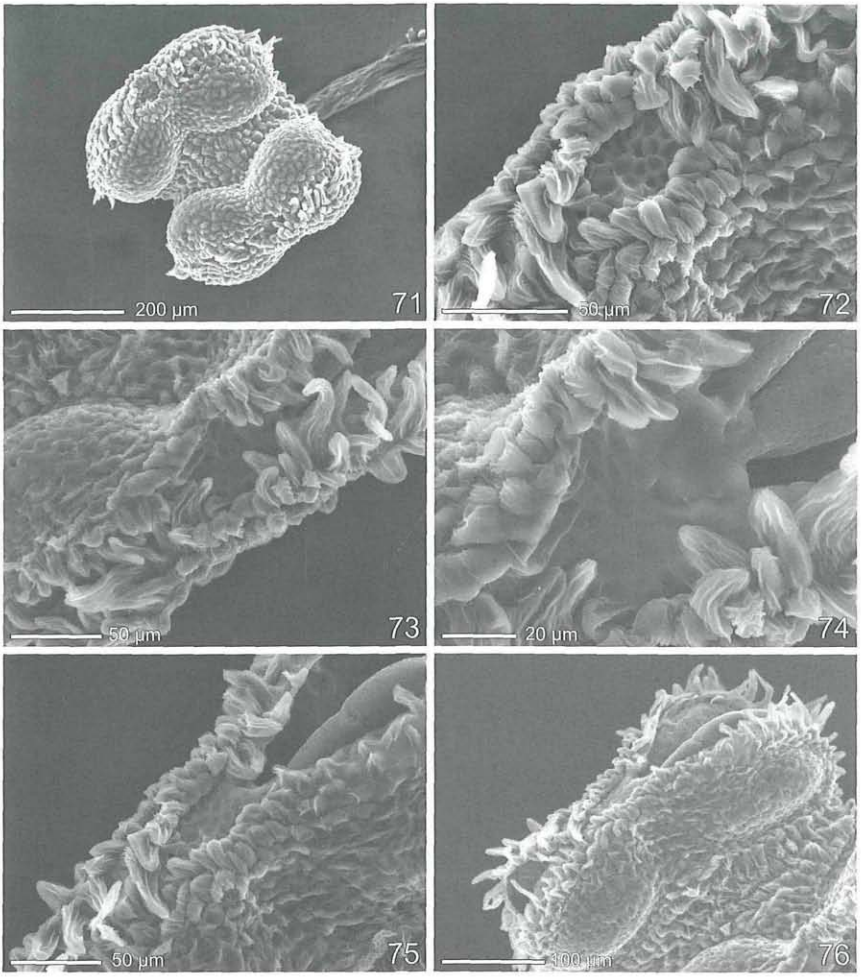
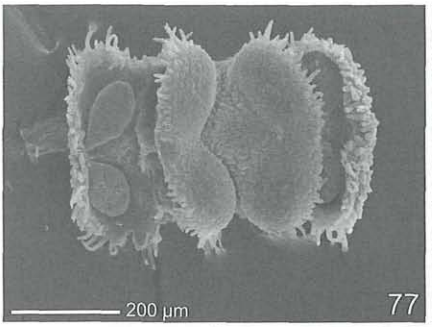
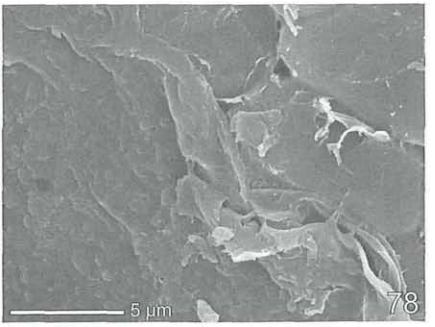


Fig. 71–76. *Calliandra angustifolia*, first stages of anther opening in the ESEM. – Fig. 71. Start of opening at the ends of the thecae. – Fig. 72. The eye with intact epidermis cells of the upper theca in Fig. 71. – Fig. 73. The slit approaches to the eye of the theca below. – Fig. 74. The theca below in Fig. 71, diffuse appearance of the eye epidermis by contact with the mucilage; above right: splitting of the eye had began and the tips of neighbour-polyads with their united mucilage appear. – Fig. 75. Splitting along the stomium reached the eye in the upper theca of Fig. 71. – Fig. 76. Upper theca of Fig. 71, stomium largely opened beginning from the theca end, the eye still intact. Further development not observed in this case because by drying up, apparently, the process was arrested. Time span between Fig. 71 and 76: c. 66 minutes.





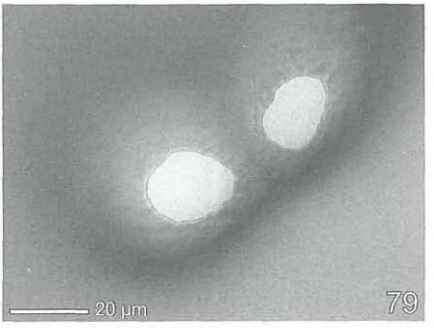


Fig. 77–78. *Calliandra angustifolia*, anther after glutaraldehyde fixation and critical point drying. – Fig. 77. Ripe, closed anther opened artificially on the stub. – Fig. 78. Detail of the inner side of the valve of an artificially opened theca. Tapetal membrane with orbicules in the left, connective protrusion right.

Fig. 79. *Calliandra angustifolia*, a polyad in air in the LM. The rounded, basal end touches the lower side of the cover slip, at the contact zones the pollenkitt forms apparent plaques.

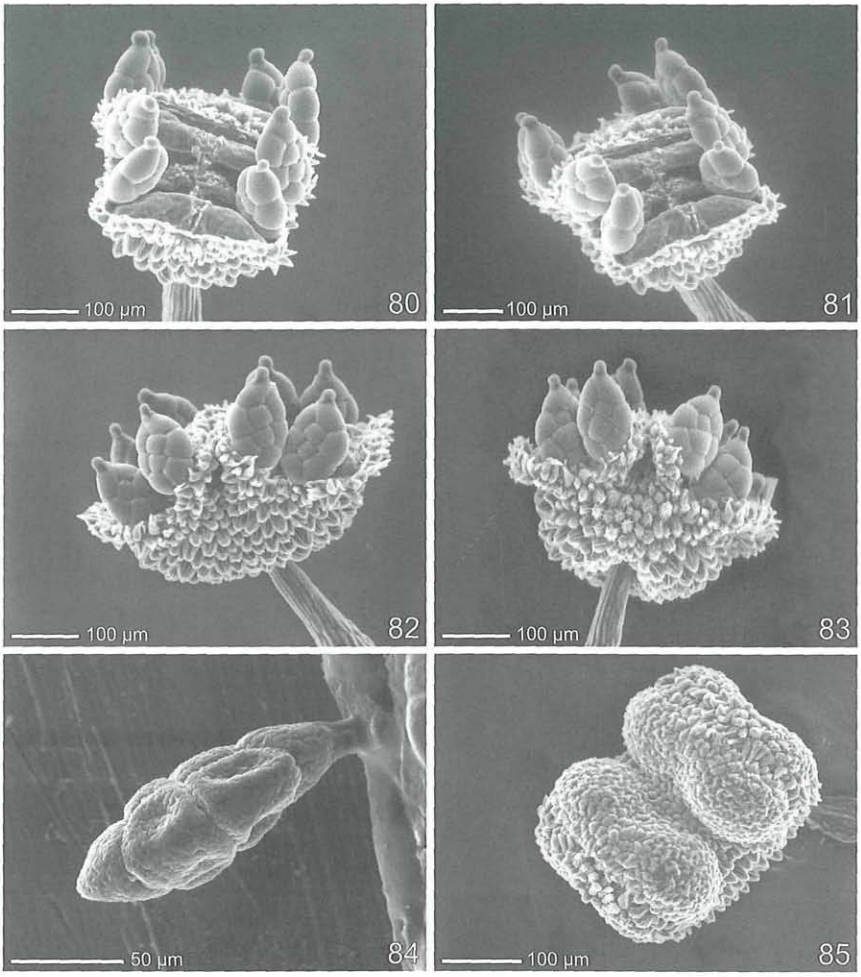


Fig. 80–83. *Calliandra tergemina,* naturally fully opened anthers with the eight, erected polyads, from the greenhouse. – Fig. 80. Side view from a little above. – Fig. 81. Diagonal view from a little above. – Fig. 82. View of an end of the anther. – Fig. 83. View of an end of the anther from a little below with the insertion of the filament and the two bent-back valves of each theca.
Fig. 84. A polyad, lateral view of the narrow side.
Fig. 85. A ripe, closed anther before the start of the opening process.

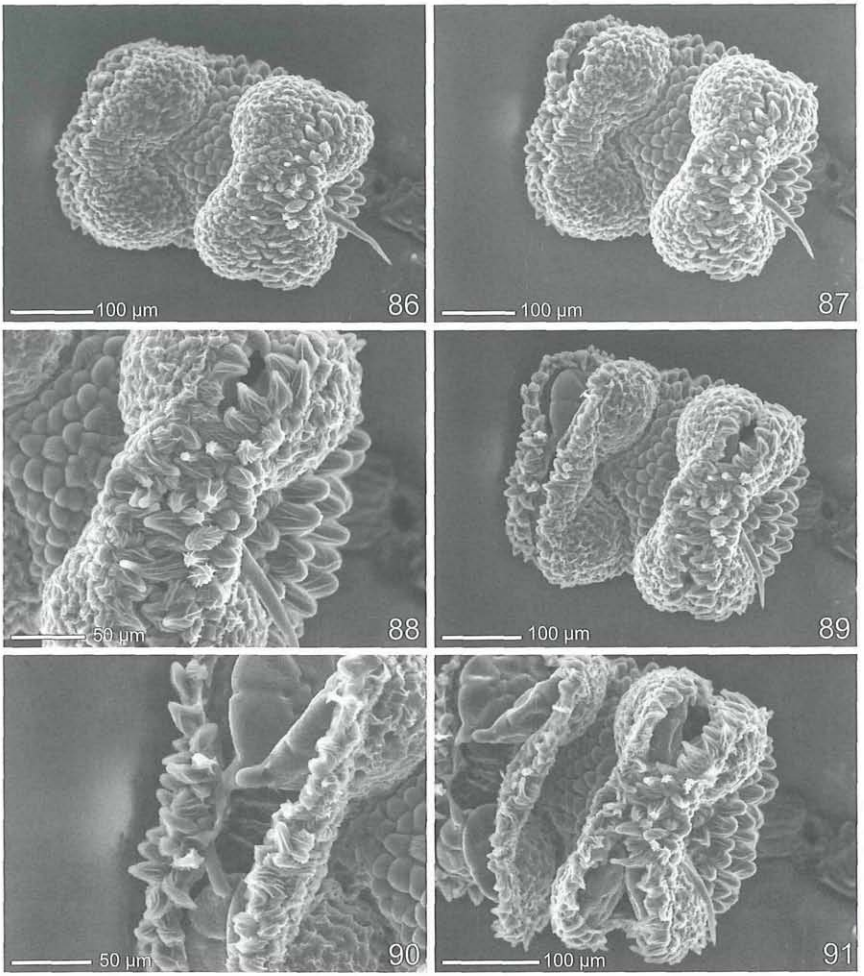


Fig. 86–91. *Calliandra tergemina,* the first phases in the opening process of an anther in the ESEM. – Fig. 86. Start of opening with the inward fold at the very base of the valves (compare Fig. 85 before the beginning of opening) and the dilatation of the ends of the thecae. – Fig. 87. Opening progressed, especially in the left theca. – Fig. 88. In the right theca the slit reaches the eye. – Fig. 89. In the left theca the eye ruptured, in the right one it is still intact. – Fig. 90. Detail of the left theca in Fig. 89. Eye split, left polyad of each locule-half connected by mucilage threads with their mucilage chamber. Lower right polyad without mucilage drop. – Fig. 91. Opening further progressed, eye still intact in the right theca. – Continuation in Fig. 92–93.

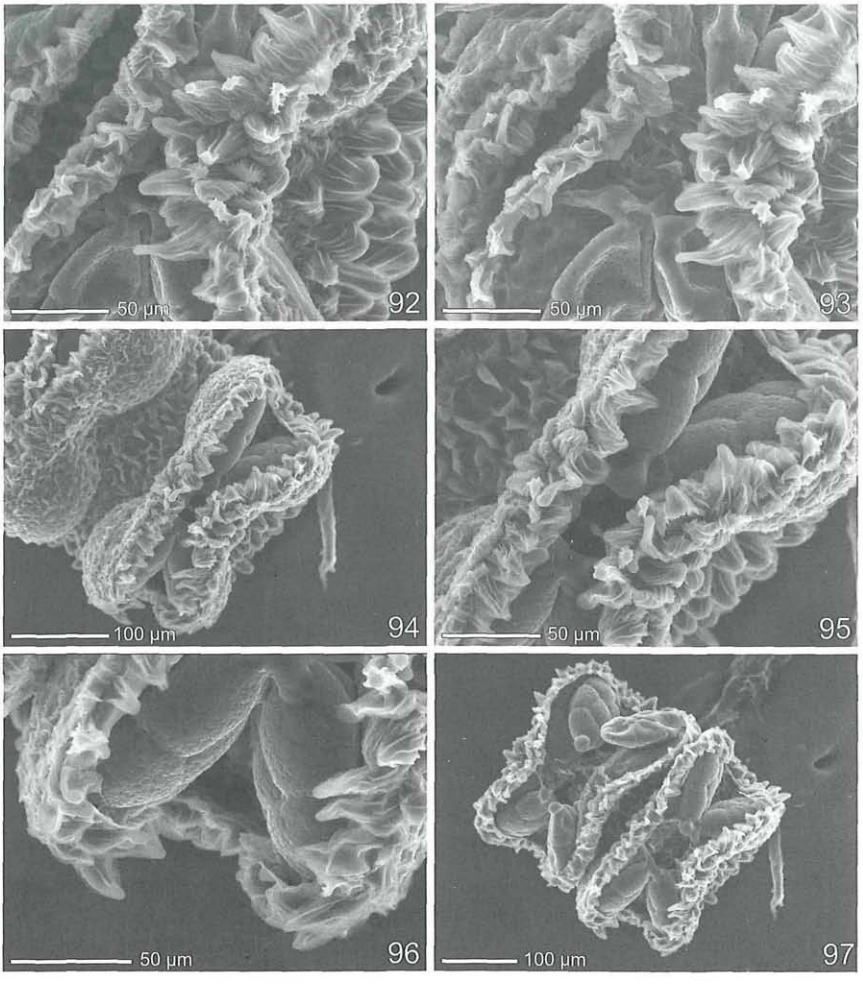


Fig. 92–93. *Calliandra tergemina*, phases in the opening process of an anther in the ESEM, right theca in Fig. 91 (continuation from Fig. 86–91). – Fig. 92. The slit in the stomium reaches the eye from both ends. Polyad tips with the mucilage drops, neighbour drops fused. – Fig. 93. Eye ruptured, opening progressed. – Time span between Fig. 86 and 93: c. 34 minutes.

Fig. 94–97. Sequence of another anther. – Fig. 94. Theca opened from the ends, eye ruptured already. – Fig. 95. Detail of Fig. 94, the central part of the theca. – Fig. 96. Detail of Fig. 94, the lower end of the theca with the two polyads, their mucilage drops connected. – Fig. 97. Whole anther, opening progressed. In the left theca the mucilage drops of the polyads are separated from each other, one polyad without mucilage. Left theca less opened, the mucilage drops of neighbour-polyads still in contact. – Time span between Fig. 94 and 97: c. 8 minutes.

ripe anther (PRENNER & TEPPNER 2005: 277, Fig. 6C; JAIN & VIJAYARAGHAVAN 1992 for *Leucaena*), the very thin tapetal membrane excepted. The anatomical details remain to be investigated. Because of the great interstice between the two thecae of an anther, also the inner thecal walls can bend back in the same manner as the outer walls (Fig. 82, 83).

The basal part of one polyad face is more or less solidly attached to the flat, slightly spoon-shaped ends of the longtitudinal bulge on the inner side of the back-bent theca walls by pollenkitt. It can not be excluded, that at the first steps of opening the margin of the theca walls with the hairs clasps the polyad sufficiently for helping to held them in the spoons (Fig. 30, 39, 94, 96) or to fix them by the pollenkitt there; in later stages, when the margin is more shrivelled (e. g., Fig. 16, 35, 80–83), adherence takes place by pollenkitt only. The polyad, fixed on the spoon-shaped part of the theca wall by the pollenkitt is obligated to follow its movements; thus the polyad is bent outward [the large face of the polyad is bent for 90° from the original position (compare, e. g., Fig. 25, 53, 64, 69 or 94 with Fig. 16, 31–37, 51 or 80–83)] and simultaneously more or less erected. The two components of the theca-wall bending, the inward moving of the longitudinal bulge and the progressively downward moving of the "spoons", are responsible for bringing the polyads in a position more or less transversal and perpendicular to the longitudinal axis of the anther (e. g., Fig. 15, 35 and 80). Finally, the apical end with the mucilage drop (pollen adhesive) is oriented directly to the pollen transporting vector (e. g., Fig. 36 and 83). The sticking of the erected polyads on the theca wall-spoons is clearly to be seen, e.g., in Fig. 17, 19, 36, 39 and 57. Finally, the erected polyads of adjacent locules stand in a very obtuse angle to each other or in the same plane (Fig. 15, 33, 35 and 80–82). Concerning the orientation of the longitudinal axis of the polyad, they are nearly perpendicular in *C. haematocephala, C. angustifolia* and *C. tergemina* usually (Fig. 36 and 80) and steeply oblique in *C. tweedii* (Fig. 15).

3.4. Variability / Deviations

3.4.1. Beginning of Opening at the Eye

In contrast to PRENNER 1998: 46 and PRENNER & TEPPNER 2005: 278 the opening of *C. angustifolia* anthers at the eye was only observed once. In the overhelming majority of the thecae opening began at the ends.

In *C. haematocephala* we had the chance to observe one case of opening in the centre, at the eye, in the ESEM (Fig. 59–64). The primary gap originated directly at the eye and progressed first slowly, then quickly in both directions to the ends. The further steps of the opening process do not differ from the cases with opening from the ends.

3.4.2. Confluent Mucilage Drops

The four mucilage drops per theca develop independently from one another in the four mucilage chambers, the narrowed, proximal ends of the lumina of the locule-halves, as stated above. Thus, under optimal conditions (from the perspective of man) the four drops on the polyad tips should be free from the very beginning. However, this is often not the case and after the breakdown of layers or cell walls the drops can fuse. This was especially abundant in *C. angustifolia* and to a lesser extent in *C. tergemina*. Sometimes all four drops are united immediately after the beginning of opening (late stage in Fig. 69). More frequently, the opposite pairs of drops are separated from the beginning and the adjacent drops only fuse side by side (Fig. 92-97). The movements of the polyads in the immediately following stages of the opening process lead to greater distance between the polyad tips and so to a renewed separation of the drops of pollen adhesive (Fig. 70, 91 and 97). These observations are an additional proof that the drop is a viscous liquid in reality from the start and does not have a solid stage.

3.4.3. Polyads without Pollen Adhesive

Rarely polyads without the drop of pollen adhesive were found (Fig. 91 and 97). One reason seems to lie in rounded, short apical grains of the polyads, which do not have contact with the mucilage chamber. Failure of the development of the pollen adhesive also seems to occur in the one or another chamber.

4. Discussion

Some papers dealing with different perspectives on *Calliandra* were published in the recent years. Lewis & Rico Arce 2005 report the current knowledge on the systematic position of this genus. Within the *Ingeae* there are many uncertainties but it seems to be sure that *Calliandra* belongs to the core *Ingeae* with an isolated position in this group. From the polyads the closest relative is *Guinetia*, but its pods appear very different (Rico Arce & al. 2000).

Barneby 1998 has written the monograph of *Calliandra* and restricted the genus to American species. Some minor inconsistencies exist in the descriptions. In *C. haematocephala* the filament tube reaches 1.5 cm in length and instead of fertile gynoecia small carpellodia may be developed. *C. tweedii:* flower number per head up to 23 flowers, in up to c. 10 central flowers a disc nectary is present, the nectary is a hollow cylinder up to 0.8 mm high and by c. half of the height connected with the stemonozone (definition: Lewis & Elias 1981: 156). The nectary is already mentioned in Loew 1904: 350. Good drawings of longitudinal sections through

stemonozone, nectary, gynophor and base of the ovary are included in ANZIBOR 1969: 133 Fig. 3A and 137 Fig. 5G. In our *C. tergemina* there was no remarkable difference between peripheral and central flowers, all flowers contained gynoeceum and nectary, the nectary is 0.5–0.8 mm high and connected with the stemonozone in the basal part.

Characteristics of the polyads are important for the delimitation of the genus and for phylogenetics, thus some papers on palynology have been published (e.g., GUINET 1965, SORSA 1969 and GUINET & HERNANDEZ 1989).

PRENNER 2004 described flower ontogeny of *C. angustifolia* in detail. Finally in PRENNER & TEPPNER 2005 and TEPPNER 2007c the structure of the anther and the origin of the pollen adhesive are well documented. However, further intensive investigations with the stereomicroscope and the observation of fresh plant material in the ESEM mode (as compared to chemically fixed and dried plant material in PRENNER & TEPPNER 2005) resulted in new details concerning the polyad presentation.

Very fascinating are the two kinds of pollen sticker (PRENNER & TEPPNER 2005: 270, TEPPNER 2007b: 234) with different functions. Pollenkitt (review: PACINI & HESSE 2005) is here – together with morphology, anatomy and movements of the anther wall – responsible for the pollen presentation (as usual in the major part of the angiosperms). The pollen adhesive (definition: PRENNER & TEPPNER 2005: 270, TEPPNER 2007b: 234; 2007c: 28; summary: VOGEL 2002) serves for the adherence of polyads to a vector. The thin film of pollenkitt is difficult to prove directly, nevertheless it is figured in GUINET & HERNANDEZ 1989: 17 Fig. 5 (unfortunately the authors did not differentiate between the two kinds of pollen sticker). Pollen adhesive in *Calliandra* is unique for *Mimosaceae* (the related *Guinetia* is not sufficiently described in this respect) and – to our knowledge – the mode of origin by lysis of cell groups in the middle layers has no parallel within the angiosperms. Because of the final position of the polyad in the open anther, we prefer to name the narrowed grain with the drop of pollen adhesive on its tip as the apical grain (PRENNER & TEPPNER 2005: 280, TEPPNER 2007b: 233).

The complex construction of *Calliandra* anthers and polyads may be derived phylogenetically from a simpler one as, e. g., in *Inga* (compare the comparison of both genera in TEPPNER 2007c: 39–40). Identical for both genera is the fine tapetal membrane, which is strongly appressed to the valves in the open anther. Very similar are the inward folded longitudinal bulges; if one suppose that a bulge roundly arched along the whole length (as in *Zapoteca* or *Leucaena*) should be primitive, than both are derived: *Inga* with nearly plane bulges and *Calliandra* with the downward bent 'spoons'. The attachment zone of the filament appears to be very small and simple in relation to *Inga*. However, from this fact alone it can not be decided if this is a primitive condition or a derived simplification by

reduction in connection with the much deeper insertion in the connective. Clearly derived characteristics of the anther in *Calliandra* are the proximally narrowed shape of the lumen in the locule half with the mucilage chamber at the end, the formation of the drop of pollen adhesive and the breaking down of the valves from the connective protrusions. The most apparent new characteristic of the polyad is the very regular, highly asymmetric shape of the polyad. Pollenkitt in *Calliandra* is present in the same manner as in *Inga* and is also responsible for the adherence of the polyad on the longitudinal bulge. The new pollen adhesive acts for the attachment to a vector in *Calliandra*.

The ESEM technique enabled the direct observation of the highly dynamic process of anther opening and polyad presentation without any sample preparation and at much higher magnifications as possible with the use of a stereomicroscope (KOLB & STABENTHEINER 2003). Direct comparison with stereomicroscopic investigations on anthers opened under natural conditions confirm the ESEM observations. Sometimes, especially in delicate anthers, the opening process stopped in the ESEM, possibly due to unripe anthers or abrupt dehydration. The method is well suited also for anthers with small, single pollen grains such as in *Leucaena leucocephala*.

Few *floral ecological* observations in nature are reported:

C. tweedii: SCHROTTKY 1908: 24 observed regularly the bee females of Augochlora nigromarginata (SPIN.), A. cupreola CKLL. and Xylocopa splendidula LEP. as well as hummingbirds on *C. tweedii* in Paraguay. He believed the bees to be the effective pollinators. LOEW 1904: 350-351 interpreted the species as ornithophilous.

C. haematocephala: On plants grown in Bogor (Java) KNUTH in LOEW 1904: 352 observed Xylocopa tenuiscapa WESTW., X. caerulea F., X. aestuans L. and one member of Pieridae as well as birds ("Honigvögel"). An impressive photo of a hummingbird in front of a *C. haematocephala* head can be found on the homepage of Dave's Garden [http://davesgarden.com/pf/showimage/40670/ (July 2006)]. The flower opening usually on the morning, the large amount of nectar and the closed access to the nectar which need force for penetration, clearly speaks for ornithophily. Bees (Apis mellifera included) and butterflies may be facultative visitors for nectar.

C. angustifolia: In Peru and in our greenhouse the flowers are visited in the night by sphingids and noctuids, which are clearly effective pollinators. At daytime the visiting hummingbirds seem not to contribute for fertilization (TEPPNER 2007a: 188).

C. tergemina: No information about visitors seems to be available. Beginning of anthesis in the morning and the large amount of nectar, e. g., makes ornithophily probable; the nectar is easily accessible, the free parts

of the filaments are relatively short, so bees and butterflies may be facultative visitors or pollinators as well.

5. Acknowledgements

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