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MYCELIAL MORPHOLOGY, MITOSPORES AND PRIMORDIUM FORMATION OF SIMOCYBE SUMPTUOSA IN LABORATORY CULTURES

H. CLÉMENÇON

Institut d'Ecologie, Université de Lausanne, CH-1015 Lausanne, Switzerland.
E-mail: Heinz.Clemencon@ie-bsg.unil.ch

In laboratory cultures *Simocybe sumptuosa* produces tiny slime drops with curved, binucleate conidia and ellipsoid, terminal or fusiform intercalary, binucleate chlamydospores. Chlamydospores occur also within the agar medium, but conidia do not. Noduli also form, and in one culture a few basidiomes developed. The mycelium is significantly denser beneath the noduli than between the noduli. Carpogenesis has been studied using stained microtome sections. *Simocybe sumptuosa* is exocarpic amphicleistoblemate.

Mycelia of *S. sumptuosa* (P.D. Orton) Sing. grow well in petridishes on various media at 20°–25°C, the most suitable ones being potato dextrose agar and oat meal agar. Two to three weeks after inoculation the agar surface is covered with a mycelium bearing innumerable, very small droplets of milky white slime that contain strongly curved conidia and ellipsoid aleuria. After another 3–4 weeks small, white pustules of aerial hyphae become visible. These are the first stages of the noduli eventually leading to primordium formation. The noduli soon overgrow neighbouring slime droplets. Most noduli cease further growth and die, but in one culture on PDA a small number of brownish primordia and young basidiomes have developed. This culture has been left unattended for about 6 weeks on a bench in the teaching laboratory where it became contaminated with bacteria that, unfortunately, have not been characterised. Despite many efforts, no other culture ever produced primordia, even when many noduli were present, but the material available allowed the analysis of carpogenesis of *S. sumptuosa,* as well as some observations on the morphology of the mycelium.

Carpogenesis of *Simocybe*-species is poorly known. So far only one single primordium of *S. centunculus* has been investigated (Reijnders, 1963: 82, as *Naucoria centunculus*). It was in a rather advanced developmental stage, and the only significant information that could be gained is the presence of a rudimentary 'partial veil' that lead to the conclusion that *S. centunculus* is 'paravelangiocarpous'. Since this primordium was collected in nature, nothing is known about mycelial morphology and mitospores that might be formed by this species.

MATERIAL AND METHODS

Basidiomes of *S. sumptuosa* (P.D. Orton) Sing. growing on deciduous wood were collected on September 6, 1995 by Dr. Beatrice Senn-Irlet in the Nijenrode Park, Breukelen, province of Gelderland in Holland. Cultures were derived from a spore-print and are kept in our laboratory under the access number BSI 95/68. It is available from the Centraalbureau voor Schimmelcultures, The Netherlands, as strain CBS 102149.

Potato dextrose agar (PDA) was prepared from dehydrated mashed potatoes following Lacy & Bridgmon (1962) and distributed in 9 cm plastic petridishes. After inoculation the dishes were sealed with Parafilm and incubated at approximately 20 °C in a 12h/12h light/dark cycle.

Small agar blocks with noduli or young basidiomes were cut out and fixed in aldehyde gas, dehydrated with methoxyethanol and embedded in a modified methacrylate mixture (Clémençon, 1990, no ethyleneglycol dimethylether, diethyleneglycol monobutylether replaced by 8% of terpineol, azoisobutyronitril replaced by 0.3% benzoyl peroxide and 0.04% N,N-dimethyl aniline). The terpineol acts as a softener, lessens the deteriorating effects of the benzoyl peroxide on the fungal cells and facilitates the removal of the gelatin capsule from the methacrylate block. The gelatin can be peeled off like an egg shell from a hard-boiled egg.

Sections were cut at 5–7 µm thickness using a tungsten carbide knife on an ordinary rotary microtome originally designed for cutting paraffin blocks. They were then placed on a drop of distilled water saturated with terpineol on a microscope slide and dried on a hot plate at roughly 60 °C. Before staining it is necessary to bake the sections onto the microscope slides (placed in a staining rack) for 1–2 h at 115–120 °C. This evaporates some of the terpineol in the embedding plastic (thus facilitating the penetration by the staining solutions) and avoids blistering of the sections during the staining at the elevated temperature used.

The most brilliant and crisp staining is with aluminium-zirconium haematoxylin resulting in blue cell content and reddish hyphal walls:

Mordant 'AZ':	Distilled water	270 ml
	Aluminium chloride	30 g
	Zirconium oxychloride	0.6 g
Haematoxylin:	Haematoxylin 10% in ethanol	3 ml
	Distilled water	300 ml
	Sodium periodate	60 mg

Staining schedule (hot = 55–65 °C; rt = room temperature; batches of 20 slides in a rack):

1	Rinse in hot distilled water	2–3 minutes
2	AZ hot	4–5 minutes
3	Rinse in distilled water rt	~ 1 minute
4	Hot distilled water I	5 minutes
5	Hot distilled water II	5 minutes
6	Hot haematoxylin	2–3 minutes
7	Rinse in distilled water rt	~ 1 minute
8	Hot distilled water	1–2 minutes
9	Hot tap water	1–2 minutes
10	Rapidly rinse in distilled water rt and dry at 55–65 °C	
11	Mount in Entellan or Eukitt	

Notes: If the washing with hot distilled water in steps 4 and 5 is too short then enough AZ stays in the plastic to react with the haematoxylin resulting in a pink grey background staining. If well washed, the methacrylate takes a yellow background staining that is easily removed in steps 8 and 9. The tap water must be slightly alkaline (if not so add a trace of sodium carbonate). If the sections have not been baked at 115–120 °C blisters will form

during hot staining. Hot staining is much quicker than staining at room temperature (which takes 24–36 h) and assures uniform staining throughout the entire thickness of the section, which is usually not the case in cold staining. The liquids (300 ml) are pre-heated in plastic staining jars using a household microwave oven at 600 W for 90–95 seconds, but the sections themselves are not exposed to microwave treatment.

Hyphal density in the agar has been estimated in microtome sections through the culture medium after staining with AZ haematoxylin. Using the 'threshold', 'make binary' and 'skeletonize' functions of the image analysis program NIH Image 1.62 by Wayne Rasband, the total mass of the hyphae present in a selected area of the cross section is estimated as the total number of black pixels. Since the hyphae are oriented at different angles with respect to the plane of observation the hyphal mass is underestimated and represents only about 88% of the real value, as detailed analyses of a few images have shown. The surface photographed for analysis measured 529 µm × 395 µm = 0.209 mm^2, and the total hyphal length contained in 1 mm^2 of the section is therefore 4.785 times bigger. Since the sections are 5 µm thick, the total biomass in 1 mm^3 amounts to 200 times the mass contained in 1 mm^2 of a section. As 1.44 pixels are equivalent to 1 µm, the mycelial mass contained in mm^3 of agar can be expressed in µm. For each estimation 11 successive microtome sections have been analysed. All estimates are made within the first 400 µm below the agar surface.

RESULTS

Mycelial morphology

The hyphae of the vegetative mycelium of *S. sumptuosa* are 2–4.5 µm wide, thin walled and roughly cylindrical. They bear clamp-connections at almost every septum, and the dolipore swelling is just visible in SDS Congo red (Clémençon, 1998), but the porus itself is too narrow to be seen. The narrow growth front on the agar surface consists of more or less parallel, radial hyphae, and the hyphal mat behind the growth front is woven from interlaced hyphae. Within the agar the hyphae are loosely arranged and run in all directions, but in cultures producing noduli the distribution is not uniform. Two different petridish cultures producing noduli have been analysed. In the first culture the hyphal masses beneath two noduli were 14.90 m/mm^3 (SD = 1.63, N = 11) and 12.48 m/mm^3 (SD = 0.97, N = 11), as compared to 7.26 m/mm^3 (SD = 1.03, N = 11) at a control site without nodulus. In a second culture there were 10.9 m/mm^3 (SD = 1.58, N = 11) beneath a nodulus and 4.41 m/mm^3 (SD = 0.73, N = 11) under a site without nodulus. This means that beneath a nodulus there are 1.7–2.5 times as many hyphae than at a location without a nodulus. These hyphal lengths may be impressive, but assuming a mean hyphal diameter of 3 µm, the total hyphal volumes amount to 10.4%, 8.7% and 5.1% of the agar volume for the first culture and to 7.6% and 3.1% for the second culture. All differences are statistically significant.

No special hyphal differentiations are visible beneath the noduli or at any other location of the mycelium, but the contents of some hyphae and of a few chlamydospores stain brown in iodine solution indicating the possible presence of glycogen. In cultures older than 4 months many empty hyphae with secondary septa can be seen.

Mitospores

Almost the entire surface becomes covered with tiny milky-white slime droplets that contain strongly curved conidia and ellipsoid chlamydospores (Figs. 1–3, 12).

Conidia are borne in short chains at the end of a hypha or in small clusters on the sides of hyphae (Fig. 4). Several conidial chains form a slimy head (Fig. 5), as the conidia are released by gelification of the wall of the mother hyphae. Conidia are binucleate and contain lipids. The nuclei are already visible in water mounts and are strongly stained by cotton blue in lactic acid (Fig. 6), iron aceto carmine and DAPI; the lipids can be stained with Sudan III dissolved in lactophenol (Fig. 7). The conidial wall is very thin, inamyloid and hardly stains in Congo red and Cotton blue. The clamp-connection often forms a small asymmetrical blister at the base of the conidium (Fig. 8).

Besides terminal aleuria some intercalary, more or less fusiform chlamydospores are present in the mycelium. Both types of chlamydospores are binucleate, contain large amounts of lipids (Fig. 10) and bear a basal clamp-connection. The two nuclei can already be seen in water mounts, become more distinct in iodine solutions and are deeply stained in Cotton blue mounts. Chlamydospore walls are inamyloid, strongly cyanophilic and stain deeply with Congo red. Toluidine blue does not stain the walls, even after heating the slides to the boiling point.

Chlamydospores and conidia frequently occur in the same head (Fig. 9), and both are present together in the same slime droplet.

Conidia are produced only on the agar surface, but chlamydospores are also formed deep within the agar medium (Fig. 11).

In situ germination of chlamydospores has been observed, but this is a rare event. No attempts have been made to estimate the germination rates on fresh media.

Noduli and carpogenesis

Noduli are formed anywhere on the mycelium except near the centre of the culture. They soon overgrow the slime drops and develop on top of them (Figs. 12, 13). Young noduli are connected to the mycelium by a few hyphae and consist of a very loose web of undifferentiated hyphae. On the surface some elongate vesicular cells are present (Fig. 13). Neighbouring noduli may fuse and produce a single primordium (Fig. 19A). Mature noduli are round cushion-shaped or roughly spherical and brown (Figs. 14, 17). They are densely woven from thin-walled, cylindrical hyphae forming an irregular context with a few small air spaces. Near the base some crystals form small clusters surrounded by a thin amorphous mass staining deeply with aluminium zirconium haematoxylin (Figs. 14, 15, 20, 21). The surface is completely covered with a layer of radially arranged, inflated hyphae with vesicular-fusiform end cells (Figs. 14, 16). This layer grows out from the nodulus context and can be named a noduloblema.

For the study of carpogenesis four representative primordia have been selected (Fig. 19). Primordium A consists of an erect shaft emerging from a nodulus and bearing a small pileus initial (in the case figured the shaft actually emerges from two fused noduli, but this is not the rule). Primordium B has grown a thin veil and a small pileus with a smooth prehymenial palisade on its underside. In primordium C general enlargement of all parts has started, and lamellae begin to develop. Primordium D has a very elongated stipe and a few gills, but the partial veil has not grown noticeably.

Fig. 18 shows a primordium intermediate between C and D, but one which has not been sectioned. The veil forms a woolly cover on the stipe and a thin fibrous cover on the pileus, and a few hyphae bridge the gap between the pileus margin and the stipe. This veil consists of a cauloblema, a pileoblema and an amphicleistoblema, as shown below.

Primordium A — Carpogenesis starts with the formation of an erect shaft growing out from the nodulus (Fig. 20). The bottom of the nodulus can be identified by the presence of some crystals and the irregular arrangement of the hyphae (Fig. 23). The shaft does not grow out from the surface of the nodulus but originates from its centre. Here the upward-growing generative hyphae are very thin and loosely arranged. The region corresponding to the centre of the nodulus shows some mucilage (Fig. 23), but higher up the shaft is exempt of mucilage.

In the upper half of the shaft the hyphae form a denser stipe initial with only a few small air spaces. Towards the periphery the hyphae have begun to inflate and to form a tube of denser context. The sides of the shaft are covered with a rudimentary cauloblema consisting mainly of isolated vesicular hyphal end cells.

On top of the shaft is a small pileus initial consisting of a dense context woven from irregularly arranged vegetative hyphae, but it is not sharply delimited from the stipe initial (Fig. 22). It generates a voluminous pileoblema consisting of two zones that are easily distinguished although they are not distinctly separated. The inner zone resembles the nodulo-blema in the radial arrangement of inflated hyphae with vesicular end cells. The outer zone consists of periclinal hyphae also bearing vesicular end cells (Fig. 22). The development of the primordia shows that the inner pileoblema becomes the pileipellis, whereas the outer pileoblema contributes to the veil.

Primordium B — Fig. 24 shows the general organisation of this developmental stage. The total length has not increased significantly, but the pileus has formed and its underside is lined with a smooth prehymenial palisade. The cauloblema and the pileoblema bridge the gap between the pileus and the stipe thus creating an amphicleistoblema and a secondary prehymenial cavity. This primordium confirms that the erect shaft (the future stipe) grows from the centre of the nodulus.

The growth direction of the hyphae of the metablemas is indicated by the orientation of the vesicular end cells and can also be determined by looking at the clamp-connections, whose open ends point away from the hyphal tips. A fine bundle of hyphae of the cauloblema growing from the stipe surface over the pileus margin and showing many upright vesicular end cells can be seen in Fig. 25. The hyphae of the cauloblema grow over a considerable part of the pileus, and they intermingle with downward growing hyphae from the pileoblema, as seen in Figs. 26A–D. The origin of the hyphae of the pileoblema is shown in Fig. 27. Just below the prehymenial cavity many young hyphae rich in cytoplasm emerge from the stipe surface and grow in the direction of the palisade. This is a young cauloblema (Figs. 28, 28A). A hypha of the cauloblema located on the pileus surface is visible in the same photographs, its growth direction being indicated by the orientation of the clamp-connection (Figs. 28, 28B, arrows). The cauloblema and the pileoblema intermingle on the pileus surface (Fig. 29) and also on the stipe surface (Fig. 30).

The pileus margin begins to be differentiated, but it is not yet present on the entire circumference of the pileus. It is only about 50 µm wide and its hyphae project beyond the level of the prehymenial palisade (Fig. 31). Hyphae of the cauloblema not only grow on the pileus surface, but some of them penetrate into the pileus margin (Fig. 32). The prehymenial palisade forms a continuous ring around the stipe. Lamellae are not yet formed (Figs. 33, 34).

In this developmental stage the hyphae begin to inflate as a means to increase the volume of the basidiome, as documented in Figs. 36–40. The stipe consists of a lateral, tube-like part consisting of short-celled, slightly inflated hyphae and a central pith with thin-walled, faintly staining hyphae.

Primordium C — The size increase is accompanied by inflation of most hyphae of the central pileus context. Air spaces have also increased somewhat in the pileus, leading to a less dense structure as compared to the primordium B (Fig. 42). The first thromboplerous hyphae appear in the pileus context (Fig. 41). Many short hyphae grow out from the stipe surface thus increasing the mass of the cauloblema (Fig. 48), but the outer pileoblema and the cleistoblema have become thinner by the expansion of the pileus. As the stipe has not yet stretched significantly, the structure of its context is the same as the one in primordium B (Fig. 47). It is interesting to note that some medullar hyphae do not reach the pileus. They bear slightly inflated terminal cells reminiscent of acrophysalides (Figs. 45, 46). The first lamellae begin to form, as indicated by the bulge of the palisade in Fig. 49. The gill trama is subregular and slightly divergent, and a few hyphae running roughly parallel to the gill edge are also present (as is frequently the case in mature gills near the edge). The palisade is evenly covering the young gills, without any indication of a differentiation on the gill edge (Figs. 50, 51).

Primordium D — The stipe has much elongated and so have the cells of its lateral part (Fig. 56). The pileus has expanded by further cell inflation and air space formation (Figs. 52, 53). The lamellae are now regular, radial, downward growing ridges with a subregular, slightly diverging gill trama of cylindrical, not yet inflated hyphae (Fig. 54). The gill edges are differentiated by the presence of vesicular cells, the future cheilocystidia. The pileoblema and the cleistoblema have become scantier, but the cauloblema has increased its mass, not by gaining in thickness, but by covering the total length of the stipe. Terminal hyphal end cells in the medulla are confirmed (Fig. 57; compare Figs. 45 and 46).

Legends to Figures 1–25, 41–48

Figs. 1–3. *Simocybe sumptuosa*, slime drops on the agar surface in a petri dish, PDA, 4 weeks, room temperature. — 1. Surface view of a living culture; 2 & 3. cross sections showing strongly curved conidia and vesicular chlamydospores (aleuria). In some mitospores two nuclei can be seen. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Figs. 4–9. *Simocybe sumptuosa*, conidia and chlamydospores. — 4. Spiral growth during conidium formation; 5. a small slimy head of conidia; 6. conidia stained with cotton blue dissolved in lactic acid. The wall is acyanophilous (or only very weakly stained), but the two nuclei stain deeply; 7. lipids stained with Sudan III in lactophenol; 8. wall stained with Congo red. Please note the empty appendices formed by the mother cell wall; 9. a slimy head with conidia and chlamydospores, stained with Congo red. The chlamydospore walls are thicker and stain strongly, the thin conidial walls do not stain well.

Figs. 10 & 11. *Simocybe sumptuosa*, chlamydospores. — 10. On the surface of the nutrient agar. Lipids stained with Sudan III in lactophenol; 11. within the nutrient agar (PDA). — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Figs. 12 & 13. *Simocybe sumptuosa*, Young noduli growing over the slimy heads of mitospores. — 12. Surface view of a living culture, PDA, 2 months, room temperature; 13. near median cross section. Under the nodulus 2 slimy heads are buried. The structure is still very loose. Vesicular, club-shaped cells are already present on the surface. Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Figs. 14–16. *Simocybe sumptuosa*, mature nodulus, near median cross section. — 14. An almost homogeneous, dense plect of cylindrical, strongly interwoven hyphae bears a well-developed metablema (that may be called a noduloblema) of radial, inflated hyphae bearing elongate vesicular cells. Near the base a few clusters of crystals are located between the hyphae (white arrow); 15. close up view of the location indicated by the arrow in Fig. 14; 16. close up view of the noduloblema. — Scale bar valid for Figs. 15 &16. Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Figs. 17–19. *Simocybe sumptuosa,* nodules and primordia. Scale bar valid for all Figs. — 17. At left two ochre brownish noduli near maturity, still without surface hyphae. At right two dark brown noduli beginning to form surface hyphae (one hypha clearly visible on the upper nodulus). Please note the dense population of slimy heads on the agar surface; 18. a primordium with light brownish stipe and ochre brown pileus, photographed to show the woolly cauloblema and the hyphae of the pileoblema; 19. near median longitudinal sections of the four developmental stages discussed: A. a conical erect shaft has been formed by two coalescent noduli. The dark spots in the base are crystals; B. the pileus has been formed, the metablemas are well developed, the prehymenial palisade and the prehymenial cavity are already present, but the lamellae have not yet begun to grow; C. the lamellae grow into the cavity. The stipe is already hollow. The cleistoblema still bridges the gap between the pileus margin and the stipe surface; D. the stipe elongates, the metablemas have slightly grown, but the cleistoblema begins to tear apart. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin. The subsequent figures showing details of each developmental stage of these primordia do not necessarily stem from the sections shown here.

Figs. 20 & 21. *Simocybe sumptuosa,* developmental stage A. — 20. The cauloblema with its vesicular cells is still poorly developed. The pileoblema consists of an inner layer of radially arranged inflated hyphae and a loose outer layer of repent hyphae. The dark spots in the plect of the nodulus are crystals (white arrow). The inner part of the nodulus shows a looser structure than the mature nodulus in Fig. 14. This loosening at primordium formation is confirmed by the other primordia studied (e.g. Fig. 24). The shaft is already differentiated into a medulla of thin hyphae and a peripheral zone of short-celled inflated hyphae. On top of the shaft and beneath the pileoblema the pileus initial takes the form of a small area of tightly entangled hyphae; 21. crystals from the base of a crushed nodulus. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

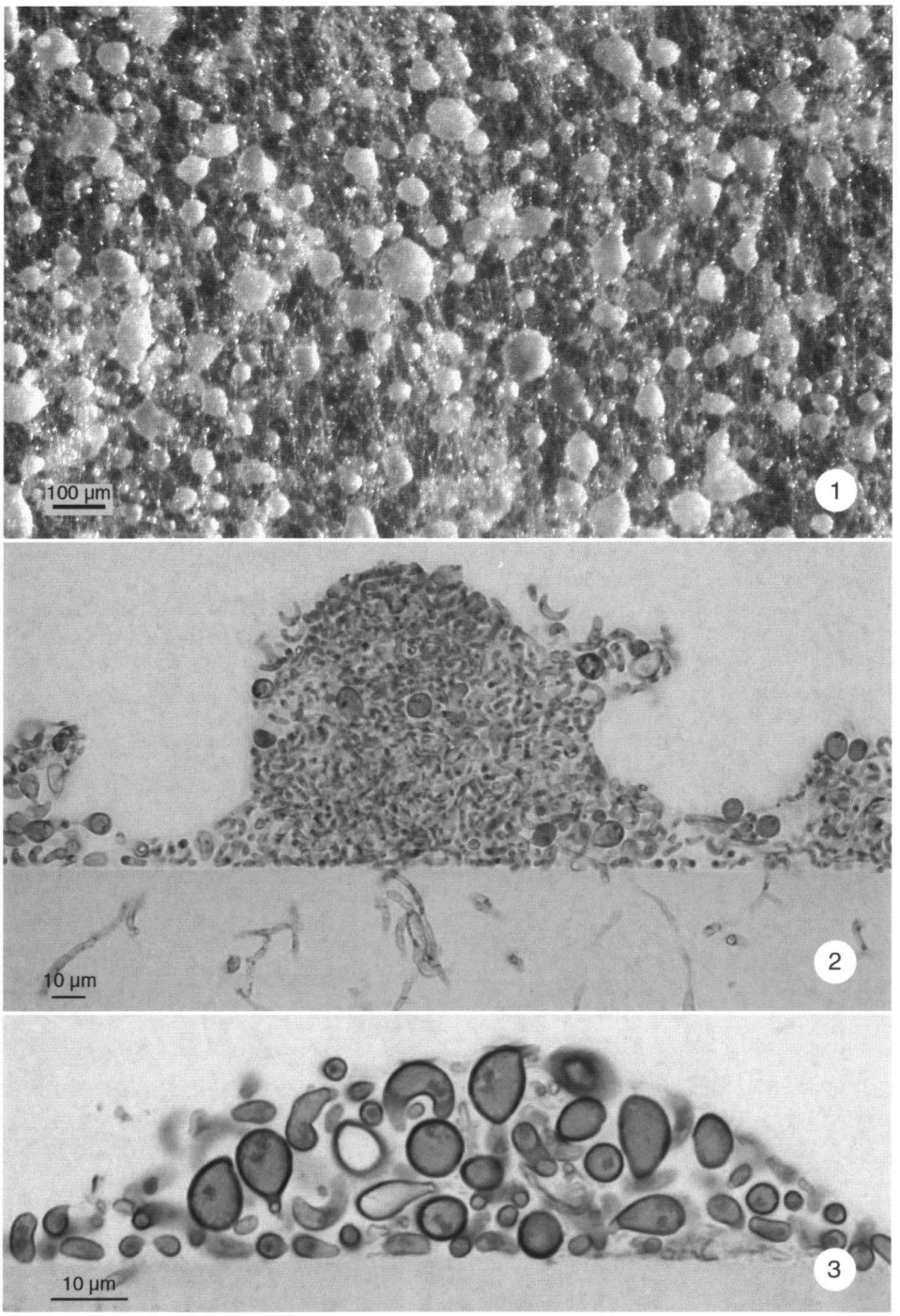
Fig. 22. *Simocybe sumptuosa,* developmental stage A, top of Fig. 20, showing the pileus initial covered with the anticlinal inflated hyphae of the inner pileoblema and the periclinal hyphae of the outer pileoblema. Below the pileus initial the upper part of the erect shaft is formed by slightly inflated, roughly parallel hyphae. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Fig. 23. *Simocybe sumptuosa,* developmental stage A, bottom of Fig. 20, showing the transition zone between the nodulus and the stipe medulla. In this zone we find some mucilage absent elsewhere. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

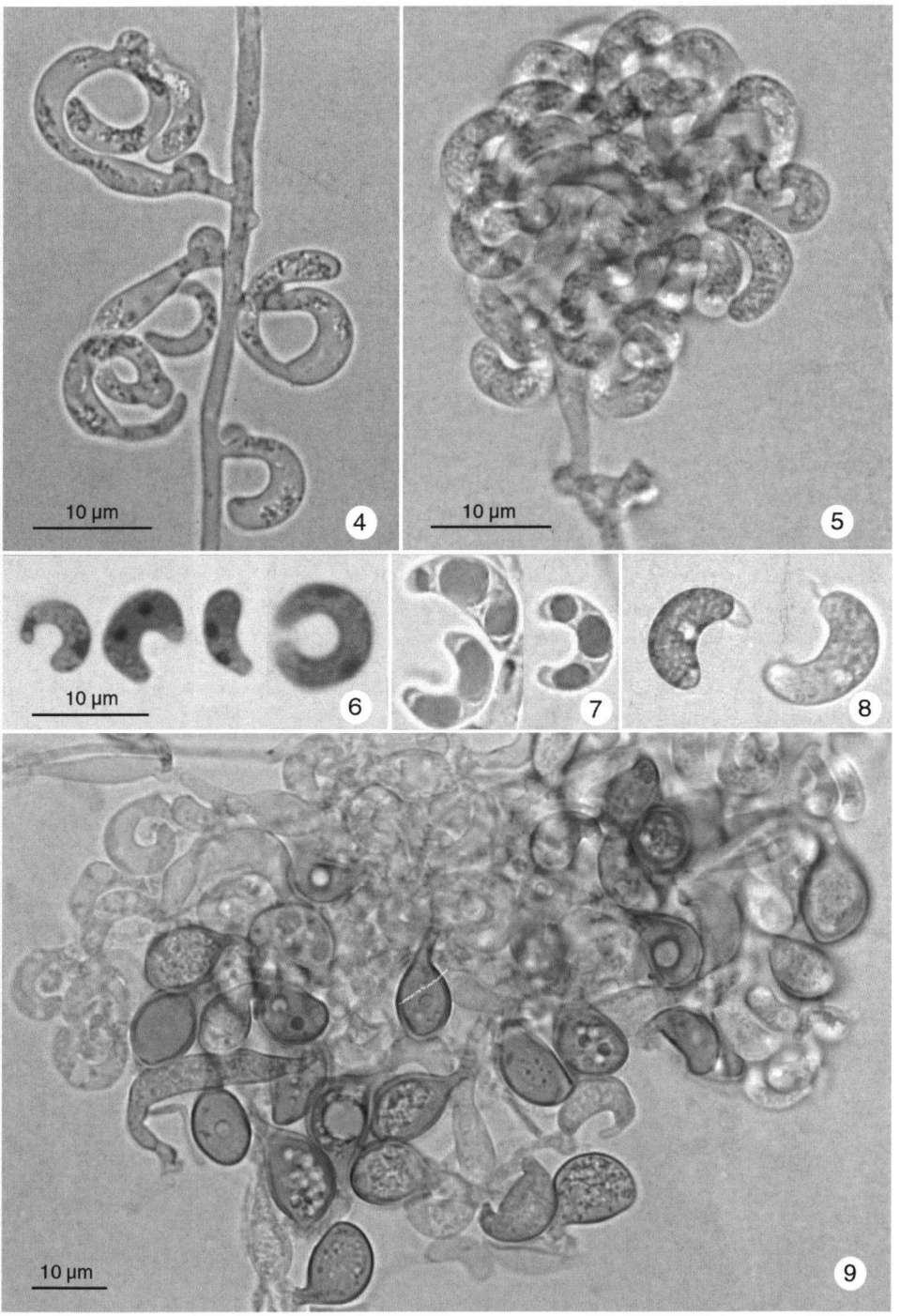
Fig. 24. *Simocybe sumptuosa,* developmental stage B, identification of the different organs. The term 'partial veil' is used here in the same way 'tree' or 'fruit' is used in botany, i.e. without any morphogenetic implications. The nodulus is not very sharply delimited from the stipe, but it can be recognised without great difficulty, although its core has a loose structure. Beneath the prehymenial palisade the cavity is clearly visible. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Fig. 25. *Simocybe sumptuosa,* developmental stage B. Analysis of the veil, a cauloblema growing from the stipe over the pileus margin. The growth direction is indicated by the orientation of the vesicular hyphal end cells. The prehymenial cavity is loosely filled with hyphae from the cauloblema. Please note that the hyphae of the stipe are already slightly inflated. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

Figs. 41–48. *Simocybe sumptuosa,* developmental stage C. — 41. Thromboplerous hyphae staining dark with AZ-haematoxylin begin to appear in the pileus trama. The zone photographed is situated near the periphery at the left side of the pileus; 42. context from the centre of the pileus. The hyphae are considerably more inflated and the interhyphal spaces bigger than in primordium B (cf. Fig. 37); 43. transition zone between the prehymenial palisade and the stipe surface. The palisade becomes disorganised and intergrades with the cauloblema; 44. context of the pileus from the centre of the transition zone from stipe to pileus. The hyphae are not yet inflated; 45 & 46. stipe medulla just below the pileus (Fig. 45) and from the central region of the stipe. The hyphae on top are more densely packed than in the central part of the stipe. The arrows indicate two slightly inflated free hyphal end cells. Some nuclei and dolipore swellings are visible; 47. lateral part of the stipe. The structure has not changed significantly from that in primordium B (cf. Fig. 38); 48. short celled cauloblema from the central part of the stipe. — The scale bar applies to all figures. Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.



Figs. 1–3 (legend on p. 412).



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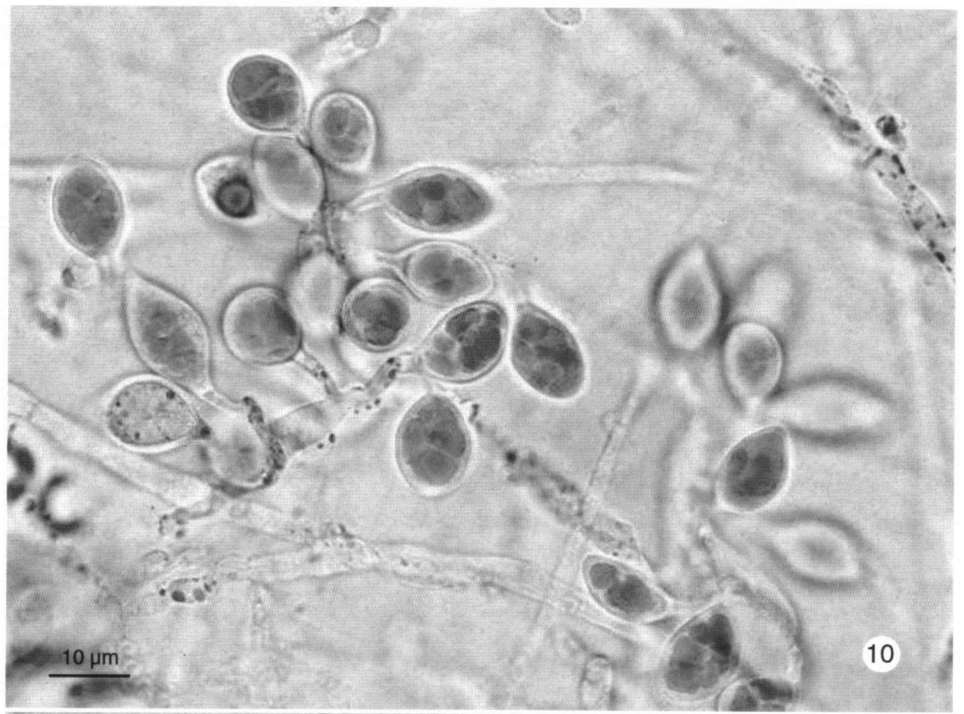

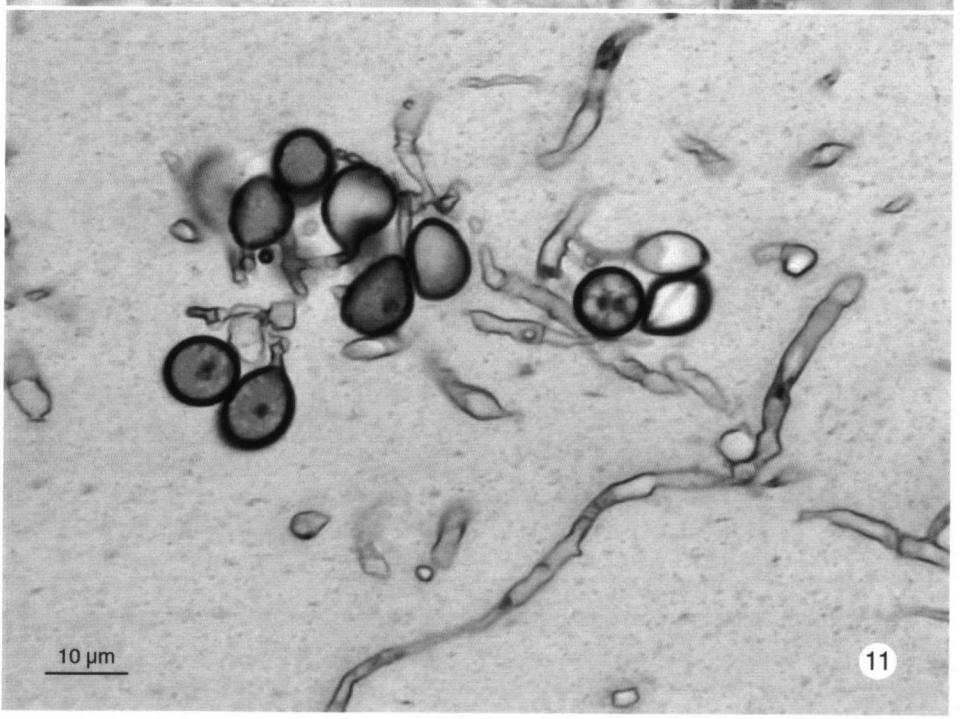

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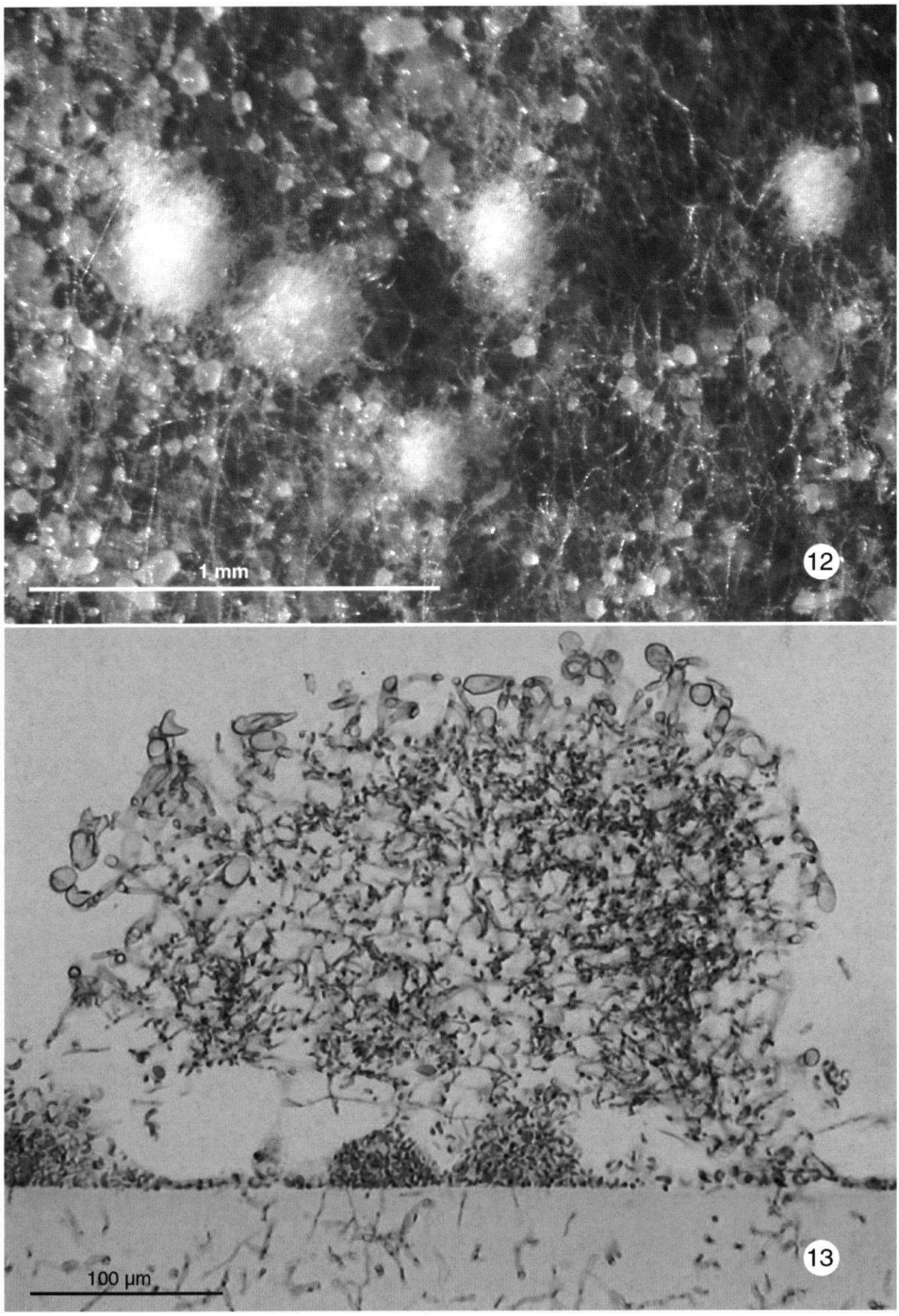

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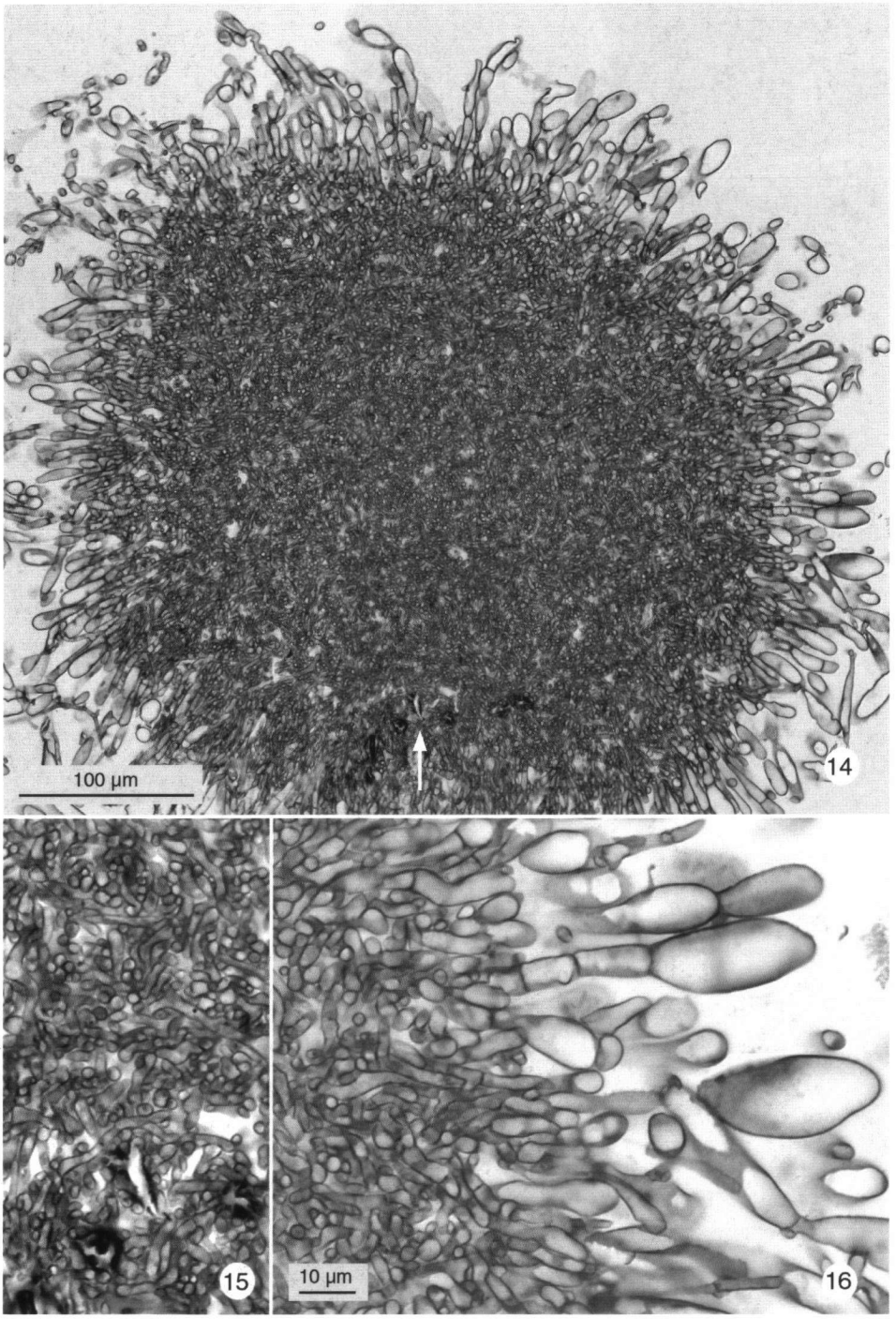

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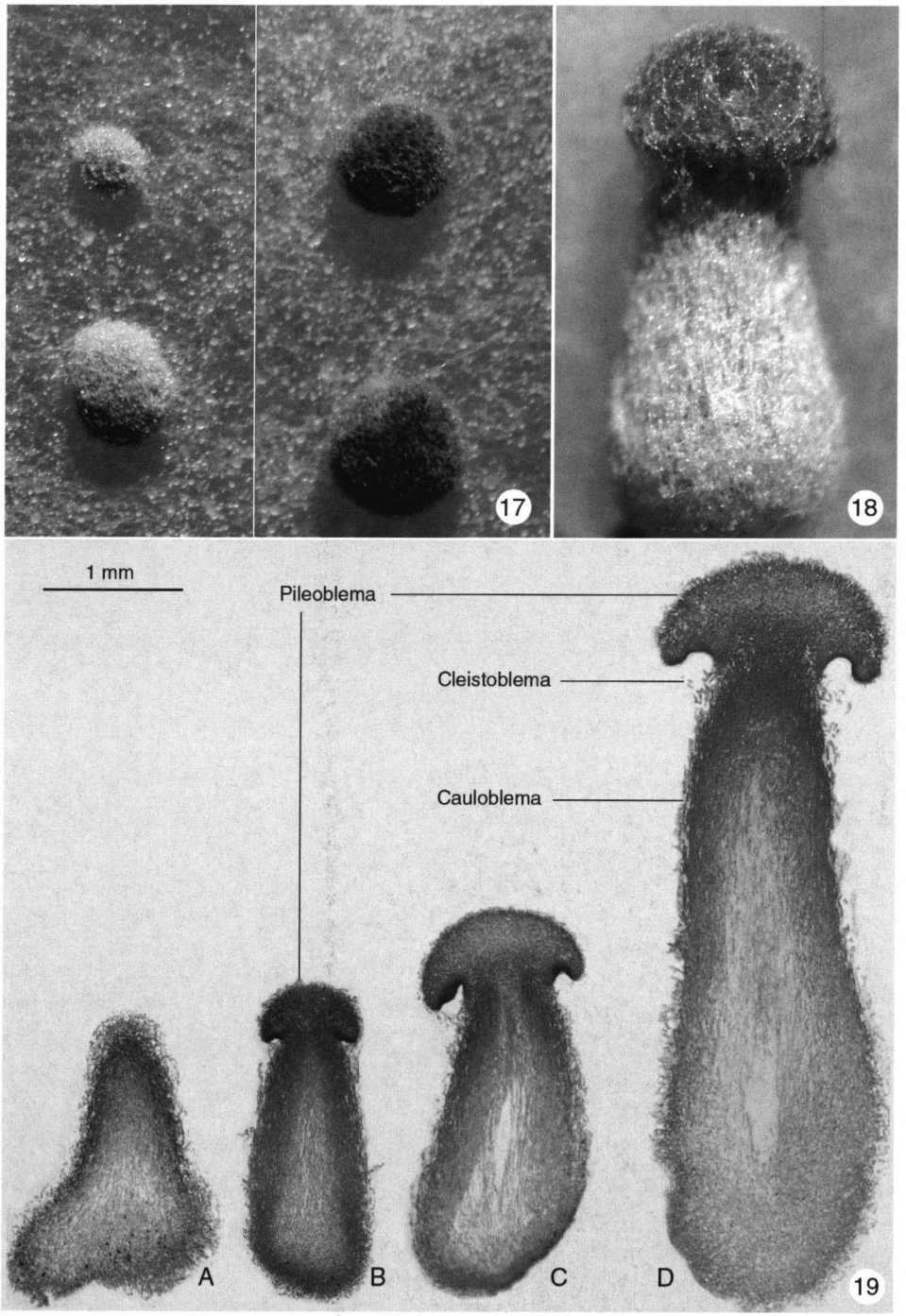

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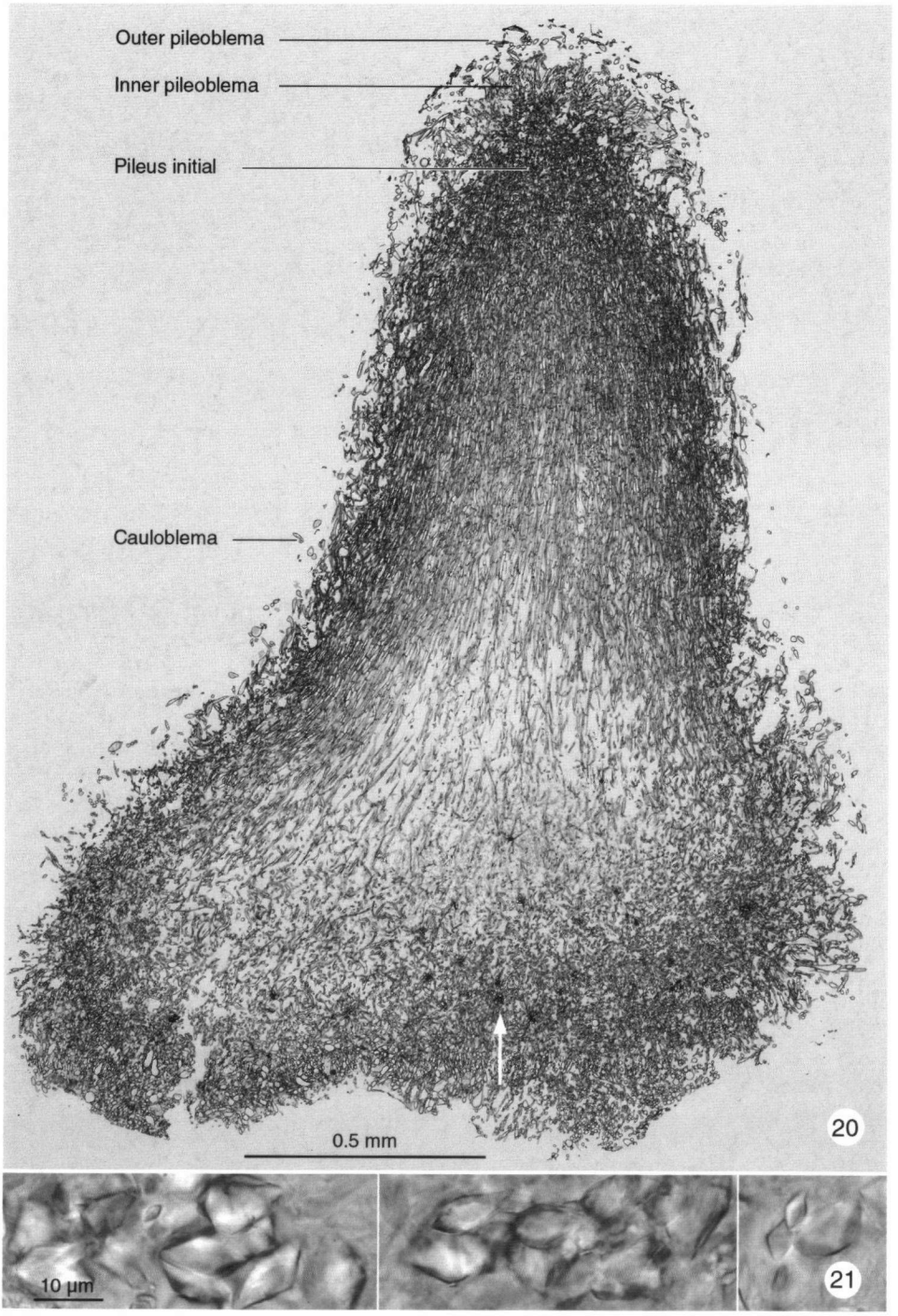

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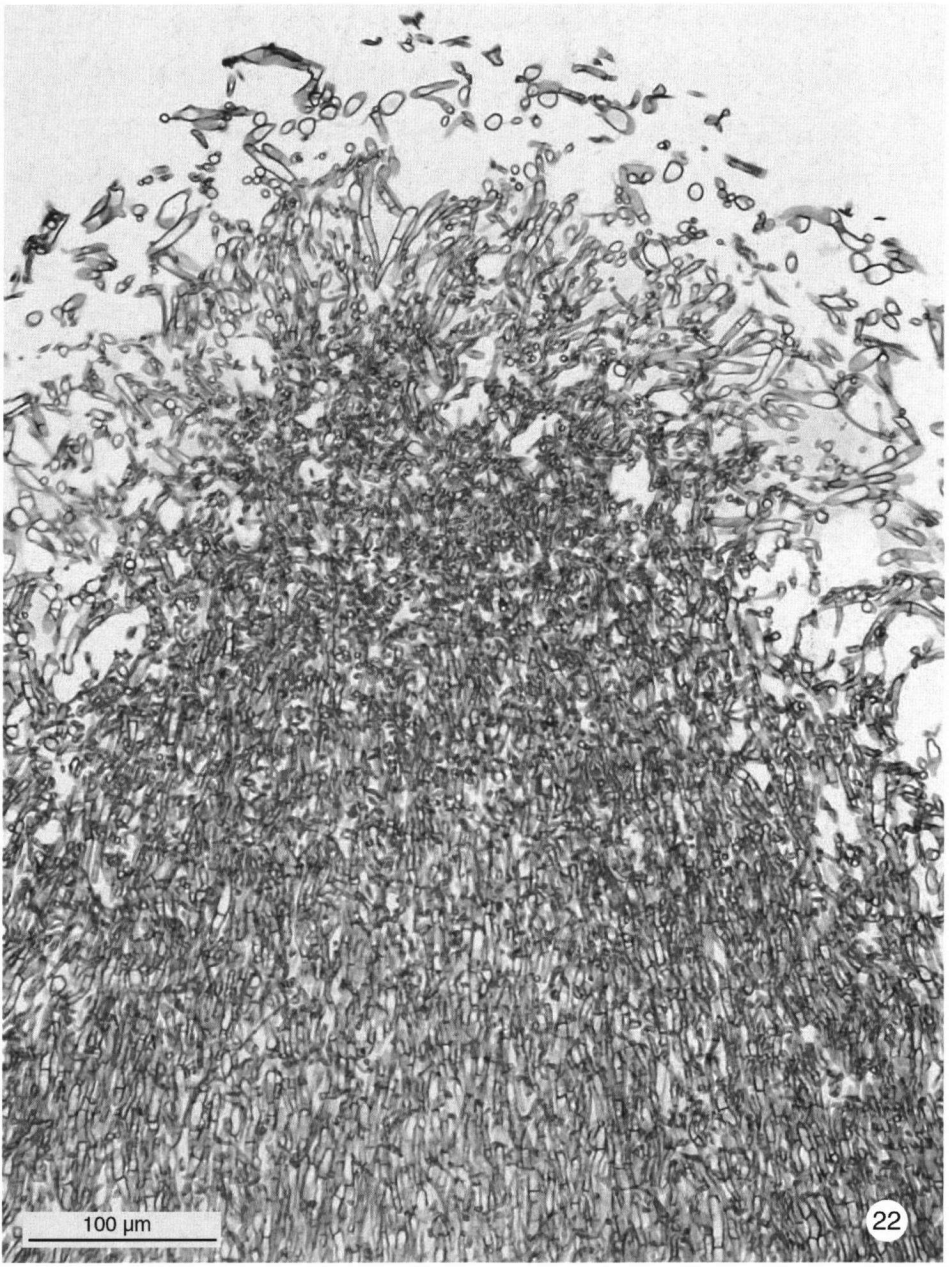

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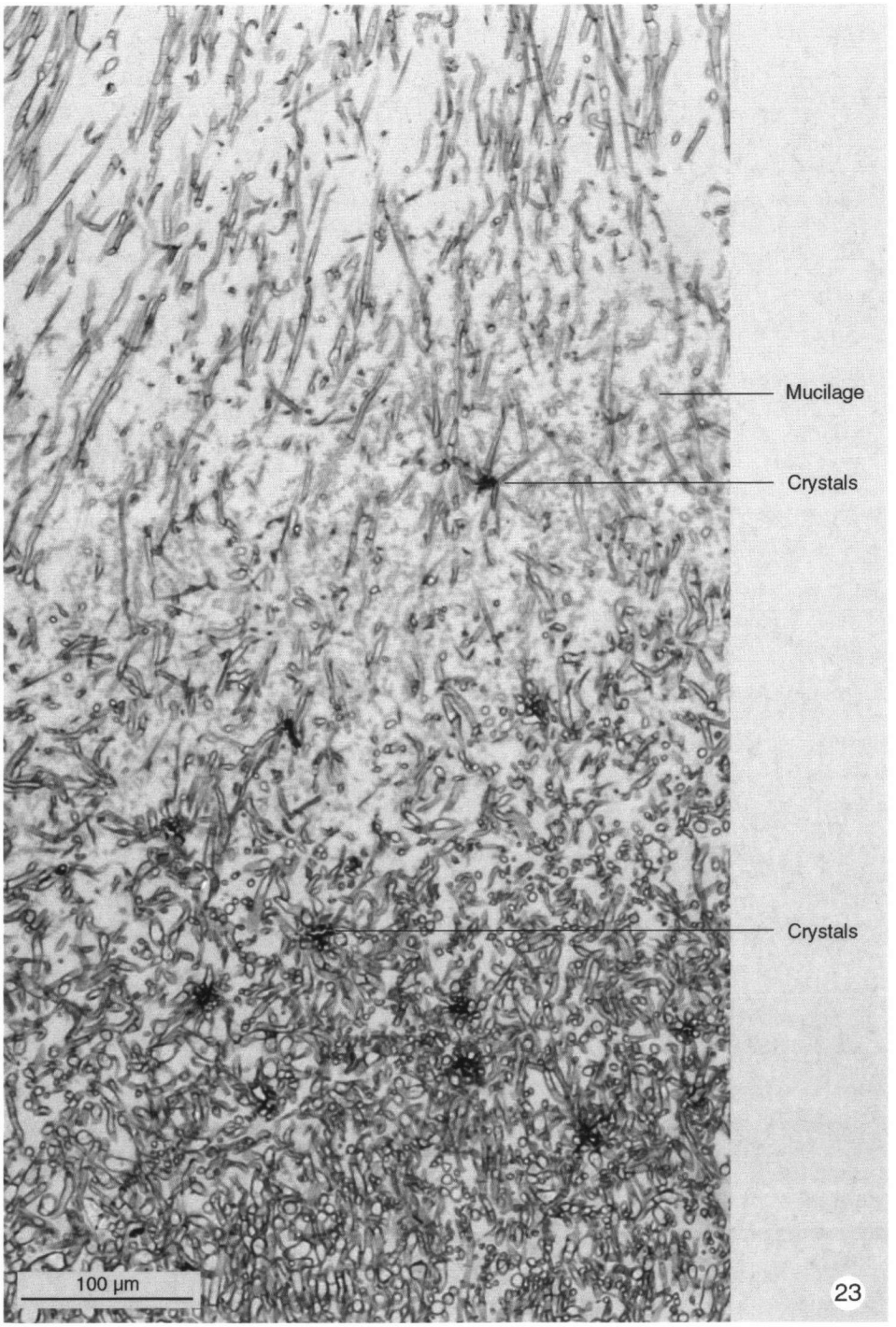


Fig. 23 (legend on p. 413).

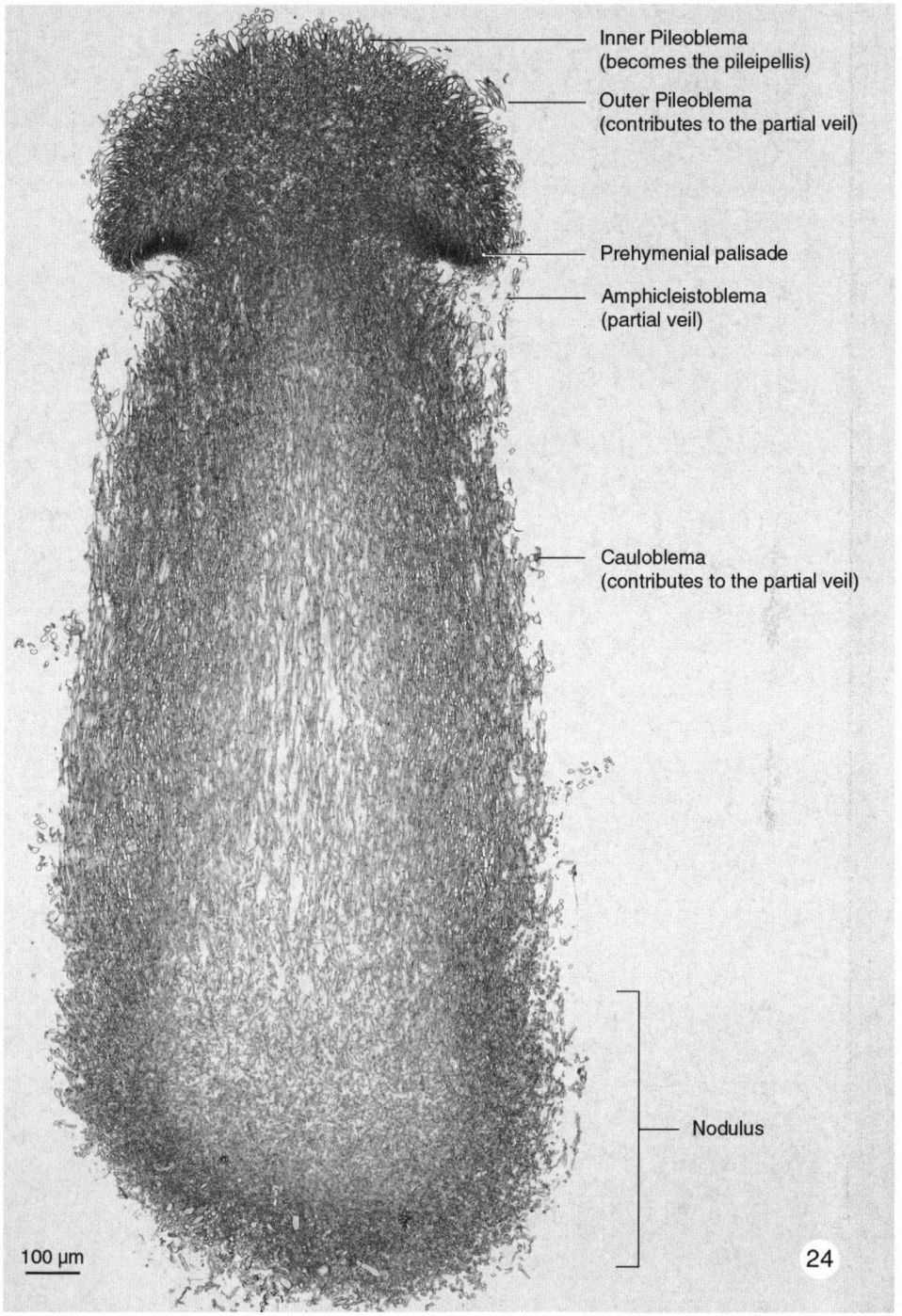


Fig. 24 (legend on p. 413).

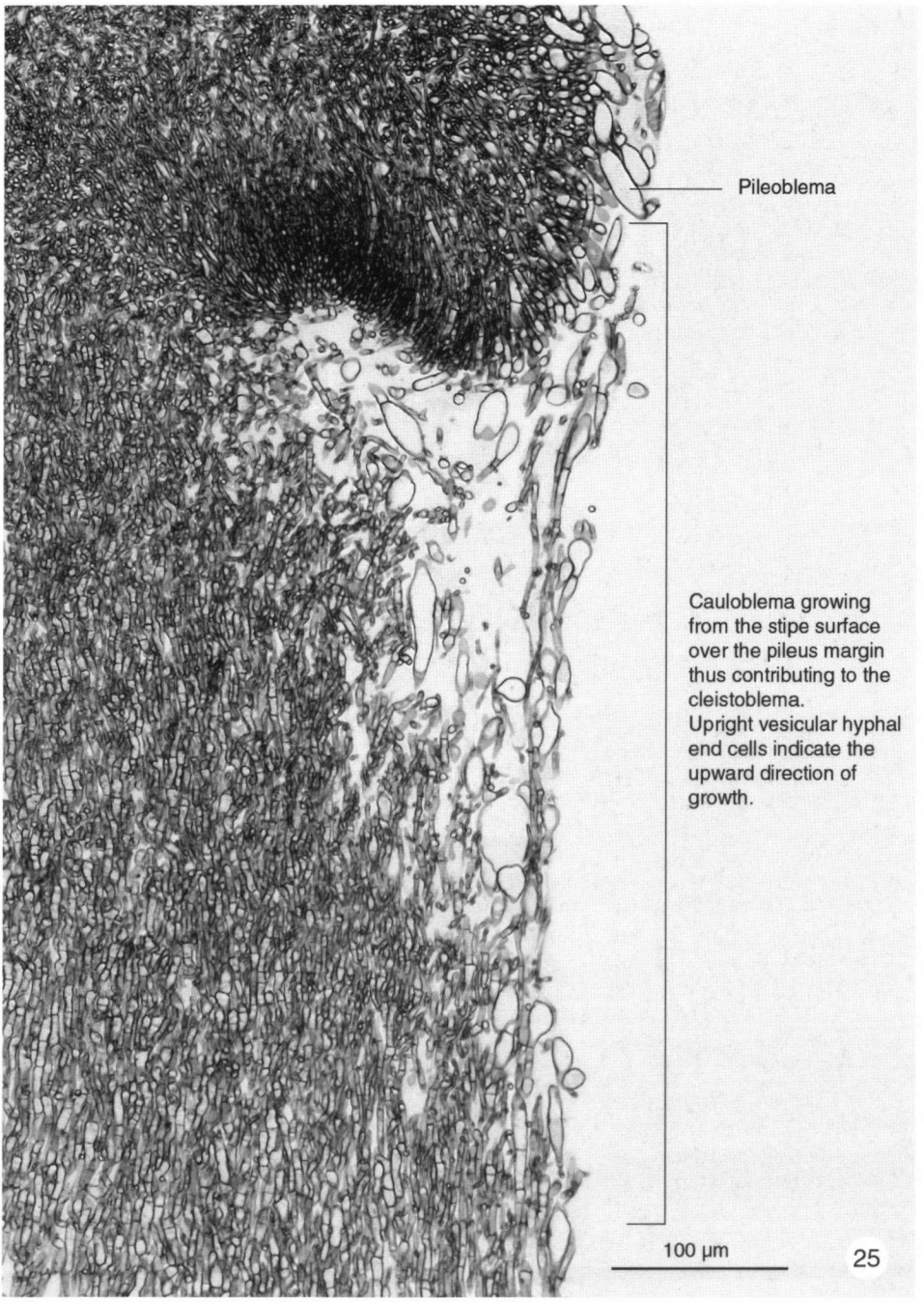


Fig. 25 (legend on p. 413).

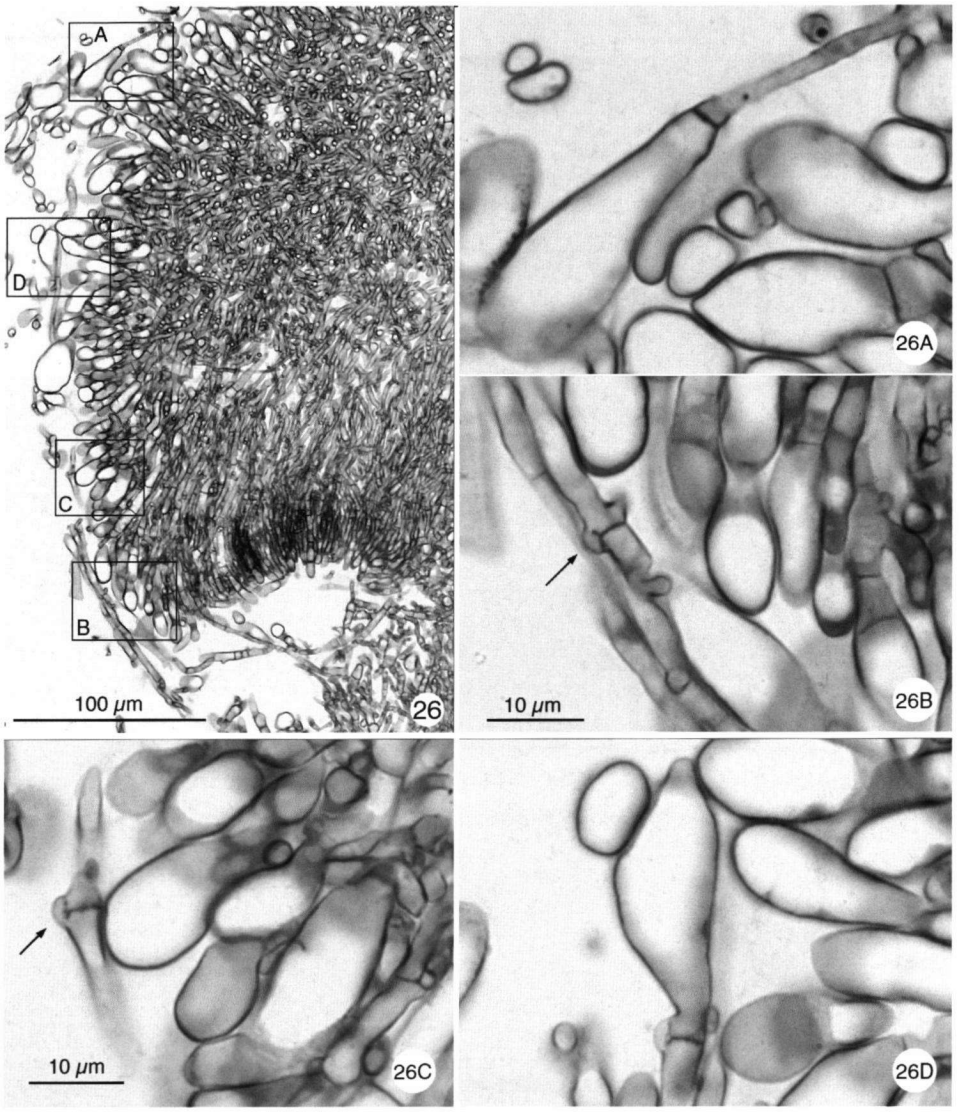
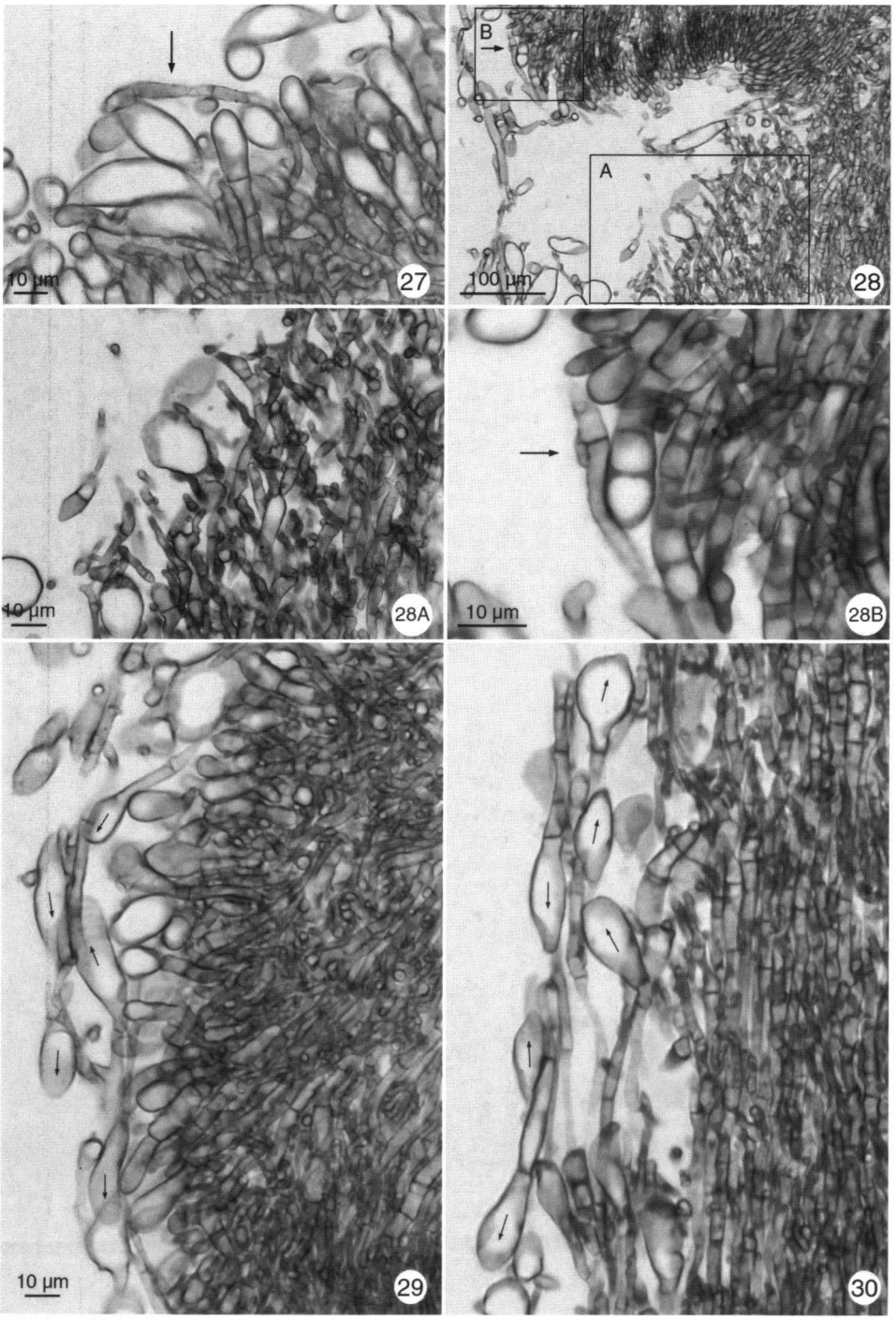
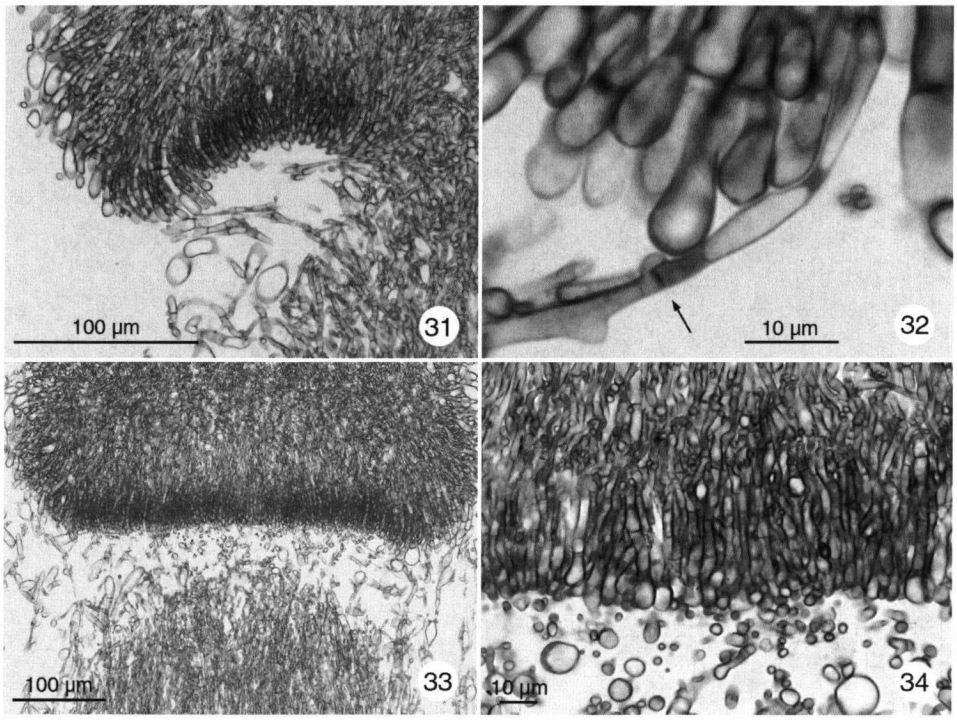


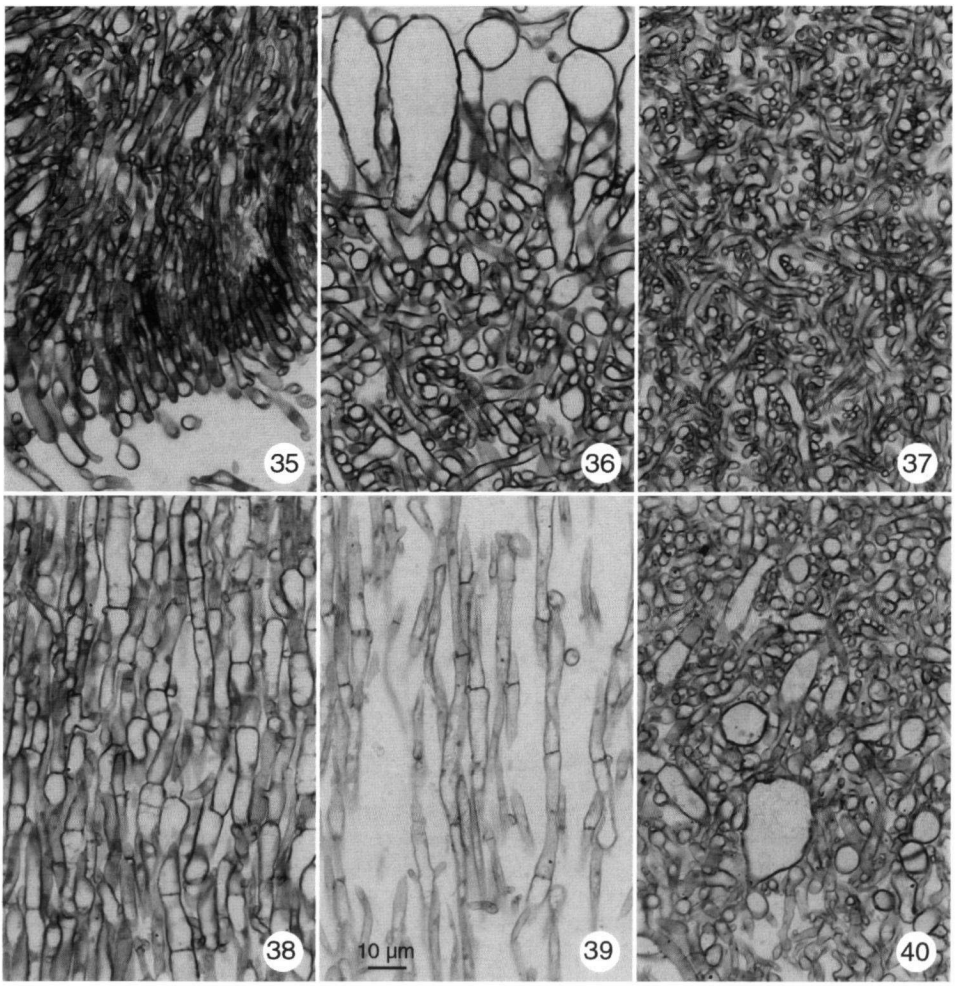
Fig. 26. *Simocybe sumptuosa,* developmental stage B. Analysis of the veil. — 26A & B. Pileoblema growing down, identified by the vesicular hyphal end cell and the orientation of a clamp-connection (arrow); 26C & D. cauloblema growing up, identified by the vesicular hyphal end cell and the orientation of a clamp-connection (arrow). — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.


10 µm
27
B
A
100 µm
28
10 µm
28A
10 µm
28B
10 µm
29
30

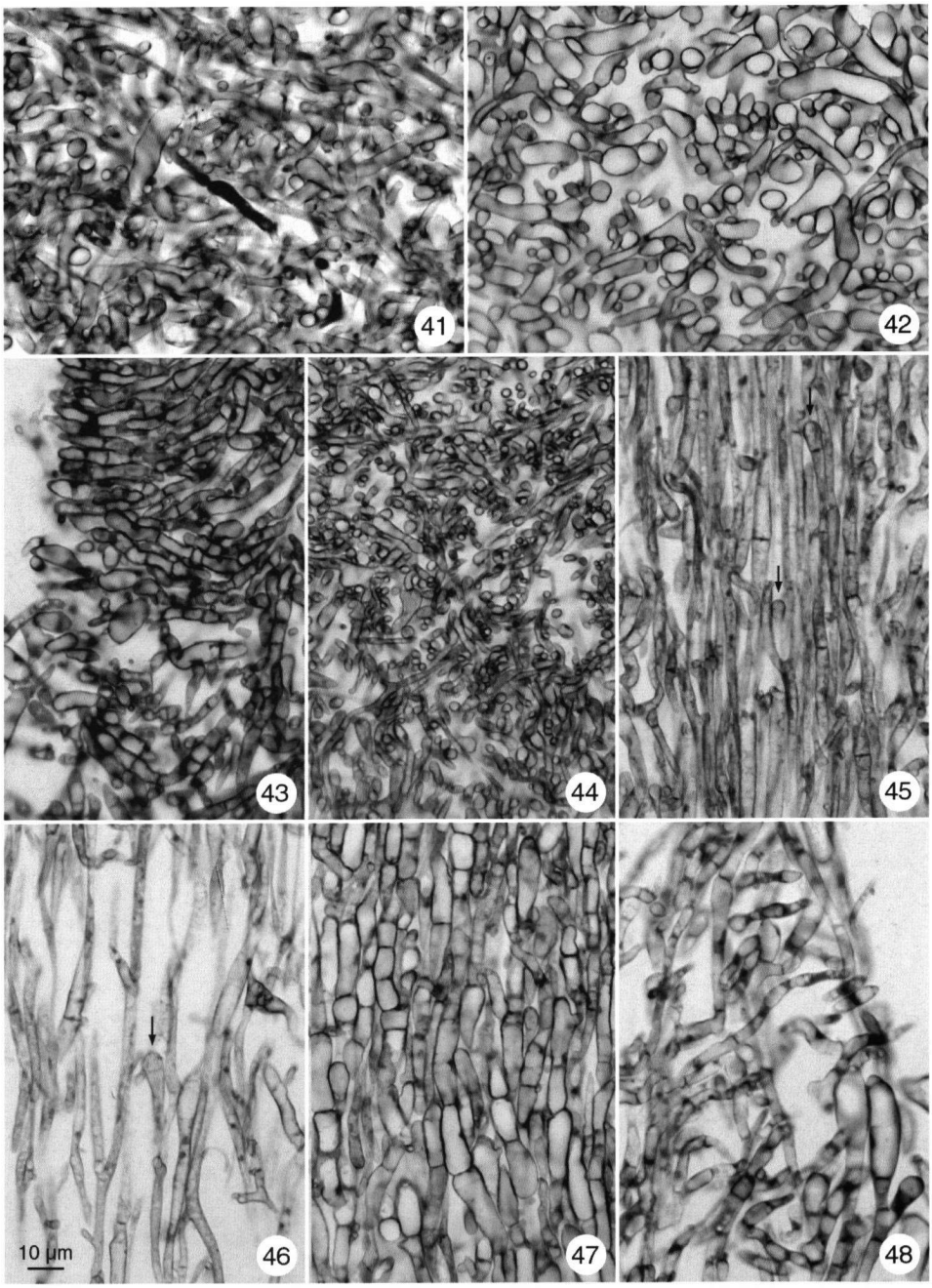


Figs. 31–34. *Simocybe sumptuosa,* developmental stage B. Anatomy of the prehymenial palisade and the pileus margin. — 31. The pileus margin is about 50 µm thick and differentiated from the prehymenial palisade by the longer hyphae that have grown beyond the level of the palisade. It contains one strikingly inflated hypha looking empty in this photograph. Elsewhere on the same primordium the pileus margin is not as nicely differentiated; 32. a hypha from the cauloblema penetrates between the hyphae of the right pileus margin. Its growth direction is indicated by the clamp-connection (arrow); 33 & 34. tangential sections through the pileus. The prehymenial palisade is still flat, lamellae have not yet begun to form. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

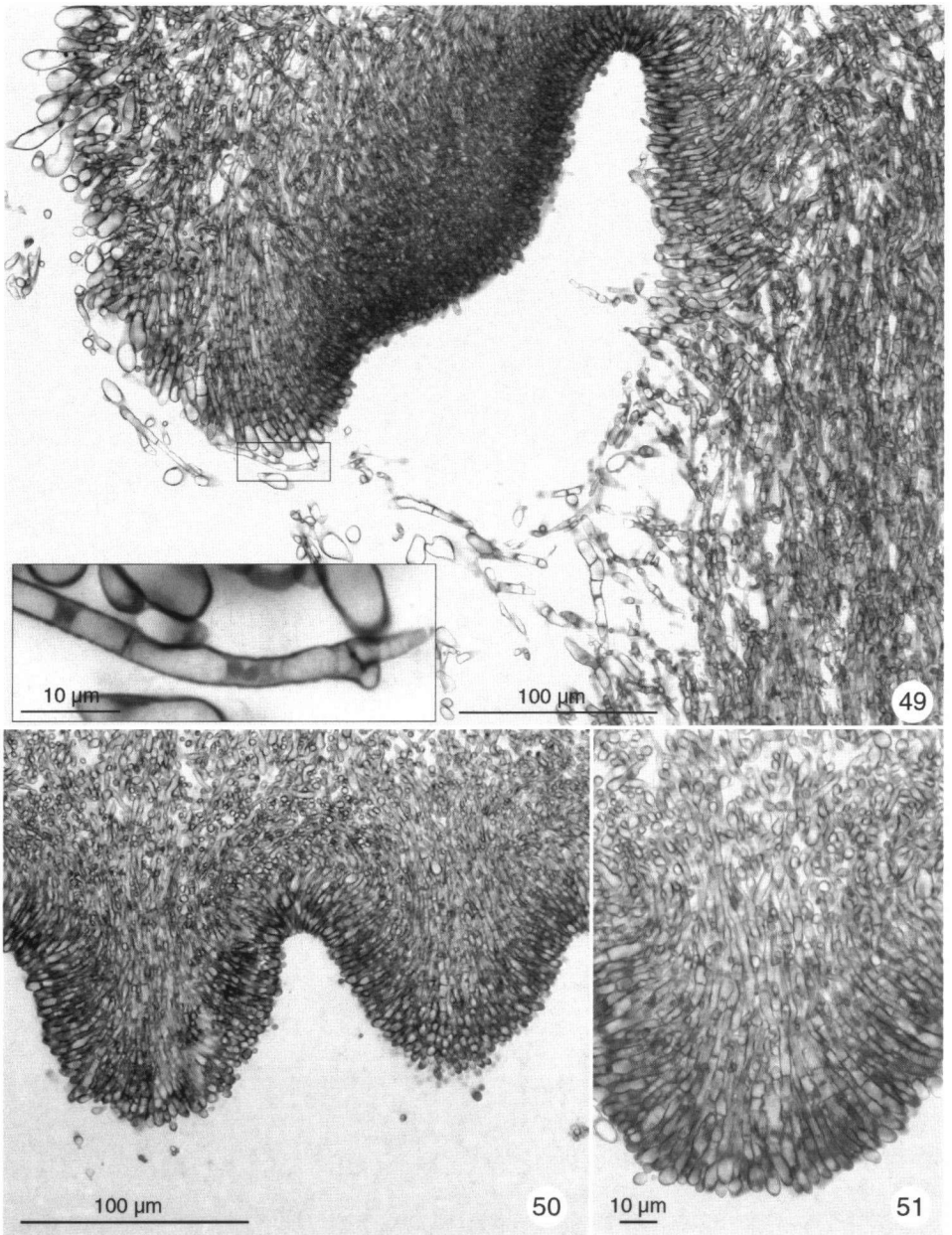
Figs. 27–30. *Simocybe sumptuosa,* developmental stage B. Analysis of the veil. — 27. A hypha of the outer pileoblema (arrow) grows out from the inner pileoblema; 28. cauloblema, general view for the next two figures; 28A. top of the stipe with many young hyphae of the cauloblema growing out from the stipe surface in the direction of the prehymenial palisade; 28B. a hypha of the cauloblema located on the pileus margin. The opening of the clamp-connection (arrow) indicates the upward growth direction; 29. pileoblema and cauloblema on the left side of the pileus. The arrows indicate the growth directions. Hyphae growing downward belong to the pileoblema, upward growing ones to the cauloblema; 30. pileoblema and cauloblema covering the left side of the stipe. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.



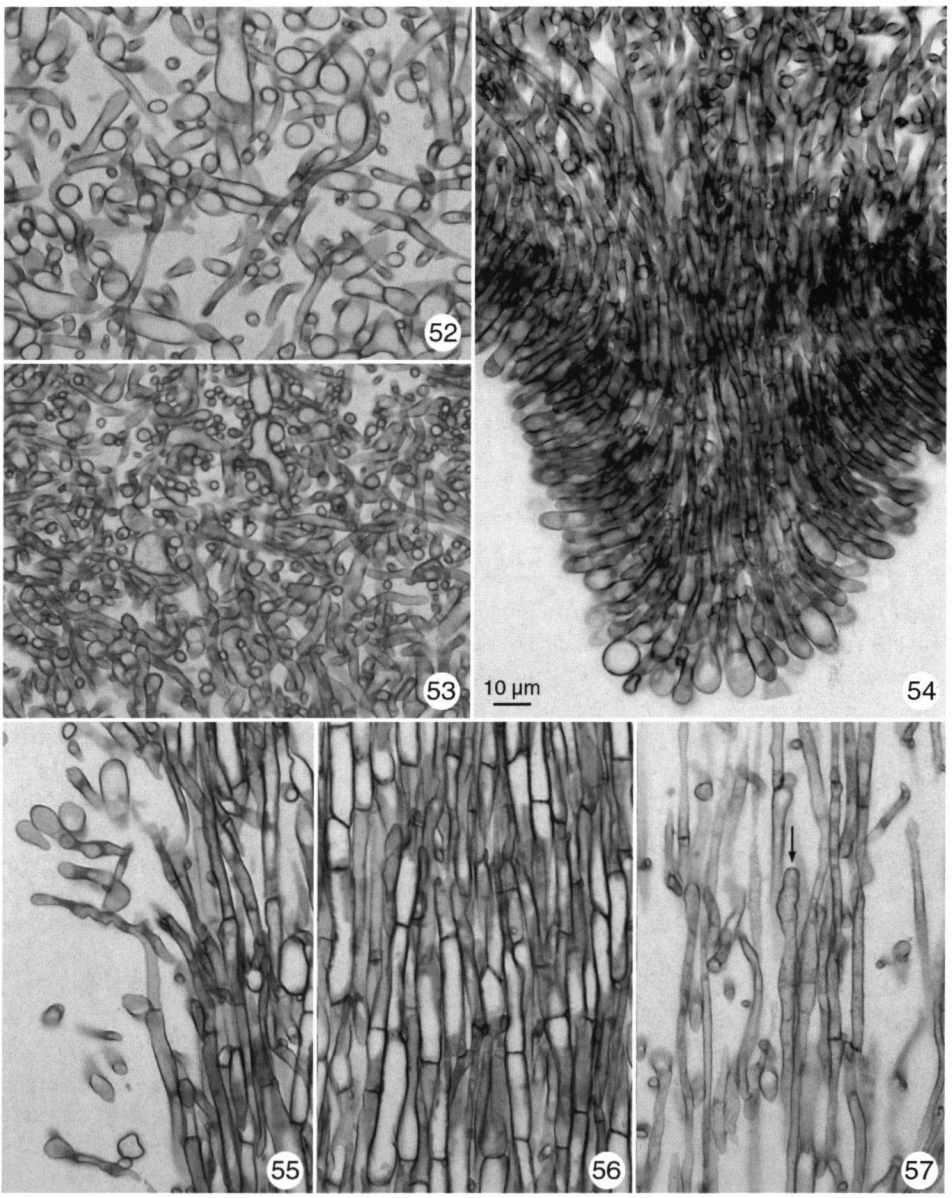
Figs. 35–40. *Simocybe sumptuosa,* developmental stage B. Beginning of the inflation of context hyphae by turgescence. — 35. Hyphae located over the left pileus margin and the prehymenial palisade. Only a few hyphal cells are inflated; 36. on top of the pileus under the pileoblema some hyphae are considerably enlarged; 37. in the centre of the pileus the context hyphae are significantly thinner than at the periphery of the pileus, but some are inflated; 38. inflated hyphae from the lateral part of the stipe; 39. some hyphae from the central pith of the stipe are slightly inflated, others are not. Some nuclei and dolipore-swellings are just visible in this photograph. The hyphal walls stain less intensely than the walls elsewhere in the primordium, maybe because they are thinner or because these hyphae do not belong to a mechanically supporting system (as compared to the hyphae of the lateral parts of the stipe); 40. basal part of the nodulus with strongly inflated hyphal cells. — The scale bar applies to all figures. Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.



Figs. 41–48 (legend on p. 413).



Figs. 49–51. *Simocybe sumptuosa,* developmental stage C. Formation of the gills. — 49. The scanty partial veil consists mostly of the hyphae of the cauloblema, but some hyphae of the pileoblema are also present making the partial veil an amphicleistoblema. The inset shows the region marked by the rectangle where a downward growing pileoblema hypha is located on the pileus margin. Please note the clamp-connection indicating the growth direction, the two nuclei and the dolipore swellings; 50 & 51. the first gills are covered with a continuous palisade. The gill trama is subregular and slightly diverging. — Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.



Figs. 52–57. *Simocybe sumptuosa*, developmental stage D. — 52 & 53. The contexts from the central part (52) and the basal part (53) of the pileus are slightly more dilated than in the previous stage (cf. Figs. 42, 44); 54. the gill edge is already differentiated from the future hymenium by the presence of inflated cells. The structure of the gill trama has not changed significantly; 55–57. stipe surface, lateral stratum (= the mechanically supporting structure) and medulla from the central region of the stipe. The arrow indicates a slightly inflated free hyphal end cell. — The scale bar applies to all figures. Aldehyde gas fixation, methacrylate, aluminium zirconium haematoxylin.

CONCLUSIONS AND DISCUSSION

Mycelial morphology is slightly changed during basidiome formation by an increase of hyphal density in the nutrient agar beneath the noduli, but special structures, such as dense masses of short cells filled with glycogen (e.g. in *Coprinus trisporus,* Clémençon, 1997: 846) are lacking.

Conidia and chlamydospores of *Simocybe* are described for the first time. The exact mechanism of conidiogenesis has not been discussed here. Therefore no attempt has been made to identify the anamorph state with a genus of the Deuteromycetes. The chlamydospores of *S. sumptuosa* are usually terminal aleuria, but some intercalary, irregularly fusiform chlamydospores are also formed. Clearly the two types are homologous variants of a single mitospore. Conidia and chlamydospores may occur in the same mitosporogenic heads, but the differentiating mechanism is unknown.

In her monograph of the genus *Simocybe* Senn-Irlet (1995) wrote that a veil is lacking or could never be seen in the material she studied. This may be true for mature basidiomes, but as Reijnders (1963) showed for *S. centunculus,* a spurious veil is present in very young fruit-bodies of this species. This is confirmed here for a species very close (if not identical) to the one studied by Reijnders. Furthermore, in *S. sumptuosa* the origin and development of the veil could be studied for the first time. This analysis shows clearly that the veil is not a residual outer part of a matrix (i.e. it is not a rudimentary innate veil) but a new formation, a metablema produced by the stipe and by the pileus. The cauloblema is independent from the pileoblema, but both meet and intermingle on the lateral pileus surface and on the upper stipe surface thus bridging the gap between the pileus margin and the stipe. The partial veil is therefore a composite structure and can be termed an amphicleistoblema. Since there is no matrix *S. sumptuosa* is exocarpic amphicleistoblemate (terminology of Clémençon, 1997), or it may be called mixangiocarpic (terminology of Reijnders, 1963). This is in strong contrast with the paravelangiocarpy proposed by Reijnders (1963: 82) for the similar *S. centunculus,* but this author could not study very young specimens, and he did not pay attention to the growth direction of the hyphae of the partial veil.

A metablema is not necessarily equivalent to a 'velum emanatum' (Reijnders, 1963), but it may also become a pileipellis, or it may result in two different organs, as illustrated by *S. sumptuosa,* since the inner pileoblema turns into a pileipellis while the outer part enters a cleistoblema.

Since the stipe initial (the erect shaft) originates from the central part of the nodulus the pileus initial could simply be the upper part of the nodulus that has been lifted on top of the shaft. The presence of the same vesicular cells on the pileus initial would point in the same direction. This 'nodulopileate' developmental type is certainly conceivable and may perhaps occur in nature, but it probably does not apply to *S. sumptuosa,* as the direct comparison of the structures of the nodulus and the pileus initial reveals differences in hyphal diameter and hyphal spacing (Figs. 22 & 23).

Figs. 19A–D suggest that after having formed the erect shaft the pileus is formed while the total length of the primordium does not increase significantly. Only after completion of the pileus formation (but without the gills) does growth continue. But since the developmental stages A–D are not from a single primordium, the suggested pattern must remain a hypothesis that should be tested using time lapse cinematography.

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