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Ascus structure and ascospore formation in the lichen-forming *Chaenotheca chrysocephala* (Caliciales)

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Abstract. – In the thin-walled pre-meiotic asci of *Chaenotheca chrysocephala* a peripheral ascospore delimiting membrane cylinder was formed by transformation of membrane material generated by the proliferating plasma membrane. The ascus wall broke in parts and was degraded soon after the onset of secondary ascospore wall formation. Numerous lysosomes were observed prior to ascus deliquescence. The ascospores grew and their secondary wall layer became melanized after their release into the mazaedium.

Introduction

The Caliciales are a group of mainly lichen-forming, rarely saprophytic or parasymbiotic ascomycetes with a particularly interesting mode of ascospore formation. Their prototunicate asci develop in stipitate or sessile, hemiangiocarpic ascomata which can be interpreted as apothecia. The relatively thin ascus wall disintegrates long before the ascospores have attained their full size, ornamentation and pigmentation. The final ripening of the ascospores occurs in the mazaedium, a powdery mass of spores intermixed with paraphyses and sometimes also with debris of dissolved asci, which covers the hymenial surface. From there the ascospores are dispersed by the wind or by insects or mites.

Prototunicate asci and a mazaedium are formed in two out of the three families of the Caliciales in their present delimitation (Hawksworth & al., 1983), namely in the Caliciaceae and in the exclusively lichenized Sphaerophoraceae. In the third family, the non-lichenized Mycocaliciaceae, unitunicate asci are formed in the stipitate ascomata. Their ascus reaches the hymenial surface at maturity and bursts with an irregular slit prior to ascospore release (Schmidt, 1970a; Samuels & Buchanan, 1983). The ascospores are ejected sufficiently far above the hymenial surface to allow their dispersal.

The development of ascogenous hyphae and asci was investigated on the light microscopical level in extensive comparative studies in numerous calicialean species by Schmidt (1970a, b). Ultrastructural studies on ascus structure and ascospore ornamentation have been carried out in the Mycocaliciaceae and Caliciaceae by

TIBELL (1975), SAMUELS & BUCHANAN (1983) and HONEGGER (1985), but no sequential studies on ascus development and ascospore formation are available. Due to the very small size of asci and ascospores in these families it is not possible to study all aspects of ascosporogenesis in light microscopic preparations. The present ultrastructural study aims to investigate ascus and ascospore development in *Chaenotheca chrysocephala,* a lichen-forming member of the Caliciaceae with circumboreal distribution (TIBELL, 1975). In symbiosis with *Trebouxia simplex* TSCHERMAK-WOESS (1978), its chlorococcalean phycobiont *Chaenotheca chrysocephala* forms bright yellow thalline crusts preferably on conifers and stipitate, strongly melanized podetia with terminal apothecium (Figs. 1–2).

Materials and Methods

Chaenotheca chrysocephala (TURN) Th. FR. was collected on *Abies alba* in the forest north of Ratenpass, Kanton Schwyz, central Switzerland; 1000 m alt.; March 1980.

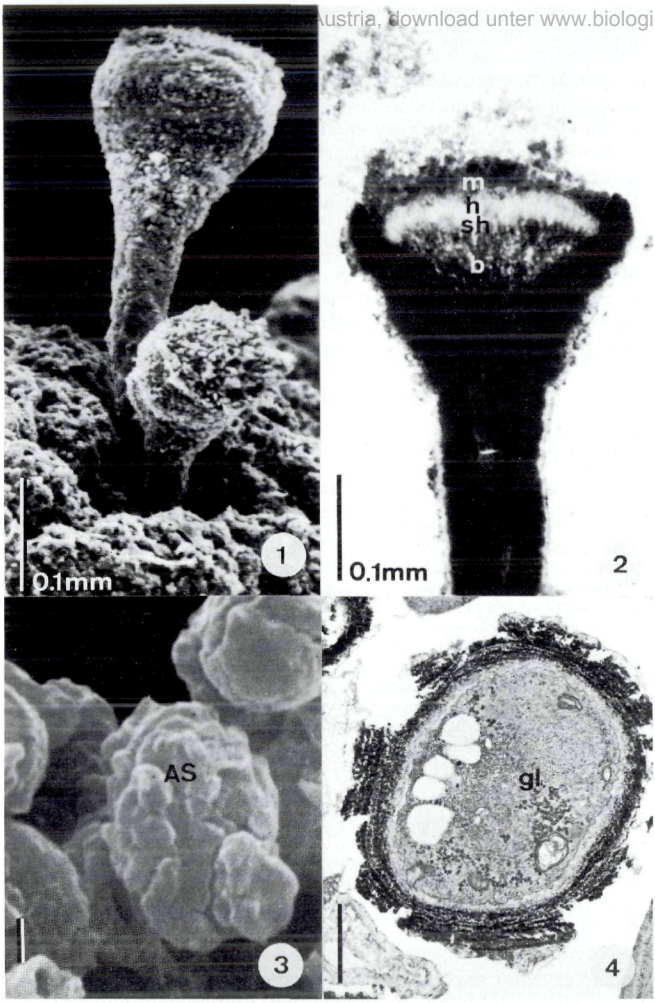
Light microscopy. – 12 µm thick serial sections were obtained with a Jung microtome in a Linde cryostat. Sections stained with lactophenol-cottonblue were examined in a Leitz M2 microscope.

Transmission electron microscopy. – The freshly collected ascomata were cut longitudinally in halves and fixed for 4 h in a mixture containing 1.5% acrolein and 1.25% glutaraldehyde in Soerensen's phosphate buffer, p. H. 7.1, at room temperature. In order to soak the specimens properly and let them sink into the fixing solution the material had to be thoroughly evacuated with a water aspirator at the beginning of the fixing process. In this preparative step which was essential for adequate specimen preservation part of the mazaedium disintegrated and was lost. After several washings in buffer the samples were postfixed in 2% buffered osmium tetroxide at room temperature overnight, then dehydrated in a graded series of acetone, infiltrated and finally embedded in the firm mixture of SPURR's low viscosity epoxy resin. Sectioning was carried out on a Reichert OM U3 ultramicrotome equipped with a diamond knife. Ultrathin sections stained with uranyl acetate and lead mixture according to SATO (1967) were examined in a Hitachi HS 8 electron microscope.

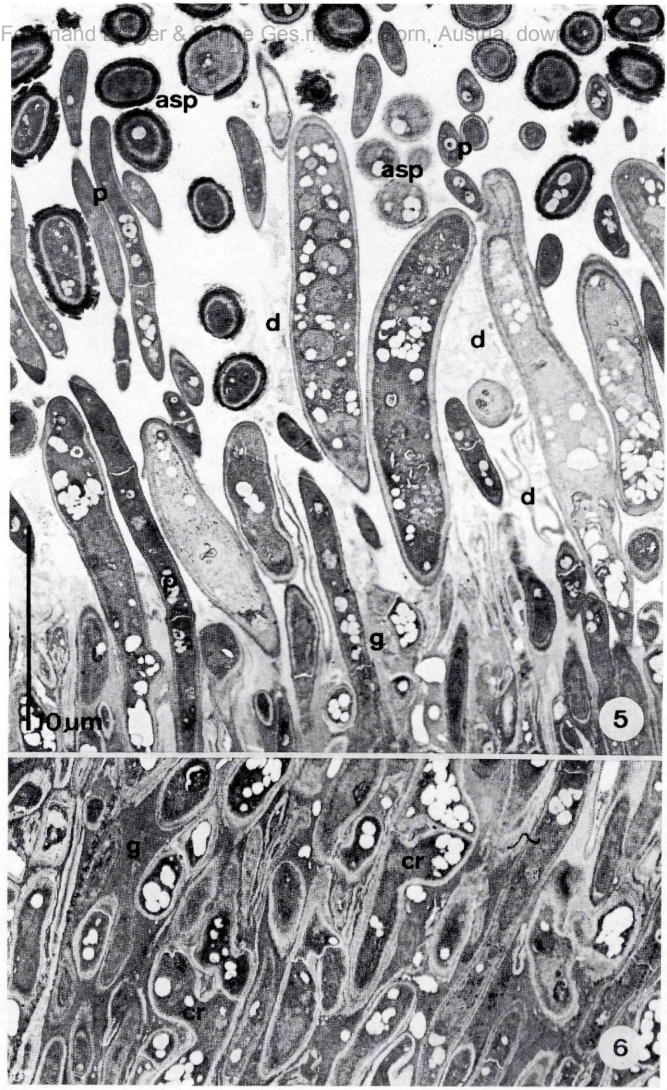
Scanning electron microscopy. – Freshly collected specimens were fixed in the vapour of a 4% solution of osmium tetroxide for 4 h at room temperature. After dehydration in a graded series of acetone and critical point drying the samples were mounted on specimen stubs, sputter-coated with a 80/20% alloy of gold and palladium, and examined in a Cambridge Stereoscan.

Results

In the cryostat sections of the ascomata the brown, pigmented basal layer, the unpigmented subhymenial and hymenial layers and the mazaedium could be distinguished (Fig. 2). Surprisingly the cottonblue did not enter either cell type. Neither in ascogenous hyphae or asci, nor in ripening or mature ascospores was the cytoplasm stained by aniline blue. The cytoplasm of hyphae and phycobiont cells of the crustose thallus, however, was intensely stained by cotton blue (not shown).



Figs. 1–4: 1. SEM preparation of two stipitate ascomata of *Chaenotheca chrysocephala* arising from the crustose epiphleodal thallus. × 180. – 2. Light micrograph of a cryostat section of an ascoma mounted in lactophenol-cottonblue. b, basal layer; h, hymenium; m, mazaedium; sh, subhymenial layer. The apothecial margin and the entire podetial complex are strongly melanized. × 180. – 3. SEM preparation of the surface of the mazaedium. AS, ascospore with coarse, irregular ornamentation. × 7'200. – 4. TEM preparation of an ascospore located at the mazaedial surface. gl, glycogen. × 13'600 (bar equals, if not otherwise stated, 1 µm).



Figs. 5–6: TEM preparations of the hymenial and subhymenial layers. Different developmental stages of asci and ascospores (asp) and debris of dissolved asci (d) are intermixed with paraphyses (p). Crozier-forming ascogenous hyphae (cr) and paraphyses are embedded in a subhymenial gelatine (g). × 3'500 (bar equals 1 µm).

The crozier-forming ascogenous hyphae and accompanying sterile elements were embedded in a gelatinous subhymenial matrix (Figs. 5–6). The hymenial layer itself contained no gelatinous material. All stages of ascus development and ascospore formation could be observed simultaneously. The wall of fully grown pre-meiotic asci was more or less equally thin from the basis to the apex (Fig. 5). The outer zone of the ascus wall was somewhat more electron opaque than the inner part (Fig. 14).

In fully grown premeiotic asci intense proliferations and invaginations of the plasma membrane were observed, leading to the formation of conspicuous deposits of membrane material in the cytoplasm (Fig. 15). These membrane pools generated the material for the peripheral membrane cylinder according to BECKETT (1981; "ascus vesicle" of the older literature; e. g. REEVES, 1967; GREENHALGH & GRIFFITHS, 1970). The peripheral membrane cylinder was built up during meiosis and was formed by transformation rather than by unfolding of the membrane deposits (Fig. 14). This process was almost completed at the end of meiosis (Fig. 14). Ascospore delimitation by the invaginating peripheral membrane cylinder around nucleate portions of cytoplasm occurred immediately after the post-meiotic nuclear division. Like in all other ascomycetes so far investigated the ascospore wall was formed between the pair of delimiting membranes, the inner becoming the plasma membrane, the outer serving as the investing membrane until completion of ascospore wall formation (Figs. 16–21).

As soon as two ascospore wall layers, an inner, more or less completed primary and a still developing secondary one could be distinguished the epiplasm of the ascus started to disintegrate (Figs. 8–9, 13). The electron-transparent vacuoles of the epiplasm seemed to play an important rôle in ascus wall degradation. In the immediate vicinity of these vacuoles which are interpreted as lysosomes according to WILSON et al. (1970), the ascus wall disintegrated first and broke in parts (Fig. 11, 17–18). Another type of vacuolar or vesicular organelle was observed in close connection with the investing membrane of the ascospores (Figs. 8–9, 17–19). It remains unclear what their function is, but it is imaginable that these

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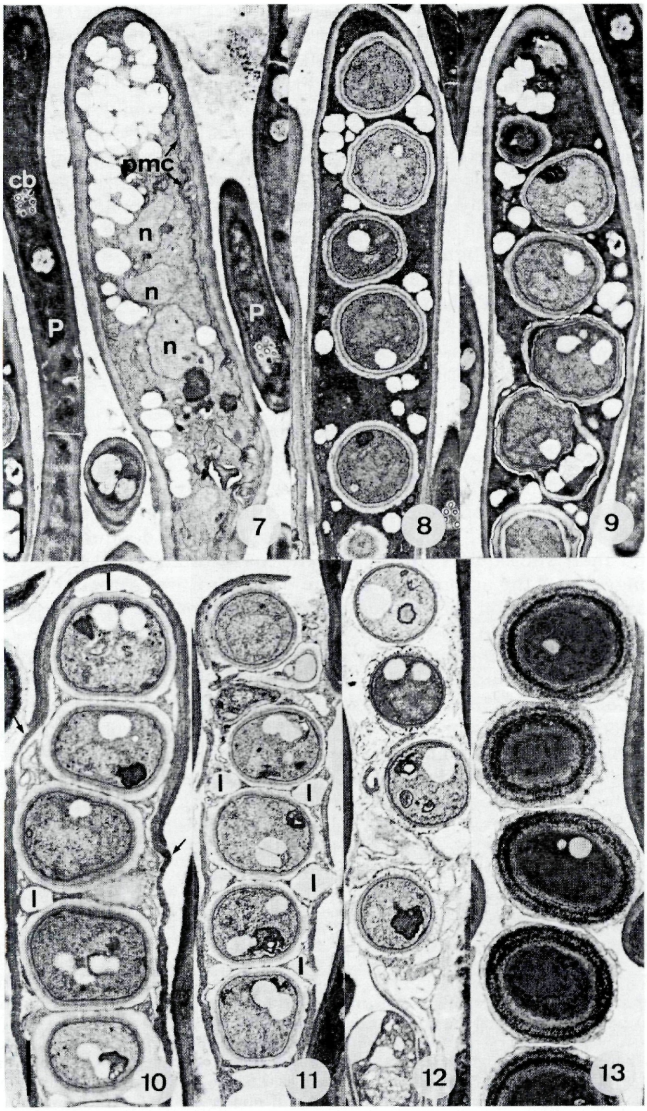
Figs. 7–13: TEM preparations of successive stages of ascospore formation and ascus deliquescence. – 7. Post-meiotic ascus; 3 out of 4 nuclei (n) and part of the peripheral membrane cylinder (pmc) are visible. p, paraphyses with clusters of concentric bodies (cb). – 8–9. Onset of ascus degradation. – 10. Ascus epiplasm nearly dissolved and ascus wall locally disintegrated (arrows) l, lysosomes. – 11. Further stage of ascus wall degradation, best visible in the vicinity of the numerous lysosomes (l). – 12. Ascus wall largely dissolved and onset of melanization of the secondary ascospore wall. – 13. Mature ascospores with fully melanized secondary cell wall and dense cytoplasm. – Figs. 7–9, 11–13: × 7'900; Fig. 10: × 9'600 (bars equal 1 μm).



organelles contain hydrolases and/or phosphatases for the degradation of the epiplasm. As soon as the ascus wall was partly disintegrated the secondary ascospore wall swelled and so did the vesicular elements which were closely associated with the investing membrane (Figs. 18–20). Debris of the ascus wall and cytoplasm were surrounding the ripening ascospores, but quite soon this material was completely degraded (Figs. 12–13, 18–20). Melanization of the secondary wall layer of the ascospores started soon after their liberation from the ascus (Fig. 19). During the ripening process the ascospores grew slightly (Figs.. 4, 20–21).

Fully melanized mature ascospores in the lower part of the mazaedium revealed a very dense cytoplasm and a relatively smooth wall surface (Figs. 13, 21). The typical coarse, irregular ornamentation was seen in those ascospores which were lying on or near the mazaedial surface (Figs. 3–4).

Discussion

Mazaedium formation is not restricted to the Caliciales, but occurs also in the Gymnoascales (HAWKSWORTH al., 1983). However, the peculiarites of calicialean mazaedium-bearing ascomata are first, the release of immature ascospores from the deliquescing asci and their subsequent ripening in the mazaedium, and second, the longevity of the ascomata. The cytological features associated with ascus degradation and deliquescence in *Chaenotheca chrysocephala* are comparable to the autolytic processes observed in the deliquescing asci of *Chaetomium brasiliense* (ROSING, 1984) or in the fruiting bodies of *Coprinus lagopus* (ITEN & MATILE, 1970). In senescing gills of *Coprinus lagopus* all cells except the basidiospores simultaneosly undergo autolysis due to the release of lysosomal hydrolases (ITEN & MATILE; 1970). The same seems to happen to the asci of *Chaetomium brasiliense* where acid phosphatase was localized with cytochemical methods in epiplasmic vacuoles and in association with the walls of mature ascospores (ROSING, 1984). In the longlived ascomata of *Chaenotheca chrysocephala* and *Coniocybe furfuracea* (HONEGGER, 1985), however, all stages of autolysis are found in immediate vicinity of growing and developing asci. Obviously the walls and cytoplasm of developing asci, paraphyses and ripening and mature ascospores remain unaffected by the lysosomal activities of neigh-

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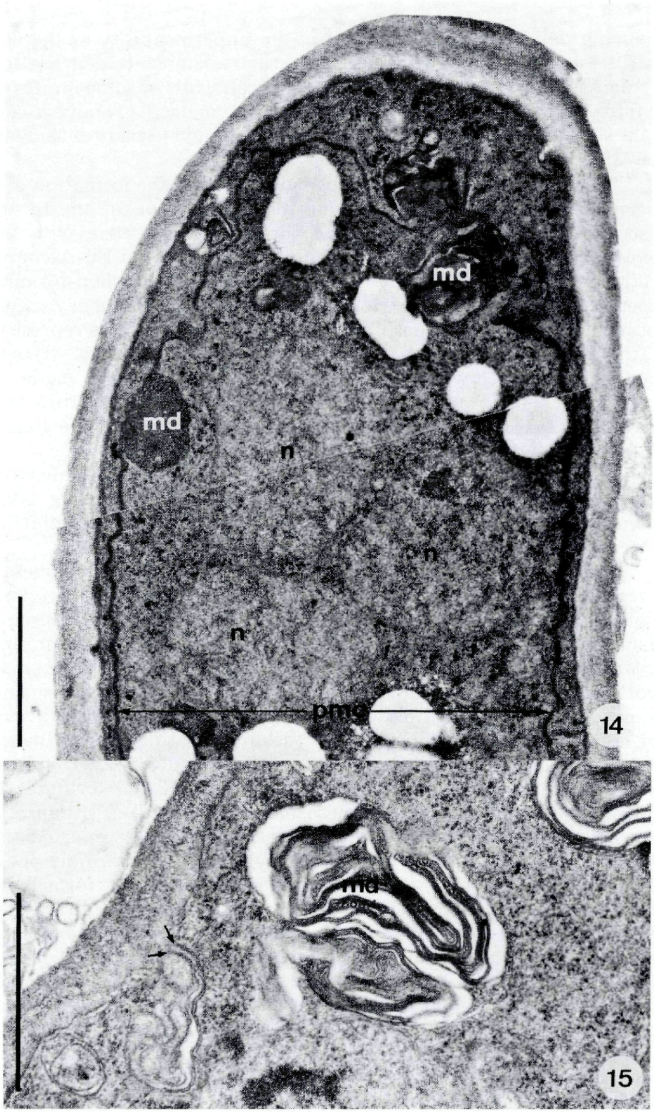
Figs. 14–15: TEM preparations of peripheral membrane cylinder formation. – 14. apical part of a post-meiotic ascus (3 out of 4 nuclei are visible) with not yet fully completed peripheral membrane cylinder (pmc). md. plasma membrane-derived membrane deposits which generate the material for the pmc by transformation. × 25'700. – 15. Part of a pre-meiotic ascus with locally invaginating plasma membrane (arrows) and prominent membrane deposits (md). × 34'000 (bar equals 1 µm).

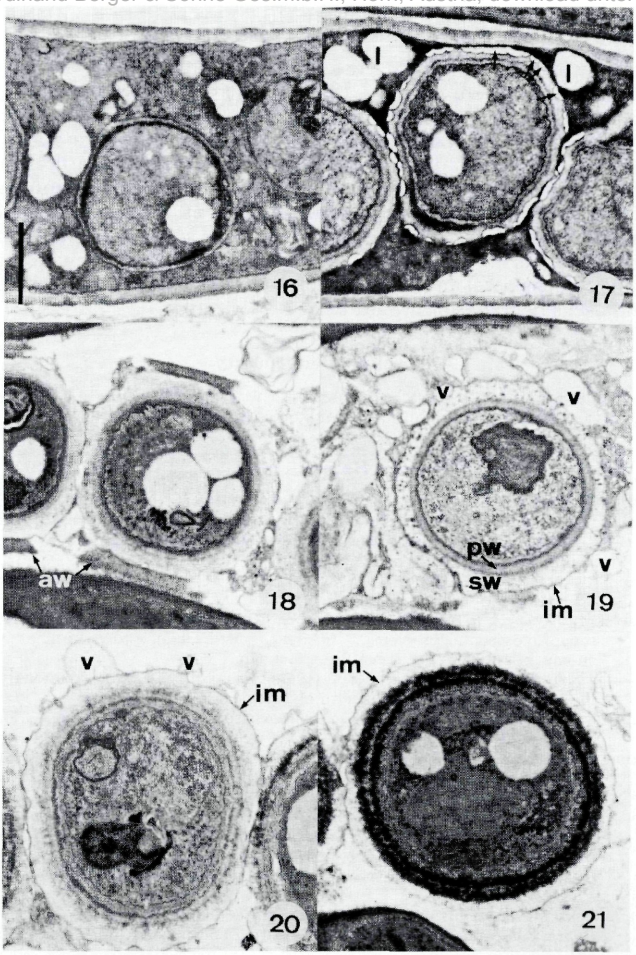


bouring deliquescing asci. Whether the impermeability of the cell walls for aniline blue correlates with the resistance to degradation remains to be investigated. The peculiar cell walls of some groups of chlorococcalean algae are impermeable for molecules above a certain size, aniline blue with mol. wt. 735 included (SYRETT & THOMAS, 1973).

The early stages of ascosporogenesis, mainly the formation of a peripheral membrane cylinder for the delimitation of the future ascospores are similar in most of the Eu-Ascomycetes. With the exception of the Tuberales all taxa of non-lichenized Eu-Ascomycetes (reviewed by BECKETT, 1981) and also the few lichen-forming species so far investigated (HONEGGER, 1982) form a peripheral membrane cylinder and so does *Chaenotheca chrysocephala,* a representative of an order which "crosses biological boundaries" (HAWKSWORTH, 1978). However, differences are noted in the mode of peripheral membrane cylinder formation within the different groups of Ascomycetes. The invaginating plasma membrane generates the material for the peripheral membrane cylinder in members of the Gymnoascales, Sordariales, Sphaeriales and Phacidiales (reviewed by BECKETT, 1981), but also in some lichen-forming taxa, namely in members of the Peltigerales and in the helotialean *Baeomyces rufus* (HONEGGER, 1982; 1983).

It may be particularly interesting to compare the early stages of ascosporogenesis in *Chaenotheca chrysocephala* with non-lichenized taxa which form either a mazaedium, or deliquescing asci. GIL (1973) investigated the origin of the peripheral membrane cylinder in *Ajellomyces dermatitidis* and *Arthroderma benhamiae,* both members of the Gymnoascales which bear unitunicate asci and a mazaedium in non-ostiolate ascomata. He observed accumulations of membrane material formed by the invaginating plasma membrane in pre-meiotic asci and compared these structures with fungal and bacterial mesosomes. The peripheral membrane cylinder is formed by unfolding of these mesosomes (GIL, 1973). In *Nannizzia gypsea* (another gymnoascalean species) HILL (1975) reported the formation of a peripheral membrane cylinder by membrane pools which he termed myelin figures and supposed to be formed by convolutions of the nuclear envelope. The plasma membrane-derived membrane pools observed in pre-meiotic asci of *Chaenotheca chrysocephala* structurally ressemble both the mesosomes according to GIL (1973) and the myelin figures according to HILL (1975). As neither term is cytologically satisfactory they were not used in the present study.

In the unitunicate asci of the sordarialean *Chaetomium brasiliense* which develop in ostiolate ascomata and are simultaneously deliquescing at maturity the peripheral membrane cylinder is



Figs. 16–21: TEM preparations of successive stages of ascospore maturation. – 16. Ascospore immediately after delimitation at the onset of wall formation. – 17. 2-layered ascospore wall with electron-transparent vesicular organelles (arrows) associated with the investing membrane. l, lysosomes in the ascal epiplasm. – 18. Ascospore after the rupture of the wall of the deliquescing ascus (as). – 19. Onset of melanization of the secondary ascospore wall (sw). Vesicular organelles (v) in contact with the investing membrane (im) and the disintegrating remains of the ascus. pw, primary ascospore wall. – 20. During differentiation of the secondary wall layer the ascospore attains its final size and shape. im, investing membrane with closely associated vesicular organelles (v). – 21. Mature ascospore with fully melanized secondary wall layer and a dense cytoplasm. The investing membrane (im) is still present. – Figs. 16–21: × 14'000 (bar equals 1 μm).

formed like in all Sordariales so far investigated (reviewed by BECKETT, 1981), directly by the invaginating plasma membrane (ROSING, 1982).

As stated by BECKETT (1981) the differences in the early stages of ascosporogenesis may prove useful for corroborating natural taxonomic groupings. From this point of view it would be most interesting to obtain more data on ascosporogenesis in other Caliciales, mainly in the Mycocaliciaceae which differ in so many respects from the neighbouring families. In addition to ultrastructural investigations cytochemical and biochemical studies on ascospore maturation and ascus degradation would be most desirable.

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References

BECKETT, A. (1981). Ascospore formation. – In: TURIAN, G. HOHL, H. R. (eds). The fungal spore. Morphogenesis and control. p. 107–129. Academic Press, London.

GIL, F. (1973). Mesosomes: their role in the delimitation of the ascospore. – Mycopath. Mycol. appl. 49(4): 243–247.

GREENHALGH, G. N. & GRIFFITHS, H. B. (1970). The ascus vesicle. – Trans. Br. mycol. Soc. 54(3): 489–493.

HAWKSWORTH, D. L. (1978). The taxonomy of lichen-forming fungi: reflections on some fundamental problems. – In: STREET, H. F. (ed.). Essays in plant taxonomy. p. 211–243. Academic Press, London.

— , SUTTON, B. C. & AINSWORTH, G. C. (1983). Ainsworth and Bisby's dictionary of the fungi. – 7th ed. Commonwealth Mycological Institute, Kew.

HILL, T. W. (1975). Ultrastructure of ascosporogenesis in *Nannizzia gypsea*. – J. Bacteriol. 122(2): 743–748.

HONEGGER, R. (1982). Ascus structure and function, ascospore delimitation, and phycobiont cell wall types associated with the Lecanorales (lichenized ascomycetes). – J. Hattori bot. lab. 52: 417–429.

— (1983). The ascus apex in lichenized fungi. IV. *Baeomyces* and *Icmadophila* in comparison with *Cladonia* (Lecanorales) and the nonlichenized *Leotia* (Helotiales). – Lichenologist 15(1): 57–71.

— (1985). The hyphomycetous anamorph of *Coniocybe furfuracea*. – Lichenologist 17(3): 273–279.

ITEN, W. & MATILE, P. (1970). Role of chitinase and other lysosomal enzymes of *Coprinus lagopus* in the autolysis of fruiting bodies. – J. Gen. Microbiol. 61: 301–309.

REEVES, F. (1967). The fine structure of ascospore formation in *Pyronema domesticum*. – Mycologia 59: 1018–1033.

ROSING, W. C. (1982). Ultrastructure of ascus and ascospore development in *Chaetomium brasiliense*. – Mycologia 74(6): 960–974.

ROSING, W. C. (1984). Ultracytochemical localization of acid phosphatase within deliquescing asci of *Chaetomium brasiliense*. – Mycologia 76(1): 67–73.

SAMUELS, G. J. & BUCHANAN, D. E. (1983). Ascomycetes of New Zealand. 5. *Mycocalicium schefflerae* sp. nov., its ascal ultrastructure and *Phialophora* anamorph. – New Zealand J. Bot. 21: 163–170.

SATO, T. (1967). A modified method for lead staining of thin sections. – J. Electron Microsc. 16: 133.

SCHMIDT, A. (1970a) Ascustypen in der Familie der Caliciaceae (Ordnung Caliciales). – Ber. Dtsch. bot. Ges., N. F. 4: 127–137.

— (1970b). Anatomisch-taxonomische Untersuchungen an europaeischen Arten der Flechtenfamilie Caliciaceae. – Mitt. Staatsinst. Allg. Bot. Hamburg 13: 111–166.

SYRETT, P. J. & THOMAS, E. M. (1973). The assay of nitrate reductase in whole cells of *Chlorella:* strain differences and the effect of cell walls. – New Phytol. 72: 1307–1310.

TIBELL, L. (1975). The Caliciales of boreal North America. Taxonomy, ecological and distributional comparisons with Europe, and ultrastructural investigations in some species. – Symb. Bot. Upsal. 21(2): 1–128.

TSCHERMAK-WOESS, E. (1978). Ueber die Phycobionten der Sektion *Cystophora* von *Chaenotheca,* insbesondere *Dictyochloropsis splendida* und *Trebouxia simplex* spec. nov. – Pl. Syst. Evol. 129: 185–208.

WILSON, C. L., STIERS, D. L. & SMITH, G. G. (1970). Fungal lysosomes and sphaerosomes. – Phytopathol. 60: 216–227.