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ULTRASTRUCTURE OF THE ASCOSPORE WALL IN PEZIZALES (ASCOMYCETES)—III

Otideaceae and Pezizaceae

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(With Plates 34–45)

The development of wall layers and ornamentation of ascospores is studied with the electron microscope in members of the Otideaceae and Pezizaceae. Primary wall, endospore, and epispore develop in the same way as in *Ascodesmis* and the Pyronemataceae; the development of the secondary wall and the formation of the patterns of ornamentation resemble that in the Pyronemataceae. Special attention is paid to specialized plasmic structures.

INTRODUCTION

In earlier papers (Merkus, 1973, 1974) I reported my electron microscopy of the ultrastructure of the ascospore wall in Pezizales and discussed the results on *Ascodesmis* and the Pyronemataceae sensu Eckblad (1968).

The present paper represents a continuation of this study and gives the results in species belonging to the Otideaceae and Pezizaceae, which are practically identical with the Otideae and Aleurieae of the Aleuriaceae of Le Gal (1947: 284).

REVIEW OF EARLIER WORK

Like in my paper on the Pyronemataceae (Merkus, 1974), a brief review is given of Le Gal's light microscopy of the ornamentation patterns of the ascospores of the Pezizaceae (1947). Though this involves duplication of some of the data, it gives a better overall insight and makes it easier to interpret new results. According to the rules of the international code of botanical nomenclature some of the names used by Le Gal (see footnotes) had to be changed.

The species of Pezizaceae studied by Le Gal develop simple spore ornamentation. In all the species a primary wall arises and ornamentation on it is present; the ornamentation consists of callose and pectine formations and is of sporal origin.

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The primary wall is covered by an "assise sous-périsporique" and a "pellicule membranaire". The "assise sous-périsporique" is formed before ornamentation develops. The "pellicule membranaire" is termed "coque interpérisporique" if it is formed at the same time as the ornamentation and is penetrated by the substance of the ornamentation; the "coque interpérisporique" and the substance of the ornamentation both grow into one, the "coque interpérisporique" also consisting of callose and pectine. The "pellicule membranaire" is termed "tunique externe de l'assise" if it is formed before ornamentation develops and is not penetrated by the substance of the ornamentation.

Pulparia persoonii (Crouan) Korf & *al.* apud Korf[1] develops simple ornamentation that is formed between the primary wall and its covering layers.

Peziza succosa Berk.,[2] *P. badia* Pers. per Mérat,[3] *P. echinospora* P. Karst.,[4] *P. trachycarpa* Curr.,[5] *P. apiculata* Cooke,[6] and *P. reperta* (Boud.) Moser[7] develop simple ornamentation, the substance of which penetrates the covering layers of the primary wall and is deposited on the "coque interpérisporique". During the development of the ornamentation a "périspore" is present on the outside of the ascospores. In the first four species the "périspore" disappears in a later stage; in the other two species it remains.

Le Gal does not give a description of the ornamentation patterns of the ascospores of the Otideaceae.

Though the ascoplasm is not the main subject of the present study special attention is paid to it. It reveals the development of globular structures, like oil bodies and other specialized globules of a still unknown substance; these were also described by Guilliermond (1904, 1910, 1920), the latter as "corpuscules métachromatiques". The development of the structures and their relation to ascospore development is discussed.

MATERIALS AND METHODS

The material of the species in the present study was collected in the Netherlands, in France, and in Germany; the following list gives some data about the specimens and their localities: *Otidea alutacea* (Pers. per S. F. Gray) Mass. – *van Brummelen 73-501*, on needles of *Picea*, "forêt de Liciat", Oyonnax, Ain, France, 7.X.1973 (L); — *Piepenbroek 837*, on soil under *Quercus*, 't Joppe, Gorssel, Gelderland, The Netherlands, 12.X.1974 (L); *O. bufonia* (Pers.) Boud. — *van Brummelen 4073*, on the ground under *Betula*, Schoorl, North Holland, The Netherlands, 3.IX.1973 (L); —

1 *Plicaria persoonii* (Crouan) Boud.; syn. *Marcelleina persoonii* (Crouan) Brumm.
2 *Galactinia succosa* (Berk.) Sacc.
3 *Galactinia badia* (Pers. per Mérat) Boud.
4 *Aleuria umbrina* (Boud. apud Cooke) Gill.; not *Peziza umbrina* Pers.
5 *Plicaria trachycarpa* (Curr.) Boud.
6 *Aleuria apiculata* (Cooke) Boud.
7 *Aleuria reperta* (Boud.) Boud.

Piepenbroek 802, on soil, among grasses under Quercus, 't Joppe, Gorssel, Gelderland, The Netherlands, 28.VII.1974 (L); *O. onotica* (Pers. per S. F. Gray) Fckl. — *van Brummelen 4638*, on the ground under oaks, Koningshof, Overveen, Bloemendaal, North Holland, The Netherlands, 2.XI.1974 (L); *Peziza ammophila* Dur. & Lév. apud Dur. — collected during a field trip of the Dutch Mycological Society, sandy dunes, Hollumerduinen, Ameland, Friesland, The Netherlands, 27.X.1973 (L); *P. ampliata* Pers. per Pers. — *van Brummelen 4074*, on dead culms of *Phragmites*, Nederhorst den Berg, North Holland, The Netherlands, 12.V.1973 (L); *P. badia* Pers. per Mérat — *Piepenbroek 806*, on sandy soil, near estate "Dorth", Gorssel, Gelderland, The Netherlands, 28.VII.1974 (L); — *Piepenbroek 811*, on sandy soil, estate "Ampsen", Lochem, Gelderland, The Netherlands, 10.VIII.1974 (L); *P. badiofusca* (Boud.) Dennis — *Piepenbroek 746*, on clay soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 23.VI:1974 (L); *P. emileia* Cooke — *Piepenbroek 556*, on burnt soil, "de Bannink", Colmschate, Overijsel, The Netherlands, 3.VI.1973 (L); *P. michelii* (Boud.) Dennis — *van Brummelen 4500*, on sandy soil, Waterdijk, Diepenveen, Overijsel, The Netherlands, 15.VII.1974 (L); *P. petersii* Berk. — *Siteur*, on burnt soil, Eindhoven, North Brabant, The Netherlands, 21.VII. 1974 (L); *P. plebeia* (Le Gal) Nannf. in Lundell & Nannfeldt — *Piepenbroek 773*, on sandy soil, Epserbos, Epse, Gelderland, The Netherlands, 21.VII.1974 (L); *P. praetervisa* Bres. — *van Brummelen 4072*, on burnt soil, 't Joppe, Gorssel, Gelderland, The Netherlands, 6.VIII.1973 (L); — *Piepenbroek 794*, on burnt soil, "Klein Noordijk", Wilp, Voorst, Gelderland, The Netherlands, 27.VII.1974 (L); *P. succosa* Berk. — *van Brummelen 4511*, on soil under *Salix*, Hengforder Waarden between Olst and Wijhe, Overijsel, The Netherlands, 15.VII.1974 (L); *P. succosella* Le Gal & Romagn. — *van Brummelen & Piepenbroek 4510*, on soil under *Salix*, Waterdijk, Diepenveen, Overijsel, The Netherlands, 15.VII.1974 (L); *P. trachycarpa* Curr. — *Piepenbroek 807*, on soil among mosses, near estate "Dorth", Gorssel, Gelderland, The Netherlands, 28.VII.1974 (L); *P. vesiculosa* Bull. per St-Am. — *van Brummelen 4080*, on soil in hot-house, Kortenhoef, North Holland, The Netherlands, 28.I.1974 (L); *Pulparia persoonii* Korf & *al.* apud Korf — *Piepenbroek 575b*, on loamy soil, Duursche Waarden near Fortmond, Overijsel, The Netherlands, 19.VIII.1973 (L); — *Piepenbroek 738*, on loamy soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 23.VI.1974 (L); *van Brummelen 4515*, on loamy soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 15.VII.1974 (L); *Pustularia cupularis* (L. per Fr.) Fckl. — *Piepenbroek 772*, on sandy soil, Epserbos, Epse, Gelderland, The Netherlands, 21.VII.1974 (L); *Sowerbyella radiculata* (Sow. per Fr.) Nannf. — *Haas*, under *Picea*, Kreis Heidenheim, Schwäbisch Alb, Germany, 4.X.1973 (L); — from exposition, Oyonnax, Ain, France, 22.X.1973 (L).

After collection from their substratum in the field, the apothecia were placed in the fixative. Further treatment of the apothecia followed much the same procedure as described in former studies but a few additional remarks are called for.

In addition to the former percentages used, 1% $KMnO_4$ or 1% glutaraldehyde was applied as primary fixative before postfixation with OsO_4 to improve results.

In order to solve the problem of inadequate impregnation of the embedding medium in the relatively hard material, in some cases the usual Epon embedding medium according to Luft (1961) was replaced by a low-viscosity embedding medium according to Spurr (1961), with the additive dibutyl phtalate (Clemençon, 1973). The material was transferred to the Spurr embedding medium via an ethanol series, 100% ethanol, 100% acetone, and mixtures of acetone and the Spurr embedding medium; polymerization lasted 12 hours at 60 °C.

The components of the Spurr embedding medium were used at a rate of 10.0 g ERL–4206, 6.0 g D.E.R. 736, 26.0 g nonenylsuccinic anhydride, 0.4 g dimethylaminoethanol and 0.8 g dibutyl phtalate.

All sections were cut with a diamond knife.

OBSERVATIONS

The species studied so far all showed practically the same ultrastructure and a general description could be given.

A review of the present data reveals that the ultrastructure of species of the Otideaceae and Pezizaceae answers to this description. This holds particularly for the younger stages of development; the delimitation of ascospores in the ascoplasm by two delimiting unit membranes, the form and structure of organels and their distribution in epiplasm and sporoplasm, and the development of the characteristic appearance of the epiplasm and sporoplasm accord completely.

The occurrence of globular structures in the ascoplasm distinguishes the ultrastructures of a number of species. Though specific details of these globular structures will be given farther on for each species separately, some general observations can be made about them first. After use of the glutaraldehyde-OsO_4-fixative all of them are electron-transparent and look alike. After use of the permanganate-OsO_4-fixative they can be ranked as oil bodies that are more or less electron-transparent and homogeneous; or else as other specialized globules of a still unknown substance, which are electron-dense or more moderately electron-dense and have a slightly granular structure (often with an unfixed center). In future this description is followed. The globular structures with moderate electron density mostly occur with both other types and it is not impossible that they represent intermediate forms; they are not found frequently.

It is not impossible that all three types of globular structures are formed in a very early stage of ascus development; at any rate for a number of species it has been found that they are present before meiosis and mitosis take place; the sites of their first formation then seem to be the zones of ascoplasm above and beneath the nucleus. Their formation probably continues during the further stages of ascus development since they abound particularly after meiosis and mitosis, during the delimitation of the ascospores; in that case the zones of ascoplasm between the nuclei are also involved in their formation. After spore delimitation they are found in both the young ascospores and the remaining epiplasm.

Though in some species the organization of the ascoplasmic zones above and beneath the nucleus before meiosis and mitosis is somewhat different from that of the zones between the nuclei after meiosis and mitosis, all of them are essentially similar in structure in all species; this changes during ascus development in the same way.

The globular structures and the ascoplasmic zones in which they arise largely predominate in the ascoplasm and, by the time spores have developed, also in the epiplasm.

In a good many species the ascoplasm also forms a large amount of the same granular or more flocky material as was found in the Pyronemataceae. There it was supposed that both types of material are related; the granular material was compared with the glycogen particles in *Ascodesmis microscopica* and *A. nigricans*. The results of the present study seem to confirm this, which has led to the use of the term glycogen for both the granular and the more flocky material. In very young stages of development glycogen is found scattered in the ascoplasm. When the asci develop further and the ascospores arise it abounds in clusters just inside the ascoplasmalemma and also appears in large quantities around the spores; even more than in the Pyronemataceae, it may finally form the ground mass of the epiplasm. Moreover it may form a large plug just beneath the ascus top, and may fill the basal part of the ascus completely. It is also present in small quantities in the sporoplasm.

The types of fixative applied determine the appearance of the glycogen. Permanganate-OsO_4 seems to preserve it adequately and gives it a fairly electron-dense, granular or more flocky structure. Glutaraldehyde-OsO_4 (or OsO_4 only; Merkus, 1973) gives poorer overall results, as was also found by Schrantz (1968); it makes the glycogen less electron-dense (to electron-transparent) and it seems to change the location of the glycogen, sometimes not fixing it at all or else concentrating it at a few places, thereby causing large vacuoles in the epiplasm.

The development of the spore walls is much the same as in *Ascodesmis* and the Pyronemataceae; between the two delimiting unit membranes wall material successively forming the primary and secondary walls is deposited; at first the primary wall is homogeneous in appearance but in a later stage of development it seems to differentiate into an outer epispore and an inner endospore; the secondary wall arises between the primary wall and the outer delimiting membrane that is now called investing membrane; the inner delimiting membrane becomes the sporoplasmalemma. Primary wall, epispore, and endospore show exactly the same development and ultrastructure as those in *Ascodesmis* and the Pyronemataceae. For a general description compare my earlier studies. The development and the ultrastructure of the secondary wall have much in common with those in the Pyronemataceae; they will be described in detail for each species separately.

As was found in *Ascodesmis* and the Pyronemataceae the epiplasm gradually loses the organels originally present; apart from the formation of glycogen, large vacuoles that often have flocky contents develop in the epiplasm of many species. When the spores mature the epiplasm disintegrates almost completely; only a thin layer of original plasm just inside the ascoplasmalemma may remain. In the sporoplasm all

organels remain present; apart from a general increase in electron density its total appearance changes in many species through the development of large oil drops.

OTIDEACEAE

OTIDEA ALUTACEA, O. BUFONIA, AND O. ONOTICA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. Of the three species *Otidea bufonia* showed the best fixation; the description of the younger stages of development applies only to this species; the mature spores at any rate do not differ.

Young asci before meiosis and mitosis show a normal ascoplasm without any special unusual structures; clusters of glycogen have already been formed. In the same stage however changes are found in the endoplasmic reticulum; at some places it widens slightly, becomes electron-transparent internally and may form circular structures (Pl. 34A); it may also widen further and form small vacuoles with vesicles of varying sizes (Pl.34B); finally a local increase in electron density may be found (Pl. 34A, B); a relation with the electron dense globules, evidently arising in this stage, is not impossible (Pl. 34A, B). Whether there is any connection between these changes and the presence of glycogen is not clear.

Following stages of development show further structural changes in the ascoplasm and formation of the spores. The epiplasm forms increasing amounts of glycogen, and vacuoles with fairly electron-dense flocky contents; the organels disappear. The sporoplasm increases in electron density and develops oil drops. The primary wall (permanganate-OsO_4 350–450 nm, glutaraldehyde-OsO_4 250 nm thick), the epispore (25 nm thick), and the endospore (permanganate-OsO_4 250 nm, glutaraldehyde-OsO_4 200 nm thick) are normal in appearance; apart from the normal striation in the epispore the endospore shows some internal differentiation (Pl. 34C, D, G). The development of the secondary wall proceeds regularly; it consists of homogeneous or more flocky material and varies from 100–1000 nm in thickness; inclusions from the epiplasm are sometimes present (Pl. 34C, D).

The permanganate-OsO_4-fixed material could not be observed in older stages. In all three species it has been found in the glutaraldehyde-OsO_4-fixed material that part of the contents of the secondary wall concentrates on the epispore. Here it forms two succeeding smooth layers that each have a thickness of about 70 nm; though connected, they remain clearly distinguishable (Pl. 34F, G). The structures that have been found in the secondary wall of *Otidea onotica* and, to some extent, also in that of *O. alutacea* possibly represent transitional forms in the development of the two layers (Pl. 34E).

By the time the spores have matured the epiplasm and the rest of the secondary wall have disappeared; the condensed layers on the epispore seem to remain.

PUSTULARIA CUPULARIS

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4.

The youngest stages present show a primary wall (permanganate-OsO_4 100–200 nm, glutaraldehyde-OsO_4 50–100 nm thick) that has a thin inner layer of an obviously loose structure and a homogeneous outer layer which appears in the slides as an uninterrupted, circular band along the spores. Separation of the investing membrane has occurred along the whole primary wall. The intermediate space, which varies in thickness from 100–1500 nm, has been filled up with fairly electron-dense flocky material forming the secondary wall; it also contains inclusions like vesicular structures that must have been derived from the epiplasm or from the investing membrane, which in both fixatives runs irregularly. Epiplasm and sporoplasm are normal in appearance (Pl. 35A).

From this stage of development on, the epiplasm disintegrates slowly; it appears loose and flocky, like the contents of the secondary wall; the organels in it disappear. Little glycogen seems to be formed. At the same time the sporoplasm increases greatly in electron density and develops oil drops. In the glutaraldehyde-OsO_4-fixed material some concentration of secondary wall material is found near the primary wall or distributed in the secondary wall (Pl. 35B).

In succeeding stages an epispore and an endospore evolve; the epispore is 30 nm thick and has normal striation; as in *Sepultaria*, the endospore (permanganate-OsO_4 150–250 nm, glutaraldehyde-OsO_4 100–150 nm thick) may show a broad electron-dense outer part and a thin and sometimes interrupted fairly electron-dense layer in the innermore parts (Pl. 35C, D).

In the mature asci the epiplasm has dissolved completely and almost disappeared, leaving only a small zone at the inner side of the ascus wall. The secondary wall has also been broken down; the remnants of the investing membrane remain the longest. The mature spores are smooth (Pl. 35D).

SOWERBYELLA RADICULATA

Fixative: glutaraldehyde-OsO_4. Though from the pictures available nothing can be said about the development of the ascoplasm and, in later stages, of the epiplasm, a few remarks can still be made about the spores.

The first stages of development show that the structures of the primary wall (250–400 nm thick) and the sporoplasm do not differ from the general description; the sporoplasm develops some oil drops. In following stages an epispore (25 nm thick) and an endospore (150–200 nm thick) have differentiated and the secondary wall has been formed between the investing membrane and the primary wall.

The secondary wall material has a granular electron-dense structure, though it is sometimes found to be more homogeneous. During further spore development the secondary wall material concentrates on the primary wall, where ornamentation crops up. Perpendicular to the primary wall an internal striation of the elements

of ornamentation is evident in the younger stages before their electron density has increased that far (Pl. 35E). This may agree with fibrous structures that have been found in the secondary wall (Pl. 35F).

The mature spores have tapering or more rounded spines about 50–250 nm high, at the ends of the spores up to 700 nm high. The spines occur at regular intervals and are connected by a smooth layer of about 10 nm thick; growing together is also to be found (Pl. 35G).

PEZIZACEAE

PEZIZA AMMOPHILA AND P. PRAETERVISA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4.

In these two species the ascoplasmic zones above and beneath the nucleus, in which the globular structures start to arise, are more or less partitioned in an early stage of development; the central ascoplasm is subdivided into a kind of membrane enveloped plasmic compartments between which glycogen is found, and the more superficial ascoplasm contains much glycogen gathered in clusters (Pl. 36A).

The organels in the compartments are: elements of the endoplasmic reticulum, which appear as tubular or vesicular structures; larger vesicles that usually show poor electron density and may be derived from the endoplasmic reticulum; and membranous structures which are globular and seem to consist of a moderately electron-dense center that might be glycogen and that is wrapped up in a varying number of membranes, possibly invaginating in the center. At many places the membranes enveloping the compartments are diffuse in appearance. The vesicles with poor electron density are also found in the superficial ascoplasm.

The globular structures, which are electron-dense in these species, are particularly evident in the glycogen of the superficial ascoplasm but are also found between the compartments in the central ascoplasm (Pl. 36A).

In later stages of development, in which meiosis and mitosis take place and spore delimitation starts (Pl. 37A), the compartments enlarge considerably and grow into large vacuoles with a flocky basic substance (Pl. 36B). Simultaneously the ascoplasm between the nuclei becomes essentially similar in appearance, whereby the glycogen and the electron-dense globular structures are conspicuous. Large vacuoles however are not found here; the elements of the endoplasmic reticulum and the membranous structures cluster in small vacuoles (Pl. 36C).

Once the spores are formed the development of the plasm proceeds in the epiplasm. Here the amount of glycogen proceeds to increase; the glycogen also fills the basal part of the ascus completely.

Both the primary wall and the sporoplasm are regular in appearance. In *Peziza praetervisa* the primary wall (permanganate-OsO_4 400–500 nm, glutaraldehyde-OsO_4 200–300 nm thick) is electron-transparent but sometimes shows a thin and fairly electron-dense intermediate layer. In *Peziza ammophila* the primary wall

(permanganate-OsO_4 400–600 nm, glutaraldehyde-OsO_4 300–500 nm thick) is completely homogeneous (Pl. 37C). In the sporoplasm both the electron-dense globular structures and an abundant endoplasmic reticulum are present; the electron density has increased (Pl. 37B, C, D).

Separation of the investing membrane occurs along the whole primary wall, in some places becoming fairly conspicuous; the investing membrane runs straight. The secondary wall that is formed between the primary wall and the investing membrane has fairly electron-dense and homogeneous contents (Pl. 37C, D).

In *Peziza praetervisa* the amount of glycogen increases in this stage so much that only a small strip of epiplasmic structures remains between the glycogen and the secondary wall. In *Peziza ammophila* large vacuoles with flocky electron-dense contents develop around the spores; but for the most part these vacuoles disappear again and are replaced by large clusters of glycogen. Once the secondary wall has reached its ultimate thickness (locally 200 nm in *Peziza ammophila* and 500 nm in *P. praetervisa*) all organels in the epiplasm will have disappeared; the epiplasm then consists only of glycogen, in which the electron-dense globular structures are no longer conspicuous; they shrivel and finally disappear. In the sporoplasm two oil drops develop; the electron-dense globular structures remain.

During the development of the secondary wall an epispore and an endospore evolve; the epispore (*Peziza praetervisa*: 35–40 nm thick; *P. ammophila*: 35–50 nm thick) shows a normal pattern of differentiation; the endospore (*Peziza praetervisa*: permanganate-OsO_4 300–400 nm, glutaraldehyde-OsO_4 150–250 nm thick; *P. ammophila*: permanganate-OsO_4 500–1500 nm, glutaraldehyde-OsO_4 350–500 nm thick) sometimes has a thin layer with increased electron density in the innermost part (Pl. 37B, D, E, F).

When the spores mature the contents of the secondary wall concentrate on the epispore in both species (Pl. 37D, E). In *Peziza ammophila* this results in a smooth electron-dense layer about 70 nm thick, in which internal differentiation can be distinguished; it shows subtle striation that is surrounded by a heavier layer (Pl. 37F). In *Peziza praetervisa* ornamentation is formed consisting of a fairly smooth electron-dense layer about 40 nm thick, at regular intervals punctuated by small rounded warts approximately 300 nm high; the elements of ornamentation do not show any internal structure (Pl. 37B). In both species the epiplasm and the rest of the secondary wall disappear.

PEZIZA VESICULOSA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4.

The appearance of the ascoplasm of this species agrees fairly well with that of the two preceding species; the structures are similar but not so abundant as in *Peziza ammophila* and *P. praetervisa*; the ascoplasm above and beneath the nucleus shows subdivision into compartments in the initial stages of development, particularly in the upper part of the ascus (Pl. 38A, B). In this species both the basal part and some

of the superficial part of the ascoplasm consist of glycogen in this stage, the basal part clearly containing the remnants of the original ascoplasm.

After delimitation of the spores, subdivision into plasmic compartments becomes more evident in the epiplasm beneath the lowermost spore. The membranes forming the compartments may show numerous invaginations; they may also separate locally and have larger areas with glycogen; in the glycogen the globular structures are found (Pl. 38C). In contrast to those in *Peziza ammophila* and *P. praetervisa* these develop as fairly electron-transparent structures with somewhat flocky contents (Pl. 38B) and become only in a later stage more electron-dense (Pl. 38C). The epiplasm between the spores develops an essentially corresponding structure, as does that in *Peziza ammophila* and *P. praetervisa*.

In following stages a primary wall (permanganate-OsO_4 500–900 nm thick) is formed (Pl. 39A, B) which seems to differentiate into an epispore (35–50 nm thick) and an endospore (permanganate-OsO_4 500–2000 nm thick) (Pl. 39C, D, E, F); the secondary wall arises between the primary wall and the investing membrane, its local thickness amounting to about 700 nm (Pl. 39B, C). The appearance of the primary wall and, at this stage, also of the secondary wall accords with those in *Peziza ammophila* and *P. praetervisa*. The structures of the epiplasm and the sporoplasm are also similar to those in *P. ammophila* and *P. praetervisa*; in the epiplasm vacuolization and formation of large clusters of glycogen are found; in the sporoplasm no oil drops are formed.

The last stages of development of the spores are the same as in *Peziza ammophila*. The concentration of the secondary wall material on the epispore (Pl. 39C, D) results in a rather smooth electron-dense layer (about 100 nm thick), which shows a similar internal structure, subtle striation surrounded by a heavier layer (Pl. 39E, F); the epiplasm and the rest of the secondary wall disappear.

PEZIZA MICHELII, P. PLEBEIA, P. SUCCOSA, AND P. SUCCOSELLA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. These four species have so much in common that they can be described together.

Young asci before meiosis and mitosis show a rather vague partition of the ascoplasm above and beneath the nucleus into a central part and a more superficial part, the latter consisting mainly of normal ascoplasm; poorly electron-dense endoplasmic vesicles and glycogen are scattered over both parts. The appearance of the central part is practically the same as that of the internuclear ascoplasm in *Peziza ammophila* and *P. praetervisa*; the elements of the endoplasmic reticulum together with numerous membranous structures cluster in small vacuoles. In *Peziza succosa* and *P. succosella* the membranous structures are globular; in *P. michelii* and *P. plebeia* reniform or dumb-bell-shaped structures are also present (Pls. 40A, B, C; 41A, B, C).

Characteristic of the four species is that the numerous globular structures prove to be more or less electron-transparent oil bodies, which are present all over the ascoplasm. Though not always clearly visible in this young stage of development the

oil bodies are embedded in the glycogen; in later stages, when the amount of glycogen increases, this becomes more evident (Pls. 40C; 41B, C). Though young asci were difficult to find it appeared that in *Peziza michelii* and in *P. plebeia* all the structures are present in asci that have just been formed (Pl. 41A).

After delimitation of the spores the epiplasm between the nuclei will have developed similar structures, and the amount of both the oil bodies and the glycogen will have largely increased. The glycogen is found in clusters all over the ascus, particularly in the apical and basal part; in the latter it has completely replaced the epiplasm. The sporoplasm contains numerous oil bodies. In *Peziza michelii* and *P. plebeia* the epiplasm does not change further in a succeeding stage of development; in *P. succosa* and *P. succosella* real vacuoles may arise.

The spores develop normally. The primary wall (permanganate-OsO_4 150–350 nm, glutaraldehyde-OsO_4 100–200 nm thick) does not show any internal differentiation before the endospore (permanganate-OsO_4 150–250 nm, glutaraldehyde-OsO_4 100–200 nm thick) and the epispore (*Peziza michelii* and *P. plebeia*: 45–55 nm thick; *P. succosa* and *P. succosella*: 50–60 nm thick) evolve. The endospore is practically homogeneous in appearance; the epispore shows the ordinary layered structure (Pls. 42; 43). The secondary wall is formed between the investing membrane and the primary wall. It has flocky, fairly electron-dense contents and may vary widely in thickness; the investing membrane runs almost straight (Pls. 42A, B; 43A). In this stage of development the epiplasm slowly disintegrates; just inside the ascus wall it remains the longest. Here the oil bodies become elongated and orientated perpendicular to the ascus wall; they sometimes develop a somewhat flocky content. Invaginations from the epiplasm into the secondary wall have been found with both types of fixative (Pl. 43A, C). The sporoplasm develops large oil drops and a sometimes conspicuous endoplasmic reticulum.

Before the differentiation of the primary wall the contents of the secondary wall concentrate into electron-dense globules, flattened globules or more continuous layers on the primary wall (Pls. 42B; 43C, F). In following stages the secondary wall material gradually concentrates, and warts and ridges connected by a smooth thin layer arise on the primary wall at regular intervals. During further maturation of the spores both the epiplasm and the rest of the secondary wall disappear.

In *Peziza succosa* and *P. succosella*, the elements of ornamentation at first show internal differentiation. In the permanganate-OsO_4-fixed material the basal parts of the elements are fairly electron-dense and maintain a rather loose structure, the condensed material that borders the epispore showing subtle striation all over the spore surface; the upper parts are fairly electron-dense and homogeneous and seem to have been added separately as a kind of cap (Pls. 42C, D; 43B). In the glutaraldehyde-OsO_4-fixed material the loose structure of the basal parts may show striation perpendicular to the spore surface; the upper parts are electron-dense and may be covered by an electron-transparent layer (Pl. 42F, G).

In later stages the internal differences in the elements of ornamentation finally disappear in the permanganate-OsO_4-fixed material and the ornamentation becomes

electron-dense (Pl. 42E). In glutaraldehyde-OsO_4-fixed material of *Peziza succosella* the electron-transparent material remains present and seems to be surrounded by an electron-dense layer (Pl. 42H).

In *Peziza succosa* and *P. succosella* ornamentation is rather similar. In *P. succosa* warts 600–800 nm high, which are sometimes slightly broadened or may have grown into ridges, are found together with smaller warts about 150–300 nm high; in *P. succosella* warts and ridges are connected by a smooth layer about 50 nm thick (Pl. 42E, H).

In *Peziza michelii* and *P. plebeia* the concentration of secondary wall material on the epispore seems to have increased more regularly since separate parts cannot easily be distinguished; only less solid parts seem to remain and form permanent gaps (Pl. 43D, E). In *P. plebeia* ornamentation develops in exactly the same way as in *P. michelii*; glutaraldehyde-OsO_4-fixed material could not be studied in the latest stages of development. The ornamentation consists of rounded warts, which vary in size from 200–700 nm and may have broadened or grown together; the connective layer varies in thickness from 60–100 nm.

PEZIZA BADIA

Fixative: permanganate-OsO_4.

Though this species closely resembles *Peziza michelii*, *P. plebeia*, *P. succosa*, and *P. succosella* in all stages of development, it has a particular amount in common with the first two species mentioned. In an early stage of ascus development differences are found in the fact that the membranous structures are almost never globular, but reniform, dumb-bell-shaped or in other ways irregular (Pl. 44A). In a somewhat later stage large clusters of glycogen take the place of the organels in the epiplasm.

The aspects of the primary wall (300 nm thick), the epispore (45–55 nm thick), and the endospore (150–200 nm thick) agree with the general description, as does the aspect of the secondary wall. Like in *Peziza michelii* and *P. plebeia* the condensation of secondary wall material proceeds fairly regularly; the different parts in the developing elements of ornamentation, clearly present in *P. succosa* and *P. succosella*, are not formed.

The ornamentation is surrounded by an electron-dense layer that is evidently formed by its outermost part; it consists of warts and ridges (100–400 nm high), which may form an incomplete network and which are connected by a smooth layer about 40 nm thick (Pl. 44B).

PEZIZA BADIOFUSCA, P. EMILEIA, AND P. PETERSII

Fixative: permanganate-OsO_4. As an incipient spore formation is present in the youngest stages studied, it could not be concluded what kind of development the ascoplasm undergoes.

The structures of the epispore (*Peziza badiofusca* and *P. petersii*: 60 nm thick; *P. emileia*: 40–50 nm thick) and the endospore (*P. badiofusca*: 200–250 nm thick; *P. emileia*: 500–800 nm thick; *P. petersii*: 250–350 nm thick) do not differ from the general description (Pls. 44C, D, E, F; 45A, B). The endospore sometimes has a thin electron-dense layer in the innermore parts; in *P. petersii* several electron-dense layers may be present (Pl. 44F).

The secondary wall has also developed; it varies largely in thickness along the surface of a single spore; its contents are fairly electron-dense and homogeneous, sometimes slightly flocky (Pl. 44C). The investing membrane runs almost straight. The epiplasmic organels have nearly all disappeared and have been replaced by glycogen; the remainder is found close to the ascus wall. Here they may occur together with the same electron-dense globular structures as those found in other species, which at this place may be elongated and orientated perpendicular to the ascus wall. The sporoplasm has an increased electron density and has developed oil drops; it often shows a rather abundant endoplasmic reticulum.

The development of spore ornamentation resembles that in preceding species. In the first stages of redistribution of the secondary wall material internal differences divide the elements of ornamentation in various parts. In *Peziza badiofusca* the basal parts of the elements are electron-dense and homogeneous, the intermediate parts have a fairly electron-dense and loose structure, and the upper parts are fairly electron-dense and homogeneous (Pl. 45A). In *P. emileia* and *P. petersii* the developing elements of ornamentation are homogeneous in structure, the upper parts being less electron-dense than the lower parts, in which in *P. petersii* subtle striation is sometimes distinguishable (Pl. 44C, D, E, F). In some places in *P. petersii* the internal structure of the elements is loose, which causes permanent "gaps" (Pl. 44E). In later stages all the internal differences disappear and the elements of ornamentation become completely electron-dense in all three species (Pl. 45B).

In the mature spores of *Peziza badiofusca* ornamentation is formed by rounded, isolated warts, 300–700 nm high and spread regularly on the spore surface; they are connected by a rather smooth layer about 50 nm thick in which small warts are sometimes found (Pl. 45B). In *P. emileia* ornamentation is formed by slender, isolated warts (500–800 nm high), which are connected by a smooth layer (about 30 nm thick) and occur at regular intervals (Pl. 44D). In *P. petersii* warts and ridges (300–500 nm high) are also found at regular intervals along the spore surface and are connected by a smooth layer (60–70 nm thick); in the oldest stages of development the tops of the warts may enlarge further and grow out laterally, the warts sometimes fusing (Pl. 44F).

Like in *Peziza badia*, ornamentation in *P. badiofusca* and particularly that in *P. petersii* is surrounded by a marked electron-dense layer (Pls. 44E, F; 45B). In all three species the epiplasm and the rest of the secondary wall have disappeared when the spores are mature.

PEZIZA TRACHYCARPA

Fixative: permanganate-OsO_4. In the youngest stages discernible the development of the primary wall is complete and a secondary wall has already started to form. Therefore, and as a result of relatively poor preservation, no conclusions could be drawn about the first stages of the ascoplasm.

The primary wall is 600–800 nm thick and has a normal aspect; separation of the investing membrane from the primary wall has made formation of the secondary wall possible; this has homogeneous and fairly electron-dense contents. In the remnants of the epiplasm glycogen is found and the same oil bodies occur as in several other species. The sporoplasm has fairly electron-dense contents with an extensive endoplasmic reticulum; it has developed one large oil drop, sometimes accompanied by smaller ones.

In a following stage of development a normal epispore (60 nm thick) and endospore (200–250 nm thick) differentiate and, though in most places the investing membrane has gone, it can be seen that locally the secondary wall increases further in thickness. The primary wall becomes extremely wavy in outline, which must be seen as an artefact.

At the same time the secondary wall material concentrates on the epispore. Like in *Peziza emileia* and *P. petersii*, at first the basal parts of the developing elements of ornamentation are electron-dense and homogeneous, while the upper parts are fairly electron-dense and homogeneous (Pl. 45C). A locally loose structure of the elements may cause permanent "gaps", as was also found in *P. petersii*. In later stages the elements of ornamentation become completely electron-dense. Together with the epiplasm, the rest of the secondary wall material disappears.

When the spores mature the upper parts of the elements of ornamentation grow out laterally, no longer forming warts, but umbrella-shaped structures (1200–1500 nm high) that sometimes fuse at the edges. In between the epispore is covered by a smooth layer (50 nm thick). Finally, their temporary "hairy" appearance is worth mentioning; this is not found in any of the other species studied (Pl. 45D).

PULPARIA PERSOONII

Fixative: permanganate-OsO_4. Preservation of this species appears to be difficult; this made a complete study of it impossible.

As regards the contents of the ascoplasm and their distribution over the ascus, young stages show close similarity to *Peziza badia*, *P. michelii*, *P. plebeia*, *P. succosa*, and *P. succosella*; particularly the first three species agree in that the membranous structures are globular or more irregularly formed. A noteworthy difference with these species is the absence of oil bodies and the presence of electron-dense globular structures (often with an unfixed center, as sometimes found in other species) resembling those in *Peziza ammophila*, *P. praetervisa*, and others.

The eventual development of vacuoles in the epiplasm is uncertain; glycogen is present in large quantities and takes the place of most of the original epiplasm. In the sporoplasm large oil drops develop. The development of the spore walls is normal; the epispore (50 nm thick) and the endospore (250–350 nm thick) seem to differentiate from the primary wall (300–400 nm thick); the secondary wall develops between the primary wall and the separating investing membrane.

At first the secondary wall material is homogeneous and fairly electron-dense. When its redistribution starts, electron-dense spots arise at regular distances from each other on the spore surface. As they gradually enlarge, the remaining contents of the secondary wall seem to be restructured into fibrous elements; these easily join and give the developing elements of ornamentation a freakish appearance (Pl. 45E). It is not clear whether these structures result from normal development or are caused by incomplete fixation. In the mature spores ornamentation is formed by irregular warts (400–800 nm high) that maintain a fibrous outer layer (Pl. 45F).

DISCUSSION

On many points the results of this electron microscopy confirm those of earlier studies. Not only does the general ultrastructure of each species agree with what is already known of Ascomycetes but the way in which the ascospores start to develop — delimitation of the spores in the ascoplasm by two delimiting unit membranes and the formation of spore walls between these two delimiting unit membranes — bears out similar developments in *Ascodesmis* (Merkus, 1973), in the Pyronemataceae (Merkus, 1974), and in other Ascomycetes (Reeves, 1967; Carroll, 1966, 1967, 1969; Delay, 1966; Wells, 1972; Schrantz, 1966, 1970).

Later stages of development reveal a strong structural resemblance to both *Ascodesmis* and the Pyronemataceae. The different appearance of two successively formed spore wall layers has again led to the use of the terms primary and secondary walls; and the processes involved in the formation of these spore walls, the differentiation of the primary wall into the epispore and the endospore, the internal differentiation of the epispore and the endospore, the various reactions of the spore wall layers on the fixatives applied, together with the structural changes of the epiplasm and sporoplasm, also agree. Perhaps, therefore, it will suffice to refer to previous discussions on these subjects.

A marked difference with the results obtained thus far is the appearance of the young ascoplasm of the genera *Otidea*, *Peziza*, and *Pulparia*. Globular structures, like oil bodies and other specialized globules, are present in most of the species; the more or less electron-transparent oil bodies are restricted to *Peziza*; the other specialized globules, which are electron-dense or more moderately electron-dense, prove to exist in *Otidea* and *Pulparia* and are found in a number of species of *Peziza*. The genus *Sowerbyella* could not be adequately studied on this point. The genus *Pustularia* is more usual in its internal structure and lacks the globular structures.

It is difficult to decide what processes are involved in the development of the

globular structures, which are often found embedded in glycogen. It is not impossible that the membranous structures in the central ascoplasm may play a role, though intervention of the poorly electron-dense vesicles derived from the endoplasmic reticulum must also not be overlooked. More knowledge about their chemistry might reveal something more about these processes. In this connection the development of the ascoplasm of species of *Otidea* seems to represent a simple form of those in the other species.

The globular structures are probably a food reserve; this was also assumed by Guilliermond (1904, 1910, 1920). They do not seem to play an active role in the formation of the spore walls. In the epiplasm they slowly lose their contents and disappear when the spores mature, not by being absorbed by the spores (Guilliermond) but by shrinkage or shriveling. In the sporoplasm they seem to persist; the oil bodies join to large oil drops, like in *Peziza badia*, *P. michelii*, *P. plebeia*, *P. succosa*, and *P. succosella*; the other specialized globules maintain their original form. When oil bodies are absent in the ascoplasm the spores may still develop oil drops in later stages, like in *Otidea alutacea*, *O. bufonia*, *O. onotica*, *Peziza ammophila*, *P. badiofusca*, *P. emileia*, *P. petersii*, *P. praetervisa*, *P. trachycarpa*, *Pulparia persoonii*, and *Pustularia cupularis*. In *Peziza vesiculosa* no oil drops are formed at all.

A food reserve is also provided in large quantities by glycogen. Though the possibility of chemical changes or the influence of the fixative cannot be precluded it seems as if other structural forms of glycogen exist apart from the typical glycogen particles that have been described (a.o. by Schrantz, 1968, for *Peziza plebeia*).

The aspect of the secondary wall and the structural changes it undergoes during the development of ornamentation is the same as in the Pyronemataceae. The secondary wall material is at first regularly spread in the secondary wall and is similar in appearance to the older epiplasm in most of the species. It seems to be redistributed in later stages and condenses or concentrates on the epispore, where it forms a complete ornamentation. Like in the Pyronemataceae, the processes involved in this condensation or concentration of secondary wall material are regular and characteristic for each species separately. A continuous addition of new secondary wall material during the formation of ornamentation is not impossible. The fate of the remaining secondary wall material and the investing membrane is unknown, though it is highly likely that it disappears with the epiplasm.

Like in the Pyronemataceae this development of ornamentation is found in all the species with ornamented spores, viz. *Peziza badia*, *P. badiofusca*, *P. emileia*, *P. michelii*, *P. petersii*, *P. plebeia*, *P. praetervisa*, *P. succosa*, *P. succosella*, *Pulparia persoonii*, and *Sowerbyella radiculata*. Apart from the striation in the developing ornamentation of *Sowerbyella radiculata*, the thin surrounding layer that is present in *Peziza badia*, *P. badiofusca*, and *P. petersii*, and the somewhat fibrous outer part of the ornamentation of *Pulparia persoonii*, no further internal differentiation in the ornamentation is found in any of the species.

In comparing the development of the smooth spores in the Pyronemataceae and in the species of this study it appears that similarities exist; in *Pustularia cupularis* some

condensation of secondary wall material is found but it disappears in a later stage so that ultimately the epispore forms the outermost part of the mature spores. In *Peziza ammophila* and *P. vesiculosa* however a permanent condensation of secondary wall material causes the formation of extra, smooth layers on the epispore. A similar addition of smooth layers on the epispore occurs in *Otidea alutacea*, *O. bufonia*, and *O. onotica* but it is uncertain whether these layers are permanent.

Though the terminology of the spore wall layers is somewhat different my results on the species *Pustularia cupularis* and *Peziza plebeia* agree with those of Schrantz (1966, 1970). His descriptions for both the development of two succeeding spore wall layers, the "couche primaire" (primary wall) and the "périspore" (secondary wall) in both species, and the formation of the "couche ornementale" in the "périspore" of *Peziza plebeia* are similar to those in the present study. He also found differentiation of the "couche primaire" into the outer "exospore" (epispore) and the inner "épispore" (endospore) in both species, and further development of the innermost "endospore" (not described as a particular layer by me) in *Pustularia cupularis*; and mentioned the presence of an "ectospore" (investing membrane) and "masses denses" (plasmic inclusions).

As on the one hand so many similarities prove to exist between the ultrastructure of the species of the Pyronemataceae, Otideaceae, and Pezizaceae and, on the other hand, Le Gal's observations on these taxa also agree, I can add no further details to the comparison of the two studies. To avoid further duplication I refer to previous discussions on the subject.

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EXPLANATION OF PLATES 34–45

ABBREVIATIONS USED IN PLATES. — AW, ascus wall; CM, condensed material; E, epiplasm; En, endospore; Ep, epispore; ER, endoplasmic reticulum; EV, endoplasmic vesicle; G, glycogen; GS, globular structure; IAM, inner ascospore-delimiting membrane; IM, investing membrane; MS, membranous structure; OAM, outer ascospore-delimiting membrane; N, nucleus; PI, plasmic inclusions; PW, primary wall; S, sporoplasm; SW, secondary wall; T, tonoplast; V, vesicle; Va, vacuole.

PLATE 34

Figs. A-D. *Otidea bufonia*, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Figs. A, B. ascoplasm, before spore development, ×18,200; Fig. C. spore development, development of secondary wall, ×29,000; Fig. D. id. also showing development of epispore and endospore, ×29,000.

Fig. E. *Otidea onotica*, spore development, temporary internal structure of condensing secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700.

Fig. F. *Otidea alutacea*, spore development, condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 18,500.

Fig. G. *Otidea bufonia*, detail of condensed secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 115,500.

PLATE 35

Figs. A-D. *Pustularia cupularis*, spore development, stained with lead citrate: Fig. A. beginning of secondary wall formation, fixed in 1% $KMnO_4$ and 1% OsO_4, × 29,700; Fig. B. further development of secondary wall, with condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4, × 29,700; Fig. C. development of epispore and endospore, fixed in 1% glutaraldehyde and 1% OsO_4, × 36,300; Fig. D. id.

Figs. E-G. *Sowerbyella radiculata*, spore development, fixed in 3.25% glutaraldehyde and 1% OsO_4: Fig. E. condensation of secondary wall material and development of epispore and endospore, stained with uranyl acetate and lead citrate, × 36,300; Fig. F. id. showing fibrous structure of secondary wall material, stained with lead citrate; Fig. G. advanced state in development of ornamentation, stained with uranyl acetate and lead citrate, × 29,700.

PLATE 36

Figs. A-C. *Peziza praetervisa*, ascoplasm, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. upper part of ascus before meiosis and mitosis, × 16,600; Fig. B. upper part of ascus after meiosis and mitosis, × 8,400; Fig. C. ascoplasm of the same ascus, between the nuclei, × 8,400.

PLATE 37

Figs. A, B. *Peziza praetervisa*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. young stage of spore development, just after spore delimitation, × 14,900; Fig. B. advanced state in the development of ornamentation, epispore, and endospore, × 18,200.

Figs. C-F. *Peziza ammophila*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. C. beginning of secondary wall formation, × 18,200; Fig. D. condensation of secondary wall material and development of epispore and endospore, × 9,900; Fig. E. id. × 18,200; Fig. F. id. detail, advanced state of spore development, × 36,300.

PLATE 38

Figs. A-C. *Peziza vesiculosa*, ascoplasm, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. upper part of ascus before meiosis and mitosis, stained with uranyl acetate and lead citrate, × 9,900; Fig. B. basal part of ascus before meiosis and mitosis, stained with uranyl acetate and lead citrate, × 17,300; Fig. C. basal part of ascus after delimitation of spores, stained with lead citrate, × 17,300.

PLATE 39

Figs. A-F. *Peziza vesiculosa*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. young ascospore during formation of primary wall, stained with uranyl acetate and lead citrate, × 18,200; Fig. B. beginning of secondary wall formation, stained with uranyl

acetate and lead citrate, ×9,900; Fig. C. condensation of secondary wall material and development of epispore and endospore, stained with lead citrate, ×18,200; Fig. D. id.; Fig. E. id.; Fig. F. id. advanced state of spore development, stained with uranyl acetate and lead citrate, ×21,400.

PLATE 40

Figs. A, B. *Peziza succosa*, ascoplasm, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. upper part of ascus before meiosis and mitosis, ×7,100; Fig. B. detail, ×16,600.

Fig. C. *Peziza succosella*, id. ×5,800.

PLATE 41

Fig. A. *Peziza michelii*, ascoplasm, upper part of ascus before meiosis and mitosis, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×7,400.

Figs. B, C. *Peziza plebeia*, id.: Fig. B. ×7,100; Fig. C. detail, ×21,400.

PLATE 42

Figs. A-H. *Peziza succosella*, spore development: Fig. A. beginning of secondary wall formation, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700; Fig. B. id. condensation of secondary wall material and development of epispore and endospore, stained with lead citrate; Fig. C. id. stained with uranyl acetate and lead citrate; Fig. D. id. stained with lead citrate, ×33,300; Fig. E. id. advanced state of spore development, stained with uranyl acetate and lead citrate, ×29,700; Fig. F. condensation of secondary wall material and development of epispore and endospore, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700; Fig. G. id.; Fig. H. id. advanced state of spore development.

PLATE 43

Figs. A, B. *Peziza succosa*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700: Fig. A. beginning of secondary wall formation and some condensation of secondary wall material; Fig. B. condensation of secondary wall material and development of epispore and endospore.

Figs. C-E. *Peziza michelii*, spore development, stained with uranyl acetate and lead citrate, ×29,700: Fig. C. condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4; Fig. D. id. also showing development of epispore and endospore, fixed in 1% $KMnO_4$ and 1% OsO_4; Fig. E. id.

Fig. F. *Peziza plebeia*, spore development, condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700.

PLATE 44

Figs. A, B. *Peziza badia*, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×23,100: Fig. A. detail of ascoplasm in upper part of ascus before meiosis and mitosis; Fig. B. condensation of secondary wall material and development of epispore and endospore, advanced state of spore development.

Figs. C, D. *Peziza emileia*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700: Fig. C. condensation of secondary wall material and development of epispore and endospore; Fig. D. id. advanced state of spore development.

Figs. E, F. *Peziza petersii*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. E. condensation of secondary wall material and development of epispore and endospore, ×29,700; Fig. F. id. advanced state of spore development, ×33,300.

PLATE 45

Figs. A, B. *Peziza badiofusca*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. condensation of secondary wall material and development of epispore and endospore, ×29,700; Fig. B. id. advanced state of spore development, ×23,100.

Figs. C, D. *Peziza trachycarpa*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. C. condensation of secondary wall material and development of epispore and endospore, ×29,700; Fig. D. id. advanced state of spore development, ×23,100.

Figs. E, F. *Pulparia persoonii*, spore development, stained with uranyl acetate and lead citrate: Fig. E. condensation of secondary wall material and development of epispore and endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4, ×29,700; Fig. F. id. advanced state of spore development, fixed in 1% $KMnO_4$ and 1% OsO_4, ×36,300.

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ON THE IDENTITY OF POLYPORUS SCHULZERI FR.

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(With three Text-figures)

The existing collection of *Polyporus schulzeri* Fr. (≡ *P. irpex* Schulzer) in the Natural History Museum at Vienna (W) agrees microscopically with Schulzer's unpublished observations and is therefore considered to represent the type specimen. A comparison of this material with specimens of *Piptoporus soloniensis* (Dub. ex Fr.) Pilát showed that both fungi are identical in structure and represent, in fact, the same species. A detailed study of fresh material and a thorough revision of the genus *Piptoporus* is needed before it can be decided if a new genus should be described for this species. In *Piptoporus* the correct name is *P. soloniensis* with *P. schulzeri* as a synonym.

Many of the species described by Schulzer still remain a mystery for present-day mycologists, since very few of his exsiccates exist. *Polyporus schulzeri*, however, remained so for exactly one hundred years in spite of an extant specimen, perhaps even because of it! It was considered to be either identical with a rare species named later *Spongipellis litschaueri*, or to represent a separate species, known only from a single locality, but neither interpretation could be proved to everyone's satisfaction.

The whole trouble started with Fries' incorrect diagnosis. In his first manuscript, now in Budapest, Schulzer described a new species, *Polyporus irpex*, with a tomentose upper surface and large, toothed, yellow pores, and published it, only as nomen nudum (Schulzer, Kanitz & Knapp, 1866). Kalchbrenner sent a copy of the drawing and surely also of the description from the manuscript to Fries, who renamed the species *Polyporus schulzeri*, characterizing it as: 'Pileo sessili, e carnoso suberoso, pulvinato, glabro, azono, pellicula albida tecto; poris rotundis, majusculis, hydnoideo-dentatis, albis...' and placing it in *Placodermei*, *Suberosi*, near *Polyporus officinalis*, *P. betulinus* etc. (1874: 556). A more extensive description was published by Kalchbrenner (1877: 53, pl. 32 fig. 1) together with a part of the original drawing, but the pellicle was emphasized here too. Both authors cite *Polyporus irpex* as a synonym.

Naturally, Schulzer was indignant because of the misrepresentation of his species, its placing in the *Placodermei* and its being given a new name without a particular reason, and he protested four times in print, giving even a Latin diagnosis (1880) in which he stressed that the fungus was 'valde tomentoso-lanatus, quod vero tomentum senio non-nihil disparet, sed superficies pilei sempre inequalis impolita sine pellicula.'

However, his protests were later forgotten and, when recently rediscovered, even added to the confusion.

In spite of Fries' mentioning the presence of the pellicle as one of the chief characters, the name *Polyporus schulzeri* was applied by Bresadola to some collections of Kmet' in Czechoslovakia for a polypore with a distinctly tomentose surface, and also to a similar collection of Linhart in Roumania (both Kmet's and Linhart's localities belonged then to Hungary). As I suppose, the reason for this interpretation was the little known, or perhaps unknown fact, that Bresadola had had the opportunity to see the duplicate description and drawing of *P. irpex* in Schulzer's second manuscript (now preserved in Zagreb). Schulzer sent this manuscript to both Bresadola and Quélet for review. Bresadola's interpretation was taken over by Bourdot & Galzin (1928), who renamed the fungus *Spongipellis schulzeri*.

A different solution was proposed by Lohwag (1931). He at first determined a specimen, collected in Austria, as *S. schulzeri* in the sense of Bourdot & Galzin, but it struck him that the tomentose upper surface, as stated by those authors, was in direct contradiction with Fries' diagnosis, which he of course assumed to be correct, not knowing about Schulzer's denials. Just at that time he was informed that there existed a specimen in the Natural History Museum in Vienna (W), collected and annotated by Schulzer himself in 1859 as *Polyporus irpex*, which he concluded to be the type. Although this specimen was old, sterile and in a poor condition, macroscopical and microscopical examination showed that its structure was widely different from Lohwag's specimen, and that these two collections represented two quite distinct species. Lohwag placed Schulzer's species in the genus *Ungulina*, as *U. schulzeri*, since he found a pellicle on the type specimen that apparently confirmed Fries' description. He examined also some of the above mentioned Kmet's and Linhart's material identified by Bresadola as *P. schulzeri*, and found it conspecific with the Austrian specimen. This taxon being now without a name, Lohwag described it as the new species *Spongipellis litschaueri*.

Schulzer's specimen in W was later examined by Kotlaba & Pouzar, who declared (1965: 76): '...we consider it most probably *Tyromyces lacteus* (Fr.) Murrill (among other characters it is d i m i t i c with skeletal hyphae).'

Igmándy (1957) was the first to draw again the attention of the mycologists to Schulzer's protests against the mention of a pellicle in *P. irpex*. He studied Schulzer's original description in the first manuscript and moreover found a specimen in the Natural History Museum at Budapest (BP) under the name of *Polyporus irpex* from Hazslinszky's herbarium with the label (in Hungarian): 'During an excursion with Schulzer collected from a plum tree in a garden in Subanya at Szava, June [1] 868.' (The locality is Županja at the river Sava, about 25 km south of Vinkovci where Schulzer lived.) Both the description in Schulzer's manuscript and Hazslinszky's specimen seemed to agree well with *Spongipellis litschaueri*, and therefore Schulzer's name would have priority. Igmándy reintroduced the original name given by Schulzer, who never acknowledged Fries' renaming of his fungus, and placed the species in the genus *Leptoporus*, as *L. irpex*. Igmándy's article seems to have been

generally overlooked, as it is in Hungarian language, with short summaries in Russian and in French.

Recently Donk (1972) reexamined the whole problem. He cited most of Schulzer's published discussions on *P. irpex* (*P. schulzeri*) including the Latin diagnosis, and argued, like Igmándy, that Schulzer was in fact describing *S. litschaueri*. Donk believed that even Fries' diagnosis applied well to this species, excepting the pellicle. He consequently again proposed *Spongipellis schulzeri* (Fr.) Bourd. & Galz. as the correct name for *S. litschaueri*. However, he could not account for the specimen in W which, having a pellicle, could not be the type, and thought that some error had crept in.

Although Schulzer's Latin diagnosis is now easily accessible, being cited by Donk (1972), it lacks some details which are to be found in Schulzer's manuscripts. The description from the manuscript in Zagreb which I have studied in the original is almost word for word identical with that in the first manuscript, a copy of which I have seen now. The spaced words were underlined by Schulzer.

'No 1323. *Polyporus irpex* Schulzer. Ich begegnete dieser Pilzform erst zweimal: im Szabarer Walde bei Mohács und später nach Jahrzehenden in Črni gaj bei Vinkovce,.... Sie gehört somit zu s e l t e n s t e n V o r k o m m n i s s e n ...

Ich sah den Pilz vom Oktober bis zum Februar an Eichenklötzen und an kränkelnden Eichen.

Der Hut ist gepolstert-halbkreisförmig, an der Basis etwas verengt, mit nicht scharfem Rande, weil das 1,2–2,5 cm dicke Fleisch daselbst zwar oft plötzlich abfällt, aber derselbe niedergebogen ist, 6,6–13,2 cm breit, oben weiss oder gelblich, am Grunde häufig aschgrau, sehr f i l z i g - w o l l i g, was im Alter verschwindet, zonenlos, auf der Unterseite sieht man anfangs kleine blassgelbe Löchlein, welche später durch Zerreissen der Ränder 0,5–1 mm breit, überaus zerschlitzt, förmlich gezähnt und im weitern Verfolge unregelmässig gewunden, lebhaft gelb, wohl auch gelb-zimmetbraun werden.

Die Röhrchen sind licht schwefel- oder ockergelb, am Hutrande sehr kurz, weiter davon 0,9–2,7 cm lang, an der Basis ausgegossen auf mehrere Centimeter verlängert, anfangs zwar fein, aber am Ende, durch Schwinden der Seitenwände und des Löcherwandes wirkliche, ungleichgeförmte, und dabei schlappe, weiche Zacken.

Das Fleisch ist erst lederartig-fasrig, dann korkartig, zuletzt mürbe und zerfallend; im Anfange weiss oder gelblich, am Ende röthlich. Die Grenze zwischen Fleisch und Röhrchen ist scharf markirt, beide aber doch nicht leicht voneinander trennbar. Sie bestehen aus langen, dichtverwebten, unseptirten, knorrigen, wenig ästigen Fadenzellen, an denen man häufig knospenartige Ausstülpungen sieht. Eine davon abweichende Hymeniumschicht fand ich nicht, sondern die Enden der Fadenzellen bekleiden die Röhrchenfläche. Geruchlos.'

In the second manuscript after the description proper, the following interesting remarks about Fries' treatment of his species are added. These remarks, hardly toned down, were published in several of Schulzer's papers:

'In seinem letzten Werke "Hymenomycetes europaei" nennt Fries diese Art *Polyporus Schulzeri*. Obschon die Widmung auf die schmeichelhafteste Weise mit den Worten folgte: "Hymenium ex icone magis *Hydnum* quam Irpicem refert, quare hanc speciem dicatam volui felicissimo fungorum investigatori," so muss ich doch die mir von dem grossen Mycologen zugedachte Ehre d a n k e n d a b l e h n e n.

Erstens ... hoffentlich wird Niemand beim Anschauen der Abbildung eine Spur von den

die Gattung *Hydnum* characterisirenden "Aculei subulati" finden, dagegen an *Irpex* lebhaft erinnernde Zähne (Dentes).

Zweitens ist es eine Artigkeit sehr, eigentlich weniger als, dubiosen Werthes, wenn Jemand eine meinerseits entdeckte und benamste Art umtauft, ihr meinen Namen beilegt und als Aufsteller den seinigen anhängt. ...

Drittens endlich, was die Hauptursache der Ablehnung ist, existirt zur Zeit kein Pilzgebilde, welches der Diagnose des Pol. Schulzeri entspräche, und da auch jene Kalchbrenners in den "Icones" Seite 53 unrichtig ist, so sah ich mich, wie erwähnt, genöthigt, in der Oest. bot. Zeitschrift die einzig wahre zu veröffentlichen....

Ich stand mit Fries nie in Verbindung; meine Arbeiten in erstem grossen Bildwerke kannte er, ohne mein Verlangen, bloss durch Kalchbrenner, und da er der deutschen Sprache, in welcher die Diagnosen gegeben sind, nicht mächtig war, musste sie ihm ein Anderer ins Lateinische übersetzen, was bei einigen Arten leider stümperhaft, bei dieser vollends ganz und gar unrichtig geschah. Welch' total falsche Vorstellung er hindurch von unserm Pilze gewann, sieht man daran, dass er ihn zu den mit einer festen Kruste bedeckten Placodermei stellte! ...'

Thus Schulzer explains Fries' error about the pellicle on the grounds of incorrect translation of the description by Kalchbrenner included with the drawing when he sent this to Fries. It is possible, however, that Kalchbrenner had already failed to copy Schulzer's work faithfully, since he too mentions the pellicle in this 'Icones' (1877), which led Schulzer to make the following comments (1880: 108): '...Mein Freund Kalchbrenner gibt die Abbildung richtig, aber im Widerspruche mit derselben spukt auch bei ihm in der Diagnose das verwirrende "glaber"....'

Igmándy's and Donk's very convincing arguments for the conspecifity of *Polyporus irpex* and *Spongipellis litschaueri* seem to be confirmed by Schulzer's original description, and also by the mentioned (but not cited here) similarity of his species with *Polyporus labyrinthicus* Schw. (= *Spongipellis unicolor* (Schw.) Murr.).

However, certain curious details, some of them not mentioned in the published Latin diagnosis, cannot be explained away. Schulzer was a most careful and painstaking observer, who would have noticed any duplex structure of the context, such as is characteristic for *S. litschaueri*. He never says a word about it, but describes the context as becoming friable ('mürbe und zerfallend') in old specimens. The tubes are flexible ('schlappe, weiche Zacken'). Also, his description of the hyphae — gnarled, non-septate, with bud-like swellings ('knorrig, unseptirt, mit knospenartigen Ausstülpungen') does not apply to the hyphae of *S. litschaueri*, even if 'Ausstülpungen' are taken to mean clamp-connections on septa which he could not notice with his lowpower microscope. There is a final problem concerning the specimens of *P. irpex*; one in W, collected and identified by Schulzer which according to those who have examined it, does not agree with the description made by its author; and the other from Hazslinszky's herbarium, collected during an excursion with Schulzer, which seemed to be identical with *Spongipellis litschaueri*.

Obviously, these doubts can only be resolved by the examination of the specimens.

Schulzer's original material, which was obtained on loan from the Natural History Museum, Vienna (W) consists of two envelopes. The larger is labelled in Schulzer's hand: '*Polyporus Irpex* Schulzer, Černi gaj zwischen Vinkovce und Jarmina, Novbr.

1859.' Underneath is added in another hand and in red ink: 'Slavonien, l. Schulzer.' In the smaller envelope, from the herbarium of V. Litschauer, the specimen is named *P. schulzeri*, but the date and locality are the same. This is obviously a duplicate taken from the original collection. The first specimen is described and shown on a photograph in Lohwag's paper (1931). Both specimens are in fragments, either context with tubes or only context or tubes; originally all fragments were glued to the paper, but now some of them are detached. They are of a uniformly dirty greyish-yellowish colour and very friable, partly even reduced to powder. It is therefore not possible to reconstruct the original shape of the specimens. The tubes are up to 1 cm long and the pores are rather large, irregular, about 1–2 per mm. Only the lower, free part of the tubes was apparently torn into teeth, and this part in the course of time has broken up. In the largest fragment with tubes, the pores are completely collapsed and only remains of the irpicoid teeth can be seen.

Microscopically, both the tubes and the context consist of sparsely branched, non-septate hyphae with up to 1 μm thick walls. They are really very gnarled ('knorrig') and their diameter is 2–4(–5) μm, mostly 2–3.5 μm. They stain metachromatically in cresyl blue, turning a wonderful deep magenta colour. Only rather short fragments of hyphae can be observed as if they fractured during drying, hence the friable consistency. No clamps were found. These hyphae are, without doubt, skeletal hyphae, as stated by Kotlaba & Pouzar (1965). The walls sometimes have irregular swellings, which would account for the 'knospenartige Ausstülpungen'. Schulzer has clearly described the hyphae exactly as he saw them, and as we can still see them now.

Lohwag (1931) declared that he had found clamps, although they were rare and not clearly visible; apart from that his description of the hyphae is very similar. However, neither he, nor others who examined the specimen, noticed that the hyphae dissolve immediately in alkaline solutions. This striking phenomenon is generally known only in *Poria cinerascens*, where it is given as a diagnostic character, but occurs also in some other species (personal communication by Dr. Z. Pouzar and my own observations).

Parts of something like a brown (not white) easily detachable pellicle are still adhering in some places to the specimen. Lohwag described this pellicle as 'eine Zone gelblich-bräunlicher Hyphen' and measured its thickness as 20–40 μm. He explained it (p. 310) as follows: 'Diese Haut ist jedenfalls nur die äusserste durch die Atmosphärilien veränderte Trama... Dass die Haut an unserem Exemplar gelbbräunlich ist, während sie in der Beschreibung als weisslich bezeichnet wird, ist bei dem Gilben und Bräunen vieler Hutoberflächen beim Trocknen sehr begreiflich. Ferner kann die Oberfläche des Pilzes tatsächlich in der Jugend flaumhaarig sein, da die Hyphen stellenweise hinausgerichtet sind.' This pellicle consists of an amorphous substance in which the hyphae of the same type as in the rest of the fruitbody are embedded or by which they are agglutinated.

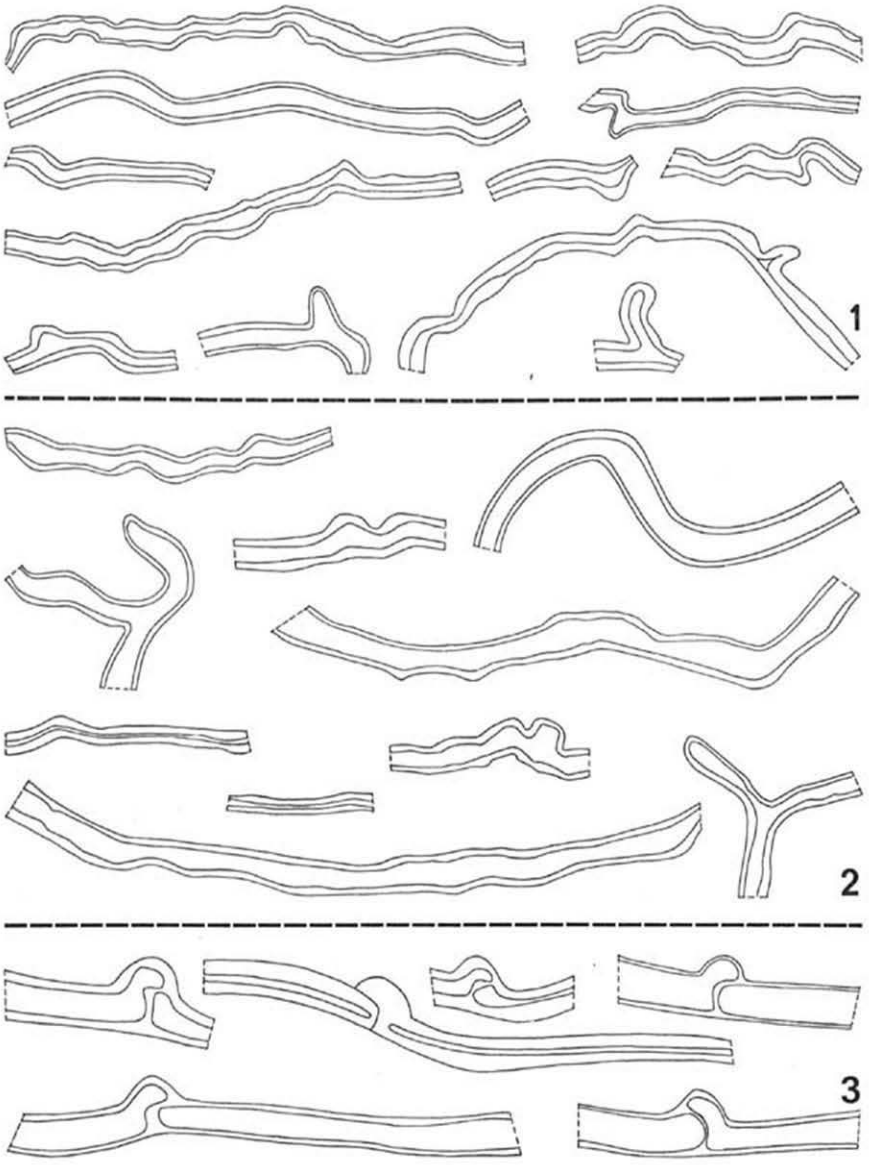
The specimens are sterile, as Schulzer observed, without traces of a hymenium or of generative hyphae.

Schulzer wrote, in a discussion on mycological herbaria (1866), that particularly during his active service he did not have the opportunity to make a collection of specimens. Also, he was of the opinion that, particularly in the case of fleshy fungi, it is far better to make a detailed description and drawing of a species than to preserve a specimen which loses its colour, shape, and other macroscopic characters (which were at that time more important for determination than the microscopical ones). Therefore, apparently he did not attach any importance to specimens which he sent to other mycologists and nowhere he mentioned the existence of a specimen of *P. irpex*. However, after repeated study of his description and of the specimen in Vienna, I came to the conclusion that the latter represents, in fact, the type material of *Polyporus irpex* (*P. schulzeri*). The most important fact supporting this view is the close correspondence of the structure of the hyphae of the specimen with the description of this structure in both manuscripts and with the drawing of it in the first manuscript, which I saw only recently (this particular figure is omitted in the second manuscript and in Kalchbrenner's work). As to the upper surface, Schulzer has repeatedly emphasized that it was 'tomentoso-lanatus' or 'sehr filzig-wollig' and I see no reason to doubt his word. The so-called pellicle can be explained as having originated from collapsing and agglutinating of the surface hairs; this can only just be guessed in one or two places. This explanation is supported by the fact that the hyphae in the tubes are also agglutinated by an amorphous hyaline substance — probably the remains of the hymenium, or, at least, of the generative hyphae. A confirmation of this supposition will be given later.

As to the specimen of *P. irpex* from Hazslinszky's herbarium, which consists of one small, thin segment: it proved to be *Laetiporus sulphureus* (Bull. ex Fr.) Murrill. The numerous spores are similar to those in *S. litschaueri*, although smaller, but the thin-walled, very broad and branched hyphae were conclusive. I do not believe that Schulzer for a moment mistook this collection for *P. irpex*. In his second manuscript he plainly says that he found this species, except near Mohács, only once near Vinkovci; moreover he denies in rather strong terms that Hazslinszky ever saw it.

So it appears that the collection in Vienna under the name *Polyporus irpex* is the only authentic collection available of Schulzer's species. Its microscopic characters prove that *Polyporus irpex* is wholly different from *Spongipellis litschaueri*. The latter name is the correct one for the species it designates.

Here must be mentioned also the specimens of *Polyporus schulzeri* collected by Kmet' and Linhart. A small part of Kmet's specimens is preserved in the Natural History Museum, Budapest (BP), whilst the majority is in the Slovakian National Museum, Bratislava (BRA). They are all identified as *S. litschaueri*, the material from BP by Lohwag and the material from BRA by Kotlaba & Pouzar. I examined these collections also and agree completely. Linhart's material (in BP) from Baie Herculane (Herkulesbad, Roumania), mentioned by Schulzer in his manuscript, and determined by Lohwag as *S. litschaueri*, consists of three envelopes, apparently one specimen cut into several thin segments. Donk (1972) suggested that it might represent, in fact, *Climacocystis borealis* (Fr.) Kotl. & Pouz. I have examined two of these specimens, one



Figs. 1–3. Hyphae (all × 1,100). — Fig. 1. *Polyporus irpex* (after Schulzer's specimen). — Fig. 2. *Piptoporus soloniensis* (after specimen collected in France in 1969). — Fig. 3. *Spongipellis litschaueri* (after collection from Jugoslavia).

of them revised by both Lohwag and Donk, and I am of the same opinion. The cystidia, characteristic of *C. borealis*, are not always abundant, but they are present all the same. They mostly have a typical fusoid form and are thin- or thick-walled, but there also exist cystidia which are rounded at the top, with thin walls. All forms often have incrustations at the top. The spores are somewhat larger than is usually cited, 6–7, 7 × 3,5–4,2(–4,9) μm. This specimen was supposed to have been collected from beech, but no trace of the substrate remained for checking — and an error in determining the substrate is frequent. Linhart's specimens therefore do not only differ completely from the specimen of *P. schulzeri*, as Lohwag pointed out, but neither are they identical with *S. litschaueri*. Therefore, as the occurrence in Roumania of *S. litschaueri* was apparently based only on Linhart's collection, this species will have to be deleted from the lists of fungi for that country, although, of course, it is probable that it will be found there sometime.

It remains now to establish whether *Polyporus schulzeri* (*P. irpex*) has ever been described under another name. After a prolonged search in the literature, I noticed in the description of another very rare polypore, *Piptoporus soloniensis* (Dub. ex Fr.) Pilát, several characters reminding one of *Polyporus irpex*. Bourdot & Galzin (1928: 607) say: 'Chapeau 8–40 cm... parfois sillonné zoné, velouté tomenteux ou strigueux hispide dans les sillons... tubes... flasques; pores assez grands, 0,5–1,5 mm, irréguliers.... Hyphes flexueuses... à parois assez épaisses... gonflées et presque solubles (sol. KOH)....' Although some other features did not agree, those cited seemed promising, and it was necessary to compare both species.

The exsiccata of *P. soloniensis* were obtained on loan from the National Museum, Prague (PRM). This material consists of two envelopes, duplicates from the herbarium of H. Bourdot, *No. 9213 bis*, collected at Massalas on 5 VIII 1911 (PRM 603632) and *No. 27907*, collected at Frégère on 5 IX 1912 (PRM 603631). The collector in both cases was A. Galzin and the specimens grew on chestnut.

In addition, a specimen of this species, collected in 1969 in France (unfortunately, the locality is unknown) was kindly sent for examination by Mme A. David (Lyon).

The specimen collected in 1912 was immature ("agé de 15 jours") and had almost non-existent tubes with only very small pores being visible in part. The surface was covered by dark brown, almost black, short tufts, incrusted with a resinous substance. The tufts merged gradually into the greyish-yellowish friable context, but, in a few places, something like a cuticle could be noted. The specimen collected in 1911 had well developed tubes, and was covered partly by fragments of a thin, brown cuticle. The context was still more friable, and the specimen presented an almost identical appearance to that of *P. schulzeri* from W. In the specimen collected in 1969 the context and tubes were white. A very thin, yellowish-brownish cuticle partly covered the surface; the tufts were missing.

Microscopically, *P. soloniensis* is made up of the same type of hyphae as *P. irpex*, with a diameter, on average, very slightly larger, 3–4 μm, but varying from 2,5 to 5 μm, whilst the walls are up to 1,5 μm thick. The hyphae were examined in water and, just as in *Polyporus irpex*, they dissolve immediately in alkaline solutions, and in

the tubes were found to be agglutinated by an amorphous hyaline substance. They too stain metachromatically in cresyl blue. The tufts from the surface of the juvenile specimen were first examined in water, but hardly anything could be seen owing to incrustations. On adding ammonia, the preparation was cleared and the tufts shown to be made up of thin-walled, clamped, agglutinated, parallel, generative hyphae, c. 3–7 μm in diameter, with brown contents. The contents of these hyphae turn blue in cresyl blue, but the walls do not seem to stain metachromatically. In the older specimen of Bourdot the brown cuticle, when examined in water, is seen as an amorphous brown substance in which thick-walled hyphae are embedded (as in *P. irpex*), but, after ammonia is added and these hyphae have dissolved, it can be observed, although only locally and with difficulty, that the substance is made up of collapsed brownish hyphae resembling those found in the immature specimen. This fact makes it probable that the cuticle in *P. schulzeri* also originated from such thin-walled hyphae. Bourdot & Galzin (1928: 608) give the measurements of basidia and spores as: '...basides 18–21 × 5–6 μ; spores oblongues ellipsoïdes... 4,5–6–7,5 × 2,5–3–4 μ, lisses ou lâchement et obscurément grènelées.'

In juvenile material, clamped, thin-walled generative hyphae, c. 2–4,5 μm broad, were observed in some places in context and tubes. In the tubes some deformed basidia were seen. Deformed particles, resembling spores, were also noticed.

If one compares only the descriptions of *Polyporus irpex* and *Piptoporus soloniensis* one may be inclined to consider them to be two different species, especially in regard to the descriptions of the pores. On comparing the specimens, however, I have little doubt about their conspecifity. The discrepancy between the descriptions may be explained either by the variability of the species, e.g. it probably can have entire or torn pores, or by different characters emphasized by the few observers who had the opportunity to study this fungus in the fresh state. The different substrata present no difficulties, since other lignicolous species with a preference for oak and chestnut but very rarely occurring on other trees are well known, e.g. *Fistulina hepatica*.

The names *Polyporus soloniensis* and *P. schulzeri* were both published by Fries in 1874. However, the basionym of the first name was published much earlier by Dubois as *Agaricus soloniensis*, and later De Candolle (1815) published the recombination *Boletus soloniensis*. Although the descriptions of species of older authors are often very scanty, giving only a few macroscopic characters, the description by De Candolle (a copy of which I obtained through courtesy of Mme A. David, Lyon) seems to me recognizable, and 'soloniensis' is therefore the oldest epithet for this species.

The question is now whether this species really belongs to the genus *Piptoporus*, which includes now also *P. betulinus* (Bull. ex Fr.) P. Karst. and *P. pseudobetulinus* (Murashk.) Pilát. In his 'Check list of European polypores' (1974) Donk includes *P. soloniensis* in the genus *Piptoporus*, but with a question mark. Indeed it differs from the other two in several respects: in hyphae which dissolve readily in alkaline solutions and stain clearly metachromatically in cresyl blue; in the form and diameter of the pores, and in the form and size of the spores. However, the spores of *P. betulinus* and

P. pseudobetulinus are also different in form and size. The dissolving of the hyphae of *P. betulinus* in alkaline solution has been recorded by Z. Pouzar, but these hyphae remain hyaline in cresyl blue, whereas those of *P. pseudobetulinus* turn violet-blue. A thorough revision of this genus is needed, but will be difficult since two of the species are very rare, and only a few herbarium specimens are available. Fresh material would be indispensable, particularly in the case of *P. soloniensis*, as its descriptions and those of *P. irpex* differ in several points.

For the moment, nothing else can be done but to leave this species where it is now, and here the correct name is *Piptoporus soloniensis* (Dubois ex Fr.) Pilát, with *P. schulzeri* Fr. as a synonym.

This temporary solution leaves *P. schulzeri* exactly where Fries put it, together with *P. betulinus*, the position in the system against which Schulzer protested so strongly! However, he would probably have consoled himself by citing his favourite maxim: 'Einen Irrthum zu berichtigen, ist weit förderlicher für die Wissenschaft als das zufällige Auffinden einer neuen Art.'

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LIGHT AND ELECTRON MICROSCOPIC STUDIES OF THE ASCUS TOP IN SARCOSCYPHA COCCINEA

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(With Plates 46–47 and three Text-figures)

The structure of the top of the ascus in live and fixed *Sarcoscypha coccinea* has been studied with different methods of light microscopy. Electron micrographs have been made of median sections of asci first fixed in 1.5% $KMnO_4$, then postfixed with OsO_4.

Light and electron microscopy give somewhat different but supplementary information on the lateral wall and the top of the ascus in *Sarcoscypha*. In the ascoplasm a funnel and a funiculus have been found. The ascus wall consists of three layers. (1) An outer layer, which after different stainings is visible with the light microscope, corresponds with the two outer strata of the stratified electron-transparent layer, and is very thin in the top. (2) A middle layer, which is formed by the inner stratum of the electron-transparent layer, continues with about the same thickness in the top. (3) An inner layer, which is anisotropic and electron-dense, is deposited on the inside of the wall after meiosis. This layer becomes very thick in the top. Its central part is separated by a conical boundary plane to form the basal part of the opercular plug.

Former studies on the structure and dehiscence of the ascus are discussed. The view that the ascus is suboperculate and characterized by having an interrupted apical ring is refuted.

The structure of the ascus of *Sarcoscypha* and related genera is considered to be of great importance to the taxonomy of the operculate Ascomycetes (or Pezizales).

On the ground of observations made by Chadefaud (1946) and Le Gal (1946a, 1946b) the structure of the apical apparatus of the asci in *Sarcoscypha coccinea* and several related species used to be considered to differ essentially from that of the other Operculati ('Operculés vrais'). They called this structure 'para-operculé' and 'suboperculé' respectively and regarded the fungi concerned as highranking taxa (order or suborder) intermediate between Inoperculati (Helotiales) and the remainder of the Operculati.

Chadefaud (l.c.) distinguished two wall-layers in the asci of *Sarcoscypha coccinea*: a cuticular outer layer which is light-refractive and an inner one which is dull and somewhat hygrophilous. At the top a ridge ('bourrelet') of the internal layer was stated to delimit a subapical space ('chambre sous-apicale'). A very extensive subapical pad ('coussinet sous-apical') covered its inner side, containing a hemispherical mass at the summit. This mass Chadefaud considered homologous with an apical ring in

the asci of Inoperculati like *Leotia*. The existence of a delicate apical tractus he could not establish with certainty. The operculum, called para-operculum by Chadefaud, and formed by the apical cap ('calotte apical') of the outer layer, exactly covers the hemispherical mass. The operculum is very narrow and very thick. A hinge he did not find and apparently the operculum was shot away as a whole from the aperture of dehiscence like a stopper. Shortly before dehiscence, from the top of the ascus a thin apical hood ('capuchon') is set free which probably consists of mucus or excreted matter. The outer layer of the ascus is shortened by folding like the bellows of a concertina. This shortening should produce a circular split around the operculum, after which the surface of the subapical space becomes exposed. The dehiscence mechanism reminds somewhat that what is called 'Jack-in-the-box' by American authors.

Le Gal (l.c.) studied *Sarcoscypha coccinea* and 14 other species, which she considers related, after exsiccati and material conserved in formaldehydic or alcoholic solution.

The problems met in studying this kind of material she expressed as follows (Le Gal, 1946b):

'Nous avons dû procéder à un grand nombre d'observations, car certains organes de l'appareil apical sont d'un examen délicat, et bien qu'existant chez toutes les thèques normalement constituées, ils ne sont souvent visible au microscope et sur matériel non vivant que sur un petit nombre d'entre elles.'

She found that the species studied have essential features in common in the apical apparatuses of their asci. On the ground of minor divergencies three types were distinguished. A large group of species, among which *Sarcoscypha coccinea*, belongs to her first type. Here two layers are distinguished in the ascus wall. Exaggerative swelling, especially of the inner layer, causes constrictions in the ascus profile between the ascospores. At a certain stage of development there is a thickening of the internal wall layer at the top of the ascus. This swelling forms the 'chambre apicale'. In this a rounded mass appears what Le Gal calls the 'coussinet apical'. This mass is more or less thick and has the appearance of an open ring of which the ends come near to each other. She considered the ascus to be a helical structure with a convex dorsal side and a concave ventral side (cf. Chadefaud, 1942). The opening of the ring she found to be situated always on the ventral side, where the opercular hinge is formed. This type of ascus she called a suboperculate ascus. Fungi with such asci belong to the Suboperculati.

According to Le Gal her 'chambre apicale' might correspond with Chadefaud's 'chambre sous-apicale'. She failed to find the very extensive 'coussinet sous-apical' in her material, but she suggested that her 'coussinet apical' was nothing but the hemispherical mass at the summit of Chadefaud's 'coussinet sous-apical'.

Studies of Buller (1934) on the influence of light on the tips of asci made it plausible that in species with quite straight asci like in the closely related *Microstoma protracta* (Fr.) Kanouse[1] there is no evident response to light even in the projecting

[1] =*Sarcoscypha protracta* (Fr.) Sacc.

parts of the asci. The curvature towards light is restricted to the extreme tip, resulting in displacement of the opercular lid towards the more strongly illuminated side of the apex. This displacement is sufficient to direct the jet of spores towards the incident light.

Eckblad (1968, 1972) criticized the cited observations by Chadefaud and Le Gal as he could not find an apical chamber with a ring-like 'coussinet apical'. Even in fresh material of four species of this family, among which *Sarcoscypha coccinea*, he failed to find the slightest indication of the said structures. Most authors in their descriptions seem to use the term 'suboperculate ascus' without defining or illustrating what structure they exactly mean by this term. Eckblad arrived at the conclusion that the Suboperculati as a whole do not possess a suboperculate apical apparatus as originally defined.

In answer to Eckblad's criticism Le Gal (1969) objected [translated]: 'One cannot put merely and simply the Sarcoscyphaceae in the operculates as done by Eckblad, on the excuse that one has not seen the apical ring of which we have spoken. We will call to mind that the place of this apical ring is easily seen at the top of the ascus and between its two wall layers, especially with a little practice, but that for observation of the ring itself, much more is involved. In fact, it is a delicate structure which presents itself as a small mass, mostly colourless, probably of more or less mucilaginous character. To distinguish it, one must have the good fortune to seek for it just at the moment when it becomes turgid. Otherwise, one runs the risk of not noticing it, which is not to say that it does not exist. Others than we have seen it. Sometimes we ourselves have looked for it during weeks and months, without becoming discouraged. So one has to perform very accurate, long-lasting and patient investigations.'

MATERIALS AND METHODS

The material of *Sarcoscypha coccinea* (Scop. per S. F. Gray) Lamb. used for the present study was handed over to me by Mr. G. D. Swanenburg de Veye on 8 March 1974. The origin of the material is unknown (probably of central European provenance). Several mature and ripening fruit bodies were growing on branches of *Fraxinus excelsior* L.

Part of the material was fixed for purposes of light and electron microscopy. The branches with the fungus were placed in an illuminated moist chamber at 12 °C in order to follow further ripening of the fruit bodies. Periods of 8 hours of light with an illumination intensity of about 5000 lux were alternated with periods of 16 hours of darkness.

Living asci were observed in squash-mounts in a slightly hypotonic solution of glucose in distilled water. The slides were examined with Zernike's phase contrast and Zeiss Nomarski's interference contrast optics. Of great value also proved to be observations on unstained asci with polarized light.

Other methods used in light and electron microscopy were the same as described earlier (van Brummelen, 1974).

RESULTS

OBSERVATIONS WITH THE LIGHT MICROSCOPE

To study fresh asci a hymenium fragment must be dissociated. The asci are not found to be arranged in bundles, like in most of the other Pezizales. Each ascus stands on the tip of its ascogenous cell without a lateral crozier. This aporhynchous type of dangeardian element (cf. Chadefaud, 1943, 1953; Berthet, 1964) gives the base of the ascus a markedly straight appearance. Moreover this base is very long and slender.

The shape of the asci is elongated-cylindrical while the top is semiglobular (Fig. 1). They measure 400–450 × 13–15 μm when mature.

In young asci up to meiosis the wall in the lateral region is about twice as thick as in the top. After the beginning of sporogenesis changes in the top occur by which the wall in the top becomes twice as thick as in the lower parts.

The outer layer of the ascus wall is strongly birefringent and rather thin. The outer surface of this layer shows a very peculiar structure. With phase contrast and interference contrast optics or after the use of certain stains (e.g. Congo red, methyl blue, cresyl blue) this surface shows a dense pattern of rarely anastomosing horizontal rings alternating with parallel depressions. In very young asci this pattern is very fine and visible only near the base. As the ripening of asci proceeds this pattern becomes more pronounced (up to about 1 per μm on the average) and extends towards the tip. At maturity the tip is almost reached. It seems as if the outer layer becomes discontinuous during this wrinkling-process (Fig. 1).

The inner layer of the wall in the lateral region of the ascus is not or only weakly anisotropic. It shows a strong tendency to swell, especially in certain fixing-solutions and diluted alkali.

Shortly after meiosis a rather thick zone is differentiated on the inner side of the wall in the ascus top. This zone, which stains distinctively with Congo red, forms the inner covering of the distal end of the ascus. Soon this zone is further differentiated in an apical lenticular body (about 1.8–2.8 μm thick and 4.5–6.0 μm in diameter), a surrounding ring-shaped fold (*c.* 0,5 μm wide), and a more lateral subcylindrical part (Fig. 2A, B).

These parts show different degrees of anisotropy. Especially the lenticular body is strongly birefringent. The boundary between the lenticular body and the ring-shaped fold becomes more distinct at maturity. In many cases the boundary between the ring-shaped fold and the surrounding more lateral zone cannot be distinguished.

From the boundary of the lenticular body downwards in the epiplasm a cylindrical or funnel-shaped structure is observed with phase or interference contrast (Fig. 2A).

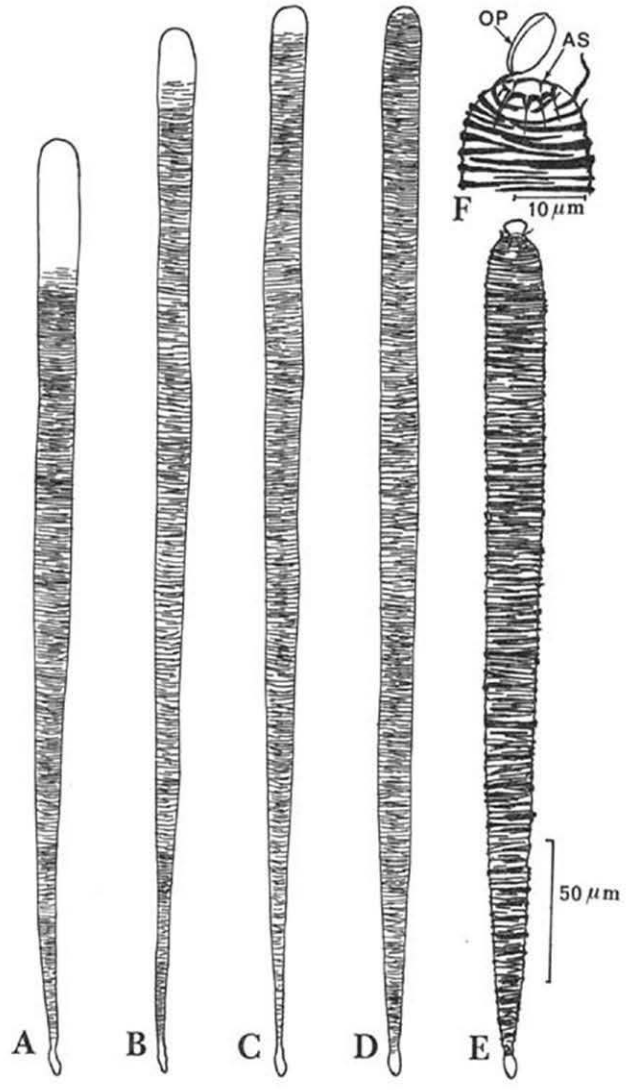

Fig. 1. *Sarcoscypha coccinea*, asci at successive stages of development as seen from the side with light microscopy. — A. Before meiosis. — B, C. Ripening asci. — D. Mature ascus. — E. Ascus after spore discharge. — F. Detail of top after discharge.

Lower down the cytoplasmic threads of this structure are united to form a funiculus which sometimes can be continued till the last spore.

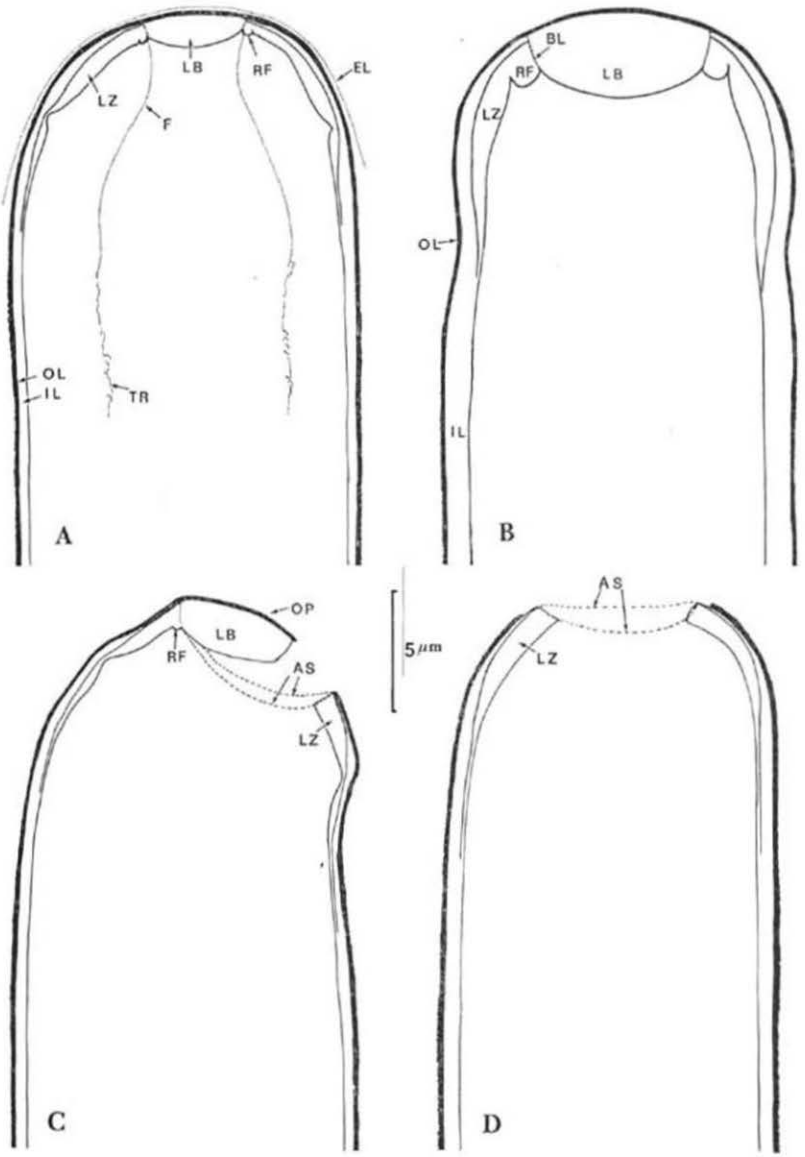

Fig. 2. *Sarcoscypha coccinea*, diagrammatic sections of ascus tops, as seen with light microscopy. — A. Almost mature ascus of which the spores have left through a rupture in the basal part. — B. Ascus in the same stage observed in a solution of Congo red in diluted ammonia, showing thickened walls and a weak ridge ('bourrelet'). — C, D. Asci after spore discharge.

Usually the epiplasm in the apex or ascoplasm is absent or very restricted. In fully mature asci the top of the uppermost spore is often found to be in contact with the lenticular body of the apex. The position of the lenticular body is mostly horizontal and exactly apical, but slight inclination is also observed.

The apical part of the ascus is often covered with a thin hood consisting of a thin layer of readily staining mucilaginous substance (Fig. 2A).

Shortly before ascospore discharge the inner layer of the ascus wall is weakened along the boundary line of the lenticular body (Fig. 2B) while the strength of the outer layer seems to be reduced when the pattern of rings in the ascus surface draws near to the tip (Fig. 1).

Ascus dehiscence is provoked by a rupture along these weakened lines. The lenticular body together with the corresponding outer layers on top form the operculum, which is shot away like a stopper (Fig. 2C, D).

The ascostome (Seaver, 1928) or the line of dehiscence (Boedijn, 1933) is more or less frayed or torn after ascospore discharge (Fig. 1F). In about equal numbers the opercular plug has been found completely severed from the ascus top and shot away or kept hanging on one side to a fringe of the outer layer. After ascospore discharge the empty ascus shrinks irregularly and collapses.

No part of the ascus wall shows a specific reaction with iodine.

Electron microscopy

Among the fixations and embeddings used in this study, the $KMnO_4$-OsO_4-fixation followed by Epon-embedding produced images with sufficient contrast in the walls of asci. All observations are based on such material.

Up to the beginning of sporogenesis the walls of asci look rather homogeneous and do not show a layered structure. The thickness of the wall in the lateral region measures 1020–1200 nm, while it reaches only approximately 340 nm at the top (Pl. 46A, B).

In these young asci the outer surface is already finely wrinkled from the base up to about 15 μm under the tip.

The plasma membrane is rather smooth in the lower part of the ascoplasm but often denticulate or irregular at the top. During the last stage of ripening of the ascospores the epiplasm disappears almost completely from the upper part of the ascus. If in rare cases there is any ascoplasm left at the top of the mature ascus this does not show any special structure.

During ascosporogenesis further differentiation of the ascus wall is observed especially near the top. Soon an electron-dense inner layer can be distinguished (Pl. 46G; 47A–H). In the lateral region of the wall this layer measures 120 to 330 nm but in the apex it may reach 1820 nm. In the top this layer is slightly less electron-transparent and more complex. Often a stratification or orientation of elements parallel to the ascus surface is visible (Pl. 46D; C–E, G).

During further ripening on the inside of the wall two circular furrows can be

distinguished, which divide the inner layer at the top in three different parts: an apical lenticular body, a surrounding narrow ring-shaped zone between both furrows, and an outer more lateral part (Pl. 46D, G; 47C, D; Fig. 3A).

At maturity the lenticular body is clearly segregated by a sharp and regular boundary-plane. The lateral side of this body is obconical in shape. At the base it measures 2470 to 3200 nm across, while the upper diameter is 3600 tot 4040 nm. In the centre it reaches a thickness of 1390 to 1820 nm.

In the ring-shaped zone the layer is 950 to 1200 nm thick and the distance between both circular furrows measures 440 to 600 (rarely 950) nm. The more lateral part of this layer gradually decreases in thickness and ends rather sharply in the middle of the inner layer of the ascus wall of the lateral region (Pl. 46G; 47A, C, G).

The outer part of the ascus wall in the lateral region consists of an electron-transparent layer 800 to 1300 nm thick. Only under favourable conditions in sufficiently contrasted sections a stratification parallel to the ascus surface is found. At least three strata are observed: an inner rather thick one which continues in the top, a middle one which becomes thin and vague in the top, and an outer one (230 to 340 nm thick) which contains the roughly wrinkled outer surface and ends where the top narrows (Pl. 46C, E, F; Fig. 3A).

In thin sections the outer surface of the ascus is accentuated by a double membrane with raised contrast, which follows continuously all folds and wrinkles of the outline (Pl. 46D; 47A, B, G).

An extra-ascan layer covers the top of the ascus where it reaches a thickness of 110 to 138 nm. (Fig. 46A, B, D, G; 47C, E, G; Fig. 3A). In lower regions it becomes thicker (160 to 250 nm) and less distinct.

At maturity the boundary of the lenticular body in the top becomes more conspicuous as an electron-dense line. The surface of the ascus becomes more roughly wrinkled also in the apical region (Pl. 46G; 47A, F–H).

At ascus dehiscence the lenticular body and the corresponding parts of the covering electron-transparent layer and the extra-ascan layer are shot away. The preformed boundary of the lenticular body is smooth while the other layers are more or less torn during dehiscence (Pl. 47E, F; Fig. 3B). In emptied asci the circular furrows in the inner layer of the top are smoothened, while the upper surface of this layer is always damaged (Pl. 47F–H; Fig. 3B). In such asci an excessive swelling of all layers of the wall is observed. This swelling produces walls that are about twice as thick as before dehiscence (Pl. 47F–H).

DISCUSSION

The pictures obtained with electron microscopy seem to differ from those seen with the light microscope in regard to the layers revealed in the ascus wall. In living asci, as well as in stained thin sections, a very thin chromophil outer layer and a thick chromophobe inner layer are visible. Electron microscopy displays a very thick

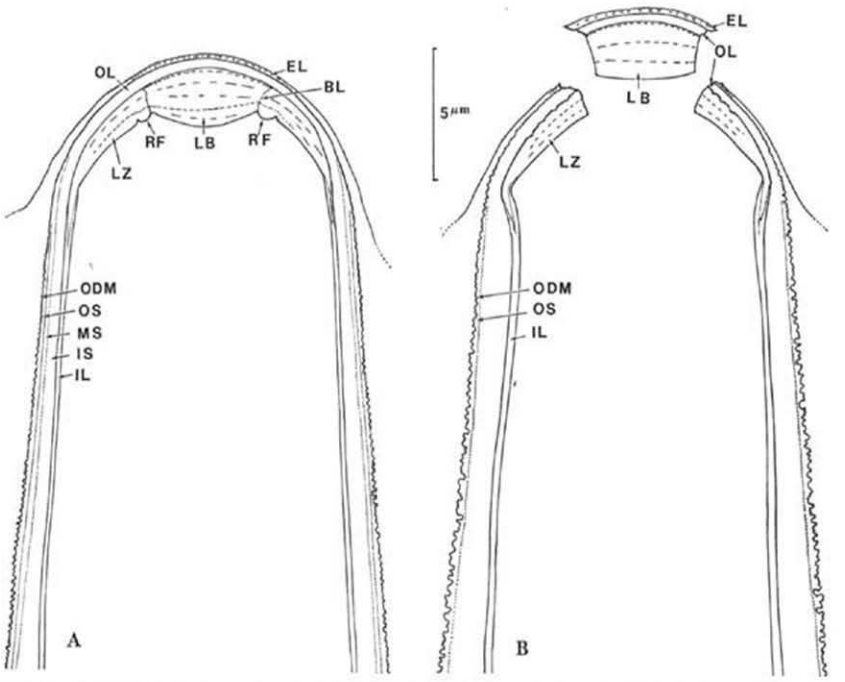

Fig. 3. *Sarcoscypha coccinea*, diagrammatic sections of ascus tops as seen with electron microscopy. — A. Almost mature ascus. — B. Ascus after spore discharge.

stratified electron-transparent outer layer and a thin electron-dense inner layer which is formed during sporogenesis.

When the boundaries of the different layers, as seen with both methods of observation, are compared, the chromophil layer of light microscopy seems to correspond with the outer two strata of the outer layer as revealed with the electron microscope. The inner rather thick stratum of this electron-transparent layer, which also continues in the top, may be regarded as an individual layer. For the time being the terms used are mainly descriptive ones. The introduction of new terms should be based on a comparative study of asci in different families of the operculate Ascomycetes.

In the living state the ascus wall is rather thin. After ascospore discharge the walls of emptied asci contract and swell considerably. The same change is observed in ripening asci in fixed and preserved material.

In live asci of *Sarcoscypha coccinea* the ascospores are free from the lateral wall and longitudinally arranged in a single row in the upper third of the ascus. At maturity one end of the uppermost spore is usually in contact with the base of the lenticular body in the top. Probably all eight spores are ejaculated more or less together, since many groups of up to eight spores are found fixed to a coverglass held closely above a hymenium with mature asci.

The disturbance of the submicroscopic structure of the upper part of the thick inner layer in the top of emptied asci may well be caused by the forcible discharge of the ascus. This damage is not found in the corresponding part of this layer in the plug. Even in living asci there is a discrepancy between the width of mature ascospores and the inner diameter of the ascostome. That the top of the ascus is damaged during discharge is also demonstrated by the frayed or torn ascostome as shown with the light microscope.

In literature many pictures are given of strongly contracted and swollen asci of *Sarcoscypha* and related genera (e.g. Le Gal 1946b, 1953; Denison 1972). Here the ascospores are depicted in close contact with the constricted ascus walls. Such pictures may lead to wrong interpretations. In his study of the genera *Phillipsia* Berk. and *Cookeina* O. K. Boedijn (1933) proved that interpretations based on post mortem material do not agree with the actual events. This is especially relevant in observations concerning the ejaculation mechanism of ascospores.

Irrespective of a few virtual or hypothetical structures that are mentioned to demonstrate a supposed phylogenetic relationship, Chadefaud's (1946) description of the ascus of *Sarcoscypha coccinea* has been confirmed in the essential points. The ridge ('bourrelet') he described in the inner layer is not found again, and his 'chambre sous-apical' is occupied for the greater part by the uppermost ascospore.

The depression in the middle of the lenticular body, giving rise to a 'chambre oculaire' and a surrounding 'pendentif', as illustrated in later publications of Chadefaud (1960: 546; 1973: 157), has not been found in the present study.

In the study of Le Gal (1946b), which has had great influence on the taxonomy of the Pezizales, a terminology is used which is partly different from that of Chadefaud (1942, 1946). In some cases Le Gal used slightly altered terms for quite different structures.

By comparison it becomes evident that the 'chambre sous-apical' of Chadefaud is a more or less hypothetical space dependent on the presence of an inner ridge ('bourrelet') in the top of the ascus. It may contain some acroplasm. The 'chambre apical' of Le Gal is Chadefaud's 'coussinet sous-apical', which corresponds with the entire thick zone formed at the inner side of the wall in the ascus top. The 'coussinet apical' as defined by Le Gal seems identical, as far as the place is concerned, with Chadefaud's 'masse hémisphérique du coussinet sous-apical'. This was also ascertained by Le Gal (l.c.) for the case of *Sarcoscypha coccinea*. In this species the place of this structure corresponds with that of the lenticular body in the apex.

Le Gal described the 'coussinet apical' as an open ring in *Sarcoscypha* and related genera. The presence of this open ring was considered the most important character for the Suboperculati. In fresh asci of *S. coccinea*, however, no indication of a ring-shaped structure is found on this place during any stage of development.

For matters of comparison asci of *Sarcoscypha coccinea* and several species of related genera have been studied after dried material. Especially when observed in a solution of Congo red in diluted ammonia, as preferably used by Le Gal, the ascus wall is altered by contraction and swelling. Considerable shrinkage of the opening at the top

is observed. In the top of asci in *Sarcoscypha coccinea*, *Phillipsia domingensis* (Berk.) Berk., *Cookeina sulcipes* (Berk.) O. K., and *Wynnea americana* Thaxt. comparable structures are found. In the last three the construction of the apex is asymmetrical. The only structure which gives the impression of a ring is the boundary plane between the lenticular body and the ring-shaped fold. This plane stains more intensely with Congo red and becomes evident only shortly before ascus dehiscence. This ring, however, is not sharply delimited on the outside.

The ring-shaped fold itself is usually not contrasted. In asymmetric apices this fold is rather conspicuous and thick at the base of the lid. This is in contradiction with Le Gal's ring which is open just at this place.

In one case only Le Gal illustrated the 'coussinet apical' in an emptied ascus. In *Phillipsia domingensis* the ring is drawn just at the border of the ascostome (Le Gal, 1946b: fig. 7, 2). There can be no doubt that here the 'coussinet apical' is identical with the ring-shaped fold or Boedijn's (1933) 'gelatinous ring'. Also the remark by Le Gal (1969) that one has to look for it just at the moment when it becomes turgid, suggests that it is a swollen structure, which might be more applicable to the ring-shaped fold than to the lenticular body in the top. The boundary plane of the lenticular body as well as the ring-shaped fold are perceptible as complete rings.

The conclusion may be drawn that Le Gal's 'coussinet apical' represents different structures in the top of the ascus. Very probably it is an artifact of swollen material which is not found in living asci.

The 'coussinet apical' is the only character of the suboperculate ascus and the original common basis for the Suboperculati or suborder Sarcoscyphineae. As already argued by Eckblad (1968, 1972) there is little left of the hypothesis that the Sarcoscyphineae form a taxon intermediate between Inoperculati and Operculati. The basis for a close phylogenetic relationship of Sarcoscyphineae and Inoperculati is absent.

On the other hand, the ascus of *Sarcoscypha* and related genera may represent a special type of operculate ascus within the Pezizales. This type could be characterized by a thick anisotropic layer at the inner side of the wall in the top, a thick stratified lateral wall and a long, narrow, aporhynchous base.

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Explanation of Plates 46–47

ABBREVIATIONS USED IN PLATES AND TEXT-FIGURES. — AS, ascostome; AW, ascus wall; BL, boundary line of lenticular body; E, epiplasm; EL, extra-ascan layer; F, funnel; IL, inner layer of the ascus wall; IS, inner stratum of outer wall layer; LB, lenticular body; LZ, lateral zone of thickened inner layer in ascus top; MS, middle stratum of outer wall layer; ODM, outer double membrane; OL, outer layer of the ascus wall; OP, opercular plug; OS, outer stratum of the outer wall layer; PM, plasma membrane; RF, ring-shaped fold in thickened inner layer of ascus top; S, ascospore; TR, tractus or funiculus.

GENERAL DIRECTIONS. — All material illustrated has been fixed in 1.5% $KMnO_4$, post-fixed in OsO_4, embedded in Epon, and stained with uranyl acetate and lead citrate. The scale markers in Plates 46 and 47 equal approximately 1 μm.

Plate 46

Figs. A-G. *Sarcoscypha coccinea*, electron micrographs of developing asci: Figs. A, B. median sections of distal portion of young asci before meiosis; Figs. C, E. longitudinal sections of the distal portion of ripening asi showing stratified outer layer of ascus wall; Fig. D. detail of

median section of apex of ripening ascus; Fig. F. detail of lateral ascus wall in longitudinal section; Fig. G. median section of distal portion of ripening ascus, with epiplasm and ascospores retracted.

PLATE 47

Figs. A–H. *Sarcoscypha coccinea*, electron micrographs of ripening and collapsed asci: Figs. A, D. detail of median section of ripening ascus near apex; Fig. B. transverse section of lateral wall in almost mature ascus; Fig. C. median section of ripening ascus near apex; Fig. F. median section through distal portion of collapsed ascus shortly after spore discharge, showing opercular plug; Figs. E, G. details of section shown in Fig. F; Fig. H. longitudinal section through collapsed ascus, showing the disturbed structure in the upper part of the lateral zone of the thickened inner layer in the top, also showing the strong swelling of the lateral ascus wall.

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RACODIUM PERS. NOT A GENUS OF LICHENES

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Racodium Pers. per Fr. 1821 (starting-point date of nomenclature, 1821) has to be typified by *Racodium cellare* Pers., which makes it a genus of imperfect fungi (Deuteromycetes). Even if *Racodium* Fr. 1829 is accepted as a genus nomenclatively distinct from *Racodium* Pers. per Fr. 1821 and as such is referred to the (imperfect) lichens (starting-point date, 1753), it still must be rejected as a later homonym. *Zasmidium* Fr. 1849 is another name for *Racodium* Pers. ex. Fr. 1821 and a later synonym of it. For the lichen genus *Racodium* Fr. 1829 the substitute name *Rhacodiopsis* Donk, nom. nov. is introduced and for its type species the name *Rhacodiopsis rupestris* (Pers. per. S. F. Gray) Donk, comb. nov.

In two recently published papers (Riedl, *1968*; Hawksworth, *1970*) their authors came to the conclusion that *Racodium* Pers. has to be typified by *Racodium rupestre* Pers., a genus of 'Lichenes imperfecti'. Riedl also concluded that *Racodium* Pers. if conceived as a genus of fungi cannot compete with the lichen genus because it was published before the starting-point date of nomenclature of the 'Fungi caeteri' of the 'Code' (1821) the starting-point date for lichens being 1753. Finally he concluded that *Zasmidium* Fr. 1849 was the correct name and genus for one of the original fungous elements, viz. *Racodium cellare* Pers. Hawksworth is less specific as to the last conclusion; he stated that "the species of non-lichenized 'Mycelia Sterilia' currently placed in *Racodium*, should therefore be transferred to other genera." He overlooked Riedl's paper.

I beg to disagree. Both Riedl and Hawksworth overlooked many facts pertinent to the subject. Most of these data were briefly mentioned in a résumé by Donk (*1962*: 96). The following lines do not contain new data in relation to typification; they include *inter alia* merely an elaborated version of my previous remarks.

Racodium Pers. 1794 (devalidated name). — The genus was already published before 1801, the date given by Riedl. It was introduced (Persoon, *1794*: 123) with three species, viz. *Racodium aluta* Pers., *R. cellare* Pers., and *R. rupestre* Pers. Of these, the undisputable lectotype species is *R. cellare*. The reasons for this explicit conclusion are the following.

(i). The genus *Racodium* was considerably restricted by Link (*1809*: 21, 22, 23), who excluded two of the three original species. One was transferred to *Xylostroma*

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Tode; this is *R. aluta*, the first of the examples mentioned when the generic name was first published. The other was transferred to *Dematium* Pers.; this is *R. rupestre*, the imperfect lichen Riedl and Hawksworth had in mind. *Racodium* itself was restricted to a single and original species, *R. cellare*: 'Hujus loci *Racodium cellare* Pers.'

(ii). That to the author of the generic name himself *R. cellare* was the principal species becomes quite evident from reading a later account of the genus published by him: —

'Un autre genre, que communément on regarde aussi comme une espèce de moissure, est cette villosité à filaments dense qui couvre les vieux tonneaux de vin.... L'espèce vulgare est le Bisse des caves, *Racod. cellare*; bien sec, il peut servir d'amadou. Le *Racodium rupestre* est d'une texture plus serrée, et il est plus noir, presque toujours mêlé avec la croute blanche d'un *Lepraria*, avec lequel il croît sur les rochers.' — Persoon (1818: 60).

The introductory sentence to the genus; the way *R. cellare* is mentioned; and the fact that *R. rupestre* is spoken of by comparing it with the former species, all these facts leave no doubt about the importance Persoon himself attached to this member of *Racodium*.

(iii). The first author to indicate a lectotype species for *Racodium* in a straightforward way completely in agreement with the present 'Code' was de Brongniart (*1824*: 545). He followed Link and indicated *Racodium cellare* as the type of the generic name '*Racodium* [Pers. emend.] Link; *Racodii* spec. Pers.'

In my opinion *Racodium cellare* has to be retained as type species as long as it has not been explained why it should be abandoned as such in agreement with the 'Code'. The selection of *R. aluta* by Hughes (*1958*: 800) cannot stand the test of the above-raised arguments.

Racodium Pers. per Fr. 1821. — The generic name was validly published on the (arbitrarily fixed) starting-point date (January 1, 1821) and in the starting-point book (Fries, *1821*: xlvi), where the genus was accepted as '*Racodium* P.,' without any description comment; the reference 'P.' (=Persoon) ensures the valid publication of the name for a genus in Persoon's o r i g i n a l sense. The genus was also accepted in the same year by Hooker (*1821*: 34), with one species, *R. cellare*; and by Gray (*1821*: 557) again with one and the same species. Other authors followed, one of them being Persoon (*1822*: 67), with *R. cellare* as the first of 18 (and 3 doubtful) species (and *R. rupestre* as the fourth). More examples of authors who did not deviate from Persoon's or Link's conception can be given from the period between valid publication in 1821 and Fries's introduction of a homonymous genus in 1829. Gray (*1821*: 556) was the first author explicitly to exclude *Racodium rupestre* from *Racodium* Pers. after the starting-point date (1821). As stated above de Brongniart in 1824 was the first author explicitly to indicate the type species (*Racodium cellare*) for *Racodium* Pers.

Racodium Fr. 1829. — In a later volume of the starting-point book for 'Fungi caeteri' of the 'Code' Fries (*1829*: 229, in obs.) all at once went his own way; as he so

often did, he ignored what his predecessors had done: 'Generis *Racodii* mihi typus est species primaria in Pers. synopsi [*1801*: 702], nempe *R. rupestre*, quod ob vitam perennem, rupicolam etc. a Fungis exclusum ad Byssaceas refero.' Donk (l.c.) concluded that 'it would seem that Fries, when rejecting Persoon's genus *Racodium*, introduced a new one, *Racodium* Fr. (non Pers.), rather than that he misapplied the earlier name.' Compare also Fries (*1832*: Index p. 151) in a foot-note appended to *Racodium rupestre*, 'Byssacea', 'Huic et similibus a myceliis saltim certe diversis nomen servo.' Even, if it were to be concluded that Fries emended the earlier generic name, then '*Racodium* Pers. sensu Fr.' will have to be corrected into '*Racodium* Fr.' because he excluded the type, which he transferred to *Antennaria* Link (Fries, *1829*: 229); This correction would be necessary to remain in accordance with the present wording of the 'Code' (Art. 48).

It will be obvious from the above that as a later homonym *Racodium* Fr. 1829 is impriorable (illegitimate) in view of *Racodium* Pers. per Fr. 1821.

Zasmidium Fr. — There is no need to adopt another generic name for *Racodium cellare* as long as the above-sketched facts are accepted as basically correct. *Zasmidium* becomes superfluous by assuming, first, that Fries had the same fungus in mind as Persoon when he published this new genus (Fries, *1849*: 407) for it, and secondly, that Riedl is correct in equating the bodies that Fries called 'perithecia' with 'amorphe Klümpchen von Exkretionen'.

Correct spelling. — As far as I am aware the variant spelling *Rhacodium* 'Link' was first used by Sprengel (*1827*: 557). It has found a wide application and was expressly defended by Guéguen (*1906*: 81 foot-note); etymologically it is the more correct spelling if it is accepted as being derived from ῥάκος, rag.

Correct names. — Judging from my notes (which may be far from complete) I would conclude that the correct name for the type species of *Racodium* Pers. per Fr. 1821 is

Racodium cellare Pers. per Hook.: Fr. — *Racodium cellare* Pers. *in* Neues Magazin Bot. **1**: 123, 1794 (devalidated name). — *Racodium cellare* Pers. *per* Hook., Fl. scot. **2**: 24. 1821; S. F. Gray, Nat. Arr. Br. Pl. **1**: 557. 1821. — *Antennaria cellaris* (Pers. per Hook.) Fr., Syst. mycol. **3** (1): 229. 1829. — &c.

In case the typification of *Racodium* Pers. per Fr. 1821 by *Racodium cellare* Pers. is accepted the lichen species will appear to be nameless for the period before 1821 because the generic appellation of the binomium *Racodium rupestre* was not validly published during that period. The first validly published name for the species seems to be *Dematium rupestre* (Pers.) per S. F. Gray 1821.

The next problem to be solved is to provide the correct generic name for this species. A generic name often cited as a synonym of the lichen genus *Racodium* is *Cystocoleus* Thwait. (*1849*: 341) but this name must be kept for a different genus as was pointed out by Hawksworth. I have also thought of *Kanta* Adans. (*1763*: 3) and

Loten Adans. (*1763*: 3) as possible recipient genera but they do not appear to be typifiable by a species that is conspecific or congeneric with *Racodium rupestre*. Since a distinct genus for this species appears to be in order I revive *Racodium* Fr. 1829 but providing it with the necessary substitute name: **Rhacodiopsis** Donk, *nom. nov.*; basionymum, *Racodium* E. M. Fries, Syst. mycol. **3** (1): 229. 1829; holotypus, *Racodium rupestre* Pers. The correct specific name becomes

Rhacodiopsis rupestris (Pers. per S. F. Gray) Donk, *comb. nov.* — Basionymum: *Dematium rupestre* (Pers.) *per* S. F. Gray, Nat. Arr. Br. Pl. **1**: 558. 1821. — *Racodium rupestre* Pers. *in* Neues Magazin Bot. **1**: 123. 1794=Tent. 43, 76. 1797 (generic name not validly published). — *Byssus rupestris* (Pers.) DC., Lam. & DC. Fl. franç., 3e Ed., **2**: 592. 1805 (generic name not validly published). — *Dematium rupestre* (Pers.) Nees, Syst. Pilze 76 *pl. 5 f. 73*. 1816 (generic name not validly published). — *Racodium rupestre* (Pers. per S. F. Gray) Pers., Mycol. europ. **1**: 68. 1822. — &c.

This synonymy presupposes that the generic name *Byssus* L. (*1753*: 1168) has as its selected type species (*cf.* Drouet & Daily, *1956*: 145) *Byssus flos-aquae* L. (type in Linnaeus's herbarium)=*Oscillatoria prolifica* (Grev.) per Gom., a species of Nostocaceae Homocysteae, of which the starting-point date is 1892–3.

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MARATTI'S GENERIC NAMES FOR FUNGI

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The generic names for fungi used by Maratti in his 'Flora romana' must be accepted as validly published. Notes are given on the validly re-published names. Of these *Agaricum* and *Coralloides* may cause some difficulties. Conservation of *Fomes* (Fr.) Fr. against *Agaricum* [Mich.] Maratti is proposed. To the nomina rejicienda of the conserved name *Ramaria* (Fr.) Bon. *Coralloides* [Tourn.] Maratti should be added.

Maratti's 'Flora romana', vol. 2[1] ends with an enumeration of fungi. Some of the fungus genera are placed in 'Cryptogamia Algae' and (together with some genera of liverworts and algae) appear with no supplementation of their names by generic descriptions or references to such descriptions. If they had been new genera these would therefore not have been validly published. They are *Botrytis* Mich. (p. 444) and *Mucor* Mich. (p. 451), belonging to the 'Fungi caeteri', starting-point date January 1, 1821; and *Lichen* L. (p. 430) belonging to the Lichenes, starting-point Linnaeus's 'Spieces Plantarum', 1753. The genus *Tremella* Dill. = *Tremella* L. (p. 443), not *Tremella* Pers. per St-Am. 1821, can in my opinion be typified by *Tremella nostoc* L.; it is now known as *Nostoc* Vauch. per Born. & Flah. ('Nostocaceae heterocysteae'), a genus of algae.

The genera of 'Cryptogamia Fungi' present a different picture. An introductory remark on page 453 makes it clear that Maratti relied in the first place on P. A. Micheli's 'Nova Plantarum genera', 1729. He followed the author very closely, for instance by copying the generic descriptions from his work. The species are listed without 'trivial names' (specific epithets). Many of Micheli's species were not included, apparently because they were not known to occur in the geographically restricted region covered by the 'Flora romana'.

The lack of 'trivial names' in certain sections of Maratti's 'Flora romana' should not be invoked as a reason for rejecting the generic names. That he did not consistently employ the Linnaean binomial system of nomenclature does not a priori outlaw his g e n e r i c names. If these comply with the 'Code' they must be accepted as validly published. There are several thousands of generic names (mainly published as the 'overflow' of the pre-Linnaean era) that were validly published even though the works in which they occur did not comply with Linnaeus's binomial system. The

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[1] J. F. Maratti: Flora romana. Opus posthumum nunc primum in lucem editum, vol. 2, Roma, 1822.

introduction to Dandy's 'Index of generic names of vascular plants 1753–1774' (*1967*) is recommended for consultation by those mycologists who hesitate to accept generic names that cannot be directly associated with binomials.

The impact on the prevailing nomenclature of the generic fungus names discussed below is not very important. Only two names cause some difficulties; these are mentioned under *Agaricum* and *Coralloides*.

Some notes on the validly re-published names follow.

AGARICUM [Mich.] Maratti, Fl. romana **2**: 455. 1822. — *Agaricum* is an ancient name for a fungus that was highly esteemed for its alleged medical properties. It occurs in Europe on species of *Larix* and has been collected so avidly that this may have seriously contributed to its near-extinction in Europe. (Another cause for its scarcity seems to be the paucity of *Larix* stands with old trees.) For a long time the fungus was known as *Agaricus* [or *Agaricum*] *sive fungus laricis*. It entered validly published nomenclature as *Polyporus officinalis* (Vill.) per Fr. 1821. The variant *Agaricus* was adopted by de Tournefort (*1694*: 441; *1700*: 562) and introduced in his binary system covering more or less pileate wood fungi in general. Micheli (*1729*: 117) preferred the old form *Agaricum*, and Maratti accepted the genus as conceived by Micheli. Micheli's (and Maratti's) first species is *Agaricum esculentum &c.* Mich. *1729*: 117 *pl. 60=Fistulina hepatica* (Schaeff.) per Fr. Another of Micheli's species is the above-mentioned '*Agaricum, sive Fungus Laricis* C. Bauh.' (Micheli, *1729*: 119 *pl. 61 f. 1*), which is *Polyporus officinalis*. I select it as the (unavoidable) type species; it is the one and only original bearer of the name *Agaricum*. Although it was not listed by Maratti (presumably because he had not found it in or around Rome) this is not essential since he did not emend Micheli's genus at all but simply copied verbatim the essential parts of Micheli's generic description. In any case it was not excluded by Maratti on purpose.

I have already accepted the consequences of this typification and replaced *Laricifomes* Kotl. & P. 1957 by *Agaricum* [Mich.] Maratti 1822 (Donk, *1971*: 25). I found it impossible to invoke the 'Code' and to treat *Agaricum* [Mich]. Maratti as a mere orthographic variant of *Agaricus* L. per Fr. 1821 (type species, *A. campestris* L. per Fr.; cf. Donk, *1962*: 11).

As long as the monotypic genus *Agaricum* [*Laricifomes*] is not generally accepted however it will not cease to be a nuisance. Authors who accept a genus *Fomes* (Fr.) Fr. 1849 in a conservative emendation inclusive of *Polyporus officinalis* will have to exchange the name *Fomes* for *Agaricum*. It is for the benefit of these mycologists that the following proposal is moved.

PROPOSAL.—Nomen conservandum: *Fomes* (Fries) Fries, Summa Veg. Scand. **2**: 319 (foot-note), 321. 1849. T.: *Polyporus fomentarius* (L.) ex Fr.=*Fomes fomentarius* (L. ex Fr.) Fr.

Nomen rejiciendum: (=) *Agaricum* [Mich.] Maratti, Fl. romana **2**: 455. 1822. T.: "*Agaricum sive Fungus Laricis*" Mich., Nova Plant. Gen. 119. 1729 [=*Polyporus officinalis* (Vill.) ex Fr.].

SUILLUS [Mich.] Maratti, Fl. romana **2**: 458. 1822. — This is *Suillus* Mich. (*1729*: 126). *Suillis* is another old name adopted my Micheli for a genus of the fungi; it corresponds with the Boleti of modern mycologists except for the first two species; these have been referred to *Albatrellus ovinus* (Schaeff. per Fr.) Kotl. & P. ('Polyporaceae').

Gray (*1821*: 646) was the first author after the starting-point date (Januari 1, 1821) to use *Suillus* and cite Micheli as its author. 'Since the one species he retained under it is at least very doubtfully acceptable as the type of *Suillus* Mich., his emendation should rather be considered a misapplication which by the introduction of the later starting-point for these fungi acquired the status of a 'new' genus; hence it is preferable to drop the author's citation Micheli in connection with *Suillus* S. F. Gray.' — Donk (*1955*: 303–304). Gray divided the Boleti (that is, *Suillus* Mich.) over three genera: *Suillus* S. F. Gray, *Pinuzza* S. F. Gray, and *Leccinum* S. F. Gray. He included only one species in the first; with *Pinuzza* his genus *Suillus* was defined as including collared (ringed) species. *Suillus* S. F. Gray is now widely used as a correct name in a much broader sense.

When Kuntze (*1898*: 534) published *Suillus* Haller he actually restored *Suillus* Mich. To Kuntze the starting-point date for generic names was 1735; after that Haller (*1742*: 29) was the first to use Micheli's name. At the same time Kuntze made his conception equivalent to *Boletus* as compiled in Saccardo's 'Sylloge Fungorum' but with the exclusion of the ringed species. The lectotype for *Suillus* [Haller] O.K. is *Suillus fulvus inferne ex flavo virescens* Haller (cf. Donk, *1955*: 304). von Haller cited *Suillus esculentus crassus superne fulvus &c.* Mich. *1729*: 127 as a synonum. Now I accept this as the lectotype for *Suillus* Mich. and *Suillus* [Mich.] Maratti. It is likely that the synonym '*Fungus suillus, esculentus* Caesalp. 617' cited by Micheli suggested the generic name to him. The species to which Micheli referred it is among Maratti's species (by error Maratti wrote 'flavus' instead of 'fulvus'; p. 459). It is difficult to be exact about the identity of Micheli's species; it may belong, or be related, to *Boletus edulis* Bull. per Fr.

Suillus P. Karst. 1882 must be considered still another and later homonym (cf. Donk, *1955*: 305).

POLYPORUS [Mich.] Maratti, Fl. romana **2**: 1822. — This is *Polyporus* Mich. (*1729*: 129), a genus also accepted by Fries in the starting-point book (*1821*: 341); I select as lectotype one of Micheli's species, viz. *Polyporus esculentus &c.* Mich. *pl. 71 f. 1* = *Polyporus tuberaster* (Pers.) per Fr. (cf. Donk, *1960*: 261); it is also one of Maratti's species. With this typification *Polyporus* [Mich.] Maratti becomes a typonym (rather than a homonym) of *Polyporus* [Mich.] Fr. or, technically, a mere application of the latter name. It is also the earliest narrow circumscription of Fries's very expansive emendation of the genus.

ERINACEUS [Dill.] Maratti, Fl. romana **2**: 463, 1822.—This genus was published first by Dillenius (*1719*: 188, App. p. 74) for a single species (the type). When

Micheli (*1729*: 132) accepted the genus he cited Dillenius's sole species as a synonym of his *Erinaceus esculentus, pallide luteus* Mich. 132 *pl. 72 f. 3*, which is also one of Maratti's species. This has usually been identified with *Hydnum repandum* L. per Fr., but I now suggest that Micheli's figure represents *Hydnum rufescens* (Schaeff. per S. F. Gray) Steud. instead.

FUNGUS [Tourn.] Maratti, Fl. romana **2**: 464, 1822.—This genus was introduced by de Tournefort (*1694*: 439; *1700*: 556) for all centrally stalked mushrooms and toadstools with gills and tubes. Micheli excluded those with tubes (*Suillus*, *Polyporus*). The selected type for *Fungus* Tourn. is de Tournefort's first species, '*Fungus campestris albus superne, inferne rubens* J. B. 3. 824' (as cited by him) (cf. Donk, *1962*: 102, 103). It is often supposed to be *Agaricus campestris* L. per Fr. When Micheli (*1729*: 174) adapted the genus he listed de Tournefort's species among his own in precisely the same form as de Tournefort did and I also accept it as the type of *Fungus* [Tourn.] Maratti. Although it is not among the species listed by Maratti, by inference it is one of his original species; he did not exclude it from the genus. By this selection *Fungus* [Tourn.] O.K. (*1898*: 477; *cf.* Donk, *1962*: 102) becomes a later typonym rather than a later homonym, that is, technically, a mere application of *Fungus* [Tourn.] Maratti.

The correct name for the genus is *Agaricus* L. per Fr. 1821.

CORALLOIDES [Tourn.] Maratti, Fl. romana **2**: 483. 1822.—This is another of de Tournefort's generic fungus names (*1694*: 442 *pl. 332*; *1700*: 564 *pl.* **332**) that was accepted by Micheli (*1729*: 209). It was introduced for a wide variety of plants but the two depicted by de Tournefort represent branched clavarias, most likely of the genus *Ramaria*. On a former occasion I felt no necessity for selecting a type (Donk, *1954*: 456) but now that the name appears to have been validly published this must be done.

In the first place it should be pointed out that Micheli excluded some of the Tournefort's species. Moreover, the two examples depicted by de Tournefort were cited by de Tournefort only in connection with his genus as such; the figures were not mentioned separately with any of his species. With this and a few other items in mind attention becomes focused on Micheli's species 1, 2 and 3 which are also among de Tournefort's. These are:—

(1) '*Coralloides flavum* Inst. R. H. 562. *Fungus ramosus, flavus* I. B. 3. 837.' This is doubtless one of the big, terrestrial species of *Ramaria*, although it would be unduly confident to equate it unconditionally with *Ramaria flava* (Schaeff. per Fr.) Quél. in the sense of Schaeffer.

(2) '*Coralloides albidum* Ibid. *Fungus ramosus, albidus* I. B. 3. 837.' This might be rather *Clavulina cristata* (Holmskj. per Fr.) J. Schroet. but I make this suggestion without much conviction.

(3) '*Coralloides dilute purpurascens* Ibid. [≡ I. B. 3. 837]. *xix genus esculentorum Fungorum ii. species* Clus. Hist. CCLXXV.' There can be little doubt that this is the

best known edible species among the ramarias, viz. *Ramaria botrytis* (Pers. per Fr.) Rick.

As the type species of *Coralloides* [Tourn.] Maratti either the first (also listed by Maratti) or the third of these species (not listed) is suggested. Selection of the latter is proposed here; this would establish the application of the generic name with a minimum of doubt. The selection of any of the above species would make *Coralloides* an earlier validly published name for a generic name now in use. The selection of species (3) makes *Coralloides* an earlier name for *Ramaria* (Fr.) Bon., a conserved name, and at the same time its typonym; apparently it is not a nomenclatural synonym (cf. Code, Art. 14, Note 3) since the ultimate type specimens of the two generic type species are not one and the same. Therefore, the following proposal is submitted.

PROPOSAL.—Add to the n o m i n a r e j i c i e n d a of the conserved name *Ramaria* (Fr.) Bon. the following generic name: (=) *Coralloides* [Tourn.] Maratti, Fl. romana **2**: 483. 1822. T.: *Coralloides dilute purpurascens* Tourn. [=*Ramaria botrytis* (Pers. per Fr.) Rick.].

In order to bring them to the attention of mycologists who are engaged in a closer study of the fungi involved the following four names are mentioned but only briefly.

PHALLO-BOLETUS [Mich.] Maratti, Fl. romana **2**: 481. 1822 ≡ *Phallo-boletus* Mich., Nova Pl. Gen. 202. 1729 (pre-Linnaean name); &c.

BOLETUS [Tourn.] Maratti, Fl. romana **2**: 482. 1822 ≡ *Boletus* Tourn., Elém. Bot. 440. 1694; Inst. **1**: 561. 1700; & Mich., Nova Pl. Gen. 203. 1729 (pre-Linnaean name); not *Boletus* Fr. 1821, &c.

The two generic names are synonyms of *Morchella* [Dill.] Fr., published in the starting-point book for these fungi.

LYCOPERDON [Tourn.] Maratti, Fl. romana **2**: 484. 1822 ≡ *Lycoperdon* Tourn., Elém. Bot. 441. 1694; Inst. **1**: 561. 1700: & Mich., Nova Pl. Gen. 217. 1729.—This corresponds to the modern genus *Lycoperdon* Pers. 1801, published in the starting-point book of the Gastromycetes.

TUBER [Mich.] Maratti, Fl. romana **2**: 485. 1822 ≡ *Tuber* Mich., Nova Pl. Gen. 221. 1729 & ≡ *Tubera* Tourn., Elém. Bot. 442. 1694; Inst. **1**: 565. 1700 (pre-Linnaean names).—The correct name for this genus is *Tuber* [Mich.] Fr., published in the starting-point book for these fungi.

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THE PERFECT STATE OF SARCOPHOMA MIRIBELII

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(With six Text-figures)

Guignardia miribelii is described as a new species. It represents the ascigerous state of the fungus of which the conidial state is known as *Sarcophoma miribelii*. Both states are described.

During the 6th European Mycological Congress in Avignon, France, in October 1974, a foray was made in a Cedar forest on the Mont Ventoux. Several interesting Ascomycetes and Deuteromycetes were collected on *Buxus sempervirens*, a common shrub in the area. One such fungus, obtained from thin twigs on withered or almost dead branches, proved to be an unknown species of the genus *Guignardia* Viala & Ravaz, with asci containing a variable number, usually 16 or 32 ascospores. Single ascospore isolates were prepared from ascospores originating from four ripe perithecia. All isolates gave sporulating colonies of a coelomycete, which was recognized as *Sarcophoma miribelii* (Fr.) Höhn. This common and wide-spread fungus frequently occurred on dead leaves and branches of *Buxus* in the area. The ascospore isolates were thus easily compared with fresh conidial isolates and subsequently with several herbarium specimens. These comparative studies showed that the two states belong to the same fungus. The present paper describes both states, with the ascigerous state as a new species.

Guignardia miribelii van der Aa, *spec. nov.*—Figs. 1, 2

Ascomata discreta, nigra, lucida, in cortice submersa, tympaniformia, sursum applanata, epidermide firmiter connexa, deorsum rotundata, margine elevata, in medio maturitate dehiscentia, 100–200 μm alta, 150–350 μm plerumque 200–250 μm diam.; paries superior 15–20 μm, inferior et lateralis ad 50 μm crassus; pars exterior e 1–4 stratis cellularum nigrarum, isodiametricarum, 10–17 μm diam. constat, interior e cellulis subhyalinis, isodiametricis vel collapsis, tenuitunicatis, 7–15 μm diam. Asci numerosi e pulvillo basilari hemisphaerico oriuntur, fasciculati, sessiles vel breviter pedicellati, bitunicati, cylindrici vel clavati, 75–180 × 15–20 μm, 16-vel 32-spori. Ascosporae continuae, ellipsoideae vel ovoideae, hyalinae, leves, maturitate nonnumquam olivaceae et minute punctatae, plerumque 13–19 × 5–7 μm. paraphyses absunt. Typus Herb. CBS 214, in ramulis morientibus Buxi sempervirentis, in Silva Cedri atlanticae, in ascensu Montis Ventosi.

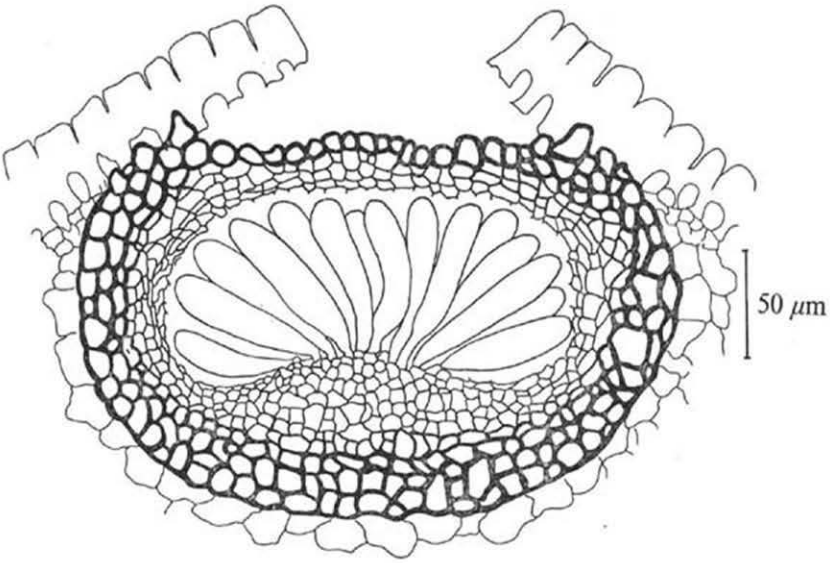

Fig. 1. *Guignardia miribelii* (holotype), ascoma, on twig of *Buxus sempervirens*.

Ascomata discrete, black, shiny, immersed in the bark and firmly grown together with the epidermis, tympaniform, apically flattened, rounded in the lower part, with a more or less raised peridial margin, without papilla, when ripe opening in the middle by dehiscence of the thin wall. Ascomata 100–200 μm high and 150–350 μm, usually 200–250 μm diameter. Wall 15–20 μm thick in the upper part and up to 50 μm thick in the lower and lateral part; the outer layer is composed of 1–4 rows of blackish, isodiametric, thick-walled cells, 10–17 μm in diameter and the inner layer of subhyaline, isodiametrical or collapsed, thin-walled cells, 7–15 μm in diameter. Asci rather numerous, arising from a hemispherical basal cushion of thin-walled isodiametrical cells, sessile or stipitate, bitunicate, cylindrical or clavate, 75–180 × 15–20 μm, usually 16 or 32 spored. Ascospores one-celled, ellipsoidal, ovate, hyaline and smooth for a long time, when young surrounded by a caducous slime layer, in age sometimes becoming olivaceous and finely punctuate, usually 13–19 × 5–7 μm, rarely with a deviating shape and size, up to 40 μm long in asci containing a smaller number of ascospores. Paraphyses absent.

On thin twigs of almost dead branches of *Buxus sempervirens*, Cedar forest, Mont Ventoux, France, 21.10.1974, *H. A. van der Aa 4434* (Herb. CBS 214).

Conidial state: SARCOPHOMA MIRIBELII (Fr.) Höhn.—Figs. 3–5

Sphaeria miribelii Fr. *in* Linnaea **5**: 548. 1830. — *Sphaeropsis miribelii* (Fr.) Lév. *in* Annls Sci. nat. (Bot.) III, **5**: 296. 1846. — *Phoma miribelii* (Fr.) Sacc. *in* Michelia **2**: 90. 1880. — *Macrophoma miribelii* (Fr.) Berl. & Vogl. *in* Atti Soc. ven.-trent. Sci. nat. **10**: 179. 1886. — *Sarcophoma miribelii* (Fr.) Höhn. *in* Hedwigia **60**: 133. 1918.

Sphaeria delitescens Wallr., Fl. crypt. Germ. **2**: 777. 1833, — *Phoma delitescens* (Wallr.) Sacc., Syll. Fung. **3**: 105. 1884.

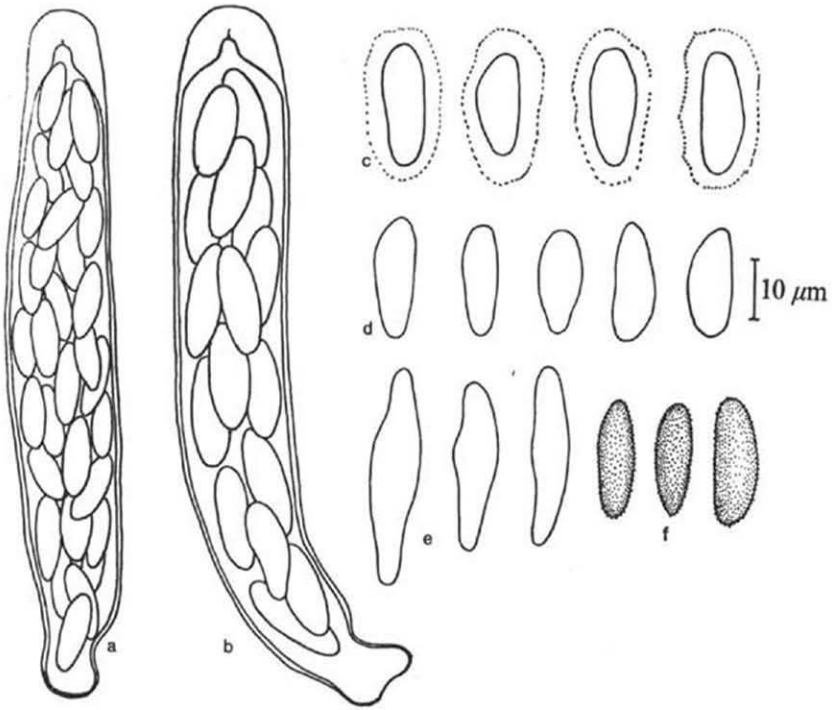

Fig. 2. *Guignardia miribelii* (holotype). — a. Ascus containing 32 ascospores. — b. Ascus containing 16 ascospores. — c. Young ascospores. — d. Ripe ascospores. — e. Some ascospores with deviating shape and size. — f. Old ascospores, pigmented and punctuate.

Phacidium buxi Lasch in Klotzsch, Herb. viv. mycol. **1**: 1154. 1847.
Gloeosporium pachybasium Sacc. *in* Michelia **2**: 117. 1880.
Phoma phacidioides Sacc. *in* Michelia **2**: 274. 1881, — *Phyllosticta phacidioides* (Sacc.) Allesch. *in* Rabenh. Kryptog-Fl. **1**(6): 25. 1898.
Gloeosporium louisiae Bäumler *in* Verh. Ver. Natur-Heilk. Pressburg N. F. **9**: 100. 1896.
Macrophoma miribelii (Fr.) Berl. & Vogl. f. *ramicola* Oudem. *in* Ned. kruidk. Arch. III **2**: 734. 1902.
Sarcophoma endogenospora Höhn. *in* Sber. K. Akad. Wiss. Wien (Math.-naturw. Kl. I) **125**: 75. 1916.
Sclerophoma confusa Petrak *in* Annls mycol. **20**: 23. 1922 = *Phyllostictina confusa* (Petrak) Petrak apud Petrak & Sydow *in* Beih. Reprium Spec. nov. Regn. veg. **42** (1): 191. 1927.

Pycnidia discrete, pale yellowish, seldom darker or blackish, immersed, subglobose to tympaniform, firmly grown together with the epidermis, not papillate, at maturity forming a wide crateriform opening by dehiscence, surrounded by the remains of the epidermis. Pycnidial wall apically and laterally 10–25 µm thick, composed of 1–4 layers of rather thin-walled, yellowish brown, isodiametric cells, 5–15 µm in size. The pycnidial base is made up of parallel rows of thin-walled, hyaline, rarely pale brown, isodiametric cells, 5–12 µm in diameter. Conidiogenous cells lining the

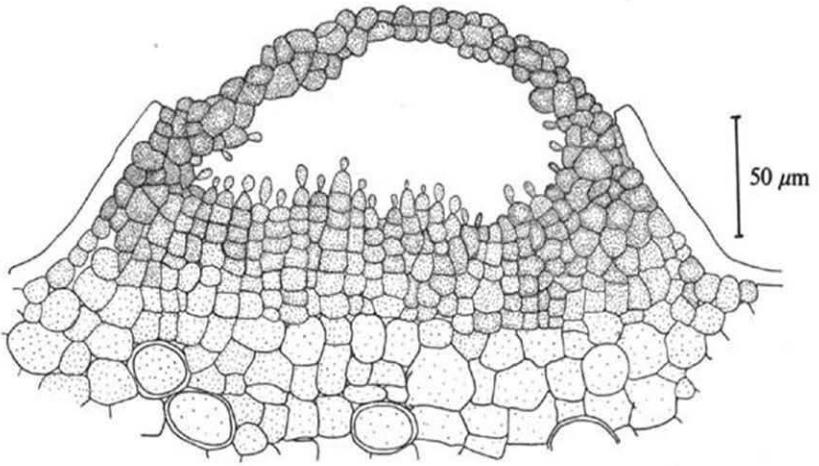

Fig. 3. *Sarcophoma miribelii* (*H. A. van der Aa 4407*), pycnidium on leaf of *Buxus sempervirens*.

cavity irregular, conical, sometimes indistinguishable from the adjacent wall cells especially in the lateral part, 9–20×7–15 µm, phialidic, forming conidia in a basipetal succession. Conidia one-celled, hyaline, ellipsoidal, ovate or pyriform, apiculate at the base, 9–19×4.5–11 µm, containing numerous small guttules and mostly one large central droplet, issuing in thick, whitish tendrils.

On twigs and leaves of *Buxus sempervirens*.

CULTURAL CHARACTERS.—Colonies on cornmeal and oatmeal agar growing moderately fast, attaining a diameter of 40–60 mm within 14 days at 25 °C, initially honey yellow, very soon turning smoke brown, fresh isolates without aerial mycelium. Mycelium composed of hyaline, thin-walled, scarcely branched, septate hyphae, 4–15 µm diameter, with intercalary and terminal chlamydospores, present from the beginning, formed singly or in long chains, in fresh isolates arranged in conspicuous dark brown rows, in subsequent cultures regularly scattered, cylindrical, globose or pyriform when terminal, olivaceous to dark brown, 12–25×10–16 µm, usually covered by a greyish or brownish, fine granular exudate (Fig. 6b). From all mycelial cells, including chlamydospores, phialoconidia similar to pycnoconidia can be formed, usually very abundantly in fresh isolates on various media but inconspicuously or lacking in subsequent cultures. This state belongs to the form-genus *Hormonema* Lagerb. & Melin, and has to be distinguished from *Aureobasidium* by its polyphialidic conidiogenous cells from which conidia arise in basipetal succession (Figs. 6a, b). Pycnidial initials are formed from about the 5th day onwards, at first subglobose to hemispherical, discrete or confluent, with structures similar to those on the host plant, except the twisted, septate, brownish hyphae, irregularly ornamented with dark brown to black exudates, which form the covering layers of the pycnidia in early stages of development in vitro (Fig. 4). In 8–10 day old colonies the pycnidia split open raggedly and develop in a melanconiaceous manner. The conidial slime is white but can turn brownish in old cultures.

MATERIAL EXAMINED (all on *Buxus sempervirens* L.).—Perfect state (*Guignardia miribelii*): On twigs, Mont Ventoux, Massif des Cèdres, France, 21.10.-1974, *H. A. van der Aa 4434* (CBS 214, holotype; single spore isolates in CBS 161.75).

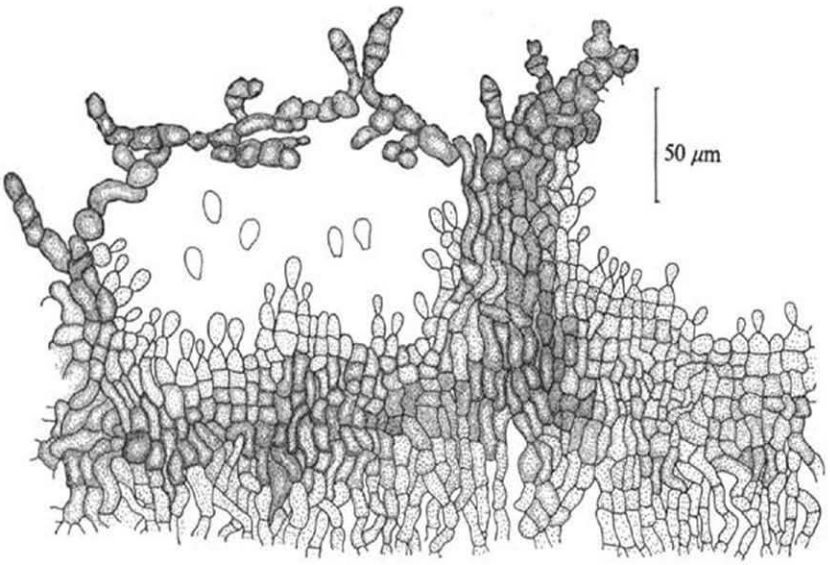

Fig. 4. *Sarcophoma miribelii* (CBS 161.75), pycnidium on cornmeal agar, 15 days old.

Conidial state (*Sarcophoma miribelii*): On dead leaves, Massif des Cèdres, Mont Ventoux, France, 21.10.1974, *H. A. van der Aa 4407* (isolate in CBS 114.75). On leaves and twigs, Solothurn, Oberbuchsitten, Switzerland, 16.7.1962, *J. A. von Arx* (Herb. CBS 125). On dead leaves and twigs, Transsylvania, Distr. Arad, Sofronea, Romania, *T. Savulescu & V. Bontea*, *in* Herb. mycol. Roman. 30: 1469 (Herb. CBS 234). On dead twigs, Nunspeet, Netherlands, *J. Beins* (two collections, *209* and *210*, designed as type of *Macrophoma miribelii* f. *ramicola* Oudem., L). Valkenburg, Limburg, Netherlands, 1901, *J. Rick* (L).

Discussion

Except for its many-spored asci, *Guignardia miribelii* fits very well in the genus *Guignardia* Viala & Ravaz (von Arx & Müller, 1954; Reusser, 1964; van der Aa, 1973; von Arx & Müller, 1975). The phenomenon of some ascospores becoming olivaceous and punctuate and others remaining hyaline and smooth at maturity even while germinating, is observed also in *Guignardia cytisi* (Fuckel) von Arx & Müller (von Arx & Müller, 1954; unpublished observations by the author).

Guignardia buxi (DC. ex Fr.) Lindau (Hilfsb. Sammeln Ascom. 21. 1903) belongs to the genus *Hyponectria* Sacc. (*cf.* von Arx & Müller, 1954: 180). *Guignardia buxicola* Camara & Luz (*in* Agron. lusit. 1: 44. 1939) differs in having eight-spored asci, while ascomata and ascospores are also aberrant.

The conidial state of *Guignardia miribelii* is found wherever the host plant occurs. The holotype specimen of *Sphaeria miribelii* Fr. is not preserved in Upsala and probably

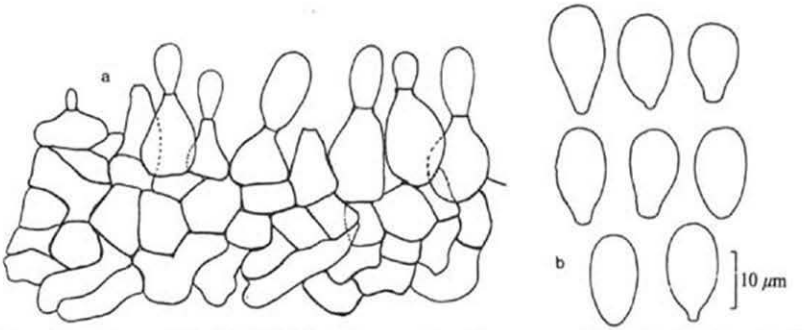

Fig. 5. *Sarcophoma miribelii* (CBS 161.75). — a. Conidiogenous cells in the basal part of the pycnidium. — b. Conidia.

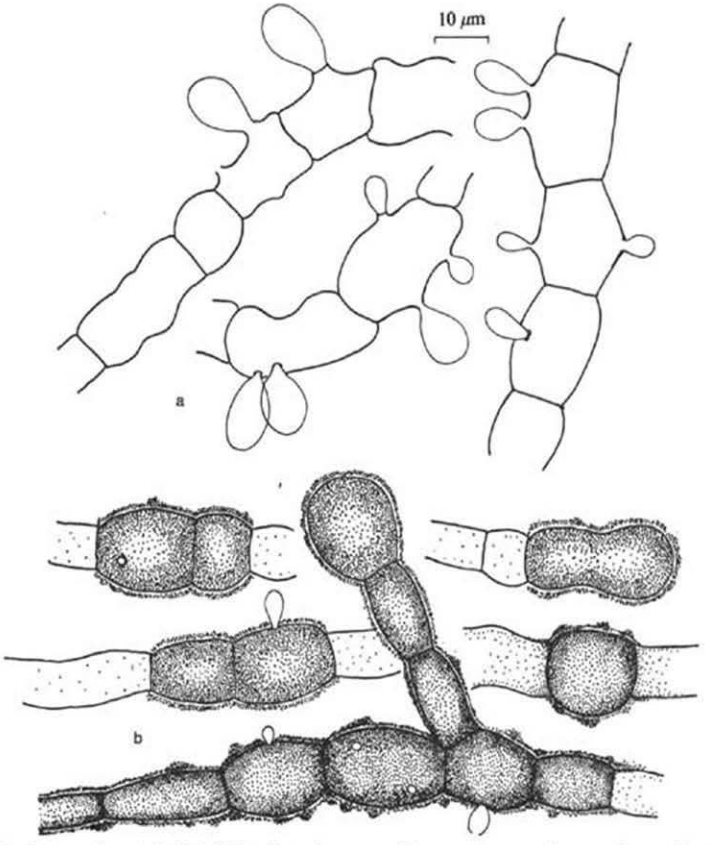

Fig. 6. *Guignardia miribelii* (CBS 161.75). — a. *Hormonema*-state in a 5 days old culture on cornmeal agar. — b. Chlamydospores with *Hormonema*-state in a 12 days old culture on cornmeal agar.

no longer extant. The present conception, including synonymy agrees with that given by von Höhnel (1918), Petrak (1922 and 1927), von Arx (1970) and van der Aa (1973). The fungus described and figured by Morgan Jones (1971) under the name *Sarcophoma miribelii*, however may represent *Dothiorella candollei* (Berk. & Br.) Petrak *in* Annls mycol. **34**: 231. 1936. In most handbooks this species is kept separate from *Sarcophoma miribelii* as *Macrophoma candollei* (Berk. & Br.) Berl. & Vogl. (Allescher, 1899; Diedicke, 1915), but Grove (1935) considered them erroneously as stages of development of one and the same species.

Fuckel (1870), Cooke (1871), and Saccardo (1883) suggested that the ascigerous state of *Sarcophoma miribelii* is *Hyponectria buxi* (Desm.) Sacc., but this was denied by von Höhnel (1918) and also not accepted by later authors. Single ascospore isolates from well-developed specimens of this fungus, collected by the author in the same area as *Guignardia miribelii* (on withering leaves, Plateau du Lubéron, France, 23.10.1974, *H. A. van der Aa 4433*, CBS), repeatedly failed to germinate on different media and as a result the connection with any conidial fungus could not be proved in this way.

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STUDIES IN RESUPINATE BASIDIOMYCETES—III[1]

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(With nine Text-figures)

Material of several species, including types specimens, have been studied. The following new genera are described: *Conohypha* (type: *Corticium albocremeum* Höhn. & Litsch.), *Membranomyces* (type: *Corticium spurium* Bourd.), and *Parvobasidium* (type: *Gloeocystidium cretatum* Bourd. & Galz.). Twelve new combinations are proposed.

ATHELOPSIS Oberw. ex Parm.

While studying the species believed to belong to the genus *Athelopsis* Oberw. ex Parm., it was found that on account of the basidial morphology two groups can be separated, viz. species with stalked and cylindrical to narrowly clavate basidia and others with very wide clavate basidia.

Basidia cylindrical.—
Corticium auriculariae (Bourd. & Galz.) Bourd. & Galz.
Corticium baculiferum Bourd. & Galz.
Corticium glaucinum Bourd. & Galz.
Athelopsis hypochnoidea Jülich
Corticium laceratum Litsch.
Corticium gemmiferum subsp. *thymicola* Bourd. & Galz.
Corticium viride (Link) Bres. apud Höhn., sensu auctt.

Basidia broadly clavate.—
Corticium confusum Bourd. & Galz.
Corticium lembosporum Bourd.
Corticium spurium f. *olivaceum* Bourd.
Corticium pausiacum Liberta
Corticium reconditum Jacks.

The first group contains the species that make up the genus *Athelopsis* sensu stricto.

[1] This study was supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

For the second group of species it was considered necessary to establish a new genus, but in the meanwhile a paper by Larsson & Hjortstam was published in which the genus *Luellia* was proposed for *Corticium reconditum* Jacks. The authors based their genus on two characters, viz. the clavate and pyriform basidia, and the brown coloration of the basal hyphae. The first character is here believed to be the essential one, whereas the last seems to me to be merely of specific importance.

To this genus *Corticium lembosporum* has to be transferred: **Luellia lembospora** (Bourd.) Jülich, *comb. nov.* (basionym: *Corticium lembosporum* Bourd. *in* Revue scient. Bourbonne **23**: 10. 1910). Good descriptions and figures of this species have been published by Liberta (*1962*) and Oberwinkler (*1965*).

The genus *Luellia* comprizes three species:

1. *Luellia furcata* Larss. & Hjortst.
2. *Luellia lembospora* (Bourd.) Jülich (synonym *Corticium confusum* Bourd. & Galz. 1911).
3. *Luellia recondita* (Jacks.) Larss. & Hjortst. (synonyms: *Corticium spurium* Bourd. f. *olivaceum* Bourd. 1922, *Corticium pausiacum* Liberta 1962).

An addition to *Athelopsis* is the following: **Athelopsis subinconspicua** (Litsch.) Jülich, *comb. nov.* (basionym: *Corticium subinconspicuum* Litsch. in Pilát & Lindtner, *in* Bull. Soc. sci. Skopje **18**: 178–179. 1938). (Fig. 1). Identical with this species is *Athelopsis hypochnoidea* Jülich 1971. The specific name 'subinconspicuum' is rather misleading since the basidiocarp of this species is of a yellowish-greenish colour and in most cases very easily seen. But the type specimen of *Corticium subinconspicuum* Litsch. happened to grow on rotten wood of almost the same colour as the basidiocarp, thus suggesting its specific epithet which suggests a *Xenasma* rather than an *Athelopsis*. The description of this species given by Litschauer (l.c.) is fairly adequate except that he observed the spores to be 'subverruculosis, minutissime punctatis usque laevibus'; in fact the spores are always smooth.

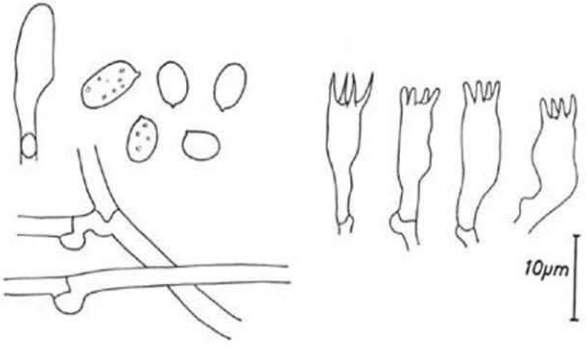

Fig. 1. *Athelopsis subinconspicua*, Macedonia, type.

Basidiocarp resupinate, effused, hypochnoid to soft-pellicular, the margin thinning out. Hymenial surface even, light yellowish with a rather faint greenish tint. Hyphal system monomitic. Hyphae hyaline, rather thin-walled (*c.* 0.3 μm thick), cylindrical, 2–3 μm in diameter, with clamps at all septa, the surface smooth or slightly covered with small granules or crystals. Cystidia lacking. Basidia stalked-cylindrical (= podobasidia), hyaline, thin-walled, 4-spored, with a basal clamp, 13–18×4–5 μm, the sterigmata about 3.5×1 μm, the contents homogeneous or slightly granulose. Spores hyaline, broadly cylindrical to broadly ellipsoidal, thin-walled, smooth, 6–7.5×4–4.5 μm, with distinct apiculus, the contents granulose or slightly guttulate, neither amyloid, dextrinoid, nor cyanophilous. Saprophytic on rotten wood.

MATERIAL STUDIED. — '*Corticium subinconspicuum* Litsch. n. sp. Macedonia: in Fagetis ad silvae limitem montis Luboten (Sar Planina), alt. 1500–1800 m.s.m., solo dolomitico. Matrix: ad ligna mucida. VII. 1937, leg. A. Pilát & V. Lindtner' (W 16529).

TRECHISPORA Karst. emend. Liberta

Of this genus Liberta (1973) has recently made an excellent revision, to which further synonyms and a species may be added:

a. *Odontia microspora* Rick *in* Egatea **18**: 39. 1933. This species is identical with *Trechispora farinacea* (Pers. ex Fr.) Liberta.

MATERIAL STUDIED. — '*Odontia microspora* Rick n. sp. Brazil, Sao Leopoldo, 1930, Rick 91' (type, K).

b. *Odontia serrata* Rick *in* Egatea **17**: 276. 1932. This species, too, is identical with *T. farinacea* (Pers. ex Fr.) Liberta.

MATERIAL STUDIED. — '*Odontia serrata* Rick n. sp. Brazil, Sao Leopoldo, 1930, Rick 130' (type, K).

c. **Trechispora lunata** (Bourd. & Galz.) Jülich, *comb. nov.* (basionym: *Grandinia lunata* Bourd. & Galz., Hym. France 410. 1928. (Fig. 2).

This species belongs to the group of smooth-spored species like *Trechispora amianthina*, *T. byssinella*, *T. confinis*, and *T. mutabilis*. It differs from all of them in having very small lacrymoid to somewhat allantoid spores (3–3.5×1.4–1.5 μm) and a thin-membranaceous, cream-coloured, grandinioid (under a lens somewhat reticulate) basidiocarp; the basidia are like those of *T. byssinella* (Bourd.) Liberta (8–12×3–4 μm), but from that species it differs by its narrower spores and the absence of rhizomorphs.

Fig. 2. *Trechispora lunata*, Sweden, lectotype.

MATERIAL STUDIED. — '*Hydnum lunatum* Romell in litt. 6. VI. 1913.', Sweden, Femsjö, 26.9.1890, in herb. Bourdot 23798 (lectotype, PC); Stockholm, 16.11.1913, *Romell*, in herb. Bourdot 23800 (PC).

d. *Corticium aegeritoides* Bourd. & Galz.

Basidiocarp minutely grandinioid, later membranaceous, whitish, rhizomorphs lacking. Hyphal system monomitic. Hyphae rather distinct, hyaline, thin-walled, cylindrical, at some places ampulliform, 1–3 μm in diameter (at the ampulliform swellings up to 6 μm in diameter, clamps present, surface smooth. Cystidia lacking. Basidia small, cylindrical to suburniform, hyaline, thin-walled, 9–11 × 4–5 μm, 4-spored, the sterigmata 3–5 × 0.8–1.0 μm. Spores hyaline, ellipsoidal, thin-walled, warted, 3–4 × 2–2.5 μm, the warts 0.2–0.4 μm long, neither amyloid, dextrinoid, nor cyanophilous. Saprophytic on petioles of ferns.

MATERIAL STUDIED. — France, Aveyron, 'près St. Sernin', 6.1.1910, *A. Galzin 5163* (Bourdot 6950; lectotype, PC).

This species is a typical *Trechispora*. The young very poorly developed state resembles a small *Aegerita* insofar as the granules are separated; they have already developed some basidia with spores. In a later state an effused, membranaceous basidiocarp with granulose-grandinioid hymenial surface is seen. This state differs in no way from *Trechispora farinacea* (Pers. ex Fr.) Liberta, and since the microfeatures are identical, I see no reason to keep *Corticium aegeritoides* apart from *Trechispora farinacea*.

e. *Corticium microsporum* subsp. *hecistosporum* Bourd. & Galz. 195–196. 1928. This subspecies is identical with *Trechispora byssinella* (Bourd.) Liberta.

MATERIAL STUDIED. — France, Allier, Forêt de Dreuille, 'sur Polytrichum', 25.10.1909, *H. Bourdot 6821* (type, PC).

Phanerochaete salmonicolor (Berk. & Br.) Jülich, *comb. nov.*—Fig. 3

Corticium salmonicolor Berk. & Br. *in* J. Linn. Soc. (Bot.) **14**: 71. 1873 (basionym).

Basidiocarp resupinate, effused, membranaceous, separable in small pieces, cracked when dry and exposing an arachnoid subiculum, the margin thinning out, no rhizomorphs present. Hymenial surface even, orange-pink when fresh, whitish to light cream-coloured when dry. Hyphal system monomitic. Hyphae cylindrical, hyaline, smooth, distinct, the basal ones 6–10 μm in diameter and with up to 1.5 μm thick walls, septate, clamps lacking. Cystidia and gloeocystidia lacking. Basidia broadly clavate when young, cylindrical to narrowly clavate when ripe, hyaline, thin-walled, smooth, densely crowded, 35–50 × 5–8 μm, 4-spored, the sterigmata *c.* 4–5 × 1.5 μm, slightly curved inwards, no clamp connection at the base. Spores broadly ellipsoidal, hyaline, thin-walled, smooth, 10–13 × 6–8 μm, with rather large apiculus, neither amyloid, dextrinoid, nor cyanophilous.

IMPERFECT STATE. — *Necator decretus* Massee. Often found together with the perfect state.

SUBSTRATE. — Growing parasitically on various frondose trees and shrubs, causing the economically important 'pink disease'.

DISTRIBUTION. — Known from tropical regions all over the world, also occurring in some southern states of the USA.

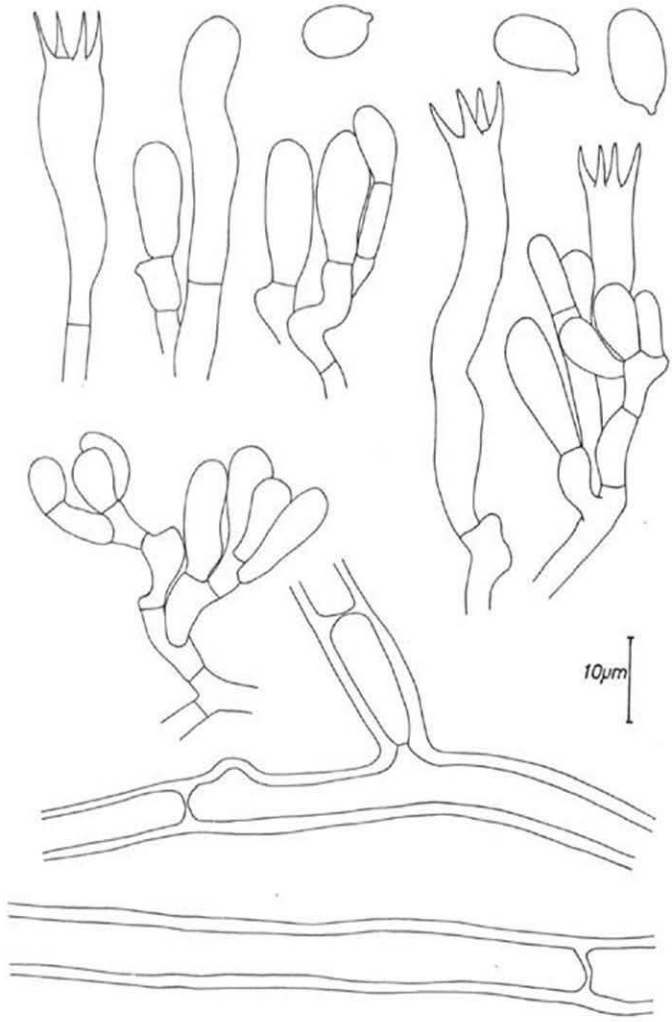

Fig. 3. *Phanerochaete salmonicolor*, Ceylon, type

MATERIAL STUDIED. — *Corticium salmonicolor* Berk. & Br., Ceylon (type, K). — Sierra Leone, Toru, Gaura, 31.10.1955, *C. T. Pyne* (K).

Byssocorticium mollicula (Bourd.) Jülich, *comb. nov.*

Poria mollicula Bourd. *in* Lloyd, Mycol. Writ. **4**: 543. 1916 (basionym). — *Poria terrestris* Bourd. & Galz. 1925.

This rare species with rather soft basidiocarp and poroid hymenial surface shows the same microstructure as *B. atrovirens* but differs from that species by its whitish to ochraceous colour.

Membranomyces Jülich, *gen. nov.*

Carposoma resupinatum, late effusum, firme membranaceum. Hymenium laeve. Systema hypharum monomiticum. Hyphae distinctae, plerumque tenui-tunicatae, efibulatae, *c.* 3–4 *µm* in diam. Basidia flexuoso-cylindracea vel anguste clavata, *c.* 30–70 *µm* longa, efibulata, tetraspora. Sporae laeves, ellipsoideae, hyalinae vel leviter flavidae, tenui-tunicatae usque ad tunicis paulo incrassatis, inamyloideae. Typus. — *Corticium spurium* Bourd.

Basidiocarp resupinate, effused, adnate, membranaceous to subceraceous. Hymenial surface even. Hyphal system monomitic. Hyphae distinct, cylindrical, thin- to slightly thick-walled, hyaline, *c.* 3–4 *µm* in diameter, lacking clamps. Basidia flexuous-cylindrical to narrowly clavate, about 30–50 *µm* long, 4-spored, lacking a basal clamp. Spores smooth, hyaline to slightly yellowish with age, thin- to slightly thick-walled (*c.* 0.4 *µm*), inamyloid.

Membranomyces spurius (Bourd.) Jülich, *comb. nov.*—Fig. 4

Corticium spurium Bourd. *in* Rev. scient. Bourbonne **35**: 15. 1922 (basionym). Synonyms: *Corticium delectabile* Jacks; *Clavulicium delectabile* (Jacks.) Hjortst.

Basidiocarp resupinate, effused, adnate, firm-membranaceous to subceraceous; margin indistinct, rhizomorphs lacking. Hymenial surface even, cream-coloured to lemon yellow. Hyphal system monomitic. Hyphae hyaline to slightly yellowish, thin- to slightly thick-walled, cylindrical, 3–4 *µm* in diameter, lacking clamps, contents homogeneous or somewhat guttulate; the subiculum rather thin, the hyphae mainly vertically arranged. Cystidia and gloeocystidia lacking. Basidia flexuous-cylindrical, often somewhat stalked and widened in the middle with the apical part often slightly narrowed, 35–75 × 6.7–9.6 *µm*, hyaline to slightly yellowish with age, thin- to somewhat thick-walled when old, lacking a clamp at the base, contents often guttulate, with four large and curved sterigmata *c.* 5–6.7 × 1.6 *µm*. Spores broadly ellipsoidal, hyaline or slightly yellowish with age, thin- to somewhat thick-walled when old, with prominent apiculus, smooth, 7.4–9.5 × 5.9–7.3 *µm*, often with numerous small guttules inside, neither amyloid, dextrinoid, nor cyanophilous.

SUBSTRATE. — On wood of coniferous and frondose trees, also in leaf litter on the ground.

MATERIAL STUDIED. — France: Aveyron, Pépisson, .11.1914, *A. Galzin 16830* (H. Bourdot 35226; K). — Aveyron, Pépisson, 22.11.1914, *A Galzin* 16838 (H. Bourdot 29742; PC). — Aveyron, Conques, 16.7.1912, *A. Galzin* 11608 (H. Bourdot 8974; type of *Corticium spurium* Bourd. (K, PC).

Sweden: Uppland, 'Djurö sn, Munkön, i norra delen av kärrets (dalgången) östra skänkel, nära ingången till "ravinen", på tallgrenar p.m.', 23.10.1949, *G. Haglund & R. Rydberg* (S).

Canada: Ontario, West of Maple, 1.10.1938, R. F. Cain (type of *Corticium delectabile* Jacks., TRTC 13684). — loc. cit., 21.9.1950, *Jackson & al.* (TRTC 23271). — loc. cit., 22.9.1940, *R. F. Cain 16432* (TRTC). — loc. cit., 22.9. 1940, *R. F. Cain* (TRTC 16432). —

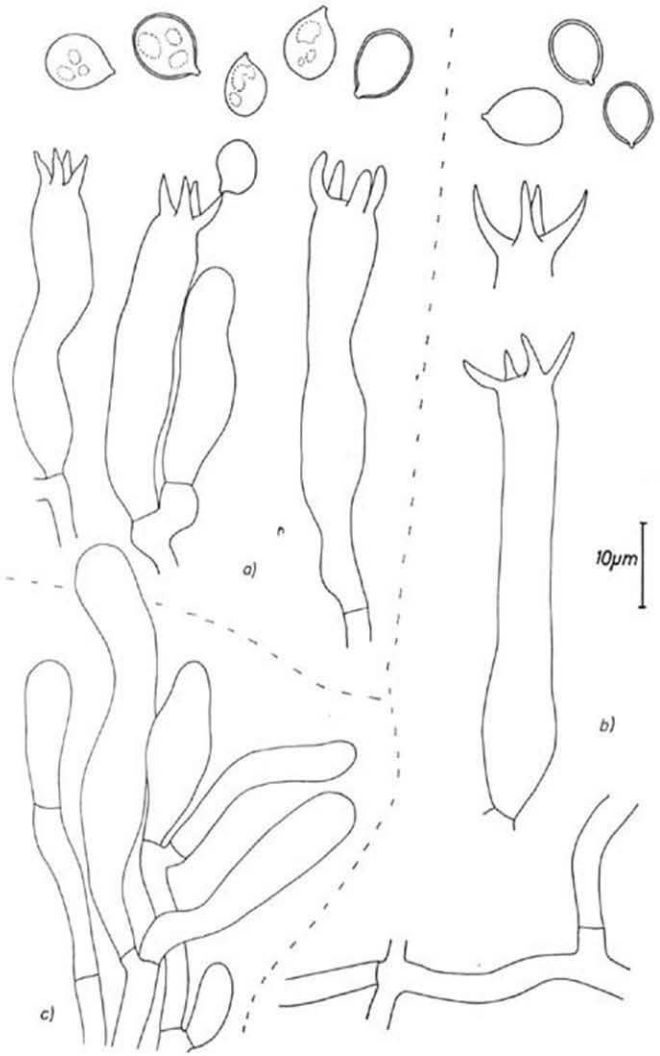

Fig. 4. *Membranomyces spurius*, France. — a. *Bourdot 8974*, type. — b. *Bourdot 29742*. — c. *Bourdot 35226*.

Woods N of Summit Golf Club, N of Richmond Hill, 6.10.1949, *H. S. Jackson* (TRTC 23032). — Don Valley, Toronto, 22.9.1934, *R. F. Cain* (TRTC 8672). — Bear Island, L. Timagami, 1.10.1948, *H. S. Jackson* (TRTC 22556). — Woods S of Aurora, York Co., 10.10.1937, *H. S. Jackson* (TRTC 16722). — Sproule Portage, Lake Opeongo, Algonquin Park, 8.9.1958, *R. F. Cain* (TRTC 33956).

Cristinia gallica (Pilát) Jülich, *comb. nov.*

Radulum mucidum (Pers.) Bourd. & Galz. sensu Bourd. & Galz. *in* Bull. trim. Soc. mycol. Fr. **30**: 247–248. 1914. — *Radulum gallicum* Pilát *in* Mykologia **2**: 54. 1925 (basionym).

Eriksson & Ryvarden (1975) proposed the following new combination: '*Cristinia mucida Erikss.* & Ryv. comb. nov. *Radulum mucidum* Bourd. & Galz., Bull. Soc. Mycol. Fr. 30 p. 247, 1914.' To this the following may be remarked, (i) they omitted to mention the bracket-authors; (ii) they based their combination on a species which had never been named by Bourdot & Galzin. When in their publication of 1914 Bourd. & Galz. made the new combination under *Radulum*, they described a fungus which they identified with Persoon's species; this description was repeated with minor additions in their book of 1928. Since they did not exclude Persoon's type, their account cannot be considered to constitute the description of a new species. Pilát (1925) was aware of the difference between the true *Hydnum mucidum* and Bourdot & Galzin's fungus and he created the nomen novum Radulum gallicum, saying: '*Hydnum mucidum* sensu autorum gallicorum (*Radulum mucidum* (Pers.) Bourdot et Galz., (Hymenomycetes de France no 318. — Bull. de Soc. Myc. de France) a *Acia mucida* (Pers.) Pilát certe diversum. Hanc speciem Gallicam nomino *Radulum Gallicum* Pilát.' There seems to be no earlier name available, at least for the moment, thus the epithet 'gallica' has to be used.

CORONICIUM Erikss. & Ryv.

When Eriksson & Ryvarden established this monotypic genus, they separated it from other genera by the following characters: 'The most conspicuous character of the new genus are the cystidia, with their incrustation which in microscope looks like a golden crown. This together with the thinwalled, indistinct hyphae, mamillate cystidia and the staining in cotton blue of the whole fungus make it characteristic.' Their description of the species *C. gemmiferum* (Bourd. & Galz.) is based on a specimen collected in Denmark by Hauerslev. They found that 'all hyphal walls as well as walls of cystidia, basidia, and spores are stained by cotton-blue.' This, however, is not the case with the type material, which I found to be acyanophilous in all parts. But apart from this, I think cyanophily is not useful as a generic character in the Corticiaceae, and so probably is the said incrustation of the cystidioles. In fact, I would rather stress some other characters of this genus, viz. the suburniform basidia (i.e. constricted in the middle), which are often somewhat stalked, the cystidioles with their apical prolongation and the indistinct, torulose hyphae. If emphasis is laid upon these features, then at least three more species will be found to have their proper place in *Coronicium*. The genus is related to *Hyphoderma*, with which it has the suburniform basidia in common, but differs from that genus by the indistinct and narrower hyphae.

1a. Spores 4.5–5.2×2.5–3.0 μm; cystidioles broadly clavate *C. proximum* (Jacks.) Jülich
1b. Spores 6–9 μm long; cystidioles narrowly clavate or fusiform 2
2a. Spores narrowly navicular, 6.5–9×2.5–3 μm *C. alboglaucum* (Bourd. & Galz.) Jülich
2b. Spores ellipsoidal or broadly navicular 3
3a. Spores ellipsoidal, 6–8×3–4 μm; hymenial surface under a lens homogeneous *C. thymicola* (Bourd. & Galz.) Jülich
3b. Spores broadly ellipsoidal to navicular, 6–9×3.5–4.5 μm; hymenial surface under a lens (50×) dotted with small particles of a brownish substance *C. gemmiferum* (Bourd. & Galz.) Erikss. & Ryv.

Coronicium alboglaucum (Bourd. & Galz.) Jülich, *comb. nov.*—Fig. 5

Corticium alboglaucum Bourd. & Galz. *in* Bull. trim. Soc. mycol. Fr. **27**: 251. 1911 (basionym).

Basidiocarp resupinate, adnate, 2–3 cm in diameter, membranaceous, cream-coloured, 50–100 μm thick, slightly thinner towards the margin, rhizomorphs lacking; no differentiation into a hymenial part and a subiculum. Hyphal system monomitic. Hyphae indistinct and mostly collapsed, some distinct, not embedded in a gelatinous matrix, mostly torulose, hyaline, thin-walled to slightly firm-walled (0.2–0.3 μm), 2–3 μm in diameter, with clamps at the septa, surface smooth, contents homogeneous; not amyloid, dextrinoid, or cyanophilous. Adjacent to the substrate is a *c.* 20 μm thick layer of parallel hyphae. Cystidioles irregularly cylindrical to ellipsoidal or subulate to ventricose-subulate, hyaline, thin-walled, with a basal clamp, surface smooth, rather often with a small elongate or subglobose projection at the apex, 15–25×3–4 μm. Basidia hyaline, suburniform, often somewhat stalked, thin-walled, with smooth surface, with a basal clamp, 15–23×4–6 μm, with four

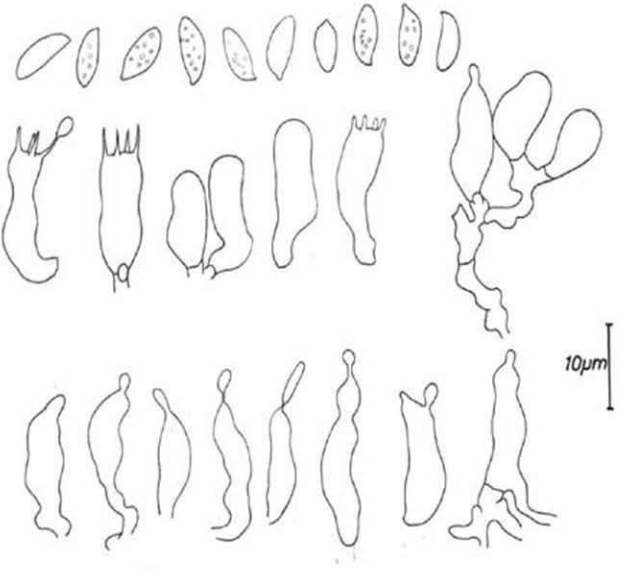

Fig. 5. *Coronicium alboglaucum*, France, type.

slender sterigmata; contents homogeneous. Spores hyaline, oblong-ellipsoidal to subnavicular or subfusiform, smooth, thin-walled, with distinct apiculus, contents with several small guttules, 6.5–9 × 2.5–3 μm, rather often two or four glued together; not amyloid, dextrinoid, or cyanophilous.

SUBSTRATE. — On decayed bark of frondose trees.

DISTRIBUTION. — In Europe known from France and Austria; recorded also from the U.S.A. and Canada (Liberta, 1960).

MATERIAL STUDIED. — France, Allier, St. Priest, 13.7.1910, *Bourdot 7349* (type, PC).

In the type material, it is extremely difficult to find clamps at the septa, since the hyphae are badly collapsed.

Coronicium proximum (Jackson) Jülich, *comb. nov.*—Fig. 6

Corticium proximum Jacks. *in* Canad. J. Res. **28**: 722. 1950 (basionym).

Basidiocarp annual, resupinate, effused up to several cm, up to 50 μm thick, adnate; consistency membranaceous, context homogeneous; margin thinning out, rhizomorphs or hyphal strands lacking. Hymenial surface even, cream-coloured or greyish. Hyphal system monomitic. Hyphae hyaline, indistinct, thin-walled, 1.5–2 μm in diameter, torulose, with clamps, crystals lacking or few. Cystidia of hymenial

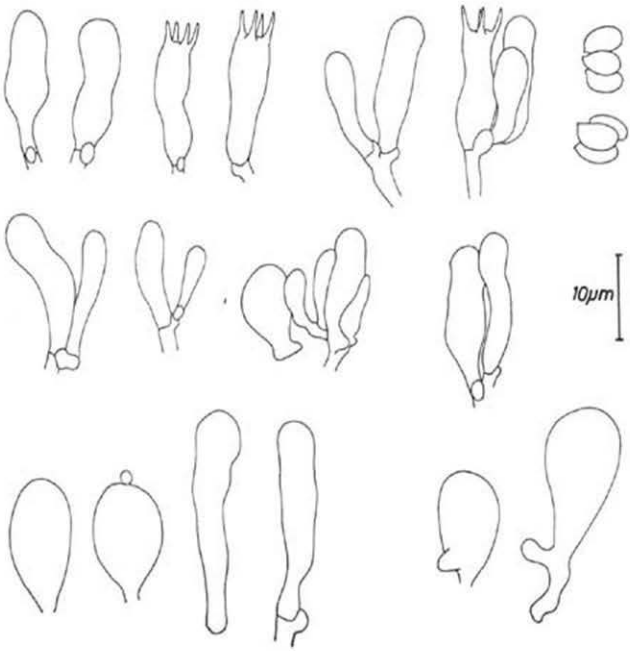

Fig. 6. *Coronicium proximum*, U.S.A., type.

origin. Cystidioles present, hyaline, broadly clavate, ellipsoidal, some with a globose projection at apex, thin-walled, smooth, enclosed or only slightly projecting, with a clamp at the base. Basidia hyaline, suburniform, somewhat stalked, young basidia cylindrical, thin-walled, smooth, 14–18×4.2–4.6 μm, with a clamp at the base, contents homogeneous, with four subulate sterigmata *c.* 3×1.2 μm. Spores hyaline, thin-walled, smooth, ellipsoidal, adaxially slightly depressed, 4.5–5.2×2.5–3 μm, with small apiculus, often 2–4 glued together, contents homogeneous or slightly guttulate, not amyloid, dextrinoid, or cyanophilous. Conidial state lacking.

REACTIONS. — No part of the basidiocarp is amyloid, dextrinoid, or cyanophilous.

MATERIAL STUDIED. — U. S. A., Vermont, Middleburg, *s. dat.*, *E. A. Burt* (TRTC).

Canada, Ontario, Bear Island, Lake Temagami, Temagami Forest Reserve, 21.8.1944, *H. S. Jackson* (TRTC).

Coronicium thymicola (Bourd. & Galz.) Jülich, *comb. nov.*—Fig. 7

Corticium gemmiferum subsp. *thymicola* Bourd. & Galz., Hym. Fr. 211. 1928 (basionym).

Basidiocarp annual, resupinate, effused up to several cm, about 50–130 μm thick, in small pieces separable; consistency membranaceous, context homogeneous; margin thinning out, rhizomorphs or hyphal strands lacking. Hymenial surface even,

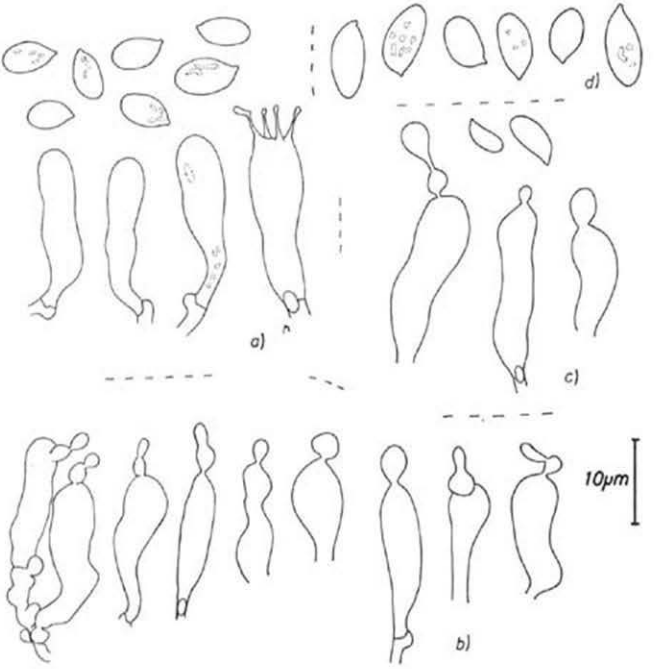

Fig. 7. *Coronicium thymicola*, France. — a. *Bourdot 14188*, lectotype. — b. *Bourdot 14184*. — c. *Bourdot 14187*. — d. *Coronicium gemmiferum*, France, type.

cracked when dry, colour cream to ochraceous. Hyphal system monomitic. Hyphae hyaline, indistinct, thin-walled and *c.* 1.5 μm wide, cylindrical to torulose, with clamps, crystals and amorphic or oily substances between the hyphae. Cystidia etc. lacking. Cystidioles present, hyaline, irregularly clavate with 1–2 apical projections, thin-walled, 15–20 × 4–6 μm, enclosed or slightly projecting, contents homogeneous, with a clamp at the base. Basidia hyaline, clavate, slightly suburniform, often somewhat stalked, thin-walled, 18–20 × 4.5–5.2 μm, with a clamp at the base, contents slightly guttulate, with four subulate sterigmata *c.* 3 × 0.5 μm. Spores hyaline, ellipsoidal, thin-walled, smooth, 6–8 × 3–4 μm, with small apiculus, contents somewhat guttulate, not amyloid, dextrinoid, or cyanophilous. Conidial state lacking.

MATERIAL STUDIED. — France: Aveyron, Navadou (Millau), 'sur Thym', 22.11.1914, *Galzin 16824* (Bourdot 14188; lectotype, PC). — Aveyron, l'Hymen (Millau), 'sur Thym', 8.5.1911, *Galzin 9067–72* (Bourdot 14184, PC). — Aveyron, 'env. de Millau, Pépisson, sur Thym', .5.1911, *Galzin 9282* (Bourdot 14187, PC).

Parvobasidium Jülich, *gen. nov.*

Carposoma resupinatum, effusum, adnatum, membranaceum. Hymenium laeve, cremeum. Systema hypharum monomiticum. Hyphae hyalinae, tenui-tunicatae, fibulatae. Gloeocystidia adsunt, hyalina, clavata. Basidia hyalina, parva, circa 8–12 μm longa, tenui-tunicata, fibulata. Sporae hyalinae, parvae, tenui-tunicatae, laeves, inamyloideae. — Typus: *Gloeocystidium cretatum* Bourd. & Galz.

Basidiocarp resupinate, effused, adnate, membranaceous. Hymenial surface even, cream-coloured. Hyphal system monomitic. Hyphae hyaline, thin-walled, with clamps. Gloeocystidia present, hyaline, clavate. Basidia hyaline, small, about 8–12 μm long, thin-walled, with a basal clamp. Spores hyaline, small, thin-walled, smooth, inamyloid.

Parvobasidium cretatum (Bourd. & Galz.) Jülich, *comb. nov.*—Fig. 8

Gloeocystidium cretatum Bourd. & Galz. *in* Bull. trim. Soc. mycol. Fr. **28**: 371. 1913 (basionym).

Basidiocarp annual, resupinate, effused up to several cm, about 50–250 μm thick, in small pieces separable; consistency membranaceous, context homogeneous; margin thinning out, indeterminate, pruinose, rhizomorphs or hyphal strands lacking. Hymenial surface even, whitish to pale cream-coloured. Hyphal system monomitic. Hyphae hyaline, thin-walled, cylindrical or somewhat torulose, with clamps at all septa, contents slightly guttulate, crystals and oily amorphic material often abundant between the hyphae. Cystidia etc. lacking. Gloeocystidia present in the hymenial and subhymenial layers, hyaline, of irregular shape (clavate, flexuous-cylindrical, fusiform), thin-walled, 17–32 × 5.2–8.9 μm, enclosed or slightly projecting, contents guttulate, with a clamp at the base. Basidia rather short, hyaline, clavate, thin-walled, 8.5–12 × 3.5–4 μm, with a clamp at the base, contents homogeneous, with four subulate sterigmata 3–3.7 × 0.5–0.6 μm. Spores hyaline, oblong-ellipsoidal, thin-walled, smooth, 4–4.5 × 1.8–2.1 μm, with small apiculus, contents homogeneous, not amyloid, dextrinoid, or cyanophilous. Conidial state lacking.

SUBSTRATE. — Growing on putrescent petioles of ferns.

DISTRIBUTION. — Known only from France.

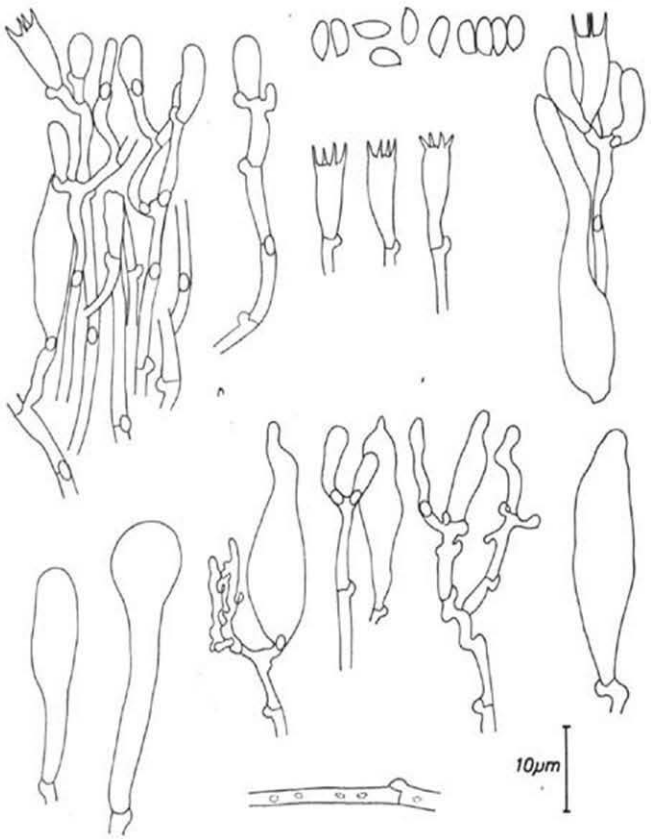

Fig. 8. *Parvobasidium cretatum*, France, type.

MATERIAL STUDIED. — F r a n c e: Aveyron, Barthe, 'sur Fougère mâle', 9.10.1910, *Galzin* 7209 (Bourdot 31670; lectotype, PC). — Aveyron, Bouisson, 'sur Polystichum filix-mas', 5.9.1909, *Galzin* 4606 (Bourdot 7341; PC).

Conohypha Jülich, *gen. nov.*

Carposoma resupinatum, effusum, membranaceum. Hymenium laeve, albidum vel cremeum. Systema hypharum monomiticum. Hyphae hyalinae, tenui-tunicatae, cellulis brevis latisque compositae, fibulatae. Basidia hyalina, circa 30 µm longa, cylindracea, tetraspora, fibulata. Sporae hyalinae, tenui-tunicatae, laeves, inamyloideae. — Typus: *Corticium albocremeum* Höhn. & Litsch.

Basidiocarp effused, resupinate, membranaceous. Hymenial surface even, whitish to cream-coloured. Hyphal system monomitic. Hyphae hyaline, thin-walled,

composed of rather short and broad cells, with clamps. Basidia hyaline, thin-walled, about 30 μm long, cylindrical, with a basal clamp, four-spored. Spores hyaline, thin-walled, smooth, inamyloid.

Conohypha albocremea (Höhn. & Litsch.) Jülich, *comb. nov.*—Fig. 9

Corticium albocremeum Höhn. & Litsch., Wiener Festschr., Wien, 61. 1908 (basionym).

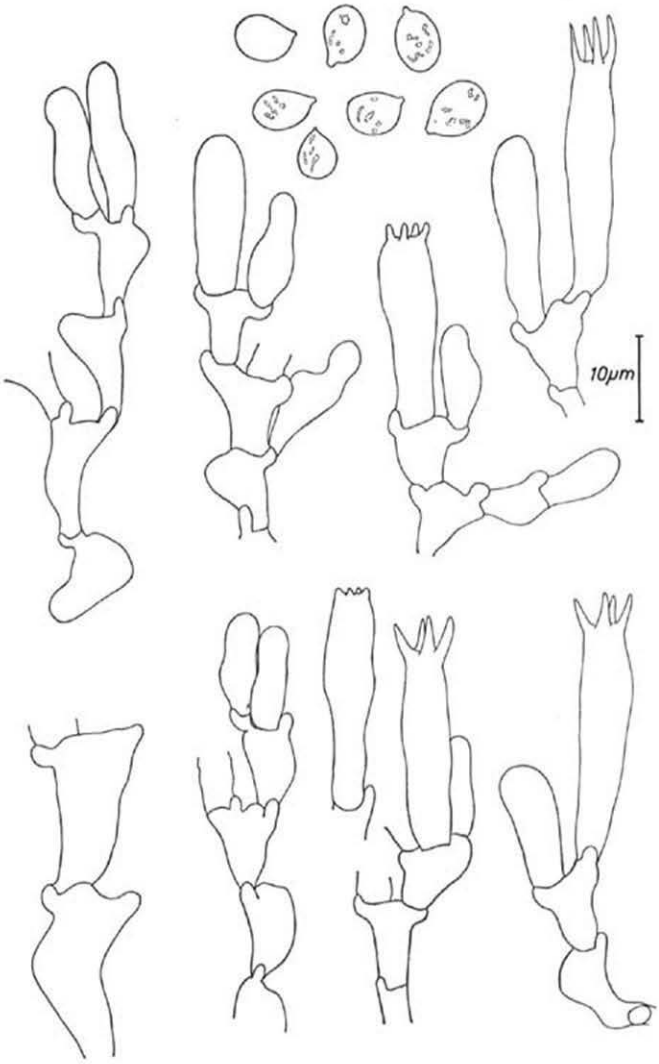

Fig. 9. *Conohypha albocremea*, Austria, type.

Basidiocarp annual, resupinate, loosely adnate, effused up to several cm, about 100 μm thick, in small pieces separable; consistency soft-membranaceous, context homogeneous; rhizomorphs or hyphal strands lacking. Hymenial surface even, cream to ochraceous. Hyphal system monomitic. Hyphae hyaline, composed of rather short and broad cells (*c.* 8–19×5–11 μm), thin-walled, inverse-conical, with clamps at all septa, branching from the top of the cells, contents homogeneous or slightly guttulate, elongated crystals present between the hyphae. Cystidia lacking. Basidia hyaline, cylindrical, thin-walled, smooth, 23–30×5.5–6 μm, with a clamp at the base, contents slightly guttulate, with four subulate sterigmata 5–7×0.7–1 μm. Spores hyaline, broadly ellipsoidal, thin-walled, 6.6–8×4.6–5.2 μm, with distinct apiculus, contents slightly guttulate, not amyloid, dextrinoid, or cyanophilous. Conidial state lacking.

REACTIONS. — No part of the basidiocarp is amyloid, dextrinoid, or cyanophilous.

CYTOLOGY. — Hyphae 2-, spores 1-nucleate.

SUBSTRATE. — On wood and bark of coniferous trees.

DISTRIBUTION. — Known from Austria and Sweden; apparently a rare species.

MATERIAL STUDIED. — Austria: Nieder-Österreich, Wienerwald, Sparbach bei Neuweg, 14.7.1902, *F. von Höhnel* (type, S). — Tirol, Schmirn-Tal 'prope St. Jodok in jugo Brenner, m. Aug.' *V. Litschauer* (L).

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THE DEVELOPMENT OF THREE SPECIES OF THE AGARICACEAE AND THE ONTOGENETIC PATTERN OF THIS FAMILY AS A WHOLE

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(With Plates 48–50 and one Table)

The developmental types within the family Agaricaceae vary considerably but it looks as if the ontogenetic pattern is not without regularity. About 20 species have been examined. On one side the stipitocarpous genera *Cystoderma*, *Phaeolepiota*, and *Chamaemyces*, on the other side genera with highly concentrated primordia like *Macrolepiota* and *Agaricus* (isocarpous, pileocarpous or hymenocarpous). *Lepiota*, *Leucocoprinus* and *Leucoagaricus* with a more concentrated development may occupy an intermediate position. Some remarks are made on developmental problems in *Squamanita*.

In *Chamaemyces fracidus* the radiating elements on the cap are not a palisadodermium sensu stricto but they belong to the universal veil. However this structure is limited to the pileus-surface, so we have to suppose that an ontogenetic factor affects in some way the formation of the pileus and that of these radiating elements. In other species a layer of tightly packed, erect elements forming part of the universal veil envelops also the pileus-margin and the upper part of the stem (with *Lepiota clypeolaria* at the outside and with *Lepiota ignipes* as an emanating veil at the inside of the universal veil). A trichodermium which is afterwards changed into a paradermium is to be found in *Leucocoprinus* whereas a true palisadodermium occurs in *Macrolepiota*.

Introduction

It was Monsieur V. Fayod (1889) who, under influence of the evolution theory, divided the Agaricales into a number of series (tribes, genera) with independent and convergent development. As an example of such convergent lines Fayod (l.c.: 399) advanced *Amanita* and *Volvaria* according to their external appearances but put them into very different series. At present, however, when we want to visualize the phylogenetic relations within this order we certainly shall not divide it into a number of independent series but we prefer to use the idea of the top of a tree of which the trunk and most of the larger branches have disappeared. Then we can admit that series with a convergent development remain and that, here and there, they are very clear amongst the tangle of tiny twigs.

Since we know that the Aphyllophorales and the Gasteromycetes are involved in the phylogenetic development of the Agaricales in a peculiar way we do not take

it for granted that the latter should have originated from one main stock. Long ago there must have been common lines of descent for certain groups of the three large divisions of higher Basidiomycetes.

Because we have examined the development of many species of Agaricales (at the moment more than 150) we have been able to affirm the experience of Fayod and to determine certain convergent lines of development, this time not concerning the appearance or habitus of the fungus but specified organs and their origin.

In our analysis of the veils we frequently came across the same structures. Thus the innate veil (i. e. the veil primarely originating from protenchyma which remains at the periphery of the primordium when the surface of the pileus and the stipe differentiate in the deeper layers) can develop, among other things, into a spherocysts-veil or it can become mucose. We have met with these developments in very different genera (Reijnders, 1963: 352). But the secundary angiocarpies, caused by emanating veils, appear in very different groups as well. Here it is especially remarkable that within one genus such veils sometimes may occur in a few species.

We shall leave out of further consideration the analysis of the veils in so far as it does not have reference to the species to be described below. However, we only want to remark that the appearance of innate veils (and therefore the primary angiocarpies as well) clearly show correlations with the sequence of development, in this sense, that few primary angiocarpies present themselves in stipitocarpous primordia. Yet a good many occur in pileo-, iso-, pileohymeno-, and hymenocarpous species (in the last ones no gymnocarpy). It is namely this sequence of development of the parts of the primordium we have come to consider the most important datum the study of the ontogeny of this group of plants has produced so far. The structure of the primordia varies considerably. Sometimes the pileus, sometimes the hymenophore differentiates first. Such development we have called concentrated.

The history of this discovery has been dealt with in our monograph (Reijnders, 1963). Singer (1975), who considers the ontogenetic structures important enough to devote ample attention to this subject, has, in the third edition of his 'Agaricales in modern Taxonomy', however not emphasized the significance of this phenomenon because: (1) so far too few species have been examined, (2) 'some well-studied genera, though undoubtedly homogenous, show a large number of different types of succession and even *Psathyrella velutina* and *P. pyrotricha* have different succession types'. So 'one is reconciled with the idea that this character has diagnostic value only when one notices that all volvate Amanitaceae and Volvariaceae are pileocarpous and that all Polyporaceae studied are stipitocarpous.'

This is true to be sure, but we distinguish different gradations concerning this sequence: pileostipitocarpous, isocarpous, and hymenopileocarpous. Besides, there is a difference whether a certain species represents a pronounced type and is e. g. pileocarpous in that sense that the pileus differentiates first very clearly and that it takes rather a long time before we can see the parallel hyphae of the stem. If, for instance, *Lacrymaria velutina* is pileostipitocarpous and *Lacrymaria pyrotricha* pileocarpous then the pileus-margin of the latter should show itself according to the definition only

somewhat earlier than the parallel stem-hyphae. But this is something completely different from the pronounced pileocarpy of, for instance, *Amanita* or *Volvaria* of which the complete pileus is clearly shaped long before differentiation of the stipe or before the hymenophore is visible. The difference between the two *Lacrymaria* or *Psathyrella* species is, in this respect, only slight and a certain latitude of variation has to be taken into consideration. Even when we distinguish many types unfortunately not all gradations can be expressed in that way. Nor do we believe that the exterior of the primordium determines the type of sequence completely. Although there is a correlation between shape and sequence one has to know the direction and differentiation of the hyphae to determine the sequence (Reijnders, 1963: 244; Moser, 1960: 32; Singer, 1975: 29).

We have to admit that, writing our publication in 1963, we still thought that there was mainly one general bifurcated line of stipitocarpous development towards pileocarpy and hymenocarpy. The first one should chiefly appear in a definite form in genera like *Amanita*, *Volvaria*, and also *Pluteus*, the latter in many chromosporous genera like *Coprinus*, *Psathyrella*, *Panaeolus*, *Conocybe*, *Bolbitius* etc. The remaining genera should show a smaller concentration and, for that matter, should group themselves between the stipitocarpy of the, in our opinion, lower Agaricales and the strong concentration as has been found in above-mentioned genera. As for this, new experiences have changed our mind. The very pronounced pileocarpy found in *Cortinarius* sect. *Scauri*, the isocarpy met in *Inocybe asterospora* (Reijnders, 1974) and the fact that pileocarpy as well as hymenocarpy is manifest in *Coprinus*, together with other reflections like the isocarpy of *Squamanita* (see the discussion under this heading) and the fair concentration of, for instance, *Deliculata* etc. have convinced us that we are dealing with parallel series in Agaricales as to the concentration of the development. However, we still consider the strongly concentrated forms as specialized because there are so many transitions from stipitocarpy to these concentrated forms occuring in different places (often coupled with innate-veil formation) and, moreover, because the concentrated primordia are to be found in genera which are considered specialized for the reason of other characteristics. Thus we are inclined to attribute phylogenetic importance to these phenomena and to speak of convergent series. We think we could demonstrate these, for instance, in the family of the Cortinariaceae, Strophariaceae, Coprinaceae and, in this case, the Agaricaceae. Of course there also are other similar series, whereas it is quite possible that concentrated forms sometimes are more or less isolated because only the top of an evolution branch remained.

In this publication we hope to demonstrate such a series in fungi which have been considered, in the course of time, as belonging to the family of Agaricaceae.

DESCRIPTIONS

CHAMAEMYCES FRACIDUS (Fr.) Donk

1. The first stage represented (Pl. 48A) consists of a slender column (length 2.5 mm, width 720 μm) which is somewhat broader towards the base. The hyphae in this

basal part are strongly interwoven (diameter 3–6.5 μm). In the higher portion of the little stalk the orientation of the hyphae is more longitudinal though always fairly intertwined (diameter 2–4 μm). The primordium is surrounded by a universal veil (width along the stem 400–550 μm) of a loose texture of somewhat wider hyphae (diameter 3–4 μm). At the tip we can see tufts of hyphae growing upwards. The universal veil, though not clearly delimited, may be somewhat broader here and its short-celled hyphae are already radiating in this stage (diameter 3 μm). This uppermost portion of the column has been photographed separately (Pl. 48B).

2. The second primordium of which we represent a median section (Pls. 48C; 49A) shows the origin of the cap (diameter at the level of the pileus 2.25 mm, diameter of the stem 1.5 mm). The pileus has arisen through the formation of a border of protenchyma (the generative tissue) at the periphery of the primordium just below the top and through the growth of these hyphae in a centrifugal and downward direction. The hyphae in the stipe run preponderantly in a longitudinal direction. A differentiation into a cortex zone and a marrow zone is perceptible, the tissue in the latter being somewhat looser, but the difference is not striking. The cells in the lower part of the stem are already inflated (diameter up to 20 μm). The stipe is surrounded by a universal veil (width 120–180 μm) consisting of loosely interlaced hyphae (diameter 2–3 μm), which are metachromatic at the periphery where the direction often is longitudinal.

Though the structure of the pileus trama is quite different from that of the stipe tissue we do not observe an abrupt demarcation between stipe and pileus, the longitudinal hyphae of the stem merging gradually into the completely intertwined tissue of the cap trama. The hyphae of the latter are more swollen in the centre (diameter 3–8 μm) but at the periphery they conserve their protenchymatic character (diameter 2–4 μm, with clamp-connections). Outwards we pass through a zone of very thin loose hyphae running in centrifugal direction and giving rise to a layer of palisades which are closely pressed together (width 30–35 μm; Pl. 48D). Although the palisades at the tip of slender hyphae are relatively short over the centre of the cap as well, they are lacking in the lateral part where the radiating hyphae still are entirely short-celled. Here and there at the outside of the palisade layer we observe fragments of the adjacent metachromatic hyphae of the outermost part of the veil. It is interesting to study the transition of the veil covering the cap into the one at the outside of the stem. This connection is to be found at the outside of the pileus margin. There we notice that the character of the veil is modified rather suddenly. The hyphae of the palisade layer lose their centrifugal direction immediately below the pileus margin and change into the interwoven hyphae of the covering of the stem (Pl. 49B). It is obvious that the palisade is limited to the cap and the question arises whether the palisade layer has to be called a trichodermium or whether it actually constitutes part of the universal veil. In the general discussion we intend to deal with this question in detail.

3. We insert a photomicrograph of a somewhat more advanced stage (diameter of the pileus *c.* 2.6 mm, length 6.4 mm; Pl. 48E) to show that here the plectenchyma at

the base has swollen into a bulb. We could call this inflated portion a primordial bulb, but this structure often is significant of the formation of the primordium as a whole since the first protenchyma arises in its interior (Reijnders, 1974). While *Chamaemyces* is clearly stipitocarpous this is not the case here and the bulb chiefly broadens later on, its cells being very inflated in this stage (diameter up to 45 μm). The demarcation between longitudinal stipe hyphae and interwoven pileus trama has become more clearly defined.

LEUCOCOPRINUS DENUDATUS (Rab.) Sing.

1. The youngest stage represented shows a slender column (Pl. 49C; width *c.* 360 μm, length *c.* 760 μm), which consists of rather intricate narrow hyphae (diameter 2–3 μm). Though these hyphae in the centre do not run strictly parallel to each other a preponderant longitudinal direction is observable. A basal plectenchyma is not yet sharply outlined. A short distance underneath the summit the hyphae are more densely interwoven and therefore this spot stands out somewhat darker. This is the rudiment of the centre of the pileus. At the outside of this dark-coloured zone the hyphae are loosely intertwined and this is also the case at the side of what will be the stem part later on. Although the universal veil is therefore not sharply outlined we meet with adjacent metachromatic hyphae at the periphery.

2. Before long there will be more differentiation in the upper part of the primordium. We photographed only the head of the column (Pl. 49D; width *c.* 610 μm, length 1.3 mm). Characteristic is a ring of chromophilous hyphae which has arisen in the upper part of the primordium perpendicular to the axis of the primordium. In the median section we observe two dark spots where the parallel hyphae are running down obliquely. These hyphae represent the rudiment of the cap margin and, more inward, of the hymenophore. The universal veil consists of two zones, the inner zone of loosely interwoven, somewhat enlarged hyphae (diameter *c.* 5 μm) and the outer zone of circumjacent periclinal hyphae. The basal plectenchyma is now obvious and composed of inflated cells (diameter up to 10 μm). There is not yet a distinct cap covering; this will differentiate later on. By short radiating hyphae with short cells crowded between the passing hyphae of the veil a chromophilous layer is formed. Afterwards this layer gives rise to a derm composed of a pseudoparenchyma (width of that layer *c.* 50 μm) of irregular isodiametric cells; consequently there is no hymeniderm. The scanty remnants of the universal veil are at the outside of the derm. At this stage the margin of the cap is still attached to the stem by the veil (width at the margin of the downward curved pileus 1.4 mm. We did not photograph this stage).

3. The only picture we insert is that of the pileus margin in a somewhat more advanced stage (diameter of the cap 1.3 mm; Pl. 49E) to show that the veil is of some more importance in this species. The lipsanenchyma is well developed and differs from the inner part of the universal veil by a denser texture of the hyphae.

The lamellae originate by folding. In this stage the derm of the pileus begins to differentiate as we described above. It is obvious that the lipsanenchyma has increased by intercalary growth and perhaps also by the hyphae coming from the stem and the pileus margin. When we compare this part of the primordium with that of similar stages of species like *Leucoagaricus naucinus* (Pl. 50F) or *Leucocoprinus cepaestipes* (Reijnders, 1948: pl. 7 fig. 30) or *Agaricus arvensis* (Atkinson, 1914: pl. 1, fig. 5, 6) we observe a striking conformation.

LEUCOAGARICUS NAUCINUS (Fr.) Sing.

This species looks much like a cultivated mushroom. We tried to obtain primordia of wild mycelia several times but it seems that even when the carpophores are still young and just coming out of the soil the superfluous primordia have disappeared already. When it turned out that they are very easily grown on about the same compost which is the substratum of the cultivated mushroom (Manz, 1971) I asked Miss G. Fritsche (Mushroom-breeding Station at Meterik, Limburg) to grow them for me; the mycelium was original from a stock present at the 'Centraalbureau voor Schimmelcultures' (Baarn).

1. The very young primordium (Pl. 50A; diameter somewhat beneath the rounded top 755 μm, length *c.* 1.4 mm) consists of a short rather bulbous or conical body with a rounded upper end. The tissue is dense, the protenchyma is composed of interwoven hyphae (diameter 2–3 μm). At the base they have the same character but, here and there, they are slightly more inflated (diameter up to 7 μm). Towards the upper end the hyphae are narrow, densely intricated and they are richer in protoplasmic content. This portion is surrounded by a universal veil (width of this layer 70–100 μm, photographed in detail on Pl. 50B). The veil has not developed at the periphery of the bulbous part, nevertheless it must be called universal veil because it will envelop, in later stages, the part where the hymenophore arises as well. The protenchymatic hyphae of the veil (diameter 1.5–2 μm) are radiating slightly but they also are intricate. At the periphery however, they are periclinal, parallel to the rounded surface of the primordium. We meet here with about the same structure as in the preceding species.

2. When the primordium is somewhat older the shape is more oblong (Pl. 50C; width beneath the tip 680 μm, length *c.* 1.6 mm). The texture is as described above, the basal part with the more inflated hyphae (diameter up to 7 μm) now taking up about one third of the primordium. Then a rather darker central portion follows towards the summit and finally we come to a chromophilous dome surrounded by the universal veil which is even less clearly outlined against the inner tissue than in the preceding stage. The chromophilous portion is, of course, the rudiment of the pileus.

3. The third stage represented (Pl. 50D; width at the level of the hymenophore 770 μm) shows the beginning of the hymenophore in two peripheral tufts of downward and outward growing hyphae under the dome. This is exactly the way in which

this structure arises in *Leucocoprinus cepaestipes* (see Reijnders, 1948: pl. 7 fig. 28). On the left side of the photograph we observe a lighter area due to the fact that some parts of these sections absorb less dye. The cause of this phenomenon is unknown; it concerns only this particular material. We did not meet with it before.

4. It is a characteristic of the material bred this way that twins are often found in it, i. e. there are two primordia united on one bulb. Leaving out of consideration the lighter spots with reduced coloration the twins develop normally and both often reach maturity (Pl. 50E; diameter of the largest specimen *c.* 1.25 mm, of the smallest one 1.15 mm). The bulb consists of rather homogenous and intricate hyphae which are much more inflated in the lower part (diameter up to 10 μm) than in the centre under the stipe (diameter up to 6.5 μm, usually 3–5 μm). The stem is rather highly coloured and still short. It consists of slender, parallel hyphae but the trama of the pileus is again composed of interwoven tissue (diameter of the hyphae *c.* 5 μm). The transition from stipe to pileus trama is not yet abrupt. Under the pileus we observe, at the sides, the downward growing protenchymatic hyphae which will form the hymenophore. The veil which, in the former stages, was very much like that of *Leucocoprinus* has undergone some changes; it now consists of more densely intricate short-celled hyphae and the difference between the radiating part and the outer part of periclinal hyphae is less striking.

Finally, over the pileus, it is almost entirely absorbed into the pileopellis but there will remain at the pileus margin a well-developed veil composed, to a large extent, of the lipsanenchyma and moreover of the universal veil at the outside. We have photographed the pileus margin and the veil of a more advanced stage (Pl. 50F; diameter of the cap 1.65 mm) to compare the structure of this part with that of *Leucocoprinus* and *Agaricus* (see there). The lamellae present themselves as folds and, at the origin, their trama is clearly divergent (Pl. 50G).

Consequently there is in this species a tendency towards pileocarpy and moreover it is bivelangiocarpous.

DISCUSSION

THE VEIL OF CHAMAEMYCES.— It appears that in the covering tissue of the Lepiotaceae often a layer of erected, radiating elements is to be found. The difficulty however is to decide whether this layer belongs to the universal veil or to the pileopellis. The universal veil is, according to the definition, a separately differentiated part on the outside of the primordium which also covers the spot where the hymenophore originates or will originate and which often covers, at least partly, the stipe (Reijnders, 1963: 27). The pileopellis is a covering layer restricted only to the pileus. The name 'pileopellis' has been proposed by C. Bas to indicate this layer in a general sense because one has attached all kinds of particular meanings to the name 'cuticula'.

Atkinson (1914) has described, in *Lepiota clypeolaria*, a universal veil already existing in a very early stage consisting of an inner zone of more or less pseudoparenchymatic

tissue and an outer layer of tightly packed, radiating hyphae. Later on it becomes concrete with the pileus because this one extends through the protenchyma. Anyway this is a universal veil for it is also present at the side of the stem. Less clear is the situation in *Lepiota cristata* (Atkinson, 1916). Here there is also a cap of radiating hyphae in the veil over the pileus but these hyphae will not extend far below the pileus-margin, even in later stages. The photographs show that they are non-existent just under the pileus-margin. The pileus becomes concrete with this layer. The veil along the stipe, actually present, has another structure. One would be inclined to consider the radiating elements here as a trichodermium, but Atkinson regards them as a universal veil, possibly in comparison with *L. clypeolaria*. Now veils with radiating hyphae developing into spherocysts-veils afterwards do really appear in Lepiotaceae: *Lepiota acutesquamosa* (Greis 1937) is a well-known example. Less distinct was the radiating development of the hyphae in the spherocysts-veils of *L. sistrata* and *Melanophyllum* (Reijnders, 1963: 106–107). But also a tricho- or palisadodermium frequently occurs in this family. *Leucocoprinus* has a similar structure dividing into many cells later on while *Macrolepiota* (*rhacodes*) has a distinct palisadodermium with slender, tightly packed palisades.

In *Chamaemyces* we found a peculiar structure which possibly throws some light on the controversy between *Lepiota clypeolaria* and *L. cristata*. Here the young primordium is unmistakably enveloped by a universal veil with a somewhat deviating, looser structure of partly broadened hyphae. Only at the top we already find radiating hyphae at the outset. Afterwards when the pileusmargin has differentiated the entire cap portion is enveloped by a veil consisting of two layers, an outer layer of erect hyphae and an inner one of interwoven hyphae with wider interspaces. The radiating hyphae however end just under the pileus-margin, so that the stem-portion of the veil exists exclusively of intricate hyphae. It is as if an organizing or determinating factor, confining itself to the pileus, asserts itself in the universal veil as well. For because of the continuation of the layer of loose hyphae of that universal veil along the stipe we have to go on calling this structure a universal veil. However, in nature no allowance is made for our classifications. This is also apparent from a structure found by Hugueney (1966) in *Lepiota ignipes* Locquin. This species from the section with spurred spores has, according to Singer (1975: 474) a 'cuticle of the pileus with a palisade'. To the opinion of Hugueney this layer however consists of part of the universal veil which here indeed continues a certain distance under the pileus-margin (but for the greater part not along the stem). At the outside of this layer there is a thin veil with periclinal protenchymatic hyphae. This was not the case in *Lepiota clypeolaria* with which Hugueney otherwise compares the veil. Here it is becoming rather difficult to tell what exactly is the veil and what the pileopellis.

LEUCOCOPRINUS.— We made researches into the development of *Leucocoprinus cepaestipes* before (Reijnders, 1948: pl. 6 figs. 25–27, pl. 7 figs. 28–33). Since it became evident that the development of the very closely related *L. luteus* (Bolt. ex Fr.) Locquin [=*L. Birnbaumii* (Corda) Sing., is homologous to that of *L. cepaestipes*, we do not consider it necessary to photograph and to describe the development of

this species once more. In this species again a structure of the young primordium which we could call pileostipitocarpous (in the earliest stages approaching stipitocarpy), again the same way of origin of the hymenophore and pileus-margin, the same structure of the veil and later on of the pileopellis. *Lepiota denudatus* (Rab.) Sing. deviates little from this type. Possibly the pileostipitocarpy is slightly more pronounced or there is even a tendency towards pileocarpy. In a very early stage which here too consists of a slender column, there is a dark spot in the upper part, being composed of interwoven tissue. Only the structure of the 'pileopellis' is slightly different in *L. denudatus*. While *L. cepaestipes* and *L. luteus* both have a pileopellis consisting of closely packed, erect elements which, in earlier stages, we could call a palisadodermium (although the palisades are divided into chains of short cells and the arrangement gets lost later on), *L. denudatus* has, afterwards, a paradermium consisting of several layers of pseudoparenchymatic cells. In both cases the adjacent hyphae of the universal veil are found at the outside.

LEUCOAGARICUS.—Singer (1975: 452) writes: 'This genus is intermediate between *Macrolepiota* and *Leucocoprinus*. The absence of clamp-connections in the trama of the pileus and the stipe makes it possible to distinguish it from *Macrolepiota*.' There should be a number of other characteristics which are more or less correlated with it. Others (Heinemann) consider this genus difficult to define since it includes a number of species not classifiable elsewhere. Be it as it may, the development does support the intermediate position between *Leucocoprinus* and *Macrolepiota*. The concentration (disposition to pileocarpy) is higher in *Leucoagaricus naucinus* than in *Leucocoprinus* but less high than in the pileocarpous *Macrolepiota rhacodes*. And how much does a section like the one in Pl. 50E resemble an analogous section of *Macrolepiota* (Reijnders, 1963: pl. 45 fig. 1). One should, for instance, pay attention to the oblique position of the tardy hymenophore. Singer gives in *Leucoagaricus* the existence of a trichodermium which however can be scattered. In *L. naucinus* the pileus is concrete with the universal veil; there are some broader elements protruding out of the veil but there is no question of a trichodermium.

THE DEVELOPMENT OF THE AGARICACEAE.—We would like to bring together, once again, the most important features of the development of the tested species (*Squamanita* excepted) in a scheme. Most of them are taken from our research of 1963. Thus we can see the *Cystoderma* species with little concentrated development on one side. The monovelagiocarpy and the lack of a differentiated pileipellis also underline the primitive character of these species among which we reckon *Phaeolepiota* as well. But there is indeed a special universal veil consisting of spherocysts originated from a matrix-layer. On the other side there are the genera with a highly concentrated development: *Macrolepiota* and *Agaricus*. The species of these genera also are often provided with a distinct bulb. In the beginning the primordia are bulbous or, at the most, oval. The pronounced bivelangiocarpy with a lipsanenchyma which, later on, develops intensively (so that the mature fungi often have a luxuriant ring) belongs herewith.

We already presumed earlier (Reijnders, 1974) that the long development under

ground enabled by the inversion of the succession leads to the origin of large specimen. These indeed are numerous in the genera *Agaricus* and *Macrolepiota*. A second reason for a prolonged subterranean development might be, we suppose, that thus we obtain species which, by inflation of cells, can rise above the soil in a short time which could be an advantage when e. g. the substratum should dry up fast or when the fungi have to make use of a shower of rain to sporulate quickly. Although *Leucocoprinus* does not show a strong concentration of development (pileostipitocarpy) the total structure of the carpophore seems to be related to a rapid unfolding; the thin sulcate pileus, the slender stipe. Possibly one thing and another are also connected with the fact that *Leucocoprinus* is to be found in warm regions or in hothouses.

In the scheme we have given once more the details of the structure of the veil and of the pileopellis accurately. Just the less concentrated forms of Agaricaceae are really specialized as to the covering layers. This is also true when the species are monovelangiocarpous or when the lipsanenchyma is underdeveloped. The difficulties in interpretation what has to be regarded as a veil and what as the pileopellis have been talked about in this discussion before. Apparently factors of development, limited to the pileus, sometimes work in the veil as well. We have to remark however that when the veil consists of a layer of closely packed elements there is no trichodermium or palisadodermium and vice versa. It is remarkable that neither the universal veil nor the pileopellis are particularly organized in the specialized genus *Agaricus*.

As to the ontogeny of the carpophores some other large taxa within Agaricales show about the same picture as the Agaricaceae. We hope to deal with the pattern of development in the Cortinariaceae and the Coprinaceae later on.

SQUAMANITA.—Finally we have to make some remarks on the genus *Squamanita* Imbach (=*Coolia* Huijsman) which we left out of consideration so far. We have examined the development of *Squamanita odorata* (Reijnders, 1952; 1963: 166–169). The ontogeny of this genus is very interesting because of the presence of 'protocarpic tubers'. Unfortunately our material was in a rather defective state and we did not have the disposal of sufficiently young stages to determine exactly the developmental succession. We stated that this succession might be at least isocarpous for the in youngest primordium available hymenophore and cap were present and the stipe was short (Reijnders, 1952: pl. 4 fig. 2). This relatively high degree of concentration is undoubtedly connected with the origin of the primordia in the protocarpic tubers which are presumably specialized primordial bulbs (Reijnders, 1974).

If *Squamanita* should belong to the Tricholomataceae, as has been suggested several times, we might consider this high concentration as a continuation of the developmental trend, met with in some sections of the Tricholomataceae with a somewhat bulbous stem.

Now Bas (1965) and Singer (1975: 481) emphasize the affinity of *Squamanita* with *Cystoderma* and their arguments are very convincing. The existence of an intermediate form, *Squamanita paradoxa* (Smith and Sing.) Bas [=*Dissoderma paradoxum* (Smith and Sing.) Sing.] is especially important in this respect. In one way the close affinity of

Cystoderma and *Squamanita* is somewhat astonishing for the species of the first genus, which have been examined, show stipitocarpous primordia.

Up to the present we believed that forms with a highly concentrated development are to be found in several sections of the system of the Agaricales and that at least in some of these genera this phenomenon presents itself without exceptions. We mentioned some of these genera before (see Introduction). There should be a gradual transformation towards the extremely concentrated models, which might prove that the latter did not arise readily in the course of evolution. As yet the instance of *Squamanita* and *Cystoderma*, where the forms with more concentrated development of the former genus are closely related to the stipitocarpous species of the latter, is still a rather exceptional case, which seems to support Singer's idea that different developmental types can occur in the same taxon. The distribution of the froms with concentrated development over the system certainly needs further study. The distribution of the forms with concentrated development over the system certainly needs further study.

Once more, we do not know exactly the degree of concentration of the primordia of *Squamanita*. However, we are inclined to think that this taxon represents the end of a short lateral branch in the pattern of evolution, which has arisen from species with non-concentrated primordia (diffuse development).

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Résumé

Les types du développement des carpophores dans la famille des Agaricaceae varient considérablement mais son patron ontogénétique n'est pas sans ordre. Environ 20 espèces ont été examinées. D'un côté les genres *Cystoderma*, *Phaeolepiota* et *Chamaemyces* dont les espèces paraissent être stipitocarpes, de l'autre côté les primordiums très concentrés de *Macrolepiota* et d'*Agaricus* (isocarpes, piléocarpes ou hyménocarpes). Les genres *Lepiota* et *Leucocoprinus* occupent évidemment une position intermédiaire comme *Leucoagaricus* dont le développement est plus concentré. Les problèmes concernant le développement chez *Squamanita* ont été traité séparément.

Les éléments rayonnants sur le pileus de *Chamaemyces fracidus* ne représentent pas un palissadoderme dans un sens stricte, mais ils font partie du voile universel. Cependant cette structure se restreint à la surface du chapeau et c'est pourquoi qu'il faut admettre qu'un facteur ontogénétique influe sur la formation du chapeau aussi bien que sur celle de cette couche vélaire. Il y a d'autres espèces où une couche a éléments dressés et serrés enveloppe

TABLE I

Species	Order of succession	Veils	Pileopellis
Cystoderma amianthinum	stipitocarpous	monovelangiocarpous a spherocysts veil, no radiating hyphae	a simple cortex
Cystoderma carcharias	stipitocarpous	monovelangiocarpous, a spherocysts veil, no radiating hyphae	a simple cortex
Phaeolepiota aurea	stipitocarpous or pileostipitocarpous	monovelangiocarpous a spherocysts veil, no radiating hyphae	a simple cortex
Chamaemyces fracidus	stipitocarpous	monovelangiocarpous, no universal veil with radiating hyphae at the outside, only over the pileus	none
Cystolepiota sistrata	pileostipitocarpous	bivelangiocarpous lipsanenchyma scanty	a cortex
Cystolepiota hetieri	probably pileostipitocarpous	bivelangiocarpous lipsanenchyma scanty	a cortex
Melanophyllum echinatum	probably pileostipitocarpous	bivelangiocarpous lipsanenchyma scanty	a cortex
Lepiota cristata	stipitocarpous	bivelangiocarpous lipsanenchyma scanty	a trichodermium?
Lepiota clypeolaria	pileostipitocarpous	bivelangiocarpous universal veil with radiating hyphae at the outside	none
Lepiota acutesquamosa	pileostipitocarpous	bivelangiocarpous, universal veil with radiating hyphae at the outside	none
Lepiota ignipes	?	bivelangiocarpous, universal veil with radiating hyphae at the inside	none
Leucocoprinus cepaestipes	pileostipitocarpous	bivelangiocarpous	at first a palisadodermium
Leucocoprinus luteus	pileostipitocarpous	bivelangiocarpous	at first a palisadodermium
Leucocoprinus denudatus	pileostipitocarpous	bivelangiocarpous	a paradermium
Leucoagaricus naucinus	somewhat pileocarpous	bivelangiocarpous lipsanenchyma luxuriant	none
Macrolepiota rhacodes	pileocarpous	bivelangiocarpous lipsanenchyma luxuriant	a palisadodermium
Agaricus comtulus	isocarpous	bivelangiocarpous lipsanenchyma luxuriant	a cortex
Agaricus arvensis	isocarpous	bivelangiocarpous lipsanenchyma luxuriant	a cortex
Agaricus bitorquis	hymenocarpous	bivelangiocarpous lipsanenchyma luxuriant	a cortex
Agaricus bisporus	hymenocarpous	bivelangiocarpous lipsanenchyma luxuriant	a cortex

aussi la marge pilérique et la partie supérieure du stipe (chez *Lepiota clypeolaria* a la périphérie et chez *Lepiota ignipes* au côté interne du voile universel comme une structure secondaire). Nous trouvons chez *Leucocoprinus* un trichoderme qui se transforme après en un paraderme; un vrai palissadoderme se présente chez *Macrolepiota*.

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EXPLANATION OF PLATES 48–50

PLATE 48

Figs. A-E. *Chamaemyces fracidus*: Fig. A. young stage ×17.5; Fig. B. top of A ×94; Fig. C. intermediate stage ×10.5; Fig. D. detail of veil on the pileus of Fig. C ×224; Fig. E. intermediate stage with bulb ×14.

PLATE 49

Figs. A-B. *Chamaemyces fracidus*: Fig. A. detail of Pl. 48C ×44.8; Fig. B. detail of pileus margin of the same stage ×179.

Figs. C-E. *Leucocoprinus denudatus*: Fig. C. young stage ×82; Fig. D. intermediate stage, upper part ×83; Fig. E. more advanced stage, pileus margin, slightly tangential ×82.

PLATE 50

Figs. A-G. *Leucoagaricus naucinus*: Fig. A. young stage ×20; Fig. B. veil on pileus of stage in Fig. A ×200; Fig. C. slightly more advanced stage ×32; Fig. D. intermediate stage, origin of the hymenophore ×80; Fig. E. twins, somewhat more advanced as in Fig. D ×20; Fig. F. pileus margin, same stage as in Pl. 49E ×94; Fig. G. the origin of the lamellae in a similar stage ×200.

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ZYGOSPORE ORNAMENTATION IN THE GENERA MUCOR AND ZYGORHYNCHUS

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(With Plates 51–57, one Table, and one Text-figure)

Zygospores of species of the genera *Mucor* and *Zygorhynchus* were studied by means of scanning electron microscopy. The types of ornamentation are described and compared with those of some species of other genera of the Mucorales. The possibility of grouping the species according to zygospore characters is discussed.

INTRODUCTION

Zygospores are an important criterium in the taxonomy of the Mucorales. If mating partners are available and the suitable conditions are known, zygospores of most *Mucor* species can be obtained in 4–7 days on one of the ordinary media. The size, shape, colour and a rough estimate of the ornamentation of the wall of the zygospores can be easily determined by light microscopy (LM) under low magnification. A detailed examination of the ornamentation by means of LM, however, is difficult because of the heavy pigmentation and thickness of the zygospore wall.

Hawker & Gooday (1968) and Hawker & Beckett (1971) studied the zygospore wall structure of *Rhizopus sexualis* by means of transmission electron microscopy (TEM). The zygospore wall is formed by deposition of material inside the original gametangium wall, which remains as torn fragments on the outside of the mature zygospores. There are two thick layers: an outer electron dense warty coating and an inner electron transparent layer, separated by a thin 'smoothing' layer. Hawker & Gooday (1968) also provided some micrographs showing the ornamentation of *Rhizopus sexualis* as seen by means of scanning electron microscopy (SEM), but no further research has been done on this subject.

The purpose of this paper is to compare the wall ornamentation of mature zygospores of all available *Mucor* species known to produce them. The zygospores of *M. plasmaticus*, recently reported by Pidoplichko & Milko (1971), were not available for study. Zygospores of the genus *Zygorhynchus* have also been included because of the close relationship between *Zygorhynchus* and *Mucor*. In addition, zygospores of some representatives of other, more or less related members of the Mucorales are compared. In some cases azygospores were also examined.

The SEM micrographs, the descriptions of zygospore ornamentation, the grouping of the species after this character, and the comparison with other genera were done by Samson and Stalpers, while Schipper provided the zygospores and gave the taxonomical comment on the grouping of the *Mucor* and *Zygorhynchus* species.

MATERIALS AND METHODS

Zygospores were obtained in matings of the tester pairs of the species as indicated by Schipper (1973, 1975) in petridishes under conditions favourable for zygospore production in the species.

STRAINS EXAMINED:
Mucor alpinus CBS 105.10
Mucor aromaticus CBS 195.71 (+) × 196.71 (—)
Mucor azygosporus CBS 292.61
Mucor bacilliformis CBS 251.53
Mucor bainieri CBS 293.63
Mucor circinelloides CBS 192.68 (+) × 394.68 (—); strain Hadlok 84 (+) × Hadlok 46 (—); CBS 192.68 (+) × 222.28 (—); CBS 479.70 (azygosporous strain)
Mucor flavus CBS 235.35 (+) × 234.35 (—)
Mucor genevensis CBS 144.08
Mucor griseocyanus f. *griseocyanus* CBS 223.56 (+) × 907.69 (—)
Mucor griseocyanus f. *jansenii* CBS 205.68 (+) × 204.68 (—)
Mucor griseocyanus f. *griseocyanus* CBS 907.69 (—) × f. *jansenii* CBS 205.68 (+)
Mucor hiemalis f. *hiemalis* CBS 200.28 (+) × 201.65 (—); CBS 972.68 (+) × 971.68 (—); CBS 978.68 (+) × 978.68A (—); CBS 975.68 (+) × 975.68 A (—)
Mucor hiemalis f. *luteus* CBS 244.35 (+) × 243.35 (—)
Mucor hiemalis f. *silvaticus* CBS 250.35 (+) × 249.35 (—)
Mucor hiemalis f. *corticolus* CBS 366.68 (+) × 365.68 (—)
Mucor inaequisporus CBS 496.66 (+) × 497.66 (—)
Mucor '*indicae-seudaticae*' CBS 104.75
Mucor lamprosporus CBS 195.28 (+) × 196.28 (—)
Mucor lusitanicus CBS 277.49 (+) × 276.49 (—)
Mucor miehei CBS 282.68; CBS 182.67
Mucor mucedo CBS 144.24 (+) × 109.16 (—)
Mucor piriformis CBS 225.29 (+) × 169.25 (—)
Mucor pusillus CBS 253.53 (+) × 354.68 (—); CBS 245.58 (homothallic strain)
Mucor plumbeus CBS 213.75 (+) × 848.72 B (—); 210.75 (+) × 246.58 (—)
Mucor racemosus f. *racemosus* CBS 124.23 (+) × 260.68 (—)
Mucor racemosus f. *sphaerosporus* CBS 115.08 (+) × 238.35 (—)
Mucor racemosus f. *racemosus* CBS 260.68 (+) × f. *sphaerosporus* CBS 115.08 (—)
Mucor saturninus CBS 974.68 (+) × CBS 137.40 (—); CBS 637.65 (+) × 521.64 (—)
Mucor rouxii CBS 226.29 (+) × CBS 422.71 (—)
Zygorhynchus californiensis CBS 402.58
Zygorhynchus exponens var. *exponens* CBS 403.58
Zygorhynchus exponens var. *smithii* CBS 404.58
Zygorhynchus heterogamus CBS 338.70
Zygorhynchus japonicus CBS 254.69
Zygorhynchus macrocarpus CBS 215.57
Zygorhynchus moelleri CBS 406.58, CBS 501.66
Zygorhynchus psychrophilus CBS 336.68

Absidia spinosa CBS 106.08
Backusella circina CBS 128.70 (+) × 129.70 (—)
Gongronella butleri CBS 227.36 (+) × 226.36 (—)
Parasitella simplex CBS 412.66 (+) × 208.28 (—)
Phycomyces blakesleeanus CBS 283.35 (+) × 284.35 (—)
Pirella circinans CBS 306.29 (+) × 367.59 (—)
Rhizopus sexualis CBS 336.62
Syzygites megalocarpus CBS 372.29.

Zygospores, usually from 7 days old cultures, were transferred to squares of double-sided adhesive tape, attached to specimen stubs for the SEM and air-dried for 24 hours. The specimens were coated with gold in a sputter coater for 2 minutes at 1.2 mV. Preparations were examined with a Cambridge Stereoscan microscope at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

TEM studies by Hawker & Gooday (1968) and Hawker & Beckett (1971) showed, that *de novo* wall formation in zygospores takes place along the inside wall of the cell after gametangial fusion combined with an increase in volume. From the present SEM studies it was concluded, that the new wall pierces the gametangial wall at various places simultaneously. Fragments of the gametangial wall can be seen at the most extended points of the ornamentation and around the suspensors (Pl. 55 fig. 27). The thick warty coating appears to be easily separable from the zygospore; gentle squashing or manipulation with a pair of needles is sometimes sufficient to remove this layer, e.g. *Mucor lusitanicus* (pl. 51 fig. 1). In contrast with *Rhizopus sexualis* (Hawker & Gooday 1968; Hawker & Beckett, 1971), the then exposed layer is nearly smooth and does not follow the shape of the warty layer.

The warts can remain rather flat and irregular in shape or in other species they enlarge to become more or less conical (Fig. 1, diagram). These conical warts have

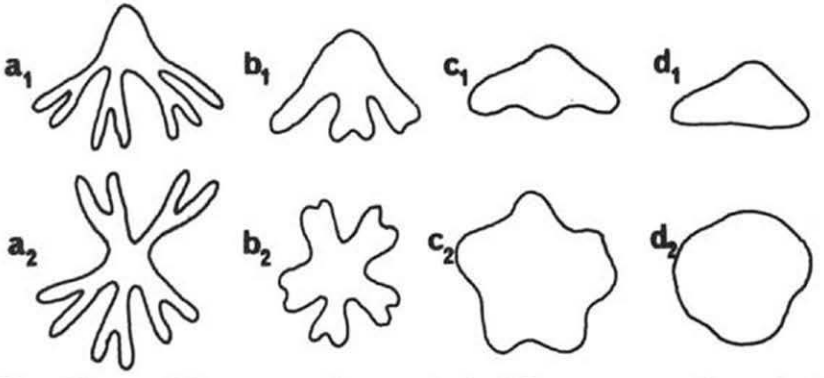

Fig. 1. Diagram of the ornamentation types in the different groups: a. Group A1; b. Group A2; c. Group B; d. Group C. (a_1–d_1 = sideview, a_2–d_2 = surface view).

the appearance of a starfish (indicated as stellate in LM descriptions); they consist of a raised central region and a number of arms, usually 4 to 5, but sometimes up to 8 or 10. The arms may interlock with those of the neighbouring warts and they may be bifurcately branched at their terminal ends. In some cases the arms tend to be free at the central region, giving the zygospore a spiny appearance (Pl. 52 fig. 10, 12), whilst in other cases arms are not actually formed, but just indicated by ridges (Pl. 53 fig. 13, 16). Length/width ratio of the arms and their number are more or less uniform within a species, but highly variable in the different species. In ageing zygospores the conical starfish-like warts may become flattened because of the increase in volume of the zygospore (Pl. 51 fig. 2–3). This feature is particularly developed in group A1.

In the species with starfish-like ornamentation the type of ornamentation is fairly constant, but in some species with irregular warts there is sometimes a tendency towards starfish-like ornamentation. This, for instance, is the case in *Mucor saturninus* (Pl. 54 fig. 24).

Three main types can be recognized in *Mucor* and *Zygorhynchus*; the delimitations, however, are not sharp and some species may eventually be placed in two groups.

GROUP A.—Zygospores covered with distinct starfish-like ornamentation which may become spiny.

1. Arms of ornamentation long and narrow, length/width=2.5–6. Warts never completely split up (Pl. 51 figs. 2–6; Pl. 52 figs. 7, 10).

   Mucor aromaticus, *M. bainieri*, *M. circinelloides*, *M. griseocyanus*, *M. lusitanicus*.

2. Arms of ornamentation rather plump, length/width=1–2.5. Zygospores sometimes spiny (Pl. 52 figs. 8, 9, 11, 12; Pl. 53 figs. 13–18; Pl. 54 figs. 19–23).

   Mucor azygosporus, *M. bacilliformis*, *M. inaequisporus*, *M. rouxii*, *M. lamprosporus*, *M. plumbeus*, *M. racemosus*, *Zygorhynchus californiensis*, *Z. exponens*, *Z. heterogamus*, *Z. japonicus*, *Z. macrocarpus*, *Z. moelleri*, *Z. psychrophilus*.

GROUP B.—Zygospores completely or at most places covered with an irregular, warty ornamentation; rarely with a tendency towards a starfishlike ornamentation.

1. Zygospores large, up to 180–290 μm in diameter. (Pl. 54 fig. 24; Pl. 55 figs. 25–27).

   Mucor flavus, *M. mucedo*, *M. piriformis*, *M. saturninus*.

2. Zygospores smaller, up to 110 μm in diameter (Pl. 55 figs. 28–30; Pl. 56 figs. 31, 32).

   Mucor genevensis, *M. hiemalis* f. *corticolus*, *M. hiemalis* f. *hiemalis*, *M. hiemalis* f. *luteus*, *M. hiemalis* f. *silvaticus*.

GROUP C.—Zygospores covered with distinct and separated warts. (Pl. 56 figs. 33, 34).

Mucor miehei, *M. pusillus*.

COMPARISON OF THE GROUPING ACCORDING TO THE ZYGOSPORE SCULPTURE WITH THAT BASED ON OTHER MORPHOLOGICAL AND INTERFERTILITY CRITERIA

GROUP A1—*Mucor circinelloides*, *M. griseocyanus*, *M. lusitanicus*, and *M. bainieri* form a natural group in view of general morphology. Interspecific mating of the heterothallic species may result in the production of zygospores. *Mucor aromaticus* differs from these species by the large dimensions of its sporogenous structures and by the colour and size of the zygospores which are produced high in the aerial mycelium. In some matings between strains of *M. griseocyanus* zygospores were observed resembling those of group A2.

GROUP A2—Besides a number of *Mucor* species this group includes all species of *Zygorhynchus*. *Mucor inaequisporus*, *M. rouxii*, and *M. lamprosporus* are rather closely related in view of the results of interspecific matings. *Mucor plumbeus* and *M. racemosus* form another group of related species. *Mucor bacilliformis* is homothallic. *Zygorhynchus exponens* and *Z. heterogamus* show a similar ornamentation to that of *M. azygosporus*, which differs from the other species of this group by the formation of azygospores. The zygospores of *Z. moelleri* resemble those of *M. rouxii* in their spiny appearance, while those of *Z. japonicus* are reminiscent of *M. bacilliformis*. The ornamentation of the zygospores of *M. bacilliformis*, *M. inaequisporus*, and *M. lamprosporus* is sometimes variable and approaches that of group B.

GROUP B1—The species classified in this group have large sporangiophores and show optimum development at lower temperatures. *Mucor mucedo* and *M. piriformis* are closely related, whilst *M. saturninus* and *M. flavus* are also related but to a lesser extend. In view of their ornamentation *M. flavus* and *M. saturninus* also have affinities with members of group B2. The taxonomy of the species of this group has been discussed in detail by Schipper (1975).

GROUP B2—The species are closely related and have been revised by Schipper (1973). A similar type of ornamentation is observed in CBS 109.10, deposited as *M. alpinus* Hansen.

GROUP C.—*Mucor pusillus* and *M. miehei* form a natural group and can be refered to as *Rhizomucor* sensu Hesseltine & Ellis (1973), a name published by Lucet & Costantin (1900) as a section of *Mucor*. The zygospores are small, up to 75 μm in diameter and only produced at temperatures between 30 and 40 °C. The ornamentation of the zygospores of *M. miehei* somewhat resembles that of *M. hiemalis*.

COMPARISON WITH OTHER GENERA

For reasons of comparison representatives of several other genera of Mucorales have been examined on zygospore ornamentation. The zygospores of *Absidia spinosa* Lendner, *Phycomyces blakesleeanus* Burgeff (Pl. 56 fig. 35) and *Syzygites megalocarpus* Ehrenb. ex Fr. (Pl. 56 fig. 36) have an ornamentation not comparable to that

Table 1. Zygospore characters of *Mucor* and *Zygorhynchus* species (mainly after Schipper, 1969, 1973, 1975, and Hesseltine & *al.*, 1959).

Name	Sexual reproduction	Max. diam.	Pigmentation	Group	Fig.
M. aromaticus Povah	het.	160 µm	black	A1	8
M. azygosporus R. K. Benjamin	azy.	180 µm	black	A2	15
M. bacilliformis Hesseltine	hom.	72 µm	black	A2	13
M. bainieri Mehrotra & Baijal	azy.	120 µm	rbr.–dbr.	A1	6
M. circinelloides Tieghem	het., azy	100 µm	rbr.–dbr.	A1	5
M. flavus Bain.	het.	180 µm	black	B1	27
M. genevensis Lendner	hom.	100 µm	brown–dbr.	B2	31
M. griseocyanus Hagem	het.	80 µm	rbr.	A1	7
M. hiemalis Wehmer	het.	100 µm	br.–black	B2	28–30, 32
M. inaequisporus Dade	het.	100 µm	black	A2	18
M. lamprosporus Lendner	het.	75 µm	brown	A2	17
M. lusitanicus Bruderlein	het.	115 µm	rbr.–dbr.	A1	1–4
M. miehei Cooney & Emerson	hom.	50 µm	rbr.–dbr.	C	34
M. mucedo L. ex Fres.	het.	250 µm	black	B1	25
M. plumbeus Bon.	het.	75 µm	dbr.	A2	14
M. piriformis Fischer	het.	240 µm	black	B1	26
M. pusillus Lindt	het.,hom.	70 µm	dbr.	C	33
M. racemosus Fres.	het.	110 µm	rbr.	A2	16
M. rouxii (Calmette) Wehmer	het.	100 µm	black	A2	12
M. saturninus Hagem	het.	180 µm	black	B1	24
Z. californiensis Hesseltine & *al.*	hom.	47 µm	brown–dbr.	A2	11
Z. exponens Burgeff	hom.	80 µm	rbr.–dbr.	A2	21
Z. exponens var. *smithii* Hesseltine & *al.*	hom.	62 µm	red–brbl.	A2	20
Z. heterogamus (Vuill.) Vuill.	hom.	70 µm	dbr.–black	A2	9
Z. japonicus Kominami	hom.	80 µm	brown–black	A2	22
Z. macrocarpus Ling	hom.	100 µm	brown–black	A2	19
Z. moelleri Vuill.	hom.	56 µm	brown–dbr.	A2	10
Z. psychrophilus Schipper & Hintikka	hom.	100 µm	black	A2	23

ABBREVIATIONS: hom. = homothallic; het. = heterothallic; azy. = azygosporous; brbl. = brownish black; dbr. = dark brown; rbr. = red-brown.

of *Mucor*; in addition the zygospores are enveloped by appendages arising from the suspensors (*Absidia*, *Phycomyces*) or by repeated ramification of zygophore branches (*Syzygites*). *Backusella circina* Ellis & Hesseltine (= *Mucor pseudolamprosporus* Naganashi) (Pl. 57 fig. 37) and *Rhizopus sexualis* (G. Smith) Callen (Pl. 57 fig. 38) have zygospores of the type of group A2. The zygospores of *Pirella circinans Bain.* (Pl. 57 fig. 39) closely resemble those of *Mucor mucedo* (group B1), except for the fact that they are much smaller (maximum diameter 140 µm). When well developed the ornamentation of the zygospores of *Parasitella simplex* Bain. (Pl. 57 fig. 40) is comparable with that of the species placed in group A2, but most zygospores have the type of ornamentation found in group B2. *Gongronella butleri* (Lendner) Peyronel & Dal Vesco (Pl. 57 fig. 41)

has regularly spiny zygospores which can not adequately be placed in one of the afore mentioned groups.

An undescribed thermophilic strain, sent to the CBS by Dr. M. J. Thirumalachar and tentatively named *Mucor indicae-seudaticae*, produced smooth-walled, brown zygospores (Pl. 57 fig. 42). Since zygospores and other morphological characters are not *Mucor*-like the classification of the species in a separate genus is advisable.

ACKNOWLEDGEMENTS

The authors wish to thank the SEM Research Group of the University of Amsterdam for their co-operation, Miss L. van Dijk for technical assistance and Miss J. H. Pannebakker for processing the photographs.

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EXPLANATION OF PLATES 51–57

Scale as indicated represents 10 μm, except in Figs. 35 and 36, 100 μm.

PLATE 51

Figs. 1–4. *Mucor lusitanicus*, CBS 277.49 ×276.49; Fig. 1. zygospore with partly removed outer wall; Figs. 2–4. different stages; Fig. 5. *M. circinelloides*, CBS 192.68 ×394.68; Fig. 6. *M. bainieri*, CBS 293.63.

PLATE 52

Fig. 7. *Mucor griseocyanus*, CBS 205.68 ×907.69; Fig. 8. *M. aromaticus*, CBS 196.71 ×196.71; Fig. 9. *Zygorhynchus heterogamus*, CBS 338. 74; Fig. 10. Z. *moelleri*, CBS 501.66; Fig. 11. *Z. californiensis*, CBS 402.58; Fig. 12. *M. rouxii*, CBS 226.29 ×422.71.

PLATE 53

Fig. 13. *Mucor bacilliformis*, CBS 251.52; Fig. 14. *M. plumbeus*, CBS 210.75 ×246.58; Fig. 15. *M. azygosporus*, CBS 292.61; Fig. 16. *M. racemosus*, CBS 260.68 ×115.08; Fig. 17. *M. lamprosporus*, CBS 195.28 ×196.28; Fig. 18. *M. inaequisporus*, CBS 496.66 ×497.66.

PLATE 54

Fig. 19. *Zygorhynchus macrocarpus*, CBS 215.57; Fig. 20. *Z. exponens* var. *smithii*, CBS 404.58; Fig. 21. *Z. exponens*, CBS 403.58; Fig. 22. *Z. japonicus*, CBS 254.69; Fig. 23. *Z. psychrophilus*, CBS 336.68; Fig. 24. *Mucor saturninus*, CBS 974.68 ×137.40.

PLATE 55

Fig. 25. *Mucor mucedo*, CBS 144.24 ×109.16; Fig. 26. *M. piriformis*, CBS 225.29 ×169.25; Fig. 27. *M. flavus*, CBS 235.35 ×234.35; Fig. 28. *M. hiemalis* f. *corticolus*, CBS 366.68 ×365.68; Fig. 29. *M. hiemalis* f. *silvaticus*, CBS 250.35 ×249.35; Fig. 30. M. *hiemalis* f. *hiemalis*, CBS 200.28 ×201.65.

PLATE 56

Fig. 31. *Mucor genevensis*, CBS 144.08; Fig. 32. *M. hiemalis* f. *luteus*, CBS 244.35 ×243.35; Fig. 33. *M. pusillus*, CBS 245.58; Fig. 34. *M. miehei*, CBS 182.67; Fig. 35. *Phycomyces blakesleeanus*, CBS 283.35 ×284.35; Fig. 36 *Syzygites megalocarpus*, CBS 372.29.

PLATE 57

Fig. 37. *Backusella circina*, CBS 128.70 ×129.70; Fig. 38. *Rhizopus sexualis*, CBS 336.62; Fig. 39. *Pirella circinans*, CBS 306.29 ×367.59; Fig. 40. *Parasitella simplex*, CBS 412.66 ×208.28; Fig. 41. *Gongronella butleri*, CBS 227.36 ×226.36; Fig. 42. *Mucor* '*indicae-seudaticae*', CBS 104.75.

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NOTES AND BRIEF ARTICLES

THE PERFECT STATE OF TILACHLIDIUM BRACHIATUM

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The morphology and nomenclature of the characteristic, probably monotypic, stilbellaceous hyphomycete genus *Tilachlidium* Preuss has been dealt with by Petch (1937) and Gams (1971: 141). A perfect state was then unknown. Colonies of the fungus in vitro are rather similar to those of *Nectria viridescens* Booth. The conidial state has now been found in nature connected with a hypocreaceous (nectriaceous) perfect state.

Pseudonectria tilachlidii W. Gams, *spec. nov.*

Perithecia in agarico putrido superficialia inter synnemata conidialia sparsa, subglobosa, ochracea, 175–185 × 160–175 μm, hyphis albidis, ad 40 μm longis, plus minusve ramosis fimbriata; paries 12–15 μm crassus, extus ochraceus, intus hyalinus; asci anguste clavati, tenuitunicati, sursum modice truncati, circa 50 μm longi, 5 μm diam. Ascosporae plus minusve biseriatae, continuae, anguste clavatae, basi truncatae, modice curvatae, tenuitunicatae, leves, hyalinae, 6–8 × 1.5–1.8 μm. Status conidialis *Tilachlidium brachiatum* (Batsch per Fr.) Petch.

Typus: *H. A. van der Aa*, prope Baarn, 10 Oct. 1974 (Herb. CBS 178).

Perithecia superficial on decaying agaric, scattered, partly aggregated, amidst conidial synnemata, subglobose, generally 175–185 μm high, 160–175 μm diam., ochraceous, covered with whitish, sometimes basitonously branched, warted, fringe-like hyphae, up to 40 μm long. Perithecial wall 12–15 μm thick, consisting of 5–6 layers of flattened cells, the outer ones slightly pigmented. Asci lining the base and sides of the perithecial cavity, slender clavate, thin-walled, with slightly truncate apex and minute apical structure, approximately 50 μm long, pars sporifera 25 μm long and up to 5 μm diam. Paraphyses scarce, filiform. Ascospores more or less biseriate, one-celled, narrowly claviform, with truncate base, mostly slightly curved, very thin and smooth-walled, hyaline, 6–8.5 × 1.5–1.8 μm.

Conidial state *Tilachlidium brachiatum* (Batsch per Fr.) Petch. The conidia are elongate, never curved and shorter than the ascospores, 3.2–4.5 μm long, in vitro up to 7.5 μm.

MATERIAL EXAMINED. — *H. A. van der Aa*, on decaying agaric near swimming pool 'De Vuursche', Baarn, 10 Oct. 1974 (Herb. CBS 178). Isolations from thoroughly washed and squashed mature perithecia yielded numerous homogeneous cultures identical with those isolated from the conidial state (CBS 697.74).

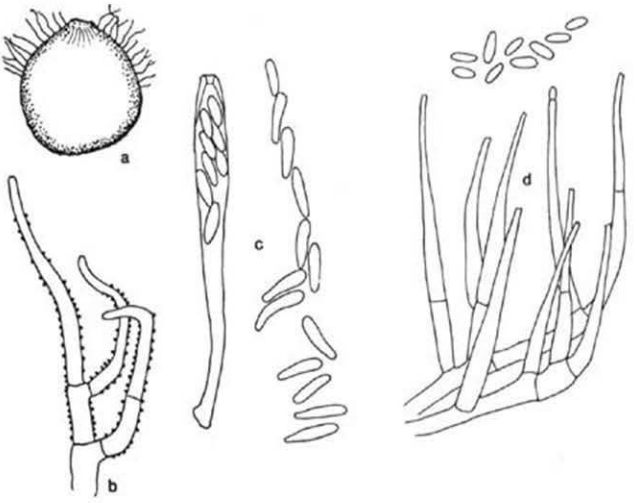

Fig. 1. *Pseudonectria tilachlidii*. — a. Perithecium × 100. — b. Perithecial hairs. — c. Ascus and ascospores. — d. Part of synnema with conidia (b-d × 1000).

Discussion

According to Rogerson (1970), von Arx & Müller (1973) and von Arx (1974), the fungus keys out as a *Pseudonectria* Seaver. Seventy-eight species have been described in this genus with its synonyms *Nectriella* Sacc. (non Nitschke) and *Notarisiella* (Sacc.) Clem. & Shear, amongst which *N. mycetophila* (Peck) Sacc. grows on decaying fungi but has spores of 12–13 × 4 μm. Because of the association with the *Tilachlidium* conidial state, a specific confusion with a described species can be ruled out. The type species, *P. rousseliana* (Mont.) Seaver apud Clem. & Shear, redescribed i.a. by Bezerra (1963), has light green perithecia covered with stiff hyaline setae which exude red droplets at their tips. *P. rousseliana* has larger perithecia, asci and spores than the present species; the ascospores are fusiform with rounded ends, usually 13–15.5 × 3.0–4.0 μm; the conidial state is *Volutella buxi* (Corda) Berk. The conidial state of *Nectriella coronata* Juel is *Sesquicillium buxi* (Schmidt apud Link) W. Gams (Juel, 1925). In other species the connection with conidial states has not been studied. *Tilachlidium* is the third genus of phialidic conidial states observed in the genus *Pseudonectria*. Another close genus is *Allantonectria* Earle apud Tracy & Earle (1901), the type species of which, *A. yuccae* Earle, has perithecia partly immersed in a basal stroma on leaves and allantoid ascospores.

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PORIA LINDBLADII FOUND IN THE NETHERLANDS

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Poria lindbladii (Berk.) Cooke

Polyporus liudbladii Berk, *in* Grevillea **1**: 54. 1872. — *Poria lindbladii* (Berk.) Cooke *in* Grevillea **14**: 111. 1886. — *Polyporus cinerascens* Bres. apud Strass. *in* Verh. zool.-bot. Ges. Wien **50**: 361. 1900. — *Poria cinerascens* (Bres.) Sacc., *in* Syll. Fung. **16**: 161. 1902. — *Tyromyces cinerascens* (Bres.) Bond. & Sing. *in* Annls mycol. **39**: 52. 1941.

Fruitbodies resupinate, initially circular, later growing together and effused up to 10 cm, readily separable, soft, fibrous, white when young, yellowish to tan when older or after drying. Grey tints not observed. Sterile margin narrow and well defined. Subiculum fibrous, 0–0.2 mm thick. Tubes about 1 mm long, pores 0.15–0.25 mm diameter, averaging 3–4 per mm, generally rather regularly circular or slightly elongated, dissepiments 0.1–0.2 mm thick, entire.

Context dimitic. Generative hyphae thin-walled, septate, with clamps, rarely branched, about 2.5 μm diameter. Skeletal hyphae thick-walled, non-septate, rarely branched, 4–6 μm diameter. A brownish, brittle, granular substance is present around and between the hyphae. The context also contains fairly numerous, irregularly shaped crystals, up to 10 μm diameter. Ripe hymenium not observed. Spores hyaline, smooth, curved, cylindric, 2-guttate or with granular contents, 6–7(–7.5) × 2–3 μm.

Skeletal hyphae, exept for their plasmatic contents, readily soluble in bases. The granular matter around the hyphae also dissolves in bases, which is attended by swelling and production of gas bubbles. The process may be followed under the microscope by first mounting the section in water, and subsequently replacing the latter slowly by a dilute (1%) base.

The Netherlands, prov. Gelderland, west of Dieren, 'Nationaal Park Veluwezoom', Hagenau, February 2, 1975, *H. F. van der Laan*, on a dead branch of *Pinus* lying on the ground.

Following Donk (1974: 157), the name *Poria lindbladii* is here used for the species generally known as *P. cinerascens*.

Poria lindbladii appears to be widely distributed in Europe. Bourdot & Galzin (1928: 667) report it from mountainous areas in France. Jahn (1970/71: 61) states that in Germany it is found in the low plains as well as in the mountains. Domański (1972: 161) lists it from the Carpathian mountains and also from the low lands in northern Poland. In the Rijksherbarium (Leiden) are collections from low altitude areas in southern England, mid-Sweden and Estland. Jahn says that in many places in Germany it is one of the most frequently found *Poria's*, but in Domański's opinion *Poria lindbladii* is a rather rare species in Poland. This also may be true for the Netherlands, because no collections of this species have been reported previously. *Pinus* and *Picea* are the preferred habitats in Europe, but the species has also been found on logs of deciduous trees (Domański 1972: 161).

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A NEW SPECIES OF GILMANIELLA FROM THE SOIL OF KUWAIT

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In the course of investigations on the fungal flora of the salt-marsh soils of Kuwait, a *Gilmaniella* species was isolated twice in 1973. Its smooth vegetative mycelium and large conidia with relatively wide germ pores indicated that it is sufficiently different from the only known species in *Gilmaniella*, *G. humicola* Barron (1964), to warrant its description as a new species.

Gilmaniella macrospora Moustafa, *spec. nov.*—Plate 58

Coloniae in agaro PDA dicto 28° C celeriter crescunt, post 7 dies ad 4-5 cm diam, laxe intricatae, velutinae, 2-3 mm altae, primum albae, deinde dilute griseae ad fuscae, margine angusta altae, primum albae, deinde dilute griseae ad fuscae, margine angusta alba circumdatae. Hyphae submersae hyalinae, leves et tenuitunicatae, 2.5–3.7 μm latae; hyphae aeriae hyalinae, deinde dilute pigmentatae, leves, septis crassis, obscuris divisae. Cellulae conidiogenae laterales orthotropicae, vel intercalares e cellulis haud differentiatis oriuntur, hyalinae, deinde obscure rubrobrunneae leves et tenuitunicatae, clavatae vel pyriformes, 7–18 × 5–7 μm;

nonnumquam conidiophora longiora, septata adsunt; cellulae conidiogenae conidia singula ad terna apicalia proferunt. Blastoconidia plerumque singula, raro catenis brevibus connexa, levia, crassitunicata, globosa, (10-)14–18 (22.5) μm diam., vel subglobosa, ovoidea, piriformia vel elongata; porus germinationis in parte superiore, conspicuus, 2.5–3.7 μm diam.; cicatrices basales in conidiis dimissis planae vel prominentes, fuscae, 2–5 μm diam.
Typus CBS 388.75, isolatus e solo halomorphico in Kuwait.

Colonies on potato-dextrose agar at 28° C growing rapidly, reaching a diameter of 4–5 cm in 7 days, consisting of loose-textured, velvety, 2–3 mm high, at first white mycelium, quickly turning into pale grey and finally dark blackish-brown with a white, narrow (less than 2 mm) margin, azonate. Reverse olivaceous-black. Exudate and odour absent. Submerged hyphae hyaline, septate, smooth- and thin-walled, 2.5–3.7 μm wide. Aerial hyphae hyaline at first, becoming subhyaline but remaining smooth-walled, with prominent, thick, dark septa. *Conidiogenous cells* arising laterally at right angles or intercalary from undifferentiated hyphae, scattered, smooth- and thin-walled, hyaline, clavate to pyriform, 7–18 × 5–7 μm; sometimes septate and elongated conidiophores occurring which are straight or flexuous, cylindrical, 18–42 × 3.7–5.0 μm; conidiogenous cells mono- or polyblastic, usually forming solitary conidia at the tips, sometimes 2 or 3 conidia arising in the apical region. In mature colonies most of the conidiogenous cells and many other cells of the vegetative mycelium turn dark reddish brown. *Conidia* blastic, mostly solitary, occasionally in short chains of 2–3, dry, smooth- and thick-walled, dark reddish brown, one-celled, spherical (10–) 14–18 (–22.5) μm in diameter, or subspherical, oval, pear-shaped to elongated, 15–22.5 (–27.5) × 10–15 μm. Germ pores in the apical region conspicuous, relatively large, 2.5–3.7 μm wide. Basal scars in detached conidia flat or prominent, dark, 2–5 μm wide.

Growth and sporulation of *G. macrospora* on other media such as malt and oatmeal agars is abundant, on Czapek's agar the colonies are very loose-textured with less sporulation.

In *Gilmaniella humicola* Barron the conidia are spherical, mostly 7–10 μm diameter (Barron, 1964) and very rarely reach 15 or 16 μm (Subramanian & Lodha, 1964), whereas in *G. macrospora* they are more variable in shape, and have larger dimensions. Moreover, the vegetative hyphae in *G. humicola* are finely roughened to verrucose while in *G. macrospora* they are smooth and remain so.

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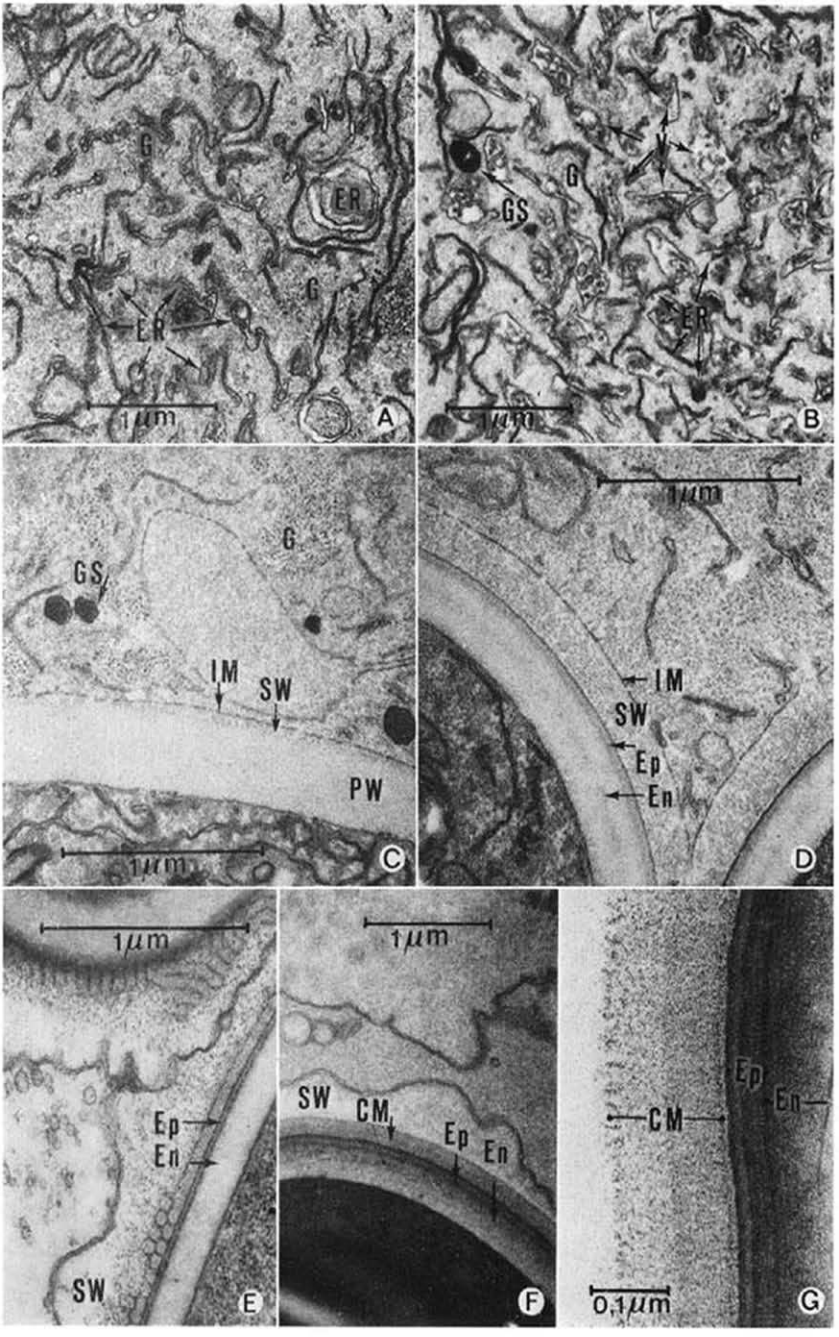
Explanation to Plate 58

Figs. A-D. Different types of conidiogenous structures of *Gilmaniella macrospora*, CBS 388.75.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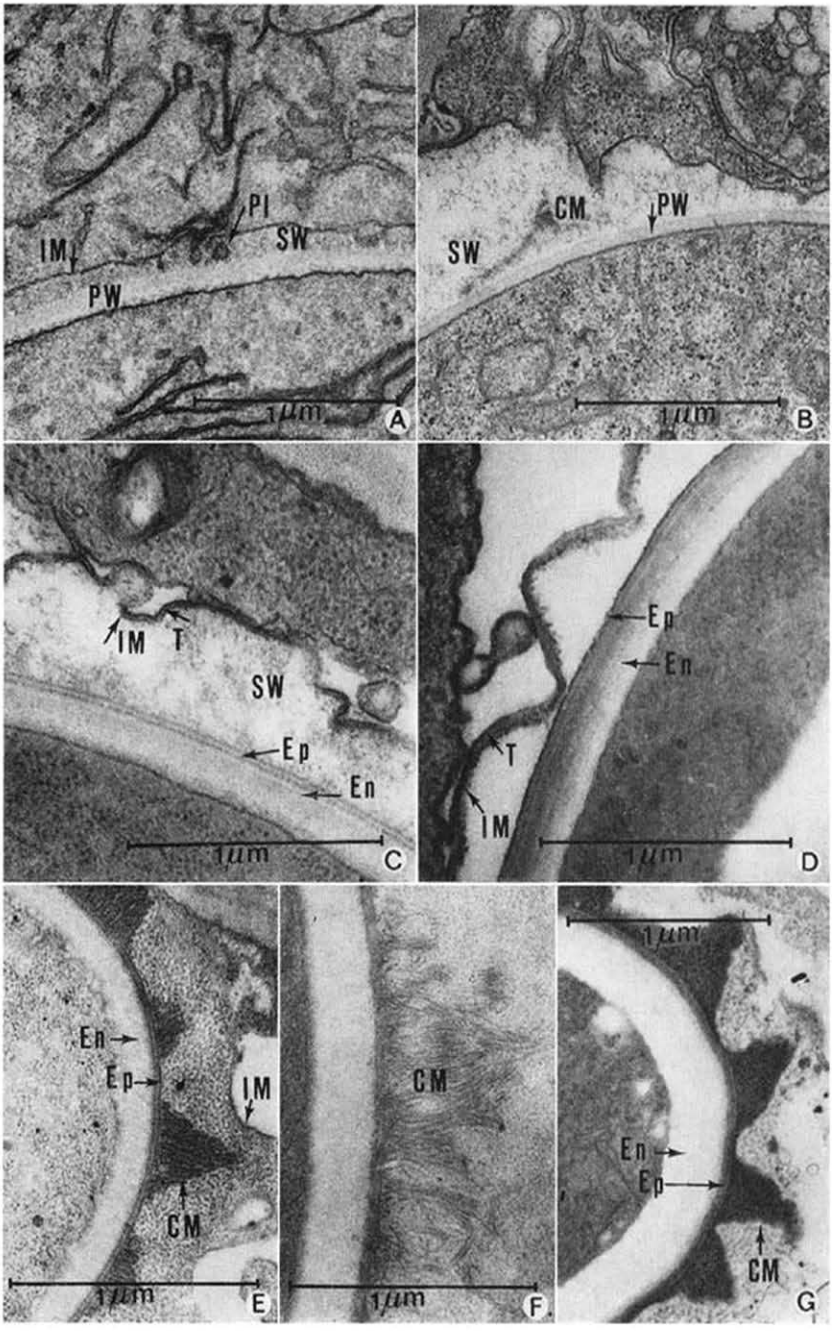
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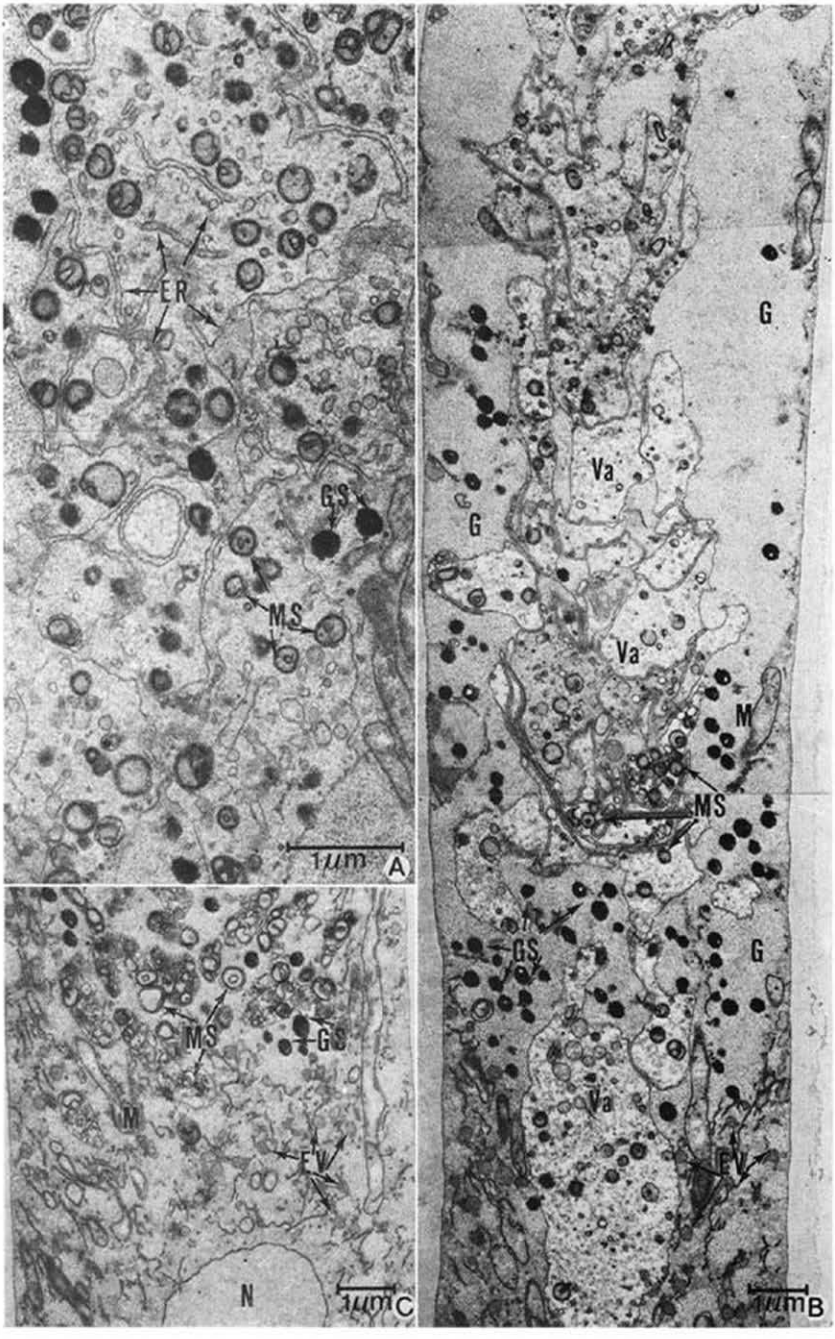
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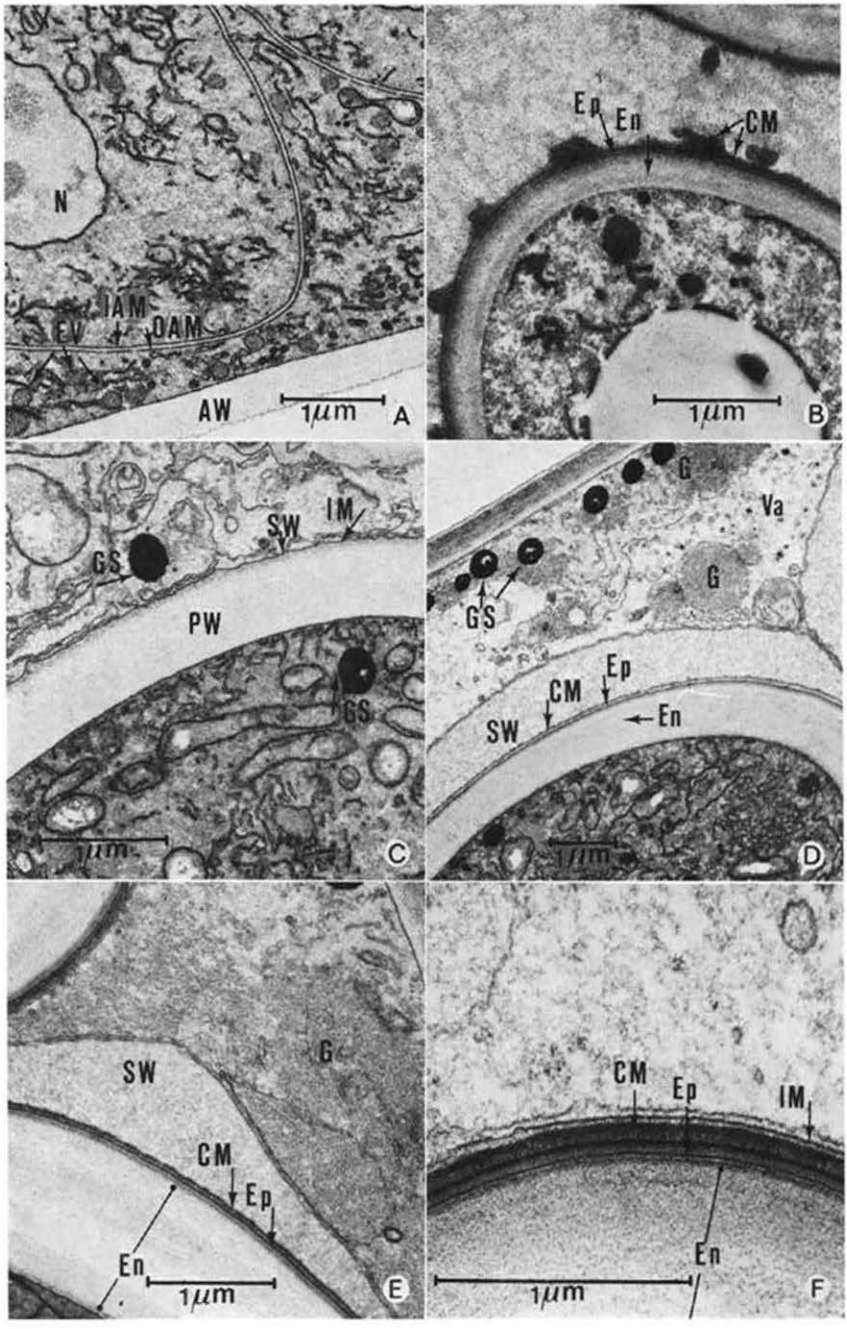
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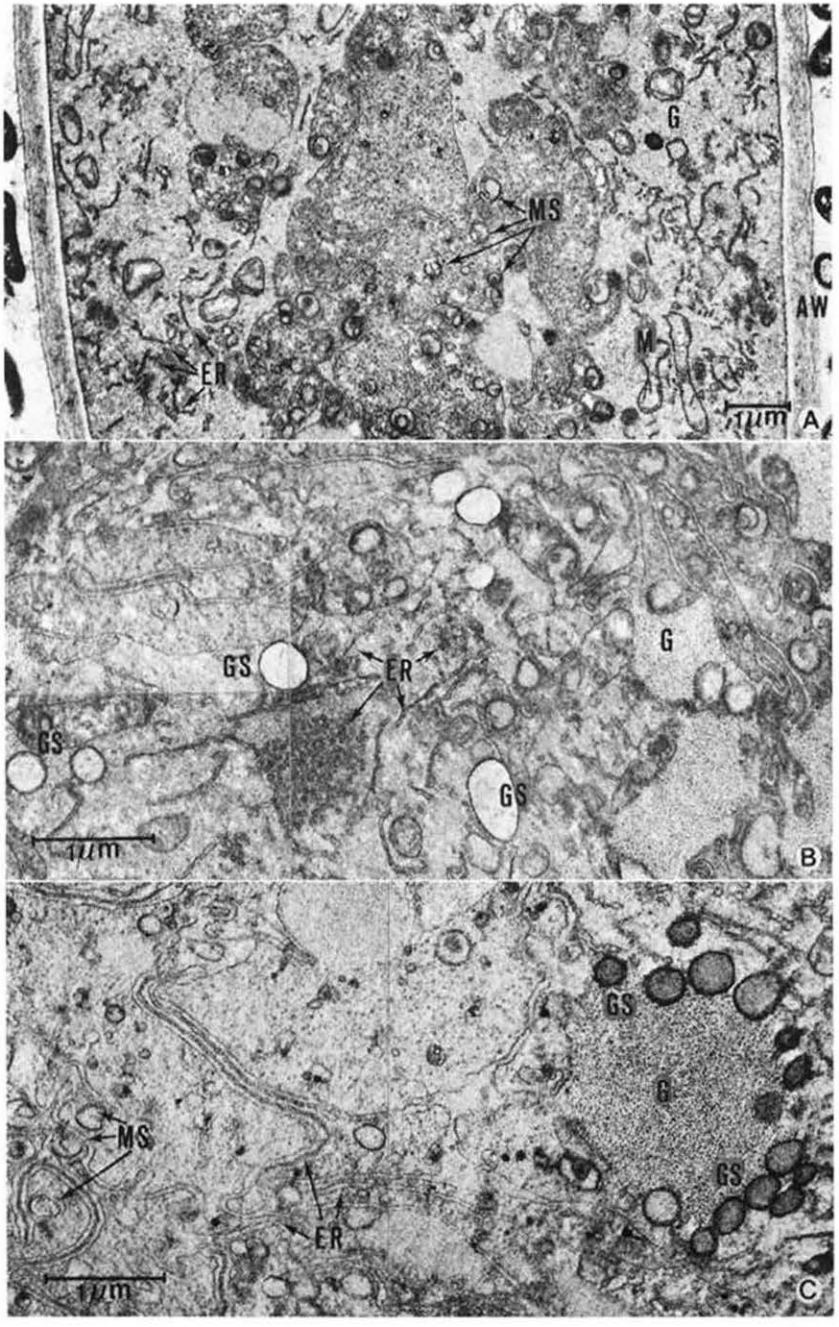
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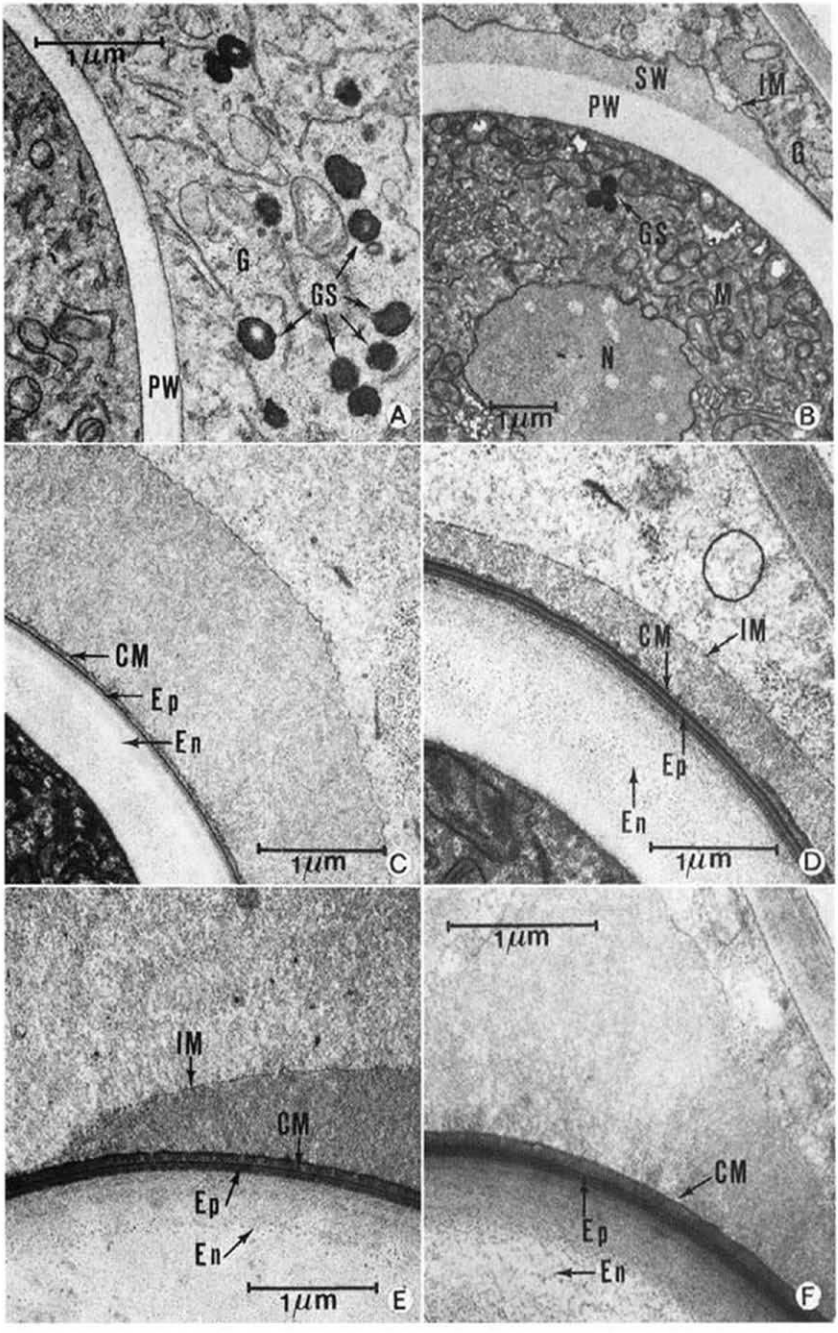
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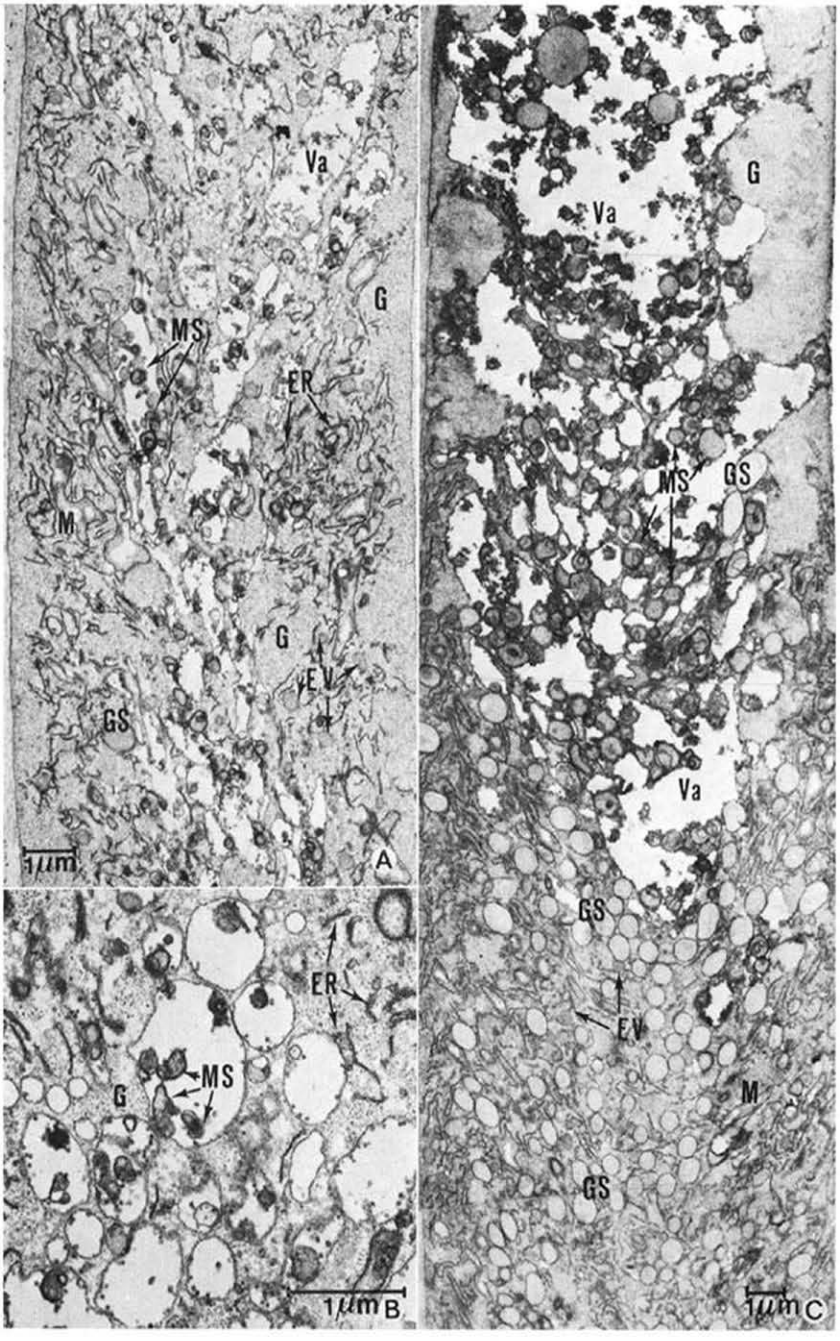
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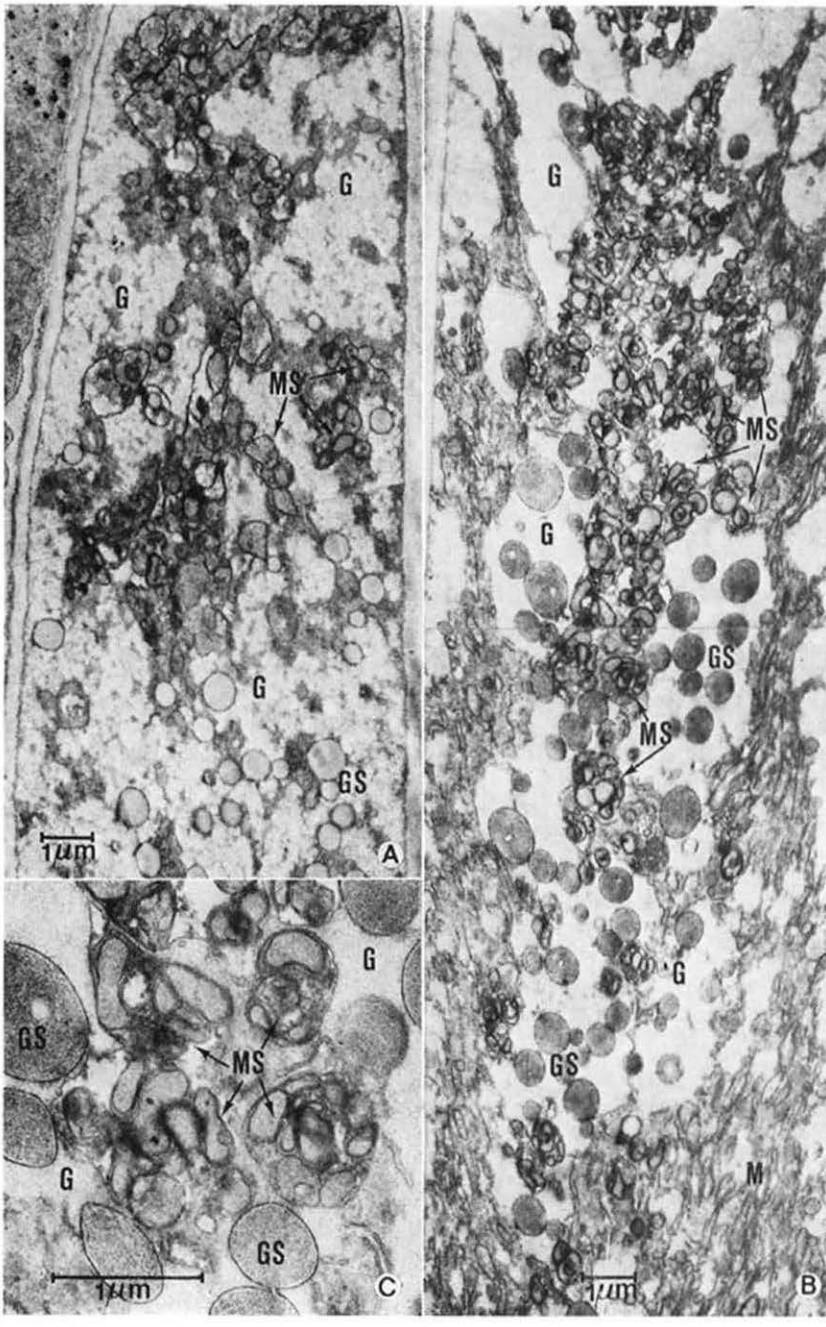
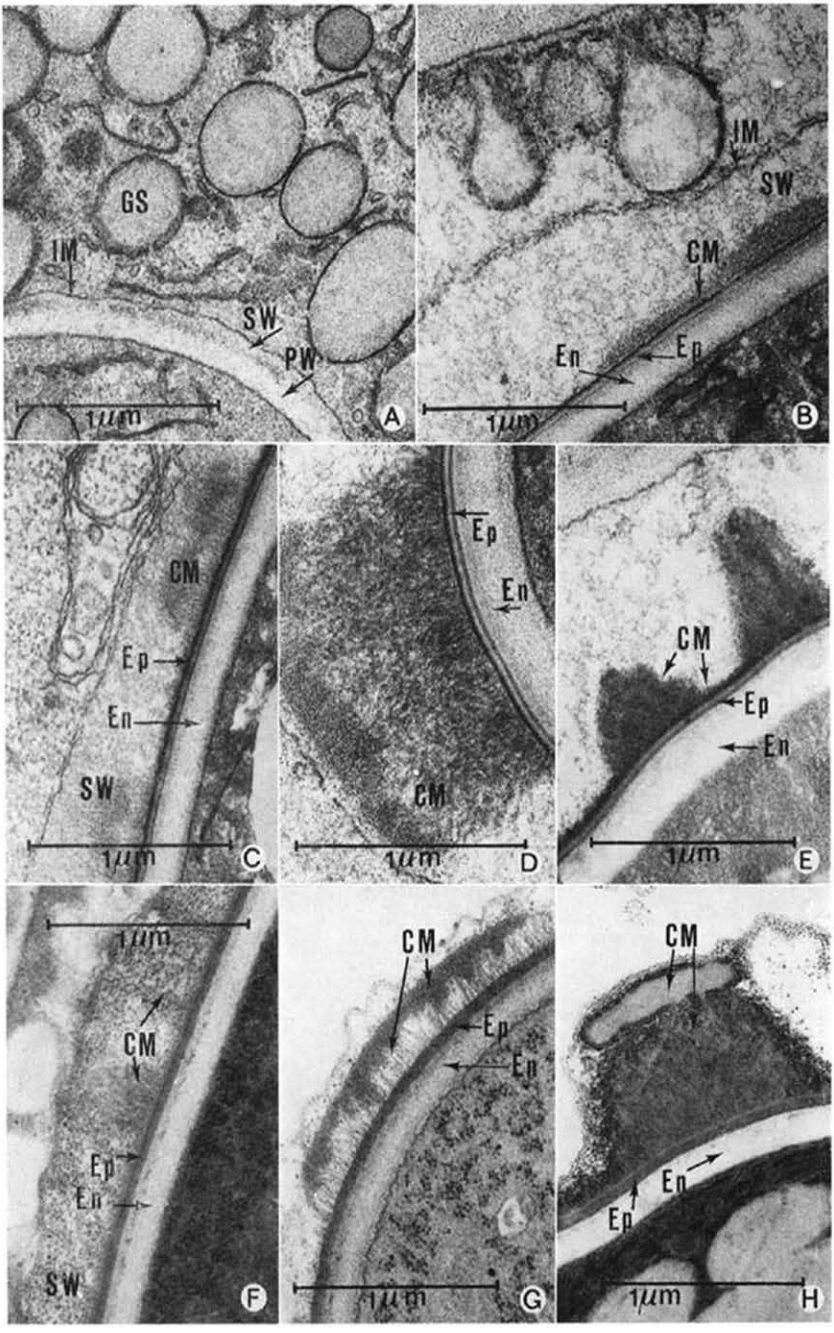
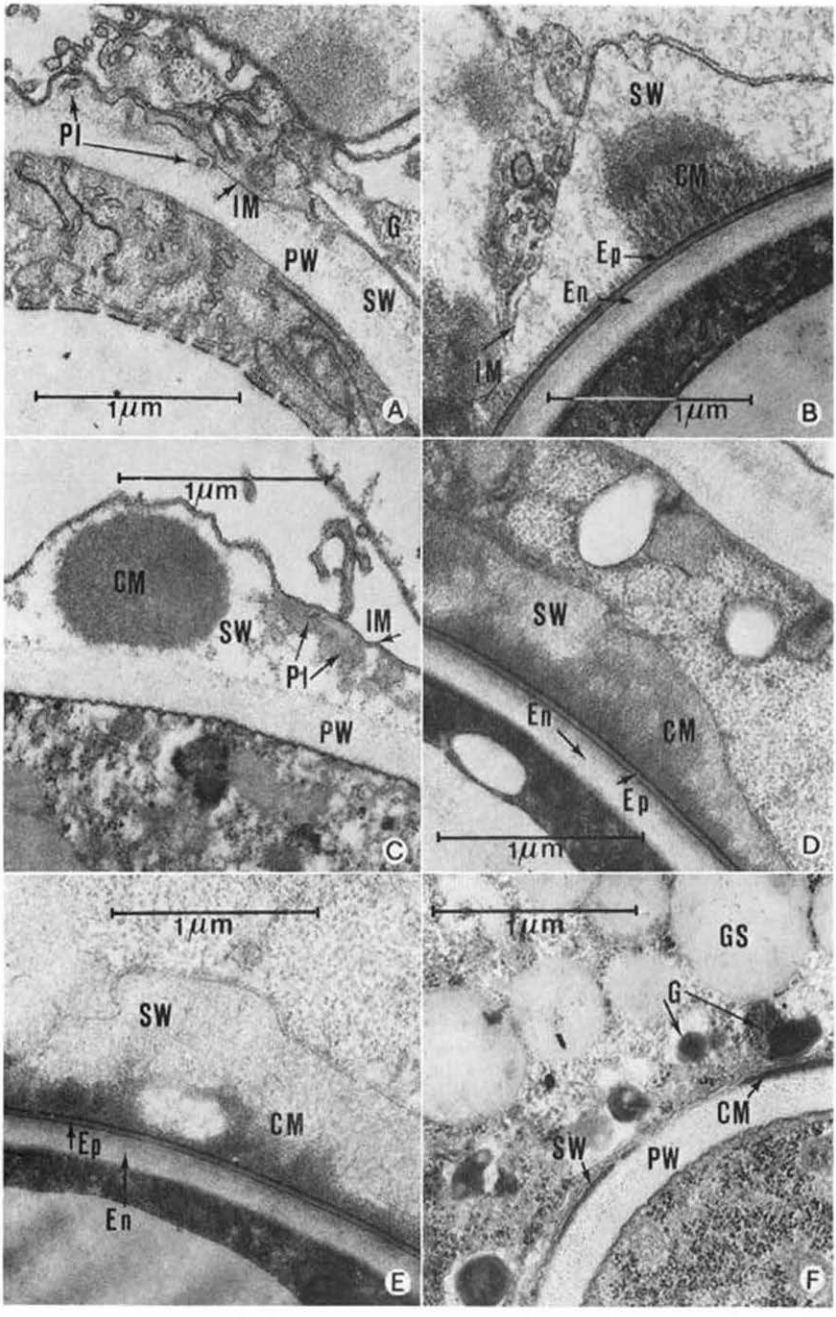

G
G
MS
G
GS
1µm
A
G
GS
MS
G
1µm
C
G
MS
GS
G
MS
GS
MS
G
G
GS
M
1µm
B


GS
IM
SW
PW
1µm
A
IM
SW
CM
En
Ep
1µm
B
CM
Ep
En
SW
1µm
C
Ep
En
CM
1µm
D
CM
Ep
En
1µm
E
1µm
CM
Ep
En
SW
F
CM
Ep
En
1µm
G
CM
Ep
En
1µm
H


Pl
IM
G
PW
SW
1μm
A
SW
CM
Ep
En
IM
1μm
B
1μm
CM
SW
IM
Pl
PW
C
SW
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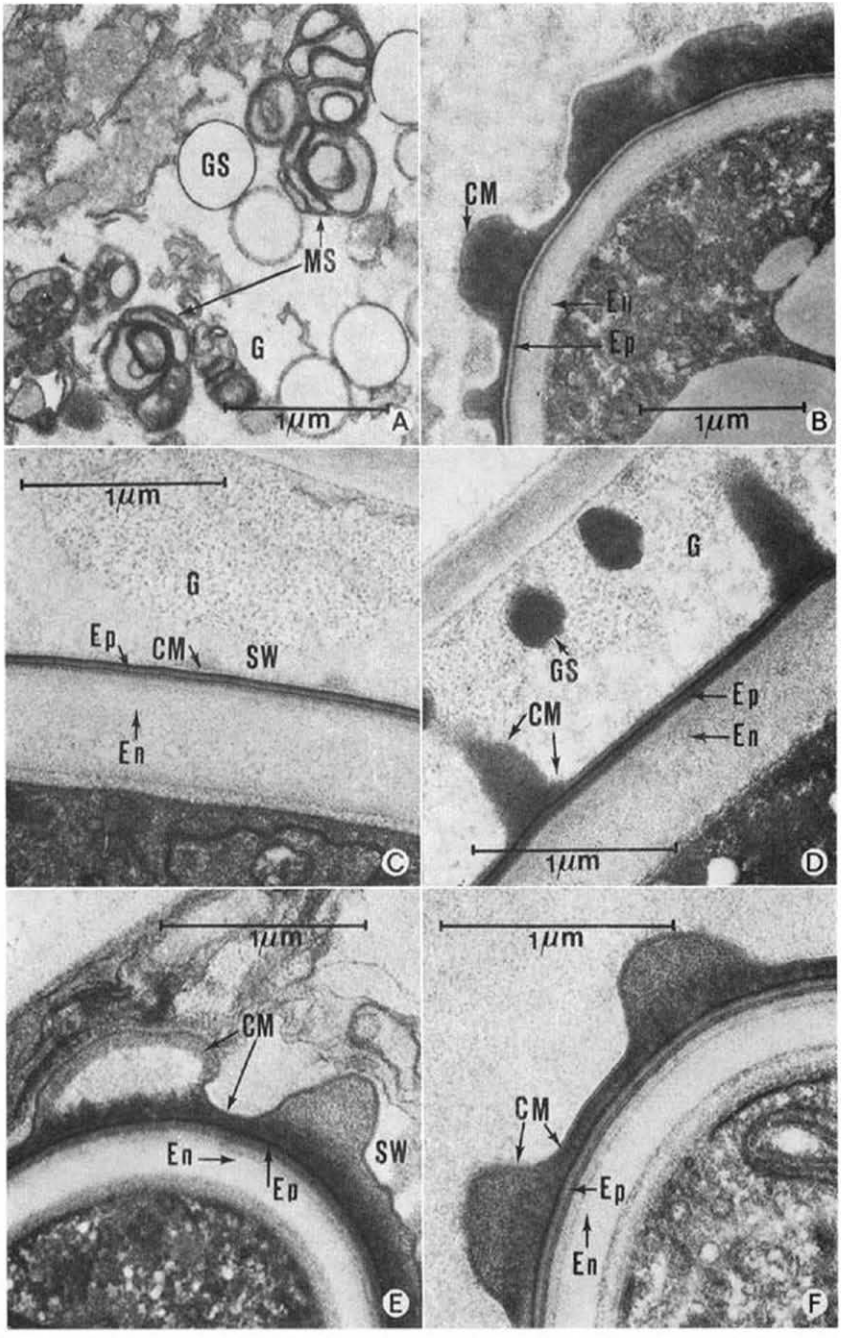
1μm
D
1μm
SW
CM
Ep
En
E
1μm
GS
G
CM
SW
PW
F


GS
MS
G
1µm
A
CM
En
Ep
1µm
B
1µm
G
Ep
CM
SW
En
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D
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SW
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CM
Ep
En
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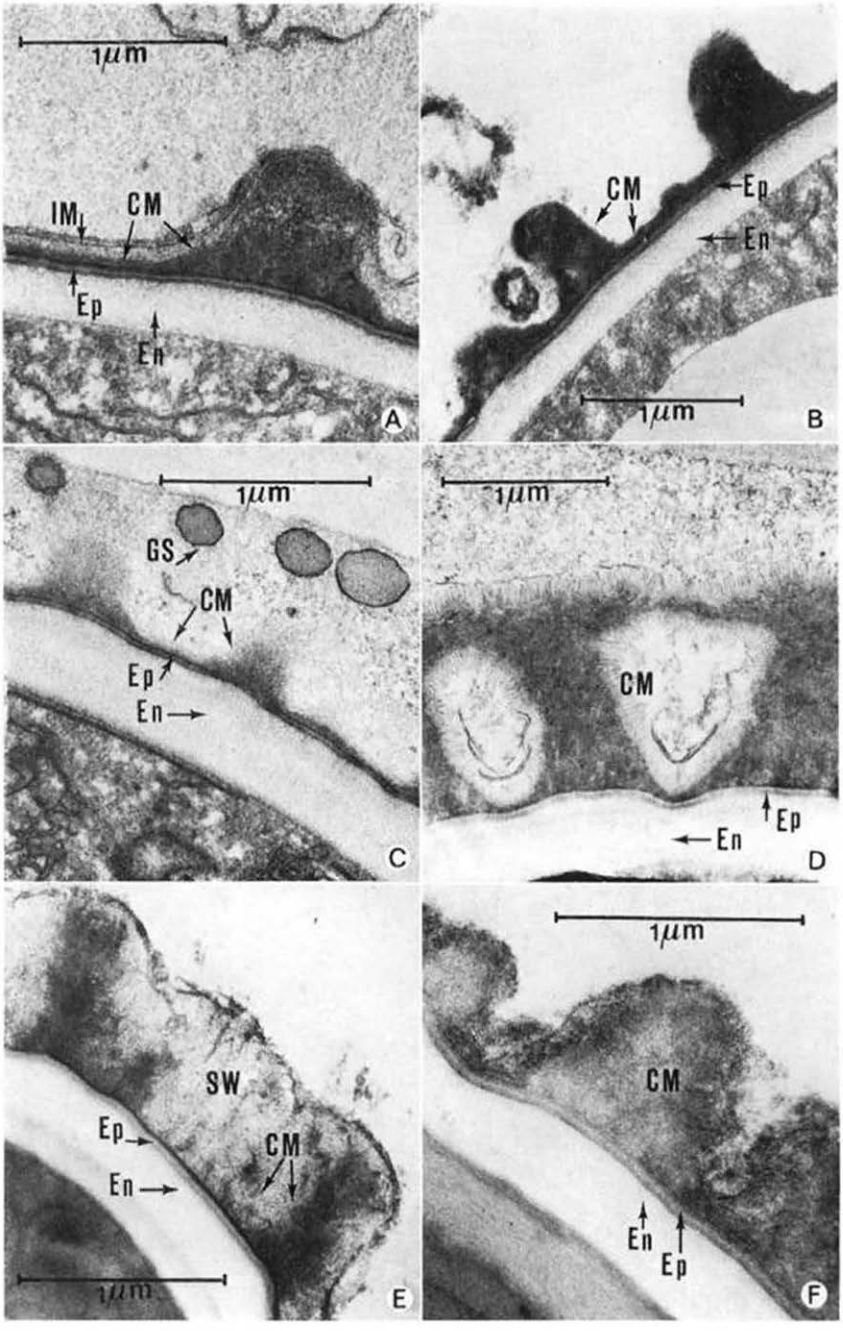
1μm
IM
CM
Ep
En
A
CM
Ep
En
1μm
B
1μm
GS
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D
SW
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CM
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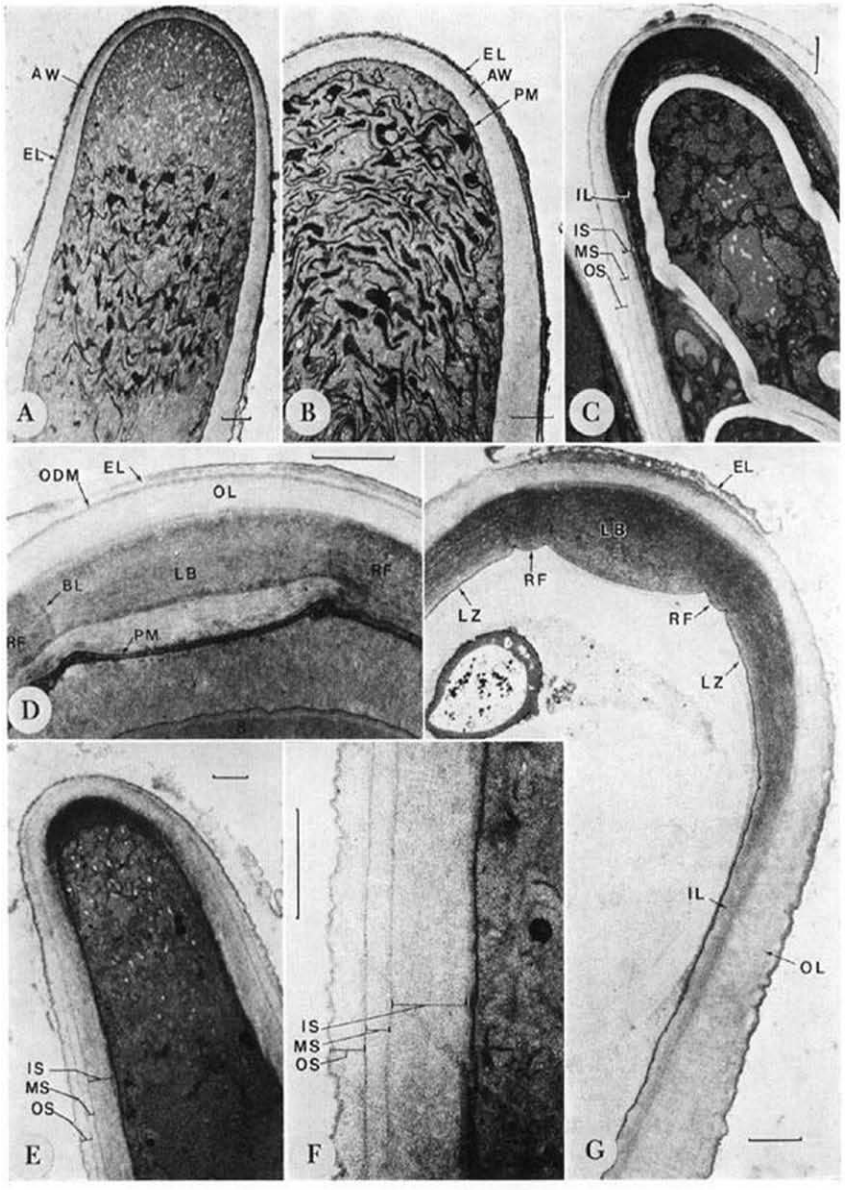
1μm
E
1μm
CM
En
Ep
F


AW
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ODM
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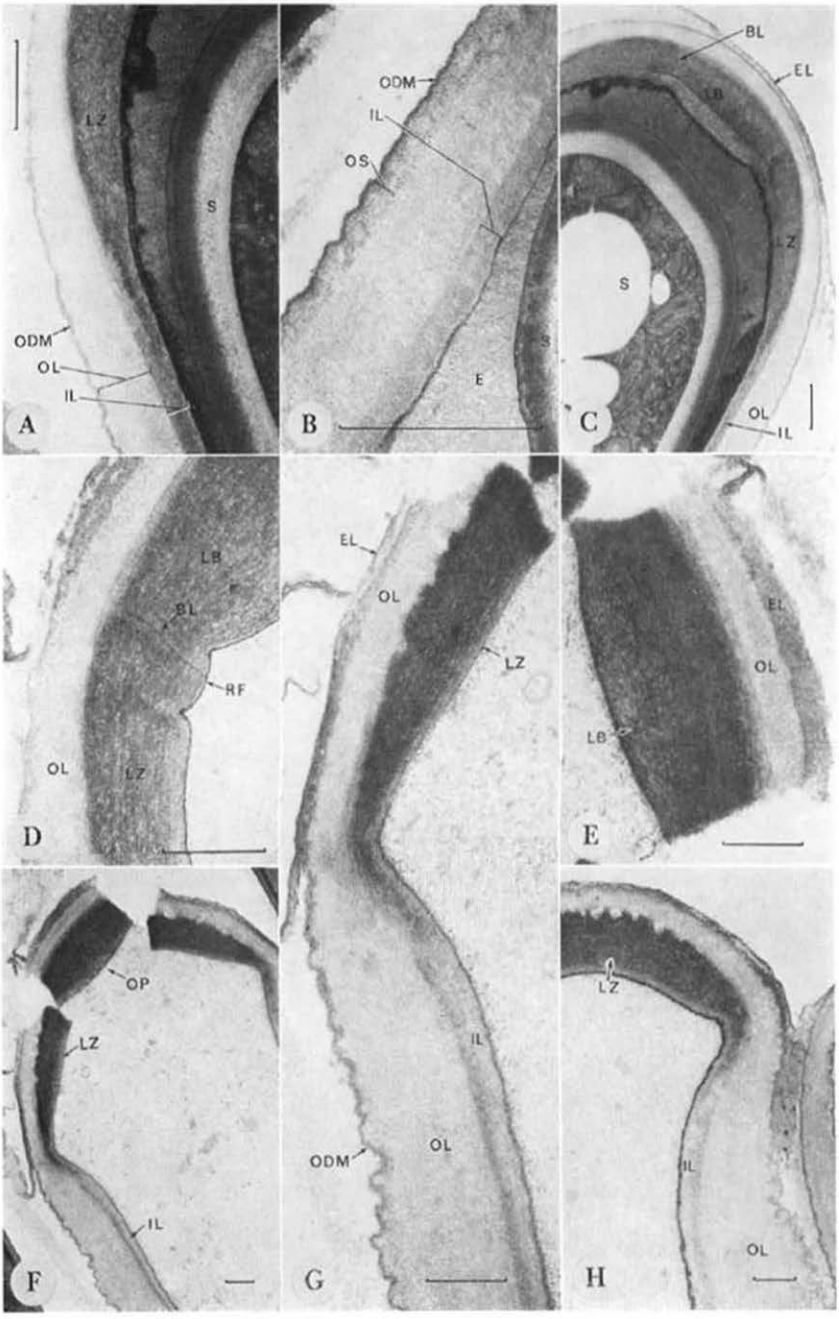
LZ
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RF
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OL
LZ
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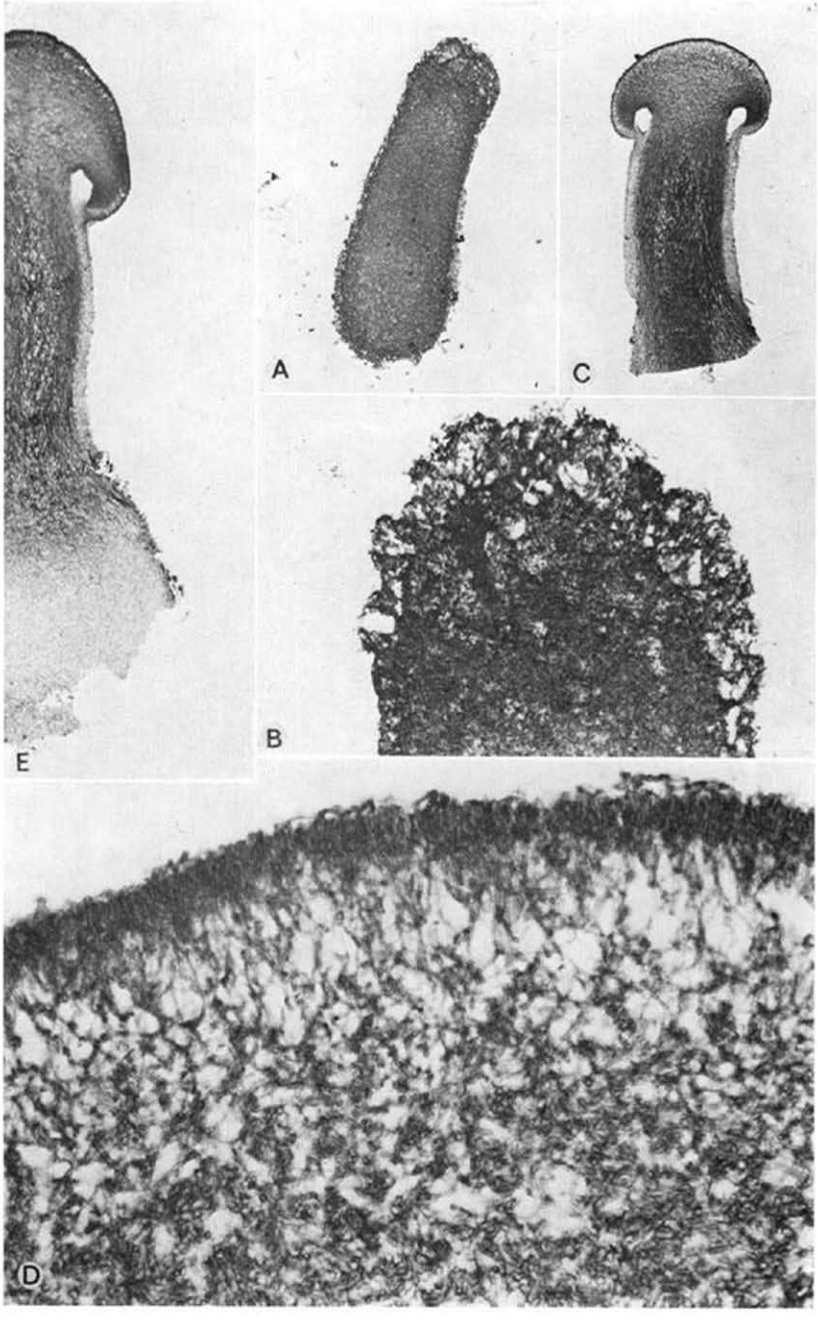
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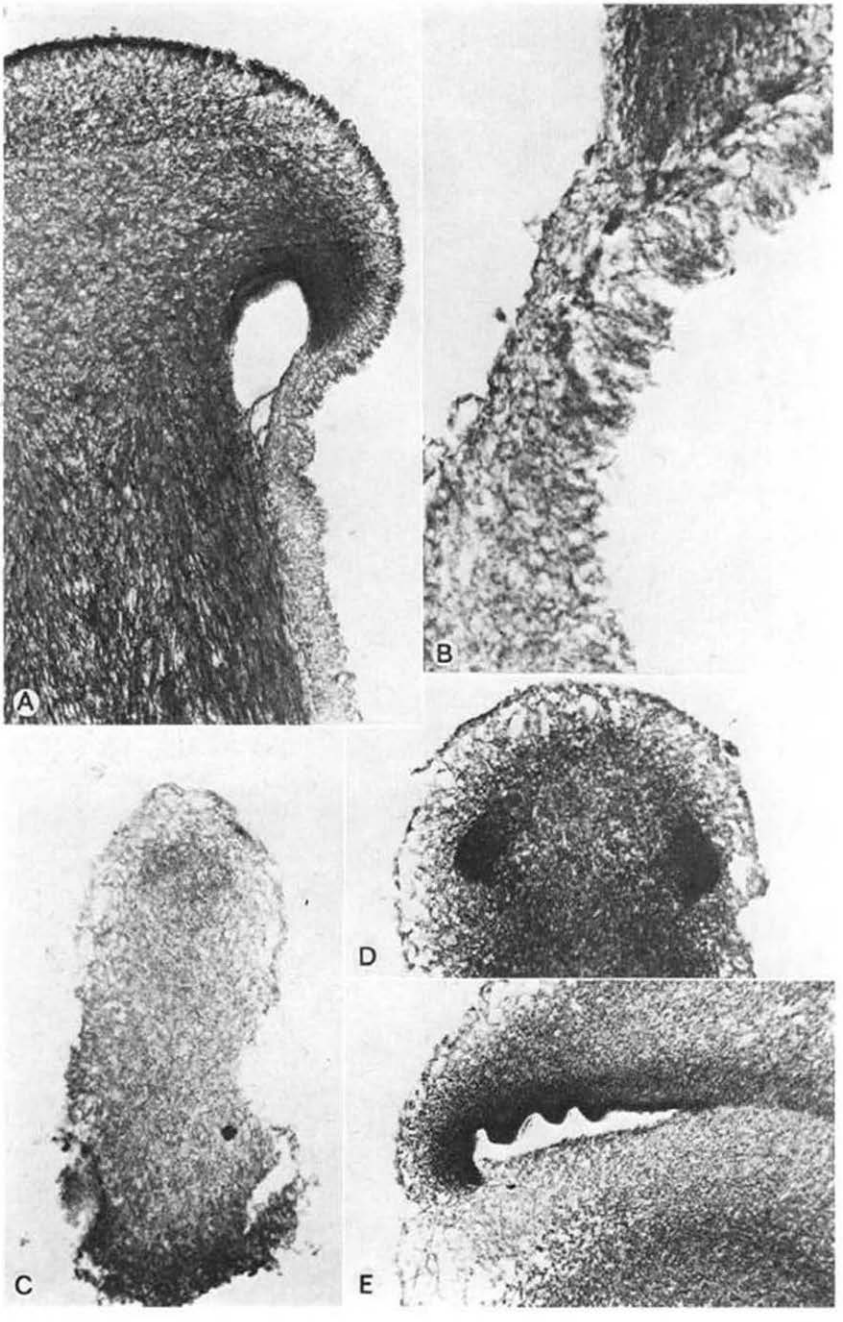
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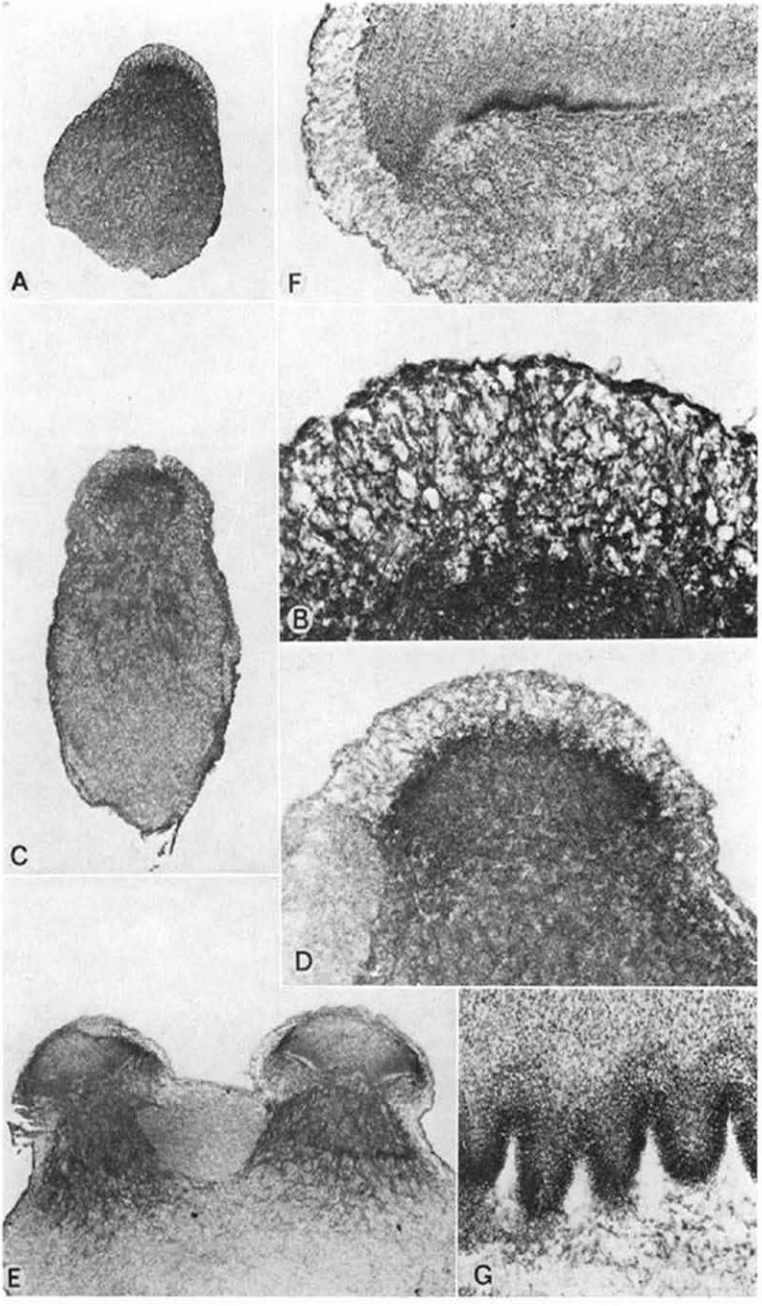
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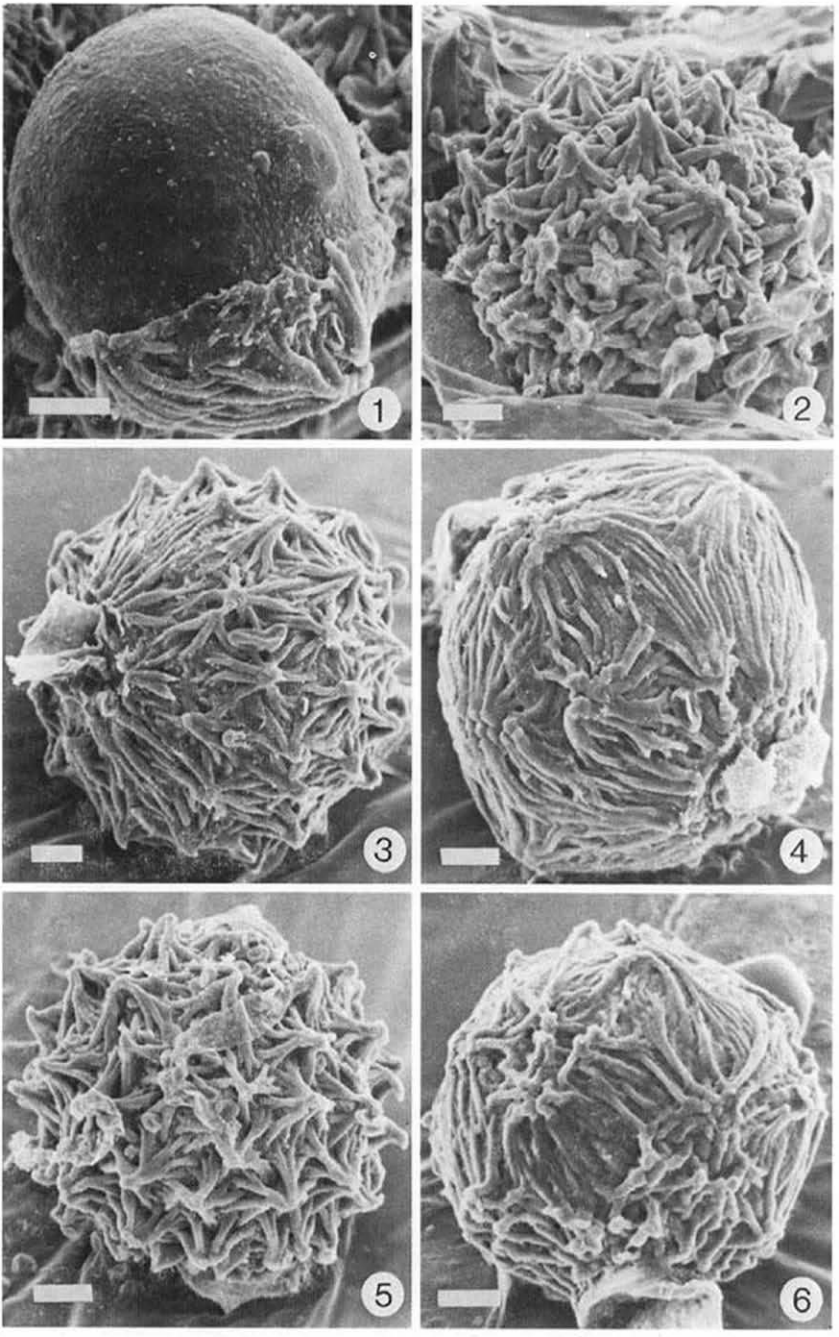
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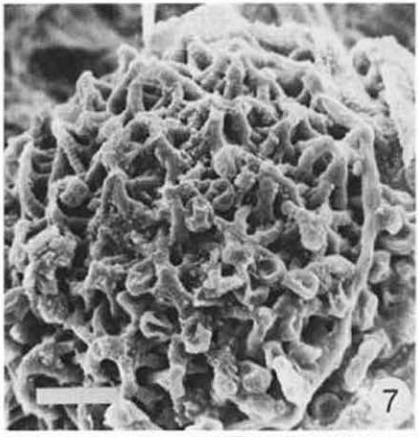
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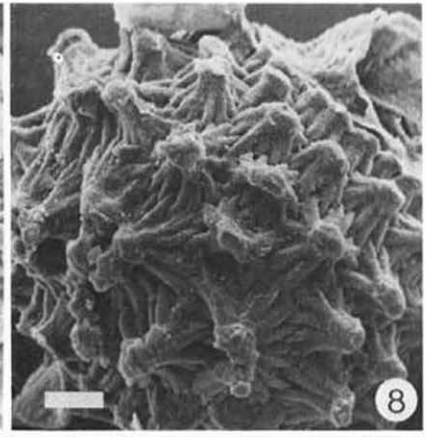
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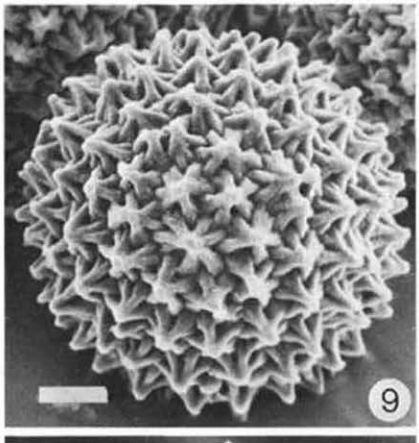
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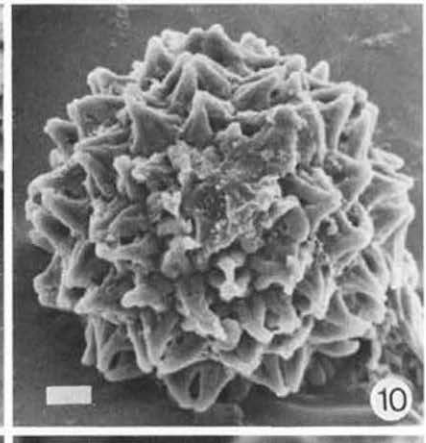
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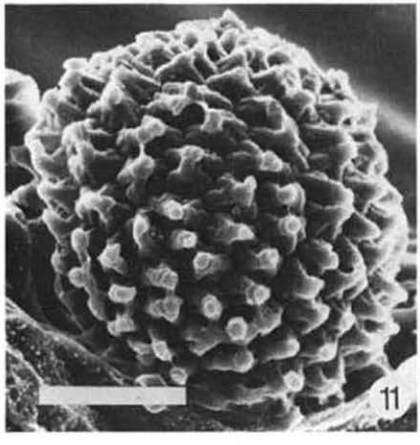
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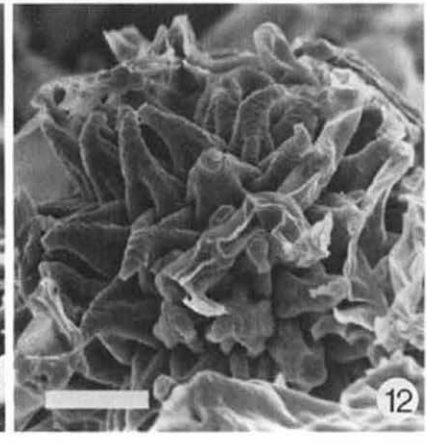
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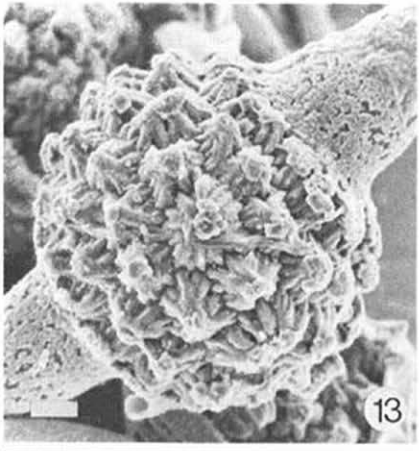
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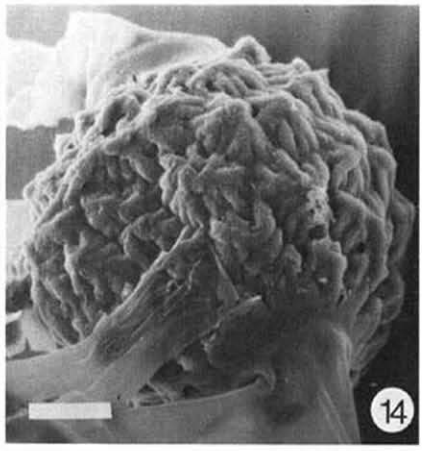
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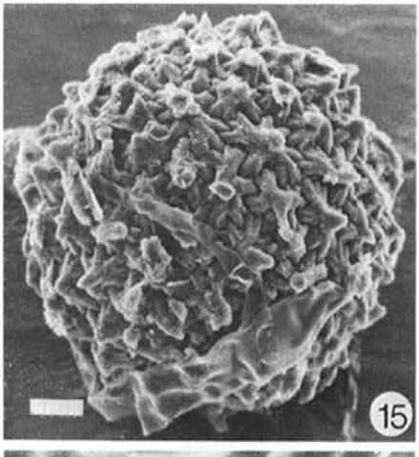
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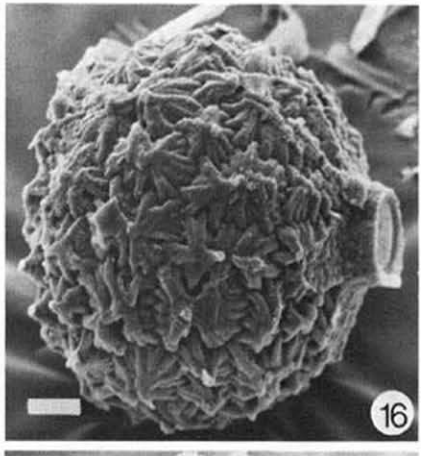
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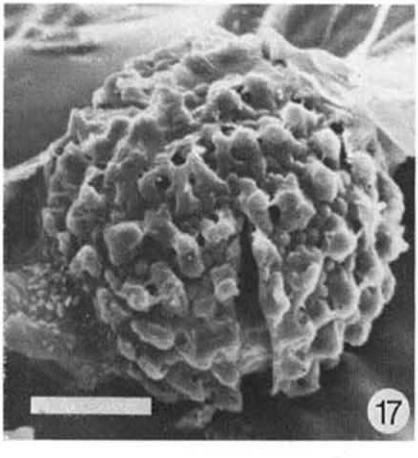
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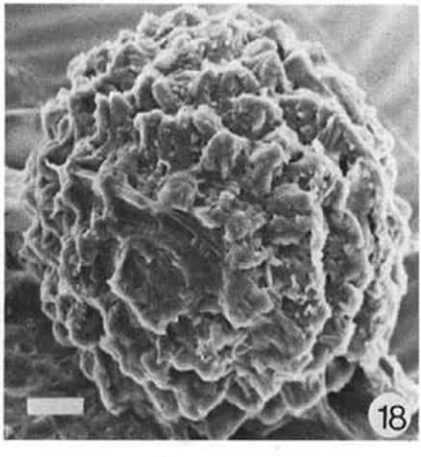
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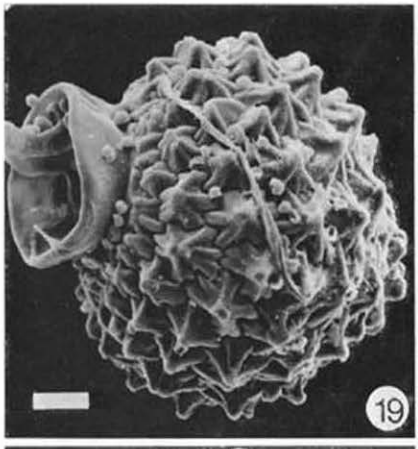
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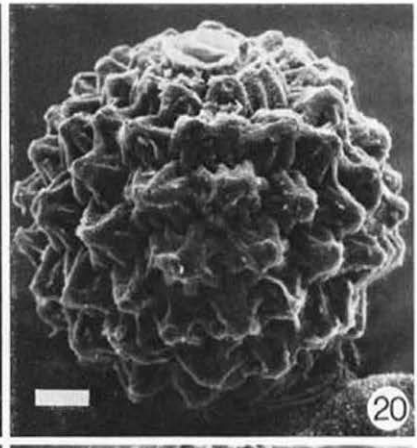
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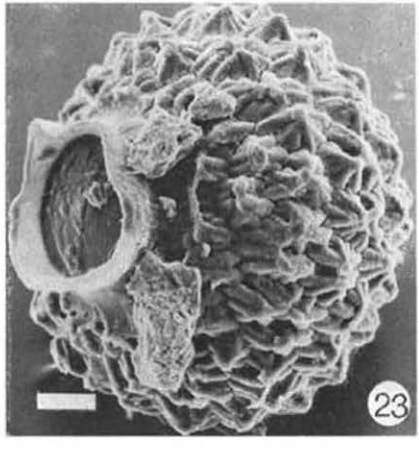
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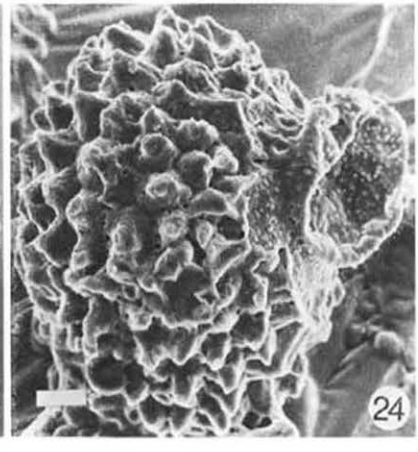
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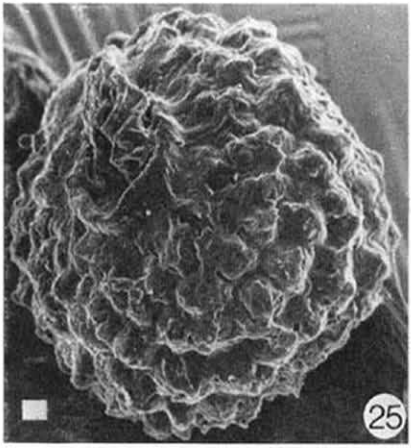
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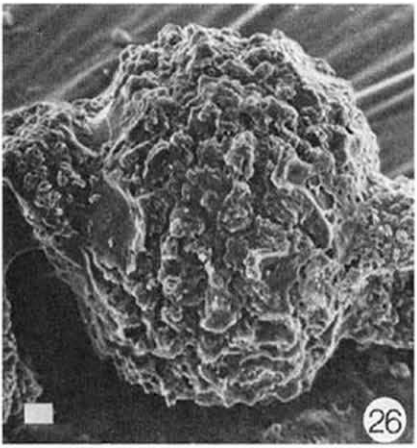
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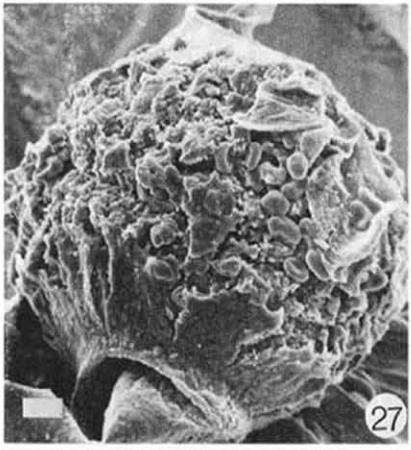
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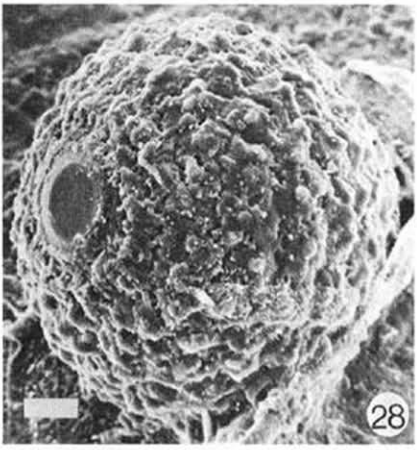
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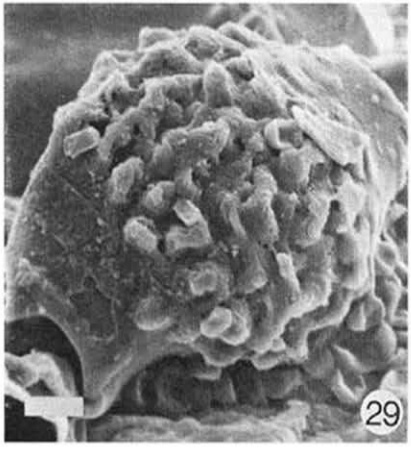
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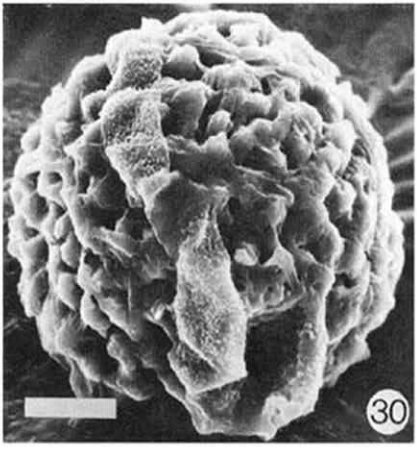
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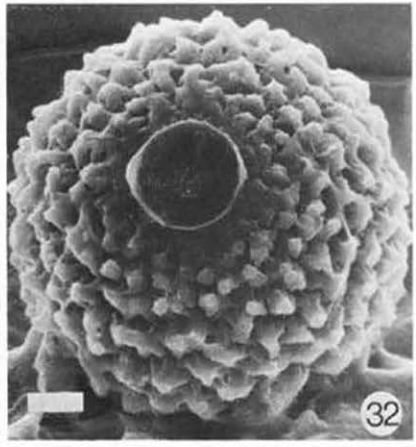
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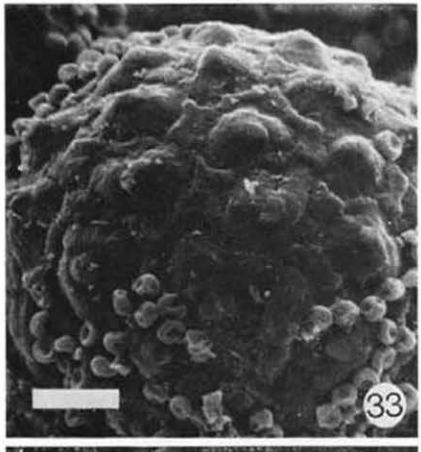
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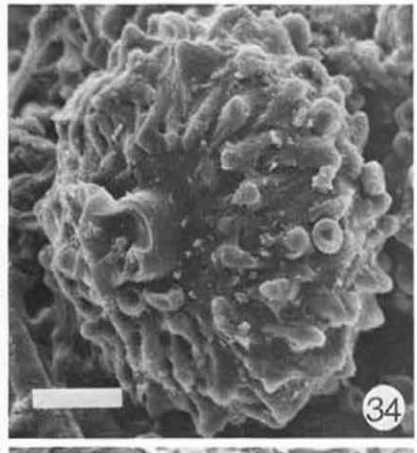
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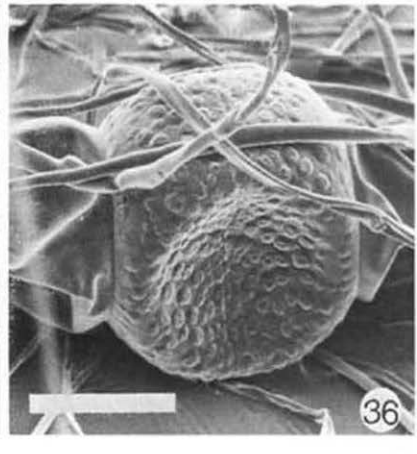
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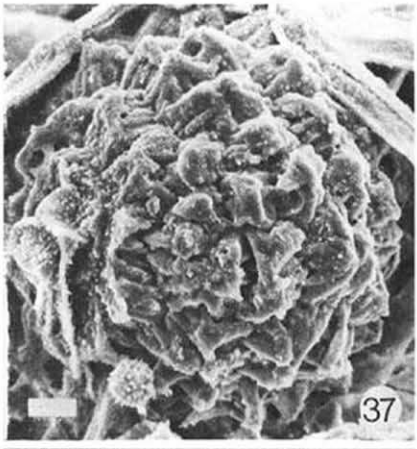
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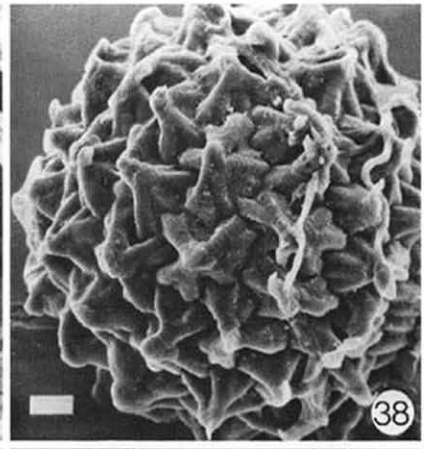
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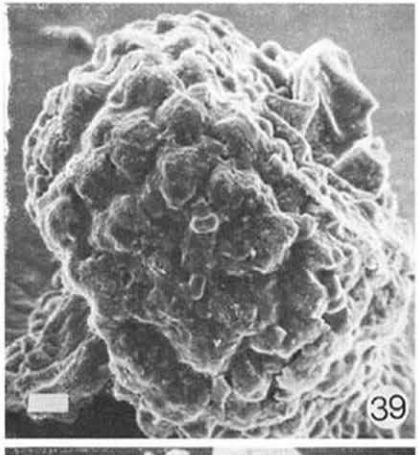
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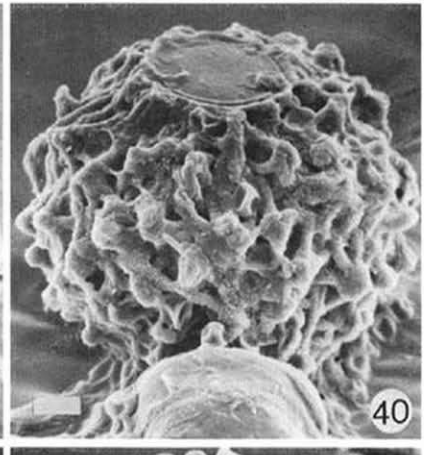
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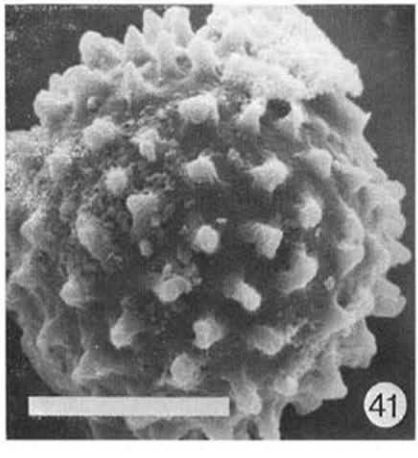
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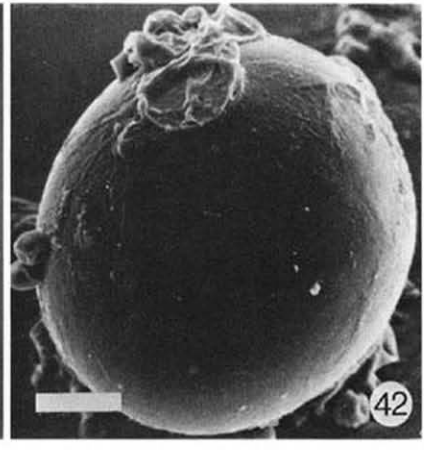
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