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THE OPERCULATE ASCUS AND ALLIED FORMS*

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The features of the operculate ascus and allied non-operculate forms are discussed. An outline of the results of recent research on fine structure and function of the different types of asci in Pezizales is presented.

When the Crouan brothers (*1857*, *1858*) discovered the operculum at the top of asci, they placed all species with asci opening by such a structure in the genus *Ascobolus*.

Boudier (*1879*, *1885*) was the first to recognize the importance of the operculate mode of ascus dehiscence for the classification of the Discomycetes. Accordingly he divided the cup fungi on the base of this character into two groups, (i) the Operculatae and (ii) the Inoperculatae.

It is a notable fact that none of Boudier's contemporaries (e.g. Quélet, Karsten, Fuckel, De Notaris, Schroeter, Saccardo, Rehm, Cooke, Phillips, Lindau) accepted this division. It became accepted only much later by Gäumann (*1926*), Seaver (*1927*, *1928*), Nannfeldt (*1932*), and Le Gal (*1947*, *1953*).

A subdivision of the cup fungi based on the mode of ascus dehiscence is now accepted by most mycologists.

All operculate Discomycetes belong to the order Pezizales, but on the other hand, not all fungi arranged within the Pezizales show operculate asci.

Already more than a century ago Boudier (*1869*) described the typical operculate ascus with an apical ring-shaped indentation delimiting the operculum. But he also found in *Peziza cunicularia* (Boudier, *1869*) asci opening at their tips by a bilabiate split.

Shortly afterwards Renny (*1871*, *1873*) described several such fungi, which he placed in *Ascobolus* section *Ascozonus*.

Other non-operculate asci were studied and described in *Thelebolus* and allied genera (Zukal, *1886*; Ramlow, *1906*, *1915*; Kimbrough, *1966*, *1972*; Kimbrough & Korf, *1967*). Here mature asci open by an irregular tear in the apical part of the ascus wall.

A special type of operculate ascus has been described by Chadefaud (*1946*) and Le Gal (*1946a*, *1946b*) as 'para-operculate' and 'sub-operculate' respectively. They regarded this

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structure important enough to distinguish a separate order or suborder between Inoperculatae (Helotiales) and the remainder of the Operculatae.

The published terminology for structural details of this type of ascus is rather confusing as both French authors used mostly different terms for the same detail and sometimes slightly altered ones for quite different elements (cf. Le Gal, *1946b*; van Brummelen, *1975*).

The term 'suboperculate ascus' as introduced by Le Gal has been generally accepted.

The suboperculate ascus was considered the most important character of the Suboperculati. It is defined by the 'coussinet apical', which Le Gal described as an interrupted ring located in the 'chambre apical' in the top of the ascus.

However, both Eckblad (*1968*, *1972*) and I (van Brummelen, *1975*) could not find sufficient evidence for the existence of the sub-operculate ascus in the original sense (cf. also Samuelson, *1975*).

From the drawings published by Le Gal (*1946b*) it was possible to conclude that the 'coussinet apical' rather represents different structures in the top of the ascus.

Although the ascus of *Sarcoscypha* and a few related genera may represent a special type of operculate ascus within the Pezizales, there is little left of the hypothesis that the Suboperculati (or Sarcoscyphineae) should represent a taxon intermediate between Inoperculatae and Operculatae.

The ascus

The shape and the structure of the asci in Pezizales show a wide range of possibilities. The shape of the asci depends on their number within each fruit body, the number of spores per ascus, as well as the pressure exerted upon the ascus by the surrounding hymenium and excipulum. In closed (cleistohymenial) fruit bodies with a single ascus this shape is often subglobular (e.g. in *Thelebolus stercoreus*, *Trichobolus zukalii*, *Lasiobolus monascus*). Obovoid asci are found in species with only a few asci within each fruit body (e.g. in the genera *Thelebolus*, *Leptokalpion*, *Ascodesmis*, *Dennisiopsis*, *Saccobolus*). Clavate and cylindrical asci, however, are most frequently met with in the Pezizales. As a rule the clavate forms prevail in the smaller fruit bodies which have a less developed lateral excipulum, whereas cylindric ones occur in the larger fruit bodies.

The ascus demonstrates a clear polarity in the distribution of its contents, which even changes during development. After the formation of ascospore nuclei from the diploid nucleus two zones can be distinguished: the upper part which becomes the sporogenous zone and the basal part filled with cytoplasm, containing reserves like glycogen. The structure of the latter was described by Schrantz (*1968*).

The ultrastructure of the ascoplasm varies considerably. As shown by the studies of Merkus (*1975*, *1976*), in some genera and groups of species it is more complex than in others, while the presence of electron-dense globular structures in the epiplasm seems to be correlated with the formation of oil drops in the sporoplasm.

During sporogenesis and the ripening of the ascospores the epiplasm becomes continuously more vacuolized. Meanwhile glycogen and organels are disappearing. Only at the end of this stage the ascus is ripe and ready to discharge its spores.

In most asci of the Pezizales one meiosis and two mitoses take place to form eight nuclei, each of which initiates the formation of a unicellular ascospore. In a few species of some unrelated genera four-spored asci seem to be a constant character (cf. Eckblad, *1968*). In some of these, four of the eight spores degenerate.

Cytological investigations by Berthet (*1964*) revealed that in most operculate Discomycetes the ascospores are uninucleate, save in Sarcoscyphaceae and Morchellaceae, where the spores are plurinuclear with maturity, and in Helvellaceae, where they are tetranuclear. In several genera of coprophilous Thelebolaceae meiosis is followed by more than two mitoses, resulting in asci having 16 to over 4000 spores.

THE ASCOSPORES

Ascosporogenesis of operculate Discomycetes was studied with electron microscopy in *Saccobolus kervernii* by Carroll (*1966, 1967, 1969*), in *Ascodesmis sphaerosporus* by Moore (*1963*) and Carroll (*1966*), in *Ascodesmis nigricans* by Bracker & Williams (*1966*), in Pyronema by Reeves (*1967*) and Griffith (*1968*), in *Ascobolus immersus* by Delay (*1966*), in *Ascobolus viridulus* by Oso (*1969*), in *Ascobolus stercorarius* by Wells (*1972*), and in *Pustularia cupularis* by Schrantz (*1966, 1967*).

While the ascospores of the Inoperculatae are always smooth, many operculate Discomycetes develop a more or less complex spore sculpturing.

Le Gal's (*1947*) detailed studies on ascospore ornamentation with the aid of light microscopy, have exercised a far-reaching influence on the taxonomy of the operculate Discomycetes. She described a great variation of the patterns of ornamentation, of which the development often seemed to be extremely complex.

Submicroscopical investigations in this field were rather fragmentary and not focused on taxonomic comparison.

Recently a comparative study of the ultrastructure and development of ascospore ornamentation with the Pezizales was published by Merkus (*1973, 1974, 1975, 1976*). She demonstrated that, in principle, the development of the ascospore ornamentation is a single common process with a certain number of variations.

In smooth-spored species an initial ornamentation is formed, which disappears again during further ripening of the ascospores. In a few other smooth-spored species a permanent smooth ornamentation is deposited on the ascospore wall (Merkus, *1976*).

It is clear from these studies that the ascospore ornamentation should be used with great caution in the delimitation of genera or families.

MATERIALS AND METHODS

Up to now over 45 species of Pezizales belonging to about 30 genera have been studied by me with light and electron microscopy to analyse the dehiscence mechanism of their asci. The light microscope revealed information about the structure and function of the living ascus. Vital and subvital observations have been made in squash mounts in a slightly hypotonic solution of glycose in distilled water. The slides were examined with phase contrast and Nomarski's interference contrast optics. Of special value proved to be the observation of unstained asci with polarized light. For light microscopy asci were also stained with a wide variety of dyes, of which Congo red, acid fuchsin, trypan blue, methyl blue, and methylene blue gave satisfactory results. Also, sections about 0.5 μm thick of material fixed in glutaraldehyde and osmium tetroxide and subsequently embedded in epoxy resin proved to be of great value. These sections were stained with toluidine blue.

When using Congo red as a stain for wall material one should be aware that this stain shows two staining mechanisms: one which is by chemical linkage (a 'true staining'), and a second which is by physical adsorption of the long dipolar molecules by the surfaces of microfibrillae where there is sufficient space for them to penetrate.

For electron microscopy material was fixed as usual in buffered glutaraldehyde or in $KMnO_4$ and postfixed in 1% OsO_4 (cf. van Brummelen, *1974*).

For studies of the ascus wall, material fixed in $KMnO_4$ proved to be most valuable.

OBSERVATIONS

The lateral wall of the operculate ascus consists of at least two layers. The outer layer is usually rather thin and strongly birefrigent. It stains red with Congo red and bluish violet with toluidine blue. The inner layer is usually thicker, less rigid and only weakly anisotropic. It stains reddish with toluidine blue and does not stain with Congo red. In the electron microscope the inner layer is more electron transparent in permanganate-OsO_4-fixed material.

It is my experience that the outer layer is the more variable in appearance. In young asci its surface is usually sharply delimited, but under other circumstances it may sometimes become swollen and mucilaginous with a rather diffuse delimitation at the outside. This is in agreement with recent findings of Reisinger *& al.* (*1977*) on the ultrastructure of hyphal walls of Ascomycetes and Basidiomycetes. In asci with thick walls (e.g. Thelebolaceae) or in strongly swollen layers (e.g. some Sarcoscyphaceae) a sublayering or lamellation is often visible.

In many Operculates the ascus wall is covered by an extra-ascan layer, the periascus of Chadefaud, which is usually more apparent in the apical part of the ascus. In genera with the ascus wall staining blue with iodine, this reaction is strictly confined to the mucilagenous substance of the periascus.

During ripening, the apical wall of the ascus develops certain structures to form an opening mechanism. The apical part of the wall tends to become more complex and may consist of three to four layers. Certain parts of the apical wall may become weakened by the formation of indentations, fracturing lines and weakened zones or by local breakdown or gelatinization of the wall. In other, often adjoining, parts the wall may become strengthened and more rigid. The moment at which these changes in the wall become manifest differs greatly. Sometimes they are scarcely visible before the moment of ascus dehiscence. The study of emptied asci is always necessary to decide with certainty on the structure and the mechanism of ascus dehiscence.

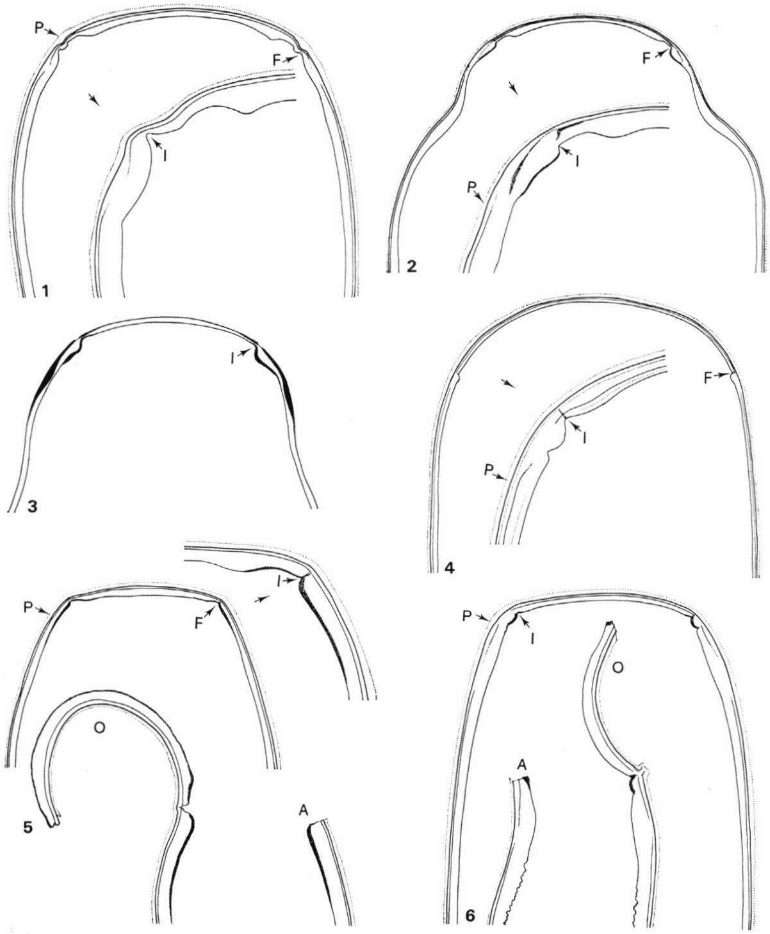
TYPES OF ASCI

The following principal types of asci can be distinguished in the Pezizales.

(1) ASCOBOLUS TYPE [= The traditional standard model of the operculate ascus].—

Operculum very large, sharply delimited by a circular internal indentation just at the inner side of a strengthened ring. The operculum and region under the ascostome are strengthened and rigid. This causes the typical sinuous outline of the apex. Ascostome smooth. Periascus of rather uniform thickness; staining blue with iodine. Funiculus present.

Examples: *Ascobolus furfuraceus* (Fig. 1), *A. sacchariferus*, *Saccobolus glaber* (Figs. 2, 3), *Thecotheus* spec. (Fig. 5), *Boudiera echinulata* (Fig. 4), and *Iodophanus carneus* (Fig. 6).



Figs. 1–6. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Fig. 1. *Ascobolus furfuraceus*. — *Fig. 2. Saccobolus glaber*. — Fig. 3. The same, as seen with light microscopy in sections stained with toluidine blue. — Fig. 4. *Boudiera echinulata*. — Fig. 5. *Thecotheus* spec. — Fig. 6. *Iodophanus carneus*.

(2) PEZIZA TYPE.—

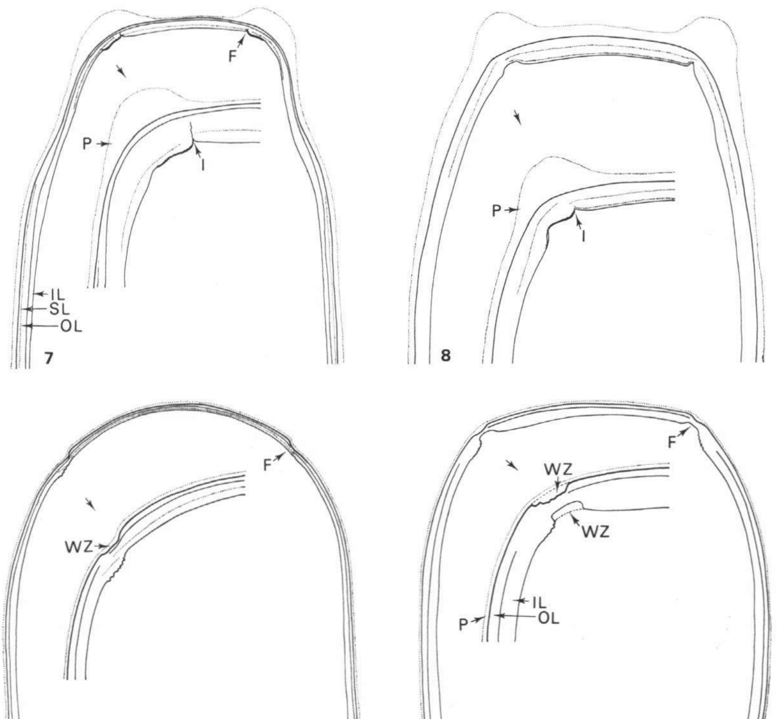
Operculum sharply delimited by a circular internal indentation just at the inner side of a weakly developed ring. The operculum and a region under the ascostome are strengthened and rigid. Wall structure rather complex. Ascostome smooth. Periascus strongly developed, with ring-shaped thickening over the ascostome, tapering towards the base; staining blue with iodine. Funiculus present.

Examples: *Peziza badia*, *P. succosella* (Fig. 7), *P. depressa*, *P. ammophila* (Fig. 8), and *P. cerea.*

(3) ASCODESMIS TYPE.—

Operculum very large, sharply delimited by a weak indentation and a circular zone of two-sided wall desintegration. Ring only weakly developed. Operculum strengthened but not rigid. Ascostome smooth. Periascus very thin; not blued with iodine. Funiculus and funnel absent.

Examples: *Ascodesmis nigricans* (Fig. 9) and *A. microscopica* (Fig. 10).

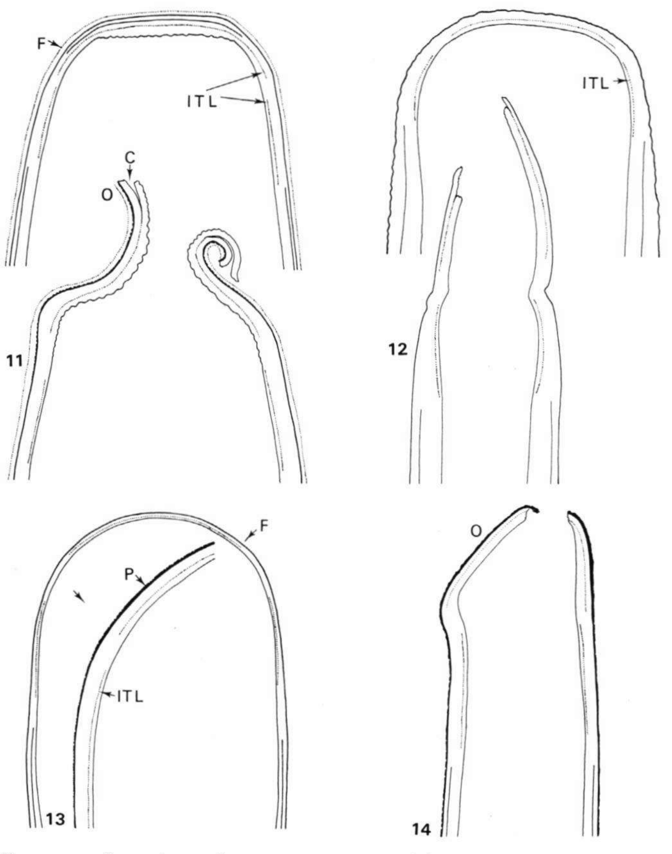


Figs. 7–10. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Fig. 7. *Peziza ammophila.* — Fig. 8. *Peziza succosella.* — Fig. 9. *Ascodesmis nigricans.* — Fig. 10. *Ascodesmis microscopica.*

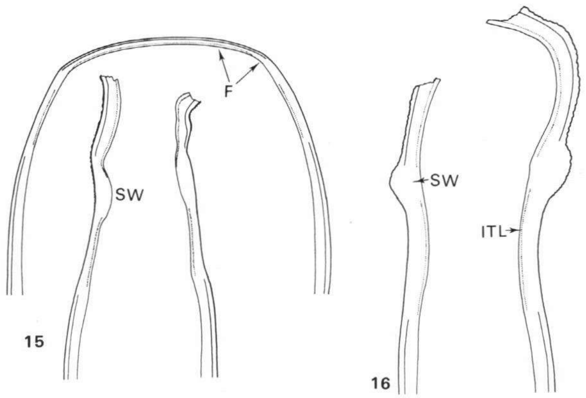
(4) OCTOSPORA TYPE..—

Operculum rather roughly delimited, without indentation or prominent ring, sometimes strengthened at its inner side by an electron-transparent layer. Apical wall with thimble-shaped electron-dense lamina interrupted by a thick ring-shaped electron-transparent zone. Dehiscence takes place in a weakened zone between the electron-transparent ring and the strengthened operculum. If the operculum is not strengthened the operculum may be torn irregularly. Cleavage of wall and operculum along the thimble-shaped lamina above the electron-transparent ring is rather frequent after dehiscence. Ascostome rough. Periascus thin if present; not blued with iodine. Funiculus present.

Examples: *Pyronema omphalodes* (Fig. 11), *Anthracobia maurilabra* (Fig. 12), *Aleuria aurantia* (Figs. 13, 14), *Otidea onotica, Coprobia granulata* (Figs. 15, 16), *Cheilymenia pulcherrima, C. vitellina* (Fig. 17), *Scutellinia armatospora* (Fig. 18), *Octospora musci-muralis* (Fig. 19), and *Sowerbyella unicolor* (Fig. 20).



Figs. 11–16. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Fig. 11. *Pyronema omphalodes.* — Fig. 12. *Anthracobia maurilabra.* — Figs. 13, 14. *Aleuria aurantia.*



Figs. 15–16. Diagrammatic sections of ascus tops, as seen with electron microscopy. *Coprobia granulata.*

(5) HELVELLA TYPE.—

Operculum sharply delimited at its basal side by a ring-shaped breaking-line just at the inner side of a narrow, scarcely proliferating ring. Apical wall with a thimble-shaped electron-dense lamina, which is only interrupted by the proliferating ring. Ascostome smooth. Periascus very thin; not blued with iodine. Funiculus present.

Examples: *Helvella crispa* (Fig. 21) and *Geopyxis carbonaria* (Fig. 22).

(6) URNULA TYPE.—

Operculum rather roughly delimited by an external ring-shaped zone of wall desintegration, without indentation or proliferating ring, not strengthened. In the inner wall layer of the top a thimble-shaped electron-dense lamina is formed, which is intercepted at the apex by a broad ring-shaped electron-transparent zone. Near the upper margin of this zone the operculum is torn loose. Ascostome rather rough. Periascus very thin, not staining blue with iodine. Funiculus not yet recorded.

Example: *Urnula platensis* (Figs. 23, 24).

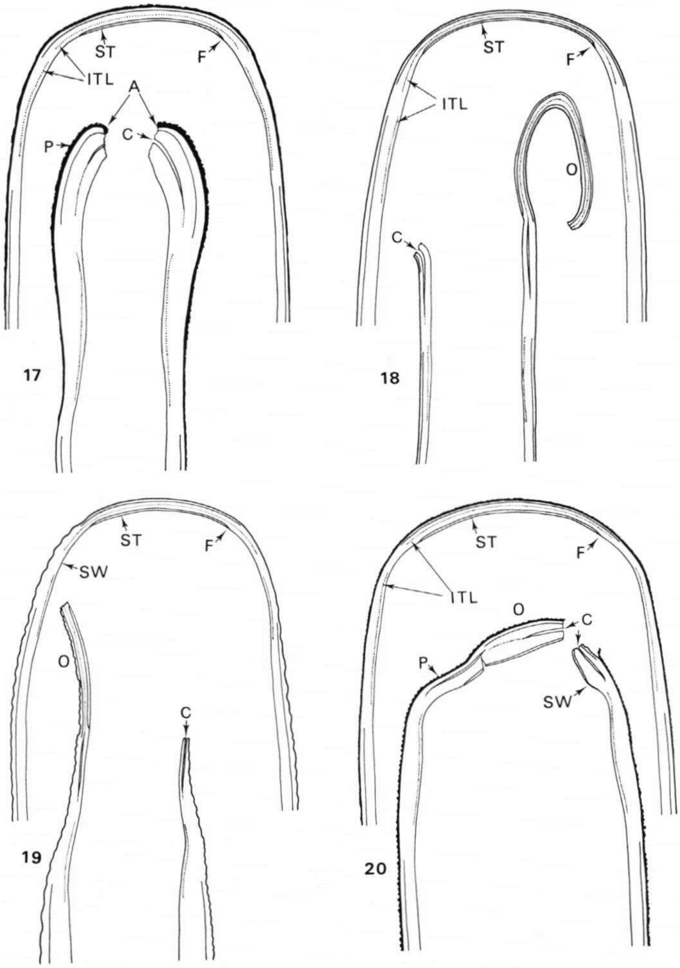
(7) SARCOSCYPHA TYPE.—

Operculum very thick, rather narrow, centrally or obliquely placed, sharply delimited in the inner layer and more roughly in the outer layer. Inner layer strongly swollen, often stratified and laminated, forming a thick lenticular body (opercular plug) at the top. Ascostome smooth. Periascus clearly developed; not blued with iodine. Funiculus present.

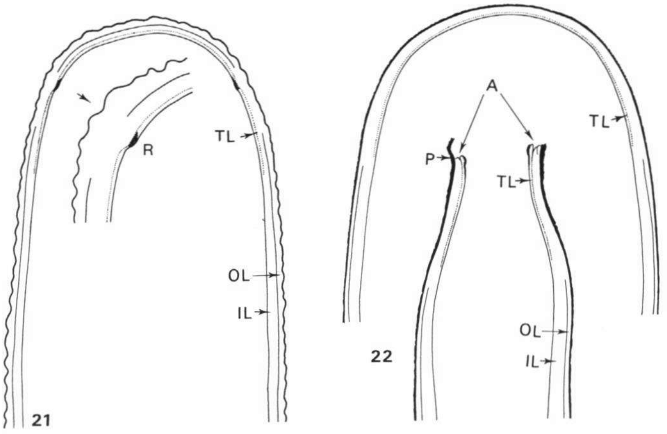
Examples: *Sarcoscypha coccinea* (Figs. 25, 26; cf. van Brummelen, *1975*), *Pithya cupressina* (Fig. 27), *Pseudoplectania nigrella* (Fig. 28), and *Desmazierella acicula* (cf. also Samuelson, *1975*.).

(8) THELEBOLUS TYPE.—

Operculum absent in multispored asci or rather roughly delimited in some 8-spored asci. Usually opening by an irregular tear or with a bilabiate split starting from the margin of a small, rigid, apical disk. Sometimes external forces produce a more or less circular tear



Figs. 17–20. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Fig. 17. *Cheilymenia vitellina.* — Fig. 18. *Scutellinia armatospora.* — Fig. 19. *Octospora musci-muralis.* — Fig. 20. *Sowerbyella unicolor.*



Figs. 21–22. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Fig. 21 *Helvella crispa*. — Fig. 22. *Geopyxis carbonaria*.

(*Leptokalpion*). A usually pronounced ring in the wall prevents the tear to pass this level. Ascostome rough (if present). Wall thick and often strongly stratified or laminated. Periascus and funiculus absent.

Examples: *Thelebolus stercoreus* (Figs. 29–32), '*Rhyparobius*' *myriosporus* (Figs. 33–38), '*R.*' *crustaceus* (Fig. 39), '*R.*' *caninus* (Fig. 40), '*Ascophanus*' *coemansii* (Fig. 41), *Coprotus* spec., *Lasiobolus monascus* (Fig. 42), *L. pilosus* (Fig. 43), and *Ascozonus woolhopensis* (Fig. 44).

The *Thelebolus* type of ascus covers a wide range of possibilities, many of which have been studied in detail by Kimbrough (*1966*, *1972*) and Kimbrough & Korf (*1967*).

As our knowledge about ascus-structure in the genera of the Pezizales is still very incomplete, it is well possible, and even probable, that further types of asci will turn up in future.

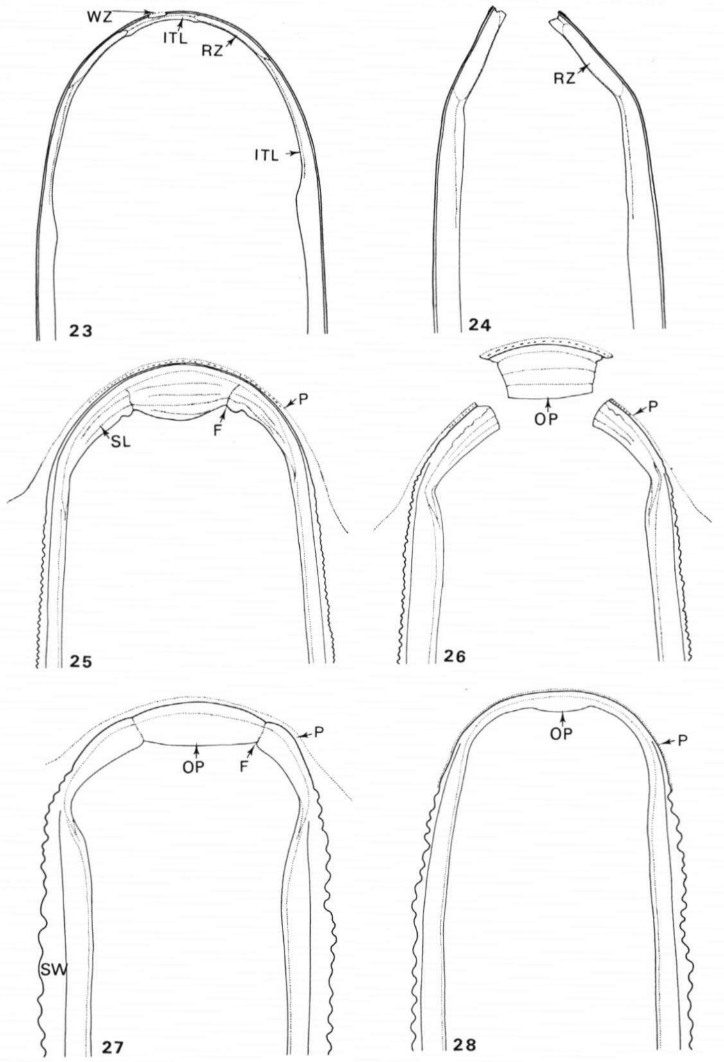
A more detailed study of the Thelebolaceae will almost certainly lead to a further subdivision of the *Thelebolus* type.

A great deal of information on the structure of the ascus top is still required from many important genera, especially from those with a tropical distribution.

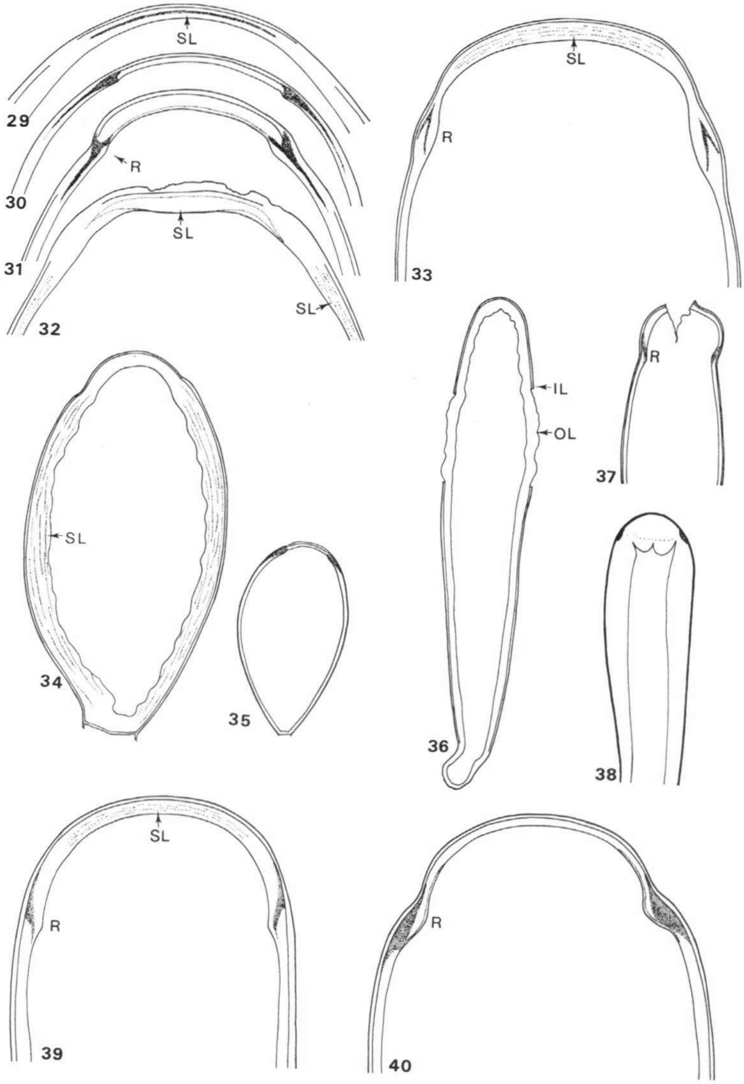
Most of the Tuberales should be classified among the Pezizales, but as these fungi have lost their mechanism of ascus dehiscence it is not possible to fit them in on this character.

CONCLUSIONS

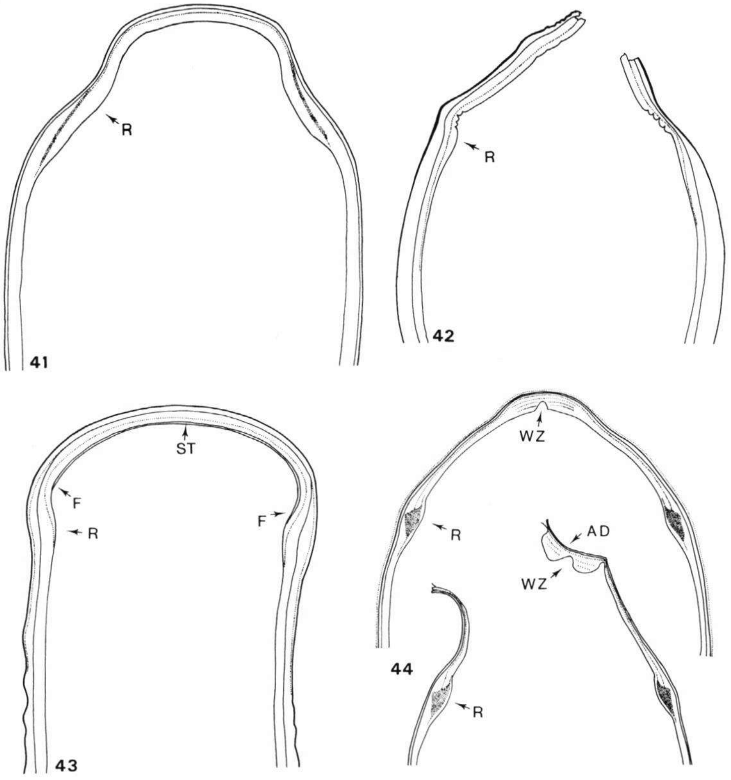
If we consider the information available and compare the different structures it is possible to recognize certain affinities and trends. This has led me in the first place to distinguish the



Figs. 23–28. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Figs. 23, 24. *Urnula platensis*. — Figs. 25, 26. *Sarcoscypha coccinea*. — Fig. 27. *Pithya cupressina*. — Fig. 28. *Pseudoplectania nigrella*.



Figs. 29–40. Diagrammatic sections of asci and ascus tops (as seen with electron microscopy, if not otherwise stated). — Figs. 29–32. *Thelebolus stercoreus* (with over 1000 spores). — Fig. 29. Very young stage, before ascosporogenesis. — Fig. 30. Ripening ascus, as seen with light microscopy in sections stained with toluidine blue. — Fig. 31. Ripening ascus. — Fig. 32. Mature ascus. — Figs. 33–38. '*Rhyparobius*' *myriosporus* (128–512 spores). — Fig. 33. Ripening ascus (with about 256 spores). — Figs. 34, 35. Asci (with about 512 spores), as seen with light microscopy, stained with Congo red. — Figs. 36–38. Ripening and mature asci (with about 256 spores), vital observation. — Fig. 39. '*Rhyparobius*' *crustaceus* (with about 64 spores). — Fig. 40. '*Rhyparobius*' *caninus* (with about 32 spores).



Figs. 41–44. Diagrammatic sections of ascus tops, as seen with electron microscopy. — Fig. 41. '*Ascophanus*' *coemansii*. — Fig. 42. *Lasiobolus monascus*. — Fig. 43. *Lasiobolus pilosus*. — Fig. 44. *Ascozonus woolhopensis*.

eight types described above, and in the second place to visualize their possible interrelationship in a scheme (Fig. 45).

The following trends can be recognized.—

(1) All asci with 'amyloid walls' studied thus far form a compact and closely related group.
(2) The *Ascodesmis* type takes a rather isolated position.
(3) There is a rather complete scale of structures ranging from *Thelebolus* via *Lasiobolus* and *Coprotus* via *Pyronema* – *Aleuria* – *Coprobia* – *Cheilymenia* – *Octospora* – *Scutellinia* – *Sowerbyella* to members of the Sarcoscyphaceae.
(4) No direct relation with the asci of Inoperculatae has been detected.

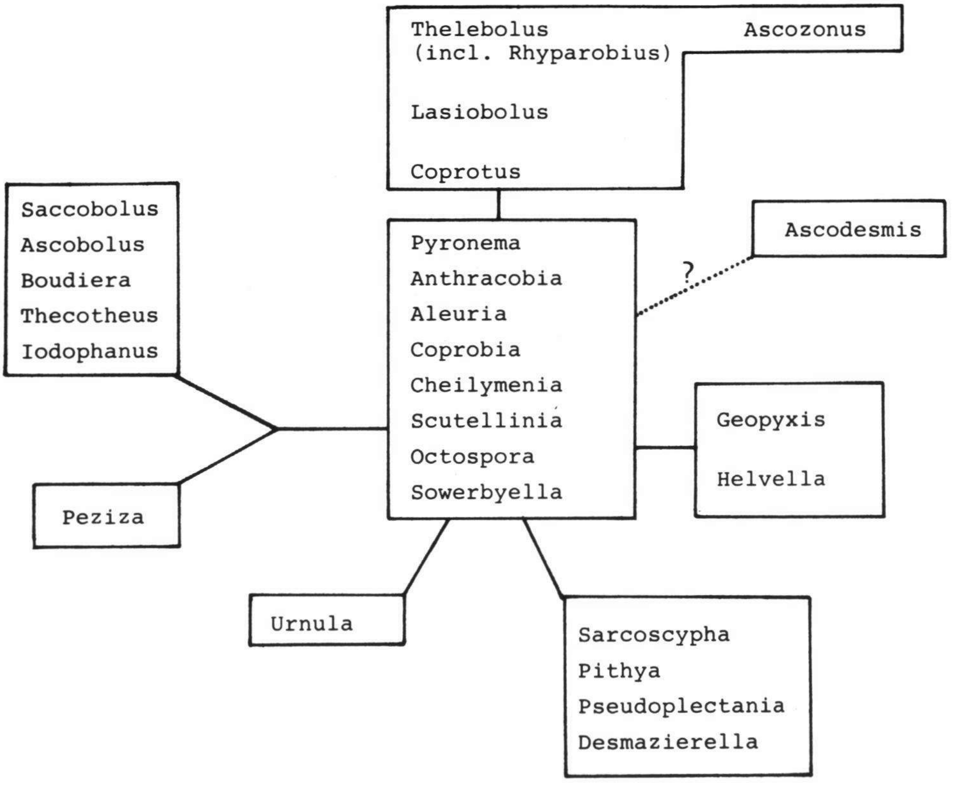


Fig. 45. Tentative scheme of possible interrelationships between different types of opening mechanisms of asci in Pezizales.

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ABBREVIATIONS USED IN TEXT-FIGURES

A, ascostome; AD, apical disk; C, cleavage of ascus wall; F, line or zone of fracturing; I,indentation of ascus wall; IL, inner layer of the ascus wall (The boundary between inner and outer layer is indicated by a continuous line.); ITL, interrupted thimble-shaped lamina (indicated by a dotted line); O, operculum; OL, outer layer of the ascus wall; OP, opercular plug; P. periascus (extra-ascan layer); R, prominent ring; RZ, ring-shaped, electron-transparent zone of the ascus wall; SL, sublayering of the ascus wall (usually indicated by dotted lines); ST, strengthened layer; SW, strongly swollen wall region; TL, thimble-shaped lamina (indicated by a dotted line); WZ, weakened zone.

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ADDENDUM

Just after receiving the galley proofs, three studies on the ascus structure of Pezizales came to my notice.

Kimbrough & Benny (1978) studied the ascus structure in *Lasiobolus monascus*.

Samuelson (*1978a*) published on the structure of iodine-positive asci in *Peziza succosa*, *Ascobolus crenulatus*, *Saccobolus depauperatus*, *Thecotheus pelletieri*, and *Iodophanus granulipolaris*. He (Samuelson, *1978b*) also published on the asci of the so-called *Otidea-Aleuria* complex. He studied the 'apical apparatus' of asci in *Otidea leporina*, *Jafnea fusicarpa*, *Humaria hemisphaerica*, *Sphaerosporella brunnea*, *Aleuria aurantia*, *Anthracobia melaloma*, *Scutellinia scutellata*, *Ascozonus woolhopensis*, *Geopyxis majalis*, and *Sowerbyella imperialis*.

Although most of their results and illustrations agree with my findings, some of their interpretations are strikingly different.