

**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% KMnO₄, then postfixed with OsO₄.

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.) Kanouse¹ 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).

R E S U L T S

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 $400-450 \times 13-15 \mu\text{m}$ 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 $1.8-2.8 \mu\text{m}$ thick and $4.5-6.0 \mu\text{m}$ in diameter), a surrounding ring-shaped fold (c. $0.5 \mu\text{m}$ 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).

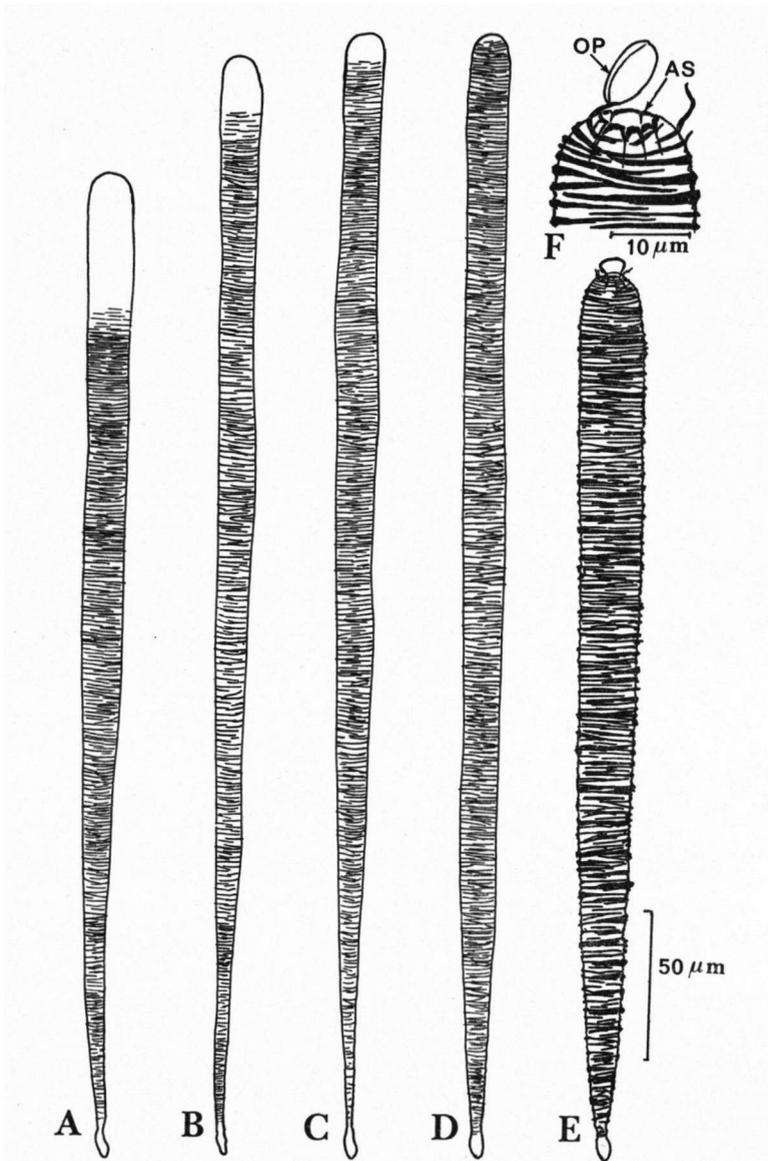


Fig. 1. *Sarcoscypha coccinea*, asci at successive stages of development as seen from the side with light microscopy. — A. Before meiosis. — B, C. Ripening asci. — D. Mature ascus. — E. Ascus after spore discharge. — F. Detail of top after discharge.

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

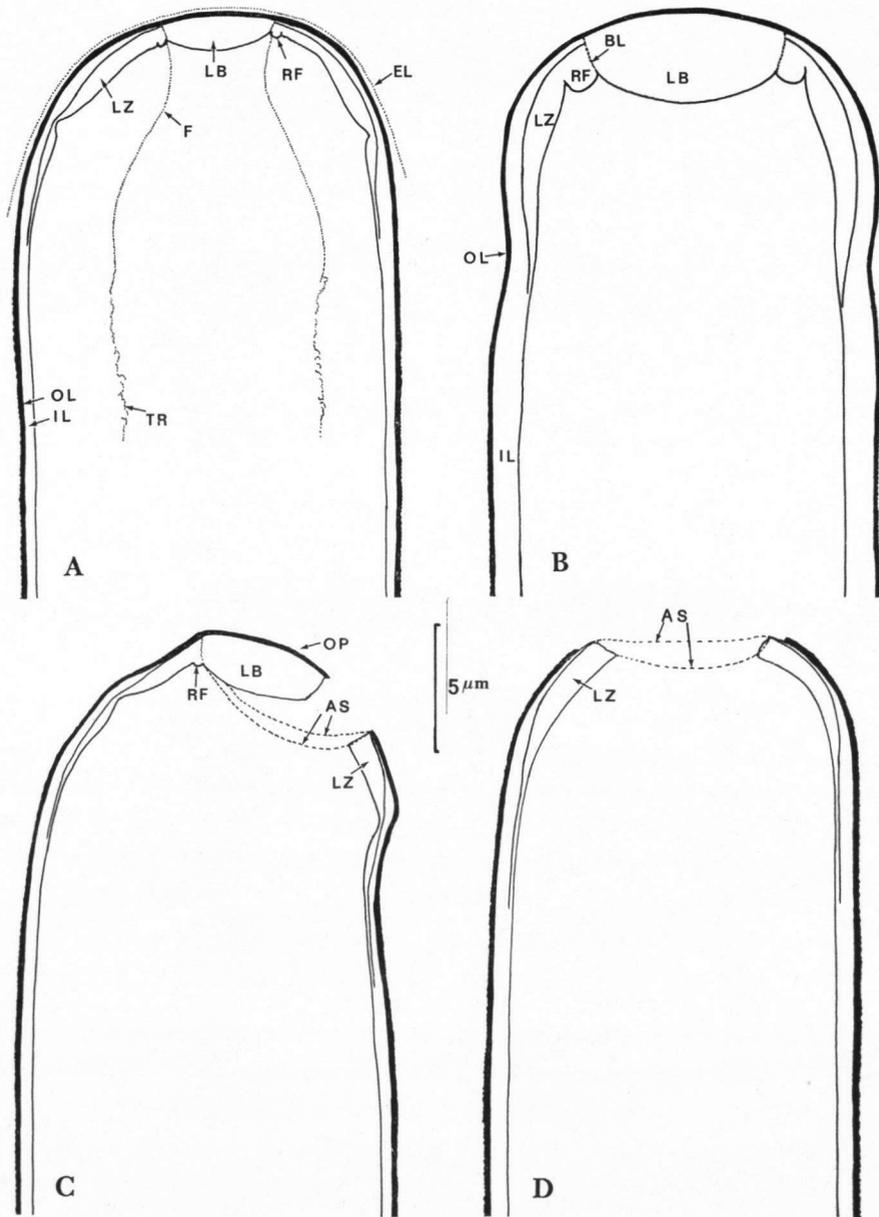


Fig. 2. *Sarcoscypha coccinea*, diagrammatic sections of ascus tops, as seen with light microscopy. — A. Almost mature ascus of which the spores have left through a rupture in the basal part. — B. Ascus in the same stage observed in a solution of Congo red in diluted ammonia, showing thickened walls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

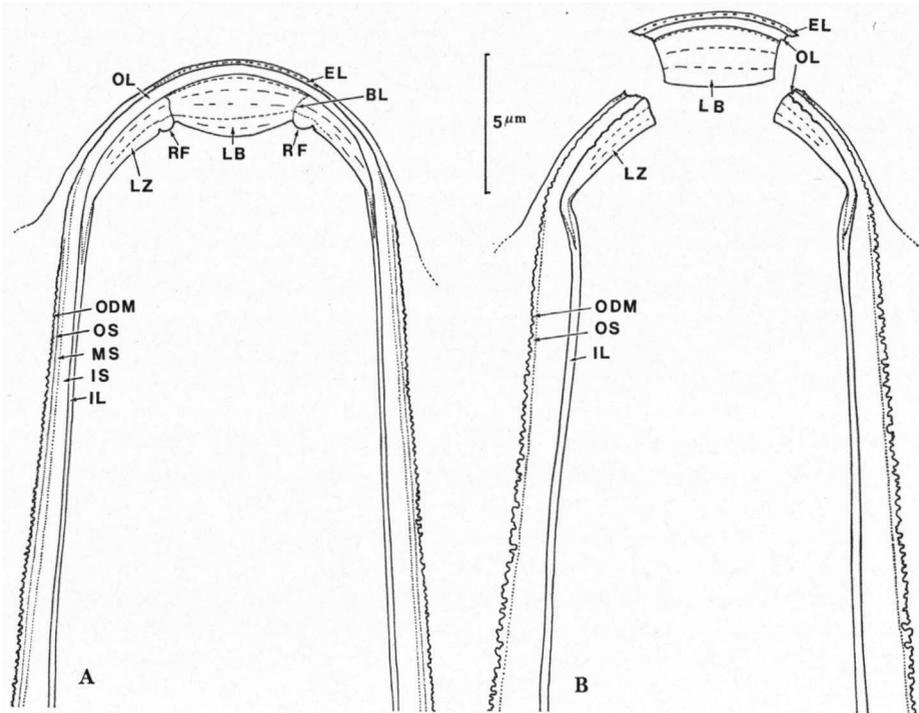


Fig. 3. *Sarcoscypha coccinea*, diagrammatic sections of ascus tops as seen with electron microscopy. — A. Almost mature ascus. — B. Ascus after spore discharge.

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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REFERENCES

- BERTHET, P. (1964). Essai biotaxonomique sur les Discomycètes. Thèse. Univ. Lyon.
- BOEDIJN, K. B. (1933). The genera *Phillipsia* and *Cookeina* in Netherlands India. In Bull. Jard. bot. Buitenz. III 12: 57-76.
- BRUMMELEN, J. VAN (1974). Light and electron microscopic studies of the ascus top in *Ascozonus woolhobensis*. In Persoonia 8: 23-32.
- BULLER, A. H. R. (1934). Researches on fungi. London. Vol. 6.
- CHADEFAUD, M. (1942). Etudes d'asques, II: Structure et anatomie comparée de l'appareil apical des asques chez divers Discomycètes et Pyrénomycètes. In Rev. Mycol. 7: 57-88.
- (1943). Sur les divers types d'éléments dangeardiens des Ascomycètes, et sur la formation des asques chez la Pézize *Pustularia catinus*. In Rev. Scient., Paris 81: 77-80.
- (1946). Les asques para-operculés et la position systématique de la Pézize *Sarcoscypha coccinea* Fries ex Jacquin. In C. r. hebd. Séanc. Acad. Sci., Paris 222: 753-755.
- (1953). Anses latérales de Eumycètes. In Öst. bot. Z. 100: 515-532.
- (1960). Les végétaux non vasculaires (Cryptogamie). In CHADEFAUD, M. & EMBERGER, L., Traité de botanique systématique I. Paris.
- (1973). Les asques et la systématique des Ascomycètes. In Bull. trim. Soc. mycol. Fr. 89: 127-170.
- DENISON, W. C. (1972). Differentiation of tribes and genera in the family Sarcoscyphaceae. In Persoonia 6: 433-438.
- ECKBLAD, F.-E. (1968). The genera of the operculate Discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. In Nytt Mag. Bot. 15: 1-191.
- (1972). The suboperculate ascus—A review. In Persoonia 6: 439-443.
- LE GAL, M. (1946a). Mode de déhiscence des asques chez les *Cookeina* et les *Leotia*, et ses conséquences du point de vue phylogénétique. In C.r. hebd. Séanc. Acad. Sci., Paris 222: 755-757.
- (1946b). Les Discomycètes suboperculés. In Bull. trim. Soc. mycol. Fr. 62: 218-240.
- (1969). Position taxonomique du genre *Phaedropezia* Le Gal et révision de la famille des Humariaceae. In Bull. trim. Soc. mycol. Fr. 85: 5-19.
- SEEVER, F. J. (1928). The North American Cup-fungi (Operculates). New York.

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% KMnO₄, post-fixed in OsO₄, 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.

