THE UNIVERSITY OF MANITOBA

STUDIES ON THE HEMIPHACIDIACEAE
ASCUS APEX AND APOTHECIAL STRUCTURE IN RELATION TO TAXONOMY

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ABSTRACT

A study of the validity of the family Hemiphacidiaceae, order Helotiales, was undertaken by examining in detail the ascus pore reaction in iodine preparations, the ascus apex structure, and the apothecial anatomy of key species of the genera comprising this family. These were the main criteria used in erecting the family.

It was shown that ascus pore reaction, in this family at least, is a direct function of the degree of complexity of the ascus apex structure with the iodine reactive species having structures, amyloid in nature, which are lacking in non-reactive species. No species which were non-reactive were found which had identical structures to reactive species. This is in contrast to reports in other families where reactive and non-reactive species are said to have identical structure.

The apothecial anatomy of the various species studied revealed significant differences between the genera.

Correlation of the nature of the apothecial anatomy with ascus apex structure and iodine reactivity prove this family is an artificial assemblage of unrelated genera, similar only in the type of host tissue they attack.

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I. INTRODUCTION

In recent years, Ascomycete taxonomy has undergone major revisions based, primarily, on characters which are thought to reflect more "natural" relationships. Consequently the systems that are now evolving supposedly reflect a degree of relatedness amongst the major groups which has previously been obscured by the artificial systems which have long been in use.

The morphological characters which are currently used in erecting taxonomic systems are the structure of the asci and the nature of the fruiting bodies in which the asci are borne.

To simply state that it is the nature of the fruiting bodies which is significant is misleading; it is actually the way in which they develop which is of prime importance. For example, the sub-division Euascomycotina can be divided into two classes, the Hymenioascomycetes and the Loculoascomycetes. In the Hymenioascomycetes the fructifications develop in response to sexual stimulation and the structural or protective tissue which encloses the asci differs from the surrounding vegetative tissue in that the latter, when present, is preformed prior to the sexual phase of the fungus being initiated. As a consequence the primary protective tissue of the ascocarp develops within the

vegetative tissue as a separate entity, frequently forming from the stalk cell of the ascogonium but always developing just prior to the development of the ascogenous hyphal system which produces the asci.

In the Loculoascomycetes on the other hand, the structural or protective tissue of the ascocarp is really nothing more than vegetative tissue which forms long before fertilization or dikaryotization of the fungus occurs and this vegetative tissue is simply modified in various ways to form the protective structure in which the ascogenous hyphal system arises and produces the asci.

Correlated with these two main developmental patterns of the fruiting bodies is the structure of the asci. In the Hymenioascomycetes the asci are unitunicate, while the asci of the Loculoascomycetes are bitunicate. These two terms, unitunicate and bitunicate, refer to the fact that the wall of a unitunicate ascus consists of a single functional layer, sometimes laminate, but usually of uniform thickness except at its apex where it is frequently typically thickened. This thickening however is not simple, for, depending upon the family under consideration, the thickening may consist of a fairly complex apparatus whose function seems to be to create a channel through which the spores are ejected and to provide various mechanisms by which this ejection can be effected.

The bitunicate asci, on the other hand are really

double walled and these individual walls are quite separate and have distinct functions. The outer wall is thin, non-extensible and protective. When the ascus is mature the outer wall ruptures variously and the thick, inner wall which is extensible elongates and a pore forms at its apex through which the spores are ejected. Furthermore in contrast to a unitunicate ascus there are no complex structures at the ascus apex.

While this correlation of fruiting body developmental pattern with the type of ascus is fairly consistent, exceptions have been noted (Booth, 1966; Luttrell, 1965).

Thus the structure of the ascus, the sac-like reproductive unit which sets the Ascomycetes apart from all other fungi, is increasing in importance in the erection of new taxonomic systems in the Ascomycetes.

Within species which have unitunicate asci, it has long been recognized that the apex structure can be used as a classification criterion. Taxonomists have long recognized the operculate ascus of, for example, the Pezizales as being structurally distinct from the inoperculate ascus of, for example, the Helotiales (both of these orders are commonly referred to as discomycetous or apothecial fungi). Unfortunately, within the discomycetous fungi it has long been accepted that once a fungus has been described as inoperculate or operculate, no further structural differences worthy of note existed in the asci of these two mutually

exclusive groups. An exception to this school of thought is evident in the work of Chadefaud (1942; 1960) who not only questioned the fundamental classification of asci into unitunicate and bitunicate forms, but has also suggested that within the inoperculate asci there is considerable variation in the ascus apex structure and that these variations in structure are of great taxonomic importance.

This study has centered on the structure of the ascus apices of the family Hemiphacidiaceae and the significance of the findings on the classification system currently used for the relatively important group of organisms found in this family. It has also uncovered some anomalies in the classification system when one considers the structure of the apothecia.

II. THE HEMIPHACIDIACEAE

The family Hemiphacidiaceae, order Helotiales was erected by Korf (1962) for a group of inoperculate discomycete genera which all attack living leaves of conifers and cause needle-blights or "snow-blights".

Korf originally included 8 genera within this family. Naemacyclus Fuckel, Didymascella Maire and Sacc., Sarcotrochila Von Höhn, Rhabdocline Syd., Fabrella Kirscht., Lophophacidium Lagerberg, Hemiphacidium Korf and Gremmenia Korf. Subsequently Reid and Cain (1962) have shown that

Lophophacidium properly belongs in the family Phacidiaceae and Reid and Pyrozinski (1968) have stated that the holotype of Gremmenia gigaspora (Gremmen) Korf, the type species of this monotypic genus, represents nothing more than abnormally developed Phacidium infestans Karst. Thus they relegated Gremmenia Korf to a synonym of Phacidium Karst. Thus, at the start of this study, the Hemiphacidiaceae comprised 6 genera one of which, Naemacyclus, was doubtfully referred to this family by Korf due to differences in the development of its apothecia and in the nature of its paraphyses and spores when compared with these structures in other genera of the family.

In general the apothecia of the members of this family range in colour from yellow to dark-brown and are formed either within the epidermis, in which case the epidermis is split as the hymenium matures, just beneath the epidermis, or just below the hypodermis of living or recently killed leaves which are still attached to the plant. The apothecia are supposed to have a poorly developed basal stratum (this, as will be shown later, is not entirely true) which gives rise to asci and paraphyses and very little excipular tissue. There is, moreover, no covering layer of fungus tissue above the hymenium and the developing hymenium is supposed to press directly against the overlying host tissue. The hymenium is then exposed by the host delimiting a circumscissile scale of tissue, composed of epidermal and hypodermal cells, which

is raised and folded back in one piece as the apothecium expands and matures.

The asci are unitunicate and inoperculate and vary from cylindrical to clavate and these are interspersed with simple cylindric paraphyses which are sometimes clavate at their apices. The only genus in which branched paraphyses are supposed to occur is in the genus Naemacyclus.

The ascospores may be variously shaped, variously septate, hyaline or coloured at physiological maturity but in Naemacyclus the ascospores are needle-shaped and as this shape is quite different from the ascospores found in other genera of the family, this also seems to set this genus apart. At least two of the genera assigned to this family, Rhabdocline and Didymascella, contain species which are of major economic importance in regions where extensive coniferous forests are found. The pathogenic potential of the species of the other genera is unclear although at least some of them can cause severe local damage under conditions favourable to the parasite.

Until fairly recently, the various genera now assigned to this family were placed in other families including the Stictidaceae and Phacidiaceae, because the apothecium was exposed by the rupture of the overlying host tissue. However many detailed investigations have shown that fungi properly assigned to the latter two families have their hymenia exposed by the splitting of a layer of fungal

tissue which overlies the developing hymenium. That is, the hymenium develops within a layer of fungus tissue. In the family Hemiphacidiaceae, as delimited, the hymenium develops on a basal stratum, not within fungal tissue, and the overlying tissue above the developing apothecium is of host origin and it is this tissue which ruptures to expose the hymenium. These differences, together with the unusually simple structure of hemiphacidiacious apothecia, led Korf to group these genera in his new family.

In earlier studies of the Helotiales sensu Nannfeldt (1932), particularly the genera of the Dermateaceae, some suggestion was made of the possible relationship of what are clearly dermateacious fungi to those now included in the Hemiphacidiaceae. However Korf (1962) has clearly shown why such relationships cannot exist and, at the moment, while other fungi may belong to this family, their identity is as yet unknown.

III. IODINE REACTION OF THE ASCI

Melzer's Reagent or various other iodine and potassium iodide solutions has long served as a useful stain for ascomycete preparations due to the fact that it turns any amyloid structure blue. Iodine in its various forms imparts a blue stain to the hymenium of many Lecideaceae and in the operculate discomycetes it is useful in separating the

Pezizazceae, tribe Pezizeae, from the superficially similar members of the Pezizaceae, tribe Otideae. In the latter case the outer layer of the ascal wall at the ascus apex turns blue in the Pezizeae but not in the Otideae - denoting starch-like compounds in the ascus wall of the Pezizeae but not in the Otideae.

In the operculate discomycetes particularly in the order Helotiales, the blue reaction of the pore plug has long been considered an important taxonomic character. In fact Korf (1962) has stated that: "It is my experience that the iodine reactivity of the ascus pore is an exceptionally stable character, throughout the discomycetes. I have never found different collections of the same species in which this character varies." It is viewpoints such as this which have led to the belief that whole families or perhaps even orders should have asci whose apices will react in the same way to iodine.

It must be clearly pointed out, however, that this colour reaction is presumed to be linked to the structure of the ascal apices, and consequently, to the mechanisms by which the ascospores are discharged from the asci.

On the basis of the reaction of the ascus apices of the members of the Hemiphacidiaceae to Melzer's Reagent, Korf (1962) divided the family into two tribes: (1) The Hemiphacidieae with iodine negative ascus apices and (2) the Sarcotrochileae with ascus apices which are iodine positive. He

recognized the anomaly of this in view of his comments reported above, but he seems to have been suggesting that the Sarcotrochileae might well be split off at some future date when more detailed studies had been completed.

The genus Rhabdocline presented an even more difficult problem. Rhabdocline psudotosugae Syd. (in Sydow and Petrak, 1922) was, according to Korf, a species in which he never encountered any blueing of the ascus pores in iodine. Weir (1917) who first described this organism, although he did not formally name it, and Brandt (1960) both stated that the pores blued in iodine. Korf (1962) tried to explain these observations as a misprint in Weir's publication which was inadvertently picked up by Brandt. Korf himself did not seem entirely satisfied with this explanation, but he could not resolve this problem.

Other workers have encountered similar problems.

Müller and Hütter (1963) and Dennis (1954) reported differing pore reactions for Chloroscypha sabina (Fuckel) Dennis, differences which cannot be explained either on maturity of the asci in the respective collections examined by these authors nor on pore size, where blue reactions in small-pored species could be easily overlooked, since C. sabina has a relatively large pore.

In addition Reid (pers. comm.) has stated that an unusual collection of Peziza repanda Pers. was drawn to his attention by Dr. R.W.G. Dennis which, when fresh, had ascal

pores which did not turn blue in Melzer's Reagent. On drying the majority, although not all, of the ascus pores of this collection turned blue in Melzer's Reagent. What, then, is the "normal" reaction in this species?

Perhaps variations of this type may be explained, as suggested by Muller (pers. comm.) by the fact that while the basic structures in such variable species may be the same, molecular orientation in the structures may vary, or change, and cause differing iodine reactions. For example, if cross-bonds were numerous between adjacent molecules, these may prevent penetration of the iodine into the intermolecular spaces and while surface molecules might give a blue colour it would be very faint, and thus missed in contrast to the structure where the iodine penetrated deeply. Chadefaud (1942) also believed that in the plugs at the tips of the asci different arrangements and condensation of the carbohydrate molecules would result in the reaction to iodine being different in asci of different taxonomic groups.

In all such rationalizations however, one point is clear: None of the variable reactions have been considered as possibly being linked to fundamental structural differences between ascus apices of different species and such differences may really be what the variable iodine reaction is reflecting. Thus what have been considered to be closely related fungi due to ascocarp structure etc., may be really quite different when one considers the presence of or absence of structures

in their ascus apices. That this is true will be proven in the members of the Hemiphacidiaceae included in this study.

IV. GENERALIZED STRUCTURE OF THE ASCUS AND APICAL APPARATUS

In view of the comparisons which must be made later, it is necessary to outline in some detail, the various structures which one can normally expect to find in an ascus.

Chadefaud (1942; 1960) has extensively investigated the apical structure of the asci of many ascomycete genera and this review is primarily based on his investigations and reports. He has shown that the structure of asci conform to a fundamental plan, variations of which may occur between different genera of the Ascomycetes. The principal function of the apical structure is to regulate dehiscence of the ascospores and, if such structures fail to develop, then other mechanisms may be present for this purpose.

An ascus is basically an elongate sac-like structure with a bounding membrane composed of two distinct layers - an outer cuticular wall layer, which is thin, non-extensible and refracting and an inner, often thicker wall layer, which is non-refracting and may or may not be extensible. At the apex of the ascus the external wall differentiates to form an apical cap or "calotte apicale" (Fig. 1) which in operculate discomycetes makes up the principal part of the operculum Underneath this, the internal wall is modified to to form a

thickened apical cushion (Fig. 1) or "coussinet apical" which fills up the tip of the ascus and adheres to the apical cap. This net may be further hollowed out to form a tract-like (?) apical canal or "punctuation apicale" (Fig. 1) which projects into the lower cavity and ends in a little "cul de sac". The internal wall thickens around the apical net to form a periapical collar or "manchon periapical" (Fig. 1) which serves for support and protection.

A thickened apical ring is differentiated and delimits the apical cap. This ring may be divided into a superior and inferior ring (Fig. 1); in some cases fused to form a single structure, or in others reduced to a plate or plug around the base of the apical canal but, according to Chadefaud the outline will always indicate its double nature. Chadefaud states that this structure may be amyloid in a number of genera, for example the genera Bulgaria, Rossellinia and Hypoxylon. He points out that in other asci there are no signs of a plug, either amyloid or non-amyloid, and no specialized structures are present in the apical region of the asci to facilitate spore discharge. Chadefaud suggests such asci may be examples of regressive evolution, a viewpoint with which it is difficult to concur because of the results obtained in this study.

Chadefaud also described four refrigent rods lying between the inner surface of the ascus wall and the cytoplasm filling the sub-apical chamber. These rods adhere to the cytoplasm rather than the ascus wall because they are

not easily separated from the cytoplasm on plamolysis. These rods, together with the epiplasm, constitute the sub-apical framework or "nasse apicale" (Fig. 1) and serve to give rigidity to the whole structure in a manner comparable to the way in which support is given by the whalebones of an umbrella. The sub-apical epiplasm ends in a toothlike structure which projects into the apical tract and encircles a circular pad, "bourrelet" (Fig. 1), found between the apical ring and the ascus wall. It must be realized that the above description represents a generalized ascus tip and that all the structures described are rarely found in one taxonomic group.

Indeed, Chadefaud seems to suggest that the more primitive the species, the greater the number of component parts which are not represented in its ascus apex, that is, its ascus has not yet evolved and developed such structures. An alternative explanation is, however, that the more primitive forms would contain all or most of the component parts represented in the generalized ascus apex and those fungi in which various parts of the ascus structure are missing represent more evolved forms.

V. ASCOSPORE DISCHARGE

The mechanism of ascospore discharge is dependent on the nature of the ascus, i.e. whether it is bitunicate or unitunicate and on the apical structures present in the unitunicate

ascus.

In the bitunicate asci, Chadefaud (1942; 1960) suggests there is a much reduced apical structure in contrast to that which is found in the unitunicate asci (whether a bitunicate ascus does indeed possess any apical structure is unclear, none has been observed in several loculoascomycetous fungi examined) but if such structures are present, their role in ascus dehiscence is unclear. As has been noted earlier, in such asci the external wall separates from the internal wall, ruptures variously, and remains as a frill about the expanding inner wall through the apex of which the spores are ejected.

In the inoperculate, unitunicate asci which possess an amyloid plug at their apices Chadefaud (1942) states that the "calotte apicale" thins out and eventually ruptures to expose the "coussinet apical". The inferior amyloid apical ring which contains nutritive reserves degenerates owing to reabsorption, then the superior ring forms a collarette or sphincter and the apical tract is transformed into a dehiscence canal. The superior ring then remains as a "doughnut-like" structure through which the dehiscence canal passes, and because of the apparent ability of the sphincter-like collarette to expand and contract it has a specific function in spore discharge. Spores would be forced into the expanding sphincter from below by the turgor pressure of the ascus and once the widest part of the spore passed through the sphincter, the contracting sphincter would squirt the

spore from the ascus apex. Spores being ejected from asci of this type would then be ejected singly and in succession.

In asci which are considered to be inoperculate but in which an apical plug cannot be demonstrated (it must be clear this does not mean asci in which there is a non-amyloid plug), the mechanism of spore discharge must be significantly different. This will be shown to be the case in at least one of the fungi studied during this investigation, Rhabdocline pseudotsugae, in which it has been shown all eight ascospores are discharged simultaneously.

VI. MATERIALS AND METHODS

The details of the specimens used in this study are given in Table I.

In order to observe the structure of the fruiting bodies, portions of infected needles bearing whole apothecia were cut out and placed in distilled water until the needle portions sank. These needle-portions were then placed overnight in a 1:1 (V/V) solution of Carter's mucilage and distilled water. They were then mounted and cut at a thickness of 5 or 10 μ on a freezing microtome and the sections so obtained were floated in water, in a watch glass, to remove any remaining mucilage. The sections were then mounted in either lacto-fuchsin mounting medium (Carmichael, 1955) or lactophenol cotton blue.

Preliminary preparation of asci in order to determine

TABLE I

Name and origin of the specimens examined

Name	Herbarium Designation[1]	Geographic Origin
Rhabdocline weirii sp. nov. Parker & Reid, in ed.	DAVFP 482; 4697; 17826; and 17834	British Columbia
Sarcotrochila balsameae (Davis) Korf	TRTC 33,607; 1,430; and 34,489	Ontario
Sarcotrochila piniperda (Rehm) Korf	MFB 6075; TFDS 81,996 (BPI)	Ontario Vermont
Rhabdocline pseudotsugae Syd.	DAVFP 17,591; 17,598; 17,798; and 17,822	British Columbia
Didymascella thujina (Durand) Maire	Herb. J. Reid, 3 collections	British Columbia
Naemacyclus niveus (Pers.) Sacc.	MFB 6470 Herb. J. Reid, 2 collections	Ontario New York
Fabrella tsugae (Farlow) Kirchstein	Herb. J. Reid, 4 collections	Two Ontario, one New York, and one Pennsylvania
Hemiphacidium convexum (Dearn.) Korf	Dearn. 5828 (Hedgcock 24,388) DAOM and (Hedgcock 43,027) DAOM Lectotype and paratype	U.S.A.
Hemiphacidium planum (Davis) Korf	Isotype TRTC, Herb. J. Reid	Wisconsin

[1] Where applicable, herbarium designations are those of Lanjouw and Stafleu (1964).

the reactivity of the ascus pores in iodine, or their structure, was as follows. Apothecia were dissected from the leaves on which they had formed with the aid of a fine scalpel and a binocular dissecting microscope. The apothecia were then placed in 5% potassium hydroxide. Such treatment was necessary in view of the statement by Erickson (1966) that what appeared to be non-amyloid ascus apices would show the amyloid reaction if the asci were treated with KOH prior to the addition of iodine to the mount.

Following this initial treatment the apothecia were treated, as follows, according to the type of microscopy to be employed.

A) Light Microscopy

In order to demonstrate the structure and iodine reaction of the ascus pores, the apothecia treated as above were mounted in Melzer's Reagent (1.5 g potassium iodide, 0.5 g iodine, 20.0 g chloral hydrate, all dissolved in 20.0 ml water) or various ink preparations.

The importance of Melzer's Reagent as an ascus stain has been discussed earlier while Shoemaker (1964) has suggested that at least some typesof commercial ink are valuable as stains in visualizing ascus apex structures. Ink under different brand names and different types of ink were compared, including Carter's Midnight Blue, Sheaffer Skrip Blue-Black Permanent, Sheaffer Jet Black Permanent Writing

Fluid, and Permanent India Ink. The Blue and Blue-Black inks stained the apical structures but as the stain faded rapidly before photomicrographs could be taken, their use was discontinued. Jet Black permanent writing fluid and India Ink proved unsuitable because of the presence of small colloidal particles which apparently prevented staining of the plug.

A combination of Blue-Black ink and Melzer's Reagent proved to be the most suitable for light microscopic observation. Here the potassium hydroxide-treated apothecia were crushed in a drop of Melzer's Reagent on a slide and covered with a cover slip. Distilled water was then drawn under the cover slip until all of the Melzer's Reagent was removed. Finally a drop of Blue-Black ink was placed at the edge of the cover slip and drawn through until the background was uniform.

As a result of such treatment it was found that a blue-stained amyloid plug would retain its colour for a number of hours. Furthermore, if no plug were present its absence could be clearly noted with no possibility of confusing a lack of iodine reactivity with plug absence.

Observations were made, and photomicrographs taken, with a Zeiss Standard Universal microscope fitted with complan eyepieces and neofluar objectives, and a 35 mm camera.

B) Nomarski Interference Contrast Microscopy

Apothecia were thoroughly soaked in distilled water, then crushed in a drop of distilled water on a slide, and covered with a coverslip.

Observations were made and photomicro-graphs taken with a polarizing filter, an achromatic aplanatic phase - contrast and Inco condenser mounted on a rotating stage, and an interfence contrast analyzing slide. Complan eyepieces, neofluar phase objectives, and a 35 mm camera were also employed.

In making observations of this type it is necessary to rotate the object under observation through 360^{o} with observations being made at 90^{o} intervals to determine the point at which maximum contrast is obtained.

C) Ultra-Violet Light Microscopy

Apothecia previously soaked in 5% KOH were thoroughly rinsed in distilled water, then crushed in a drop of 13:1 (V/V) glycerine-formaldehyde mixture (refractive index 1.452) on a quartz glass slide. The preparation was then covered with a quartz glass coverslip and mounted on the microscope for observation.

The microscope was fitted with a monochromatic ($2537A^{o}$) glycerine immersion objective, x81, an immersion condenser, and a quartz resonance lamp. Focusing was facilitated by a U.V. to visible image-converter tube, RCA model 7404.

Photographs were taken employing Ilford IFF film with an exposure time of five seconds. The films were developed with Microphen solution for five minutes at 20°C, washed in running water for twenty minutes and fixed in Edwal Quick Fix.

D) Electron Microscopy

Ascocarps, together with a portion of the leaf tissue, were cut into portions about one millimeter square and these were then placed in water, in McCartney bottles, under vacuum until all the air was removed from the fruiting bodies. The apothecia and adhering needle tissue were then fixed according to a modification of the method described by Hess (1966). The water was removed from the bottles containing the apothecia and replaced immediately by a fixative consisting of a 1:1 mixture of 3% acrolein and 3% glutaraldehyde and the material was then left under vacuum for 1 - 2 hours. The acrolein - glutaraldehyde mixture was then removed, the apothecia washed with 0.2M cacodylate buffer with a pH of about 7.4 (4 changes, 15 minutes each) and then placed in a 1% osmium tetroxide solution. The bottles containing the apothecia were then placed in an ice bath on a variable speed rotator for two hours. The apothecia were washed again with the cacodylate buffer and placed in 0.5% uranyl acetate in the cold for one day. They were then washed thoroughly in distilled water and dehydrated

in graded concentrations of ethanol (15 minutes each) and finally in two changes of absolute alcohol (30 minutes each).

The apothecia were then treated with four changes of a mixture of x-linked methacrylate, 5% divinyl benzene, and 0.8% benzoyl peroxide, prepared at least twenty-four hours prior to use, for two hours.

Individual apothecia were then placed in a gelatin capsule filled with the methacrylate mixture, then the capsule closed and placed in a hardening oven, for one to two days, at 50°C.

Sections were cut on LKB Ultrotome Type 4801A, using glass knives. The optimum thickness for the sections was 350-400A°, as indicated by the pale-gold colour of the sections under reflected light. A small amount of xylene was passed over the sections in order to stretch them, and they were then mounted on carbon-coated grids, 150-200 mesh.

It was occasionally necessary to stain some sections in order to obtain adequate contrast. For this purpose a lead stain in the form of lead citrate was used. Lead stains have a high density and a great affinity for many cellular structures, making them particularly suitable as stains. Alkaline lead citrate offers the further advantage in that it does not form precipitates on the sections on the grids. The grids with the mounted sections were placed section-side down on a drop of the stain in a wax-coated petri dish for five to ten minutes. They were then removed, dipped into a weak solution of sodium hydroxide for a few seconds, rinsed in distilled water and then dried.

Electron photomicrographs were taken on a Phillips EM, model 75B, at 60 KV, with an exposure time of two seconds using fine grain positive film. Films were developed in Microphen solution for five minutes at 20°C, washed in running water for twenty minutes and fixed in Edwal Quick Fix.

VII. RESULTS

As mentioned earlier, Korf was in doubt as to the true reaction of the ascus apices of *Rhabdocline pseudotsugae* in Melzer's Reagent. However, he decided that they should be considered non-reactive and therefore assigned this, to him, monotypic genus to the *Hemiphacideae*.

This investigation, and concurrent studies by Parker and Reid (in press) have clearly shown that this genus does not consist of a single species but of two species and several sub-species based on reactivity of the ascus apices in Melzer's Reagent. In this study, however, it has been further shown that the failure of *R. pseudotsugae* ascus apices to blue in Melzer's is due to a vastly different apical structure than that found in the *Rhabdocline* species (*R. weirii* sp. nov., ined.) which is iodine positive.

Because of this, it is necessary to describe the results obtained for the genus *Rhabdocline* under both the *Hemiphacidieae* and the *Sarcotrochileae*.

1) The Genera and Species with Iodine Reactive Ascus Apices (Sarcotrochileae sensu Korf.)

A) Rhabdocline weirii sp. nov., ined.

The ascus wall of R. weirii (Fig. 9) is clearly composed of two layers, a thin electron dense outer layer and a much thicker, less electron dense inner layer (Fig. 9). The outer layer appears to be of uniform thickness and is continued over the ascus apex. Electron photomicrographs show that this outer layer is slightly thickened, in the area immediately over the apical ring, although this is not apparent on an examination of photographs taken as a result of other types of microscopy (Fig. 4). This apparent thickening may have resulted from the action of the chemicals used in fixation and treatment in preparation for electron microscopy. The inner layer varies considerably in thickness and, at the apical end of the ascus, it is interrupted by other structures in the ascus apex which will be described in the following paragraphs.

The apical ring seems to develop within, or be attached to, this inner layer and appears as a hollow cone-shaped structure projecting inward into the ascal cytoplasm from which it is separated by the cytoplasmic membrane (Fig. 4,5). This apical ring does not seem to be divided into a superior and inferior ring, as suggested by Chadefaud (1942; 1960) (see Fig. 4,5), a feature which according to Chadefaud is supposed to be typical of asci of members of the Helotiales. Instead, it is a single entity enclosed between the inner

wall and the cytoplasmic contents of the ascus.

The crown of the ascus within which the annular ring is contained, is a circular collar or ridge of material which projects down into the lumen of the ascus, and this completely encloses the hollow cone-shaped apical ring (Fig. 4). Within this crown or ridge the apical ring, which appears as a solid blue mass in Melzer's Reagent, is not uniform in structure since in electron and ultra violet photomicrographs, it seems to be composed of smaller, somewhat discrete masses which are packed together and, perhaps, are radially arranged (Fig. 9). This is the material which is presumably responsible for the blue pore reaction in Melzer's Reagent.

There is no clear indication of an inferior annulus but in ultra-violet photomicrographs there appears to be a ring-shaped structure which is positioned below the apical ring (Fig. 8). The presence of this structure may, perhaps, be explained in one of three ways: a) it may indeed be a very reduced inferior apical ring, since it is in the position where such a structure is located when it is present b) it may represent the base or ending of the apical ring where it is appressed to the cytoplasmic membrane of the ascus cytoplasm c) it may be the thickened lining of the apical cushion.

Which of these, if any, is the correct explanation could not be decided due to the very small size of the structure involved.

The apical canal appears to be lined by a continuation of the inner wall which extends around the base of the apical ring and projects up into the canal (Fig. 4). It

does not extend right to the apex in the canal and there is a suggestion that a space exists between the apex of the apical ring and the inner surface of the outer wall. In this fungus there is no differentiated layer which may be interpreted as an operculum and the outer layer of the ascus wall appears to function as the enclosing membrane over the apical canal. In mature asci, a small refractive apical spot is visible in this overlying layer (Fig. 8), and it may be from this point that the outer layer tears to expose the apical canal instead of its being pushed off to one side as a small flap or pseudo-operculum as occurs in the Hemiphacidieae. There is no firm suggestion of the presence of an apical cushion, although this might be present in greatly reduced form, as evidenced by the slightly thickened area at the upper outside flanks of the apical ring in Melzer's stained asci, or the thickened portions evidenced in electron photomicrographs; this however is continuous with the ascus wall and not distinct from it. This thickened area could also be the periapical thickening or "manchon periapical". The sub-apical epiplasm projects up into the apical tract forming a slight "mucron" or projection and this is surrounded by the apical ring.

B) Sarcotrochila balsameae

The asci of this fungus are almost identical in structure to those of R. weirii. There is present a thin, dark-staining, outer layer which adheres to the less electron dense inner layer, and they both continue over the apical

portion of the ascus.

In these asci there is a structure similar to that described by Chadefaud as the apical cap (calotte apicale) of the Helotiales (Fig. 2) and the thin outer layer continues over this cap. Below this, necessarily so because of the bulging apical cap, is what may be interpreted as an apical cushion (Fig. 2). However, there is no differentiation which would suggest a manubrium below this apical net. In one photomicrograph (Fig. 13) there is a slightly thickened area below the apical cushion which may represent the thickened lining of the apical cushion, an inferior apical ring reduced to a small lining, a reduced manubrium, or the remains of a structure called the "corps ombilique" and apical tract. Figure 13 represents a young ascus and here it is evident that there is a triangular-shaped structure in the position of the "corps ombilique" and apical tract. It is evident that there are differences between mature and immature asci, and this may result from the fact that as an ascus matures, structures like the "corps ombilique" may be obliterated, reabsorbed or reduced.

Around the apical cushion, in the position of the apical ring, are two areas of non-uniform thickness which, as the photographs indicate (Figs. 10 and 11) blue in Melzer's Reagent. Like the apical ring of R. weirii this structure also takes the form of a hollow cone-shaped mass and the electron photomicrographs show it also is not solid

but is composed of small discrete masses (Figs. 14,15).

A "mucron" or projection from the cytoplasm of the ascus projects into the apical canal as in R. weirii but there is no evidence of an apical dome in this ascus.

(C) Sarcotrochila piniperda

The structure of the ascus apices of this fungus is similar to that found in R. weirii and S. balsameae. The ascus wall has a thin electron dense outer layer which extends over the apical end of the ascus, while its much thicker, less dense inner layer is thinned out at the ascus apex (Fig.20). As a consequence neither an apical cushion nor a manubrium may be assumed to be present, but it is possible that one or the other of these structures may be present in a greatly reduced form.

Iodine stained asci of this fungus exert an apical ring whose colour is much less intense than that obtained in comparable asci of either R. weirii and S. balsameae. This blue area is also thinner and only at its apical end where it is joined to the inner layer of the ascus wall, does it appear a very deep blue. Electron photomicrographs show this area is smaller and less well defined than is the case in either R. weirii and S. balsameae (Fig. 19). In comparison in R. weirii, the apical ring as seen in electron photomicrographs is a discrete mass, and seems to be composed of a material similar to that of which the inner wall is composed (Fig.20).

The species of the Sarcotrochileae which have been investigated and reported on in this study show no evidence of the presence of the "nasse apicale" or sub-apical framework as described by Chadefaud (Fig. 1). As can be seen from this figure it should be in evidence as four birefringent rods located close to, or adhering to, the cytoplasmic membrane of the ascal cytoplasm. However it is quite clear that, based on ascal apex structure, these fungi must be considered to be very closely related. The apical structures of their asci are so similar that an inexperienced observer probably would not be able to distinguish them one from another. There is no differentiated layer which could be interpreted as an operculum (Fig. 1) but the overlying outer layer of the ascus wall appears to serve as the enclosing membrane of the ascus apex and, when it ruptures, it does so by an irregular tearing from what may be a preformed point of dehiscence, but the torn portion is never pushed off to one side as an intact flap in the manner of an operculum.

There is no evidence of an inferior apical ring and the apical ring which is present in this group seems to correspond to the superior apical ring (Fig. 1) or, possibly, it may represent a fusion of superior and inferior apical rings (Fig. 2) in which the fusion, and a reduction in size, make it impossible to distinguish between these two separate components of the apical ring.

The differences between the ascospores and the apothe-

cial structure of these three species are such as to prevent confusion as to their separate identities. The spores of R. weirii are eventually two-celled, with at least one cell turning brown prior to germination, while the ascospores of S. balsameae and S. piniperda are one-celled and hyaline at physiological maturity but of distinctive shapes. In both these latter two species however, the spores become 1-2-septate, and brown-celled with age but these species still could not be confused with R. weirii or with each other.

The apothecial structure of R. weirii is quite distinct from that of either S. balsameae and S. piniperda but, again, with the latter two species the similarities are quite pronouced it requires some familiarity with the organisms to recognize the subtle differences.

The mechanisms of ascospore discharge in all three species discussed above conforms to the pattern described in section V of this thesis, for asci which are inoperculate, unitunicate and possess an apical plug at their apices. Thus the fungi assigned to the Sarcotrochileae eject their ascospores from the asci singly and in succession.

2) The Genera and Species with Iodine Non-reactive Ascus Apices (Hemiphacidieae sensu Korf)

Based on the structure of their apothecia and the nature of their ascal apices, the genera of this tribe do not constitute a homogeneous group as suggested by Korf.

As will be shown later, there is a considerable variation in the amount and type of tissue which forms the apothecia in the various species studied. Furthermore, in contrast to those species assigned to the Sarcotrochileae, the members of the Hemiphacidieae, with one exception, do not have any apical structures produced in either the inner or outer layers of the ascal wall. There are structures present at the apical end of the ascus, but they are located just within the cytoplasmic membrane of the ascus and the nature of these structures varies from species to species.

A) Rhabdocline pseudotsugae

This fungus differs markedly in ascal apex structure when compared to the organism described as R. weirii.

The outer layer of the ascal wall of R. pseudotsugae appears, in electron photomicrographs, to be extremely thin, consisting of but a single layer (Fig. 30). Where this layer extends over the ascus apex it becomes slightly thickened and this area usually stains quite intensely with the lead stains (Fig. 30). The inner layer of the ascal wall is, in contrast, a massive, thickened layer which is not homogeneous. This layer continues to the apex of the ascus where it thins

out to approximately 1/3 of its width along the lateral walls of the ascus (Fig.30). The electron photomicrographs suggest that this layer is interrupted at the apex (Fig.30) and if this interpretation is correct, then this would be the region where one would expect to find a very reduced "cous-sinet" or apical cushion. However, there is no evidence of this in photographs obtained as a result of light or interference microscopy but these photographs do suggest the presence of a small refractive spot in the outer wall in that region (Figs.28,29). This spot may represent a pore or a point of weakness from which the ascus walls rupture during ascospore ejection. The ascal cytoplasm is directly below this point and no further differentiation of apical structures can be discerned.

Chadefaud has stated that in some asci in which the apical structures are very reduced, one may observe a "nasse apicale". Electron photomicrographs of the asci of this fungus do not reveal the presence of such a structure (Fig. 30) but photographs obtained by interference microscopy (Fig. 28) reveal a slight dimpling in and coalescence in a small area to one side of the ascus. This could represent a modified "nasse apicale".

The apical ascal structures in this fungus are greatly reduced in comparison to those found in R. weirii.

B) Didymascella thujina

This species differs from the others investigated during this study in that there are only two ascospores found in the mature ascus instead of the eight normally found in the asci of the other species.

The outer layer of the ascal wall, in electron photomicrographs, is very thin and quite electron dense, i.e. it appears dark and homogenous (Fig. 40). In contrast, the inner layer of the ascal wall is much thicker, at least laterally, but towards the ascus apex it becomes thinner, about 1/3 of its lateral thickness, and it continues at this thickness over the apex of the ascus (Fig.40). In some mounts, there appears to be an apical invagination on either side of the ascus tip (Fig. 40), but whether this invagination truly exists, or is simply an artifact induced during fixation, could not be completely resolved. It was seen in a number of mounts, but not in others but other types of microscopy (see below) suggest it is not an artifact.

Ultraviolet and interference contrast microscopy reveal the presence of a dark, thickened area in the position of these "flaps" or invaginations noted above in the electron photomicrographs, further support for the suggestion that some structural difference exists in these areas (Figs. 38 and 39), the details of which, however, remained obscure.

There was a suggestion that a small pore exists in

the outer layer of the ascal wall and that a canal passed from the inner face of the inner ascal layer and connected to this pore (Fig. 39). The area on the outer wall layer where this pore is present is well defined during light and interference contrast observation.

Although electron photomicrographs show no general thickening of the outer layer of the ascal wall at the apex a slight localized, thickening may represent a reduced apical cap (Fig. 41).

Within the ascal cytoplasm, a group of structures of various shapes are seen about midway between the uppermost ascospore and the anterior end of the sac (Fig. 41). These may function as support structures for the end of the ascus or, they may represent nothing more than the remains of one or more of the six degenerate ascospores which routinely fail to mature in this species.

C) Naemacyclus niveus

The ascospores in this species are elongate and needle-shaped and, as such, stand in sharp contrast to the more ovoid ascospores found in other species of this family.

The outer layer of the ascal wall appears in the electron photomicrographs as a dense, dark layer, extending over the apex of the ascus, surmounted by a detachable "coiffe" or cap similar to that described by Chadefaud (1942) (Fig. 2). This cap appears to extend over the outside of the outer layer of the ascal wall, just at the apex, and while it is very easily detached it may have some protective function.

The inner layer of the ascal wall is much thicker, of less electron dense material, but here again this layer, at the apex of the ascus, thins out to about 1/3 of its lateral width. There is no real evidence of a passage or canal running through the inner layer to the outer layer but structures are present, although in this species they are located either between the inner surface of the inner layer of the ascus wall and the bounding layer of the ascal cytoplasm or just within the ascal cytoplasm.

A non-amyloid apical ring is present in this region and appears to be formed directly on, or in, a thickened apical cushion (Fig.36). This apical ring has a darkened area on its upper surface and this probably represents a reduced superior apical ring while the lower or inferior apical ring is of normal size.

In immature asci, a broad thickened cap consisting of an electron dense material is found over the anterior part of the membrane which encloses the ascal cytoplasm (Figs. 35,37). This may be the apical cushion surrounding the developing apical ring and this apical cushion is reabsorbed as the ascus matures. When the ascus is mature the remains of the apical cushion appear as strand-like fibres extending to the inner layer of the ascal wall.

Chadefaud (1942) in describing the structure of the ascus of Microglossum lutescens Boudier reported the presence of a sub-apical globule of indeterminate nature. It appeared to be hollow and stained darkly with iodine. Electron photo-

micrographs of asci of N. niveus showed a similar structure (Fig. 36), although here it did not stain darkly with iodine.

Photomicrographs taken with the aid of the ultraviolet microscope clearly revealed the presence of a thickened cap which probably corresponds to the "nasse apicale" (Fig. 1). Its presence was confirmed in electron photomicrographs (Fig.36).

D) Fabrella tsugae

The outer layer of the ascal wall is thin, rather electron dense, and continues over the top of the ascus without interruption (Fig. 44). The inner layer of the ascal wall is thicker, less electron dense, and it, too, continues over the ascus apex.

A number of photomicrographs were made, but there was no evidence of apical structures in the asci.

Since the other types of microscopy employed did not reveal the presence of structures in the ascus apex, one must conclude that this ascus represents a very reduced type.

E) Hemiphacidium convexum

The ascal apices of this fungus are amongst the simplest of all those studied.

The ascus wall is two layered (Figs. 48 and 49) with the outer layer being thinner and denser than the inner layer. The inner layer is uniform in thickness both laterally and at the apex of the ascus (Fig. 49) but regardless of the number of preparations made, no structures of any type could be seen in the ascus apex.

This suggests that spore discharge is accomplished simply by bursting of the ascal wall due to increased osmotic

pressure within the ascus and not with the aid of any special structures.

F) Hemiphacidium planum

A study of the ascal apex of this fungus shows no specialized apical structures. The outer layer of the ascal wall as seen in electron photomicrographs appears to be extremely thin and continues over the apex without any apparent thickening. The inner layer of the ascal wall is in contrast a more massive layer, which is composed of a greyish electron dense substance. This layer continues over the ascus apex, where it widens slightly in comparison to its size along the lateral walls of the ascus. The cytoplasm at the anterior end of the ascus appears to form a network which projects down between the elongate ascospores. The use or relationship of this network of cytoplasm is not evident and might only represent an artifact resulting from methods of preparation for electron microscopy.

As fresh material could not be obtained for all the species studied in this section, it is only possible to report, with certainty, on the spore discharge mechanism in R. pseudotsugae and D. thujina.

In these two species the ascus apex, where it thins out, bursts in an irregular manner. It may tear in a bilabiate or irregular fashion or resemble an operculum of an operculate discomycete.

Whatever the manner of splitting, however, the result

is that the end of the ascus is widely torn and all ascospores are discharged from an ascus at once. There is nothing resembling the pumping mechanism found in R. weirii and its allies and thus the mechanism of spore discharge appears to be quite different in the two tribes of this family.

It seems likely that all genera of the Hemiphacidieae, with the exception of Naemacyclus, discharge their spores in this fashion.

3) Apothecial Structure

Korf (1962) stated that the genera which he included in the family Hemiphacidiaceae were of very simple structure, consisting essentially of a poorly developed basal stratum giving rise to asci and paraphyses with scarcely any marginal excipular structure and, except for Naemacyclus pinastri (Fuckel) Fuckel and Lophophacidium hyperboreum Lagerberg, completely lacking any covering layer of fungous tissue.

As noted earlier, Reid and Cain (1962) have shown that L. hyperboreum is truly a phacidiaceous fungus but Korf's statement implies that the apothecial structure of all other genera in his family is similar.

Detailed examination of numerous apothecial sections indicate that Korf has confused smallness of size with simplicity of structure and, in fact, although the apothecia of these genera are uniformly small, they vary greatly in structure and complexity. This makes it difficult to believe that fungi showing such diverse apothecial structures can be included within the same family.

A) Rhabdocline, Fabrella, Didymascella

The apothecia of these genera, together with those of Hemiphacidium planum which will be discussed more fully later, conform to the concept of simplicity for fungi Korf assigned to his family (Figs. 22, 23 and 27). In these genera the apothecia consist of a layer of asci and paraphyses

arising from a poorly developed hypothecium or subiculum. If excipular tissue does develop it is very rudimentary and is made up of nothing more than a marginal band of paraphyses which are essentially hyaline but may, in some cases, be very pale-brown in colour. There is never any fungal tissue present above the developing hymenium which is protected prior to exposure only by the overlying host tissue.

There is some suggestion that either the apices of the paraphyses are coated with a fine mucilaginous sheath or that the paraphyses and the asci are both embedded in a mucilaginous matrix, however this point cannot be resolved satisfactorily.

B) Naemacyclus niveus

Korf only doubtfully referred this species to the Hemiphacidiaceae, with the key point being that there is a very thin layer of hyaline hyphae beneath the host epidermis and above the hymenium.

Darker (1932) stated that ascocarps of this fungus develop beneath the hypodermis of the leaf and the overlying host tissue which consists of epidermis and hypodermis bursts along stomatal lines. The two halves of the covering layer so formed then become widely opened so that the hymenium is exposed due to a central fissue which forms in the overlying host tissue and remains as two flaps flanking the hymenium. An interesting point in Darker's description

is a statement referring to the fungal tissue overlying the hymenium: "covering layer of colorless pseudoparenchyma 12-18 μ thick in centre, towards margins consists of short loose threads projecting down from hypodermis".

This statement clearly suggests that in the developing apothecia there is a differentiated layer of fungous tissue above the developing hymenium, a layer which does not consist simply of a layer of hyaline hyphae as Korf suggested.

The apothecial sections examined during the course of this study show the presence of the pseudoparenchymal tissue to which Darker refers (Fig. 31), but this tissue is not always made up simply of hyaline cells. In some of the sections studied, pigmentation was present in the cell walls and while this never resulted in the formation of a dark stromatic mass, this tissue varied from pale to dark-brown in color (Fig. 32). At the margins the tissue becomes much thinner, and does seem to consist primarily of hyphae, as Darker indicated (Fig. 32). This finding seems to be analogous to that reported by Reid and Cain (1962) for L. hyperboreum which lead them to believe that this fungus L. hyperboreum, was truly a number of the Phacidiaceae; Korf apparently agreed with this decision (see Korf (1962) addendum added in proof).

The presence of this overlying stroma in the apothecia of N. niveus, even though it is relatively thin and may not be formed in all apothecia, indicates that this fungus is truly

a member of the Phacidiaceae, a family to which it is presently referred by some mycologists, e.g. Dennis (1968).

The confusion as to the true nature of this fungus may have arisen because persons studying this organism may not have examined a sufficient number of sections in order to locate those in which the stroma was present or, perhaps, the techniques used in the preparation of the sections resulted in the breaking off or falling out of this pseudoparenchymal tissue. If this is so, then such workers would only have been aware of the marginal portion of the overlying tissue and, since this is hyphal like, it would explain the reports that the overlying fungal tissue consists simply of a few hyphal elements.

C) Hemiphacidium planum and Hemiphacidium convexum

Korf (1962, pages 26-27) in erecting the genus Hemicidium designated H. planum as the type species. He only doubtfully referred H. convexum to this genus. As Korf pointed out the ascospores in H. convexum are shaped rather like those of Sarcotrochila and are of a different shape than those of H. planum, but he made his decision that H. convexum and H. planum were congeneric primarily because in neither of these two species did the ascus apices turn blue in Melzer's Reagent. He does state that the genus closely resembles Sarcotrochila, and this statement must, presumably, be based primarily on what Korf considered to be

very similar apothecial structures.

Earlier in this thesis it has been shown that in spite of the fact that the ascus apices of these two species do not blue in Melzer's Reagent, they do have a very different ascus apex structure. This suggests that the similarity of reaction in Melzer's Reagent might not be as important as Korf believes.

The results of this study also show that these two species have completely different apothecial structures (Figs. 22 and 45) and neither of them have apothecia whose structure could in any way be considered similar to those of the species of the genus Sarcotrochila studied herein (Fig. 16).

The apothecia of H. planum (Fig. 22) are very simple in structure consisting of nothing more than a feebly developed basal stratum which gives rise to asci and paraphyses. The marginal excipular tissue consists of a few parallel hyphal elements which may, in some cases, be lightly pigmented, but are frequently hyaline and then are very similar to, and indistinguishable from, paraphyses. The apothecia of this species are therefore very similar to those found in the genera Rhabdocline, Fabrella and Didymascella.

In H. convexum the apothecia, while small in size, have a more complex structure than those of H. planum. Here the apothecia are erumpent and subtended and anchored into

the host tissue by a well developed stalk (Fig.45). This stalk consists of both textura globulosa and undifferentiated hyphal elements and the presence of these globose cells recalls the type of structure commonly associated with fungi of the family Dermateaceae. In addition to this, the marginal ectal excipular tissue is strongly developed (Figs. 46 and 47) and does not simply consist of a few paraphyses-like elements. Some of the hyphae which make up this excipular tissue have shortened and thickened cells suggesting the development of a more extensive pseudoparenchyma - like tissue.

The interascal elements are unlike those found in H. planum or in either of the two species of Sarcotrochila examined. In this species, H. convexum, the paraphyses are filiform, septate and hyaline towards their bases but towards their tips they are brown in colour and are either agglutinated due to a mucilaginous substance or they are fused together creating an epithecium-like layer. In this they seem to resemble the paraphyses-like structures found in Korfia tsugae (Cash and Davidson) Reid and Cain (1963). In fact the entire apothecial structure of H. convexum has more similarities with the genus Korfia than any other genus which has been placed in the Hemiphacidiaceae.

D) Sarcotrochila piniperda and S. balsameae

In this genus it is obvious that once again smallness

of size has been confused with simplicity of apothecial structure.

The apothecia of these two species are entirely unlike those of either Rhabdocline or those genera similar in structure to Rhabdocline.

In Sarcotrochila (Fig. 16) the apothecia are strongly erumpent, but lack a well developed basal stalk. The exciplum, however, is well developed with an ectal excipulum consisting of parallel rows of dark walled, septate hyphae, adnate at their bases, but free at their distal ends. There is a tendency for the free ends of the hyphae to turn outwards and this imparts a fine, wooly appearance to the surface of the excipulum. The medullary excipulum is composed of thin-walled, septate, hyaline interwoven hyphal elements which are loosely packed together.

These apothecia have a degree of complexity similar to that found in representatives of a number of families of the Helotiales and are quite distinct in apothecial structure from the other genera of the Hemiphacidiaceae.

DISCUSSION

As was suggested in the introduction, classification systems within the Ascomycetes are not fixed, and cannot be so, until we fully understand the true nature of the various characters which have been used as criteria in classification systems of the past.

The family Hemiphacidiaceae clearly illustrates this problem with respect to two major criteria used in Ascomycete taxonomy: (1) apothecial structure or anatomy and (2) the iodine reaction of the ascus apices.

Apothecial anatomy, ie. the arrangement and types of tissues within the apothecium, is often of great importance in classifying discomycetes. Unfortunately, however, in order to assess the true anatomical structure of the apothecia of any one species one must section a number of apothecia for any single apothecium may lack constituent portions normally found in apothecia of that species.

Korf made such an error when dealing with the genera Lophophacidium and Gremmenia (see Korf (1962) addendum added in proof and Reid and Pirozynski (1968)) and these studies show that with respect to the genera reported on herein he has also confused small size and simplicity.

In this family, the apothecia actually range from a very simple structure with little or no hypothecium and but a small amount of feebly developed marginal excipulum, as exemplified by the genus Rhabdocline, e.g. R. pseudotsugae

(Fig. 27), but also found in Didymascella, Fabrella, and Hemiphacidium planum, to the more well developed, cup-shaped apothecia of the genus Sarcotrochila.

In the latter genus there is a well developed hypothecium and excipulum, clearly separable into their constituent parts, and thus the apothecium of this genus differs markedly from those listed above.

Korf, in his description of the Hemiphacidiaceae, states that genera with well developed apothecia composed of a strongly differentiated ectal excipulum, a distinct covering of stromatic tissue, and an ectal layer composed of dark-brown rather thick-walled, globose cells are invariably lacking in this family. Thus, Hemiphacidium convexum with its well developed though small apothecia subtended by a stalk consisting of comparatively massive tissue containing globose cells and having a strongly differentiated excipulum enclosing the hymenium, must be unrelated to the other genera of the Hemiphacidiaceae. It seems to have its affinities with the family Dermateaceae sensu Nannfeldt (1932), but whether it represents an undescribed genus of that family or should be assigned to some known genus could not be resolved during this study.

Naemacyclus, which Korf doubtfully referred to the Hemiphacidiaceae, has an apothecial structure similar to that of the Phacidiaceae, sensu Nannfeldt (Terrier, 1942). This is so because of the finding of the scale of fungous tissue over the developing hymenium (Figs. 31 and 32). This development of stromatic tissue over the hymenium is contrary to

Korf's concept of his family but is normal in various members of the Phacidiaceae. Thus the genus Naemacyclus, like H. convexum, based on apothecial anatomy, does not belong in the Hemiphacidiaceae.

Thus these studies have shown that the apothecia of the genera comprising this family are separable into several groups, similar in smallness of size, but differing greatly in structure from very simple to quite complex. If this family were a valid taxonomic unit, then one would have expected a far greater similarity in apothecial structure.

When one considers the ascus pore reaction in Melzer's reagent of the various genera studied and the relation of this reaction to actual ascus apex structure, the results obtained support the belief that this family is an artificial one. Furthermore, the implications of some of the findings call into question some traditional taxonomic ideas.

Korf, like many others, regards blueing of ascus apices in Melzer's reagent to be an exceptionally stable character, and this belief was reflected in his separation of this family into the tribes Hemiphacidieae and Sarcotrochileae. The former have asci whose pores do not blue in Melzer's; the latter do.

In the genus Rhabdocline, R. weirii reacts positively while R. pseudotsugae does not and this, if we follow Korfs concept, should prevent them from being placed in the same tribe let alone the same genus. As noted earlier both Müller and Hütter (1963) and Parker and Reid (1969) have drawn

attention to species whose ascus pore reactions vary. In these cases, however, the structure of the ascus apex was apparently constant, the reaction differences being unexplainable, but in the species of Rhabdocline the explanation is quite apparent.

In R. pseudotsugae there is no pore structure at all at the ascus apex and for ascospore discharge to occur, the ascal apex simply bursts, the wall being thinner there, and the spores are ejected due to pressure which has apprently built up within the ascus. It is this pressure which also must burst the apex and, as a result, the ascospores are ejected in a clump, all eight spores at once. Thus it is not a matter of the ascus apex structure not being amyloid, but there is no structure present which could possibly turn blue.

In R. weirii, on the other hand, there is an amyloid apical ring, which turns blue in Melzer's reagent and is perforated by a canal which is closed by the outer layer of the ascal wall. When the ascus is mature, this outer layer breaks, and the apical ring acts as a sphincter forcing the ascospores out singly by means of a pumping action, although in fairly rapid succession.

Now if R. pseudotsugae and R. weirii are retained within the same genus then it is impossible to have two species of one genus placed in two different tribes of the same family as would have to be the case in this family. Thus, this variability of pore structure, and consequently Melzer's reaction, would argue against the validity of tribes within this family.

This difficulty could be overcome, however, if we were to consider that R. weirii and R. pseudotsugae actually

represent two distinct genera, one of which would belong in the tribe Sarcotrochileae, the other in the tribe Hemiphacidieae.

R. weirii is known only in North America and has an imperfect state in its development, while R. pseudotsugae is found in both Europe and North America and lacks an imperfect state. They cause similar diseases on the same host, but at different times of the year and have very similar apothecia and mature ascospores. Thus the main points in arguing for different genera would be the differences in the ascus apex structure and, consequently, differences in method of spore discharge, and the presence or absence of imperfect states. Thus, on the basis of these latter points, one would have to argue, that these two species represent different genera whose similarities are but the result of convergent evolution in relationship with the same host species over a long period of time. This would imply that ascus apex structure is a far more important taxonomic criteria than apothecial anatomy, spore shape, etc., a fact which may be unacceptable to most discomycete taxonomists at the moment and perhaps for all time.

There is, of course, the alternative explanation that one of these two species evolved from the other, probably R. pseudotsugae from R. weirii by loss of its ascus apex structure and imperfect state. This is less likely since R. pseudotsugae would have been less efficient when it lost the conidial state, than R. weirii, and it should not have become as wide spread as it has.

Therefore the genus Rhabdocline poses a problem for taxonomists interested in using pore reactivity as a taxonomic character.

That the structure of the ascus apex and Melzer's reactivity is clearly linked to the method of ascospore discharge, can be seen in the two different methods as noted in these studies. Only R. weirii and the genus Sarcotrochila have Melzer's reactive pores, and in them discharge is as described for R. weirii. In the Melzer's non-reactive species and genera, the ascus apices show a variety of structures but none have an apical ring, or sphincter, and all eject their spores in a clump, at once, as in R. pseudotsugae.

The interesting point here is that traditionally one would have assumed that having stated that this second group were Melzer's (or iodine) non-reactive, there was nothing more of interest and that they automatically had the same ascus apex structure. That such is not the case in the members of the tribe Hemiphacidieae has been clearly shown in this study and if we give the importance to ascus apex structure which Chadefaud (1942; 1960) suggests is warranted, then this supports the contention that the Hemiphacidiaceae is not a natural grouping.

Naemacyclus which was suggested to be a member of the Phacidiales on apothecial anatomy, also stands apart in that its ascus apices are the only one of the Melzer's non-reactive group, which appears to have an inferior apical structure. This is present in the cytoplasm at the ascus apex and seems

similar to that noted in some Phacidiales.

This study has shown that the Hemiphacidiaceae is not a valid taxonomic family and thus does not show the true relationships of the organisms included within it. More interesting, however,are the implications of ascus structure which should be considered by all future discomycete taxonomists in their attempts to set up taxonomic systems which will show the true relationships of this most interesting group of organisms.

SUMMARY

The genera included in the Hemiphacidiaceae (Korf, 1962) were examined with respect to apothecial structure, ascus pore reactivity in Melzer's reagent (an iodine preparation), and ascus apex structure using different preparative techniques and types of microscopy. The types of microscopy employed were standard light, ultraviolet, Nomarski interference contrast, and electron microscopy.

The results show that the apothecia of the taxa placed in this family are not all similar in structure, but that each taxon can be placed in one of the four apothecial types into which, on the basis of anatomy, this family can be divided. They further show that the four apothecial types cannot be considered to fall within the scope of a single family, and that some of these taxa have their affinities with other, well known families.

Pore reactivity and ascus apex structure is not constant in this family and when the results obtained in the study of these two features are correlated with the anatomical studies, they support the belief that the Hemiphacidiaceae is an artificial assemblage of, for the most part, unrelated organisms.

These results also stress the need for further studies on the significance of ascus apex structure in relation to taxonomy and point out that some criteria thought, in the past, to be stable and useful may be of doubtful value in erecting taxonomic systems.

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Plate I.

Fig. 1. Generalised ascus apex structure after Chadefaud (1942).

Fig. 2. Generalised structure of an ascus representative of the Helotiales after Chadefaud (1960).

The following abbreviations are used for Figs. 1 and 2 and all subsequent figures:

a...apical cap..'calotte apicale"

b...apical invagination

c...periapical collar..'manchon periapical'

d...apical cushion..'coussinet apical'

e...superior apical ring

f...double lining of apical cushion and tract

g...inferior apical ring

h...apical framework..'nasse apicale'

i...epiplasm..'cytoplasm'

j...internal wall layer

k...external wall layer

l...apical ring

m...projection..'mucron'

o...apical tract

p...apical dome

s...detachable apical cap

v...ascus wall (unitunicate)

w...refractive spot in the internal wall layer

y...modified apical ring

z...pore in the external wall layer

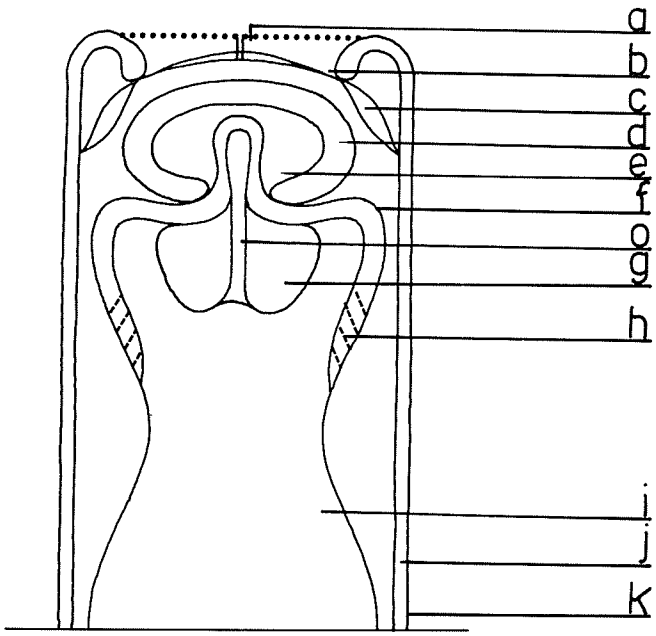


Fig. 1

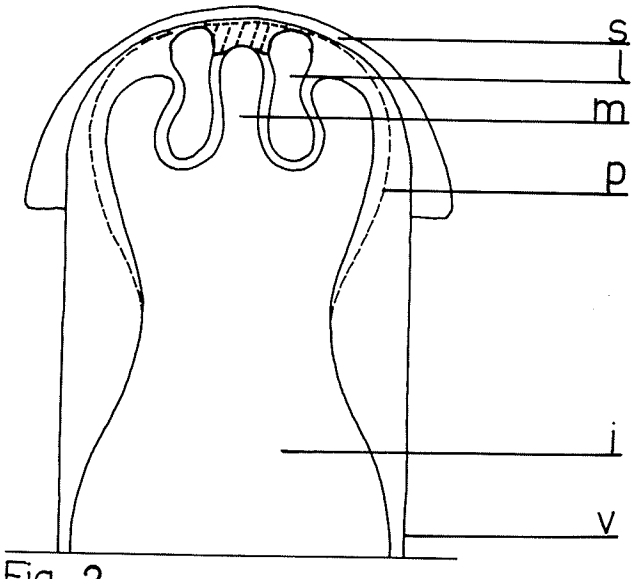


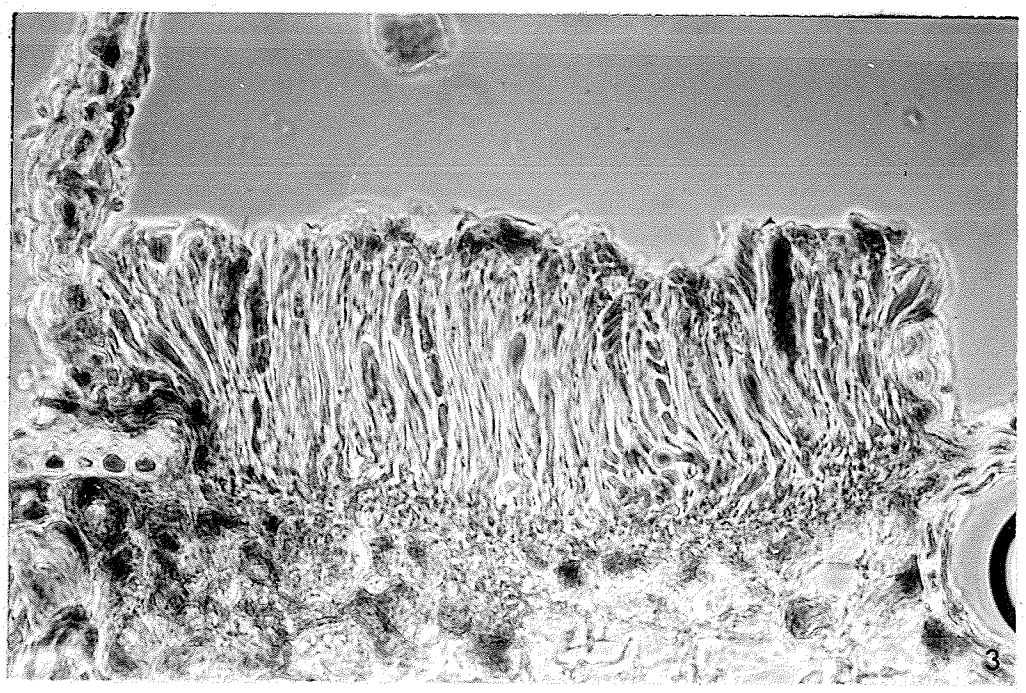
Fig. 2

Plate II

Rhabdocline weirii

Fig. 3 Photomicrograph taken with the light microscope of a cross section of an apothecium. Note the poorly developed basal stratum and marginal excipulum.
Magnification x240

Fig. 4 and 5 Photomicrographs taken with a light microscope of iodine and ink stained asci. Note the presence of the cone-shaped apical ring (l) perforated by a canal.
Magnifications x4,400.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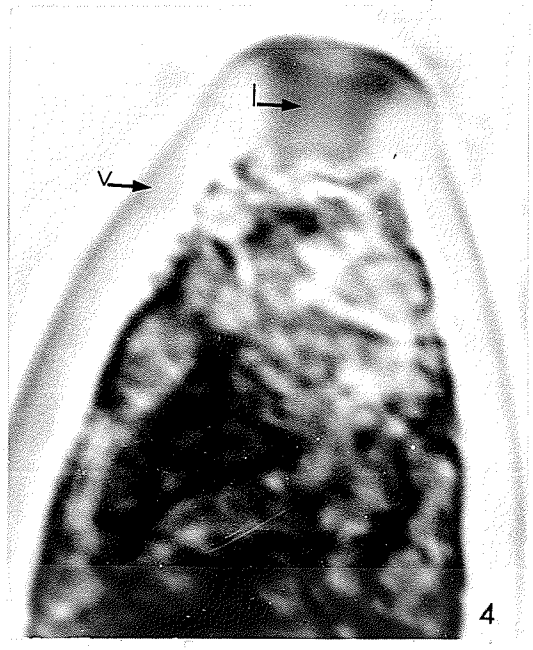
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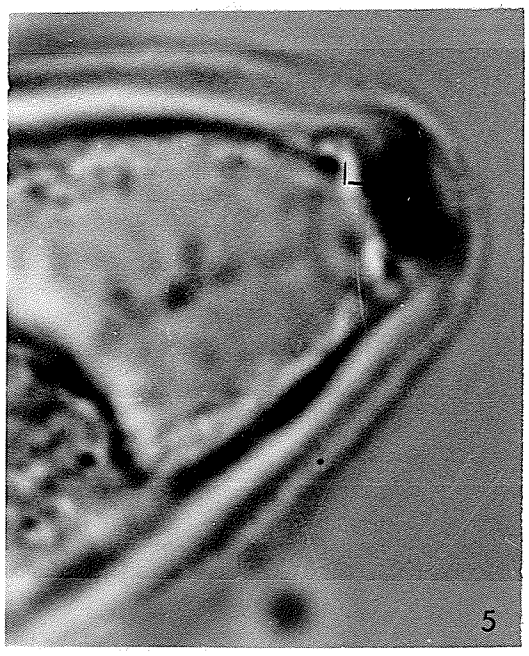
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Plate III

Rhabdocline weirii

Fig. 6 Interference contrast photomicrograph. The apical ring appears as two dark structures on either side of a central canal. Magnification x4,400.

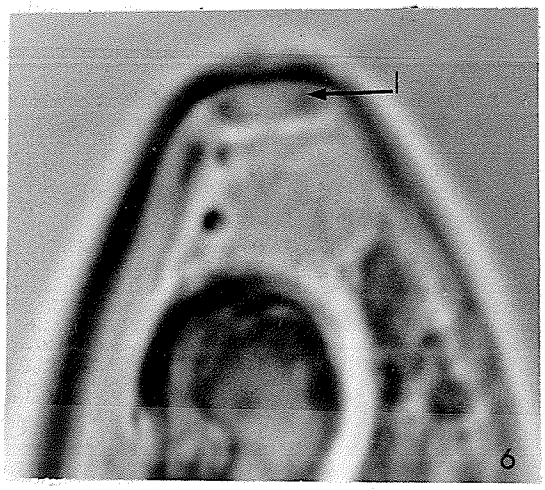
Fig. 7 and 8 Ultra-violet photomicrographs of asci showing the presence of;

(a) the double layered wall at the ascus apex.

(b) the pore in the external wall layer at the ascus apex.

(c) the doughnut-shaped ring at the anterior end of the cytoplasm.

Magnifications x4,400.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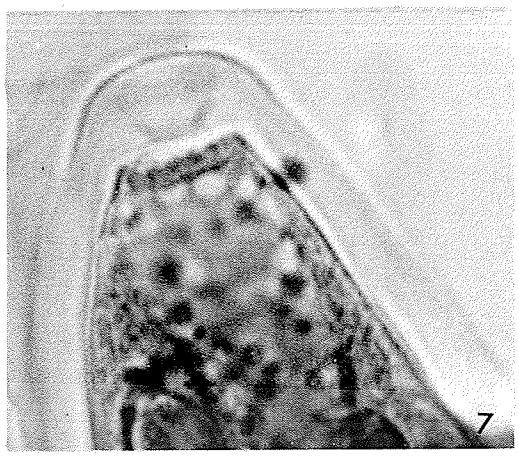
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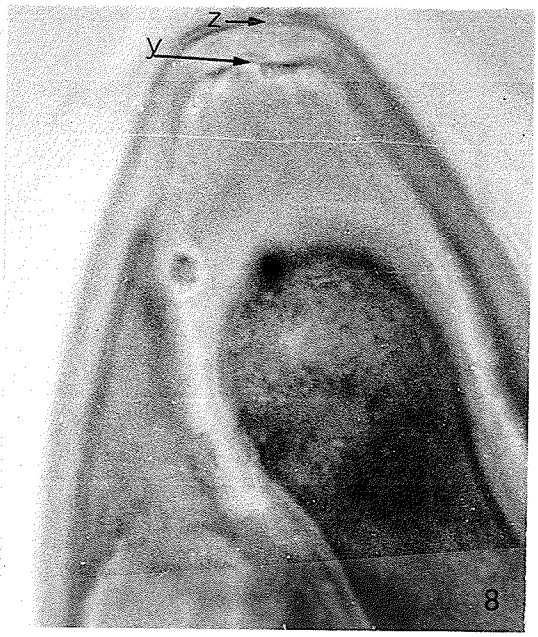
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Plate IV

Rhabdocline weirii

Fig. 9 Electron photomicrograph showing:

(a) the double nature of the ascus wall,

(b) the projection of the cytoplasm, the 'mucron', and

(c) the apical ring composed of small discrete masses closely packed together.

Magnification x8,800

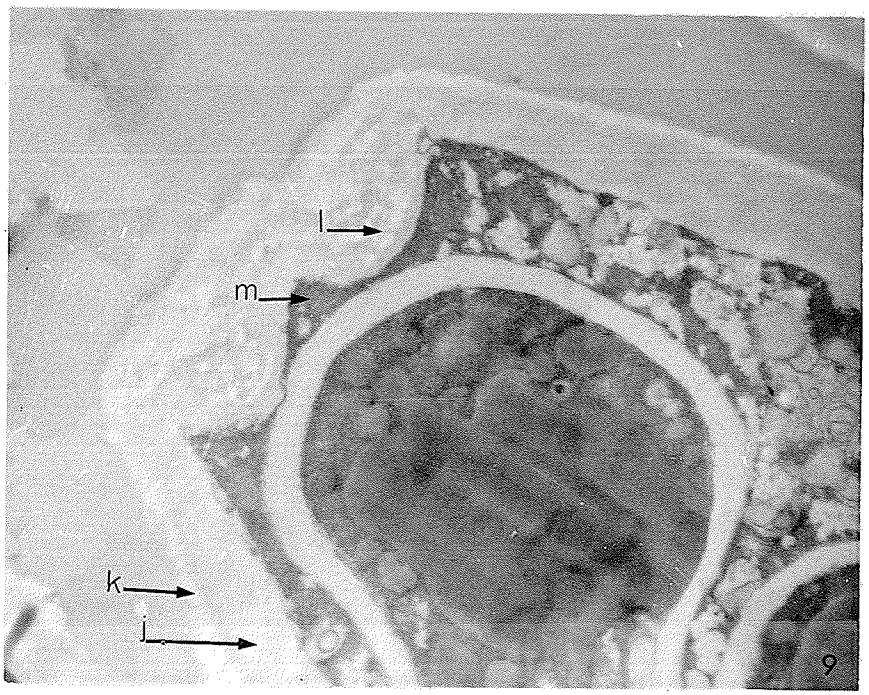
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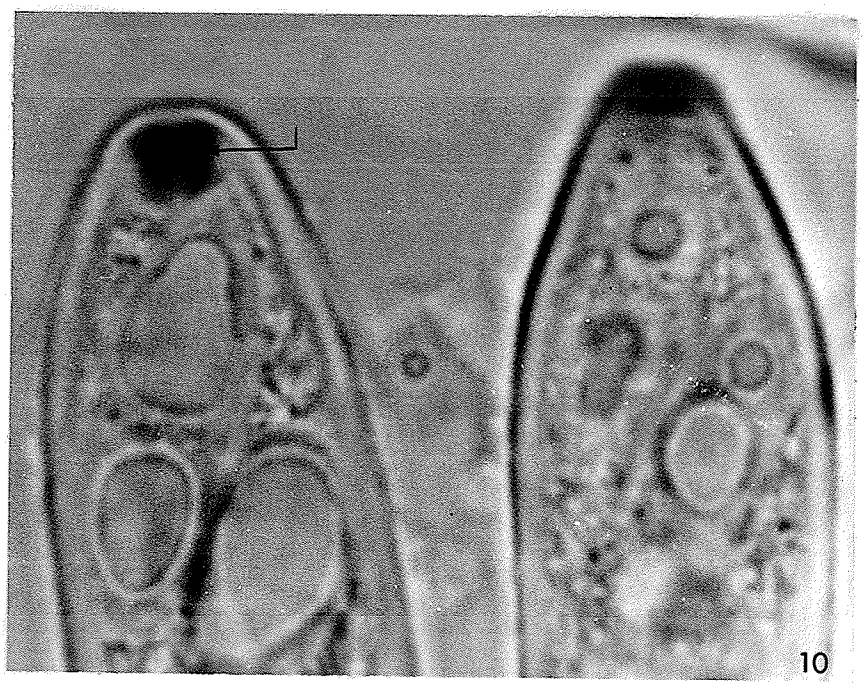
Plate V

Sarcotrochila balsameae

Fig. 10 Light microscope photomicrographs of iodine and ink-stained asci. The apical ring appears as a dark staining body located in the inner layer of the ascal wall at the apex and perforated by a central canal. Magnification x4,000.

Fig. 11 Ultra-violet photomicrograph of an ascus apex. The position of the apical ring appears as two dark areas leading into the cytoplasm. Magnification x4,400.

Fig. 12 Interference contrast photomicrographs of an ascus apex. This type of photography did not reveal any special structures in the ascus apex. Magnification x4,400



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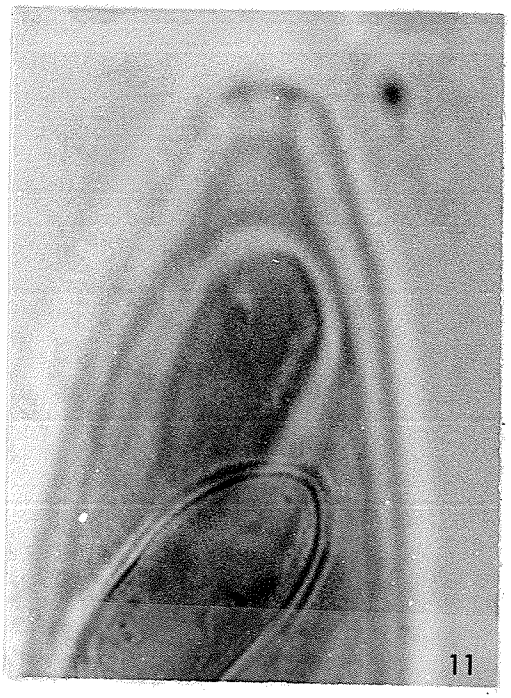

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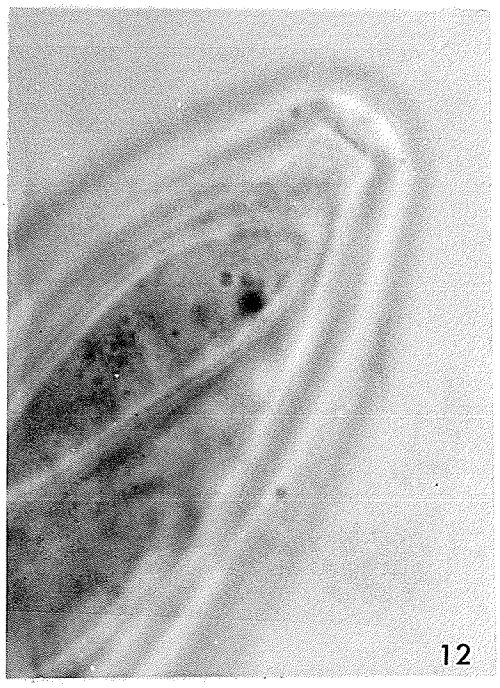

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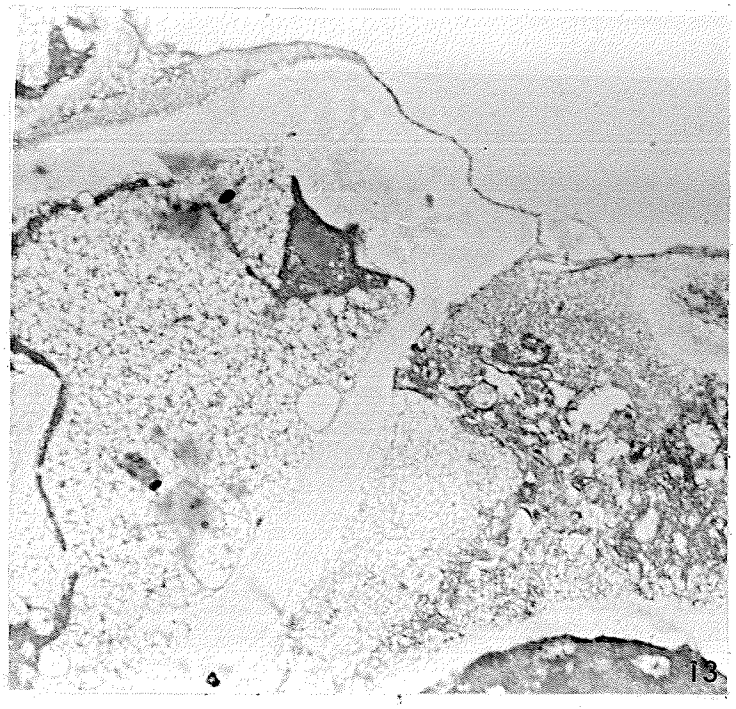
Plate VI

Sarcotrochila balsameae

Fig. 13 Electron photomicrographs of a young ascus. Note the triangular shaped structure in the position of a "corps ombilique".
Magnification x8,800

Fig. 14 Electron photomicrograph of the tip of an ascus. In the position of the apical ring there are two areas of non-uniform thickness, taking the form of a hollow cone shaped mass.
Magnification x8,800.



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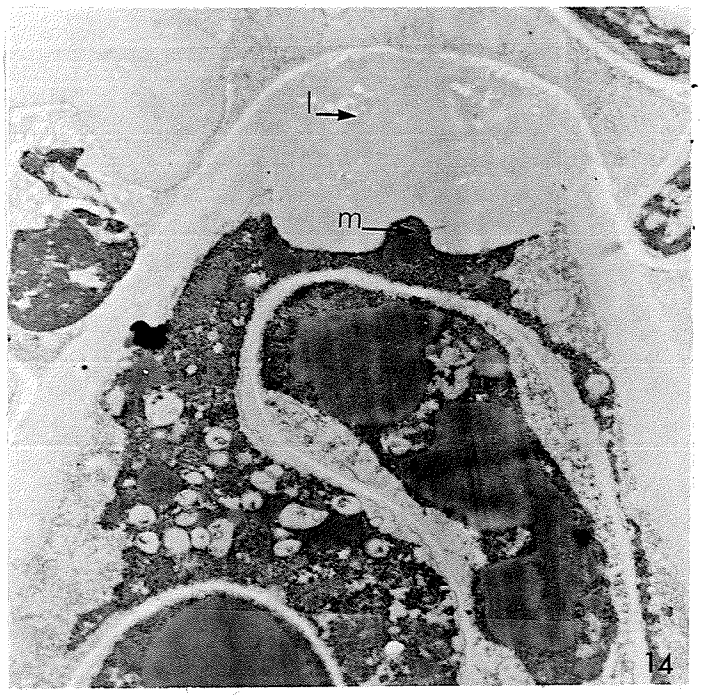

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Plate VII

Sarcotrochila balsameae

Fig. 15 Electron photomicrograph of the tip of an ascus. In the position of the apical ring there are two areas of non-uniform thickness, taking the form of a hollow cone shaped mass.

Magnification x10,100

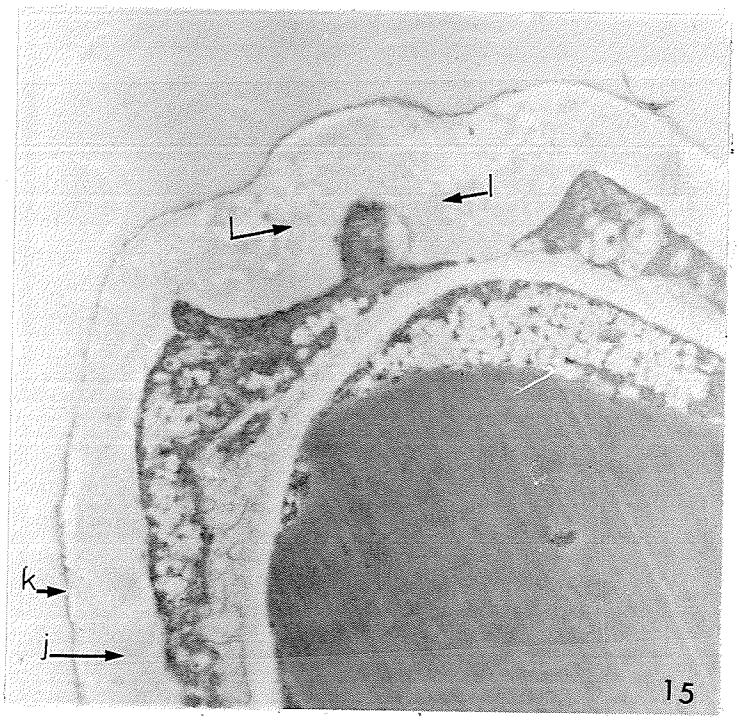
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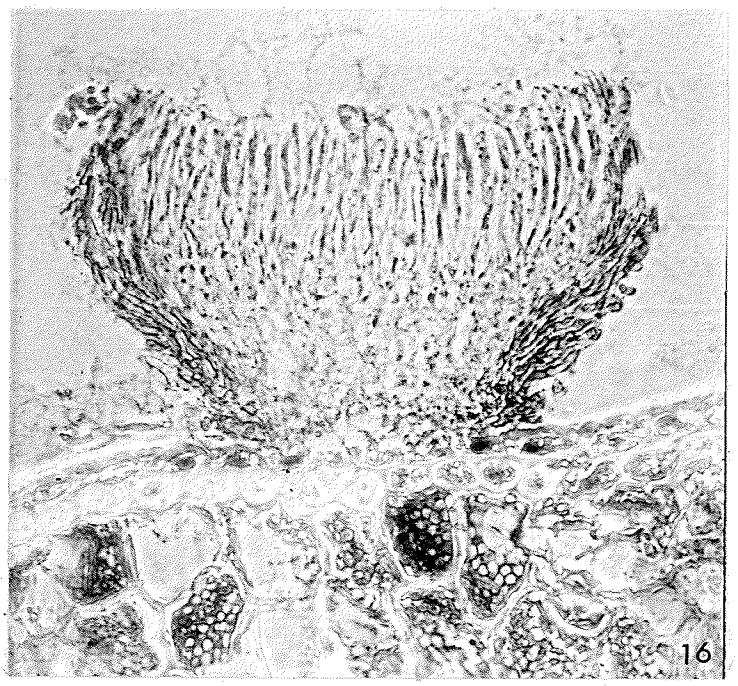
Plate VIII

Sarcotrochila piniperda

Fig. 16 A cross section of an apothecium. This is a well developed cup-shaped apothecium with a marginal excipulum and well developed hypothecium and basal stratum.
Magnification x240.

Figs. 17 and 18 Light microscope photomicrographs of an ascus apex. The apical ring appears as two darkly staining bodies between the inner wall layer and the cytoplasmic membrane.
Magnification x3,400.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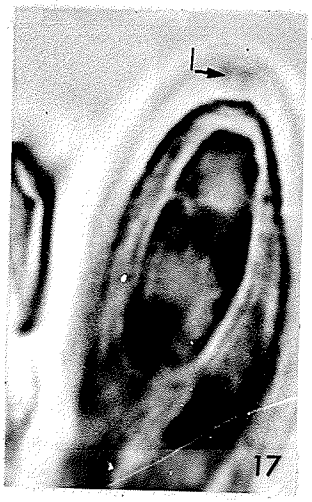
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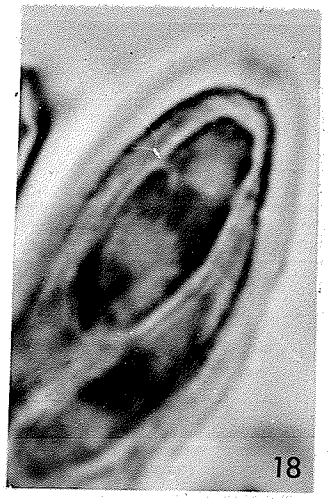
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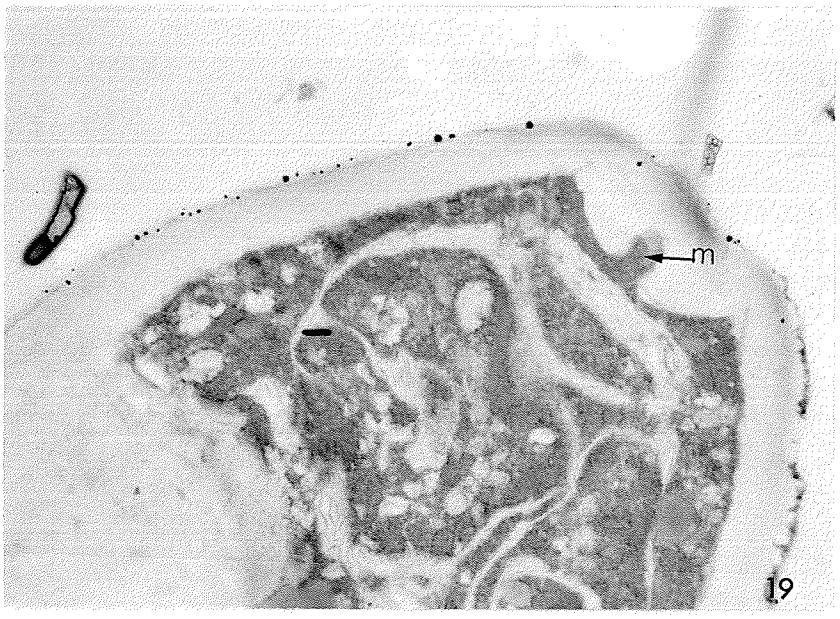
Plate IX

Sarcotrochila piniperda

Figs. 19 and 20. Electron photomicrographs of ascal apices. Note the double layered ascal wall, internal and external, and the projection of the cytoplasm into the internal wall layer.

Magnification x10,100



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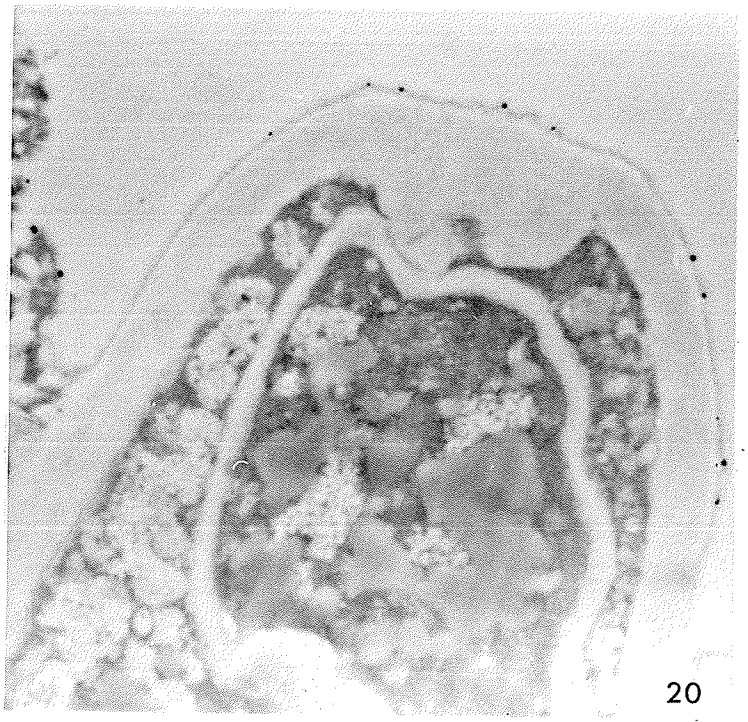

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Plate X

Sarcotrochila piniperda

Fig. 21 Electron photomicrographs of ascal apices. Note the double layered ascal wall, internal and external, and the projection of the cytoplasm into the internal wall layer. Magnification x10,100.

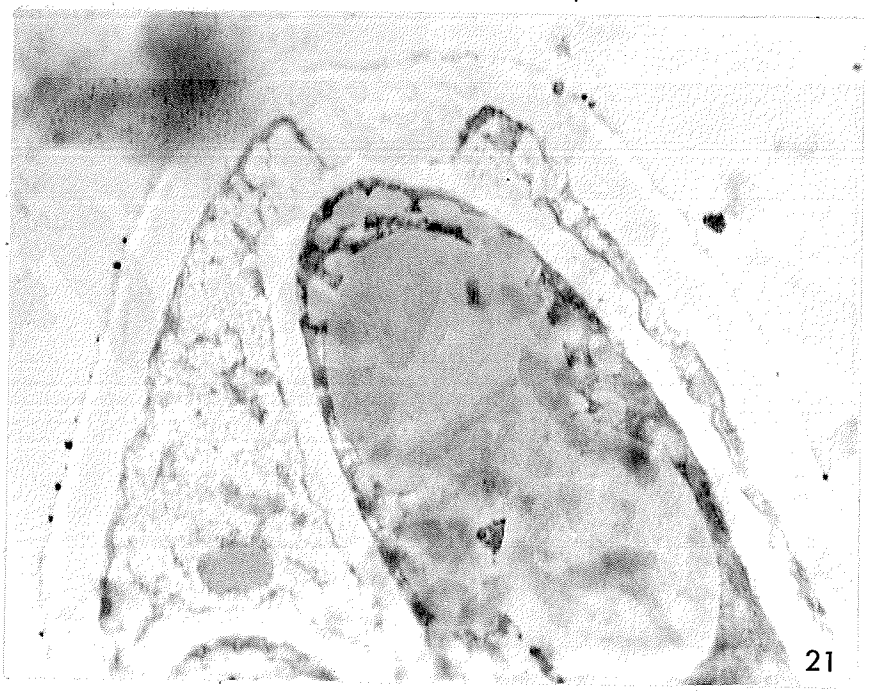
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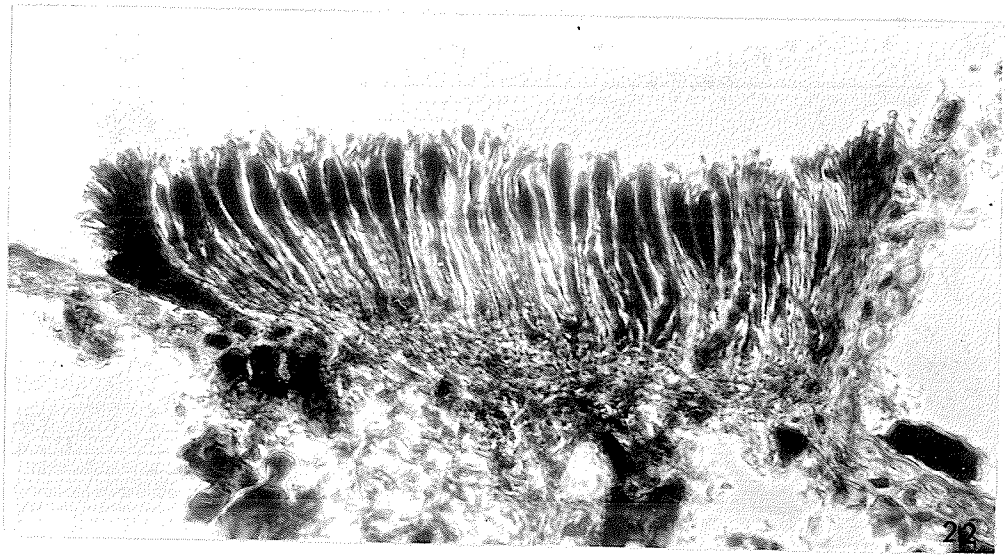
Plate XI

Hemiphacidium planum

Fig. 22. Cross section of an apothecium. Note the very simple structure, which closely resembles that of the two species of *Rhabdocline*, with the poorly developed basal stratum and marginal excipulum, and with the epidermis torn back as an adhering flap.
Magnification x240.

Fig. 23. An enlarged portion of the margin of the above apothecium, showing the marginal excipular tissue, epidermis, and the basal stratum.
Magnification x500.



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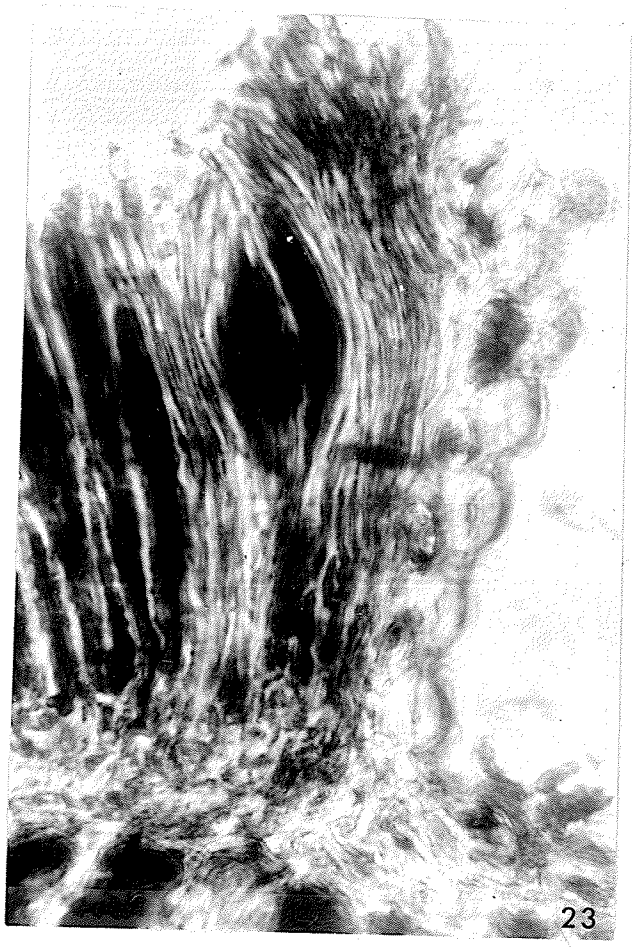

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Plate XII

Hemiphacidium planum

Fig. 24. Interference contrast photomicrograph of the ascus apex. These did not reveal any special structures present in the ascus apex.
Magnification x4,800.

Fig. 25. Ultra-violet photomicrograph of the ascus apex which, too, did not reveal the presence of any structures in the ascus apex.
Magnification x4,000.

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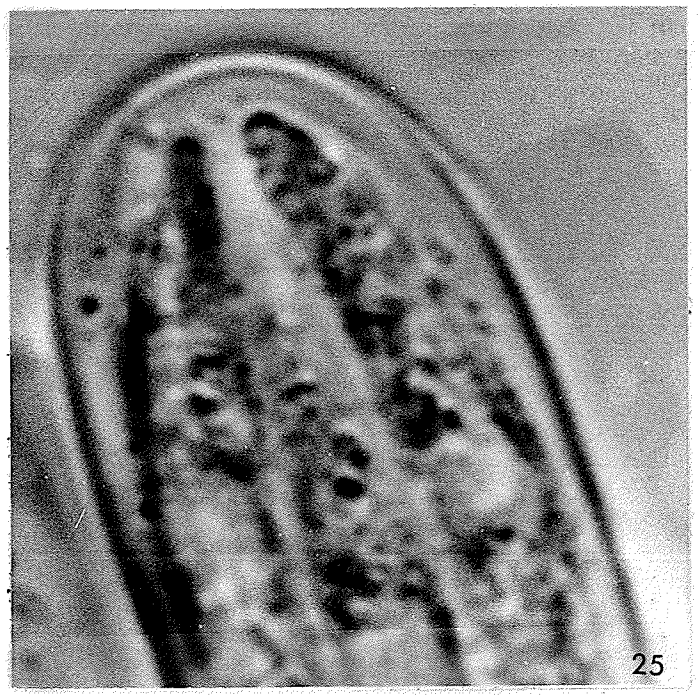

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Plate XIII

Hemiphacidium planum

Fig. 26 Electron photomicrograph of an ascus apex. The cytoplasmic membrane adheres directly to the inner layer of the ascal wall and no special structures appear to be present.
Magnification x10,100.

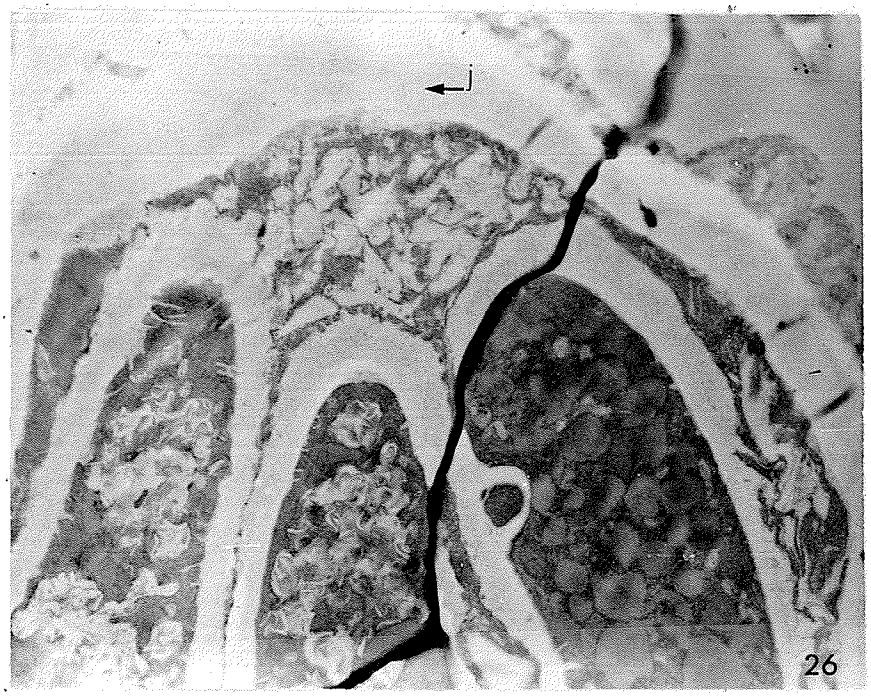
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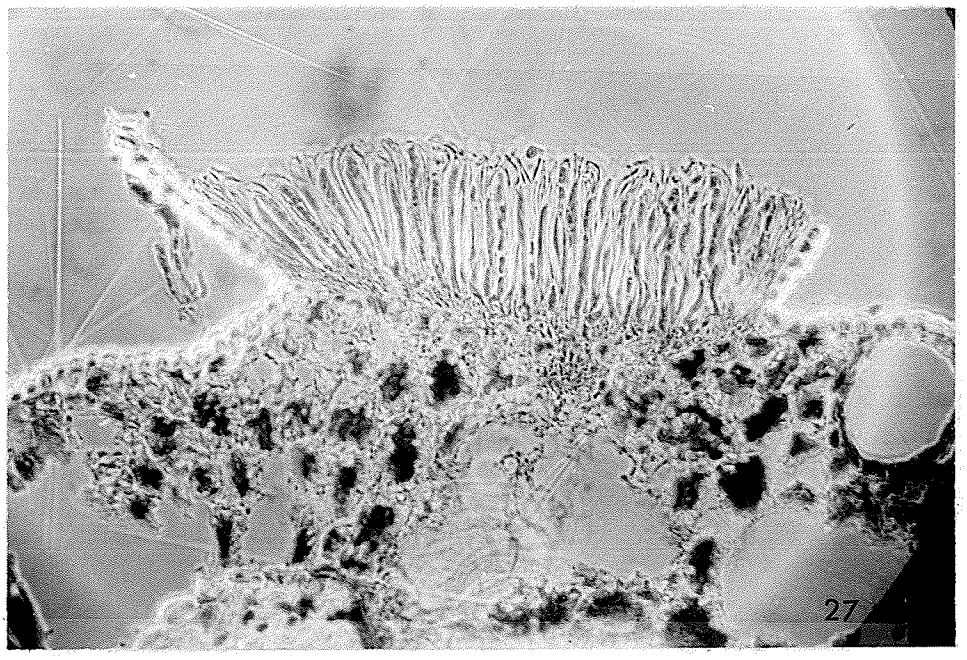
Plate XIV

Rhabdocline pseudotsugae

Fig. 27 Light microscope photomicrograph of the cross-section of an apothecium. The apothecium is of very simple structure with little or no basal stratum nor marginal excipulum. The epidermis remains attached on both sides of the apothecium. Magnification x240.

Fig. 28 Light microscope photomicrograph of an ascus apex. A small refractive pore appears at the ascus apex just inside the inner layer of the ascus wall. Magnification x3,200.



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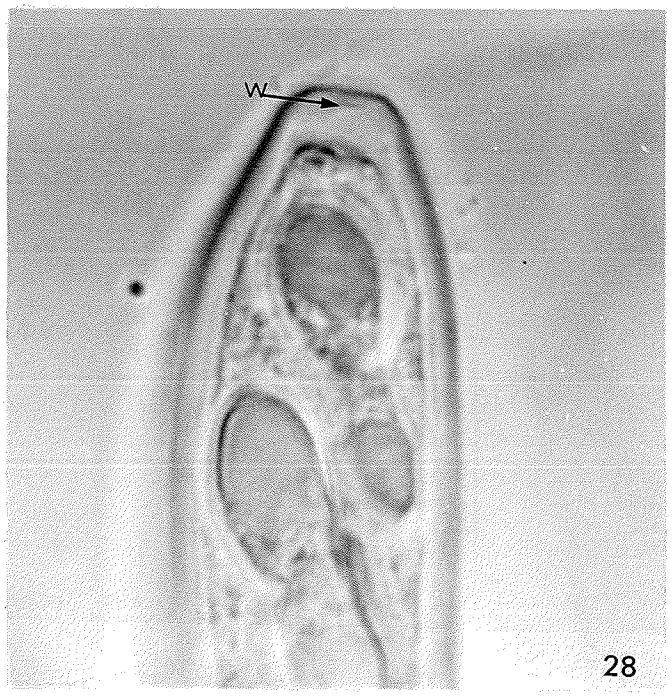

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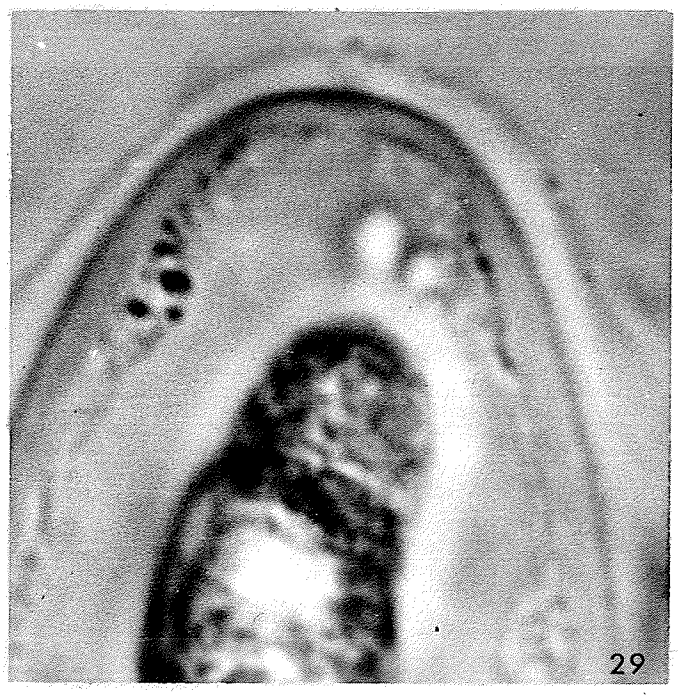
Plate XV

Rhabdocline pseudotsugae

Fig. 29 Interference contrast photomicrograph of an ascus apex. There are no defined structures present in the apex, but along the cytoplasmic membrane there are droplets which tend to coalesce.
Magnification x5,400.

Fig. 30 Electron photomicrographs of an ascus apex. Note that the internal wall layer thins out at the apex and the external wall layer thickens slightly over the apical region.
Magnification x10,000



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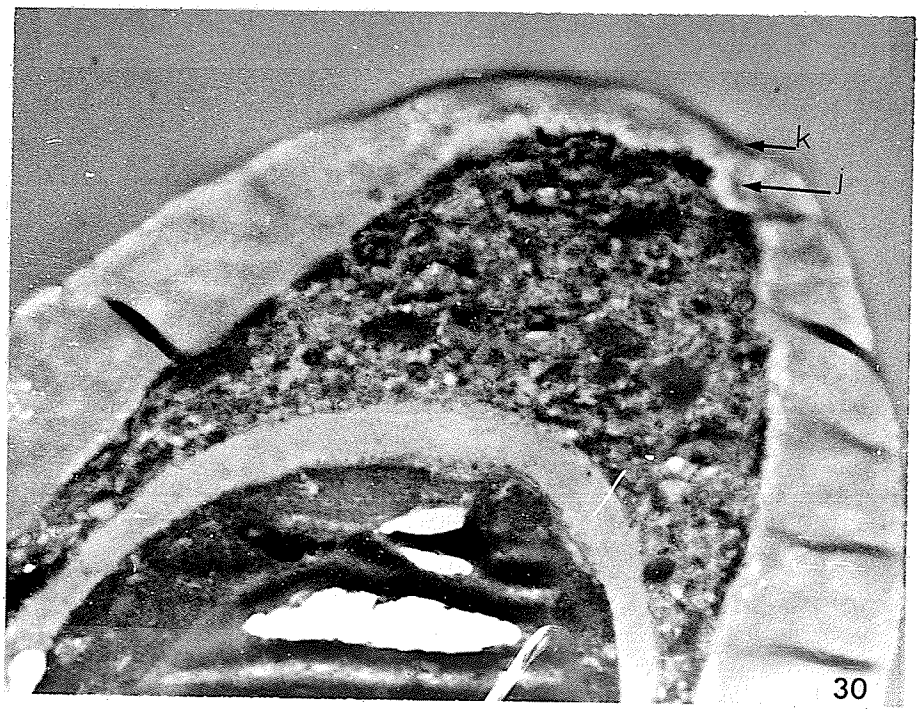

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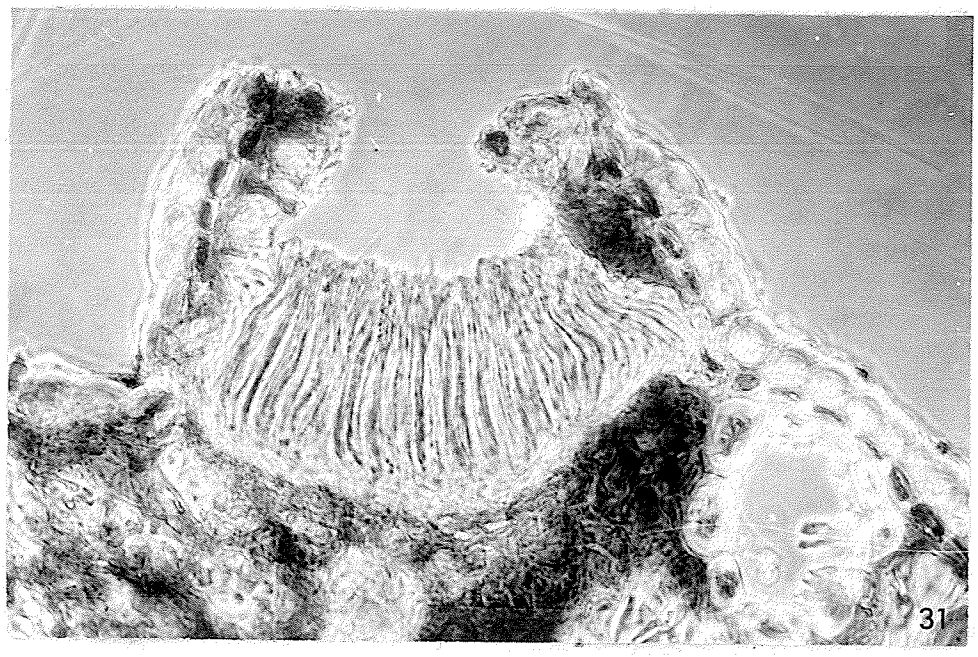
Plate XVI

Naemacyclus niveus

Fig. 31 Cross section of an apothecium showing the layer of fungous tissue over the asci and paraphyses and the poorly developed basal layer upon which the asci are formed. Magnification x240.

Fig. 32 Enlarged portion of the pseudoparenchymatous tissue formed by the fungus as a layer overlying the hymenium. Magnification x960.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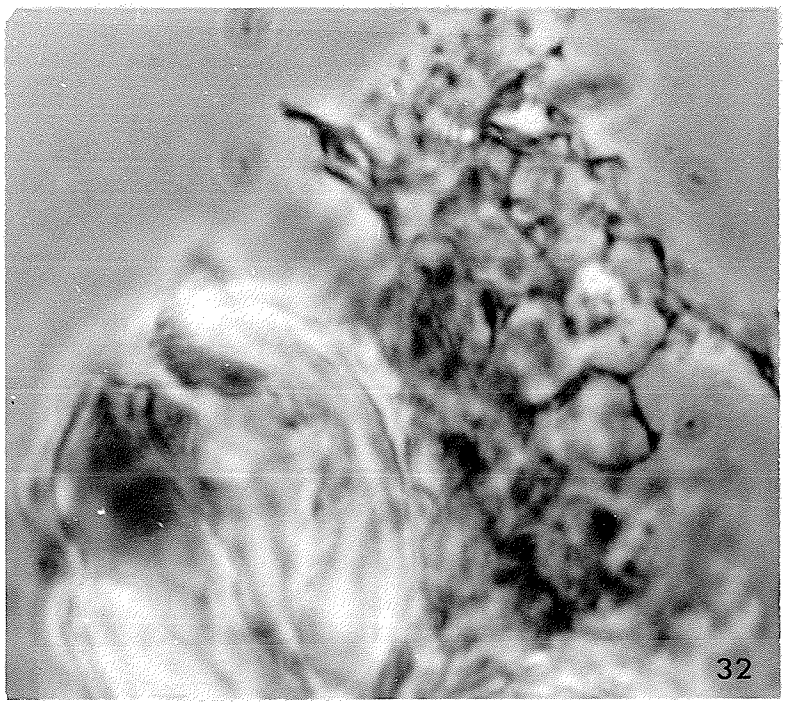
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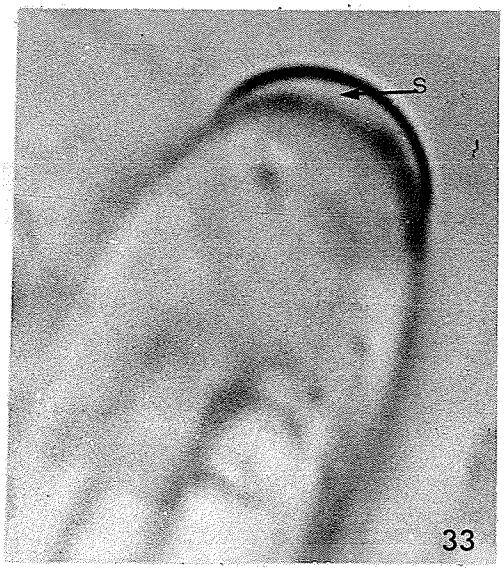
Plate XVII

Naemacyclus niveus

Fig. 33. Light microscope photomicrograph of an ascus apex, showing the detachable apical cap.
Magnification x4,800.

Fig. 34. Ultraviolet photomicrograph of an ascus apex. The dark staining areas in the apex shows the position of the apical ring and another structure in the position of the apical framework.
Magnification x4,400.

Fig. 35. Electron photomicrograph of the apex of a young ascus. The two wall layers are very well defined and just below the inner wall layer in the ascus apex, a thickened dark lining is evident.
Magnification x8,800.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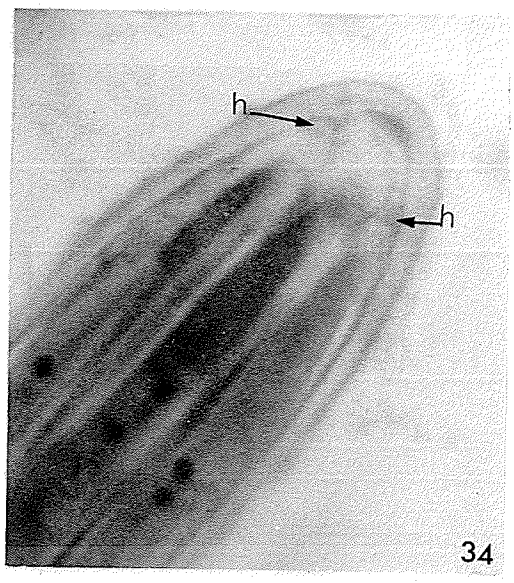
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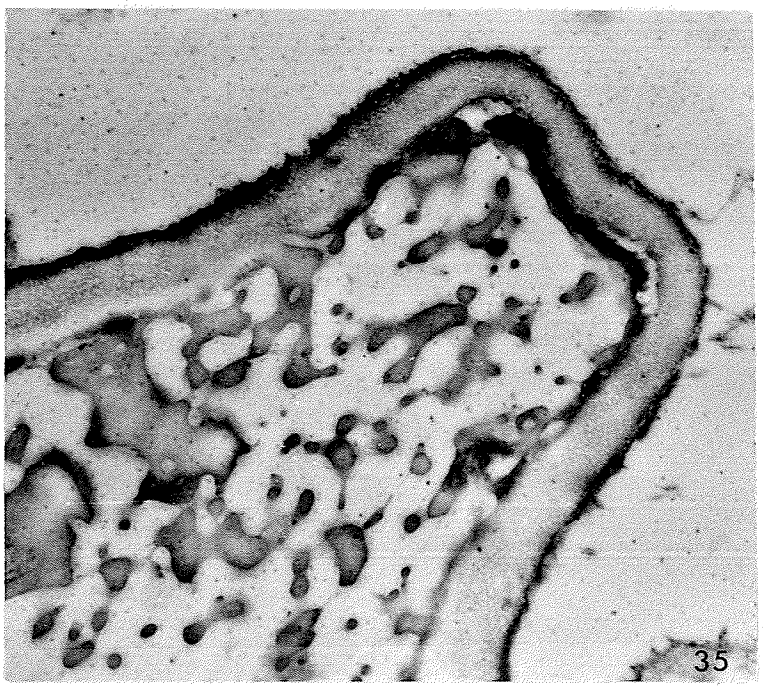
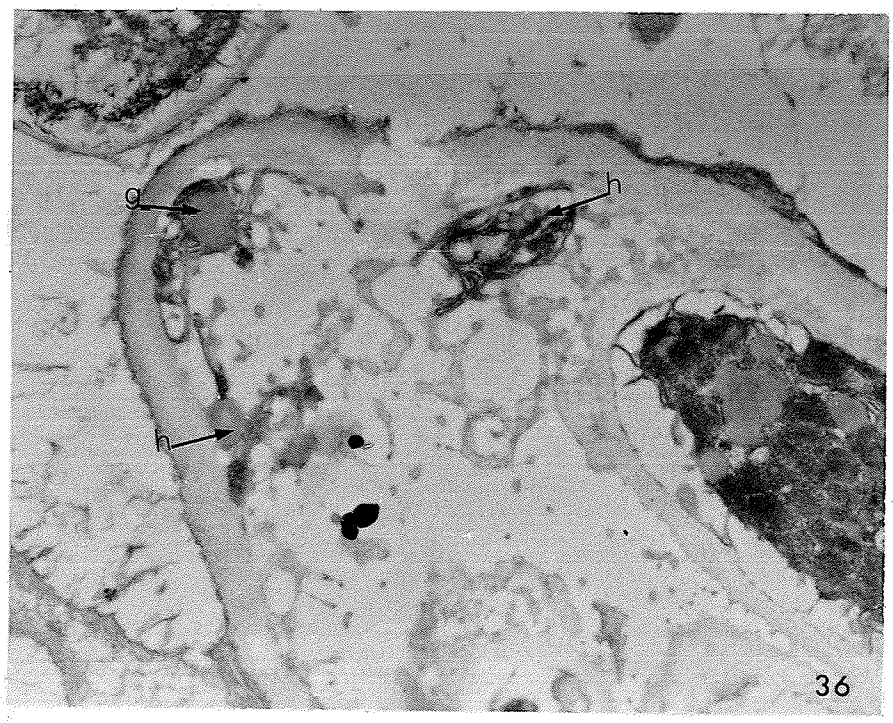

35

Plate XVIII

Naemacyclus niveus

Fig. 36 Electron photomicrograph of the anterior end of the ascus. Note the double layered ascal wall, internal and external and the round electron dense structure in the position of an inferior apical ring. Magnification x10,100.

Fig. 37 Electron photomicrograph of the apex of a young ascus. A broad band of very electron dense material is present at the anterior end of the cytoplasm, which probably represents the immature inferior apical ring.
Magnification x10,100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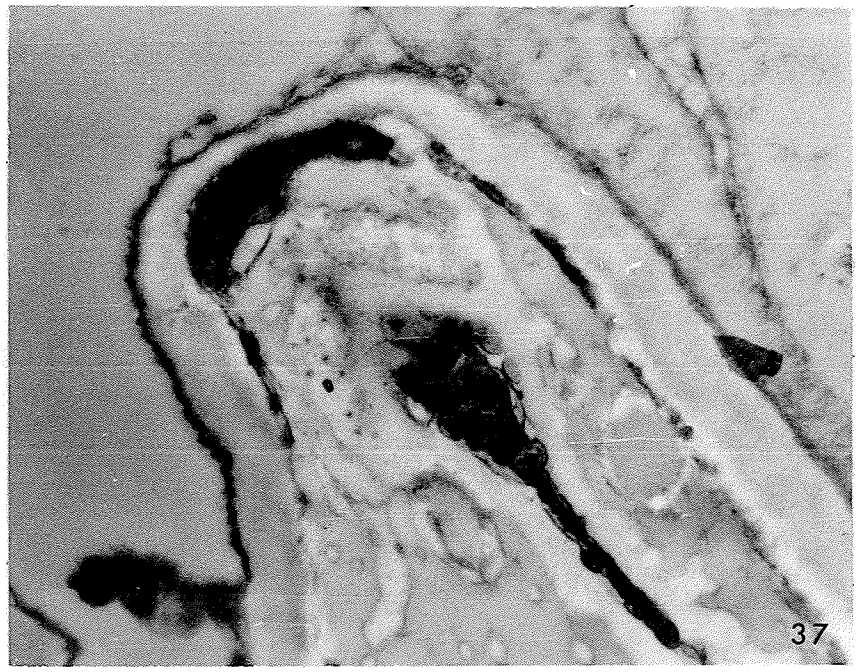
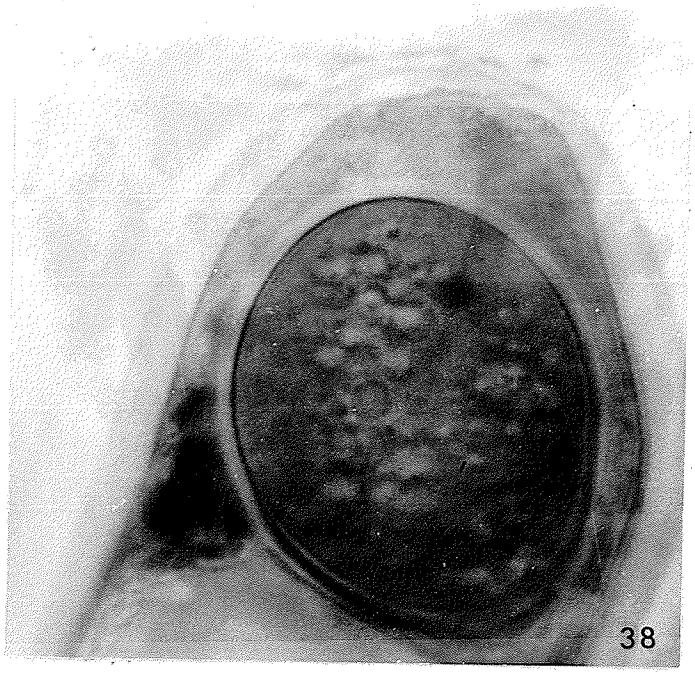

37

Plate XIX

Didymascella thujina

Figs. 38 and 39

Photomicrographs taken with an ultra-violet microscope of ascal apices which suggests the presence of a small refractive pore in the anterior end of the outer wall of the ascus and, in Fig. 39, a darkly staining area at the ascus apex in the internal wall layer. This might represent a canal leading to the external wall layer. Magnification both x4,400.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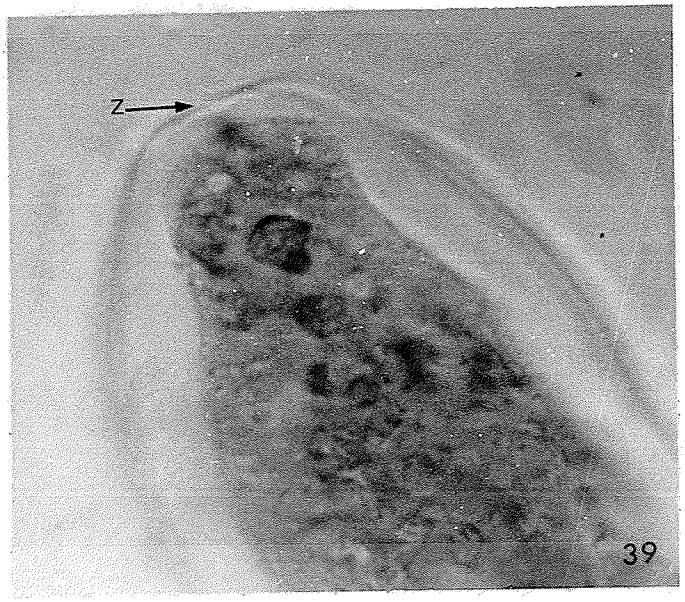
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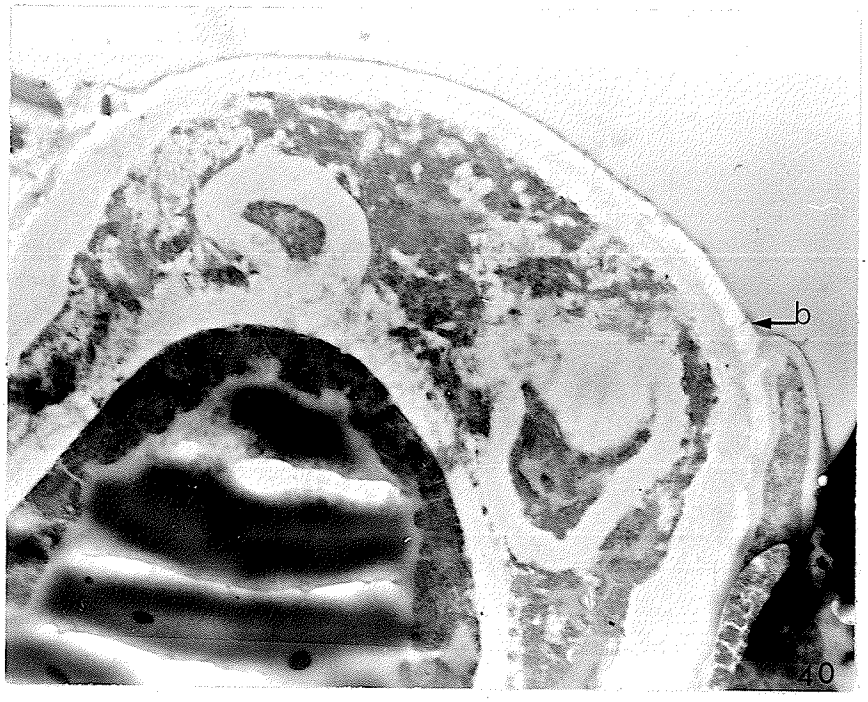
Plate XX

Didymascella thujina

Figs. 40 and 41

Electron photomicrographs of ascal apices. The internal wall layer is thinned out at the ascus apex and this suggests presence of an apical invagination. Note the electron dense bodies present above the ascospore which probably represents degenerate ascospores.

Magnification both x8,800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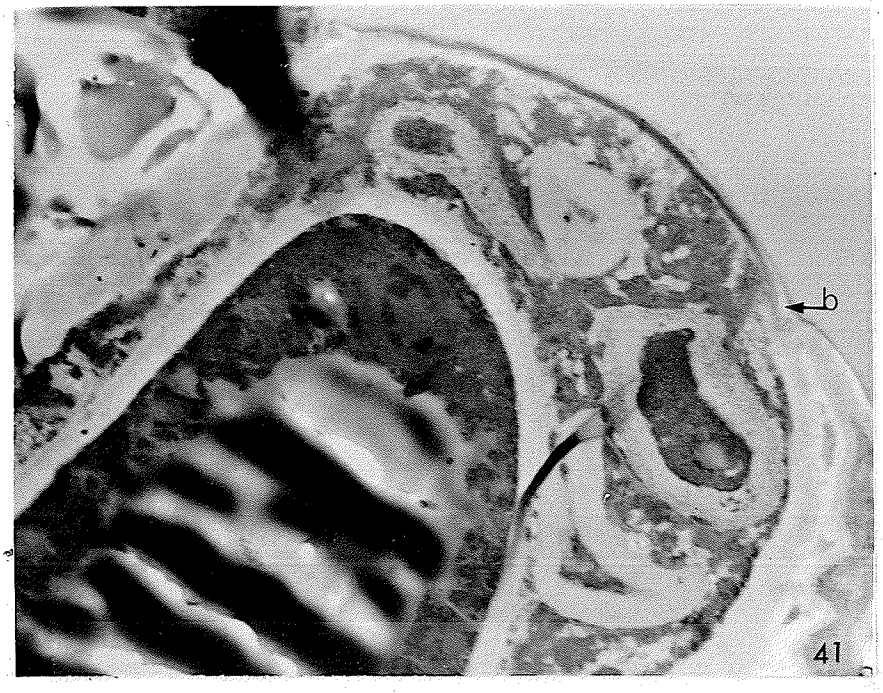

b
41

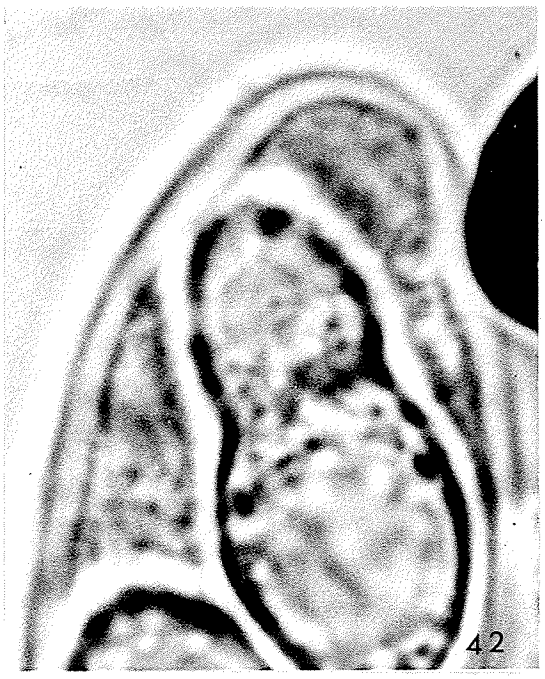
Plate XXI

Fabrella tsugae

Fig. 42 Photomicrograph taken with a light microscope. This did not reveal the presence of any special structures in the ascus apex.
Magnification x4,400.

Fig. 43 Photomicrograph of young ascus taken with a light microscope. There are no specialised structures present.
Magnification x4,400.

Fig. 44 Electron photomicrograph of an ascus apex which shows that there are no special structures present in the ascus apex.
Magnification x10,100.



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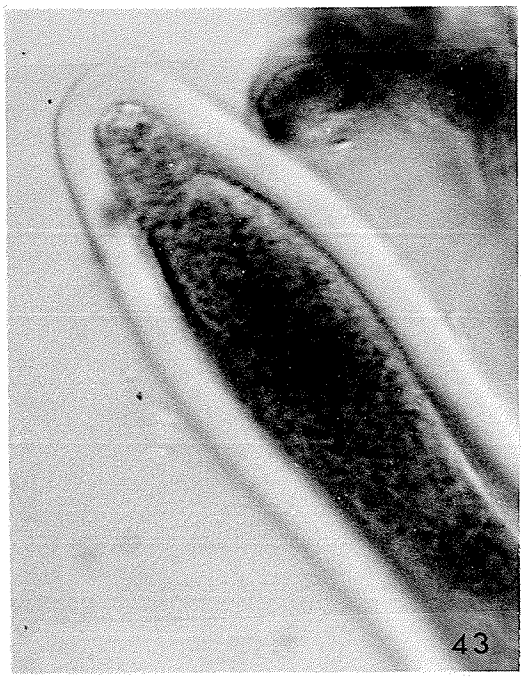

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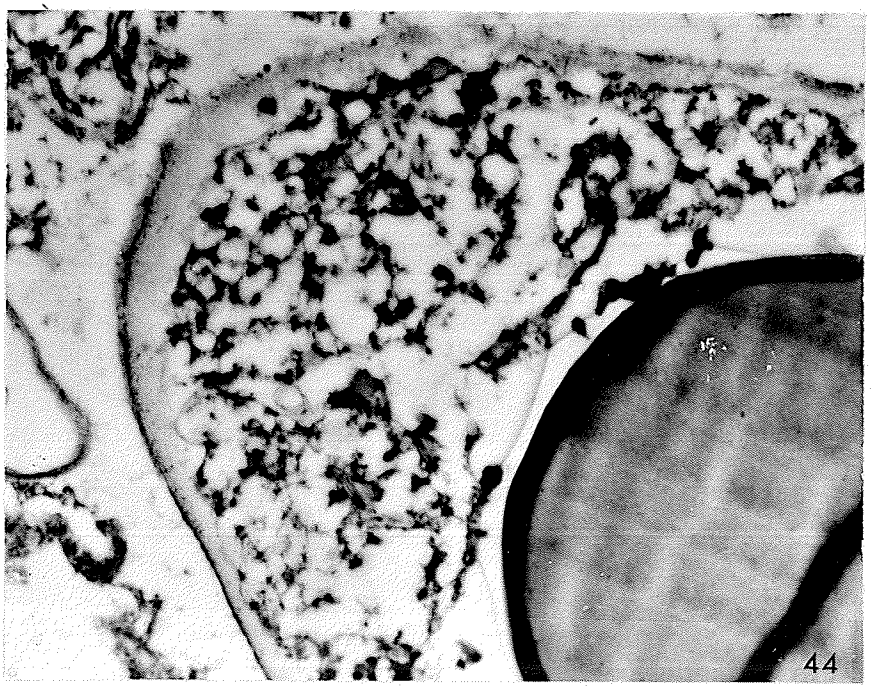

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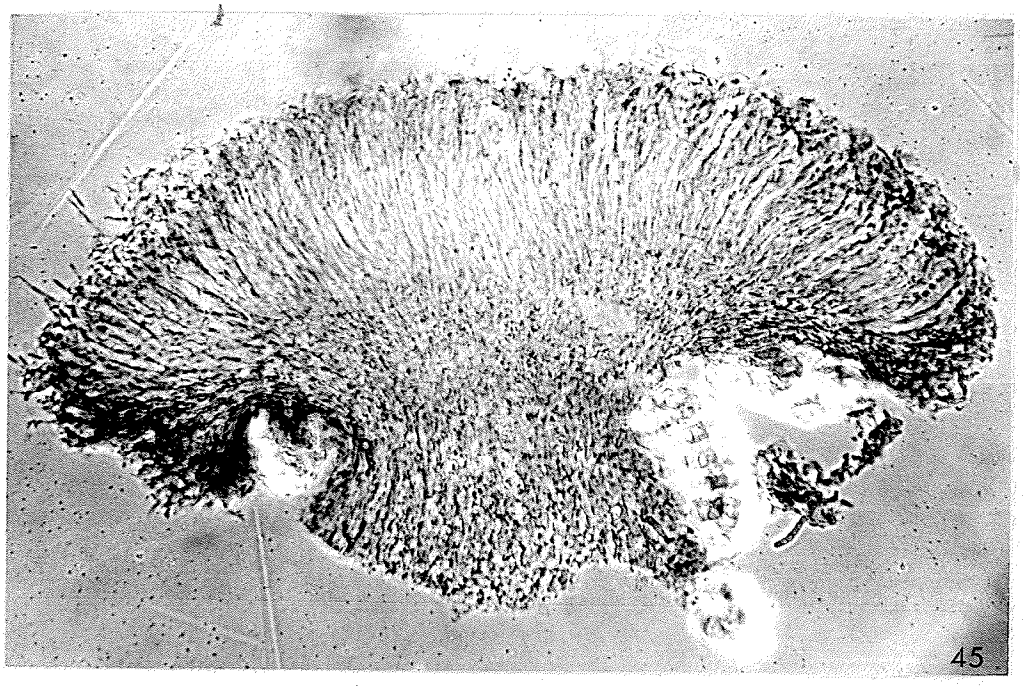
Plate XXII

Hemiphacidium convexum

Fig. 45 A cross section of an apothecium. Note that it is strongly erumpent and with a well developed stalk.
Magnification x240.

Fig. 46 An enlarged section of the stalk and excipular region of the apothecium showing the strongly developed marginal ectal excipulum, and dark-staining elongate hyphal elements comprising the stalk.
Magnification x500.



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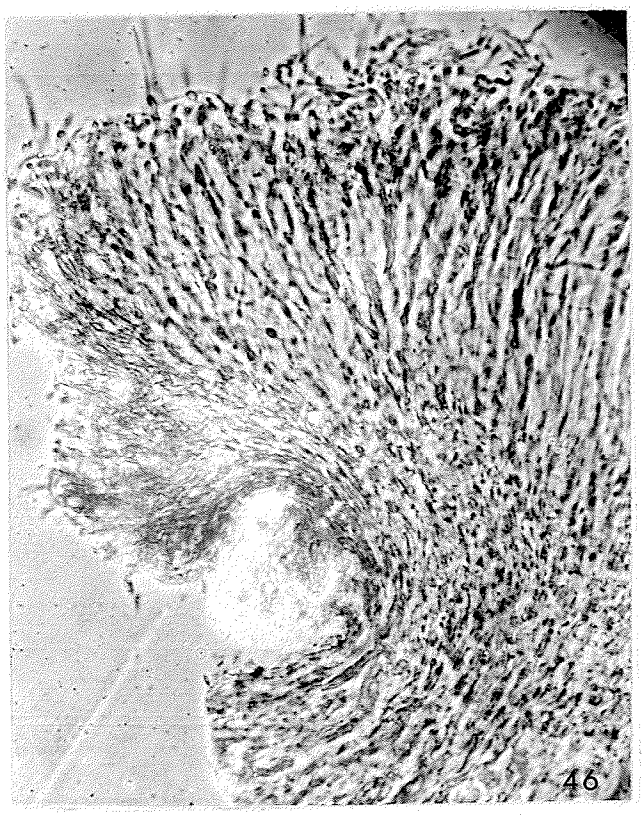

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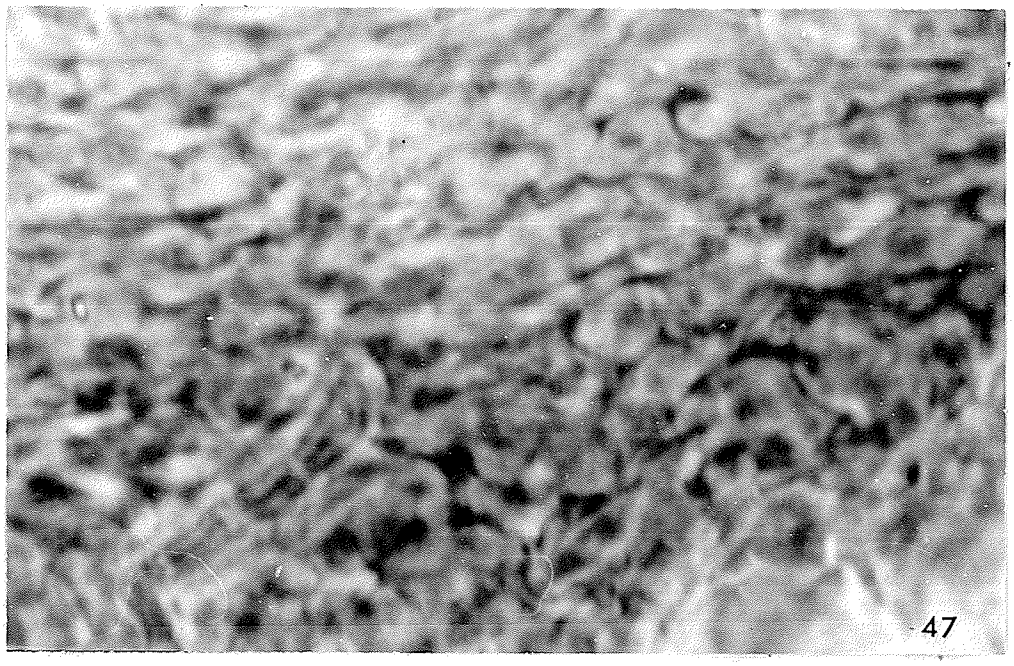
Plate XXIII

Hemiphacidium convexum

Fig. 47 Enlarged portion of an apothecium which make up the excipular tissue. These have shortened and thickened, suggesting the development of pseudoparenchymal-like tissue.
Magnification x2,200 app.

Fig. 48 Interference contrast photomicrographs of an ascus apex. This type of photography did not reveal the presence of any structures in the ascus apex.
Magnification x4,400.



47

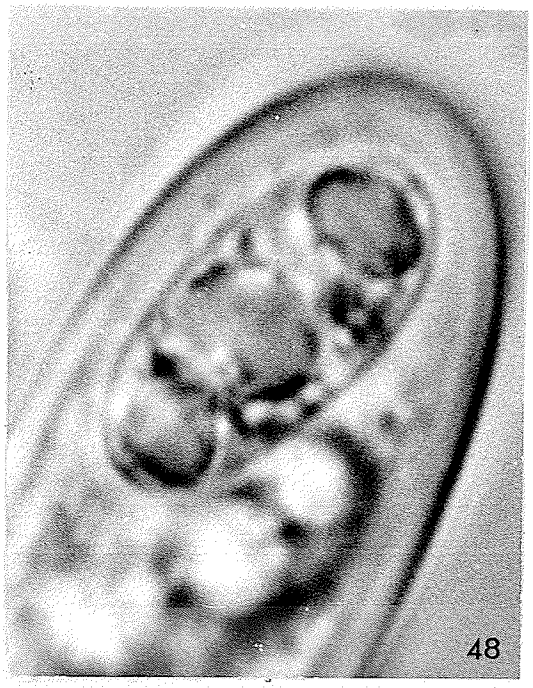


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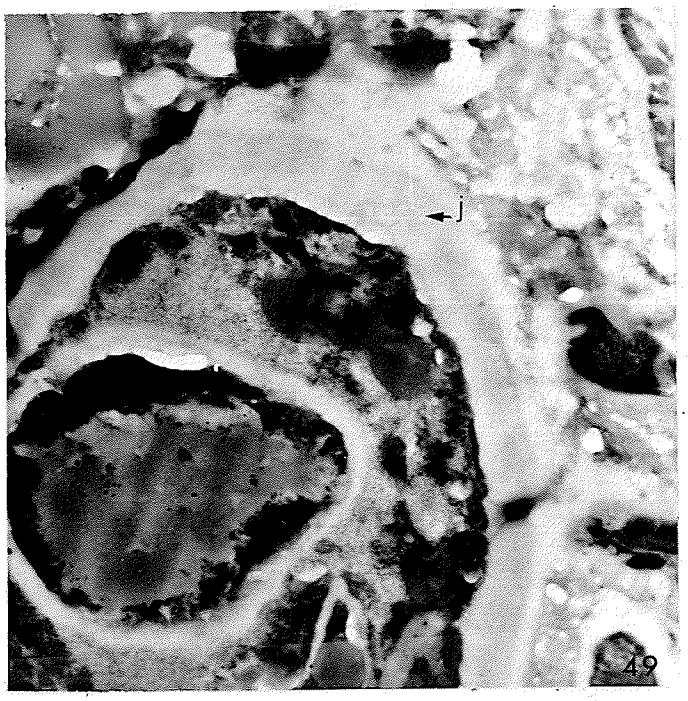
Plate XXIV

Hemiphacidium convexum

Fig. 49 Electron photomicrograph of an ascus apex. The external wall layer is not evident, probably as a result of preparation for electron microscopy. The inner wall layer appears an electron dense non-homogenous layer, which continues over the ascus apex and thickens slightly. There are no other structures present in the ascus apex.
Magnification x10,000.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