STRUCTURE, DEVELOPMENT AND CYTOLOGY OF APPENDICULELLA AND ALLIED GENERA

by

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SUMMARY

In this thesis two members of the ectoparasitic Meliolineae were studied: *Appendiculella* and *Asteridiella*. By the serial-sectioning of the fungi on their leaf hosts, the life-history was determined. This was compared with previous work on the allied genus, *Meliola*, and the conclusion drawn that a combined and generalised description could be given of the important events in the life histories of all three genera.

The fungus develops a stroma-like ascocarp in which two cells become modified and later fuse: these two cells can be regarded as very rudimentary sex cells. Septate ascogenous hyphae arise from the resulting binucleate cell, branch, and give rise to asci by the crozier formation. A cavity develops, mainly by dissolution. Cell-elongation, however, is also an important factor in the enlargement of the interior of the ascocarp, for true paraphyses are absent. The ascus is very thin-walled, deliquesces early, and leaves the spores lying free within the ascocarp. Normally only two spores mature. Spore dispersal is probably effected by the rupture of the ascocarp near its base, although a periphysate ostiole does develop.

The presence of "abortive asci" is the unusual feature of the fungi studied. Seemingly paraphysis-like structures, they are, however, binucleate and arise from ascogenous hyphae late in ascocarp development.

The taxonomy of the Meliola group is discussed in relation to the classifications of Luttrell, Martin and Miller. With the life-history of these fungi now determined, their taxonomy must be revised, the first step being in their removal from those orders in which the true perithecium exists. Suggestions are made as to their positions in the classifications of those authors mentioned above.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge and belief the thesis contains no material previously published or written by another person except when due reference is made in the text of the thesis.

STRUCTURE, DEVELOPMENT AND CYTOLOGY OF APPENDICULELLA AND ALLIED GENERA.

INTRODUCTION.

In this project investigations were carried out to determine the sequence of events inthe development of the perithecium in the Heliola group of fungi. The presence or absence of sex organs has long been a controversial point. They have been recorded by Graff but their presence has been denied by Toro (these two authors being among the main workers in this field). There has never been any definite pronouncement on the presence of ascogenous hyphae and paraphyses, the origin of the ascus itself or the number of nuclei in the spore cells. In the present investigation an attempt has been made to elucidate these problems.

PREVIOUS WORK.

Reference to the literature revealed that, although there has been adequate morphological investigation, there has indeed been very little work done on the life cycle of the Heliola group.

The first thorough investigation into any of the Meliola group was done by Ward in 1883, although in 1825 (Ward, 1883) Fries had introduced the name, and vaguely delimited the group as one comprising certain tropical fungi. Bornet too had presented a morphological description of the genus Meliola (Bornet 1851); but even so he was concerned as much with the macroscopic appearance of the fungus on the host leaf as with a description of the hyphopodia and perithecia and its contents. He described a few species and very briefly discussed the taxonomical position. Ward delimited the group as one "composed of mycelium which supports appendages and perithecia" (Ward 1883), and described the morphology and vegetative development of the fungus in minute and accurate detail. But he made no mention of ascogenous hyphae, crozier formation, periphyses or upright hyphal tissue. He believed that the group, with its apparent lack or reduction of sexual organs, would "probably fill up yet more completely the gap... between the lower and higher Ascomycetes" (Ward 1883).

Many years later, in 1932, Graff worked on the life history of Meliola circinans Earle, and described most confidently a definite oogonium and antheridium, around which

vegetative hyphae had grown to produce a shield-like stroma. It is obvious from the present work however that there are no sex organs in Appendiculella and Asteridiella. Nevertheless, his observations on the branched ascogenous hyphae giving rise to asci by hooks, the presence of periphyses, the detailed description of nuclear behaviour within the ascogenous tissue, and a discussion on terminology are most pertinent.

In his "Taxonomy of the Pyrenomycetes" (1951), Luttrell uses Graff's description in placing the group within his classification. But he was not really convinced by it, and felt that the data on perithecial development, until then, was insufficient to place the Meliola group correctly.

Since 1932 very little effective work has been done to determine the life history. Toro (1952), like Luttrell, was concerned largely with taxonomy, and there are no detailed observations of the life-history in his study; one important fact is that, unlike Graff, he failed to observe any sex organs. He approached the subject from a more physiological and ecological viewpoint, while, in describing the morphology of several genera of the Meliola group and related groups, he attempted a review of their taxonomy.

Hansford (1957) has dealt only with morphology and that mainly from the viewpoint of classification within the Heliolineae. In his monograph he states: "The details of the internal structure at the various stages of growth of the 'perithecium' as well as the origin of the asci, must await further investigation. Until these are accurately known, the relationship of the Heliolineae to other orders of the Ascomycetes remains doubtful". (Hansford 1957). With these two statements in mind, I have endeavoured to incorporate in the present paper the work of all previous authors, where relevant, together with present observations, into a more complete and comprehensive study of some genera allied to Heliola.

In the description of the life history which follows later, the relevant parts of previous works (often quoted verbatim) have been added to my own observations to provide a more comprehensive picture of the development and a better comparative study of the many disagreements than could be gained by leaving these to a Discussion. Also, much unnecessary repetition has been avoided by this means. And I have been able to confine to the Discussion only the marked discrepancies, particularly those pertaining to taxonomy. Also included in the Life History are those morphological details adequately and correctly described by previous authors, which did not form part of my work (as I was concerned mainly with the internal development of the stroma), but which have been included in order to present a clearer and more complete picture.

MATERIALS

SPECIMENS RECEIVED

The Meliola group is poorly represented in South Australia, so specimens were requested from various outside sources. Those received and examined were as follows:—

1. Appendiculella calostroma (Desm.) von Höhnel on Rubus sp. provided by Dr. Lilian Fraser from New South Wales in December 1956.

2. Asteridiella eucalyptorum (Hansf.) Hansford on Choricarpia leptopetala sent from Mount Coot-tha, Queensland in November 1956.

3. Asteridiella sp. on Nauclea latifolia, received from Mr. Pyne, Sierra Leone: collected from Njala (Kori chiefdom) in December, 1956, fixed in Acetic alcohol 1:4 and preserved in 70% alcohol.

4. Asteridiella sp. on Alchornea cordifolia from the above source and collected in September, 1956.

5. Asteridiella glabra (B&C) Hansford on Coffea canephora sent from Kawanda, Africa and collected there in November 1956.

6. Echidnodes diospyri Hansford on Diospyros australis from Mount Glorious, Queensland, and collected there in January 1957.

7. Asterina radiofissilis (Sacc.) Theisn on Acalypha nemorum from the same source as No. 6.

8. Meliolina mollis (B & B) Hoehnel on Leptospermum scoparium from Shaw Road, Oratin, New Zealand, and collected there in November 1956.

(The above species were identified by Dr. C. S. Hansford)

TAXONOMIC DISCUSSION

Although the taxonomy of these ecto-parasitic Ascomycetes is in a great state of flux, many of the above specimens are, at the present, considered to be members of the Meliola group. However, Echidnodes and Asterina have been placed in another group, the Microthyriales, due to the presence of, among other characteristics, radially arranged cells in the outer wall.

Similarly too, with the Dimerium which was parasitising Asteridiella glabra. Although the Dimerium was present in

a sufficient number of stages to enable its life history to be followed, it was soon realised that these stages varied from those seen in the Asteridiella sp. and in the Appendiculella calostroma: contrast Figure 78 (Dimerium) with Figures 58 or 61, and Figure 79 (Dimerium) with Figure 60. It was therefore considered to belong not to the Meliola group, and was therefore not studied. Comparison can be drawn, however, between some vegetative characteristics of the two groups. Compare the development of the peritheciun in Dimerium (Figure 73) with that of Appendiculella (Figures 5 and 9-13), and the structure of the periphyses in both — Figures 80-82 (Dimerium) and Figures 62 and 67 (Appendiculella). At the present time Dimerium is a "genus confusum", but with its two-celled spores and hyperparasitism it is quite probable that it does belong to another group.

The Meliola group is a strict leaf-parasitic one, with the fungus dependent for its nourishment on small haustoria developed in the host cell and produced from a filament extension of the head cell of a capitate hyphopodium (a lateral branch of the external mycelium); there is no internal mycelium. The perithecia are more or less globose, with an opaque, black outer wall of non-radiating cells, and the early-evanescent asci which

line the base normally contain two or four coloured spores each. Genera have hitherto been differentiated on such characteristics as the presence or absence of mycelial setae, stromatal appendages, stromatal setae, and so on; for example, according to Toro, Meliola ischaracterised by the presence of mycelial setae, Irene by the absence of such setae but the presence of stromatal vermiform appendages, and Irenina by the absence of both appendages and setae (Toro 1952).

HYPERPARASITES

Only three of the specimens received could be used for the present study, namely Appendiculella calostroma, Asteridiella eucalyptorum and Asteridiella sp. (No. 3), and these were examined in detail. Of the other specimens, some were discarded due to insufficient quantity of the fungus, or insufficient number of representative stages. For example, Meliolina mollis was present mainly as mycelium with a few very young fructifications, hence its taxonomic position could not be checked, and in any case, it probably does not belong to the group because, although it is a leaf parasite, it has no hyphopodia. It may be pertinent to remark here that identification of the fungi, despite inadequate material,

is often madepossible by the fact that members of this group have a very limited host range. Confirmation is then obtained by checking such species characteristics as hyphal configuration and shape and size of the capitate hyphopodia.

Other specimens were useless because of heavy parasitism. This is a common occurrence, and is effected by members not only of the Fungi Imperfecti and Ascomycetes but even other Pyrenomycetes themselves. Consequent on parasitism is the apparent reduction in number of the fruiting bodies and the possibility of developing sterility. It is of interest to note that these parasites were a source of confusion to earlier workers, e.g. Bornet (1851) could not find the 'conidial stages' of Meliola described by Lindley, Berkeley and Léveillé; in all probability these structures belonged to some parasitic Hyphomycete.

Asteridiella eucalyptorum, although studied later on because it demonstrated various developmental stages, was parasitised so heavily by a Helminthosporium that the colonies appeared velvety under the stereoscopic microscope. Asterina radiofissilis was so extensively parasitised by a Cicinnobella that the material was useless, and in any case, Asterina does not belong to this group. Similarly with Asteridiella glabra, which proved to be an

interesting specimen in that the Dimerium which was parasitising it was itself parasitised by a Naemosphaera sp.; examples of this are shown in Figures 73-76. In these cases of parasitism, the host hyphae may be completely surrounded by the hyphae of its parasite; or, if the host is attacked at a later stage, e.g. during perithecial formation, the hyphae of the parasite will penetrate the host tissue itself. According to Toro (1952) the restriction of the developing ascocarp of the host fungus is consequent on the increased amount of nutrients present in this region.

SPECIMENS STUDIED

The two species finally studied were Appendiculella calostroma and Asteridiella sp. (No. 3). A. calostroma was used for the majority of the drawings as its host, a Rubus sp., was soft-leaved and easy to cut. Nauclea latifolia, on which the Asteridiella sp. was growing had a very tough leaf, and some of the fungus material was dislodged during the cutting of the very thin sections required.

A general morphological description of Appendiculella calostroma is considered relevant at this stage before proceeding to an account of its life history. It must be remembered that the genera of this group are differentiated from one another on

purely vegetative characteristics. Thus the presence of e.g. appendages on Appendiculella calostroma will distinguish it from an Asteridiella or a Meliola, but its development will show no real difference. While the following morphological description applies only to A. calostroma, the life history is virtually the same for each member of the group.

Colonies mostly epiphyllous, rather thin, up to 2 mm. diam., sometimes causing a red leaf-spot on host, sometimes numerous and widely confluent. Mycelium of dark brown, substraight to undulate hyphae, 6 - 8 µ thick, the cells mostly 20 - 30 µ long, branching opposite or irregular, loosely reticulate. Capitate hyphopodia alternate, more or less antrorse, straight or bent, 20 - 35 µ long; stalk cell cylindric, 5 - 18 µ long; head cell sometimes subglobose to pyriform and entire, more usually rounded-anguloso to irregularly and shallowly lobed, 12-20 x 11 - 17 µ. Mucronate hyphopodia mixed with capitate, fairly numerous, opposite or alternate, ampulliform, 14 - 24 x 6 - 9 µ, neck upturned, 3 µ thick. Setae none. Perithecia usually in a central group, black, globose, rough, up to 250 µ diameter, the surface cells conic to mammillate, but some growing out into

larviform appendages 60 - 110 μ long, 20 - 25 μ diameter at the base, recurved above and tapering to obtuse apex, brown, somewhat translucent, transversely striate. Spores dark brown, cylindric, straight or somewhat bent, obtuse, 3-septate, slightly constricted, 38 - 45 x 13 - 15 μ. (Hansford 1953).

METHOD

FIXATION AND PRESERVATION

All the specimens werefixed before being sent, and arrived preserved in 70% alcohol.

PREPARATION OF MATERIAL

The fungus forms dark, discrete, easily detachable colonies approximately 1 - 5 mm. in diameter, spread over the surface of the host leaf — on the upper side in the case of Asteridiella sp. (No. 3) and Appendiculella calostroma, on the lower in the case of Asteridiella eucalyptorum.

Portions containing single colonies were cut out under the stereoscopic microscope to ensure a minimum edge of uncolonised leaf tissue and thereby avoiding unnecessary and time-consuming sectioning and examination.

EMBEDDING

As these colonies are easily dislodged from their superficial attachment to the leaf, only two or three of these small leaf portions were taken through the dehydrating solutions at a time in order to minimise movement of the colonies against one another. The portions were placed in specimen jars where they remained throughout the transfers through the different concentrations of alcohols and xylols. The material had been adequately dehydrated, so there was no difficulty in getting it to sink to the bottom and remain there as the successive solutions were poured off.

The portions were finally embedded in 56° M.P. Paraffin Wax (Gurr's); the method used here was to add very small shavings of the wax to the xylol from time to time as this was found to be the most suitable method for the material, and the most practicable in view of the delicate nature of the leaf-fungus attachment. To facilitate sectioning during the winter months, it was necessary to cover the wax blocks with a thin layer of 54° MP. wax.

SECTIONING

Sections were cut 3 μ thick. This thickness was decided on, after experimenting with various thicknesses, as being the best for the material used and for the detailed nuclear examination necessary in the study. Graff (1932) chose 5 - 7.5 μ as the best thickness considering the condition of his material, Heliola circinans, and the Carex sp. on which the fungus was growing.

STAINING

At first, Flemming's Triple Stain was used. This, however, produced inadequate contrast between purple nuclear material and pink cytoplasm, and seemed dependent on precise timing and on quantities employed in order to obtain a marked differentiation. Also, the fact that the specimens had probably been fixed in alcohol when gathered may have affected this staining, as Flemming's reagent is best in tissue fixed in a chromic-osmic acid mixture; this probably accounts for its successful use by Graff (1932) in the staining of his sections of H. circinans, which had been fixed in Flemming's medium fixative when gathered. Since well over a thousand slides had to be prepared to ensure the presence of a sufficient number of stages, this stain was later abandoned, and Safranin-Fast Green combination (7 g. / 500 ml. aq. Safranin: 1% alcoholic (95%) Fast-Green) used instead. This was lesssubject to personal

error, gave quicker results, and was more reliable in the contrast staining ofthe red nuclei against the surrounding green matrix, any extraneous red dye being removed from the matrix when the Fast Green was introduced.

EXAMINATION AND RECORDING OF OBSERVATIONS

The stained and mounted slides, each having two or three rows of sections, were then filed in serial order. Later each was examined under low power, if it were found to contain an important and clearly differentiated stage, this was ringed with Indian Ink, and its position on the slide was noted on a card, accompanied by a brief description of the section. Detailed examination of useful sections was then carried out, using a 90:1 or, where necessary, a 100:1 oil-immersion objective lens.

— DESCRIPTION OF THE LIFE-HISTORY —

As previously mentioned, the main species studied was an Appendiculella due to the wider range of stages available. The various stages seen in the Asteridiella species, however, were so similar that one description will serve them both.

The life histories of Asteridiella and Appendiculella (as found in this study) were then compared with that of Meliola (as taken from the literature). The following is a table of such comparisons:

Organ	Appendiculella	Asteridiella	Meliola
Pre-formed stromatic ascocarp.	+	+	+ (W) + (R)
Reprod. cells.	+ (r)	+ (r)	+ (G) - (T)
Fusion of cells	+	+	+ (G)
Ascog. hyphae	+	+	+ (G)
Crozier	+	U	+ (G)
Solution cavity	+	+	+ (W) - (T)
2 mature ascospores	+	+	+ (G)
All spore cells equally nucleated	+	+	- (G)
'Paraphyses'	+	+	+ (W)
Periphyses	+	+	+ (G)
Ostiole	+	+	+ (W) + (G)

where + = recorded
- = recorded as absent
U = not seen
r = rudimentary

W = Ward (1883)
G = Groff (1932)
T = Toro (1952)

Note:- The recorded details in the formation and composition of the various stages were virtually identical in the three genera.

From this comparison it was felt the development of one of the Meliola group could now be taken as representative of the whole group. Accordingly, Appendiculella is described, but references from outside sources are incorporated to make one complete story for the group. To cite one example, see Figures 15a and b where a binucleate stage of Appendiculella is represented, and compare this with an equivalent stage of Asteridiella as seen in Figure 15c. Appendiculella is, however, slightly more stromatic than Asteridiella in all stages (Compare figures 16a and 22), and no doubt there are other such unimportant differences among the remaining members of the group. All diagrams therefore, except where mentioned otherwise, are of Appendiculella calostroma.

This section, dealing with the life-history of the fungus, can be divided into two main parts: general morphology and the development of the perithecium, both of which are further subdivided and the text discussed within these sub-divisions.

GENERAL MORPHOLOGY

(The investigations by Ward (1883) pertinent to the first part

of this study, namely the description of the morphology and the vegetative development of Meliola cannot (and must not) be overlooked. For minute and detailed description of actual observations his work has not been excelled by later mycologists in this particular field. (The preponderance of quotations (from his work) which follows is due to this).

The life-history of the fungus may be considered to begin with the spore and its germination on the leaf surface.

THE MATURE SPORE

The mature spores, as released from the perithecium, are elliptical-spindle shaped, brown-walled with brown septa, and between each cell is a pore in the wall. In Asteridiella and Appendiculella each spore was four-celled and mostly four-nucleate, with one nucleus per cell. Sometimes, however, and it is of too frequent occurrence to be overlooked, these four nuclei become eight nuclei either before, during or after septation of the spore (Figs. 43, 48, 44, 45 and 46), and a four-celled, eight-nucleate spore is thus seen. Similarly too, many two-celled spores occur (Figs. 49 and 54), a fact also recorded by Ward (1883) in his description of the occasional

presence of eight two-chambered spores in the asci of Meliola sp. As this condition occurs in both old and young spores, its significance is difficult to explain. Possibly these binucleate cells can immediately give rise to a hyphopodial head and stalk cell on germination of the spore, without the necessary cell division which must precede the germination of a uninucleate cell to give the two cells ofthe hyphopodium.

Graff (1932) described the four-septate spores of Meliola as having single nuclei in the terminal and subterminal cells but with two nuclei present in the central cell (Meliola having 5 cells, as distinct from Asteridiella and Appendiculella). In the present work no such arrangement of spore nuclei was ever observed; either all spore cells were uninucleate or all were binucleate. To substantiate this is the view that, in spore germination, all the cells of the spore appear to be of similar nature; in the great majority of spores examined by Hansford the two terminal cells of the spore each produced a single capitate hyphopodium, while vegetative hyphae later grew out from the central cells and sometimes also from one or both of the end cells. There appears to be no essential difference between the potentialities of the central and end cells of any spore, and indeed Ward (1883) described 1 or more simple

protuberances arising from any one of the cells, and Graff himself (1832) attributed the same potentiality to all.

GERMINATION OF THE SPORE

The spores germinate on the leaf surface. The first stage is an outgrowth, usually from one of the terminal cells. Bornet (1851) connected the point of exit of the outgrowth with a small line, like a split, of "couleur foncée", pointing obliquely inwards from the outside wall of each cell; he compared it to the "fente" of the Puccinialos, for on germination he noticed the hypha arising from this position (Bornet 1851).

Capitate Hyphopodia

This short tube, a simple bulging out of the lateral wall, very soon becomes divided by a septum into the terminal 'head cell' and the short 'stalk cell' of a 'capitate hyphopodium'. From the head cell a haustorium is rapidly formed in the epidermal cell of the host immediately beneath it. In the formation of this primary hyphopodium two nuclear divisions take place. The first results in one daughter nucleus remaining in the parent spore-cell, the other passing to the rudimentary hyphopodium. The second nuclear division is bound

up with the differentiation of the two hyphopodial cells. Often the first of these two divisions occurs before germination of the spore, accounting for the mature spores which may contain four binucleate cells (Fig. 56).

Subsequently hyphae are developed from the same or different cells of the spore. These hyphae run over the leaf, usually following closely the epidermal outline (Fig. 4). The hyphae branch as they radiate out from the parent spore and consist of dark-walled, uninucleate cells of equal diameter all along separated by dark septa. At regular intervals, and in an arrangement characteristic of the species, the hyphopodia or 'pyriform' outgrowths, are given off, and, according to Ward (1883) are "undoubtedly of the nature of arrested branches" (See Fig. 3a).

Different functions have been attributed to these structures; Ward (1883) declared they became detached and acted as conidia, a vegetative means of reproduction, and this was also agreed to by Bornet (1851); but no proof of this has been found in this study. In 1908, Maire believed them to be no more than the first rudiments of perithecia (Toro 1952), which idea is at least partly correct although it may be an incidental

function, Toro (1952) pointing out that in this region more nutrients are likely to be present than elsewhere on the mycelium. Ryan too, (1926) saw the swollen ends of the hyphopodia as substitutes for sex organs, for of the 40 species he studied all species showed perithecia as originating from hyphopodia. But the answer to all these controversies lies in the presence or absence of haustoria.

Until the presence of haustoria was realised, capitate hyphopodia could not be attributed with an absorbing function. In 1892, Gaillard had declared the Meliolae to be entirely superficial (Graff 1932), and in 1908, Maire rejected the idea even of an osmotic interchange between hyphae and epidermal cells (Doidge 1921), although it must have been observed that the hyphae do at least cause discolouration to the host. Graff (1932) attempted an explanation of this in describing an hydrolysis of cutin by direct contact of the hyphae in the absence of haustoria, and indeed some physical and chemical change must occur.

The hyphopodial head cell has a small pore (Fig. 3b), a "spot where no colouring matter is deposited in the cell-walls, and where the contained protoplasm is placed more nearly in connection with the outside" (Ward 1883). Graff (1932), who

did not see haustoria in the M. circinans with which he worked, maintained that a thinning of the wall of the undersurface of the hyphae occurred; but in the study of Asteridiella and Appendiculella the wall thinned only where the filament emerged, as seen in diagrams already mentioned above. They are a very rudimentary type of haustorium and connect the fungus to the leaf by a very fine thread of protoplasm, the p enetrating filament; this "becomes paler as it grows away from external hyphae" (Doidge 1921), and expands just within the host cell to form a vesicle (Figs. 6a, b and d) which is described by Doidge as being "delicate, hyaline and with a single central nucleus" (1921). The filament may become thicker and darker where the cuticle is deeper, but otherwise its form is constant for a given species. Possibly the "oil globules" seen by Bornet (1851) in the head cell were the emission points of the haustoria, as this is a logical assumption from observati ons if one were unaware of the presence of haustoria (See again fig. 3b). It was held originally (and even later by Toro) that haustoria of this group penetrated only as far as the epidermal cell, and such was the case in the study of Appendiculella and Asteridiella (Fig. 7). Doidge however, (1921), described the penetration of the mesophyll cells by haustoria of Meliola sp., either the palisade or the spongy layers depending on which leaf surface was parasitised.

Mucronate Hyphopodia

As well as the capitate hyphopodium, another structure appears on the hyphae as a second form of lateral branchlet. It is flask-shaped, single-celled and sometimes open at the apex. In 1896 Thaxter remarked on its resemblance to characteristic antheridial cells of the Laboulbeniaceae, observing that they developed no further whereas capitate hyphopodia may develop into ascocarps (Graff 1932). But this is hypothetical analogy only. In agreement with Ward, who wrote that he had "never succeeded in observing anything emitted from the pore" (Ward 1883), and with Hansford (1957), these mucronate hyphopodia always appeared empty in examination, and their function remains thus unknown.

DEVELOPMENT OF THE PERITHECIUM

VEGETATIVE DEVELOPMENT

The vegetative development of the young perithecium from a lateral branch has been described by Hansford (1957), by Ward in 1883 in much detail, by Toro in 1952 and by Bornet. Bornet believed perithecia to be confined to the lower surface of the leaf, the mycelium in the upper surface being sterile (Bornet 1851); but this is certainly not so and perithecia

were found on both sides of the leaf. The lateral branch from which the perithecium develops is short and becomes two-celled, with the upper cell round (Figs. 5a and b). Because of its resemblance to a capitate hyphopodium it has caused much confusion as to the origin of the perithecium, although much earlier Ward had noticed that not all hyphopodia had the "bright spot" and had indeed suggested the presence of two different types of these lateral branches (one for reproduction and one for absorption) (Ward 1883). The perithecium does not arise from a hyphopodium, and the absence of the light-coloured, refractile, round structure (from which the haustorium grows) confirms this. Perithecial development usually occurs only when the maculae are about 3 mms. in diameter (with as much as 5 mms. in H. circinens as studied by Graff (1932), or occasionally a little less than 3 mms. in other species).

Division

As Ward has so accurately described the division of the lateral branch to form a perithecium, this part of the life-history is best treated by quoting his relevant passages: "The simple pyriform body, after becoming more swollen, has suffered division into two portions or cells by a septum,

usually vertical to the plane of the mycelium a nd leaf, and passing diagonally across the cavity with a slight curve, so as to abut on the outer walls at right angles, or nearly so. The originally unicellular protuberance becomes in this mannef divided into two more-or-less unequal cells, and it will be shown in the sequel that these two cells have, from the first, each a different destiny in the formation of the fruit. The more apical cell, which is smaller.....produces thé central ascogenous tissue of the young perithecium, while the other..... originates the outer portions of the case or perithecium wall..... a septum appears across the larger of the two cells.... rapidly followed by another septum and so the larger cell becomes cut up into three.... a number of further divisions in planes at right angles to the preceding are soon established, and at the same time, though much more slowly, one or two more division walls are formed" in the more apical cell (Ward 1883).

Differentiation

Thus the division of the more rapidly growing cell (the inner one) results in the production of a sheet of cells affixed to the few-celled mass resulting from the slow division

of the more apical cell. The cells resulting from this latter become gradually enveloped more and more in those resulting from the more rapidly dividing one, the mass of cells enfolded being termed the 'ascogenous core'. This story was later confirmed by Ryan (1926); it is exemplified, in a few of its stages, in figs. 8 b and 11 a - c.

The young perithecium becomes hemispherical in shape, and tangential and radial divisions give an internal mass of uninucleate, hyaline, thin-walled cells surrounded by the enfolding layer of larger, dark- and thick-walled cells (Fig. 13). Ward (1883) saw these outer cells to be "extending as a curved layer over the 'core' of cells". When parasitised by other fungi (a very common occurrence), the central core becomes absorbed, as shown in Figs. 12 a and b, and the perithecium is hollow.

REPRODUCTION

On the sequence of events from this purely vegetative stage until the formation of spores virtually no work has been done by any previous worker. Ward (1883) vaguely describes the central lower part of the perithecium as one in which "certain cells with very delicate outlines and finely granular refractive contents, maintain their larger size and upright arrangement, and are.....readily distinguished by their special peculiarities

and no question can be entertained as to their significance in the formation of the essential parts of the fruit-body. This group of cells is the forerunner of the young asci and may be termed the 'ascogonium'" (Ward 1883). Hansford merely mentions this core as a "central mass of hyaline, thin-walled parenchyma , at the base of which a number of asci are formed" (Hansford 1957). The present work endeavours to bridge this gap.

Fusion

The earliest stage seen in reproduction consisted of two densely protoplasmic cells with larger nuclei than those in the surrounding cells, thus indicating reproductive cells — 'sex'cells (Fig. 14); these were contained in very young perithecia with few other cells. These cells then fuse to give a single binucleate cell, as seen in Fig. 15c and also in 15 a and b. This seems a very logical conclusion from close examination of many sections, for these cells so described were the only such types seen in their respective perithecia (examined in serial sections), and were surrounded by cells of a vegetative nature with very small nuclei.

Graff described two short stalked cells arising within the stroma; they were oval in shape and attached to the side wall of the perithecium.. These were considered true sex organs. But the description is unconvincing and could not be substantiated for the two genera under consideration (Graff 1932). His diagrammatic representation of this stage, shown as containing many binucleate cells, is possibly a far more advanced one and the 'oogone' could easily be the first ascus.

Toro (1952) could find no sex organs in M. capsicola although he believed they might be present in a very reduced form. He saw no nuclear fusion until the young ascal stage. Toro's report, like Ward's, suggests that the interim stages were not seen. Ward (1883) described stages involved in the ascogenous tissue only after the development of wall cells below the ascogenous body. At the stage of cell fusion there are none of these lower cells (Figs. 14 - 16), a fact also substantiated by Graff's illustrated section of perithecia at this early stage. (Note the beginning and developing of thick-walled cells at the base of the perithecium in fig. 19 b — a stage at ascal development (NOT at cell fusion) — and a more advanced stage in Fig. 55).

Ascogenous Hyphae.

From this original cell arise, by cell division, septate ascogenous hyphae, which penetrate the original stromatic centre of the peritheciun and spread over the enlarged ascocarp base as rather enlarged cells so that at this stage many binucleate cells are seen among the uninucleate stromatic cells (Figs. 16 a - e). The nuclei of these cells are larger than those of the vegetative cells. In older perithecia the ascogenous hyphae can be seen as hyphae (definite attached cells — Figs. 20 and 16 a) and usually lining the floor of the peritheciun.

The ascogenous hyphae may branch (Fig. 16 c). Although Ward was unable to decide whether any branching did take place he thought it highly probable. Branching in ascogenous hyphae may not always be seen; however, it was at least indicated by the ascal arrangement (Figs. 31, 34, and 35). Graff did record branched ascogenous hyphae although, in his observations, they were coming from the cells arising from the fertilised "oogone" (Graff 1932).

Crozier formation.

From studies in older perithecia it is evident that crozier formation precedes ascus formation. That none were seen in the very young perithecia in which a single ascus was developing, may be due to the immediate fusion of the two nuclei within the cell to give the ascus. But that the ascus is produced by crozier formation in older perithecia is certainly evident. A complete hook was seen in one section (Fig. 17 a), and evidence of it in many others, particularly the later stage of the hook, in which the tip had fused with the penultimate cell to form another binucleate cell below the prospective ascus (Fig. 17 c). Within this upper cell, the two nuclei fuse to give a very large fusion nucleus (Figs. 23 a, b and 35). Crozier formation was also recorded by Graff (1932), but his ascogenous hyphae were uninucleate except for, what he described as "temporary increases"; his illustrations clearly show binucleate cells, but these are not discussed as such.

The Ascus.

The first ascus in the young perithecium is alwaysin a cavity (Fig. 18). In some sections the presence of a gelatinous, structureless mass around this ascus suggests the digestion and absorption by the developing ascus of the strom-

cells around it to allow of its enlargement, as though these cells are especially for nourishing (Cf. Fig. 61 with Figs. 55 and 58). Ward (1883) describes this space as "filled with an almost transparent semi-fluid mucus;.....a jelly-like mass of swollen and fused cell-walls". It is however, a break-down of the cellular material, not a distortion of the cells as no such cells as described were seen in the present study.

The inner stromatic cells simultaneously elongate in the horizontal plane and also divide. This results in the formation of a perithecium, which by this time is rounding up, with darker-walled cells both below and above. The formation of this shape leads to a separation of the core cells of the perithecium, assisting in the enlargement of the space for the developing ascus. The appearance of new, dark, thick-walled cells below the ascogenous mass, as a continuation in development of the original wall cells, occurs after early ascal development. Ward (1883) however, records variants, wherein complete enclosure of the inner mass occurs before ascogenous development; such statements are probably due, as was remarked earlier in this section, to his inability to find, or recognise, early ascogenous stages.

Toro, too, observed this cavity, formed because "as more cells are produced, in a centripetal fashion, the central cells, which are the oldest, become detached and a cavity begins to form"; but his following statements — that "those cells that have detached and become free, round up and then elongate, becoming the initials of the asci", and that the asci are "formed consecutively as small protuberances of the nurse cells adjacent to the wall surrounding the centrum" — are based on very incomplete observations (Toro 1952).

In young perithecia a few asci appear to arise simultaneously (Fig. 19 b), but later they are produced continuously so that at any one time all stages of ascal development may be seen. Even in old perithecia there are always some young asci developing around the periphery, while in the inner and older portion of the perithecium the ascospores may be maturing or matured.

The fusion nucleus is extremely large and must grow considerably before its first division, since the two daughter nuclei are almost as large as the original fusion nucleus (Cf. Fig. 23c with 25 a or b). This apparent enlargement in the fusion nucleus was also noticed by Graff (1932). The first division of the fusion nucleus, a meiotic division, is by a

vertical spindle near the centre of the ascus (Figs. 33, 36 and 23 c). Graff (1932) described the first nuclear division as being parallel with the long axis or slightly oblique, and Ward (1883) described a sharp division line forming on the first division of the ascus which was oblique to the long axis. This was certainly not seen at such an early stage in division in the species studied, but it may be a feature characteristic of the genus Meliola with which both authors worked.

The second division is at right angles to the first (Figs. 40 and 38), to give four nuclei lying in a horizontal plane but often at different vertical levels (diagrammatically shown in Fig. 26 e). At this stage the protoplasm segments around these four nuclei (Figs. 25b, 21 and 38). It is not uncommon however, for eight nuclei to be present (after the third division) before segmentation begins. In certain squash preparations of a perithecium of Meliola eight young spores, hyaline and four-septate, were present in some asci; this is not at all unusual in this genus, but normally only four are originally formed, of which two usually (or one or three) eventually mature, the others degenerating (Figs. 31, 25 a and d, 21 b and 50).

The protoplasm cleaves into rather wedge-shaped portions in each of which one nucleus lies; these portions later re-arrange themselves and become the spores (Fig. 39a), the residual ascal protoplasm, the epiplasm, remaining very dense (Figs. 26, 28 and 46).

The asci, thin-walled and densely protoplasmic in their younger stages, are quite irregular in outline at first (Figs. 30, 32, 23 and 25 e), but later tend to the more typical clavate shape (Fig. 51). They have only one wall and this probably accounts for, in part, the early dissolution of the ascus following its distension during spore development. However, an account from Toro's work on "Tropical American Black-Mildews" (1952) is very interesting in that it describes an ascus-wall of two membranes. He writes: "Sometimes in specimens from dry places, as represented by M. nigra Stev..... the asci do not deliquesce too early and then it is possible to observe that the wall is made up of two membranes, the inner one closely surrounding the ascospores, the outer one thicker in the upper part and terminating in a short pedicle in the lower". This is apparently not at all analagous with the "two-walled" condition of Luttrell's Bitunicatae, since Toro had earlier (in the same article) denied the presence of a definite elastic inner wall.

Ascospores

Spore development could be followed only in the genus Appendiculella (which has a fourcelled spore), as the specimens of Asteridiella were either not well developed past the ascal stage or else parasitism had prevented further development in the material available.

Commonly two spores reach maturity in an ascus. Following the second division, the four nuclei are already the initials of the ultimate spores, and the third division gives the second nucleus of each spore around which the protoplasm is collecting (Fig. 53); however, usually two spores only continue to develop. Graff (1932) records the presence of four cells in the beginning, but that later two grow at theexpense of the others andenlarge as the others become thinner. When eight nuclei develop before actual cleavage into spores, they usually belong to two spores, each then having four nuclei (Figs. 34 and 25 d). In some asci one of the first or second division nuclei will give rise to a spore ahead of the other nucleus or nuclei (Figs. 29 and 43), and a single-spored ascus has been seen (Fig. 28) among the many asci examined. Of fairly common occurrence is the cleavage of the ascal contents into three portions around eight nuclei; one of these segments usually degenerates, leaving two spores to mature (Figs. 26, 30, 21 a and b.).

Occasionally four spores develop (Figs. 53 and 17), usually all lying parallel with the vertical axis, suggesting the second division spindle to be in the horizontal plane. Bornet (1851) described occasional occurrences of spores lying crossed or at an acute angle within the ascus of the Meliola sp. with which he worked; this is shown to a slight extent in Fig. 27. Apparently the spores elongate vertically soon after being cut off from the ascal protoplasm, as in a few cases these four spores are seen arranged in a tetrad fashion in their very early stages (Fig. 40), where later extension of one axis only would bring them into the common arrangement (Fig. 41), and one in which they lie following the curve of the enlarging ascus wall on their outer walls (Figs. 45 and 52).

Following another division of the now binucleate segment, the young spore is thus usually four-nucleate on its complete separation from the surrounding ascal protoplasm (Figs. 48, 29 and 42). Cross-walls are then formed, the first one being the central one (Figs. 47 and 28), although it can appear first as cutting off the two end cells and leaving a four-nucleated central cell (Figs. 48 and 26 b). This latter method is also recorded by Graff (1932) in his description of the nuclear division which forms the 5-celled

spore characteristic of some of the genus Meliola (he having studied M. circinans - Earle). Here the spore reaches a four-nucleate stage, and has two end cells cut off. The remaining two nuclei divide into four. But this central portion is divided by only two septa so that ~~only~~ two nuclei are left in the newly-formed central cell (and only one nucleus in each of the other four cells). This anomaly on unequally nucleated cells is thus accounted for by seemingly normal nuclear behaviour. As a four-celled spore only was studied in this present work, the presence of a central binucleated cell in a 5-celled spore cannot be entirely discarded as erroneous; however, access to mounted specimens of Meliola species certainly showed no such condition. Toro either accepted the fact as stated by Graff, or did actually observe this phenomenon, but there is no evidence of this observation in his work as stated by him, on the "Black Mildews" (Toro 1952).

Finally, a four-celled, four-nucleated spore results (Fig. 52), later developing thickened and darkened walls (Fig. 57). The nuclei appear to lie in no fixed area of the cell; Graff (1932) however, records the confinement of each nucleus to the outer side of the spore. The cells of the spore become more

rounded as the spore matures, changing from a spindle-shape to an almost plano-convex shape with constrictions at each septum; oil drops may then accumulate. At this stage, or even while the spore is still immature, the thin-walled asci, containing now a very small amount of epiplasm, disintegrate and the spores lie free within the perithecium (Fig. 58 c).

'Paraphyses'

Simultaneous with early ascal formation is the development of 'paraphyses'. The original central space has enlarged as the outer lining cells become elongated — the side-walls vertically and the upper cells thus horizontally (Fig. 55) — and then break away (Fig. 65), and the hyphal structures grow into this, arising in the base of the perithecium. Although Ward (1883) described only very briefly the few paraphyses which he saw scattered through the perithecium, he did at least note their origin as being in the perithecial base. They are rather wide — almost half as wide as an ascus — usually branched in an irregular manner and often arising from a bulbous base (Fig. 63). Because of their similarity in origin to an ascus they were immediately assumed to arise from ascogenous hyphae; and this was later confirmed (in better fixed specimens) where two nuclei were seen in some of these upright hyphae (Fig. 60); in fact, in some cases it appears as if the asci

are developing into 'paraphyses'. The binucleate fragments of hyphae in the middle region of the perithecium, as seen in Fig. 61, are assumed to be the turned-in tips of other 'paraphyses', from which they had been severed in sectioning. Those ascogenous hyphae which were scattered above the original binucleate cell also appear to give rise to hyphae which line the perithecial wall and dip into the cavity, having elongated into narrow cells in the vertical direction, but still often retaining their two nuclei (Figs. 55 and 58).

In view of the binucleate condition of these 'paraphyses' and of their probable origin from the ascogenous hyphae, they could be regarded as 'abortive asci'. They do not correspond to either 'paraphyses' or 'paraphysoids' as hitherto known, the former arising after formation of ascogenous hyphae but from cells adjacent to ascogonia, the latter formed from cells of the internal stroma prior to development of ascogenous hyphae. Both are uninucleate.

The 'paraphyses' are distinguished in their early stages from asci by their narrower width together with their, as yet, large nuclei. Apparently some ascogenous hyphae form asci, others form 'paraphyses'. This certainly does not agree with Toro's conception of the interthecial tissue which he describes as

"multicellular threads which are connected above and below, separating the individual asci...especially at the time of maturity of the ascocarps" (Toro, 1952). Such connected filaments were never seen in any stage later than that of the formation of ascogenous hyphae in Asteridiella sp. (Fig. 16 e); at maturity the threads grow upwards from the base only, and each thread is one elongated cell only (and, indeed, Toro's photographs, even though of Meliola, seem to indicate this). Note: the presence of intrathecial filamentous tissue connected to the top and bottom is a characteristic of the Dimerium group of Ascomycetes (shown in Figs. 77 - 79 as parasitising an Asteridiella sp.). There is more paraphysoid tissue in Asteridiella than in Appendiculella, and it remains for a longer time after spore maturation, finally forming rather structureless-looking strands. These probably correspond with Graff's "paraphyses" which he described as becoming irregularly cytoplasmic and often lacking nuclei in the older perithecia (Graff 1932).

These 'paraphyses' appear to have no real function due to their rather late appearance in perithecial development — they are certainly not present in the pre-ascal stage. In Asteridiella eucalyptorum however, they are more evident around

the inner edge of the perithecium than in Appendiculella; the inner portion of the perithecium develops first (as spores appear here much earlier than those towards the periphery), and perhaps in this outer, more confined and shallower space, where asci are still forming, the presence of upright filaments is necessary to enlarge the space for ascal development.

Periphyses.

The development of the periphyses precedes that of the apical opening, the ostiole. The periphyses are very thin-walled and sparingly protoplasmic and they disappear in the very old perithecial stage. They are clearly seen in serial transverse sections of the perithecium, growing near the top (Fig. 67) where they fringe the ostiole. They arise from the wall cells in the uppermost part of the perithecium (their position of origin as reported in Graff's paper (1932) is "slightly over half way up the inner portion of the perithecium") and give off branches near the ostiole (Fig. 62). These stromatic wall cells have a rather large nucleus (Fig. 66) and apparently cut off the filamentous uninucleate hypha of the periphysis which has a small nucleus. The periphyses lie almost parallel to the top of the perithecium and are a few cells deep below the ostiole. In most of the literature periphyses are unrecorded; they may, however, be equivalent to Ward's

"papilla". Graff (1932) and Fischer, 1897 (Graff 1932), have been the only ones to mention them under this name, and both described them as protruding from the ostiole. At maturity the periphyses protrude slightly, the part so exposed usually becoming thicker and darker-walled.

The Ostiole and Dehiscence of the Peritheciun.

The Ostiole itself is formed while the spores are semi-mature; it is very narrow in comparison with spore width and the presence of very old perithecia with their top quarter broken open to leave a more cup-shaped structure, Bornet's "cupule", suggests that the ostiole alone may be an insufficient outlet for spore dispersal. This has also been described by Bornet, who wrote, as early as 1851, that "dehiscence takes place below, the whole of the upper part of the peritheciun becoming broken away by a circular rupture at the base" (Ward 1883); but he also described a "minute and dilatable" pore at the apex through which the spores escape. In 1897, Fischer described a well developed ostiole as appearing only at maturity (Graff 1932), and Graff himself agreed with this, while also mentioning the rupturing of the peritheciun around its base. Presumably both methods are used in the dispersal of the ascospores.

Sometimes, in the older perithecium, upright cells with unthickened walls are seen around the ostiole as if the cells of the upper wall have elongated upwards (Fig. 71) as they are much wider and paler than protruding periphyses; it is more probable that this is what Ward referred to when he described "a slight protuberance" (Ward 1883).

The old perithecium has only a thin layer of flattened cells lining the inside edge, with perhaps hyphal remnants on the inner lower surface. The outer surface of the perithecium appears as an embossed pattern due to the angular shape of these thickened cells. Spores may remain inside the perithecium for a long time and may even germinate there.

Almost as soon as the young stromatic perithecium is formed, both appendages (in Appendiculella only) and radiating hyphae develop. These radiating hyphae (Figs. 68 and 70 a) appear to support the sessile perithecium, as its attachment to the hyphae and to the leaf is rather insecure particularly when it enlarges. They resemble the ordinary vegetative hyphae and are seen to arise from an outer cell of the perithecial wall (Figs. 12 a, 13 c, 14 and 18; and 70 c in T.S.); that they are certainly growing away from the perithecium is evidenced in the outward-directed hyphopodia (Fig. 70 b);

In Appendiculella, appendages arise quite early and are visible, although small, in perithecia with as yet only few cells (Figs. 9 and 13); but they usually enlarge greatly towards the end of perithecial development. They are dark-brown structures, tapering but with a blunt end, and are striated in the walls only, due to the fact that the growth outwards is in successive stages (Fig. 68).

DISCUSSION

This study of the life-history of the Heliola group has brought to light some interesting facts (particularly from the taxonomic point of view), some of which are new and some different from those already described. We have here a fungus with a rudimentary 'ascogonium' developing in an already differentiating perithecium; and one where the 'ascogonium' is derived from the fusion of two cells modified for this purpose, so-called 'sex' cells. From this binucleate cell, binucleate ascogenous hyphae develop which branch and give rise to asci through a crozier formation; ascospores are consequent on the fusion of the two nuclei and the subsequent mitosis. Upright binucleate filaments, alias 'paraphyses' also develop from these ascogenous cells but appear to be 'abortive' asci rather than to function as paraphyses. No spores containing dissimilar numbers of nuclei in their cells were seen, there being, usually, one nucleus or, occasionally, two nuclei per cell. At maturity the spores are either released through a pore lined with periphyses (the ostiole) or the perithecium may also rupture at the base.

Many of the controversies arising from these results have been discussed contemporaneously in the main text of this thesis; the reason for this was explained in the early part of the

work and was, basically, to avoid tedious repetition. As classification should express phylogeny, the knowledge of the life-history of the fungus is very important and indeed necessary for an accurate assessment of its position. In previous work on this group classification based mainly on morphology.

Apart from the earlier ignorance of the life-history itself of the Heliola group, the wide variance in definition of terms in fungal anatomy on the whole, particularly in the Ascomycetes, has led to much confusion in taxonomy. Perhaps the most frequent error is in the misinterpretation of the term "perithecium": this was originally to be restricted to the products of true sexual reproductive organs in which cells from the oogonial stalk are the initials of the enclosing body. 'Ascocarp' should thus be applied to the ascus-bearing fruit until its origin is definitely determined. In this Discussion, therefore, 'ascocarp' can now be used without causing confusion, and the nature of the ascus-bearing fruit of the Heliola group certainly warrants another term than 'perithecium'.

Important taxonomic characters are the nature of the sex organs and the distribution medium of the asci (whether it is a vegetative stroma or sterile tissue from the reproductive cells); also, the origin itself of the sterile tissue is relevant.

Among the purely morphological characteristics originally used, such as the ostiole, ascal shape, spore septation etc., a recent addition is the structure of the ascal wall; this character was introduced by Luttrell and used as the basis of his classification of the Euascomycetes (Luttrell 1951). Luttrell's classification is the main one used in this Discussion later for determining the taxonomy of the Meliola group.

The Meliola group were originally considered as the tropical representatives of the Erysiphaceae; Fries described Meliola as a "genus in tropicis vulgatissimum ut Erysiphes in terris temperatis" (Toro 1952). Toro (1952) has shown that, provided the appropriate host is present, constant humidity rather than high temperature is probably responsible for the abundance of some species in temperature latitudes. Ward (1883) considered the Meliola group as a branch from the Erysiphe stem, and which had developed along lines more or less parallel with the Erysiphe stem. But the union of either two seemingly vegetative hyphae or of definite oogone and antherid prior to ascocarp formation in the Erysiphe group is far removed from the findings of the life-history of Meliola group in this thesis. In addition, the Erysiphe group lack ostioles. From his work on detailed morphology and some internal development however, Ward did realise, even from the little information obtained, that the development of the fruiting

body is important and should be taken into account.

The Meliola group forms, with two other genera, the slightly larger group, the Meliolineae, distinguished from all other members of the Meliolaceae by their multiseptate spores — the "Phaeophragmeae" in the classification used by Hansford (1946). The Meliola group itself comprises those genera with globose perithecia, for example Meliola, Asteridiella and Appendiculella, while Actinodothis and Amazonia are the other two members of the Meliolineae but separated from theformer group by their applanate perithecia. Hansford's placing of the Meliolaceae in the order Myriangiales is based on the formation of asci within a stroma where, in the Meliolaceae, the stroma is reduced to a unilocular ascocarp. In this way it is separated from the Erysiphaceae only by virtue of its hyphopodia and dark-walled cells and its lack of internal mycelium.

Perhaps the first classification to deal with the ascus structure and the character of the centrum was devised by Miller in an endeavour to produce a more natural system (Miller, 1949). He divided the Ascomycetes into four, perhaps five, large groups based on ontogeny as well as morphology, and ignored such characters as position of ascocarp relative to the substrate and colour of the stroma, believing them to be not fundamental.

Thus, from the developmental point of view, Meliola would fit well into his Plectomycetes, for in this group the asci are without a pore and lie at irregular levels in loose or pseudo-parenchymatous tissue composed of ascogenous hyphae (and with a tendency later for the asci to lie in monascal locules whose partitions are then remnant stromal elements), and the spores are liberated within the ascocarp. In his "Tentative key", however, the Plectomycetes are distinguished by closed ascocarps, so that, while according to him, Erysiphales is accommodated, Meliolales is not. Meliolales would, therefore, in this key, have to be placed within the Pyrenomycetes, and indeed a few of the morphological characters do fit this group. But not all characters of any one family within the Pyrenomycetes are satisfied by the Meliola group — his first group of the sessile ascocarp subdivision demands a true perithecium, and his second group, although stromatic, has neither periphyses nor differentiated wall to the ascal centrum.

But, even so, in this way, the purpose of Miller's classification is defeated, namely, a classification based on ontogeny as manifested in ascal and centrum structure. Erysiphales arise from union of sex organs pri-or to ascocarp development and produce a true perithecium, albeit a closed one. They are placed, however, in the Plectomycetes even though their spores are liberated

outside the ascocarp. The Heliolales on the other hand, show stromatic development of the ascocarp within which the ascospores are liberated, yet they are ostiolate (and due to this, cannot be even considered in the Plectomycetes in which they would more logically fit even to the exclusion of the Erysiphales). They would thus appear to have no position in this scheme as it stands; the fact that Nannfeldt (Miller, 1949) did include ostiolate groups in his Plectoscales on the comparison of early deliquescing asci unfortunately did not influence Miller in including them in his analagous group. Toro, too, has suggested a similar scheme (Toro, 1952), in that, if Nannfeldt's classification is followed, the Heliolaceae could be included in the Plectascales, his arguments being based on the perithecium-like ascocarp of the Heliola group and the clearly differentiated opening for dehiscence. This then would seem to be the best of all classifications both in its origin and application. And in including the Myriangiales among the Plectomycetes as Miller has done, then such a revised scheme of Miller's as just suggested (i.e. Heliolales placed in the Plectomycetes) would correspond with Hansford's placing of the Heliolaceae in the Myriangiales (Hansford 1946).

Of course, Miller did realise that Meliola and its related genera needed more investigation before they could be satisfactorily fixed in the system, and on this belief too, he separated the Erysiphaceae from the Meliola group and raised them to an order, Erysiphales. Similarly, Arnaud in 1918 (Toro, 1952) had created the new tribe, Meliolinées for the group containing Meliola to separate them from the other Perisporiales. This concept of Arnaud was followed later by Hansford (and others) but the tribe was raised by Hansford to the status of family, Meliolaceae, on a level with the Erysiphaceae, disregarding in his classification, the type of ascocarp.

The most recent classification of the Pyrenomycetes was compiled by Luttrell in 1952. Although based on newer concepts, it represents perhaps, at the moment, more a basis for discussion than a radically new system for classification. The fundamental criteria are ascus structure and centrum structure. The possession of two or one ascal walls divides the Euascomycetes into the two large series, Bitunicatae and Unitunicatae, and these are sub-divided according to ascocarp development and the consequent formation of characteristic centrum structures. The centrum comprises the asci and sterile tissues, and variations in centrum structure depend to a great extent on differences in the sterile tissue in which the asci develop; originally thought of as a nourishing

tissue only, it has recently been suggested (and it is considered by Luttrell) that this sterile tissue may serve more for creation of locules in which the asci develop.

As mentioned earlier in this discussion, Luttrell's taxonomy, which is the most recent, would be used here in endeavouring to classify the Heliolineae; not because it is the best nor even the most accommodating, but because its ideas merit such an attempt.

In the following, the relevent character of the Heliola group is described, (a), and its 'a priori' position within Luttrell's classification is then discussed, (b).

1a The ascus of the Heliolineae has one wall which is extremely thin, but uniform, and deliquesces early in the life-history of spore development.

b This places the group in the Unitunicatae. Although such ascal walls are usually thickened at the apex and laminated in structure, this need not always be so. Typically too, ascospore discharge is simultaneous, but this is certainly not so in the Heliolineae.

2b The Unitunicatae is subdivided into eight types, of which the Onhiostoma type would well include the Heliola group and would be the only one of the eight to do so. Here the asci are "globoid, non-stipitate, uniformly thin-walled, and lack a pore. The ascus wall deliquesces to liberate the ascospores within the ascocarp". (That Luttrell considers this type to be characteristic of the Plectomycetes further confirms the ideas suggested earlier). The separation of the Heliola group from the Erysiphe group is very marked at this stage, for in the latter type the asci swell at the time of spore discharge and rupture the ascocarp; they protrude and burst and the spores are forcibly discharged.

3a The ascocarp is of a stromatic nature in that it develops by proliferation of a purely vegetative cell; following absorption of the thin-walled "centrum" by the developing asci, the mature ascocarp is left as an outer layer of dark-walled tissue only. The presence of binucleate upright filaments (in the position of paraphyses) — "binucleate paraphyses", or preferably, "abortive asci" — presents the main difference between the Heliolineae

and other groups. The presence of periphyses inside a definite apical pore (ostiole) also separates the group from others such as Erysiphaceae, etc.

b. In discussing the morphology of the Centrum, Luttrell again obtained eight different types, but it is proposed here, to discuss only those which are relevant to the Meliola question.

(1) although there is close agreement between the ascus of the Meliola group and the Ophiostoma group, the analogy does not hold so well between the centrum development of the two: in the Ophiostoma type of Centrum (type V), ascogonia are free upon the mycelium and are enveloped by branches from their stalk cell or by neighbouring vegetative hyphae. Most of the consequent development, however, is similar.

(2) The stromatic types must unfortunately be discarded as prospective groups for Meliola and its allied genera, on the basis primarily of bitunicate asci, and then on such characters as pseudo-paraphyses attached to the top and bottom

of perithecia (See Figs. 77 - 79 of Dimerium) as, for example, in the Pleospora type (type 11), the lack of ostiole and periphyses in the Eleince type (type 111) and so on.

(3) The true perithecial types must also be discarded as these presume the development of the outer wall from the stalk cell of an ascogonium. However, periphysate ostioles, as in the Heliolineae, do occur in this group.

(4) Luttrell discusses Heliola under the Phyllactinia type (type V111), basing this on Graff's description of the life-history of Heliola circinans. Although he was not convinced by these descriptions he felt there did exist a few similarities and that, in any case, there were even less resemblances shown to other types. But the ascogonia are free on a superficial mycelium, a true perithecium is developed and it is non-ostiolate.

The difficulty in fitting in Heliola appears to arise from its lack of true sex organs, or conversely the presence of greatly reduced sex organs. The two cells which become modified and fuse, lying within a pseudo-parenchymatous mass of cells could supposedly be called rudimentary sex cells; but they must be considered as developing within a pseudo-parenchymatous stroma — a perithecium-like stroma whose outer walls have become thickened. The fungus cannot, therefore, fit into the Pyrenomycetes but only into a stromatic group — a stromatic group in which the stroma is the ascocarp itself.

With these facts in mind, the Heliola group could now be placed in Luttrell's subseries, Plectomycetes. The fact that Luttrell has included the group, as the family Meliolaceae, together with the family Erysiphaceae within the order Erysiphales of the Pyrenomycetes is wrong in the light of present knowledge.

Because of its periphysate ostiole and abortive asci, "binucleate paraphyses", the group could not however be considered under any of the orders designated by Luttrell within his existing Plectomycetes; to take one example, in none of the eight types of centrum are there described abortive asci (or other corresponding term). Thus, another order, the Meliolales, would have to be introduced and included in this subseries.

Martin's classification, dissatisfying to Luttrell, seems very satisfactory in the case of Meliola. His Pyrenomycetes include all ascomycetes bearing asci in cavities with or without a small opening. This large group is divided immediately into orders, of which the Meliolales is one; it is characterised by stromata frequently resembling perithecia, a largely superficial mycelium, and a unilloculate stroma not notably flattened. Although this classification is based on morphological characters only, the result seems more comprehensive than Luttrell's. It can be noted here, too, that under this system, the Erysiphales are separated off at the level in which true perithecia occur, and later characterised from other orders by their superficial mycelium and lack of an ostiole.

However they may be classified, it is obvious that the Meliola group are of equal importance with the Erysiphe group, and markedly different from them; they are not, as Fries, Lóveillé and Bornet believed, mere "tropical representatives" of the Erysiphes.

LIST OF ILLUSTRATIONS.

Appendiculella calostroma

1. Germinating ascospore, showing progressive stages from a - d. (x 730)

2. T.S. of germinating spore through the cell which is producing the outgrowth. (x 730)

3. Hyphopodium formed

   a) immediately after germination of spore and showing septation into head and 'stalk' cell. (x 470)

   b) along hyphae, and showing, from above, the pore (indicating point of emission of penetrating filament) (x 730)

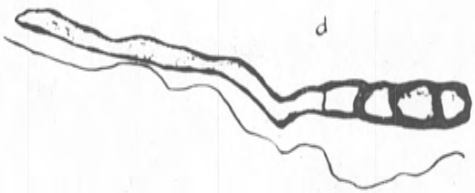
4. Mycelium, showing hypha following configuration of epidermal cells. (x 470)

1a

b

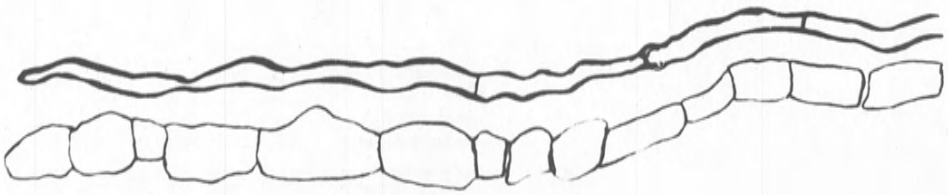
2

c



d

3a



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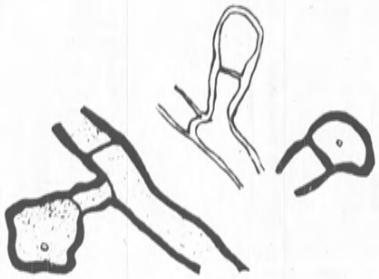


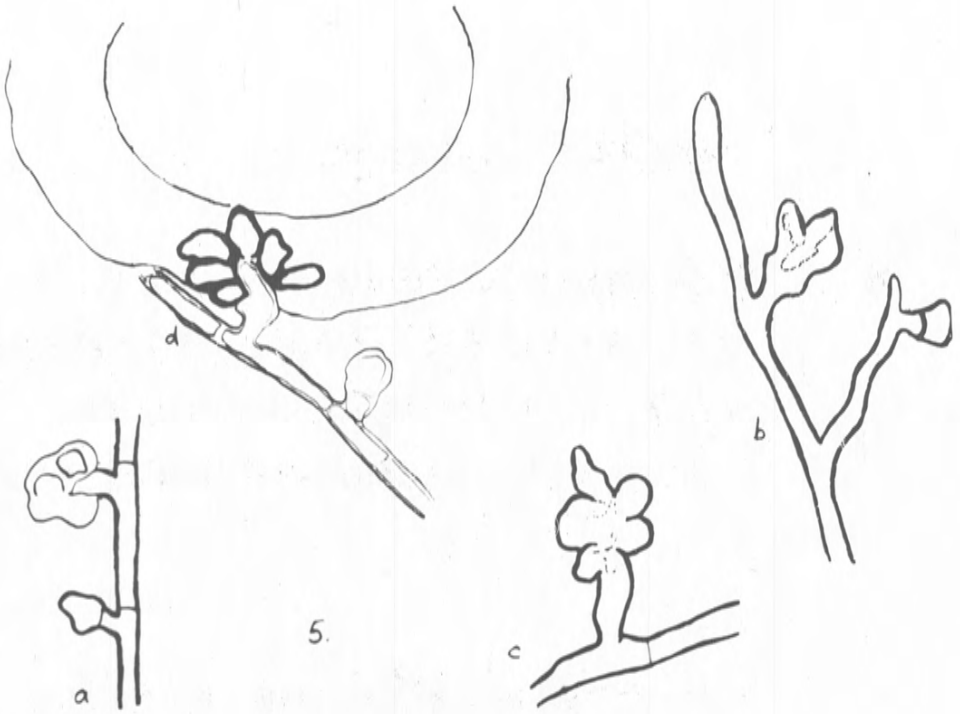
3b

Appendiculella calostroma

5. Development of perithecium from hyphopodium head cell. a - c show the flat plate of cells being formed. d T.S. perithecium, having been formed by further division of these cells (x 470)

6. Cross section of hyphodial head cell showing haustoria being sent into epidermal cell (x 1730) and in c, into wall between epidermal cells (x 730) Note penetrating filament in b.

7. T.S. of hypha, and L.S. of stalk and head cell and the haustorium. (x 730)



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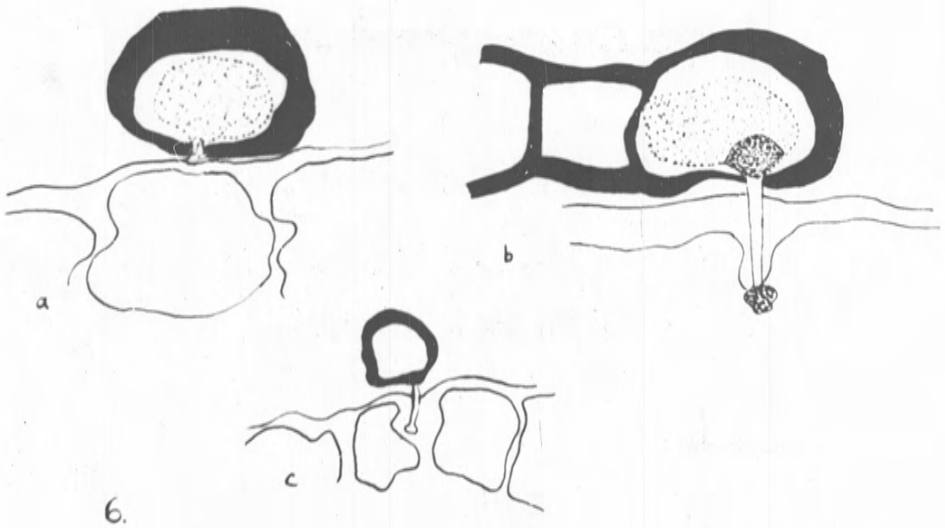

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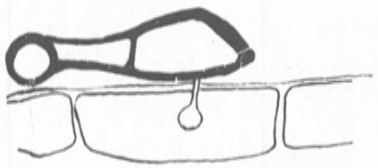


7.

Appendiculella calostromæ

8. T.S. of young developing perithecium showing
   a. initial cell and b - c the subsequent cells from its division.
   b. view from the side
   c. view in section. (x 470)

9. Outside lateral view of very young perithecium showing formation from hyphopodium, and appendage on top. (x 470)

10. L.S. through very young perithecium showing upper thick-walled cells and lower thin-walled cells. (x 470)

11. Diagrammatic L.S. of young perithecium in successive stages of development, showing, in c, an upper and lower layer of cells both of which have developed simultaneously. Note: all cells of vegetative nature — see also Fig. 13. (x 470)

12. a. L.S., and b. T.S. of perithecia showing hollow centre following parasitism. (x 470)

13. L.S. of young perithecium showing an inner mass of thin-walled uninucleated cells at a stage later than 11c. Note development of thickened walls in the outer cells. (x 470)

9.

a

8.

b

10

c

11.

a

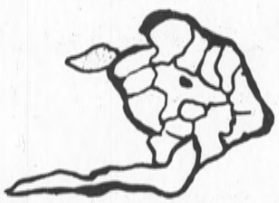
b

c

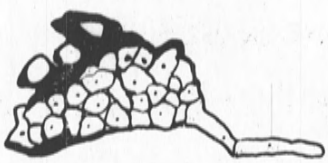
a.

12.

b



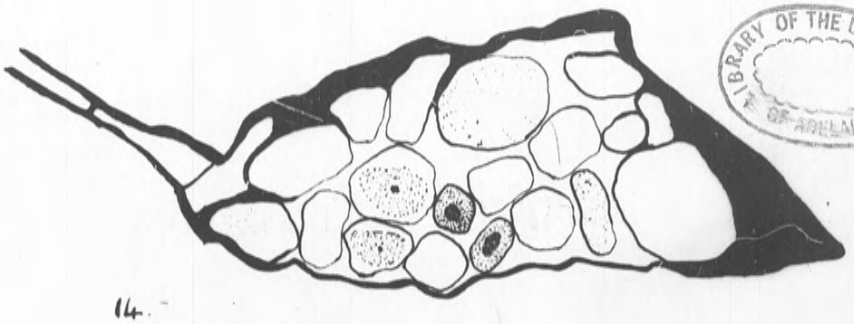
13.



Appendiculella calostroma

14. L.S. of perithecium showing (possibly) the two modified or 'sex' cells of the inner stroma (densely protoplasmic with large nuclei) prior to their fusion. (x 1730)

15. a. and b. L.S. of perithecium at the stage immediately following 14, showing the one binucleate cell resulting from fusion of the 2 'sex' cells, in a central cavity.
Note: large nuclei in comparison with that of an adjacent vegetative cell. (x 1730)



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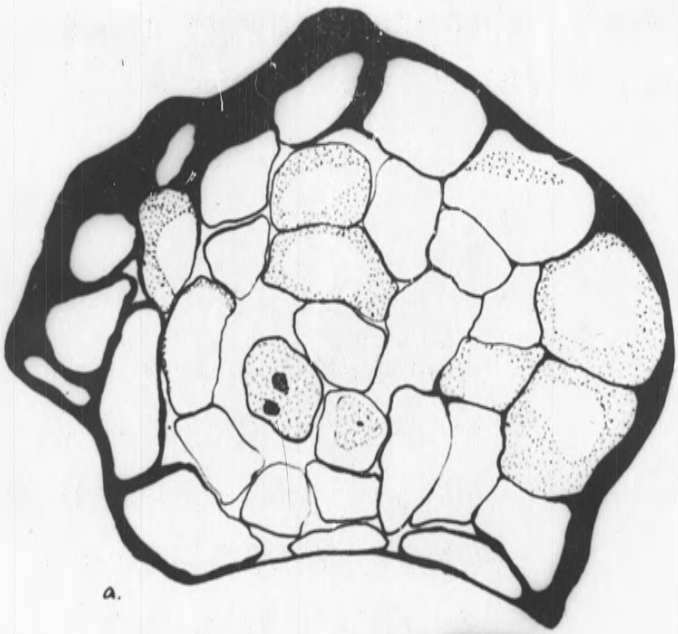
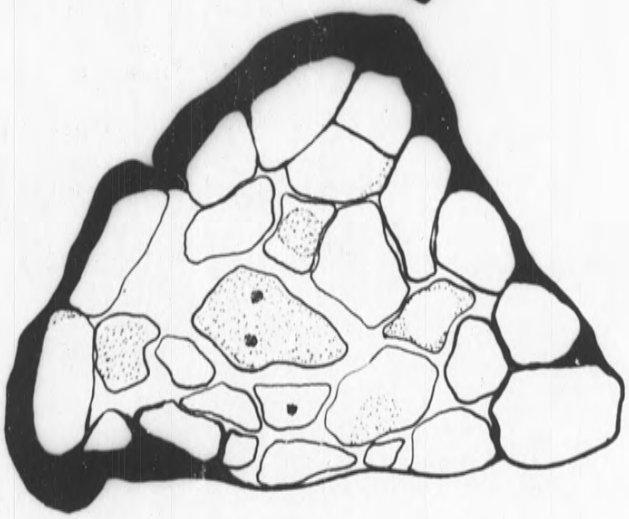


a.

15.



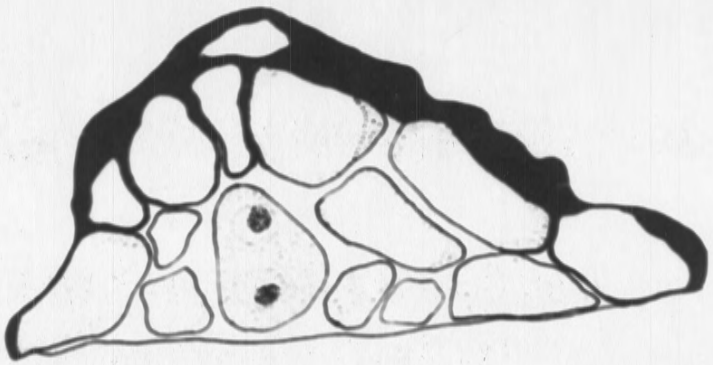
b.

Appendiculella calostroma and Asteridiella sp.

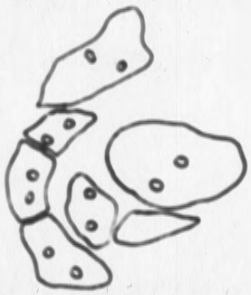
15. c. L.S. of perithecium of Asteridiella sp. (specimen No. 3) showing stage comparable to 15a and b in Appendiculella. A binucleate cell in the centre of a mass of vegetative cells. (x 1730)

16. a. Ascogenous hyphae — binucleate cells arising from original binucleate cells. (x 1870)

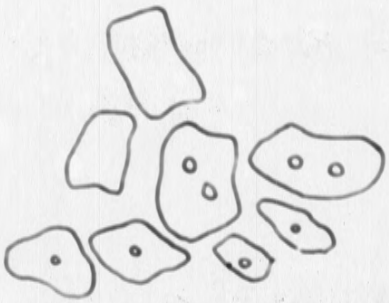
b. do. in Asteridiella sp. showing also (possibly) the original binucleated cell. (x 1730).

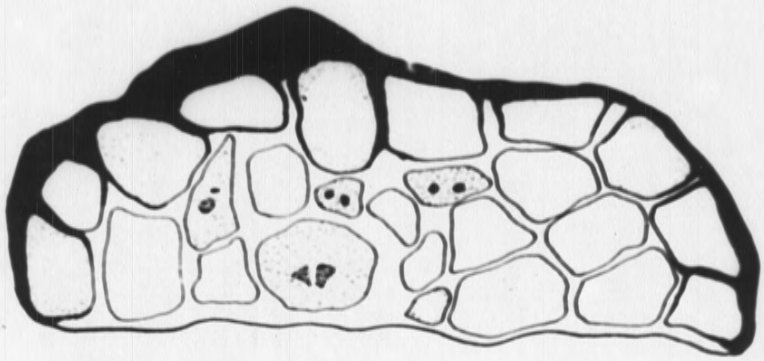


c.



16 a





b

Appendiculella calostroma and Asteridiella sp.

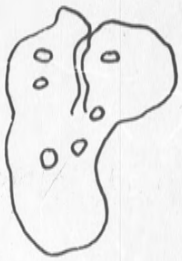
16. c - d. Ascogenous hyphae from original binucleate cell.

e do. in Asteridiella sp. showing vegetative cells between ascogenous hyphae still connected to each other as stromatic tissue. (x 1730)

17. Crozier formation.

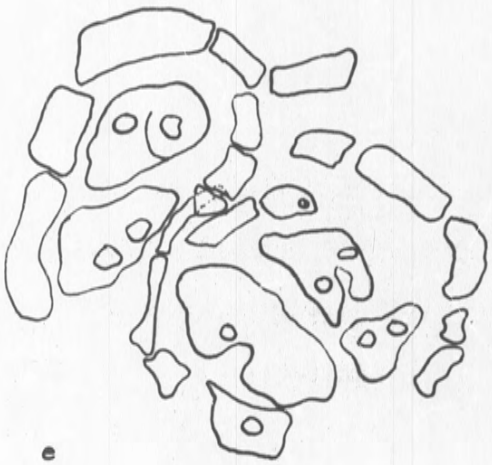
a, b. before fusion of tip of hypha to antepenultimate cell.

c. after cell fusion, with potential ascus above. (x 1870)

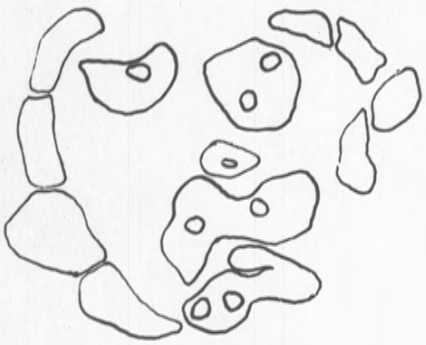


c.

d



e



17 a.

c.

b.

Appendiculella calostroma.

18. L.S. of perithecium showing first ascus in a cavity, (Note radiating hyphae). (x 730)

19. a. Inset of a cavity showing potential asci formed from crosiers. (x 1730)

b. do. in a perithecium. L.S. (x 730)

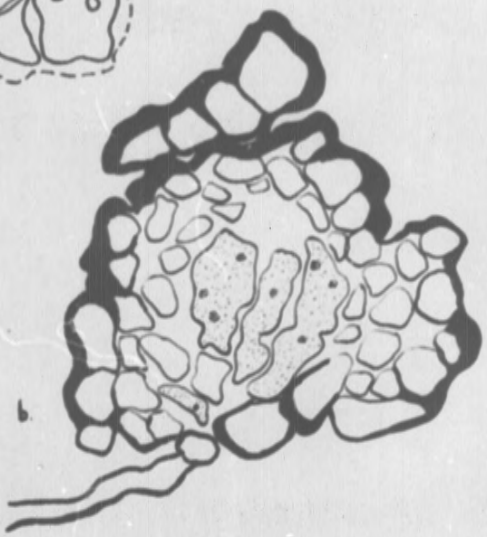
20. Row of asci lining base of perithecium and developed from ascogenous hyphae. (x 730)

21. Cleavage of nucleated asci into subsequent spores. (approx. x 670)

18.

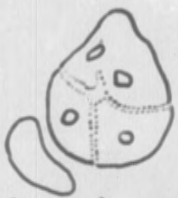
a.

19

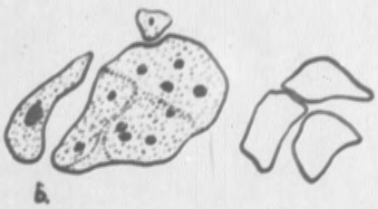


b.

20.



21. a.



b.

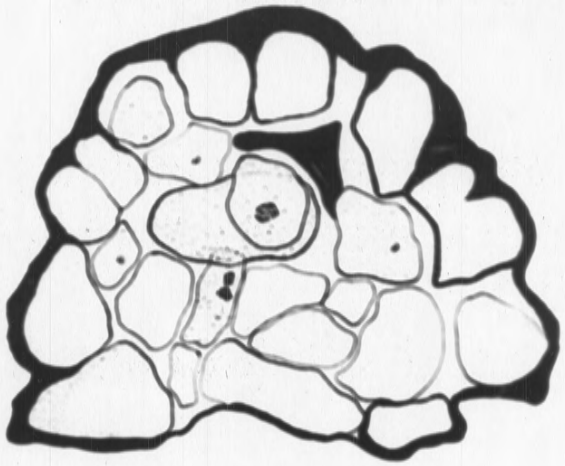
Appendiculella calostroma and Asteridiella sp.

22. L.S. of perithecium showing ascus (with fusion nucleus) formed from an ascogenous hypha.
This stage follows stage 17c. (x 1730)

23. a and b. Young ascus with enlarged fusion nucleus, following their formation by crosiers from ascogenous hyphae (seen below each ascus.)

c. First division of fusion nucleus in ascus in Asteridiella. (x 1900)

24. L.S. of perithecium of Appendiculella calostroma showing ascus with first division nuclei.
Compare equivalent stage in Figure 23c.
(x 1730)



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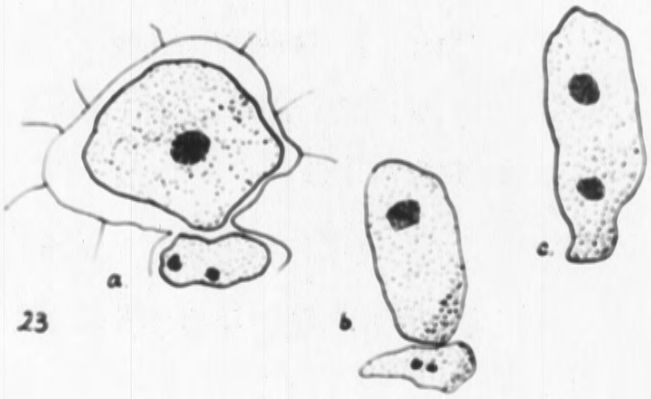

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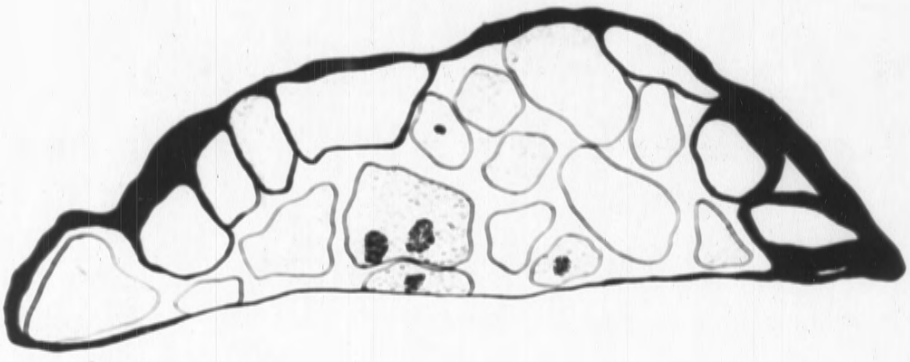


24

Appendiculella calostroma

25. Sections in cavity showing

a. segmenting asci and ascus with 2 nuclei, surrounded by ascogenous hyphae.

b. 4-nucleated ascus at stage of segmentation.

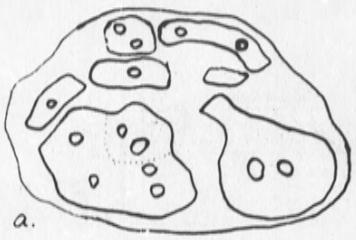
c. 8-nucleated ascus before segmentation.

d. Ascus becoming 8-nucleated and beginning to segment with one wall.

e. 4-nucleated ascus before segmentation and its ascogenous hyphal cell below (x 1730)

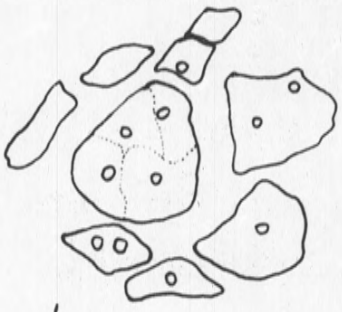
26. a. T.S. of ascus with 2 functional spores, and one disintegrating.

b. L.S. of an ascus in a similar condition to a. (x 730)



a.

25.



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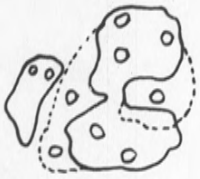

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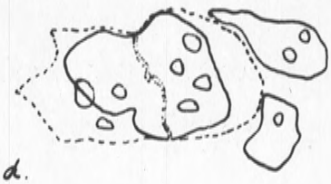
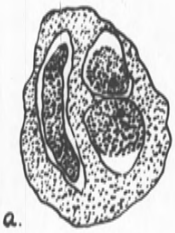
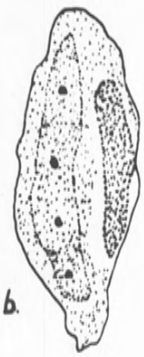


d.

e.



a.



b.

26.

Appendiculella calostroma

27. 4 spores in an ascus. (x 1730)

28. 1 spore in an ascus, showing 4 nuclei, and as yet, 1 septum (x 1730)

29. Ascus, with 1 spore containing 4 nuclei before septation, and one nucleus in surrounding protoplasm. (x 1730)

30. Ascus formed from an ascogenous hypha and containing a large fusion nucleus. (x 1730)

31 - 34. Group of asci developed from ascogenous hyphae and seen at various stages of nuclear division. (x 1730)

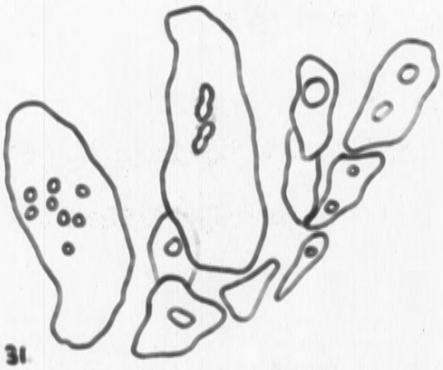
Note in 33: Beginning of a "paraphysis" by elongation of a binucleate cell, from an ascogenous hypha.

28

27

29

30



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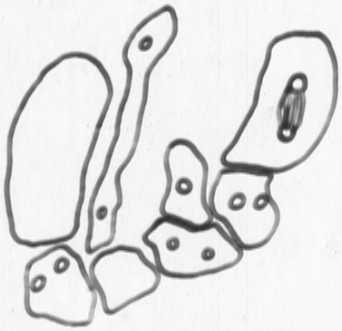
32



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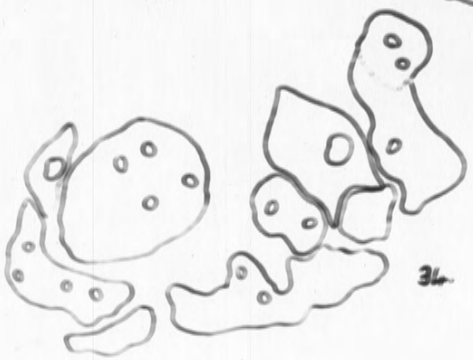


34

Appendiculella calostroma

35. Young ascus with fusion nucleus with the fused cells of the ascogenous hypha below it following crozier formation. (x 1870)

36. Ascus with fusion nucleus dividing into 2. (x 1730)

37. Ascus containing 2 asco- spores.

38. Group of asci, at different stages in nuclear division, with ascogenous hyphae. (x 1730)

39. a. T.S. of ascus showing 4 spores developing, following segmentation of protoplasm. (x 1870)

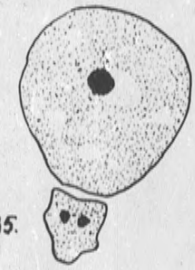
40. L.S. of ascus showing 4 young spores in tetrad arrangement. (x 1730)

41. Similar to 40, but at a later stage with spores arranged parallel to each other, following elongation of the longitudinal spore axes. (x 1700)

42. Ascus containing 2 ascospores, each 4 nucleated, before septation. (x 1730)

43. Ascus with 1 spore and 1 nucleus undeveloped. (x 1730)

44 and 45. Asci with 2 spores, 8 nucleated each, at stage of septation. (x 1730)



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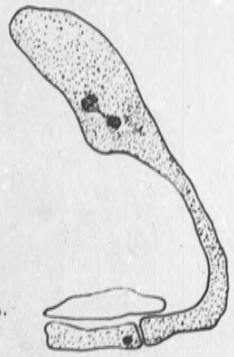

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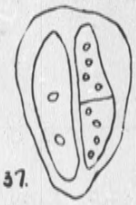

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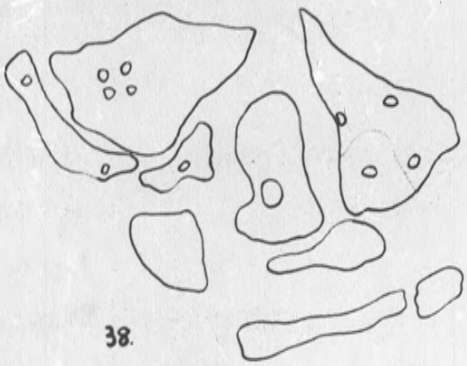
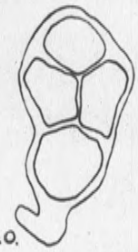


38.

39 a.



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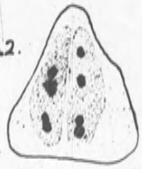


42.

43.

44.

45.

Appendiculella calostroma.

46. Ascus containing 4 young spores in tetrad arrangement, surrounded by epiplasm. (x 1730)

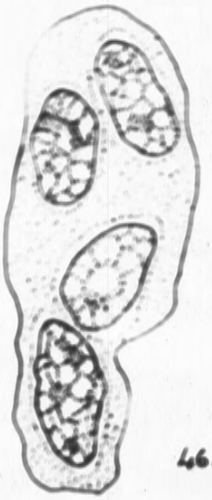
47. Ascus containing 2 spores, each 4 nucleated with the 1st median septum developing (x 1730)

48. 2 ascospores in a disintegrating asci, the left spore having developed 8 nuclei prior to septation. (x 1730).

49. A 2-celled ascospore, with thickened walls and septum dark in colour. (x 1870).

50. T.S. Ascus with 8 nuclei but with protoplasm cleaving into 3 parts only (x 1730)

51. Arrangement of asci from a branched ascogenous hypha. (approx. x 850).



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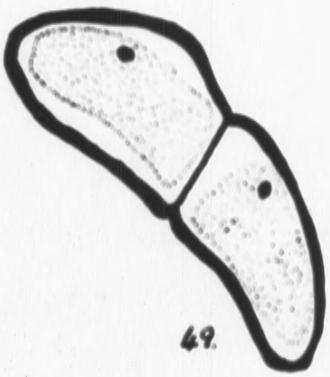

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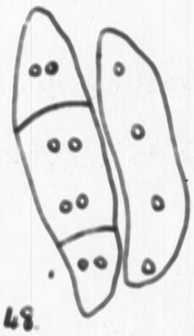

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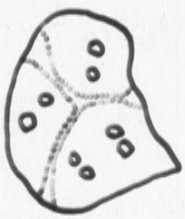

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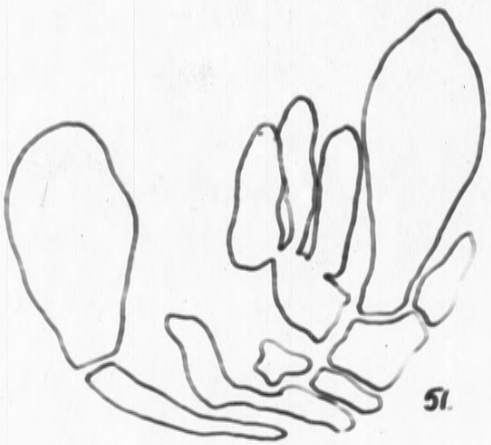


51.

Appendiculella calostroma

52. Ascus with 2 spores, each 4-nucleate and 4-celled (x 730)

53. T.S. of a 4 spored ascus (x 730)

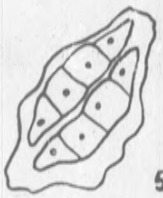
54. A 2-celled spore (x 730)

55. L.S. of a perithecium, at procstiole stage, showing young asci (deeply staining), "paraphyses" and stromatic cells lining the wall and becoming elongated and separated at top of perithecium (x 470)

56. Mature ascospore showing 2 nuclei in each cell except the upper cell which still has a larger single nucleus, before its division. (x 1870)

57. A mature 4-celled 4-nucleated spore, the end cells more rounded than in earlier stages (compare fig. 52) the shape still plano-convex, and one end cell slightly wider than the other (in this particular species). (x730)

58. a. 'Paraphyses' (diagrammatic).
    b. Ascus from ascogenous hypha (diagrammatic).
    c. L.S. of a perithecium, at a later stage than 55, showing asci lining the base, young ascospores, and a disintegrating old ascus and a free mature ascospore, also 'paraphyses' branching, side filaments dipping into cavity and pariphyses around the ostiole. (x 730)



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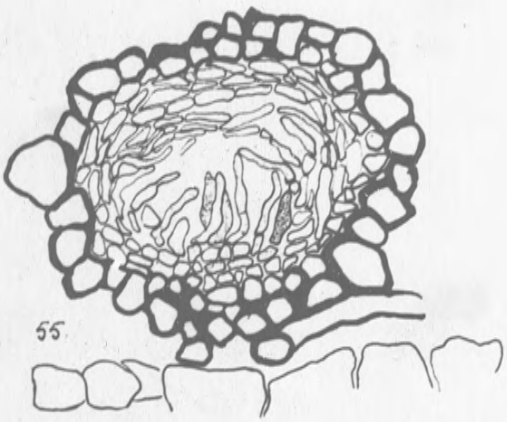
53.

54.



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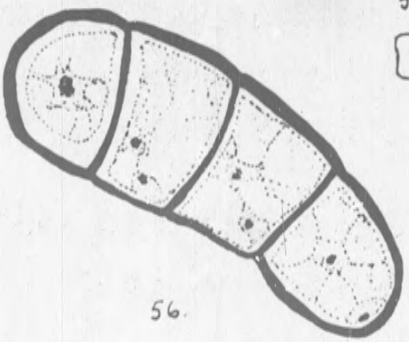

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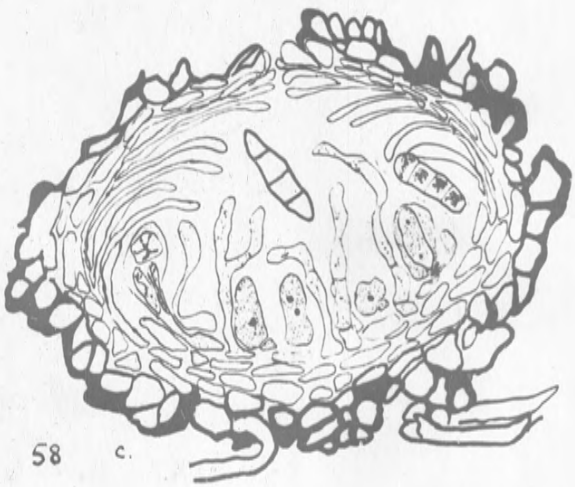
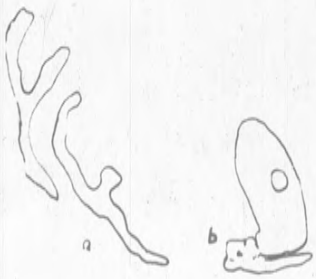


58 c.



a b

Appendiculella calostroma.

59a. Hypha-like extension of side walls — upper ascogenous hyphae. (x 1730)

b. Ascus in cavity surrounded by ascogenous hyphae. (x 1730)

60. Group of 'paraphyses' showing branching and their formation from ascus-like cells and ascogenous hyphae (approx. x 670).

61. L.S. of a perithecium, at a stage similar to 58, showing ascogenous hyphae and separating upper cells, etc., and disintegrating tissue below ascal layer and within the perithecium in general. (x 730)

59

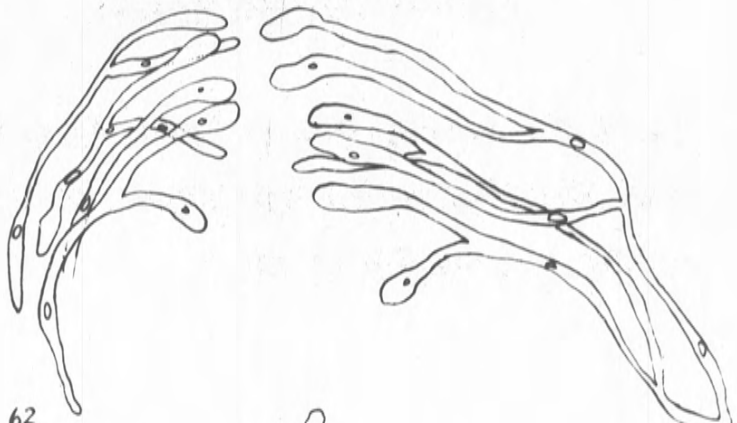
60

Appendiculella calostroma.

62. Enlarged view of periphyses showing their origin from wall cells (with a big nucleus) and their branching. (approx. x 1700)

63. Layer of 'paraphyses' developed from ascogenous hyphae, showing their width and the branching. (approx. x 1700).

64. Diagram of ostiole and the cells surrounding it. Note the thickening of the exposed parts of some periphyses. (approx. x 1700)



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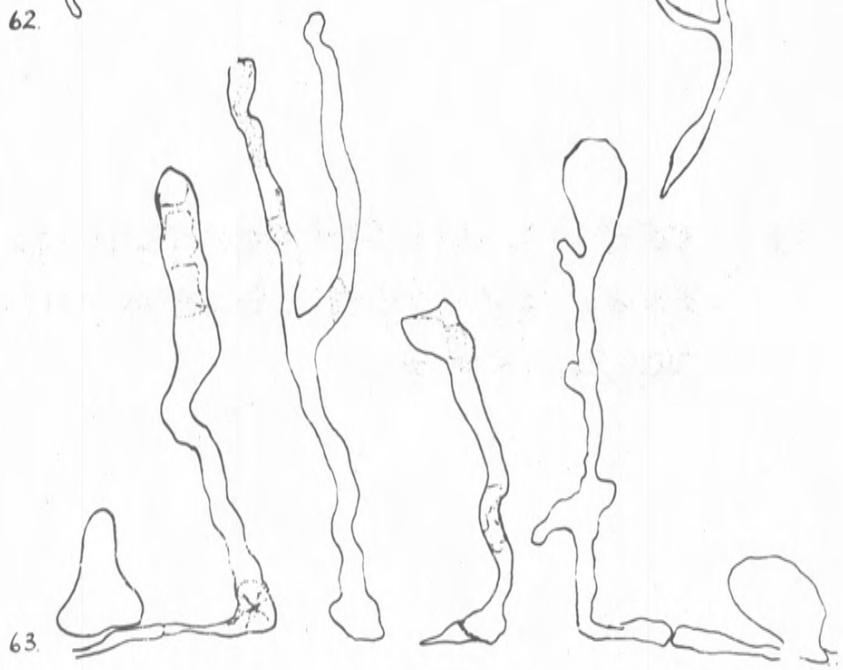

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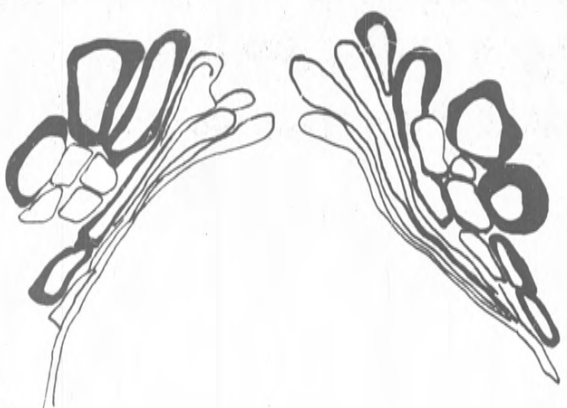


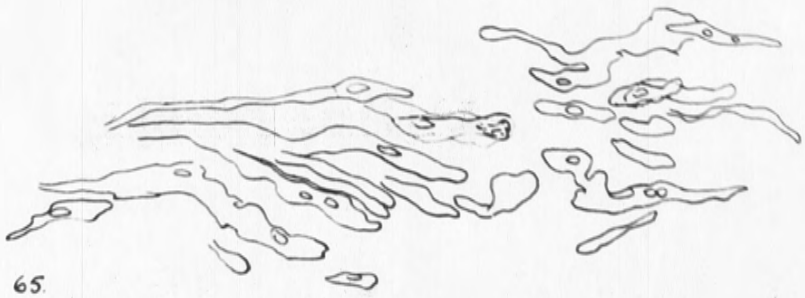
64.

Appendiculella calostroma

65. Cells of upper region of a growing perithecium at time of separation and disintegration prior to their absorption by developing asci. (x 1730)

66. Section through perithecium showing cells lining the wall and their large nuclei. (x 730)

67. Top view of perithecium showing ostiole and periphyses surrounding it and a few inner wall cells (thin-walled). (x 730)



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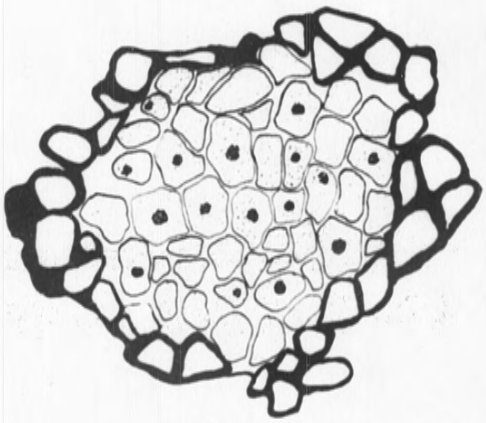

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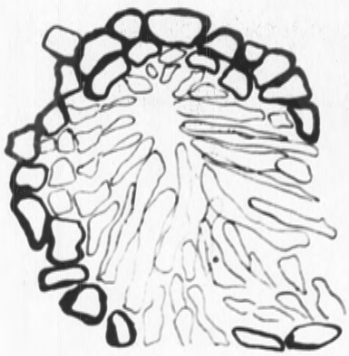


67.

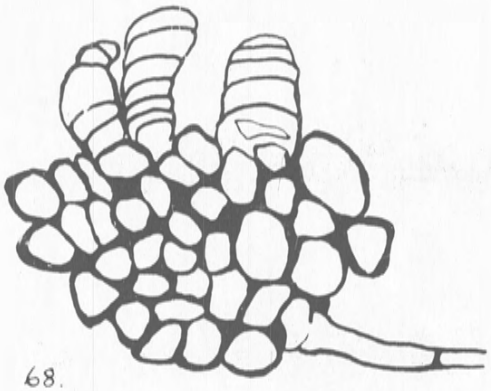
Appendiculella calostroma

68. Perithecium from outside showing dark thick-walled cells, striated appendages, and a radiating hypha. (x 730)

69. A radiating hypha. (x 730)

70. a, and b. Radiating hyphae from outside cells of perithecia. (x 730)

c. do. shown in T.S. (x 470)



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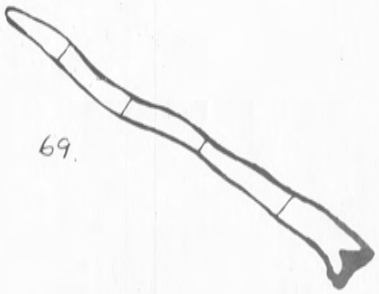

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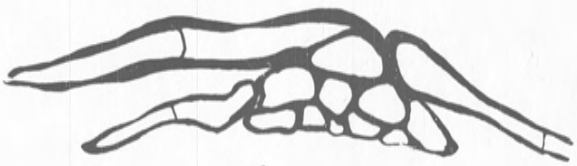
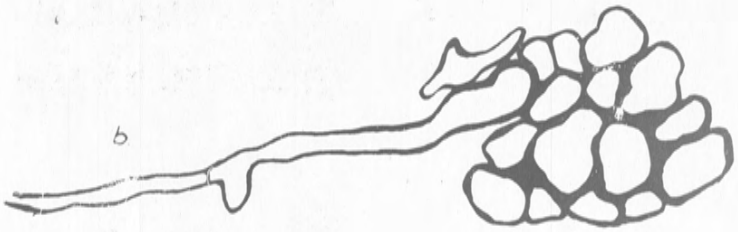


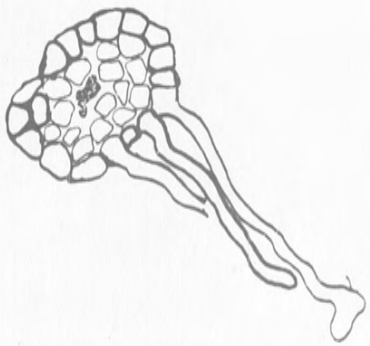
a.

70.



b

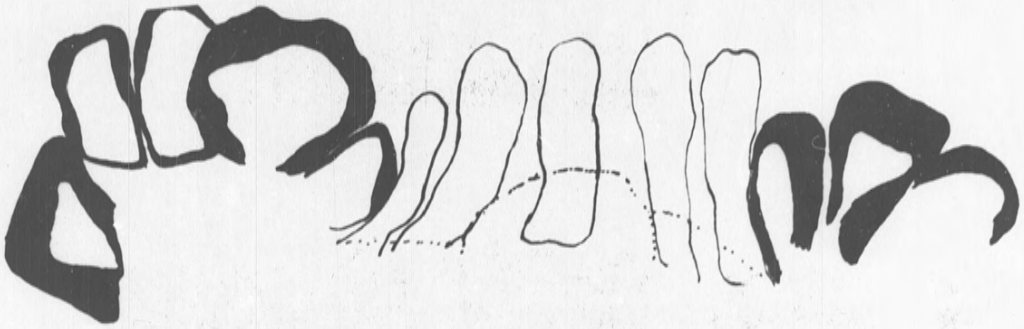
c.



Appendiculella calostroma

71. Upper cells around ostiole which have elongated vertically. (x 1730)

72. Section through perithecium showing a long appendage. (x 220)



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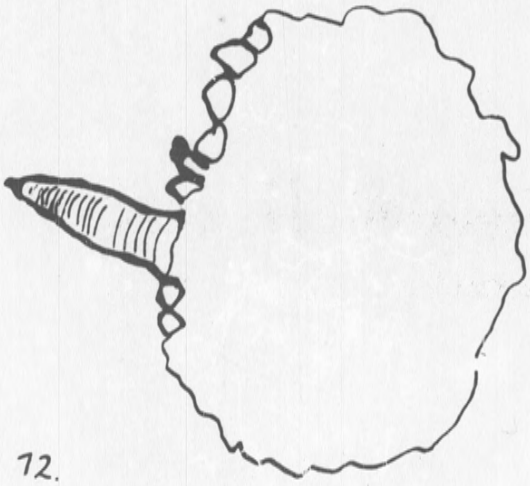


72.

Dimerium sp.

(A parasite on Asteridiella glabra, an ecto-parasite of Eriffon cancphora).

73. (1) Beginning of perithecial formation showing

a. outside and inside

b. L.S. through the middle — a mass of vegetative cells. The perithecium and hyp hae are growing on mycelium of Asteridiella. (x 730)

(2) a and b. L.S. of very young perithecium showing its origin from the hyphal cells parasitising the Asteridiella (x 730)

(3) T.S. of perithecium of Dimerium showing thick-walled outer cells, thin-walled inner cells and hyphae of both Dimerium and Asteridiella (x 730)

(4) Transverse sections showing Dimerium hyphae growing over Asteridiella hyphae as in b, and giving rise to the perithecium. (x 730)

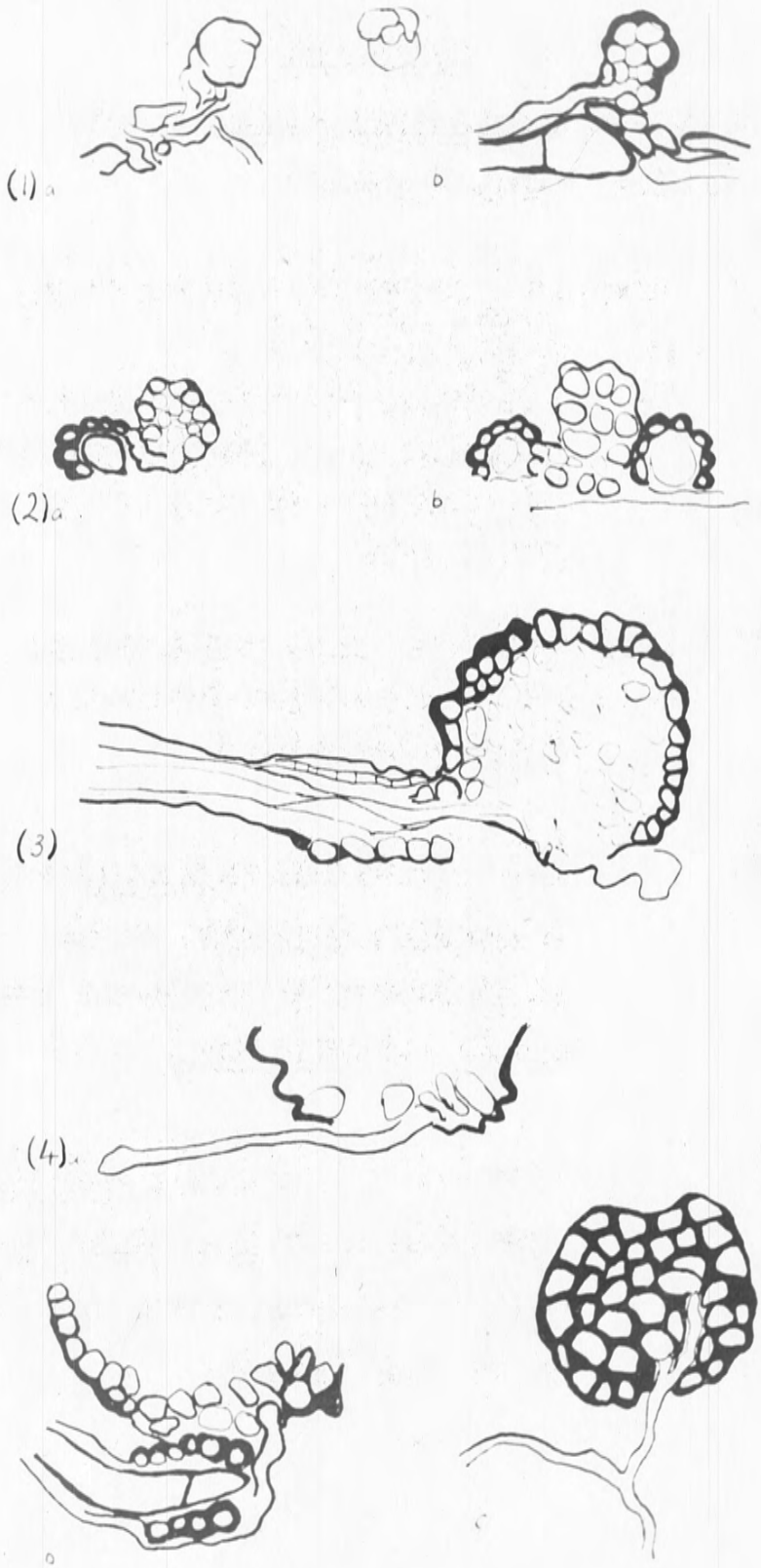
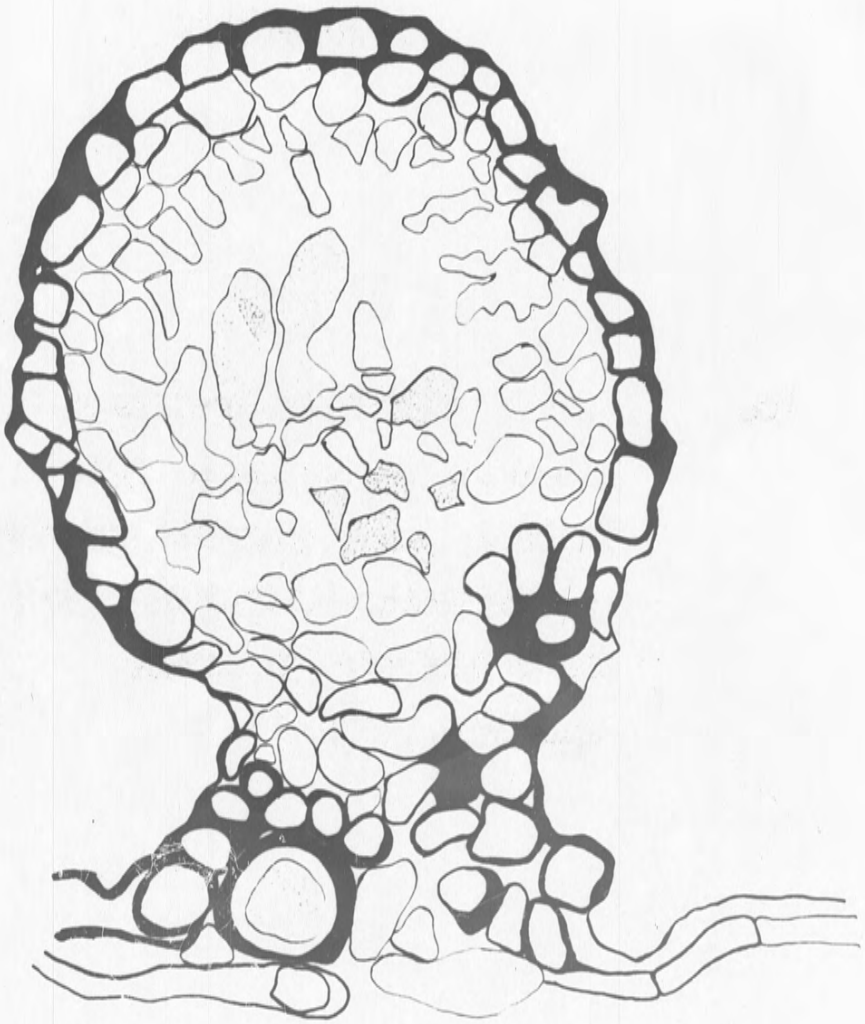


Fig. 73

Dimerium sp.

74. L.S. of perithecium of Dimerium showing parasitism on Asteridiella. hypha (shown in T.S.), asci (granular) and hyphal strands becoming disconnected, due to further extension and possibly absorption. (x 1730)

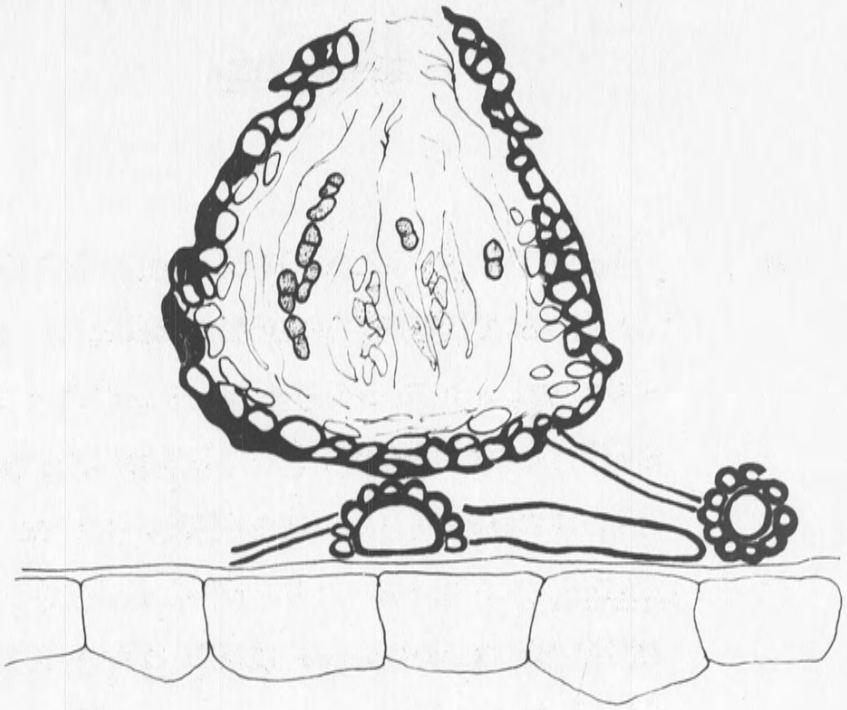


74

Dimerium sp.

75. L.S. of mature perithecium showing interthecial tissue reduced to filamentous strands, no periphyses, and stromatic tissue of a few cells mainly squashed and disintegrating. Most ascospores are lying freely inside. The Dimerium hyphae are seen (in T.S.) completely encircling the larger Asteridiella hyphae also in T.S. (x 730)

76. View in L.S. of a young Haemosphaera parasitising a Dimerium perithecium and sending down its dark hyphae into it.
N.B. Asteridiella hyphae, in L.S., below the Dimerium perithecium. (x 730)



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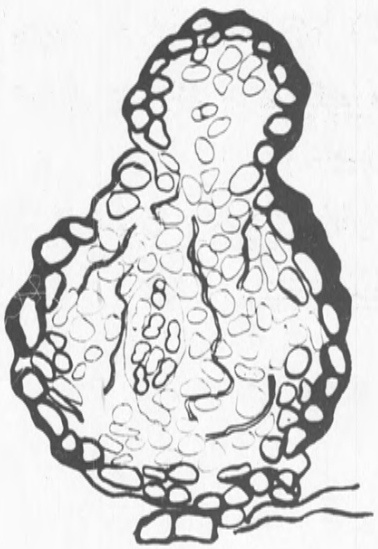


76.

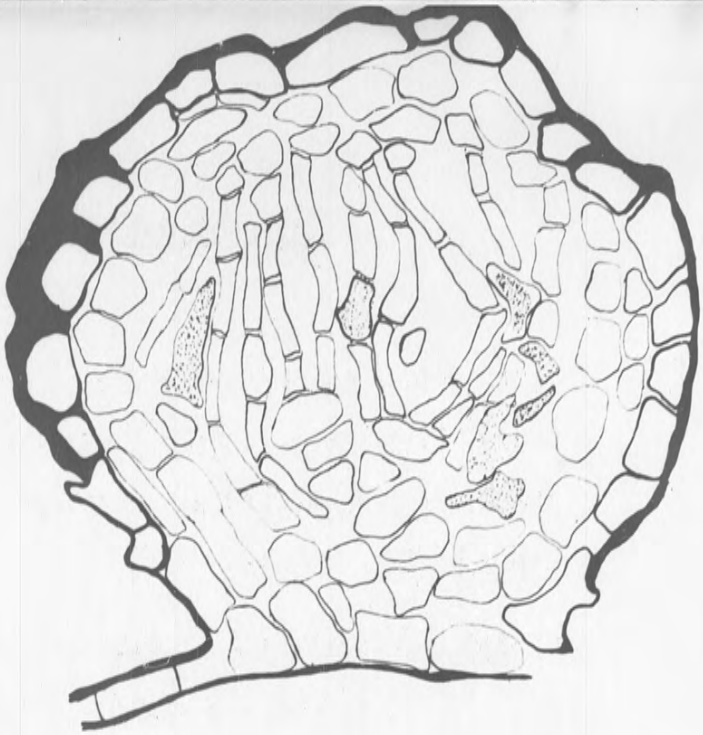
Dimerium sp.

77. L.S. of perithecium showing hyphal strands attached to top and bottom, young asci (granular) and ascogenous hyphae (also granular) (x 1730)

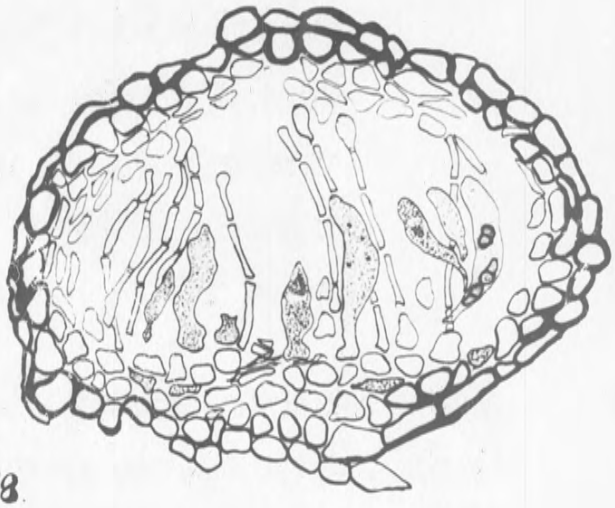
Note: The hyphal strands are originally parenchymatous cells. These are rounded at first but gradually become elongated in a vertical direction and eventually form parallel rows. By this means they create gaps in the perithecium in which the young asci can grow.

78. L.S. of perithecium showing septated inter-thecial hyphae (i.e. elongated stromatic cells still joining) which are becoming separated, asci (granular) and ascospores. (x 730)

79. Further details of interior of above showing, in b, the septated strands becoming aseptate by the dissolving of the cross-walls. (x 730)

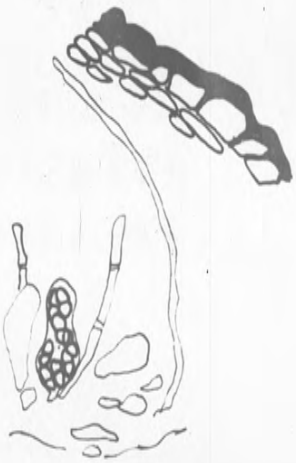


77.



78.

79 a.



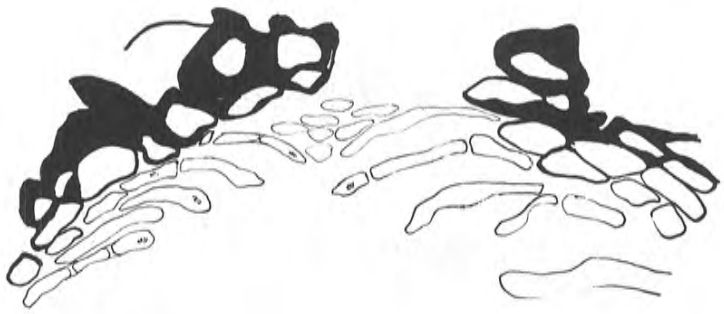
b.

Dimerium sp.

80 and 81. Detail of periphyses, showing origin from inner stromatic wall cells by extension inwards. They are more cytoplasmic than the remaining stromatic tissue at this stage, are short and unicellular and fringe the ostiole. (x 1730)

82. View of perithecium from the top, showing the periphyses lining the ostiole.

(x 730)



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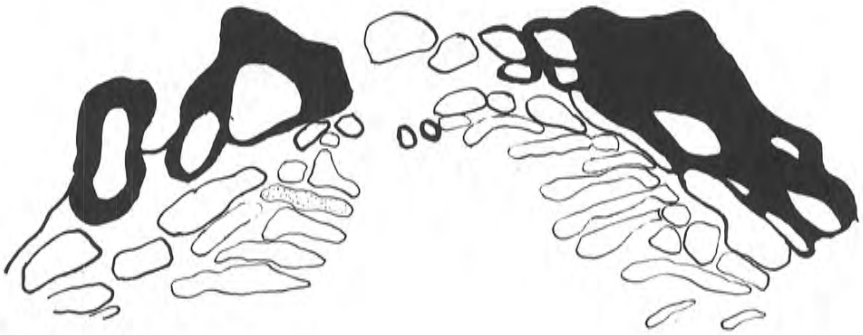

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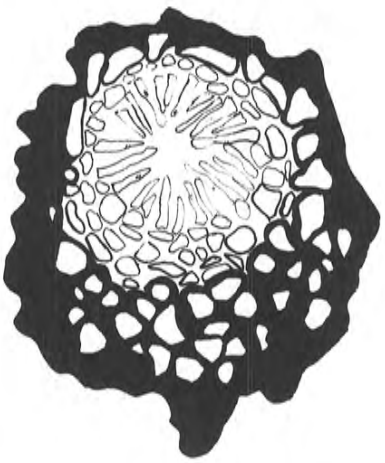

82.

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