DEVELOPMENTAL STUDIES OF CERTAIN SOUTH AFRICAN ASCOSTROMATIC ASCOMYCETES

by

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TABLE OF CONTENTS

Page No.

5. FORMAL DESCRIPTIONS:

i) The morphology and development of APHYSA RHYNCHOSIAE (Kalchbr. et Cooke) Theiss. et Syd.

ii) The morphology and development of TRICHOTHYRIUM ASTEROPHORUM (B. et Br.) v. Höhn.

iii) The morphology and development of MICROCYCLUS AMPHIMELAENA (Mont.) v.Arx.

Page No.

iv) The morphology and development of
<u>SCUTELLOIDEA CONCENTRICA</u> Tim,
gen. nov., sp. nov.

v) The morphology and development of
<u>ANTENNULARIA ENGLERIANA</u> (P.Henn) v.Höhn.

vi) The morphology and development of
<u>TRIPOSPORA TRIPOS</u> (Cooke) Lindau.

SUMMARY

1. A review of the literature pertaining to the use of ascus structure and ascocarp development in the taxonomic studies of the Ascostromatic Ascomycetes is presented.

2. Detailed studies of the morphology and development of the following species are given separately:-
   - i) *Aphysa rhynchosiae* (Kalchbr.et Cooke)Theiss.et Syd.
   - ii) *Trichothyrium asterophorum* (B.et Br.)v.Höhn.
   - iii) *Microcyclus amphimelaena* (Mont.)v.Höhn.
   - iv) *Scutelloidea concentrica* Tim, gen.nov., sp.nov.
   - v) *Antennularia engleriana* (P.Henn.)v.Höhn.
   - vi) *Tripospora tripos* (Cooke) Lindau.

3. A discussion of the taxonomic position of each of the six species is included at the end of each study.

4. A new developmental type, the *Corynelioid Type*, has been described under the section on *Tripospora tripos* (Cooke) Lindau.

PREFACE

This dissertation has been bound in two parts to facilitate reference to the Figures while the text is being read. Both photomicrographs as well as drawings are referred to as 'Figures' and these have been numbered in sequence.

Each of the six developmental studies form discrete units on their own. In preference to a general discussion at the end of the thesis, separate ones for each of the studies has been included.

In most cases, relevant measurements have been excluded from the main text and have been included under the heading 'Diagnosis' at the end of each study.

TECHNIQUES

In all cases, the process of fixation, dehydration and imbedding are as described in Johansen (1940). For fine sectioning of material, the Leitz Minot Rotary and Cambridge Rocking Microtomes were employed whilst for woody stems, the Reichert Sledge Microtome was resorted to.

Staining with Heidenhain's iron haematoxylin was universally employed as the principal stain with the schedule of Johansen again followed. A number of counter stains were tried such as Erythrosin, Bismarck Brown, Fast Green and Orange G, all in clove oil. Orange G was found the most satisfactory. Other stains included Safranin and Fast Green, Picro-Aniline Blue and Cotton Blue in Lactophenol. Canada Balsam was used as the mountant.

A squared eyepiece grid was used to obtain proportions in drawings. Photomicrographs were taken using the Zeiss Photomicroscope and Kodak Pan X film. Prints were made on Agfa Brovira BN I or BH I.

In all cases where photomicrographs and/or drawings were made from permanent slides, reference to the latter are included in the legends. The catalogued (accession) number is followed by a fraction. The numerator refers to the number of the row of sections, counting these from top to bottom, and the denominator, the number of the actual section, counting from left to right, when the slide label is to the right.

GENERAL INTRODUCTION

Toward the last half of the nineteenth century, the structure and mode of development of the ascocarp has aroused much interest. De Bary (1887) recognised the fruiting bodies of the Ascomycetes as 'compound sporophores' made up of interwoven hyphae or of pseudoparenchyma consisting of a peripheral layer separate from an inner tissue. These fruiting bodies included the discocarp or apothecium, the pyrenocarp or perithecium and the cleistocarp or cleistothecium.

The name, Pyrenomycetes, has been variously applied to a group of Ascomycetes but mainly to the perithecial types, inclusive of the true perithecia and the loculate forms. The perithecium itself was described as a 'cup-shaped discomycetous' sporocarp with margins incurved to form a pyriform structure (de Bary, 1887). The presence or absence of a stroma had been long considered as a basis for the separation of the major groups of the Ascomycetes. Separation on these grounds was found unacceptable as it grouped together clearly unrelated species or separated related ones. As the subdivisions of the Pyrenomycetous Ascomycetes are dependent upon the morphological features of the ascocarp, it would be pertinent to discuss the following:

a) ... The Stroma.

b) ... The various forms of ascocarp which may or may not be associated with such a stroma.
    i) .. Perithecia: free or immersed in a stroma.
    ii) .. Uni- or multiloculate stromata.

c) ... The centrum, the details associated with the centrum and the use of these details in the taxonomy of the Pyrenomycetes.

The Stroma:

The application of the term, stroma, has been highly variable. This is illustrated in Orton's (1924) review of the literature pertaining to the usage of this term. An early attempt had been made by Fuisting (1867) and later Ruhland (1900) to distinguish between the various zones in stromata. Fuisting's epistroma, as applied to Nummularia, consisted of the outer, conidia-bearing part with the hypostroma as the deeper layer in which perithecia were found. Ruhland used the terms ecto- and entostroma to depict the same zones. Wehmeyer (1926) retained ecto- and entostroma, with the latter consisting of both host or substrate plus fungal tissue. He admitted these terms were of use only in those stromatic Sphaeriales which first develop conidia-bearing stromata and later, fertile portions with asci and spores. Orton (1924), followed by Wehmeyer (1926), suggested the exclusion of all tissues arising as a result of a sexual stimulus from the definition of a stroma.

The stroma can thus be considered as a mass of vegetative hyphae or a mixture of host tissue and fungous cells. The fungal tissue may be pseudoparenchymatous or prosenchymatous. Ascigerous locules may or may not be developed in or on this tissue.

Perithecia:

As early as 1887, Winter distinguished between fruiting bodies consisting of unwalled locules in a stroma and those with walled perithecia embedded in a stroma. However, this distinction was not generally accepted by other mycologists. Miller (1928) realised the difference between such fruiting bodies and in his paper on the

Sphaeriales, was primarily concerned with separating out fungi with sunken perithecial fruiting bodies from the loculate stromata of the Sphaeriales. He especially drew attention to the difficulty of determining the difference between uniloculate perithecia and uniloculate stromata. Gwynne-Vaughan and Barnes (1927), for example, did not recognise uniloculate stromata or single perithecia immersed in a stroma in their definition of stromatic ascocarps. This tendency to interpret uniloculate stromata as perithecia had resulted in the grouping together of morphologically unrelated forms, as for example, in the Sphaeriales. Miller (1928) was convinced of the separateness of the wall in perithecial forms and demonstrated that it was possible, after prior maceration, to isolate intact perithecia from stromata containing one or more walled perithecia. Being aware of the danger of basing diagnoses on superficial grounds, Miller's main contribution to this concept of the perithecium or loculate stroma was that more stress should be laid on the importance of considering the actual origin of the wall. For the identification of the type of stroma, Miller (1928, 1949) stated that where there was more than one perithecium in the stroma, the presence or absence of a perithecial wall (i.e. walls which have arisen from the ascogonium and/or the sterile supporting cells) was sufficient. The distinction between single perithecia and uniloculate stromata could not be similarly resolved but Miller (1949) and Luttrell (1951a) pointed out the possibility of using the character of the ascus to differentiate these two types ......see later.

Holm (1958) questioned the actual definition pertaining to the origin of the wall. Miller (1928) derived the wall from the 'archicarp' i.e. the ascogonium plus supporting cells. As quoted by Holm, work on Sphaerostilbe aurantiicola (Luttrell, 1944a) indicated that the perithecial wall arose from the surrounding vegetative hyphae. This would not alter the fact of the morphological distinction between the wall and stroma.

Uni- or Multiloculate Ascostromata:

The order Dothideales was established by Lindau (1897) in an attempt to group together genera with unwalled locules in a stroma as opposed to those in the Sphaeriales with perithecia borne separately or immersed in a stroma. Nevertheless, both perithecial and loculate forms were included in the order and some of the earlier workers such as Gäumann (1926), Nannfeldt (1932) and Bessey (1935) doubted whether the Dothideales constituted a natural group. Because of the gradation of ascocarps from mutiloculate stromata to uniloculate, perithecium-like forms, the latter types had been easily confused with the simple perithecia. Consequently, the distinction between loculate and perithecial forms, on a developmental basis, was advocated by Miller (1928, 1949), Nannfeldt (1932) and Luttrell (1951a).

Orton used the term, ascostroma, to describe that part of the stroma bearing asci. Luttrell's (1951a, 1965a) use of ascostroma (or ascostromatic ascocarp or pseudothecium) is confined to the stroma bearing cavities or unwalled locules in which asci arise. In ascostromata (sensu Luttrell, 1951a), one or more stromal cells become differentiated into one or more ascogonia. From the ascogenous hyphae produced from the ascogonium, asci arise and these come to lie in unwalled locules in a stroma. In some cases, structures may arise in the centrum before or concommitant with the development of the asci. Groenhart (1965a) proposed the term, tichus, for the innermost layer of the stroma bordering on the locule. His use of 'wall' in his description of this layer ('distinctive protective wall') is unfortunate and apt to confuse ascostromal with walled perithecial types.

The Centrum:

From the characters associated with the presence of absence of a wall to the ascigerous locules, attention was drawn to the centrum - the 'nucleus' of von Höhnel (1907) - consisting of the ascogenous hyphae, asci and sterile tissues found within the perithecial cavity or locule. It was Miller (1928) who proposed a correlation of centrum characters with those of the perithecia or loculate stromata. He distinguished two main types:

Type I.	Perithecial wall absent. Pseudoparenchymatous centrum present. No paraphyses or periphyses. Convex, flat or concave hymenium. Lysigenous opening to the fruiting body.
Type II.	Perithecial wall present. Pseudoparenchymatous centrum absent. True paraphyses and periphyses. Concave hymenium. Schizogenous ostiole. Wall arising from the basal cells associated with the ascogonium.

The presence or absence of periphyses, mode of opening of the fruiting body (schizogenous or lysigenous) and the presence or absence of pseudoparenchyma in the centrum have been found to be inconsistent with the respective types. The form of the hymenium was also of limited value in this separation. However, the details of the interascal structures, if any, had aroused interest and attention was focussed on these.

In 1907, von Höhnel distinguished between the compound ascostromata as found in Lindau's (1897) Dothideaceae and the uniloculate forms such as Wettsteinina and Pseudosphaeria. He grouped the latter genera in the Pseudosphaeriaceae. Since the asci in the uniloculate stromata were found to be separated by strands of tissue, von Höhnel conceived the idea that the asci arose in monascous locules and were separated from each other by compressed stromal tissue. Although the Pseudosphaeriaceae, subsequently raised to the ordinal rank by Theissen and Sydow (1918) was rejected by most mycologists due to the heterogeneity of the included forms, the idea of considering the tissue between the asci had been established. Petrak (1923) proposed that if the asci in a Myriangium - type ascocarp became restricted to a single layer, the stromal tissue between the asci could become so compressed, as a result of the increase in number of asci, that 'paraphyses' could result from these. The perithecial wall could come about from the lateral compression by the expanding asci and 'paraphyses'. Such a gradation of development was not accepted especially when it was later shown that the wall was ontogenetically distinct and separate from the stromal tissue - where this existed.

In his terminology, Petrak (1923) called the thread-like stromal tissue between the asci the true paraphyses ('echte Paraphysen') if these were thinnish and thread-like. Thicker, somewhat cellular types were dubbed 'paraphysoids'. Threads with free ends and which were thick and slimy were 'pseudoparaphyses' whilst similar ones, but thinner and non-slimy, the 'metaphyses'. Nannfeldt (1932) did not agree with this terminology as it was confusing. The differences between some of the forms were not great enough to separate these easily. He proposed the following: Thread-like structures free at the apices were paraphyses. Paraphysis-like structures in the Pseudosphaeriaceae were the 'paraphysoide Interthecialfasern' or interthecial paraphysoids. Structures identical with the latter had been named earlier as the 'interascicular pseudoparenchyma' by Stevens (1919). The term 'metaphysis' was discounted altogether.

Having defined these interascal structures, Nannfeldt (1932) differentiated two groups among the Pyrenomycetous Ascomycetes. These were the Ascoloculares and the Ascohymeniales. In the Ascoloculares, conforming somewhat to Miller's (1928) type I category, there is a performed stroma in which loculi develop containing the asci. As opposed to this, the Ascohymeniales (Miller's type II ascocarp) includes forms where the ascogenous system arises somewhere on a superficial hypha or in a stroma. In this latter case, the fertile portion is clothed by a wall which is independent of the stromal tissue. The asci are interspersed with paraphyses with free ends which arise in an hymenium from the tissues associated with the ascogonium. Groenhart (1965), in attempting to distinguish the ascostromata of the Ascoloculares from the stromal forms in the Ascohymeniales (e.g. Xylaria, Hypoxylon) proposed the term 'carpostroma' for the latter type. This has not found acceptance among mycologists. Further correlations with the ascocarp concern the asci themselves. In the Ascoloculares, Nannfeldt drew attention to the fact that the asci were nearly always thick-walled and bitunicate. He related the thin-walled asci with the Ascohymeniales and showed that the tips of these asci also show some distinction in being thicker than the rest of the wall and that some stain blue with iodine due to the presence of an amyloid substance.

Gradually it was realised that the variations in construction of the centrum were significant enough to provide characters for separating groups of fungi. The Dothideaceous or Ascolocular forms were especially involved and Miller (1938) apportioned these into two subdivisions:-

Ascocarps Uniloculate or Multiloculate.

a) Those forming a fascicle of asci growing up into more or less pseudoparenchymatous tissue, e.g.
    i) Uniloculate form = Members of the Mycosphaerellaceae.
    ii) Multiloculate form = Dothidea spp.
b) Asci in a concave layer with locule traversed by sterile threads attached to the top and bottom with asci growing up between these. These threads are the 'Paraphysoiden' or the paraphysoids and appear in the locule before the asci arise and "develop from the coiled archicarp". Characteristic of the species of Pleospora, Botryosphaeria, Cucurbitaria, Dibotryon and others of the families Dothioraceae or Pseudosphaeriaceae.

Of interest was that the Myriangiales, with monascous locules, was not considered closely related to the Pyrenomycetes and Miller (1938) aligned them nearer the Plectascales (sensu

Nannfeldt, 1932). Some attempt was made by Nannfeldt to correlate the colour and form of the ascospores and conidia with the ascolocular and ascohymenial types but these characters were not consistent. Miller (1949) discounted characters such as the position of the ascocarp relative to the substrate and the colour and extent of the stroma as not fundamental enough and was perhaps the first to make an effort to compile a comprehensive classification of the Ascomycetes (mainly Pyrenomycetes) on the basis of ascus structure and centrum structure. The following were distinguished:-

(Extracted from Miller, J.H. 1949, Mycologia 41, p.102-103)

"1. The globose or broadly clavate asci without a pore lie at irregular levels throughout loose or pseudoparenchymatous tissue composed of ascogenous hyphae. As evolution proceeds the asci are brought into one locule and each ascus grows up into the stroma. The monascal locule partitions are then the remnant stromal elements. These tendencies are exhibited in the Plectomycetes.

2. The clavate asci with no pore arise in a basal fascicle and in growing upward dissolve out a stromal locule. These are multi-ascal locules, with no interthecial threads. This is the central tendency of the Dothideales.

3. The clavate to cylindrical asci with no pore arise from an entire wall-layer in a stroma and grow upward between filiform threads, which are connected at the top and bottom of the locule. Following the presence of the archicarp there is a fan-shaped array of deeply staining threads that grow downward toward the base of the locule, ultimately appearing as a palisade of vertical threads. Then the asci arise from a position at their bases and grow up between them. These

> threads are present before the asci arise and are here designated as pseudoparaphyses. They are characteristic of the Pseudosphaeriales, Microthyriales, Hysteriales, and Lophiostomales.
>
> 4. The clavate to cylindrical asci with a pore arise between apically free threads, which usually precede them in development. This is the writer's (Miller, 1949) concept of paraphyses. The ascocarp wall is a special structure developed from the basal cells of the archicarp and is not part of the stroma. This type of centrum is characteristic of the Sphaeriales and the Discomycetes.
>
> 5. This type has been discovered by Luttrell (1944) in Sphaerostilbe aurantiicola of the Hypocreales. There is a true wall to the perithecium and periphyses in the ostiole, but vertical threads or pseudoparaphyses precede the asci as in the Pseudosphaeriales. The asci are as in type 4. This is a combination of types 3 and 4 in part. Should this prove characteristic of many of the species of the Hypocreales it would provide a 5th trend in Ascomycete development."

These various points including ontogeny with morphology were considered by Miller (1949) to comprise 'criteria of relationship'. Although he did not stress the actual bitunicate nature of the asci in the ascostromal forms, he did refer to the double wall in his Dothideales, Pseudosphaeriales and Hysteriales.

Luttrell (1951a) laid great emphasis on utilising the ascus structure in the classification of the Ascomycetes. On the basis of the difference in wall structure of the ascus, he divided the Pyrenomycetes into the Bitunicatae and the Unitunicatae. With respect to the previous stress on the presence or absence of a wall to the locule, Luttrell reasoned as follows:- Perithecia could have been derived from the ascostromata by the conversion of the outer stromal layers into a perithecial wall - as was proposed by Petrak (1923) and Gäumann (1926). On the other hand, ascostromata could have arisen from perithecial forms by the reduction of the walls. Luttrell favoured the latter hypothesis on the grounds of the

> "striking correspondence in the centrum structure in groups of ascostromatic forms with that in groups in perithecial forms. Consequently, it might be concluded that Nannfeldt's (1932) conception of the ascostromatic and perithecial forms as representing two distinct series of Ascomycetes is invalid and that the ascostromatic forms may have been derived from the perithecial forms at many points, being more closely related to certain groups of perithecial Pyrenomycetes than to each other."

He goes on to say that, bearing in mind the correspondence between bitunicate asci only with ascostromatic forms, if the ascostromatic forms were derived from the perithecial ones, it seems highly unlikely that where relevant, the loss of a perithecial wall could have been accompanied by the origin of a bitunicate ascus. Forms with ascostromatic ascocarps corresponding with bitunicate asci were thus proposed as representing a monophyletic line among ascostromatic Ascomycetes (Luttrell, 1951a, 1955).

Luttrell was aware of some objections to assigning such importance to the bitunicate ascus. Conflicting reports of ascostromata coinciding with unitunicate asci have come from Sphaerognomonia carpineae (Miller & Thompson, 1940); Ophiobolus (Jones, 1926); Caliciopsis (McCormack, 1936); *Tripospora* (included in this thesis); members of the Nitschkiaceae (Miller, 1949); *Didymocrea sadasavanii* (Kowalski, 1965). These findings tend to cast doubt on the validity of dividing the Pyrenomycetes on ascus structure. However the idea does become acceptable if one accepts that these seemingly anomalous forms have developed from perithecial ones, yet on a line running parallel with that which produced bitunicate asci as well. But, as pointed out by Luttrell (1951a), the separation of the Pyrenomycetes on the uni-/bitunicate nature of the ascus would result in a similar classification as that based on ascostromal/perithecial criteria. Also in favour of Luttrell's proposal of the origin of the ascostromal type from the perithecial is the fact that there are no verified reports of Ascomycetes with true perithecia having bitunicate asci. The reports that certain Discomycetes might have bitunicate asci is losing ground as in some of these cases, as in *Keithia* (Pantidou & Korf, 1954), the nature of the ascocarp as an apothecium has been questioned or proved otherwise. On the practical side, Miller (1949) has pointed out that the ascus characters are decidedly easier to utilise in separating the Pyrenomycetes especially in relation to the single perithecial forms from the unilocular stromal ones, where the young stages are not available.

Emphasis was further placed on details of the development of the centrum as a taxonomic criterion. The variation in these details was wide enough to be used in the separation of genera within the perithecial and ascostromatic forms and Luttrell (1951a), using data accumulated from previous workers, recognised a number of

It has been recommended in the International Code of Botanical Nomenclature, 1966, p.25, Article 16, Recommendation 16A, that the name of a class in the fungi should end in -mycetes and a subclass in -mycetidae. Since Luttrell uses the ending -mycetes for the subclass Loculoascomycetes in the majority of cases, the ending -mycetes will be used in subsequent references to this subclass.

developmental types based on centrum morphology.

In 1955, Luttrell proposed that the series, Bitunicatae and Unitunicatae be given definite taxonomic rank and the subclasses, Loculoascomycetes and Euascomycetes respectively were brought about. Further, the centrum structure was used for dividing these subclasses into orders. The classification of the Pyrenomycetes proposed is presented in the following summary. The developmental types together with the details of the structures associated with these types are as found in Luttrell, (1951a, 1955 & 1965):-

Subclass: Loculoascomycetes: (syn. Ascoloculares, Bitunicatae.

		DEVELOPMENTAL TYPE:
Order I.	Myriangiales.	Elsinoe type.
Order II.	Dothideales. (syn. Pseudosphaeriales, Capnodiales, Dothiorales).	Dothidea type.
Order III.	Trichothyriales.	? see Trichothyrium in this thesis.
Order IV.	Pleosporales.	Pleospora type.
Order V.	Hysteriales.	
Order VI.	Microthyriales. (syn. Hemisphaeriales, Asterinales).	

Subclass: Euascomycetes: (syn. Ascohymeniales, Plectascales, Unitunicatae).

Four subseries are recognised by Luttrell (1951a) but only the Pyrenomycetes will be considered here.

Order I.	Xylariales.	Xylaria type.
Order II.	Hypocreales.	Nectria type.
Order III.	Diaporthales.	Diaporthe type.
Order IV.	Erysiphales.	Phyllactinia type.
Order V.	Coronophorales.	? Diaporthe type?
Order VI.	Coryneliales.	? see Tripospora in this thesis.

This proposal for the classification of the Ascomycetes by Luttrell (1951a, 1955) has met with some opposition. The ontogenetic approach was criticised by Müller and von Arx (1962) because of the apparent difficulty of applying this system for practical ends. Also, they claimed that it does not readily provide for intermediate types bridging major groups separated apart on developmental lines. Nevertheless, the Luttrell and Müller/v. Arx classifications follow each other reasonably closely. A phylogenetic approach forms the main basis behind the classification adopted by Müller and von Arx. In Luttrell's scheme, whilst phylogenetic facets are not entirely ignored, the main stress is placed on the strict definition - and therefore the developmental details - associated with the variety of centra.

In 1965, Luttrell (1965a) introduced the 'Pseudosphaeria developmental type'. As opposed to the Elsinoe type where the stromal tissue between the monascous locules remains relatively unaltered, that in the Pseudosphaeria type becomes much compressed into strands or disintegrates. It thus becomes possible to envisage intergradations between the Elsinoe and Dothidea developmental types by way of the Pseudosphaeria type. Consequently, a slightly modified classification of the Loculoascomycetes was presented by Luttrell (1965a) which catered for intergrading species. This classification is as follows:

Subclass: Loculoascomycetes.		DEVELOPMENTAL TYPE:
Order I.	Myriangiales	Elsinoe type.
Order II.	Pleosporales.	Pleospora type.
Order III.	Hysteriales.	
Order IV.	Dothideales.	Dothidea/Pseudosphaer types.
Order V.	Capnodiales.	
Order VI.	Hemisphaeriales.	Pleospora/ Pseudosphaeria types.

With this emphasis on ascus and centrum construction, it becomes apparent that all details associated with centrum structure and development will have to be examined carefully. In some cases, structures have been considered synonymous because of their superficial resemblance to each other. A case in point concerns the difference between interthecial tissue and pseudoparaphyses (Luttrell, 1965). Until the details of the actual origin of structures are elucidated, will it be possible to say whether or not the structures are the same or different.

On this basis of 'mode of development', the following descriptions of the morphology and development of a number of Ascomycetes are presented. With this approach, it is hoped to substantiate recognised developmental types or else to point out details not in agreement with ones already known. It is also hoped that these details will go toward the characterisation of genera and will indicate the natural affinities between various genera and species of Ascomycetes.

THE MORPHOLOGY AND DEVELOPMENT OF APHYSA RHYNCHOSIAE (Kalchbr. et Cooke) Theiss. et Syd.

Introduction:

Aphysa rhynchosiae (Kalchbr. et Cooke) Theiss. et Syd. is a parasitic fungus found predominantly on the upper leaf surfaces of Rhynchosia caribaea DC. in the form of colonies of extremely small and shiny ascocarps (fig. 1.). The latter are of a flattened, hemispherical type which has resulted in their inclusion in the Hemisphaeriales, an order which, according to Luttrell (1951a), can hardly be described as a natural group. Since A. rhynchosiae has been variously classified and named, it is felt that developmental work might help toward establishing its natural affinities.

In 1880, Kalchbrenner and Cooke assigned a fungus growing on the leaves of Rhynchosia sp. to the genus Stigmatea Fr. which included fungi with paraphysate or pseudoparaphysate ascocarps and called it Stigmatea rhynchosiae Kalchbr. et Cooke. Under this latter name, Saccardo (1882) grouped it in the Hyalodidymae of the Sphaeriaceae.

Theissen (1913) erected the order Hemisphaeriales in which was included the Hemisphaeriaceae, Microthyriaceae and Trichopeltaceae and later also the Polystomellaceae and Stigmateaceae (Theissen & Sydow, 1917a). The feature common to the members of this order is the dimidiate ascocarp which was subcuticular or superficial in position and which, except in the Hemisphaeriaceae, was covered by a shield of hyphae, radiate in arrangement. In 1917 Theissen and Sydow (1917b) described a fungus growing on the hosts Rhynchosia elegans and Vigna sp. This fungus was called

Parodiella schimperi P.Henn. and was in the Stigmateaceae. Theissen and Sydow considered that as the ascocarp was subcuticular and hemispherically shaped as opposed to the superficial and usually globose nature of the other members of the genus *Parodiella*, it was incorrectly assigned to this genus. Consequently, *P.schimperi* was removed to a new genus in the Stigmateaceae, this being *Aphysa*. The older binomial, *Stigmatea rhynchosiae* and *Parodiella schimperi* were considered to be synonymous so that the correct name for the species was *Aphysa rhynchosiae* (Kalchbr. et Cooke) Theiss. et Syd.

Hansford (1946) published a description of *A. rhynchosiae* and placed it in the Microthyriales on the characteristics of the flattened ascocarp and radiate arrangement of the hyphae making up the upper wall, and in the Stigmateaceae because of the subcuticular nature of the ascocarp. Doidge (1950) also listed this species and followed the classification adopted by Hansford.

In 1962, Müller and von Arx incorporated this species into the family Venturiaceae and into the genus *Coleroa* Rabenh., in this case a new combination *Coleroa rhychosiae* (Kalchbr. et Cooke) E.Muell., not only on the features of the subcuticular and flattened ascocarp but also on the presence of thread-like paraphyses surrounding the asci. However, in view of the fact that this investigation casts doubt on the existence of paraphyses, the name, *Aphysa rhynchosiae*, has been retained at any rate until developmental studies of *Coleroa* sensu lato, as well as other species of *Aphysa*, have been undertaken.

This investigation is primarily concerned with the type of development of the centrum exhibited by

Aphysa rhynchosiae with the object of ascertaining to which group of fungi this species is most closely related.

Materials & Methods:

Material for this study was collected at Nature's Valley near Plettenberg Bay, Cape Province during July and August, 1965 and April, 1966.

Both hand-cut and microtomed sections were employed. In the latter case, material was fixed in form-acetic-alcohol, carried through a butyl alcohol series and embedded in paraffin wax. Sections were cut at 6 - 10µ on a rocker-type microtome and stained in Heidenhain's hematoxylin and counterstained with Orange G (Johansen, 1940). Other stains included safranin and fast green, aniline blue in picric acid and cotton blue in lactophenol.

Host-Parasite Relations:

Ascocarps, 140 - 170µ in diameter and 60µ in height at maturity are conspicuously shiny in surface view and lie in close proximity to each other in scattered colonies. The latter are circular in extent and of a diameter 1.5 - 3.0 mm. Superficial mycelium is entirely lacking and hyphal growth is confined to the immediate subcuticular zone of the leaf and this only in the region of the colonies of ascocarps. The hyphae are either in the form of closely applied, hyaline threads of short, uninucleate cells which branch and anastomose and appear as narrow, radiating bands of hyphal material or in the form of reticulated pseudoparenchyma (fig. 2). This subcuticular layer is, for most part, one cell in thickness though occasionally there is an overlapping of hyphae. The outline of the underlying epidermal cells is easily distinguishable through the mycelium except beneath the older ascocarps. Deeper penetrating hyphae have not been observed although Hansford (1946) claims the presence of intercellular hyphae extending just beyond the palisade layer. With both microtome and hand cut sections, no evidence for the presence of haustoria into the epidermal cells from the subcuticular hyphae can be found. Though infection appears confined to the subcuticular zone, it seems possible that toxic substances must diffuse out from the fungus into the host tissue as the leaf does succumb in large infections with concommitant collapse of the mesophyll tissue. Confluent ascocarps are commonly found and in some cases, ascocarps and microconidial locules are seen to be united.

Ascigerous Locule:

As mentioned before, the subcuticular hyphae are hyaline and form a layer mostly one cell in thickness (fig. 3). The initial stages in ascocarp development may be easily followed in surface view. Certain parts of the hyphal layer consist of clusters of cells which are distinguishable from the surrounding somatic cells by their larger size and darker coloration (fig. 8). These are the ascocarp initials. Whether or not more than one cell is involved in the early process of ascocarp formation is not certain due to the transparency, smallness and close proximity of the hyphae and the difficulty in distinguishing these. Such initials are found predominantly toward the edge of the colonies resulting in the centrifugal spread of the new ascocarps. However, these initials are occasionally found to occur among the more mature ascocarps.

As an increase of cells constituting an ascocarp takes place, a stratification into layers becomes evident, an upper and lower layer being distinguishable with an intervening tissue of pseudo-parenchyma. The upper layer, as seen in surface view, assumes a compact pseudo-parenchymatous nature (figs. 9 & 10) and only in localised areas toward the edges can a radiate effect of hyphae be discerned. At an early stage, the future pore area toward the apex of the ascocarp can be clearly made out as the cells here are thin-walled, less carbonaceous and lighter staining relative to the rest of the cells (fig. 10). Such a pore area is present even before any appreciable differentiation has occurred within the centrum.

The darker appearance of the outermost wall of the ascocarp can be seen to be the result of the outer tangential and radial walls of the cells, as seen in vertical section of the ascocarp, being thicker and darker. Ultimately, cells of the lower layer do become filled with dark staining material and this gives rise to the thick, carbonaceous appearance of the walls surrounding the centrum. This, coupled with the over-lying cuticle, gives the shiny, black appearance to the ascocarps in surface view. The lower layer is also composed of pseudoparenchymatous tissue but these cells as opposed to those of the upper layer, have irregularly thickened walls (fig. 7) and this tissue is deeply set into the intercellular grooves between the cells of the upper epidermis.

There is evidence to show that tangential division of the early centrum cells takes place, this being indicated by walls having been laid down in a tangential plane, i.e. parallel to the leaf surface (figs. 4 & 5). Increase in the height of the ascocarps can be thus accounted for. Radial division of cells must also take place as an increase in diameter of the ascocarps also results.

Ascogonial formation is initiated by certain cells in the centre of the pseudoparenchymatous centrum becoming darker staining and showing prominent nucleii. Figure 6 shows the youngest stage observed with a two-celled ascogonial initial. These early initials are always seen to lie in a central position in the fruiting structure which, at this stage of development, is somewhat lens-shaped. A compact mass of ascogonia is eventually formed which, being positioned in the centre, results in the ascocarp assuming a conoid form. Angular pseudoparenchyma fills the rest of the ascocarp.

From these early ascogonial cells arise elongate trichogynes which pierce the upper dark layer of the ascocarp and protrude beyond the cuticle. Trichogynes and attendant ascogonia consist of either two or three cells in series. Figure 11 shows a three-celled structure whilst figure 12, the diagrammatic representation of figure 13, likewise shows a multicellular arrangement of ascogenous cells and a trichogyne. More than one trichogyne may be present. Figures 14 & 15 show two and three respectively. The protruding portion of the trichogyne is rounded and slightly bulbous and presents a prominent surface to the exterior. However, attached microconidia were not seen and thus the cytological details of fertilisation have not been elucidated.

After the central ascogenous mass is initiated, basal spread of ascogenous cells takes place in two ways. Firstly, new ascogenous cells develop independently in more lateral positions producing their own trichogynes and are quite separate from the central mass and secondly, derivatives from this central ascogenous mass spread out laterally and, in turn, appear to produce their own trichogynes. Up to six trichogynes have been accounted for in one locule. From the ascogonia, short ascogenous hyphae are produced along the base of the locule into which nuclei appear to migrate in pairs (fig. 18). As croziers have not been detected, it would appear as if the ascus initials, produced from these binucleate ascogenous hyphae, merely elongate upwards into the pseudoparenchyma. Fusion must occur between the two nuclei in the terminal cells, each of which becomes clearly distinguishable with a single, dark and large nucleus and these are seen to line the base of the ascocarp (figs. 16 & 17). Three successive divisions take place in these initials as indicated by the various stages present in the developing asci (fig. 18). The eight resulting nuclei become delimited from each other and the rest of the ascus by the formation of ascospore walls.

The most significant part in the development of the centrum becomes apparent at this stage for it can be seen that narrow plates or walls of material are present between the asci (figs. 16, 17 & 18). For the most part, these walls do not seem to have any definite structure, appearing quite homogenous in section though a cellular make-up is initially apparent (fig. 16). This cellular nature is lost as the asci expand and then their appearance in vertical section can be described as little more than thin threads. These hyaline walls are the cells of the centrum compressed by the growth of the expanding asci and will be referred to as the interthecial tissue (Luttrell, 1965). In horizontal section of the ascocarp, the partitioning of each ascus from its immediate neighbours is obvious, the intervening interthecial tissue forming a network in section. Around the periphery of the ascocarp where mature asci are more abundant, the monascous state is most conspicuous (figs. 19 & 20). Pseudoparenchyma is evident as a layer lining the outer limits of the centrum.

As the asci reach maturity, the interthecial tissue becomes even more compressed and disappears from most parts. With the asci in a common locule, there is a tendency for these asci to converge toward the apex where a pore is formed (fig. 21). At spore ejection, the asci elongate, usually one at a time, through the pore. After the spores are shot away, the ascus contracts (fig. 25) and another takes its place. Pseudoparenchyma lining the fruiting structure also becomes compressed and disorganised and is wanting to a great degree in the mature ascocarp (fig. 21).

Asci are globose to broadly ovate, 40 - 50µ x 10 - 16µ (figs 22 & 26). Observations of asci in distilled water show these to be bitunicate, the extended ascus being up to 110µ in length (figs. 23, 24 & 27). Ascospores number eight per ascus and are two-celled, each with a septum which is unequally placed and very slightly constricted in this region. The ascospores are light brown in colour and of dimensions 10 - 13µ x 4 - 5µ, and are shot out in quick succession when mounted in distilled water.

Microconidial locule:

The initial stages in the formation of microconidial locules are identical with those of the ascocarps. However, in these, the central pseudoparenchymatous tissue becomes disorganised and bacilliform bodies, 5 - 8µ x 1.0 - 1.2µ can be seen to fill this zone. The origin of these microconidia was not determined as microconidia expressed from the locules in squash preparations were never accompanied by the surrounding pseudoparenchyma from which the microconidia are formed. At maturity, the microconidia also fill the whole locule and only a thin layer of pseudoparenchyma remains around the edge of the locule. These microconidia, each uninucleate, are eventually extruded through and form a globular mound just outside the ostiole (fig. 28). Since the presence of microconidia together with ascogonia with receptive trichogynes have been found, though no sexual fusions have been seen, it has been deemed reasonable to assume that fertilisation of ascogonia by microconidia via trichogynes does occur. Furthermore, attempts to germinate these microconidia in water have failed and this would appear to substantiate their spermatial nature.

Discussion:

Since the ascogonial cells are to be found subsequent to the differentiation of the enclosing layers of the ascocarp, the latter would be an ascostroma. This fact, together with the bitunicate nature of the asci, would indicate that the fungus should be placed in the subclass Loculoascomycetes (Luttrell, 1955).

Of significance to the further taxonomic status of Aphysa rhynchosiae, the following is relevant. As greater recognition was given to the developmental rather than the stress on superficial characters in the assigning of species to specific rank, it was found that species with diverse internal structure were grouped together. This can be illustrated in the Hemisphaeriales in which Luttrell (1944) distinguished two separate developmental types.

Type I: Species with asci in flask-shaped locules in a dimidiate ascostroma, the asci dispersed with pseudoparaphyses. The Pleospora development type was indicated here.

Type II: Species with a dimidiate ascostroma aparaphysate, with the Elsinoe developmental type.

On this ontogenetic basis, the Hemisphaeriales were divided into two families, the Myiocopronaceae (Type I development) and the Microthyriellaceae, (Type II development) and the latter were included in the Myriangiales (Luttrell, 1951a) the former in the Pleosporales.

This study has indicated that the centrum development in A. rhynchosiae appears to be that of the Elsinoe type (Luttrell,1951a) as shown by the asci being produced in monascous cavities or locules with the "walls" between these cavities consisting of compressed pseudoparenchymatous tissue. The monascous state is not obvious at maturity as the interthecial tissue breaks down or disappears as a result of the mutual pressure exerted by the maturing asci. The latter then appear quite contiguously placed within a common cavity or 'pseudolocule' (Luttrell, 1951^{a}).

The interthecial tissue never assumes any structural condition which can be equated with the true paraphyses as delimited by Luttrell (1965). Hence, the presence of the 'fädigen paraphysoiden' of Müller and von Arx (1962) is questioned and consequently, the validity of incorporating Aphysa with Coleroa. It must be stressed, however, that it is not implied that the 'paraphysioden' of Müller and von Arx are the same as the 'paraphyses' of Luttrell but the presence of distinctive or well-defined structures between the asci appears to be definitely wanting in this fungus studied.

On Luttrell's scheme (Luttrell, 1951a,1955)Aphysa rhynchosiae is best placed in the Myriangiales and possibly in the family Stephanothecaceae.

Previous studies, though incomplete, have indicated other species which appear to conform in their development with the Elsinoe type. Working on Desmotascus portoricensis Stevens, Stevens (1919) described interascicular pseudoparenchym composed of thin-walled, hyaline cells. It was implied that asci occurred singly and from the base of the fruiting body and were separated by pseudoparenchyma. Yet another significant work was that by Luttrell (1940), on Morenoella quercina (Ellis & Martin) Theiss. where it was found that the asci, which arose singly within the stroma, were separated by sterile tissue which eventually became crushed and absorbed. But at no time did this stromal tissue resemble pseudoparenchyma (Luttrell, 1944). Arnaud (1918, 1925, 1931) on Protothyrium salvadore (Cke.) Arn., Campoa pulcherrima Speg. and Microthyriella rickii (Rehm.) v. Hoehn. respectively, also described the monascous state in these species.

Diagnosis:

Aphysa rhynchosiae (Kalchbr. et Cooke) Theiss. et Syd. is a parasitic fungus on the leaves of Rhynchosia caribaea DC and consists of small, circular and flattened, subcuticular ascostromata and microconidial locules (spermogonia). The ascocarps range from 140 - 170µ in diameter and up to 60µ in height. Colonies of ascocarps are somewhat circular in extent and are of an overall diameter of 1.5 - 3.0 mm. The centrum is aparaphysate, of the Elsinoe developmental type, containing a basal layer of globose, bitunicate asci of dimensions 40 - 50µ x 10 - 16µ. Each ascus contains eight, two-celled ascospores which have an unequally placed septum, are very slightly constricted, are light-brown and are 10 - 13µ x 4 - 5µ in size. Spermogonia 90 - 95µ in diameter and up to 40µ in height. Spermatia bacilliform, 5 - 8µ x 1.0 0 1.2µ.

Taxonomic position:

O. Myriangiales.

F. Stephanothecaceae (syn. Microthyriellaceae).

THE MORPHOLOGY AND DEVELOPMENT OF TRICHOTHYRIUM ASTEROPHORUM (B.et Br.) v.Höhn.

Trichothyrium asterophorum is an hyperparasitic fungus reported on various fungal hosts. In this study, the host is Asteridiella scabra (Doidge) Hansf. (Meliolaceae) which in turn is parasitic on Trichocladus ellipticus E.et Z. (Hamamelidaceae).

The genus Trichothyrium was established by Spegazzini (1889). Von Höhnel (1909) suggested that Trichothyrium had affinities with the Perisporiaceae but Theissen (1914) disagreed with this classification. The distinctness of the upper and lower walls of the ascocarp and the presence of a definite ostiole were characteristics not shared with other members in the Perisporiaceae and Theissen suggested it be placed in a separate family, the Trichothyriaceae.

Nannfeldt (1932) removed the Trichothyriaceae, placed in the Perisporiales by Theissen and Sydow (1917a), to the new order, Trichothyriales, included in the Ascoloculares or Ascostromatic Ascomycetes. In 1945, Martin placed ~~delimited~~ species with flattened, dimidiate ascostromata in the Microthyriales (Hemisphaeriales) and included the Trichothyriaceae in this order.

The incorporation of the Trichothyriaceae in the Hemisphaeriales (Microthyriales) by Martin (1945) and Wolf and Wolf (1947) was considered by Luttrell (1951a)to be on the purely superficial lines of the gross morphological structure of the ascocarp as found in genera grouped in the Microthyriaceae and Trichopeltaceae. Also, due to his assumption that members of the Trichothyriaceae have closer affinities with the Dothideales, he was not satisfied with the inclusion of this family among species with a paraphysate centrum. He consequently removed this family to the separate order, the Trichothyriales in the Loculoascomycetes (syn. Bitunicatae)(Luttrell, 1951a & 1955).

Müller and von Arx (1962) retain the family Trichothyriaceae and include this in the Pseudosphaeriales, an order established from von Höhnel's (1907) Pseudosphaeriaceae by Theissen and Sydow (1918). In the Pseudosphaeriales (sensu Müller and von Arx, 1962) are species having a sharply defined pore to the ascocarp, either spherical or dimidiate ascocarps and cylindrical or shortly ellipsoidal asci with a short stalk. Centrum structure is not clearly defined but 'paraphysoids' are said to be present. The use of 'paraphysoids' is confusing as it can be interpreted to include both compressed interthecial tissue and vertically arranged hyphae or pseudoparaphyses (sensu Luttrell, 1965).

To date, the developmental morphology of <u>Trichothyrium</u> has been little elucidated. Theissen (1914), Petrak (1950) and others have been hampered in their investigations either by inadequate material or the unavailability of suitable developmental stages. Whilst materia collected from local sources has proved to be far from ideal, this investigation has been carried out mainly to attempt to clarify the interpretation of structures associated with the fungus, especially the sexual and asexual ones and to try to ascertain, as far as possible the type of interascicular structure present and the ontogeny of such.

Material and Methods:

Infected material of Trichocladus was collected from indigenous forest margins both at Wolfridge, near Alice and at Rabbits' Wood, Grahamstown, during the period October to May of 1967 and 1968.

Infected leaves were placed in trays lined with moist filter paper and covered with sheets of glass so as to maintain humid conditions around the material. In this way, the fungi were freshened up. Sectors of infected leaves were fixed in form-acetic-alcohol, carried through a butyl alcohol series and imbedded in 60° wax. Sections were cut at 6 - 10μ. In some cases, as the host leaf became rather hard and brittle and proved a hindrance in sectioning, this was carefully removed from the wax block and the fungus alone sectioned.

Staining was carried out in Heidenhain's haematoxylin with a counterstain of orange G, or with a double stain of safranin and fast green.

Macroscopical surface features of both fungal host and Trichothyrium were best seen in dehydrated squares of infected leaves which were cleared in cedar wood oil and examined and photographed directly.

Especially in the elucidation of the initial phases in the development of the asexual spores and ascocarp, suitable material was lifted from the host leaf and transferred to a dilute solution of cotton blue in lactophenol or in some cases, bleached in a dilute solution of sodium hypochlorite before being stained in the cotton blue.

Host-Parasite Relations:

In the further description, except where otherwise specified, Asteridiella will be referred to as the 'host', Trichothyrium as the 'parasite'. In this way, it is hoped to avoid any confusion as to which host is being referred to since Asteridiella is again parasitic on Trichocladus.

Parasitism is initiated by the germination of the ascospores of Trichothyrium close to a hypha of the host. From the start of infection the parasite-hyphae do not develop randomly but closely follow the course of and, for most part, are adpressed to the host's mycelium.

The mycelium of the parasite is in the form of tight, parallel bands of hyphae, dark brown in colour and tightly investing the host (fig. 29). Especially toward the tips of the hyphae of the host, it becomes obvious that the parasite-hyphae grow in a parallel fashion along the centre of the host-hyphae. Toward the edges, the hyphae fan out particularly over each of the host's hyphopodia, giving the margin a lobed effect. The hyphal ends at the margins are seen to be free and usually with indented apices. In forming this fanned effect, these hyphae divide dichotomously but their elongation is limited. As the host grows in length, established hyphal threads of the parasite also continue to grow but at a slower rate than those of the host, so leaving the termini of the hyphae of the latter free (fig. 30). Individual hyphal threads of the parasite can be seen to travel along the host marginally and appear to invest the hyphopodia prominently, especially the capitate types. At no point, except in the mat subtending the asexual stages, is the mycelium of the parasite more than one cell in thickness.

The parasite-mycelium may be in contact with the host only by a few cells (fig. 31). Occasionally, those cells of the parasite not in contact may produce downwardly growing protruberances which act as stilts and raise the parasite away from the leaf surface (figs. 32 & 33).

As regards the actual contact between the host and parasite, nothing conclusive has been reached using the light microscope. At the points of contact, the cell walls of the two fungi appear to merge. During the microtoming process, the parasite mycelium was occasionally torn away from the host. In such cases, parts of the parasite could be seen still attached to the host (fig. 34), thus supporting the assumption that there is intimate coalescence between the walls of the two fungi. In view of the contact being made only in the regions of the hyphae growing actively, the possibility of such coalescence seems feasible as the cell walls of the two organisms are, at that stage, still relatively soft. In no cases were any haustorial projections seen penetrating the host cells. It is only the cap cell of the hyphopodium of Asteridiella which has an haustorial connection with the epidermal cells of Trichocladus. From these haustoria, nutrient is absorbed into the Meliolaceous host and further exchange between the two fungi is presumed to occur at the points of contact.

The host is usually parasitised from an early age with the formation of perithecia by the host being almost completely suppressed. The vegetative growth of the host, however, is not checked at all. In a fungus so parasitised, the only sexual stages present are those arising from the parasite. If parasitisation occurs on an already well established host, it is mainly the growing tips of the host hyphae which are attacked. In such cases, there are host perithecia in the centre of the colony with parasite ascocarps on the periphery.

Asexual Spores:

The generic name, Isthmospora was established by Stevens (1919) with I. spinosa, the asexual spore stage of Trichothyrium asterophorum, being the type species. These spores are also referred to as 'isthmospores' (Hughes, 1953). In this description, the terminology as employed by Hughes (1953) will be used as the observations made in this study correspond closely with those of this author. In an exploded view of an isthmospore (figs. 35 & 36), the following parts can be distinguished:-

Four prominent ECHINULATE CELLS (e) make up the bulk of the spore and these lie in one plane. These are subglobose but may appear turgid or somewhat shrunken, brown in colour and are echinulate with short spinous outgrowths and are attached in pairs. Between the two pairs of cells are five separating cells which are also brown in colour. Firstly, separating the two pairs of echinulate cells are two OUTER SEPARATING CELLS (os). Encompassed by all these cells are two other layers, the uppermost composed of a single cell, the UPPER CENTRAL SEPARATING CELL (uc) and below this, two LOWER CENTRAL SEPARATING CELLS (lc). Supporting the whole structure and being positioned below the outer separating cells are the two basal cells which are merely the subglobose and swollen terminal portions of the subtending hyphae from which the whole isthmospore arose initially. This terminal portion is possibly delimited from the rest of the hypha by a thin wall and it is at this point at which the spore abscises from the rest of the hypha. Various views of the whole or sectioned spores are represented in figures 37 - 40.

Isthmospores arise in tight clusters in localised patches on the edge of the mycelium of the parasite (fig. 41). In the ontogeny of these spores, the hyphae of the parasite grow outwards from the edge of the investing weft and form a prosenchymatous mat, two to three cells in depth, with the hyphal threads clearly evident (figs. 42 & 43).

Development of the spores is, strictly speaking, of a thallosporous nature (sensu Vuillemin, 1910). However, as the spores as a group arise in the form of sporodochia, the asexual phase ~~is~~ has been placed in the Tuberculariaceae of the Moniliales. Any new hypha is capable of giving rise to a spore, especially toward the distal regions of the prosenchymatous hyphal weft. An oval swelling becomes evident in an intercalary position and these are prominent in that they take up more stain - e.g. cotton blue in lactophenol - than the surrounding mycelium (fig. 44). In many cases, it appears as if a bridge forms between two closely situated hyphae with a swelling forming on this bridge (fig. 49).

These oval swellings are soon cut off from the rest of the hypha by cross walls (fig. 45). The remaining parts of the original hypha in contact with the spore initial would thus be the sporophores. Two further walls are laid down in line with the sporophores (fig. 46) making the spore three-celled. Up till now, the young spore is still imbedded in the surrounding hyphae but gradually, it becomes raised above this as the sporophores elongate (fig. 50).

Already, changes in the wall can be noticed. The walls become progressively thicker and darkly coloured and the surface of the two outermost cells become gently undulate (fig. 46). Each of these two outermost cells then divides once at right angles to the plane of the sporophores and this results in the formation of four echinulate cells (fig. 47). The middle cell becomes delimited into three cells as two walls are laid down, with the outermost two cells being the outer separating cells. The central cell undergoes further divisions; the first of these is in an horizontal plane so giving rise to two cells, one on top of the other. The uppermost cell is the upper central separating cell. The lower divides once more, with the wall being laid down in line with the sporophores(fig. 48), and this accounts for the two lower central separating cells.

The sporophores in the meantime increase in size just below their attachment to the outer separating cells and it is this rather bulbous part, the basal cell, which becomes an integral part of the whole spore. At spore dehiscence, a break occurs just below the two bulbous parts and the remaining upright sporophores are left still sttached to the prosenchymatous base. The mature spore is composed of eleven cells in all.

Sexual and asexual phases are very seldom present in the same colony. Their respective appearances may be of a seasonal nature.

Microconidial Locules:

Due to the scarcity and small size of the microconidial locules, it was not possible to test for the possible germination of microconidia in water. Attached microconidia to trichogynes were also not observed. However, from the small size of the microconidia as well as the fact that asexual isthmospores were also present, it seems feasible to believe that these microconidia are spermatial in function. Early details of the development of the microconidial locules follows that for the ascocarp. The wall surrounding the locule is dark in colour and pseudoparenchyma lines this wall internally (fig. 51). In the centre are a number of small bacilliform microconidia. Details of their mode of origin was not determined.

Ascigerous Locules:

Ascocarps are spaced singly or in small groups along the edge of the mycelium of the parasite. The ascocarp initial arises as a small swelling of the terminus of one of the hyphae which fan out at the edge of the infection (fig. 52) This swollen portion soon becomes cut off by a wall. Within this single cell, smaller cells become delimited around the periphery as a result of a number of anticlinal and radial divisions. At this early phase in development, the remaining part of the initial cell can be still detected as an attaching hypha connected to the top of the young fruiting body (fig. 53). In some cases there may be an uparching of the attaching hypha and this features prominently above the young ascocarp (fig. 57). Certain hyphae in the immediate vicinity of the original ascocarp initial may also take part in the construction of the young ascocarp. As far as can be seen, these do not contribute substantially toward the make-up of the structure but ramify very superficially over the upper part of the ascocarp. In the mature ascocarp, their presence cannot be detected easily.

Further growth and divisions among the peripheral cells follows an ordered pattern and a radiate arrangement of cells is achieved (figs. 54 - 56). At an early stage, the ascocarp becomes two layered, having an upper and lower plate of cells. Both of these are of the same radiate structure and in a vertical section of an ascocarp at such a stage in development (fig. 58) it can be seen that the thickening of the cell walls is confined to the other tangential walls.

As the ascocarp approaches maturity, a clearer area becomes discernable in the centre of the uppermost layer. This finally presents itself as a prominent ostiole placed on a slightly convex part of the upper wall. From this ostiole, the cells constituting the wall radiate outwards and appear very thick walled. The lower wall also shows a radiate arrangement of cells but is typically less heavily thickened than the upper and the individual cells tend to be larger. Even in the mature stage the whole ascocarp is very flattened and slightly convex in the centre. Such a two-layered effect of the wall led Spegazzini (1889) to emphasize the separateness or distinctness of the "halves" of the ascocarp.

The first signs of further development occur in the centre of the ascocarp where the neat, bi-layered effect is disrupted by the pseudoparenchymatous cells dividing and the derivatives crowding up into the area of the future pore. These are the first cells constituting the centrum. The nucleii of these cells exhibit far more prominence in stainability than the surrounding cells (fig. 58). The actual nuclear state of the cells was not elucidated as the cells in the ascocarps tended to be very crowded and the techniques employed were not entirely adequate for cytological work.

From this central mass, a trichogyne pushes through the upper wall to the outside, the protruding portion being somewhat bulbous (fig. 59). In some cases, it appeared as if there was considerable disruption of the tissue in the immediate region of the protrusion with a number of pseudoparenchymatous cells also pushing through around a central prominent trichogyne. The origin of the trichogyne was by no means clear. Due to the distinctness of this central mass of cells relative to the ordered, layered effect of the rest of the ascocarp tissue, it seems feasible to assume that at least some of these cells of the central mass are ascogonia.

In further development, a number of prominently staining cells are seen to occupy a basal portion in the ascocarp and these are presumed to be the components of the ascogenous system (fig. 60) and asci arise from these. The cells of the centrum including the wall cells now divide at random and the ascocarp becomes filled with compacted pseudoparenchyma. The cells, however, remain small and the flat nature of the ascocarp is retained. Throughout further differentiation of the centrum, the central mass of cells remains evident but their ability to take up more stain than the surrounding cells becomes less marked.

Various difficulties arise in the further interpretation of development. The extreme flatness of the ascocarp results in the asci and related structures being somewhat crowded and compressed. This flatness also precluded obtaining a wide enough range of sections in the paradermal aspect. Hence, the developmental type exhibited could only be arrived at by inference mainly from sections through very young ascocarps. In an horizontal section through such a fruiting body, represented disgrammatically in figures 61 and 62, only ascal initials are present each composed of a prominent nucleus in the process of division, showing a nucleolus and chromosomes. Between these young asci, compressed material can be seen, parts of which can be shown to possess a cellular nature. This is the interthecial tissue made up of compressed pseudoparenchyma.

In a vertical section of the fruiting body, this compressed tissue may appear thread-like but on closer examination, it is seen, in some cases, to lack a cellular structure. This tissue merges with the pseudoparenchyma of the top and bottom of the locule where cellularity is apparent (fig. 63). It can also be seen that the asci develop centripetally, with the oldest toward the periphery, the youngest toward the centre and these all point toward the ostiole.

Figure 62 also brings out another significant feature. Here it appears that each ascus is surrounded by interthecial tissue, thus a number of monascous locules occupy the centrum. This latter state is transient. As asci mature and disintegrate after dehiscing their spores, new asci grow up and infiltrate into the centrum and compress the existing interthecial material further. At the same time, some of this interthecial material breaks down and the monascous state becomes less evident (fig. 64). The central mass of pseudoparenchyma cells remains throughout the growth of the ascocarp (figs. 64 & 65) and it is from this part that new ascogenous cells are produced outwardly.

It must be stressed that the resemblance of the interthecial tissue to pseudoparaphyses (sensu Luttrell, 1965) in a vertical section is purely superficial. It is only in an horizontal section that this material can be seen to be stromal. It may be argued that the disorganised nature of the interthecial tissue is the result of pseudoparaphyses disintegrating or becoming slimy. However, the earliest stages in development show that the interascicular material is pseudoparenchymatous and becomes compressed to the point of resembling strands by the expanding asci. Such interthecial material is not homologous with pseudoparaphyses.

Eventually, the state is reached when all the asci have released their spores and have disappeared (fig. 66). Only interthecial material remains and the outlines of locules are still discernable. The central mass of cells is still evident but shows signs of disorganisation.

The asci are obclavate (fig. 67) and bitunicate with pronounced thickening toward the apex. In distilled water, these extend up to three times their original length (fig. 68) and the spores are shot out in quick succession through an elastic pore at the terminus of the endoascal wall. The spores are unequally bicellular, the wider cell always pointing toward the ascus tip.

Discussion:

The very young ascocarp is composed of little more than an upper and lower wall, the cells of which are thickened tangentially. Toward the centre, cell divisions give rise to a mass of small cells, the first cells of the centrum, in which ascogonia become differentiated and from which trichogynes are produced. Such development would make the ascocarp of Trichothyrium an ascostroma. This feature, together with the bitunicate asci, would place the fungus in the sub-class Loculoascomycetes (Luttrell, 1955).

Within the centrum many asci arise amongst the thin-walled pseudoparenchyma each ascus being surrounded by interthecial pseudoparenchyma. The first set of asci to arise are formed around the periphery of the ascostroma. As more form, their mutual expansion results in the compression of the intervening pseudoparenchyma or interthecial tissue. This tissue has no strict structure after compression and in parts, when viewed in the vertical section, may even appear thread-like. Hence it is not at all surprising that Hughes (1953) has called these "paraphyses".

The interthecial tissue has been interpreted variously. The term "paraphysoid" employed by Müller and von Arx (1962) in describing Trichothyrium has too wide a meaning to be used where the strict specificity of structure is of significance. Interthecial tissue cannot be equated with pseduoparaphyses (sensu Luttrell, 1965). The latter are definitely hyphal in structure and represent newly differentiated parts in the centrum. Asci are formed before or concommitant with these pseudoparaphyses and grow up amongst these. This interthecial tissue, as found in Trichothyrium, is progressively compressed as the asci enlarge. In fact at maturity of the asci, the compression proceeds to such an extent that in a vertical section through the ascocarp, the interthecial material may present a thread-like facies. True pseudoparaphyses would be present as many, distinctive hyphae running vertically through the centrum. It would be highly unlikely that any section would present the view of only a single pseudoparaphysi occurring between individual asci. But where monascous locules are found compressed interthecial tissue may appear as individual 'threads' between adjacent asci. This is the case in *Trichothyrium*.

A further difficulty in interpretation arises if too old a stage in development is examined as the interthecial material either shrinks markedly or may even gelatinise so that it often disappears altogether. Such seems to have been the case in material examined by Müller and von Arx (1962) who, when characterising the genus *Trichothyrium*, described the paraphysoids as being sparse and often becoming slimy at an early age. In such an old stage in development, the monascous state may become polyascous due to the intervening tissue between asci disintegrating.

Theissen (1916) assumed that the confluence of monascous locules could develop into the polyascous condition of the Dothidea type. However, work on *Dothiora* (Luttrell, 1960) together with other reports in *Mycosphaerella* (von Arx, 1939; Barr, 1958; Higgins, 1920, 1936; Jenkins, 1930, 1938, 1939; Kerr, 1961; Webber, 1967), *Dothidea* (Luttrell, 1951) seem in agreement that the Dothidea-type locules do not arise as a result of the disintegration of a number of adjacent monascous locules but are polyascous from the start. In *Trichothyrium*, the interthecial tissue appears very disrupted in the mature state but the persistence of the central mass of cells even when all the asci have disintegrated would preclude its grouping with genera exhibiting the Dothidea type. In view of the above, it would appear that the developmental type in *Trichothyrium asterophorum* is more closely allied to the Elsinoe type (Luttrell, 1951a), since the asci occupy monascous locules at least in the youngest stages.

In 1965, Luttrell (1965, 1965a) expanded his characterisation of the Elsinoe type by instituting the Pseudosphaeria developmental type, a type only hinted at in his earlier paper (Luttrell, 1955). This catered for cases where the interthecial tissue between the monascous locules becomes so compressed as to appear strand-like or disintegrated The development in Trichothyrium would conform with this type rather than the Elsinoe type where the intervening pseudoparenchyma is of a more substantial nature - as in Aphysa rhynchoisiae included in this thesis.

On the characters of the Pseudosphaeria developmental type and the flattened, dimidiate-scutate ascostroma, Trichothyrium asterophorum would be best assigned to the order, Hemisphaeriales in Luttrell's (1965a) revised classification of the Loculoascomycetes.

Regarding the inverse development of the ascocarp described for certain members in this family (von Höhnel, 1917) this is not the case in Trichothyrium asterophorum. This observation confirms those of Petrak (1950), Arnaud (1918) and others. It is possible for an ascocarp in Trichothyrium to have one or more hyphal connections with the main mycelium and which may be attached either to the top or bottom of the ascocarp. These positions are of no significance whatsoever. In both cases, the asci are formed along the base of the fruiting body which is nearest the leaf surface and the asci always point toward the ostiole which is situated on the wall furthest away from the leaf.

Diagnosis:

Trichothyrium asterophorum (B. et Br.) v. Höhn. is a parasitic fungus on Asteridiella scabra (Doidge) Hansf. (Meliolaceae).

The mycelium of the parasite invests the exposed portions of the Melioline host forming central strands of parallel-running hyphae. Hyphae along the edge spreading out in a fan-like manner.

Asexual isthmospores, formed in sporodochia, found along the edges of the mycelium. These spores rounded-oblong in surface view, 12 - 18 x 16 - 21µ. Each spore made up of four dark brown, echinulate cells, approximately 8.8 x 8.8µ., attached in pairs laterally. Two outer separating cells are situated between the two pairs of echinulate cells. These six cells (4 echinulate plus 2 separating cells) box in three cells which are placed in two planes. In the upper plane is a single upper central separating cell and below this, two lower central separating cells with the septum dividing these latter two cells in line with the outer separating cells. Two subspherical basal cells attach the outer separating cells to the sporophores

Microconidial locules flattened, black, 40 - 45µ in diameter, 15 - 19µ in height. Microconidia bacilliform, 4.5 x 0.8µ.

Ascocarp an ascostroma, black, flattened, dimidiate-scutate, upper and lower walls showing radiate development, 130 - 160µ in diameter, 35 - 48µ in height with a prominent, central ostiole. Centrum of the Pseudosphaeria developmental type (Luttrell, 1965, 1965a).

Asci ovate, bitunicate, 35 - 47 x 14 - 16µ, arising in monascous locules. Interthecial tissue usually disintegrated when ascocarp mature. Ascospores two-celled, slightly constricted at the septum with the one cell subglobose, the other of a narrower diameter and tapering.

Taxonomic Position:

The taxonomic position of Trichothyrium asterophorum appears to be confused. According to Luttrell (1965a), the Capnodiales includes fungi with the Pseudosphaeria and Dothidea developmental types and states:

> "Ascocarps small, spherical, uniloculate, opening by a pore or by dissolution of the peripheral tissue, ... The most comprehensive classification of this miscellany is that of Müller and Arx (1962) who treated the families Capnodiaceae, Chaetothyriaceae, Trichothyriaceae, Dimeriaceae, Perisporiopsidaceae (Parodiopsidaceae), and Englerulaceae. Batista and Ciferri (Batista, A.C., and R. Ciferri. 1962. The Chaetothyriales. Beih. Sydowia. Ser.2, 3:1-129; Batista, A.C., and R. Ciferri. 1963. Capnodiales. Saccardoa 2:1-296) have monographed the Chaetothyriaceae and Capnodiaceae but these treatments serve to reveal rather than resolve the complexities of classification in this group."

In the same paper, Luttrell defines the Hemisphaeriales as:

> "Ascocarps superficial or rarely subcuticular, flattened, dimidiate-scutate, ..."

and includes, in Family 2, the Brefeldiellaceae in which he states:

> "Ascocarps with hemispheric, ostiolate locules, developed beneath a common, round to irregular, radiate thallus. The family name Brefeldiellaceae is used in place of Trichopeltaceae because Trichopeltis is congeneric with Trichothyrium and Trichopeltaceae therefore becomes a synonym of Trichothyriaceae (Müller and von Arx, 1962).

There appears from the above quotations some confusion as to where Luttrell proposes to place the Trichothyriaceae. By a process of elimination and indirect inference, it appears that he would include the Trichothyriaceae in the Capnodiales. However, the characters of Trichothyrium do not appear to closely conform with those of the order Capnodiales. On the other hand, it would appear that Luttrell states that the Brefeldiellaceae are synonymous with the Trichothyriaceae and it is unclear whether he intends placing the members of the Trichothyriaceae in this family, the characters of which, as stated for the Hemisphaeriales and Brefeldiellaceae, could describe those for Trichothyrium.

It seems, therefore, that only a personal communication with Luttrell on his true intentions can resolve the matter. It is therefore proposed not to make any definite statement as to the taxonomic position of Trichothyrium at this stage.

Note: According to Müller and von Arx (1962), the Trichothyriaceae are included in the Pseudosphaeriales.

Erratum:

In the light of the argument given on pages 58 and 59, it is best to retain the name Diplochorella amphimelaena (Mont.) Theiss. et Syd. instead of Microcyclus amphimelaena (Mont.) v. Arx until further developmental studies have been completed on the species in the two genera.

THE MORPHOLOGY AND DEVELOPMENT OF MICROCYCLUS AMPHIMELAENA (Mont.) v. Arx.

The genus *Diplochorella* was established by H. & P. Sydow (1913). Müller and von Arx (1962) incorporated this genus with that of *Microcyclus*. Hence the former *Diplochorella amphimelaena* (Mont.) Theiss. et Syd. is now listed as *Microcyclus amphimelaena* (Mont.) v. Arx and placed by the authors who effected the change in the family Mycosphaerellaceae of the Pseudosphaeriales.

In this study, the features of the infection will be described ~~investigated~~ together with the origin of the locules and the type of centrum development exhibited.

Materials and Methods:

Infected shoots of *Colpoon compressum* Berg. (Santalaceae) were collected from Belmont Valley and Highlands, Grahamstown from July to February, 1966 - 1967 and 1967 - 1968.

Squares of infected leaf tissue were cut out, fixed in form-acetic-alcohol, carried through a butyl alcohol series and imbedded in 60° wax. Sections were cut at 8 - 12μ and stained either in Heidenhain's hematoxylin with a counter stain of orange G or in Safranin and fast green.

Host-Parasite Relations:

The lesion manifests itself as a mound of hypertrophied host tissue, appearing mainly on the leaves but also found on petioles, stems, especially the young tips, and the fruits.

Early stages in infection of the leaves appear as a slightly yellowish patch on the surface, circular in outline. Hyperthrophy is evident almost immediately with these patches becoming raised above the surrounding leaf tissue. During these initial phases, the detection of a fungus from external and superficial characteristics is not obvious because the tissue, besides being slightly discoloured relative to the surrounding green tissue, does not show any brown tint usually associated with the presence of hyphae. Eventually, such a subepidermal darkening does become evident. Finally, the disc-like lesion becomes very dark brown to black in colour and is raised well above the normal leaf surface (fig. 69). Also evident is the gradual cracking of the cuticle and epidermis which is finally sloughed off from the whole zone. The surface is then seen to be covered by raised punctae, indicating the positions of the ostioles of the locules. This infected area may be double or more the thickness of the uninfected part of the leaf (fig. 70).

From serial sections, both in the horizontal (paradermal) plane as well as the transverse, the following was recorded. At the phase when slight humping of the leaf tissue is observed, hyphal invasion is fairly extensive in the leaf tissue. Hyphae are entirely intercellular, ramifying through the mesophyll tissue of the host (figs. 71 & 72). Between adjacent cells, the hyphae may branch profusely, sending out short, contracted arms in localised patches (fig. 73). Especially from such arms, tiny projection grow up against the walls of the mesophyll cells. The wall of the host cell, at this point of contact, is deeply indented and often, densely staining material may be seen to occur within the host cell at such an interface. However, there is no evidence, using the light microscope, that there is any actual penetration through the wall.

Early fungal invasion is seen to be confined to one side of the leaf but ultimately, the hyphae penetrate to the opposite side. Stromata are not initiated at both surfaces from the start but appear on one side at first and may arise on the other only well after ascostromata with asci have developed on that side.

The presence of the fungus causes the mesophyll cells to enlarge up to four or more times the size of normal cells and they do not stain up so darkly as the uninfected mesophyll (figs. 70 & 74). Chloroplast numbers are much reduced due possibly to the shading afforded by the dark stroma. The mesophyll cells also increase in number by these cells dividing with walls being laid down mainly in a plane ~~horizontal~~ parallel with the leaf surface (fig. 75). With such divisions, hypertrophy manifests itself as raised discs of leaf tissue. The vascular system remains unaltered and as a matter of fact, hyphae are absent from the region of the xylem and phloem.

Certain cells in the hypertrophied area show a thick deposition of tanniniferous substance in the cell walls and the contents within the cell are evident as dark brown globules of material with some amorphous, brown matter also present. The relationship of these to the fungus is not at all understood. Similar observations were also made during the earliest phases of infection. In some cases, a wide band of necrotic mesophyll cells may traverse the leaf from one side to the next in the hypertrophied area (fig. 76). Amongst this broken down tissue are some of the aforementioned cells with globular, brown contents. It can only be suggested that these form as a result of a reaction of the host tissue to the fungal invasion. Necrosis may reach a stage where the leaf tissue in the infected zone breaks down completely leaving a space between the ascostromata on opposed leaf surfaces.

Even before much hypertrophy in the host leaf is evident, the fungal hyphae have ramified considerably through the tissue. The first signs of a stroma forming appear on one side of the leaf. Between the mesophyll cells below the epidermis, compact masses of pseudoparenchyma become aggregated (fig. 77). As each of these masses increases in size, the adjacent mesophyll cells become much compressed and are filled with dark staining homogenous matter (fig. 78). Each of the larger patches of tissue - the future ascigerous locules - are connected by narrow bands of tissue penetrating between the dark mesophyll cells.

Microconidial locules:

Well before the individual ascigerous locules become distinguishable, tiny patches of lighter staining cells appear among the darker fungal tissue either just beneath the epidermis or deeper in the tissue. These are the young microconidial locules and are encircled by an outer layer of thick walled cells. The cells within the microconidial locules have nuclei which stain up clearly and each cell is seen to be uninucleate, whereas the enclosing dark cells appear to be enucleate or their nuclei are not readily stainable. In the locules, the cells closely associated with these enclosing layers undergo at least two divisions so giving four nuclei . Each of these becomes cellular whilst still ensheathed within the original cell wall (fig. 79). The further development of these multicellular aggregations could not be decisively determined but it appears as if the surrounding cell wall breaks down so exposing the enclosed cells. From each of these, a sterigma is produced. It is presumed that the nucleus in the mother cell divides, one daughter passing out into the bacilliform microconidium. New mother cells form progressively in a centrifugal way so increasing the size of the locule. Toward the apex, the cells become disrupted and the microconidia are gradually extruded to the outside (fig. 80). These reach the exterior after the cracking and sloughing off of the epidermis.

Microconidial locules remain functional even when the surrounding ascigerous locules reach maturity but their numbers per stroma do decrease somewhat.

Ascigerous Locules:

The initial stages in the formation of ascigerous locules are very similar to those for the young microconidial locules. Subepidermally, in each mass of pseudoparenchyma, differentiation takes place with the result that a distinct thin-walled central mass of cells becomes delimited by thicker walled cells. To begin with, there are many of these locules embedded within a stroma of host and fungal tissue, with each locule separated from its neighbours by non-functional mesophyll cells and being connected by narrow bands of stromal tissue (fig. 78). With further increase in size, each of the ascigerous locules comes to lie with its neck raised slightly above the rest of the stroma. Each locule tends to become almost separated from its neighbours (fig. 81). In effect, there is an approach here from a multiloculate stroma to the case where there are many uniloculate ones on a basal stroma.

The darker staining tissue making up the outer layers of the ascostromata is much compacted beneath the locules and the component cells link up with the hyphae which ramify through the leaf. All the mesophyll cells trapped beneath the stroma become squashed and appear as a series of plates (fig. 81).

Further details of interest in the stromal cells concern their nuclear state. Whilst the nuclei in the cells of the outer layers of the ascostromata are not readily stained, those in contact with the intercellular hyphae penetrating the leaf display darkly stained and apparently lobed nuclei (fig. 82). This lobed condition progresses to the state where the cells become multinucleate and this state is continued into the rest of the hyphae through the leaf.

Usually, only after the ascostromata on the one leaf surface are well developed does the same pattern of stromal development repeat itself on the opposite leaf surface. This results in opposed fertile layers on the hypertrophied area (fig. 70).

Each of the lighter staining areas of pseudoparenchyma destined to become an ascigerous locule assumes a somewhat flask-shaped appearance. These are filled with thin-walled cells, each uninucleate. The future apical region becomes apparent as the apex becomes beaked. In this beak, elongated cells grow up toward the apex. These persist throughout the further development of the ascocarp and are the periphyses. Figure 83 exemplifies such an early ascigerous locule with the centrum composed of compact pseudoparenchyma alone.

Toward the basal portion of such a locule, one or more cells becomes darker staining, is larger than the rest and has a prominent nucleus (figs. 84 & 85). These are the ascogonia. It appears as if more than one ascogonium per locule is the rule and these are either in a close complex or are spread out widely especially in a layer a little way up from the base. From each of these ascogonia, a bulge appears, as can be seen in the single ascogonium in figure 84. These grow upwards forming long, thread-like hyphal outgrowths, the trichogynes, which make their way through an intercellular path (figs. 86 & 87). From a few suitably stained preparations these trichogynes are seen to be multinucleate but septa are not clearly distinguishable. As the trichogynes reach the thick-walled cells at the apex, they curve between the cells to the outside (fig. 88). Toward the apical region, the trichogynes also tend to be thicker and to have darkly staining contents and in a few cases, branch shortly. By the time trichogynes have traversed the whole locule, the epidermis of the host has already cracked considerably and much of the loose tissue has broken away.

Although a number of trichogynes reach the apex, usually only a single one actually grows beyond the outer dark layers of the ascostroma. Such protruding portions are somewhat bulbous and are invested with a wide, hyaline ensheathing material (fig. 89).

Details of fertilisation were not elucidated but it was noted that in some cases, a film of debris-like matter which included bacilliform bodies of the same shape and size as the microconidia, was aggregated over each ascostroma. It is therefore assumed that sexual fusions between trichogynes and microconidia do take place.

After fertilisation of the ascogonia, ascogenous cells proliferate in a flat plane toward the base of the locule (fig. 90) and stain darker than the rest of the pseudoparenchyma. These cell are binucleate and give rise to asci directly. In each ascus initial, the two nucleii fuse and the cell housing the fusion nucleus becomes larger than the rest of the cells of the ascogenous system and elongates into the pseudoparenchyma. In conjunction with the upgrowth of asci, changes occur in the centremost cells of the pseudoparenchymatous centrum. The cells become lighter staining and cell to cell contact is lost so that these cells lie loosely in the centre of the ascostroma. This loose aggregation of cells may persist with the ascus initials growing up into the disintegrating tissue (fig. 91). In some instances, disintegration of these cells has already proceeded to a marked degree before the upgrowth of the young asci (figs. 92 & 93). In any event, whether this disintegration occurs concurrently with or a little later than the early development of the asci, an area almost completely devoid of pseudoparenchyma surrounds the asci when these have reached maturity (figs. 94 & 95). Each ascus is cylindrical in shape, astipitate and bitunicate, containing eight ascospores (figs. 96 & 97). Each of the latter is bicellular, slightly waisted at the septum, with the cell directed toward the apex being broader and with a more rounded end relative to the other hemicell. The lower cell is more tapering and slightly longer than the upper cell.

Disintegration of the central pseudoparenchyma progresses to the point where a thin layer of thin-walled tissue lines the sides of the locule. The apex remains prominently encircled by inwardly growing periphyses which, by their growth, leads to the separation of the outermost layers of the ascostroma at the top and finally to the ostiole. Each ascus in turn extends upwards and the tip of the endoascus protrudes through the ostiole. The ascospores are shot out in quick succession through the elastic pore. The spent ascus then contracts back into the locule, its place being then taken by another ascus. Collapsed asci soon disintegrate.

Discussion:

The centrum in Microcyclus amphimelaena is, initially, a mass of thin-walled pseudoparenchyma. Within this, cells (or a single cell) subsequently become distinct from the surrounding ones in that they stain darker, are larger than the rest and are prominently nucleated. These are the ascogonia which give rise to the ascogenous cells after fertilisation. Thus the fruiting body in this fungus is validly termed an ascostroma. The fungus can be described as consisting of sunken ascostromata, each closely adpressed to one another laterally and situated almost entirely in a stromal tissue which incorporates both host and fungal tissue. Since the fungus has an ascostroma and a bitunicate ascus it belongs in the subclass Loculoascomycetes (Luttrell, 1955).

With the growth of the asci, the pseudoparenchyma in the centre of the locule disintegrates and disappears with the result that a space surrounds the cluster of asci in the mature ascostroma. Such development conforms with the Dothidea type (Luttrell, 1951a) and Microcyclus would be best placed in the order, Dothideales (sensu Luttrell, 1955). The lack of any stromal tissue between the asci together with the fact that the asci are not in a broad ascocarp occupied by a single broad palisade of asci would further include Microcyclus in the Dothideaceae (Luttrell, 1951a).

The character of the arrangement of asci may be further pursued here. Considerable stress is placed on the 'fasciculate' or 'non-fasciculate' state of the asci especially in taxonomic keys. In Microcyclus amphimelaena, there are as many cases where the asci arise from a small area, therefore fasciculate, as ones which arise from a broad plate in the fruiting body. These observations are in accord with those of Webber (1967) on Mycosphaerella ascophylli. It is also suggested by Webber that the proximity of asci may be merely a reflection of the number of ascogonia per locule produced. For example, in Mycosphaerella tulipiferae with one to two ascogonia per locule, the asci are fasciculate (Higgins, 1936) whereas in M. berkeleyi (Jenkins, 1939) with many ascogonia, the asci line the base of the ascocarp. These observations coincide with those in Microcyclus where there may be one to many ascogonia per locule as well as a fasciculate or broad basal arrangement of asci. Munk (1953) also considers the criterion of the arrangement of asci to be of doubtful value. Hence it is suggested that less stress, especially for purposes of taxonomic distinctions, be placed on the term 'fascicle' and that it should be used for general, descriptive purposes only.

Finally, some comments on the institution of the new combination, Microcyclus amphimelaena (Müller & von Arx, 1962). The use throughout this text of the generic name, Microcyclus, is purely one of convenience since the taxonomic work by Müller and von Arx provides the most recent review on this fungus.

The characteristic features of the genus, Microcyclus, as noted by Müller and Sanwal (1954) and Müller and von Arx (1962) are a prominent, superficial stroma which is raised above the host leaf and typically, is connected with the host tissue by means of a narrow, central foot, with the parasite-hyphae ramifying through the tissue of the leaf. A number of loculi develop in the stroma. The illustrations for the various species of Microcyclus (Müller and Sanwal, 1954) are shown to have stromata which conform with the above description with the portion in which the sunken ascigerous locules are situated being made up entirely of fungal pseudoparenchyma.

In *Diplochorella amphimelaena*, the stroma is never a raised structure connected to the leaf by means of a foot. In fact, the stroma is almost entirely integrated with the host tissue so that it consists of a mass of hyphae as well as incorporated mesophyll cells. In construction, this fungus conforms with the description for *D. fertil~~l~~issima* (H. & P. Sydow, 1903) which was shown to have a stroma occupying the whole thickness of the leaf producing ascigerous locules on both sides. In view of the fact that the stroma in *Diplochorella amphimelaena* does not conform with that of *Microcyclus*, it may be better to retain this fungus in the genus *Diplochorella* until developmental studies of other *Microcyclus* species have been made ~~investigated~~.

Diagnosis:

Microcyclus amphimelaena (Mont.) v.Arx is a parasitic fungus on the leaves, stems and fruits of *Colpoon compressum* Berg.

Circular stromata, 2 - 5mm. in diameter, form raised discs on both sides of the leaf. The stromata originate in a subepidermal position. Due to the hypertrophy of the leaf tissue in the areas of the lesions, the stromata eventually become erumpent and exposed to the outside with the epidermis and cuticle cracking away.

The infected part of the leaf may become over twice the thickness of the normal leaf. The outermost zone of the stroma is made up of a number of closely aggregated, sunken ascigerous locules and some microconidial locules. The latter are arranged mostly around the periphery of the raised lesion. Both types of fruiting bodies are separated from each other by compressed host mesophyll cells and are situated on a compact mass of host and fungal tissue. The area between the stromata of the upper and lower leaf surfaces is normally free of any compact stromal tissue as such but hyphae, running intercellularly through the mesophyll, traverse the leaf tissue from side to side.

Microconidial locules 37 - 45 X 57 - 63µ, containing bacilliform microconidia, 2.8 - 3.4 X 0.8µ.

Ascigerous locules are perithecium-like in form, 85 - 120 X 65 - 90µ, each with a periphysate ostiole. Centrum of the Dothidea type with asci growing up into a space resulting from the disintegration of the pseudoparenchyma of the centrum.

Asci are short cylindrical, 35 X 12µ and bitunicate. Paraphyses, pseudoparaphyses and interthecial tissue between the asci are lacking. Ascospores are brown, two-celled, 13 X 4µ, constricted at the septum with the lower cell slightly longer than the upper one.

THE MORPHOLOGY AND DEVELOPMENT OF SCUTELLOIDEA CONCENTRICA, gen. nov., sp. nov.

Introduction:

This fungus described here is parasitic on the stems of Euphorbia bothae Lotsy & Godd. Uniloculate ascostromata, interspersed with microconidial locules form superficially, in concentric rings, upon the epidermis (figs. 98 & 99).

Communication with the National Herbarium, Pretoria, (Marasas, 1969) has revealed that the only Ascomycete recorded on E. bothae is one with a pulvinate stroma with ascigerous locules arranged in a peripheral layer on this stroma and has been labelled as 'Dothidea sp. indet'. These stromal characteristics do not compare with this fungus being studied.

In the keys of Clements and Shear (1957), this fungus cannot be accommodated easily into any of the genera listed in the phaeodidymous Sphaeriaceae or Dothideaceae. Employing the keys of Müller and von Arx (1962) this fungus is best placed in the Pseudosphaeriales and either in the Dimeriaceae or the Mycosphaerellaceae. However, in the genera in both families closest to this fungus in description, these are invariably featured as having 'paraphysoids' among the asci. As interascicular structures are lacking in this fungus, it cannot be accommodated in either family.

Since, after an examination of modern literature, this species could not be placed in a suitable genus, it was decided to describe it as a new genus and species.

SCUTELLOIDEA Tim, gen. nov. (Dothideales, sensu Luttrell, 1955)

Etymology: L. scutellum = shield.
Gk. eidos = form.

Stromata superficialia, unilocularia, atrobrunnea sive nigra. Asci cylindrici, octospori, bitunicati. Sporae brunneae, bicellulares.

Type species: S. concentrica Tim.

Stromata superficial, uniloculate, brown to black in colour. Asci cylindrical, eight spored, bitunicate. Ascospores brown, bicellular.

(The generic name, Scutelloidea, was chosen because the whole infection presents a shield-like facies.)

SCUTELLOIDEA CONCENTRICA Tim, sp. nov.

Etymology: L. cum = together; centrum = centre.

Stromata caulicola, superficialia, uniloculata, saepe confluentia, 65 - 113 x 110 - 175µ, ex medio evoluti plus minusve concentrice dispositi. Asci octospori, cylindrici, 75 - 88 x 20 - 25µ, bitunicati. Sporae medio vel paullo supra medium septatae, leniter constrictae, 25 - 30 x 6 - 8µ.

Stromata caulicolous, superficial, uniloculate, may be confluent, 65 - 113 x 110 - 175µ, developing centrifugally and arranged in concentric rings. Asci eight spored, cylindrical, 75 - 88 x 20 - 25µ, bitunicate, Spores two celled, medially septate or cells subequal, very slightly constricted, 25 - 30 x 6 - 8µ.

(The specific epithet refers to the concentric arrangement of the ascocarps on the host stem.)

Habitat: Stems of Euphorbia bothae Lotsy and Godd.

Type: South Africa, Cape Province, Albany District,
Hell's Poort, grid bearing 3326 A (Grahamstown).
Tim 198 (PRE, holo,; K, iso)

Materials and Methods:

Infected stems of Euphorbia bothae were collected on a farm in Hell's Poort, Grahamstown during the period July to January, 1965 - 1966, 1966 - 1967. The earliest stages in development were usually evident during August whilst the mature stages were present throughout the year.

Slices of tissue containing stromata, 1/8" in thickness, were removed and the excess latex wiped away. It was important that the latex did not cover the stromata as this would have trapped particles of grit among the fruiting bodies which would interfere with the process of sectioning. Small squares of infected tissue were fixed in form-acetic-alcohol, carried through a butyl alcohol series and imbedded in 55 - 60° wax. Sections were cut at 6 - 10µ and stained in Heidenhains haematoxylin with a counterstain of Orange G. Temporary preparations were mounted in cotton blue in lactophenol.

For the examination of the details of spore germination on the stem, infected areas were coated with two layers of clear nail varnish and left to dry for 12 hours. The strips were carefully removed after freeing the edges with a scalpel and mounted in cotton blue in lactophenol.

Host-Parasite Relations:

Ascospores are produced in abundance from the numerous ascocarps which cover large areas of the stem. However, very few of these spores actually germinate on the stem, and of those which do, only a small proportion gain entrance into the host. To elucidate some of the factors governing spore germination, the following was carried out: Segments of infected stem were positioned about 5cm. above clean stems selected from the tops of the plants. These were left overnight in a moist atmosphere to induce spore ejection onto the uninfected stems. After this treatment, the stems were placed outdoors in a protected position and at three day intervals, a nail varnish strip was made of the newly infected part.

The number of spores germinating with time did not increase significantly. Spores which germinated near stromata exhibited a more precocious development than those which germinated away from stomata. The same was found for spores germinating near a crack in the cuticle. It was thus concluded that the unusually thick cuticle afforded an effective barrier against invasion by this fungus. The fact that germ tubes ramified between cracks in the cuticle seemed to indicate that it was unlikely that invasion through stomata was due to stimulation by a specific factor or set of factors associated with stomata as spore germination occurred near to as well as away from stomata. When germ tubes were produced some distance from a stoma, they showed randomised growth and were light in colour. Those tubes near a stoma were darker in colour, penetrated into the opening directly and produced a small knot of dark cells above the guard cell (figs. 100, 101 & 102). From such a knot, a hypha would grow inward between the guard cells (fig. 102) and invade the substomatal cavity, filling this with hyphae. Invasion of the host takes place from this part and hyphae penetrate between the cells, entering the deeper tissues of the host. At all times, the hyphae, which are composed of multinucleate cells, remain intercellular (figs. 103 & 104), do not have haustoria and do not penetrate very deeply into the tissue; the infected area remains relatively superficial and there is no attendant hypertrophy evident.

Subsequent to the hyphal invasion of the internal tissues, dark aggregations of hyphal cells concentrate near a number of stomata in close proximity to each other. These fungal cells increase and displace the guard cells in the process (fig. 105) and are the initials of the fruiting bodies. In other words, the fungus gains access into the host by way of the only natural openings, the stomata. After invasion of the tissue, these stomata serve as the exits for the fungus (fig. 106) and it is on the surface of the stem that further development takes place (fig. 107).

Each stromal initial proliferates, forming a cone of pseudoparenchyma (fig. 105). From this cone, the outermost cells in localised areas divide, forming paradermal plates of tissue mostly one cell in thickness and these lie in parallel sheets (figs. 105 & 107) raised a short distance from the surface of the stem. The appearance of a young stroma in vertical section is very much that of a pagoda.

Proliferation of the hyphae of the plates is rather unique. In each cell, the protoplasm is seen to cleave into a number of separate portions (fig. 108). Each portion may again cleave so resulting in a bulging-out of the wall of the parent cell. In this way, the prosenchyma becomes propagated in all directions (fig. 109). The plates retain their hyphal construction for most part and coalesce with adjacent plates. At any point between stomata, the cells of these plates merely proliferate and form a knot of pseudoparenchyma which also gives rise to a fruiting body and hence the number of fruiting bodies per unit area becomes considerable (figs. 107 & 110). In the formation of a stroma, a few of the hyphae of the plates grow upward and undergo a series of intracellular cleavages to form a knot of cells. The process continues, with more cells being added to the developing stroma. Even when the fruiting structures, whether ascocarps or microconidial locules, are mature, the surface cells continue this protoplasmic cleavage (figs. 114 & 117) so resulting in the surface being covered with these muriform aggregations of cells. However, they differ from the cells forming the parallel plates in that these cells which have undergone protoplasmic cleavage do not go on dividing indefinitely to form plates of tissue but remain as loose clumps of cells which add to the bulk of the fruiting bodies and are easily brushed off from the surface. Their role, if any, in the biology of the fungus is unknown.

A feature for which no totally adequate explanation could be arrived at concerns the arrangement of the fruiting bodies in relation to spore invasion. To begin with, a dense mass of such bodies comes to occupy a circular patch up to 3mm. in diameter. Eventually, the infection spreads, still in a circular manner, with a relatively clear zone between the original infection and the next band. This process may continue till the area of fruiting bodies is some 2cm. in diameter, consisting of from four to six separate bands. The concentric banding itself may be due to seasonal effects. From previous observations, only ascospores germinating near stomata produce an infection. The chances of a number of stomata in close proximity being infected is highly likely even under natural conditions, as the volume of spores produced from such abundant stromata must, logically, be tremendous. What remains puzzling is why a considerable area of stem is not covered by fruiting bodies from the start and why coalescent circles of infection are relatively rare. Old parts of the stems do show a considerable intergrading of circles of the fungus but this is the result of the original rings increasing in number with time. Perhaps there is some sort of inhibitory reaction whereby the first spore to gain access into the plant sets up a barrier which inhibits the further development of other germinated spores in the vicinity.

Microconidial locules:

The first fruiting bodies to be produced during the early phases of infection are the microconidial locules. In the cone-shaped mass of pseudoparenchyma formed above each stroma, a lighter staining portion in the centre becomes obvious. On closer examination when the structure is of a reasonable size, fertile portions producing microconidia can be seen to arise in a number of parts within the central, lighter staining part. As each of the parts producing microconidia develops at a different rate, the structure appears to be multiloculate. Because of its amorphous shape (fig. 111) the microconidial locules are clearly distinguishable from the ascigerous locules (fig. 110). The same procedure in locule formation takes place in the stromata developed on the hyphal plates between stomata.

The formation of microconidia takes place centrifugally. Microtome sections did not provide conclusive information as to the origin of the microconidial mother cells; squash preparations of young stromata proved far more satisfactory here. Internally, in each locule, the pseudoparenchyma takes on a loose appearance and each cell can be seen to have dense protoplasm with a number of nuclei . Each cell destined to form microconidial mother cells becomes somewhat elongated and points toward the centre of the locule. The protoplast undergoes cleavage so forming a row of segments (fig. 112). Cell walls eventually form and the whole structure consists of a row of cells, each with a single nucleus. The squash preparations showed most of the developmental stages as individual portions in each of the cleared cells stained up with the cotton blue, with the wall of the original cell investing the portions.

After wall formation, each portion becomes a mother cell. Microconidia are first produced from the terminal cell. The tip of this cell becomes drawn out to form a symmetrically placed sterigma from which a bacilliform microconidium is cut off. The microconidia are uninucleate and even when still attached to the mother cell, the latter is seen to contain a prominent nucleus. It can only be presumed that each mother cell is capable of giving rise to more than one microconidium. Cells lower down the series may also produce sterigmata. It seems logical to conclude that after a number of microconidia have been formed, each mother cell disintegrates, their role being continued by other cells further down the series. Cells deeper in the pseudoparenchyma lining the locule assume similar functions after their protoplasts cleave. This would account for the progressive enlargement of the locule with the concomitant growth in size of the stroma.

The microconidia fill the locule and ooze out through a number of irregular openings which break through the outer layers of the stroma. The actual role of these microconidia in this fungus is unknown but it presumed to be spermatial. Their small size and abundant production are features similar to structures in other fungi where their spermatial function has been ascertained.

Ascigerous Locules:

Ascostromata are initiated in the same way as the microconidial locules. Mounds of pseudoparenchyma arise either above the stomata or form as a result of the proliferation of the prosenchyma lining the surface of the host. Whilst stromata destined to become microconidial locules show the presence of microconidia at a very early age in the lighter staining central mass of cells, ascostromal masses appear more compact to begin with (fig. 113). The central mass soon loses its compactness and the cells appear almost separated from one another (fig. 114). Among these cells, a loose, partially coiled, multinucleate structure arises with a projection reaching up toward the apex. In a few cases, these projections appeared to penetrate the outer, dark layers of the stroma and to reach the outside (fig. 115). This structure is possibly the ascogonium with trichogyne. Should this interpretation be correct, such an ascogonium is unusual since the cells do not take up more haematoxylin stain than the surrounding tissue as has been observed in other genera with similar structures.

The area around the ascogonium becomes progressively more open and soon, is surrounded by a space with the cells lining the inner part of the ascocarp pointing inwards (figs. 116 & 117). These latter cells are elongate and appear to consist of a number of fragmented portions of the protoplast of single cells with each fragment having its own nucleus. Cell walls eventually delimit these portions in a linear series in the same way as was noted for the microconidial mother cells. The formation of the space around the ascogonium comes about as a result of the breakdown of the centrum cells. Remains of these cells in the form of extremely fine cell walls, devoid of contents, can be detected around the ascogonium. These often appear as fine threads connecting the ascogonium to the surrounding stromal layers.

Figures 116 and 117 show the lobed ascogonium as having a number of pairs of nuclei , indicating that plasmogamy has already occurred. Cell walls are not apparent and the ascogonium is still multinucleate. At the next stage of development, cell walls become clearly defined and it can be seen that the ascogonial complex is made up of a number of cells in a chain like series, somewhat reminiscent of the cells from which the microconidia arise (fig. 118). In effect, the delimitation of these bicellular segments also entails protoplasmic cleavage. Short lateral branches grow out from some of the individual cells and the binucleate condition becomes perpetuated into these arms. These constitute the ascogenous hyphae and radiate out from a complex of cells toward the base of the locule (fig. 119). Such a complex of ascogenous cells from which asci arise, was also recorded in Dothidea collecta (Luttrell, 1951).

In each of these short ascogenous hyphae, the two nuclei fuse directly, giving rise to the fusion nucleus in the ascus initial. Croziers are not formed. This ascus initial become much enlarged and vacuolate and the single fusion nucleus stands out prominently (figs. 120, 121 & 122). As ascus development progresses, the whole locule enlarges and the clear area around the ascogenous system becomes wider allowing for the ingrowth of asci to carry on without hindrance from extraneous tissue. At the apex, a beak develops and is lined with rows of cells similar to those which line the rest of the locule. These, however, are longer and persistent and remain as the periphyses whereas the others in the main body of the locule soon lose their identity, possibly as a result of the crowding by asci (figs. 122 & 123). A pore finally breaks through the dark layers of the ascocarp. Eight bicellular ascospores are produced in each ascus. The spores are dark in colour and are composed of two equal to subequal cells with a waist at the septum. The asci are cylindrical (fig. 124) and bitunicate and on extension, the endoascus of each ascus in turn reaches through the pore and the spores are shot out in quick succession. Figure 125 shows an ascus after the expulsion of spores.

Discussion:

The early ascigerous locule consists of a compact mass of pseudoparenchyma composed of thick walled peripheral cells devoid of stainable contents and thin-walled, nucleated cells of the centrum. Within the latter, an ascogonium becomes differentiated and such development would make the fruiting structure an ascostroma. This, together with the presence of the bitunicate ascus would place Scutelloidea concentrica in the Loculoascomycetes (Luttrell, 1951a

The fact that this fungus is described in a new genus, the specific definition of the general stroma is important. The initial stromata emerge from the stomatal openings and are positioned superficially on the surface of the host. From these, plates of prosenchyma grow out, invest the surface of the stem and further stromata form as a result of the proliferation of cells of these plates. These parallel plates would therefore also constitute the stroma. The ascigerous locules would be correctly described as many uniloculate ascostromata situated on a stromal base of parallel plates of prosenchyma.

Disruption of the centremost cells of the centrum progresses to the point where the asci grow up into a space. Interascicular structures such as paraphyses, pseudoparaphyses, interthecial tissue (sensu Luttrell, 1965) are absent. Such development would conform with the Dothidea type and would place *Scutelloidea* in the Dothideales (Luttrell, 1951a). It would also be conveniently included in the Dothideaceae due to the perithecium — like locule and fasciculate arrangement of asci.

The frequent occurrence of protoplasmic cleavage as a form of cell division in *Scutelloidea concentrica* is a feature shared with *Dothidea collecta* (Luttrell, 1951). It appears that almost all the cells, to begin with, are multinucleate. As a result of intracellular divisions, the uninucleate condition is brought about in all cells with the exception of the ascogenous hyphae where binucleate cells are delimited.

Diagnosis:

Scutelloidea concentrica Tim is a parasitic fungus on the stems of *Euphorbia bothae* Lotsy & Godd.

Uniloculate ascostromata interspersed with microconidial locules found superficially, in concentric rings, upon the epidermis. Adjacent rings may coalesce. Whole infection 5 - 18mm. in diameter. Ascostromata perithecium - like in form, 88 - 113 x 110 - 175µ, arising through the stomata or from the paradermal plates of stromal tissue which connect the various ascostromata. Centrum of the Dothidea developmental type. Asci fasciculate, each being cylindrical, bitunicate and eight-spored, 75 - 88 x 20 - 25µ. Spores two-celled, dark, medially septate or cells subequal, slightly constricted at the septum, 25 - 30 x 6 - 8µ. Paraphyses, pseudoparaphyses, interthecial tissue lacking. Each microconidial locule irregular in shape, consisting of a number of separate locules in contact with each other, 50 - 110 x 65 - 135µ. Microconidia bacilliform to ovate, 1.8 - 2.5 x 0.7 - 0.9µ.

Taxonomic Position:

S.C. Loculoascomycetes.
O. Dothideales.
F. Dothideaceae.

THE MORPHOLOGY AND DEVELOPMENT OF ANTENNULARIA ENGLERIANA (P. Henn.) v. Höhn.

Antennularia engleriana is a fungus which is parasitic on the stems of species of Erica (Ericaceae). This fungus has been assigned to various genera over the last 70 years. It was perhaps Doidge (1941) who described it most comprehensively under the name Dimerosporiopsis engleriana, a combination proposed by Hennings (1901). Von Höhnel (1909) transferred it to the genus Antennularia, whilst retaining the epithet of Hennings, hence Antennularia engleriana (P. Henn.) v. Höhn. In 1928, v.d. Byl proposed a new combination for this fungus, this being Gibbera engleriana (P. Henn.) v.d. Byl.

According to the taxonomic characteristics followed by Müller and von Arx (1962), this fungus must be placed among the Venturiaceae on spore characteristics and the presence of an hypostroma. However, the possession of superficial hyphae would preclude its inclusion in the genus Gibbera and the fungus has thus been transferred back to Antennularia under the original combination of A. engleriana. The use of the latter name here is one of convenience. In the light of the lack of detailed developmental studies on genera noted as synonyms for this fungus it seems futile to debate the validity of the assignation of any particular name. This description is, therefore, offered purely from a developmental point of view with the object of finding out whether or not the formation of the centrum conforms with one of the types proposed by Luttrell (1951a) and, on this basis, to suggest its taxonomic position.

Material and Methods:

The material for this study was found growing on the stems of species of Erica. Collections of parasitised Erica brownleea Bol. were made during April 1965/6 and also in March, 1966 from the Hogsback area (nr. Alice, E. Cape) and of Erica nemorosa Kl. ex Benth. in the Belmont Valley, Grahamstown, E. Cape, and also approximately eight miles from Grahamstown on the road to King William's Town. Collections of E. nemorosa were made during November, 1965 and February/March, 1966.

For developmental studies, portions of cortical tissue plus fungus were fixed in formalin-acetic-alcohol, dehydrated in a butyl alcohol series, embedded in paraffin wax (55°C.) and sectioned at 8µ on a rocker type microtome. For certain sections, a sledge microtome was also employed. The sections were, in most cases, stained in Heidenhain's hematoxylin and counterstained in Orange G (Johansen, 1940). Temporary squash preparations were made from fresh and fixed material. The early stages of ascocarp formation were deduced from the superficial hyphae mounted in lactophenol but for studies of the ascogenous hyphae, the asci and cytological stages, the preparation and staining techniques according to Wittman (1962) were employed.

Host-Parasite Relations:

Fungal invasion is initiated on very young shoots of the host plant resulting in the hypertrophy of these areas (fig. 126). The peridermal layers become cracked and the entire area is covered with a brown to black mass of intertwining fungal material. On the stem surfaces, prominent cushions of a cellular construction, brown in colour, are present and from these arise tufts of erect hyphae (figs. 127 & 128) which, due to the closeness of the

Normal, unparasitised stems were also sectioned and observed for comparison with infected stems. The observations on page 77 were also confirmed here.

cushions, appear to completely clothe the infected parts. Numerous ascocarps are to be found associated with the erect hyphae.

With regard to the hypertrophy of the tissues, it was decided to investigate ~~elucidate~~ to which tissues the increase in girth could be attributed and if fungal hyphae penetrated throughout the stem or were confined to a certain tissue. In this investigation, embedded material was sectioned in traverse, radial and tangential planes at 10µ - 20µ on a sledge microtome. The transverse and tangential sections were cut from a piece of stem which was partially infected and showed hypertrophy over half its circumference with the other half un-infected. It was thus possible to compare both parasitised and unparasitised tissue in the same section. As indicator for extent of infection, sections stained in cotton blue in lactophenol proved to be best, the hyphae taking up the blue stain. But for clear microscopical details of hyphal growth in the tissues, sections stained in safranin and fast green were resorted to.

Hyphal growth is most extensive in the cortex of the stem. Pockets of hyphae, consisting of threads or compacted pseudoparenchyma and homologous with the cushions of tissue occurring around the stem periphery, contrast with the surrounding yellow to hyaline tissue (fig. 129). Interconnecting hyphae between adjacent hyphal pockets consist of multicellular threads. Such threads, made up of short, uninucleate cells, extensively traverse the primary and secondary phloem as well as most of the secondary xylem. These fungal threads, clearly seen in radial section, are either single strands or a number closely applied to and running parallel with each other. A point of note is the fact that the hyphae are entirely intercellular.

Although cortical tissues and phloem in the infected zones are far wider than those in the uninfected areas, hypertrophy is essentially the result of an excessive production of secondary xylem, this tissue being almost double the width of that in the uninfected area. The strands passing through the secondary xylem are continuous and are either separate from the parenchymatous rays (fig. 130) or else intermingled with the ray cells (fig. 131). Of comparatively infrequent occurrence are the presence of vertically interconnecting strands from one radial hyphal 'ray' to an adjacent one (fig. 130). Transverse interconnecting strands in the secondary tissues have not been seen.

To validate the observation of the entirely intercellular nature of the invading hyphae, the tangential sectional view (fig. 131) of the secondary xylem was carefully examined. Without exception, the hyphae, whether single threads or in groups are seen to lie in the region of the middle lamella between tracheids or vessels or ray components. Interconnecting hyphae in the vertical system are also intercellular as these have been observed not to pass through any cell/s in the tangential view. Haustorial projections into the cells themselves, especially those of the phloem, cambium or cortex have not been seen. Due to their position it appears that the growth of the fungal threads keeps pace with the radial growth of the secondary tissue. This conclusion has been reached not only from the fact that the fungus occurs in continuous bands throughout the secondary tissue but also because fungal invasion does not extend beyond a clearly defined line in the secondary xylem, this line indicating the stage of growth at which infection took place.

Ascigerous Locule:

De Bary (1884) designated the term 'symphogenous' to the type of pycnidial development where young hyphal branches interwove to give rise to a compact knot of tissue, this ultimately forming the fruiting body. Zopf (1890) called a structure so formed a 'Knäuelfrucht'. Though these terms were applied to pycnidia, they might equally be used to describe the initial stages in the development of the ascocarp as encountered in this fungus. The erect hyphae clothing the surface of the stem in the infected area do not normally branch. However, some form short loops (figs. 132, 133 & 140). It appears as if there may be some mechanism governing the formation of ascocarps for it has been observed that only contiguous hyphae contribute to the formation of any specific fruiting body and that looping of hyphae only occurs among closely applied strands. Eventually these hyphae mass and intertwine and form a knot of compacted tissue (figs. 134 - 137) which, in the early stages, shows a pseudoparenchymatous internal structure (figs. 138 & 139). In very young stages, these knots are raised well above the surface of the stem (fig. 128). This was noted by Doidge (1941) who , it appears, did not connect the early stages of their development with the looping and anastomosing of the erect hyphae. These ascocarps are gradually drawn downward toward the surface of the host stem and a range of intermediate stages between the very young ascocarp ~~to~~ and the mature ones is present at any one time. In the case of the mature ascocarps, these are closely applied to the cushions of pseudoparenchyma mentioned previously (fig. 127). Even in this latter state, very short, intertwined hyphae supporting the ascocarps are still evident. Size increase of the ascocarps are the result of the more proximal hyphae anastomosing with the initial mass and also the division and differentiation of the pseudoparenchymatous cells making up the young ascocarp.

Initially, no fusion of the contributing hyphae takes place, as seen in a section of a very young fruiting body measuring some 40µ at its largest diameter. In an older ascocarp, 65µ - 70µ in diameter, the early stages in differentiation are detectable with the tissue in the centre being decidedly pseudoparenchymatous and consisting of closely compacted, polygonally shaped cells (figs. 138 & 139). Cell walls of the latter also tend to be lightly staining relative to the darker walls of the outermost cells of the ascocarp. This central tissue of thin-walled cells constitutes the early development of the centrum.

Within the central pseudoparenchyma, changes in structure soon become apparent. From some of the cells, hypha-like outgrowths are produced which, by their elongation, disrupt the neat, contiguous arrangement of cells seen in the previous stage. Concomitant with this development, a number of cells, centrally placed, can be distinguished from the others. These are conspicuous by their prominent nuclei composed of clearly defined chromosomes and nucleoli (fig. 141 - 144). This is the reproductive initial and is lobed but the compactness of the constituent cells did not allow for distinguishing between a separate ascogonium and antheridium. Consequently, the structure is considered a lobed ascogonium. The surrounding thread-like outgrowths are now more distinct and can be seen to arise from some of the pseudoparenchymatous cells. These are the initials of the pseudoparaphyses (Luttrell, 1965) and they already show septation into component cells.

The transverse section of the ascocarp from which figures 141 or 144 were taken does not indicate whether or not the pseudoparaphyses arise from any specific part of the fruiting structure. However, in the vertical section of the ascocarp (figs. 142 = 145), the outgrowths can be seen to arise from the uppermost cells of the centrum and are most prominent in the region immediately above the ascogonium. The nuclear state in the latter is better defined and its multinucleate condition clearly apparent. Some of the nuclei are paired, suggesting a dikaroytic phase. The details of plasmogamy and karyogamy have not been elucidated.

In its further development, the ascogonium gives rise to a number of branches, the ascogenous hyphae, which extend up among the downwardly growing pseudoparaphyses (fig. 146). The ascogonial branches are distinctly multinucleate (figs. 143 = 147) with dikaryons apparent here and there. The structurally distinct pseudoparaphyses consist of almost parallel-running strands of cells which traverse the locule from top to bottom, are freely branched (fig. 148) and appear to be connected to the pseudoparenchyma at both ends. These pseudoparaphyses remain evident throughout the further stages of centrum development. The suggestion by some authors (Cavara & Mollica, 1907 and Arnold, 1928) that the elongation of the pseudoparaphyses is instrumental in the size increase of the fruiting structure has not been ascertained in this study. Nevertheless, it is significant that the growth in length of the pseudoparaphyses does keep pace with the general growth of the ascocarp.

As a result of the rapid elongation of the pseudoparaphyses, the ascogonial complex becomes confined to the lower half or base of the centrum (fig. 149). Young asci, each conspicuous by a large fusion nucleus, grow upward amongst the pseudoparaphyses (fig. 151). A transverse section taken toward the base of a young ascocarp (fig. 150) lends prominence to the developing asci. The asci do not arise from many individual fasicles but eventually line the whole base of the locule with a tendency to be more concentrated toward the periphery. Finally, in more mature ascocarps filled with well-developed asci containing ascospores (figs. 152 & 153), the pseudoparaphyses are still clearly evident with the asci positioned between the strands. Most obvious at this stage is the point of origin of these pseudoparaphyses. They arise from the uppermost part of the centrum especially toward the region of the future ostiole and fan out in a downward direction from this point (figs. 149, 151 & 152).

In dry conditions, the globose ascocarps are flattened in the ostiolar area. The apical cells of the outer, dark, thick-walled layers of each of the ascocarps gradually disintegrate to form a pore of irregular outline. The thin-walled pseudoparenchyma and pseudoparaphyses remain as a continuous layer beneath the pore even when the opening has been formed. On wetting, the ascocarp absorbs water rapidly and swells up.

From the ascogenous hyphae, asci arise by typical crozier formation. Though the nuclear state of the ascogenous hyphae could not always be definitely determined, another observation substantiated crozier formation. In figure 154, the ascal initial is connected with the cytoplasm of the subtending basal and terminal cell of the crozier complex by two narrow strands of cytoplasm. These strands are especially conspicuous at the junction between the ascal initial and the subtending cells by the presence at this point of two dark spots. These are probably plugs of refractive substance in an intercellular position occurring between the cells involved in ascus formation.

As regards plugs in parts other than below the ascal initial, these could have been formed by any nucleus in the ascogenous hypha dividing, one of the daughter nuclei passing into a lateral projection produced from the side of the cell and a well forming between the nuclei . The uninucleate tip cell could fuse with the preceding uninucleate cell and after nuclear fusion, give rise to a new ascus. Such ascus formation has been described in Glomerella (Wheeler et al, 1948) and this could account for curved lateral projections which are evident all along the ascogenous hyphae of A. engleriana.

Asci are clavate (fig. 155) and bitunicate and contain eight spores each of which is unequally bicellular, up to 16µ in length and 5 - 6µ in width, slightly constricted at the septum and pluriguttulate (fig. 156). Observations of the bitunicate effect were made on a clump of asci either squashed or dissected out of locules. After extension, the ascus exceeded the length of the original by as much as two and a half times (fig. 156). Often, extension was abnormal in that the ectoascus was inclined to split in two or more places with the result that a number of collars or constricting rings were evident (fig. 157). Such a result has also been observed by Luttrell (1951) in Dothidea collecta and is considered an atypical condition resulting from extension in water. In normal extension, only a single collar of the ectoascal wall remains (fig. 156).

On spore ejection, the spores are arranged in a single file in the upper half of the ascus (fig. 156) and are shot out in quick succession. The orientation of each of the spores is also a consistent feature in that the shorter, blunter hemicel always points toward the apex. After all the spores have been shot out, the ascus contracts, revealing a thick endoascal wall with the ectoascal wall still apparent.

Discussion:

The ascocarp in its youngest form is a mass of interwoven hyphae. These hyphae eventually coalesce and give rise to a pseudoparenchymatous structure which is divided into an outer layer of dark, thick-walled cells and a central zone of thin-walled cells. One of these centrally placed cells becomes differentiated into the ascogonium. The latter has thus arisen in a pre-formed stroma and the ascocarp is essentially an ascostroma. This fact, correlated with the bitunicate ascus, would place Antennularia engleriana in the subclass Loculoascomycetes (Luttrell, 1955).

The term, pseudoparaphyses, used here to describe the hypha-like structures which traverse the centrum from top to bottom is as defined by Luttrell (1965). As far as can be deduced from the sectioned material, these structures originate as outgrowths from the stromal cells especially those above the ascogonial complex. With subsequent downward growth, which may be mainly intercalary, they take on an almost vertical orientation and are attached at both ends. Their distinct, thread-like, branched and cellular make-up has been emphasized. According to Singer and Gamundi (1963) the term 'paraphysoid hyphae' has been ascribed to threads which are originally attached to the top of the ascocarp and which grow down but remain free from the bottom, and 'paraphysoid threads' for hyphal outgrowths attached at both top and bottom of the ascocarp from the beginning of development. Ontogenetic details of these two types do not strictly apply to the structures encountered in this fungus. Gäumann (1926) applied the term 'pseudoparaphysis' to the thread-like structures in the Loculoascomycetes, noting 'paraphysoid' (sensu Petrak, 1923) as a synonym. The objection against the use of 'pseudoparaphysis' is twofold. Firstly, it has been applied to an hymenial element in certain Basidiomycetes. Secondly, Petrak (1923) employed 'pseudoparaphysis' to describe hypha-like outgrowths which were free at the tips but these are better known as true paraphyses. The use of 'paraphysoid' has been avoided due to its application to both pseudoparaphysis and interthecial tissue. Further emphasis on the use of 'pseudoparaphysis' has been made by Luttrell (1965) who stresses these are the only distinctive structures in the Loculoascomycetes for which a special term is warranted. The mode of origin and development of the structures as found in this fungus corresponds with Luttrell's description and the term 'pseudoparaphysis' has been employed here.

The pseudoparaphysate centrum and the perithecium-like ascostroma places A. engleriana in the order Pleosporales (as delimited by Luttrell, 1955). Müller and von Arx (1950, 1962) have retained the order Pseudosphaeriales, established by Theissen and Sydow (1918), in which are included forms with uniloculate, perithecium-like ascostromata. The order Pleosporales has been used here instead of Pseudosphaeriales because of the indiscriminate inclusion in the latter of forms in which the centra contain either pseudoparaphyses or interthecial tissue. These structures, as indicated by developmental evidence, are not homologous.

In 1950, Müller and von Arx proposed the family Venturiaceae which included genera with two-celled, ovoid or ellipsoid ascospores which were hyaline or pale green and which usually became olive brown or grayish green at maturity. Since the spores of this fungus studied conform with this description, *A. engleriana* would be best placed in the family Venturiaceae. However, it must be reiterated that the use of the generic name, *Antennularia* in preference to *Dimerosporiopsis* is tentatively agreed upon until other forms included in the former genus have been investigated. ~~In fact,~~ Only until investigations are carried out over a broader range among species grouped in the Pleosporales (of Luttrell) and the Pseudosphaeriales (of Müller and von Arx), can more definite criteria for subdivisions into various families be proposed.

Diagnosis:

Antennularia engleriana (P. Henn.) v. Höhn. is a fungus parasitic on the stems of species of *Erica* (Ericaceae), causing hypertrophy of the host tissues. Infected parts are covered on the outside with tufts of erect, dark-brown hyphae. The individual hyphae may be up to 400µ in length and 7µ wide. Mature ascocarps (ascostromata) numerous, nestling amongst the erect hyphae. Ascocarp wall grossly verrucose and coriacellate when wet. Ascocarp globose but somewhat flattened to concave at the top. No true ostiole but a pore develops as a result of the tissue breaking down at the apex. Centrum of the Pleospora developmental type with asci growing up between vertically arranged pseudoparaphyses. Asci cylindrical, of dimensions 100µ x 12µ and contain eight ascospores. Each spore unequally bicellular, up to 16µ in length, 5 - 6µ wide, slightly constricted at the septum, pluriguttulate and pale green in colour.

Taxonomic position:

S.C. Loculoascomycetes.
O. Pleosporales.
F. Venturiaceae.

THE MORPHOLOGY AND DEVELOPMENT OF

TRIPOSPORA TRIPOS (Cooke) Lindau.

Introduction:

Tripospora tripos (Cooke) Lindau is a parasitic fungus consisting of stromata of parallel rows of flask - shaped ascocarps with microconidial locules situated between these (fig. 158). The host range for this fungus has been reported to include various species of Podocarpus (Doidge,1950). However, in this study, parasitised leaves of Podocarpus falcatus R.Br. have been used.

The family, Coryneliaceae, established by Saccardo (1891) was included in the Sphaeriales of the Pyrenomycetineae by Lindau (1897). The Pyrenomycetes were considered to have an hymenium enclosed within a shell (or perithecium), the latter usually opening by means of an apical ostiole. The hymenium itself either lined the base of the ascocarp or also, the sides of the inner surfaces. With such a broad characterisation, the Pyrenomycetes included genera with various types of ascocarp including those with locules in a stroma, each locule lacking a wall of its own (ascostroma). Some of these ascostromal forms had been recognised earlier and grouped in the Dothideaceae, a family established by Nitschke and Fuckel (Fuckel, 1869). Lindau (1897) recognised a few such types and attempted to separate these in his Dothideales. Nevertheless, this order contained some members with true perithecia while his Sphaeriales included ascostromatic forms.

The significance of the presence or absence of a wall was further stressed by Miller (1928) who considered the Myriangiales, Pseudosphaeriales, Perisporiaceae, Coryneliaceae and Dothideales all to be related. Gäumann (1940) also recognised the distinctness of the ascostromatic forms and from the findings of McCormack (1936) that the Coryneliaceae are ascostromatic, included this family in the Pseudosphaeriales. Martin (1945) grouped the Coryneliaceae among the Dothideales.

It was Luttrell (1951a) who emphasized the correlation of ascus structure with ascocarp structure and on this basis, came to recognise two main sub~~orders~~classes, the Loculoascomycetes and the Euascomycetes (Luttrell, 1955). In the former are species with a bitunicate ascus and an ascostromatic ascocarp whereas in the latter, a unitunicate ascus and a walled ascocarp (perithecium, cleistothecium) is present, the wall "arising as a result of a stimulus derived from the presence of an ascogonium".

The unitunicate asci in the ascostromatic ascocarp of Caliciopsis pinea (Coryneliaceae) (McCormack, 1939) seem to constitute an example which breaks away from the scheme proposed by Luttrell (1951a). With this anomaly in mind, the primary aim of this morphological study of Tripospora tripos is to elucidate the details of the mode of origin of the stroma and the ascigerous locules of this Coryneliaceous fungus.

Materials and Methods:

Infected leaves of Podocarpus falcatus were collected at intervals mainly between August and April in the years 1966 - 1967 and 1967 - 1968 from the Belmont Valley, Grahamstown. Most of the young stages were found during the early summer period (mid-October to early December) but a range of developmental stages was present throughout the warmer months.

Material was fixed in form-acetic-alcohol, carried through a butyl alcohol series and imbedded in either 55° or 60° wax. Sections were cut at 8 - 10μ, stained in Heidenhain's haematoxylin and counterstained with orange G.

Host-Parasite Relations:

Ascospores are produced in great abundance throughout the year especially during the summer months from November to February. As the asci are early deliquescent, the free spores are pushed up the neck of the ascocarp and aggregate in densely packed mass in the funnel-like ostiole. The spores are easily dislodged by the wind and are distributed widely over the whole plant.

Infection takes place through the surface of the young leaves of P. falcatus. Germ tubes from the ascospores grow out over the leaf surface and enter through the stomata which are in rows running longitudinally along the leaves. Epidermal and mesophyll cells, but not the sclerenchyma, are invaded intracellularly(fig. 160). An early reaction to such invasion is the appearance in some of the mesophyll cells of a number of closely aggregated, dark brown globules which take the place of the cell contents. Such a reaction has also been noted in the host plant, Colpoon compressum after invasion by Microcyclus amphimelaena, details of which are also included in this thesis.

Initially, a number of discrete stromata are found in the area immediately below the stomatal points of entrance and, characteristically, are arranged in rows. These rows may appear along the edge of a leaf or anywhere on the surface of the lamina both adaxially and abaxially (fig. 159). No entirely satisfactory explanation for the occurrence of stromata in strict rows has been arrived at. Perhaps, after infection through one or more of the stomata, the passage of least resistance is taken, this being a channel along such a row of stomata because, whilst the substomatal cavities are not in direct communication, the intervening tissue between successive stomata is of a very few thin-walled mesophyll cells. Lateral spread of the hyphae, which initially do appear to be mainly subepidermal, would possibly be hindered due to the flanking sclerenchyma of the hypodermis on either side of the stomata (fig. 160). While the above hypothesis provides some sort of possible explanation for the row-formation of stromata, it does not explain why individual leaves are not far more heavily covered in fruiting structures than they normally are. Logically, random infection from such abundantly produced ascospores should open the leaves to being almost entirely covered with stromata. This is hardly ever achieved. Although the individual stromata coincide with stomatal openings (fig. 159) these ultimately become confluent and longitudinally continuous (fig. 158).

Microconidial locules:

The invading hyphae below a stoma divide forming a compact mass of combined fungal and host tissue (figs. 161 & 162). This mound of tissue expands and finally becomes erumpent with the cuticular layer splitting away and exposing the stroma to the outside (fig. 163). Parasitised mesophyll cells, now discernable only by their cell walls,as well as thick-walled sclerenchyma cells, are incorporated into the stromal tissue and may even be moved some distance from their original positions by the upgrowth of fungal tissue (figs. 164 & 167).

Toward the upper part of the stroma, a lighter coloured area in the dark, thick-walled pseudoparenchyma, becomes apparent. This is the first sign of the formation of the microconidial locule. As this area enlarges it can be seen that the inner wall is made up of closely packed, columnar to flask-shaped conidiophores which arise from a thin layer of thin-walled pseudoparenchyma (fig. 165). Each conidiophore is uninucleate and uninucleate microconidia, ranging from bacilliform through elliptical to almost ovate forms are produced at the tips (fig. 166). The centre of the locule is filled with a mass of microconidia and these find their way to the exterior by way of a pore which breaks through the upper wall (fig. 164). Extruded microconidia cluster around the opening and eventually find their way all over the surface of the stroma. Most abundant production of microconidia occurs when the locules are newly formed. At this time and in the unstained state, the microconidial locules are filled with pure white microconidia but at a later stage when the ascospores are being produced in abundance, the microconidia appear dark and are not produced in the same quantities as before; they also seem somewhat vacuolate with the nuclei staining up indistinctly. The production of abundant and translucent microconidia seems to coincide with the young stages in development of the ascigerous locules, when ascogonia are present.

Ascigerous Locules:

Figure 164 shows the earliest recognisable stage in the formation of ascigerous locules. The latter appear as mere buds around the periphery of the individual stromata, pointing mainly toward the margin of the leaves.

Within these buds, a lighter staining zone can be seen, entirely enclosed by the dark pseudoparenchyma of the stroma. This is the early centrum. The only structural distinction between this centrum and the rest of the stroma is the presence of a number of thread-like hyphae, forming a prosenchyma, occupying the bulk of the locule. Each of the buds bulges out progressively, producing at first a short neck with a slightly swollen basal portion merging with the stroma housing the microconidial locule (fig. 167). These bulges are extensions of the existing stroma. While this growth is going on, changes are taking place in the locule. Some of the threads of the prosenchyma can be seen to have free ends and become orientated upward toward the apex of the locule. These eventually line the whole length of the neck. The darker staining stromal cells in contact with these hyphal threads also exhibit a basically prosenchymatous nature especially toward the apical zone and merge with the rest of the pseudoparenchyma of the stroma.

In the interior of the basal portion, the tissue is compacted pseudoparenchyma and this grades into the periphysate neck cells through an intermediate zone of prosenchyma. Some of the cells of the pseudoparenchyma in this basal part appear more distinctive in that they are lobed and the individual cells tend to be larger than the rest. By examination of transverse and longitudinal sections through the basal portion of the ascigerous locule, it was possible to piece together the construction of the lobed cell masses. Each of the latter consists of a somewhat coiled complex of multinucleate cells, each cell being irregular in shape and lobed (figs. 168 & 169). Each mass constitutes an ascogonial complex and up to seven of these per locule are present and are arranged randomly in this basal portion.

From each ascogonial coil, a thread like trichogyne grows upwards, taking an intercellular course through parts offering least resistance (fig. 170). These invariably emerge through the area around the pore (figs. 171, 172 & 173) where the hyphae and periphyses are rather loose ; some also appear along the side of the neck. The trichogynes may grow a short distance after emerging and may even branch shortly (fig. 172) or else, especially where penetration has been effected through more compact tissue as along the sides of the neck of the locule, the termini remain flush with the rest of the tissue (fig. 174).

A coating of microconidia covers the whole stroma and contact of these with emerging trichogynes can be effected easily. Figure 175 shows a microconidium attached to the tip of a trichogyne. Cytological details of fertilisation were not elucidated as the trichogynes, especially at the points of emergence at the surface of the ascocarp, stained rather densely and the nuclear state of the trichogynes was not at all clear. Indications that fertilisation had occurred were twofold. In the basal portion, a number of heavily stained hyphal structures became apparent (fig. 176) but more typically, these hyphae could be seen to encircle a mass of lighter staining pseudoparenchyma (figs. 177 & 178). These hyphal structures are presumably the ascogenous hyphae which grow out centrifugally from the ascogonial complexes after fertilisation. Secondly, at the stage when these ascogenous hyphae had formed, the trichogynes had either completely disappeared or else were present but were far less conspicuous than previously.

The concentration of stain in the ascogenous hyphae again made the nuclear state very indistinct. Although some parts did seem to show a binucleate condition, it was not possible to say how prevalent this state was. As the techniques employed here were inadequate for cytological work, the mode of ascus formation was not determined.

With further growth of the ascocarp, a separation of the elements of the prosenchyma above the ascogenous system can be seen (figs. 178 & 179). This is followed by a breakdown of the tissue so resulting in a space forming above the ascogenous hyphae (fig. 180). The delicate nature of the tissue was exemplified by the fact that this tissue frequently tore during the process of sectioning whereas that of the rest of the stroma would remain perfectly intact. This disintegration of internal prosenchyma progresses upwards toward the apex of the ascigerous locule. At the same time, the periphyses lining the length of the neck elongate, resulting in the widening of the pore running up the neck.

At the same time as these changes are taking place in the neck, the asci, arising from a flattish plate of ascogenous hyphae supported on a compact pseudoparenchymatous cushion in the base of the locule, grow up into the clear space above the fertile layer. The disintegration of tissue of the locule progresses in two directions. Laterally, this occurs almost to the level of the darkly staining outer layers of the stroma while acropetally, the periphyses gradually disappear. The asci, standing at various levels, also undergo changes. A gradation from youngest ones at the base, with fusion nuclei , to mature asci can be seen (fig. 181), the stratification being possible due to the long ascal stalks. However, at the level of the beginning of the neck, the asci disintegrate and free ascospores are pushed upward through the canal along the neck (figs. 182 - 184). As mentioned previously, there is a concomitant disintegration of almost all the periphyses along the neck. Those periphyses which remain become adpressed to the wall as the ascospores, forced up by the developing asci beneath, travel up the neck. The whole process continues until the apex of the ascocarp is reached (figs. 183 & 184). The area around the pore then splays out and is lined with loose periphyses which are persistent. The ascospores accumulate amongst the periphyses in the funnel-shaped pore and form a conspicuous mound of spores broader than the diameter of the basal portion of the ascocarp (fig. 185).

Spore production is considerable with the tissue below the bed of ascogenous hyphae becoming progressively thinner with the production of more asci. The original encirclement of a pseudoparenchymatous mass of cells by the ascogenous hyphae together with the fact that this mass does diminish as asci are formed seems to indicate that this tissue is nutritional in function and can be considered tapetal in nature.

The asci do not form an hymenium as was proposed by Arnaud (1912, 1913, 1930) for Caliciopsis (Coryneliaceae). In each locule, a number of fascicles of asci are formed without any intervening structures occurring between them. One such fascicle is shown in figure 186. The ascospores are tetra-radiate, having four lobes which are produced from a central, globose core in which a single nucleus is found. The four lobes may point out in the form of a regular tetrahedron (fig. 187) or the whole structure may be somewhat flattened (fig. 188). Abnormal spores with up to six arms were found occasionally (fig. 189). All spores are a dark brown at maturity.

The asci are globose and have very long stalks. The wall of the ascus is uniformly thin and easily deformed by the points or arms of the ascospores pushing against the wall (fig. 186). The ascal wall soon breaks down and the spores, now light in colour, are released into the locule and mature further during their passage up the neck of the ascocarp, becoming progressively darker.

Discussion:

The ascocarp arises as a locule in a stroma. There is no formation of a distinctive wall around the ascigerous locule. In certain logitudinal sections (figs. 167,178 & 180) the intermediate zone between the thin pseudoparenchymatous lining of the neck of the locule and the outer layers of the stroma stains darkly. However, this has the same basic prosenchymatous structure as the outer layer and is in fact continuous with the outer layers and ontogenetically, is not independent.

Luttrell (1951a,1955) places greater emphasis on the bitunicate or unitunicate nature of the ascus than on the ascocarp. Thus, since Tripospora tripos has a unitunicate ascus with uniformly thin wall, it should be placed among the Euascomycetes (Luttrell, 1955). The possession of an ascostroma is most unusual in this subclass. Luttrell (1955) does suggest that the "ascostromatic forms in the Euascomycetes apparently have been derived through reduction of the perithecial walls from true Pyrenomycetes". It was not possible to detect any such ontogenetic evidence to substantiate this in this study. Until more evidence is accumulated especially from forms which are reported to have much reduced perithecial walls, the position of the ascostromata as found in the ascostromatic Euascomycetes cannot be defined in terms of intergrading forms between the purely ascostromatic and non-ascostromatic types.

The asci in Tripospora grow up into a space formed by a lysigenous action on the pseudoparenchyma as well as some of the periphyses in the locule. Interascal structures such as paraphyses, pseudoparaphyses and interthecial tissue (sensu Luttrell, 1965) are lacking. Hence it is not at all surprising that the early workers recognised the affinities of the Coryneliales with the Dothideaceous types. As a matter of fact, the Coryneliales were grouped in the Dothideales by Martin (1945) and in the Pseudosphaeriales by Gäumann (1949). On the basis of the pseudoparenchymatous centrum which disintegrates and leaves a space into which asci grow, Tripospora would fit into the Diaporthe type of development (Luttrell, 1951a). However, the ascostroma would make this fungus an exception among a number of genera such as Diaporthe (Luttrell, 1947), Gnomonia (Miles, 1921), Melanconis (Wehmeyer, 1937), Melanospora (Vincens, 1917; Cookson, 1928; Kowalski, 1965) and others where the ascocarps are perithecial. Besides this, it appears that in some genera conforming with the Diaporthe type, (Wehmeyer, 1926; Miller, 1949; Mainwaring and Wilson, 1968) paraphyses are present. Paraphyses are lacking in Tripospora.

It is therefore proposed that the development of the ascostroma ~~elucidated~~ established for Tripospora, as that for Caliciopsis (McCormack, 1936), be called the CORYNELIOID TYPE. A separate order, the Coryneliales, as suggested by Seaver and Chardon (1926), in the subseries Pyrenomycetes (sensu

Corynelia uberata Fr.ex Ach. was investigated (data not included in the thesis) and found to have the same developmental type as found for *Tripospora tripos*. Since *Tripospora* and *Corynelia* both belong to the Coryneliales, Coryneliaceae, it seems likely that all the members of the family, if not the order, may have the same developmental type. Consequently, the developmental type was called the *Corynelioid Type*.

Luttrell, 1951a)should be retained to accommodate the above genera. This developmental type may be characterised as follows:-

CORYNELIOID DEVELOPMENTAL TYPE:

Ascocarp an ascostroma. Centrum, at least that in contact with the ascogonium, pseudoparenchymatous. The pseudoparenchyma eventually disintegrates with the result that the asci grow up into a clear space. Fascicles of asci line the base of the ascigerous locule. Paraphyses, pseudoparaphyses and interthecial tissue between the asci lacking. Schizogenous, periphysate pore present. (The character of the long neck may have to be included).

Diagnosis:

Tripospora tripos (Cooke) Lindau is a parasitic fungus on the leaves of Podocarpus falcatus R.Br. (Podocarpaceae) Ascocarp an ascostroma. Stromata are black and arranged in definite rows up to 10mm. in length and consist of ascigerous and microconidial locules. Two more or less parallel rows of flask-shaped ascigerous locules grow out from a basal stromal portion housing the microconidial locules. The entire stroma raised above the surface of the host leaf and connected with the latter by a central, longitudinal foot. Some cells of the leaf may be incorporated in the stroma above the leaf surface.

Each flask-shaped asigerous locule is up to 900µ in length and consists of a basal portion up to 340µ in diameter. The neck alone may be 570µ in length. At maturity, the apex of the neck becomes somewhat concave in shape and is made up of loosely interwoven hyphae and may have a diameter which exceeds that of the basal portion. The outer layer of the neck is composed of dark, prosenchyma whereas the basal portion is made up of thick-walled pseudoparenchyma cells which merge with similar tissue of the stroma in which the microconidial locules are found. Centrum of the Corynelioid Type with asci growing up into a space resulting from the disintegration of the pseudoparenchymatous tissue in the basal portion.

Asci are eight-spored and unitunicate and are composed of an ovate part, 25 - 35µ in diameter, with a projection at the apex, with thin-walls and are evanescent. The ovate part is connected to a long stalk up to 145µ in length. Ascospores dark, normally tetraradiate, with four conical projections radiating from a central, globose portion. The distance from tip to tip of adjacent projections is 25 - 35µ A mass of these ascospores come to lie in the funnel-like cavity of the apex of the ascocarp.

Size and shape of the microconidial locules very variable. Microconidial conidiophores 6-9µ in length, columnar to flask-shaped. Microconidia bacilliform to elliptical, 6.2 - 7.0 x 1.0 - 1.2µ

Taxonomic Position:

S.C. Euascomycetes.
O. Coryneliales.
F. Coryneliaceae.

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REFERENCES:

ARNAUD, G. (1912 - 1913). Contribution à l'étude des fumagines III. Ann. École Nat. Agric. Montpellier 12:23-54.

ARNAUD, G. (1918). Les Astérinées. Ann. École Nat. Agric. Montpellier 16:1-288.

ARNAUD, G. (1925). Les Astérinées IV. Ann. Sci. Nat. Bot. 7:634-724.

ARNAUD, G. (1930). Les Astérinées V. Ann. Épiphyt. 16:235-275.

ARNAUD, G. (1931). Les Astérinées. VII. Ann. Crypt Exotique 4:74-97.

ARNOLD, C.A. (1928). The development of the perithecium and spermogonium of Sporormia leporina Niessl. Amer. Jour. Bot. 15:241-246.

ARX, J.A. von. (1949). Beiträge zur Kenntnis der Gattung Mycosphaerella. Sydowia (Ann. Mycol., ser. II) 3:28-100.

BARR, M.E. (1958). Life history studies on Mycosphaerella tassiana and M. typhae. Mycol. 50:501-513.

BESSEY, E.A. (1935). A text-Book of Mycology. Blakiston's Sons & Co. Philadelphia.

BYL, P.A. van der. (1928). Aantekeninge oor einige Suid Afrikaanse swamme. S. Afr. J. Sci. 25:181-184.

CAVARA, F., and MOLLICA, N. (1907). Richerche intorno al ciclo evolutivo di una interessante forma di Pleospora herbarum. Ann. Mycol. 5:119-149.

CLEMENTS, F.E., and SHEAR, C.L. (1931). The Genera of Fungi. Hafner Publishing Co. New York.

COOKSON, I. (1928). The structure and development of the perithecium in Melanospora zamiae Corda. Ann. Bot. 42:255-269.

DE BARY, A. (1887). Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria. Clarendon Press. Oxford. (Trans. & Rev. by Garnsey & Balfour).

DOIDGE, E.M. (1921). South African Ascomycetes in the National Herbarium. Bothalia I:5-32.

DOIDGE, E.M. (1941). South African Ascomycetes in the National Herbarium. Bothalia 4:206-207.

DOIDGE, E.M. (1950). The South African fungi and lichens. Bothalia 5:1-1030.

FUCKEL, L. (1869).	Symbolae Mycologicae. Wiesbaden.
FUISTING, W. (1867).	Zur Entwicklungsgeschichte der Pyrenomycetes. Bot. Zeit. 25:177-181; 185-189; 193-198; 305-311.
GÄUMANN, E. (1926).	Vergleichende Morphologie der Pilze. Gustav Fischer. Jena.
GÄUMANN, E. (1940).	Neuere Erfahrungen über die Entwicklungsgeschichte der Ascomyceten. Zeitschr. f. Bot. 35:433-513.
GÄUMANN, E. (1949).	Die Pilze, Grundzüge ihrer Entwicklungsgeschichte und Morphologie. Birkhäuser. Basel.
GROENHART, P. (1965).	Studies in ascostromatic lichen-fungi - I. The problem of Ascohymeniales and Ascoloculares Persoonia 4:1-7.
GROENHART, P. (1965a).	Studies in ascostromatic lichen-fungi - II. Types of ascostromata. Persoonia 4:9-13.
GWYNNE-VAUGHAN, H.C.I., and BARNES, B. (1927).	The structure and Development of the Fungi. Cambridge Univ. Press. Cambridge.

HANSFORD, C.G. (1946). The foliicolous Ascomycetes, their parasites and associated fungi. CMI Kew, Mycol. Paper 15:1-240.

HENNINGS, P. (1901). Fungi australiae occidentalis I. Hedwigia 40:173.

HIGGINS, B.B. (1920). Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia. Amer. Jour. Bot. 7:435-444.

HIGGINS, B.B. (1929). Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia II. Amer. Jour. Bot. 16:287-296.

HIGGINS, B.B. (1936). Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia III. Amer. Jour. Bot. 23:598-602.

HÖHNEL, F. von. (1907). Fragmente zur Mykologie III. No. 92-155. Wettsteinina n.g. Sitzb. Akad. Wiss. Wien 116:83-162.

HÖHNEL, F. von. (1909). Fragmente zur Mykologie, No. 325 Sitzb. Akad. Wiss. Wien. 118:1157-1246.

HÖHNEL, F. von. (1917). Ueber die Trichothyriaceen. Ber. Deutsch. Bot. Gesell. 35:411-416.

HOLM, L. (1958). Some comments on the ascocarps of the Pyrenomycetes. Mycol. 50:777-788.

HUGHES, S.J. (1953). Fungi from the Gold Coast. CMI Kew. Mycol. Paper 50:77-97.

JENKINS, W.A. (1930). The cherry leaf-spot fungus, *Mycosphaerella cerasella* Aderh., its morphology and life history. Phytopath. 20:329-337.

JENKINS, W.A. (1938). Two fungi causing leaf spot of peanut. Jour. Agric. Res. 56:317-332.

JENKINS, W.A. (1939). The development of *Mycosphaerella berkeleyii*. Jour. Agric. Res. 58:617-620.

JOHANSEN, D.A. (1940). Plant Microtechnique. McGraw-Hill Book Company, Inc. New York.

JONES, S.G. (1926). The development of the perithecium of *Ophiobolus graminis* Sacc. Ann. Bot. 40:607-629.

KALCHBRENNER, K., and COOKE, M.C. (1880). South African Fungi. Grevillea 9:32.

KERR, J.E. (1961). The life history and taxonomic position of *Venturia rumicis* (Desm.) Wint. Trans. Brit. Mycol. Soc. 44:465-486.

KOWALSKI, D.T. (1965). The development and cytology of *Melanospora tiffanii*. Mycol. 57:279-290.

KOWALSKI, D.T. (1965a). The development and cytology of *Didymocrea sadasavanii*. Mycol. 57:404-416.

LINDAU, G. (1897). Pyrenomycetineae & Dothideales. In Engler und Prantl. Die Natürlichen Pflanzenfamilien. l. Wilhelm Engelmann, Leipzig.

LUTTRELL, E.S. (1940). *Morenoella quercina*, cause of leaf spot of oaks. Mycol. 32:652-666.

LUTTRELL, E.S. (1944). The morphology of *Myiocopron smilacis* (De Not.) Sacc. Amer. Jour. Bot. 31:640-649.

LUTTRELL, E.S. (1944a). The morphology of *Sphaerostilbe aurantiicola* (B. & Br.) Petch. Bull. Torr. Bot. Club 71:599-619.

LUTTRELL, E.S. (1947). *Diaporthe phaseolorum* var. sojae on crop plants. Phytopath. 37:445-465.

LUTTRELL, E.S. (1951). The morphology of _Dothidea collecta_ Amer. Jour. Bot. 38:460-471.

LUTTRELL, E.S. (1951a). Taxonomy of the Pyrenomycetes. Univ. Mo. Stud. 24 (3):1-120.

LUTTRELL, E.S. (1955). The Ascostromatic Ascomycetes. Mycol. 47:511-532.

LUTTRELL, E.S. (1960). The morphology of an undescribed species of _Dothiora_. Mycol. 52:64-79.

LUTTRELL, E.S. (1965). Paraphysoids, pseudoparaphyses, and apical paraphyses. Trans. Brit. Mycol. Soc. 48:135-144.

LUTTRELL, E.S. (1965a). Classification of the Loculoascomycetes. (Symposium on Taxonomy of Pathogenic Fungi). Phytopath. 55:828-833.

MAINWARING, H.R., and WILSON, I.M. (1968). The life cycle and cytology of an apomicitic _Podospora_. Trans. Brit. Mycol. Soc. 51:663-677.

MARASAS, W.F.O. (1969). Personal communication.

MARTIN, G.W. (1945). Key to the families of Fungi. In Ainsworth, G.C., and G.R. Bisby. A dictionary of the Fungi, 2nd Ed. Imperial Mycol. Inst. Kew.

McCORMACK, H.W. (1936). The morphology and development of _Caliciopsis pinea_. Mycol. 28:188-196.

MILES, L.E. (1921). Leaf spots of the elm. Bot. Gaz. 71:161-196.

MILLER, J.H. (1928). Biologic studies in the Sphaeriales. Mycol. 20:187-213 305-339.

MILLER, J.H. (1938). Studies in the development of two _Myriangium_ species and the systematic position of the Myriangiales. Mycol. 30:158-181.

MILLER, J.H. (1949). A revision of the Classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycol. 41:99-127.

MILLER, J.H., and THOMPSON, G.E. (1940). Georgia Pyrenomycetes I. Mycol. 32:1-15.

MILLER, J.K. (1935). A new species of _Keithia_ on red cedar. Jour. Elisha Mitchell Sci. Soc. 51:167-171.

MÜLLER, E., and VON ARX, J.A. (1950) Einige Aspekte zur Systematik Pseudosphaerialer Ascomyceten. Ber. Schweiz. Bot. Ges. 60:329-397.

MÜLLER, E., and VON ARX, J.A. (1962). Die Gattungen der didymosporen Pyrenomyceten. Beit. Kryptogamenflora Schweiz II (2):1-885.

MÜLLER, E., and SANWAL, B.D. (1954). Über die Gattungen *Microcyclus* Sacc., *Coccoidella* v. Höhn., *Coccodothis* Theiss. et Syd. und *Coccodothella* Theiss. et. Syd. Sydowia (Ann. Mycol., Ser. II) 8:231-244.

MUNK, A. (1953). The system of the Pyrenomycetes. Dansk bot. Ark. 15 (2):1-163.

NANNFELDT, J.A. (1932). Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Soc. Scient. Upsaliensis Ser. IV. 8:1-368.

ORTON, C.R. (1924). Studies in the morphology of the Ascomycetes I. The stroma and the compound fructification of the Dothideaceae and other groups. Mycol. 16:49-95.

PANTIDOU, M.E., and KORF, R.P. (1954). A revision of the genus *Keithia*. Mycol. 46:386-388.

PETRAK, F. (1923). Mykologische Notizen V. No. 200, Über die Pseudosphaeriaceen v. H. und ihre Bedeutung für die spezielle Systematik der Pyrenomyzeten. Ann. Mycol. 21:1-69.

PETRAK, F. (1950). Über *Loranthomyces* v. Höhn. und andere Gattungen der Trichothyriaceen. Sydowia (Ann. Mycol., Ser. II) 4:163-174.

RUHLAND, W. (1900). Untersuchungen zu einer Morphologie der stromabildenden Sphaeriales. Hedwigia 39:1-79.

SACCARDO, P.A. (1882). Sylloge Fungorum, Vol. I.

SACCARDO, P.A. (1891). Sylloge Fungorum, Vol. 9.

SEAVER, F.J., and CHARDON, C.E. (1926). Botany of Porto Rico and the Virgin Islands. Scientific survey of Porto Rico and the Virgin Islands 8:1-208. New York Acad, Sci. New York.

SINGER, R., and GAMUNDI, I.J. (1963). Paraphyses. Taxon 12:147-150.

SPEGAZZINI, C. (1889). Fungi Puiggariani, Bol. Ac. Nac. Cien. Cordoba. 11:556.

STEVENS, F.L. (1918). Some Meliolicolous parasites and commensals from Porto Rico. Bot. Gaz. 65:227-249.

STEVENS, F.L. (1919). Perithecia with an interascicular pseudoparenchyma. Bot. Gaz. 68:474-476.

SYDOW, H., and SYDOW, P. (1913). Novae fungorum species IX. Ann. Mycol. 11:54-65. (See also: Ann. Mycol. 11:p.408.)

THEISSEN, F. (1913). Hemisphaeriales. Ann. Mycol. 11:468-469.

THEISSEN, F. (1914). Die Trichothyriaceen. Beih. Bot. Centralbl. 32:1-16.

THEISSEN, F. (1916). Mykologische Abhandlungen. Verh. K. Zool.-Bot. Gesell. Wien 66:296-400.

THEISSEN, F., and SYDOW, H. (1915). Die Dothideales. Ann. Mycol. 13:149-746.

THEISSEN, F., and SYDOW, H. (1917a). Synoptische Tafeln. Ann. Mycol. 15:389-491.

THEISSEN, F., and SYDOW, H. (1917b). Die Gattung Parodiella. Ann. Mycol. 15:125-134.

THEISSEN, F., and SYDOW, H. (1918). Vorentwürfe zu den Pseudosphaeriales Ann. Mycol. 16:1-34.

VINCENS, F. (1917). Recherches organogénique sur quelques Hypocréales. Thése. Lons-Saunier, Paris.

VUILLEMIN, P. (1910). Matériaux pour une classification rationnelle des Fungi Imperfecti. C.R. Acad. Sci., Paris. 150:882-88

WEBBER, F.C. (1967). Observations on the structure, life history and biology of Mycosphaerella ascophylli. Trans. Brit. Mycol. Soc. 50:583-601.

WEHMEYER, L.E. (1926). A biologic and phylogenetic study of the stromatic Sphaeriales. Amer. Jour. Bot. 13:575-645.

WEHMEYER, L.E. (1937). Cultural life histories of *Melanconis* and *Pseudovalsa*. III. The genus *Prosthecium* Fres. emend Petrak. Papers Michigan Acad. Sci., Arts, Lettrs. 23:233-242.

WHEELER, H.E., OLIVE, L.S., ERNEST, C.T. and EDGERTON, E.W. (1948). Genetics of *Glomerella* V. Crozier and ascus development. Amer. Jour. Bot. 35:722-728.

WINTER, G. (1887). Die Pilze Deutschlands, Oesterreichs und der Schweiz. Rabenhorst's Kryptogamenflora, 2.Aufl., Bd.I, Abt.2. VIII, 929 S.

WITTMANN, W. (1962). Aceto-iron-haematoxylin for staining chromosomes in squashes of plant material. Stain tech. 37:27-30.

WOLF, F.A., and WOLF, F.T. (1947). The Fungi. Vol. I & II. John Wiley & Sons. New York.

ZOPF, W. (1890). Die Pilze, in Schenk's Handbuch d. Bot., Encyklopaedie der Naturwissenschaften, 4:271-781.