STUDIES IN SOME INDIAN ASCOMYCETES AND FUNGI IMPERFECTI

A Thesis
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DOCTOR OF PHILOSOPHY (AGRICULTURE)
IN
MYCOLOGY AND PLANT PATHOLOGY

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PREFACE

The detailed account of the investigations carried out by me on some Indian Ascomycetes and Fungi Imperfecti has been presented in I-IV Parts respectively. The V Part is devoted to a summing up and discussion of the results obtained and contributions made with special reference to new findings brought out in the course of these studies.

All literature cited in five parts including general introduction is placed together under bibliography at the end of this compilation. The part on research publications gives a short account of the research papers published by me during the course of this study.

Legends of illustrations and photographs are typed on separate pages for each plate and are arranged opposite to the plates. Figures are referred to in the text by a composite decimal numeration, e.g. 22.4 where an integer refers to the Plate Number and the decimal denotes the Figure Number in the corresponding Plate. The Figure 22.4 denotes Fig. 4 of the Plate 22. This method dispenses with the repetition of Plate Number and Figure Number separately in every context.

All the collections of the writer that are referred in the text have been deposited at the M.A.C.S.Herbarium under appropriate accession number.

Latin diagnosis for new species and genera will be provided at the time of publishing the results as required by the Code of International Botanical Nomenclature.
GENERAL INTRODUCTION

An extremely heterogenous group of fungi the Ascomycetes with very little in common in the extremes, except producing their spores in the ascus and with varied pattern and morphology, the mycologist is often confronted with the intricate problem in proper arrangement of the various groups comprising them with a phylogenetic relationship. Lindau (1897) was the first to attempt a classification of this heterogenous group of fungi on the basis of gross morphological characters such as the type of the ascocarp, its colour, consistancy, habitat manner of opening and other structural characteristics. He recognised three types of ascocarps while proposing his classification. (1) Cleistothecium: the ascocarp is completely closed and the a-ci and ascospores are released by the disintegration of the ascocarp wall. (2) Peritheciurn: flask shaped ascocarp with a true perithecial wall and a true ostiole for the release of the ascii and ascospores. (3) Apothecium: cup or disc shaped ascocarp in which the ascii are arranged in parallel rows on exposed surface. These ascocarp types formed the main basis of the three series within the Euascomycetes i.e. Plectomycetes, Pyrenomycetes and Discomycetes respectively.

It has been recognized, however, through the works of von Hoehnel (1907-1923), Arnaud (1918-1925), Orton (1924-1944), Petrak (1925-1947), Nannfeldt (1932), Hansford (1946), Wehmeyer (1948-1954), Miller (1949), Chadefaud (1942-1965), Munk (1953), Luttrell (1951-1965), Arx and Mueller (1954), Holm (1959), Martin (1961), Mueller and Arx (1962), Korf (1952-62),
Kobayashi (1970), Kamat and Anahosur (1971), Kamat and Pande (1971) and many others that the details of development of ascocarp and centrum characters and their associated structures are of fundamental importance in establishing and evolving a natural system of classification of Ascomycetes based on truly phylogenetic concepts.

The present trend is to recognize in this class two distinct series viz. Ascoeculareae and Ascohymeniales of Nannfeldt (1932), Pseudothecium and Peritheciurn of Miller (1949) and Bitunicatae and Unitunicatae of Luttrell (1951) and again Hemiascomycetes, Ascomycetes and Loculoascomycetes of Luttrell (1955) and Prototunicatae and Eutunicatae of Gaumann (1964).

Recently Carr and Olive (1958) have suggested the use of chromosome morphology and correlation of ascus development with chromosomal events as indicators for phylogenetic relationships though on a limited scale, between different genera like Sordaria and Neurospora which they investigated.

The cytological aspects of the mycelial system in the Discomycetes has been thoroughly summarised by Berthet (1964b). He reported that the mycelia of Pezizales were coenocytic; coenocytic mycelia have also been observed in many species of inoperculate families. The studies conducted by Berthet (1961, 1963, 1964a, 1964b) have greatly added to our understanding of the Phylogenetic relationships between members of Discomycetes as a whole.
Berthet (1964b) was able to differentiate four main groups of species by the number of nuclei per ascospore.

In the current trend of fungal taxonomy the aspects which drew attention in tracing Phylogenetic relationships should not go unnoticed such as Metabolic patterns (Vogel 1960, 1961), Serology (Coons and Strong 1928, Tempel 1957, Kalyansunderam et al. 1966, Brock 1959) and Numerical taxonomy (Sokal and Sneath 1963, Kendrick and Proctor 1964, Ibrahim and Threlfall 1966).

Recently Hall (1969), and Tyrel (1969), reviewed the molecular approaches to taxonomy of fungi and biochemical systematics and fungi respectively.

To quote Korf (1969): "The year 1966 must surely go down in the history of systematics of the Discomycetes as one of the most significant ever, with the appearance of three major works dealing primarily with Operculate Discomycetes and the appearance of the second edition of British Cup Fungi (now more aptly titled). That those come so soon on the heels of the stimulating cytological survey of the Discomycetes by Berthet in 1964,"..... "The results which this study reveals are heartening, in that for the most part they confirm the classification which have evolved in the past decade, starting here from a wholly different premise. Much the same could be said for Berthet's studies of the distribution of nuclei in this group, four years earlier. It is not mere coincidence
that we find chemical and biochemical similarities, and cytological similarities, within those taxa already segregated by more traditional anatomical methods. The "coincidence" is a bright beacon that we are indeed on the right track in eliciting true relationships, and one step closer to a natural classification. The three major works in reference were those of Rifai (1968) on the Australasian Penizales, Ekblad (1968) on the taxonomy, phylogeny and nomenclature of the Penizales and Arpin's (1968) Chemotaxonomic essay on the carotinoides of Discomycetes.

Recognising the importance and significance of such studies and above mentioned characters in the taxonomy and classification of the Ascomycetes, many workers from India also, have contributed to these aspects in many ascomycetous genera, Tilak (1959), Kulkarni (1964), Ananthanarayanan (1964), Kalani (1965), Patwardhan (1966), Seshadri (1967), Muthappa (1967), Jagtap (1967), Alka Chiplonkar (1969), Pande (1969), Anahosur (1969) and Tendulkar (1971) all from this laboratory have studied ascomycetous genera like Parodiella, Phyllachora, Erysiphe, Elsinoc, Myriangium, Cyclotherca, Mycosphaerella, Tryblidiella, Claviceps, Lambosina, Trybliaria, Lecanidion, Microcyclus, Glomerella, Flagioastigme etc.

Hence it was proposed to undertake an intensive investigations into two of the following commonly occurring Indian Ascomycetes genera viz. Balsamia claviceps Speg. (F. Clavicipitaceae) and Cryptomyces muelleri Ullms (F. Hypodermataceae),
which have not been worked out previously, both collected from Coor, District of Mysore State, India at an elevation of 4000 ft. above the mean sea level.

These two genera were particularly selected for these investigations since no attempt has been made in the past to study them in respect of their fundamental aspects such as developmental pattern of ascocarp "Centrum", nature and origin of asci, cytology and sexuality, cultural behaviour and life-cycle studies etc.

Large collections of Ascomycetes and Fungi Imperfecti were also made by the writer at Coor, District of Mysore State and near and around Poona, Maharashtra State, India, during the period of this research project regularly at different seasons and various genera collected were studied taxonomically in respect of their morphology and species characters.

Imperfect fungi collected were studied more critically since they usually represent conidial or asexual states of Ascomycetes in their life cycle. The conidial state should also play important role in the taxonomy of the Ascomycetes though they have an independent status.

Mode of development of conidia or conidium ontogeny has been assuming increasing importance as taxonomic criterion in the system of classification of Hyphomycetes replacing purely artificial Saccardian system, based on conidial characters, septation and colour through the pioneer work of Hughes' (1953).
Dr. Hughes' far reaching taxonomic studies on Hyphomyceses by which he obtained refined knowledge about the modes of conidium formation and opened up new possibilities to classify and identify Hyphomyceses in a more related and natural way is well recognized through award of the third Jakob Eriksson gold medal to him at the XI International Botanical Congress, Seattle on 2nd Sept. 1969.


Thus in view of this modern trend in the study of Fungi-Imperfecti, it was decided to work out the details of conidium ontogeny in some of the Indian Hyphomyceses and Coelomycetes.

The entire research project undertaken over a period of 3½ years is presented here in five parts:

**Part I**: deals with taxonomy, internal morphology, structure and development of ascocarp, sexual reproduction, origin of asci, nuclear behaviour, chromosome complement, ascospore organisation, ascospore germination and cultural behaviour in *Balansia clavipes* inciting the 'agarbatha'disease of *Cyrtococcum oxyphyllum* Stapf.
Part II: deals with the taxonomy, internal morphology, structure and development of asccarp, sexual reproduction, origin of asci, nuclear behaviour, chromosome complement and ascospore organisation in Cryptomyces muelleri inciting leaf spots of Salix tetrasperma Roxb.

Part III: is based on the taxonomic studies into some Indian Ascomycetes collected from Coorg District of Mysore State and few from Mahabaleshwar and Khandala Forests of Maharashtra State.

Part IV: includes taxonomic studies into some Fungi Imperfecti collected from Coorg District of Mysore State and in and around Poona, Maharashtra State with special reference to conidium ontogenetic studies.

Part V: includes general discussion and evaluation of results.

Part VI: Research Publications.

MATERIAL AND METHODS

Balansia claviceps infecting Cyrtococcum oxyphyllum Stapf. was collected during September-October 1968-69 from a small patch of 50 sq. yards in a valley at Cottebetta, Pollibetta, Coorg, Mysore State where cardamom is grown along the water course during which period the host plant comes into flowering. Writer's observations over a period of 3 years in Coorg region revealed that this fungus occurred only in that particular region of Coorg and infects Cyrtococcum oxyphyllum and makes its appearance during the month of September-October every year when the host plant comes into flowering.
The *Cryptomyces muelleri* infecting living leaves of *Salix tetrasperma* Roxb. occurs throughout Coorg District of Mysore State. The medium sized trees grow along the river sides and damp places. The fungus first makes its appearance during the month of May-June and are available till October-November at various stages of development. These two fungi were fixed in various fixatives on the spot in the field at different intervals to secure adequate stages of development and nuclear divisions. The various fixatives used during this research work are presented in brief in the following pages.

**Fixation** : The following fixatives were used and the results so obtained are also presented in brief.

1) **Formalin-aceto-alcohol (F.A.A.)** :
   - Ethyl Alcohol 95° ............... 50 cc
   - Glacial Acetic Acid ............... 5 cc
   - Formaldehyde (37-40%) ............ 10 cc
   - water .......................... 35 cc

2) **Cornoy's fluid** :
   - Ethyl Alcohol ................. 60 cc
   - Chloroform ........................ 30 cc
   - Glacial Acetic Acid .............. 10 cc

3) **Navashin's fluid** :
   - **Solution A** :
     - Chromic Acid ................. 1 gm
     - Glacial Acetic Acid ............ 7 cc
     - Distilled Water ............... 92 cc
Solution B:

Neutral formalin ... 30 cc
Distilled water ... 70 cc

Amongst these fixatives F.A.A. and Cornoy's fluid gave excellent results in the fixation of nuclear divisional stages and various developmental stages. The chromosomes and nuclear divisional stages were best obtained in Cornoy's fluid and F.A.A. in case of Balansia and Cryptomyces.

Pre-treatment: The following solutions were used for pre-treatment to secure clarity in the divisional stages.

1) Cornoy's fluid - The fungus materials were treated for 5-10 minutes and then transferred to F.A.A.

2) Paradoxchlorobenzene - The materials were allowed to remain in this solution for an hour and then transferred to F.A.A.

3) Methyl alcohol - Fungus materials were treated with 2-3% solution for 5 minutes at cool temperature and then transferred to F.A.A.

Materials pretreated with Cornoy's fluid, paradoxchlorobenzene and methyl alcohol gave good results for the study of chromosome complements. In general, the fixatives F.A.A. and Cornoy's fluid and pre-treatment with paradoxchlorobenzene and methyl alcohol were found to yield good fixation for the detailed cytological studies.

Staining: The following stains and combinations were employed for the studies.
1) **Heidenhein's Hematoxylin**:

It was prepared according to Sass (1951), Johnson (1940) and Purvis *et al* (1966) as follows:

- **Haematoxylin** .... 0.5 gm.
- **Distilled water** .... 100 cc.

By keeping in light for a week, a well riped brick-red coloured nuclear stain was obtained which gave excellent results and was, therefore, used during this research project.

2) Aceto-carmine and Aceto-orcein were prepared according to the formulae prescribed by Smith (1947), Buck (1935), Cutting (1946), McIntosch (1954) and Sass (1951) used for the squash and smear techniques.

3) **Counter-stains**:

- Light green 1% in 90% alcohol. Orange G in Clove oil 1%.

4) **Mordent and destainers**:

- Ferric chloride - 4% and 2% as mordant and destainer respectively.
- Iron alum - 4% and 2% used as mordant and destainer respectively.

The fixed materials were dehydrated as usual according to Johnson (1935-1940), Sathe (1967) and embedded in 56° - 58° M.P. Paraffin wax.
Special treatments:

1) Melzien's reagent:

<table>
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<tr>
<td>Iodine</td>
<td>1 gm</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>1 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 cc</td>
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This solution is used for determining the tunicate nature of the ascus wall.

2) As the ascocarps of *Balansia* sometimes become hard, the sections were not intact and hence the following solutions were used to soften the material.

   a) Hydrofluoric acid (concentrated): The material was kept in this acid for 4 days and 7 days but no satisfactory sections were obtained.

   b) Glycerol ..... 1 part
   Phenol ..... 1 part

   The material was allowed to remain in this solution for a period of one month. Satisfactory results were obtained with this method.

Sectioning: Sections at 6-10 μ are cut with a Rotary Microtome during cool hours for the nuclear and developmental studies.

Smear technique: For obtaining smear preparations, young ascocarps fixed in F.A.A. and stored in 70% alcohol were teased between two slides previously coated with egg albumin and after drying for 24 hours washed in running water for 15-30 minutes, transferred to 4% Ferric chloride (Mordant) for 4 hours, washed by two changes in distilled water and then kept in Heidenhain's hematoxylin for 12-16 hours, slides were then washed in running
water followed by two changes of distilled water, differentiated in 2% Ferric chloride solution and washed thoroughly in running water. The slides were gradually dehydrated in alcohol series, counter stained with light green in 90% alcohol, cleared in Clove oil by two changes of Xylol and mounted in balsam as done in case of microtome sections.

This method yielded better and clear picture of the sex apparatus and nuclear events in case of Balansia claviceps.

Single Ascospore Cultures: Individual ascosporae were picked out from freshly collected material and treated with 0.1% HgCl₂ solution for one minute to get rid of external contamination and saprophytes and were given several washings in sterile distilled water. Using aseptic techniques, the ascosporae were picked with a needle and placed over a slide in drops of water containing traces of Terramycin and ascosporae were teased out with the help of another sterile glass slide. A dilute suspension of ascosporae was obtained by adding Terramycin water which was then spread over the surface of the solid water agar in Petridish. Even distribution of ascosporae was secured by gently rolling the Petridish. Periodical observations were made for ascospore germination. Individual germinating ascosporae were located under low power objective of microscope, and marked with a glass marking pencil on the corresponding lower side of the Petridish then transferred with a sterile needle to Potato-dextrose-agar plates or slants to obtain single ascospore cultures.
Germination studies: Germination studies were made in hanging drop cultures using VanTieghen cells (Wang 1934) and condensed water method after Thirumalachar (1940); Ullasa (1969). Potassium permanganate solution (0.2%) treatment for 1 minute helped in inducing germination and breaking dormancy in thick walled ascospores like Parodiella (Ullasa, 1969).

In addition to above technique, hand sections of the materials were employed for the study of morphological characters and conidium development of various collections of ascomycetes and fungi imperfecti, respectively for the purpose of taxonomic and ontogenic studies. Observations were made in distilled water or with lactophenol. Staining was done with Cotton Blue.
STUDIES INTO BALANIA CLAVICEPS SPEG.

PART I
The genus *Balansia* (F. Clavicipitaceae) was established by Spegazzini in 1885 for a Clavicipitaceous fungus with *Balansia claviceps* as type (Fig.1.2), and differentiated this genus from the genus *Claviceps* Tul. by black colour of the stroma and its position in the inflorescence of the host plant from which it arises. Spegazzini's (1885) description of the genus *Balansia* recognized its close relationship to the genus *Claviceps* Tul. and the affinity of the two genera has never since been questioned. This genus was accepted by Saccardo (1891) in Sylloge and a few years latter Lindau (1897) classified it along with ten other genera in his sub-family Hypocreaceae which was continued to be recognised as a group typified by the well known genus *Claviceps*. Atkinson (1905) revising the genus *Balansia* drew special attention to the fact that the pseudomorph from which the ascostromata arise contained both host and fungus tissue. He termed pseudomorphs as pseudosclerotium and distinguished the genus on that basis. He noted in species of his new genus *Pothlchloc* described by him in 1894, that the homologous sclerotic structure was less developed nearly forming an effuse layer on the limited surface of host tissue. Atkinson's genus has not been accepted by Moller (1901) who has studied this genus extensively. The genus *Ophiodothis* was proposed by Saccardo (1883) based on *Dothidie vorax* Berk. & Curt. Atkinson (1894) noted the
possibility of the relation of his genus Dothiohloe to Ophiodothis of Saccardo and latter (1905) considered the likelihood of Ophiodothis and Balansia of Spegazzini (1885) being synonyms, although he erroneously credited Spegazzini's publication to the year 1880 as of prior date. However, he concluded since the type specimen of Ophiodothis vorax (Berk. & Curt.) Sacc. on which the genus was based was in poor condition and inadequate for descriptive purposes and the genus Ophiodothis was invalidated and the decision since been accepted by most taxonomists of these genera. Theissen and Sydow (1915) in their monograph on Dothidiales have accepted Atkinson's (1894, 1905), allocation of Ophiodothis to Balansia and of Dothiohloe to the Hypocreaceae.

In Seaver's (1910a, 1910b, 1911) treatment of Hypocreales the four genera Gymnotheca, Barya, Typhodium (Epichloe) and Hypoorella are placed in his tribe Hypocreæ and Cordyceps, Spermoedia (Claviceps) and Balansia in his tribe "Cordycipitæ" under the family Hypocreaceæ Order Hypocreales. Von Hohnel (1910), however, recognized the Clavicipitæ characterized by scolecosporic asci provided with an apical cap and perithecia embedded in a stroma and considered the tribe to be closely related to Hypocreaceæ.

Gaumann (1926), like Spegazzini, Atkinson and Von Hohnel recognized the family Clavicipitæ under the Hypocreaceæ on the basis of presence of slime cap of the scolecosporic asci. This character is a distinctive feature of all the genera included in this group which, therefore, needs separation from
the Hypocreaceae into a separate family "Clavicipitaceae"
as had been proposed by Earle (1901) and by Nannfeldt (1932),
and later recognized by Bessey (1935), and Martin (1961) under
Hypocreales, by Miller (1949) under Sphaeriales and by Luttrell
(1951) under Xylariales (Sphaeriales)

Nannfeldt (1932) included the majority of Hypocreales in
the Sphaeriales but retained the Clavicipitaceae in a separate
order Clavicipitales. Since then this order has been recognized
through the works of Gaumann (1952), Arx and Mueller (1954),
Dennis (1968), Alexopoulos (1962) and Rogers (1970) as a
sharply defined closely related group of fungi.

Diehl (1950) who has extensively worked on the genus
Balansia recognized three sub-families viz. (1) Oomycetoidae,
(2) Clavicipitoideae and (3) Cordycitoideae, following
Gaumann's (1926) sub-divisions but under the family
Clavicipitaceae and recognizing it under Sphaeriales. His
sub-family Clavicipitoideae consisted of three tribes viz.
(1) Clavicipitae with a single genus Claviceps, (2) Balansiae
with Balansia, Atkinsonella, Epichloe, and Belensiopsis and
(3) Ustilaginoidae with Munkia and Ustilaginae. Diehl
(1950) further recognized two subgenera viz. Em祓ansiae and
Dothichloae under the genus Balansia. The subgenus Em.flipansiae
was proposed for those species having patellate to cupulate
epheidial fructifications with a well defined peridia and
only incidentally for those with ascostromata that are
directly capitata or pulvinata. The subgenus Dothichloae with
the concept of Atkinson's genus *Dothiohloe* (1894) was proposed for those species having effuse to irregularly hysteriform epithelial fructifications and with ascostromata typically effuse but varying from effused to capitate to stipitate. However, in both the sub-genera he had included both capitate and effuse forms, since epithelial types were given main emphasis.

Govindu and Thirumalachar (1963) recognized two distinct groups. Group I contains capitate and stipitate forms while in Group II Sickle shaped effuse forms were included. Srinivasan and Thirumalachar (1969) proposed to maintain Atkinson's genus *Dothiohloe* for effused stromal members on the basis of cultural characters.

Writer's studies on cultural behaviour of *Balansia claviceps* Speg. (Ullasa, 1969) and ascospore germination of *B. claviceps* and *B. sclerotica* (Ullasa, 1971) confirm the findings of Srinivasan and Thirumalachar (1969) and that the fungus *Balansia sclerotica* may be treated as species of *Dothiohloe* Atk. occupying an intermediate position between the genera *Balansia* and *Epicnloe*.

The closely related and sharply defined group the members of Clavicipitaceae had been generally placed under the Hypocreales earlier because of the bright coloured stroma. It has been suggested that the differences in the colour and consistancy of the stroma are insufficient basis for separating
Sphaeriales from Hypocreales and the two orders have accordingly been merged (Clements and Shear 1931, Miller 1941, Gaumann 1949).

However, majority of the Hypocreales exhibit Koetria type of centrum development. The centrum development on the other hand, in the Clavicipitaceae appears to be related to Xylaria type, on the basis of which Miller (1949) and Luttrell (1951) have placed this family under the Xylariales. However, Luttrell (1951) found that this group differs from the Xylariales in fasciculate arrangement of paraphysate asci as against other families of Xylariales where the asci are arranged in a continuous wall layer and are interspersed with persistent paraphyses. Consequently Luttrell (1951) considered that there may be some justification for placing this family in a separate order as originally proposed by Nannfeldt (1932).

From the foregoing discussion, it is clear that the taxonomic position of this group of fungi has been a bone of contention and subject of conflicting interpretations having been treated differently by different workers. Besides, no work has been reported into the developmental pattern, centrum characters, sexuality, cytology, and cultural behaviour in any species of the genus Balansia. An intensive investigation was, therefore, undertaken into a typical Indian species, Balansia claviceps, with a view to determine the developmental pattern so as to facilitate comparison with the other members of the family and ultimately to determine the relationship of and the exact taxonomic position of the fungus within the group and
also to determine cytological similarities if any among the closely related members of this family as suggested by Rogerson (1970), the results of which are presented in the following pages.

**Diagnosis and identity of the fungus** (Figs.1.1,1.3 to 1.7)

The infected plant can be recognised from a distance on account of the spreading panicle being converted into a compact prosenchymatous cylindrical structure with all the spikelets and rachis glued together. The stroma is subglobose to subspherical and capitate, surface is olivaceous brown to almost black; perithecia are crowded, lageniform, 170–220 x 100–130 μ; asci scoleosporic, cylindrical, straight or bent, short stipitate attenuated at one end, 120–170 x 4–6 μ, octosporous; ascospores hyaline filiform, septate, 100–150 x 1.0–1.5 μ. Leg. B.A.Ullasa, collected at Coorg District of Mysore State, October 1968, M.A.C.S. Herb. No. 618.

*Balansia claviceps* on *Cyrtococcum oxyphillum* has been earlier reported by Ramakrishnan et al. (1953) from Kerala and from Agumbe, Mysore State by Govindu and ThirumalaSchar (1963). This fungus has been reported on many graminaceous hosts and considered to be in the nature of a composite species with different races, forms and varieties (Déthl, 1950).

A comparative study was, therefore, undertaken between the writer's collection and the type species with the following results:
Table I

Comparison between the Indian collection and the type species

<table>
<thead>
<tr>
<th>Species</th>
<th>Asccarc</th>
<th>Asci</th>
<th>Ascospoire</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. claviceps</td>
<td>200-260 x 106-195 x 100-180 x</td>
<td>120-140 μ</td>
<td>5-6 μ</td>
<td>1-1.5 μ</td>
</tr>
<tr>
<td>Type (Fig. 1.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. claviceps</td>
<td>170-220 x 120-170 x 100-150 x</td>
<td>100-130 μ</td>
<td>4-6 μ</td>
<td>1.0-1.5 μ</td>
</tr>
<tr>
<td>Indian Collection (Fig. 1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On comparison, the Indian collection infecting Cyrtococcum oxyphyllum Stapf. was found to have some differences in respect of dimensions of ascocarp, asci and ascospores being comparatively smaller in size. The Indian collection, however, was significantly different from the type in not having stipitate ascostromata which is a prominent character in the type species. The present collection is, therefore, treated as a variety of the type species, in view of the consistency of the non-stipitate characters besides being collected on a new host.

Balsamia claviceps var. indica var. nov. (Figs. 1.1, 1.3 to 1.7)

Hypothallus 4-6 x 60-80 mm. in diameter, enclosing undeveloped inflorescence of the host; context white to gray, waxy when moist, and compactly prosenchymatous, exterior at first white becoming purple to black with age. Ascostromata subglobose to sub-sphaerical to capitate, sub-sessile and non-stipitate, perithecia lageniform, 170-220 x 100-130 μ; asci subcylindrical straight or bent, short stipitate attenuated at one end, secoleosporic, octosporous, 120-170 x 4-6 μ; ascospores filiform, hyaline, septate 100-150 x 1.0-1.5 μ.
Plate 1
(Figs. 1.1 - 1.7)

*Balansia claviceps* Speg.

   Habit showing sessile ascocarps x 5.

2. *Balansia claviceps* Speg. (Type)
   Habit showing stipitate ascostromata x 5
   (after Diehl 1950)

3. Section through the young maturing ascocarp,
   showing definite perithecial wall.
   *B. claviceps* var. *indica* x 350.

4. Asci squeezed out of perithecium showing
   fasciculate arrangement in a single basal cluster;
   *B. claviceps* var. *indica* x 350.

5. Individual asci of *B. claviceps* var. *indica*, x 350.

6. Upper half of the mature ascus showing mature
   ascospores, and hyaline apical crown.
   *B. claviceps* var. *indica* x 1000.

7. Photomicrograph showing a portion of septate
   mature ascospore. *B. claviceps* var. *indica* x 1200.
PLATE I
Habit: Parasitic on the inflorescence of *Cyrtococceum oxyphyllum* Stapf, collected at Cottebetta, Pollibetta, Coorg District (Mysore State), India, in October 1968 by B. A. Ullasa, M.A.C.S. Herb. No. 618.
CHAPTER - II

INTERNAL MORPHOLOGY, STRUCTURE AND DEVELOPMENT OF ASCOCARP,
SEXUALITY AND ORIGIN OF ASCI

The fungus Balansia claviceps Speg. var. indica Ullasa infecting inflorescence of Cyrtococcum oxyphyllum Stapf. has a very limited distribution but was abundantly available at Coorg District of Mysore State, India and occurs every year during the months of September to November at various stages of development. This variety of Balansia claviceps was specially selected for a detailed study into the internal morphology, the development of ascocarp centrum, sexuality and origin of asci and other related phenomenon since no work on this phase had been reported previously in the genus Balansia. Besides easy availability of this fungus in all its developmental stages and its typically tropical nature induced the writer to undertake an intensive investigation into the above mentioned aspects.

Historical Review

In this family studies on morphology and development have been carried out on two species of Claviceps viz.
C. microcephale (Wallr.) Tul. (Vineens 1917, Kulkarni 1963) and C. purpureus (Fr.) Tul. (Killian 1919), in two species of Cordyceps viz. C. militaris (L.) Lint. (Varitchak 1931) and C. agariciformis (Bolt) Seaver (Jenkins 1934) and in two species of Epichloe viz. E. typhina (Pers) Tul. (Vineens 1917, Doguet 1960) and E. bambusae Pet. (Gaumann 1927). Miller (1949)
concluded, based on partial developmental studies of members of *Asco poly pous*, *Balanasia*, *Cordiceps*, *Cordyceps*, *Dothichloce*, and *Epi chloce* that in addition to characteristics of the aeci and ascospores stressed by Mannfeldt (1932) for the order *Clavicipitales*, these genera have sharply defined perithecial walls, aeci arising from a basal plectanchyma, never in wall layers, and paraphyses which deliquesce early in most species. On the basis of previous reports in literature, Luttrell (1951) considered that there was evidence that two species of *Cordyceps* and two species of *Claviceps* had the "Xylaria" type centrum. Doguet (1960) made a detailed study of *Epichloce typhina* and reported that it also had 'Xylaria' type of centrum similar to that in *Claviceps* and *Cordyceps*. He concurred with Miller's and Luttrell's placement of the Clavicipitaceae. Very recently Rogerson (1970) has given a complete taxonomic account of the Hypocrealean fungi in the format presented in his paper offered by him as a possible model for the genera of fungi. He has recognized the order Clavicipitales with a single family Clavicipitaceae.

The mode of sexual pattern so far observed in this group shows progressive decadence of sexuality ranging from autogamy as reported for *Cordyceps* (Varitchak 1931; Jenkins 1934) through parthenogamy as shown in case of *Epichloce* (Vincens 1917, Gaumann 1927), to gametangy as in case of *Claviceps* (Vincens 1917, Killian 1919, Kulkarni 1962).
Development of Ascoarp

At an early stage of development the stromal heads of this fungus consist of loosely interwoven prosenchymatous hyphae at the centre and pseudoparenchymatous towards the periphery. As the stromal heads enlarge at their sub-peripheral region deeply staining meristematic cells develop, where at regular intervals perithecial primordia are produced in which special sexual organs could be located. These organs are surrounded by loose meristematic hyphae with free tips directed towards the sexual organs arising from the inner meristematic stromal cells of the cavity (Fig.2.1). As the sexual organs develop, the inner cells of the cavity surrounding them are progressively pushed apart along with the free hyphae and due to the pressure exerted by the developing central ascogenous system (Figs. 2.2, 2.3), the inner cells of the cavity form a definite pseudoparenchymatous perithecial wall of 2-3 layers of compressed cells (Figs.2.3, 2.6, 2.7,3.1). The free hyphae which are true paraphyses develop from the cells of the inner wall of the developing perithegium (Figs.2.4 to 2.7), are progressively pushed towards the wall due to the developing ascogenous system, which soon become evanescent and disappear at maturity (Fig.3.1). The cells of the meristematic region surrounding the ascogenous system show a definite polarity towards the periphery of the stroma and the perithecial cavity which was globular to oval in the early stage attains a pyriform shape with deeply staining meristematic region through which the lysogenic ostiole develops later (Figs.2.4,2.5,3.2 to 3.4). Following expansion of fasciculate ascogenous system
Plate 2
(Figs. 2.1 - 2.7)

Stages in the development of peritheciurn in
Balsamsia claviceps var. indica.

1. Photomicrograph showing deeply staining perithecial
   primordium in the subperipheral stromatic region x 500.

2. Photomicrograph showing development of sexual organs
   in the young perithecial primordium x 500.

3. Photomicrograph showing section through the young
   developing peritheciurn showing pyriform shape.

4-7. Photomicrographs showing sections through the
   different stages of developing asccocarp showing
   nature and development of paraphyses, arrow indicates
   the definite perithecial wall of the peritheciurn x 450.
Plate 3
(Figs. 3.1 - 3.4)

Balansia clavipes var. indica

1. Photomicrograph of maturing ascocarp showing definite perithecial wall and fasciculate arrangement of asci in a single basal cluster x 350.

2. Photomicrograph showing development of ostiole through the deeply staining meristematic region x 700.

3. Photomicrograph showing section through the ascocarp of further development of Schizogogenous ostiole x 1,300.

4. Photomicrograph of mature ascocarp showing numerous perithecia in the sub-peripheral region x 80.
within the developing stroma and simultaneous increase in the size of the perithecial cavity, the meristematic cells of the ostiolar region which show intercalary elongation break in the centre and appear to hang back into the perithecial cavity and ostiolar canal (Figs. 2.4 to 2.7) and constitute the ostiolar periphyses near the apical region.

Mechanism of Sex and Origin of Asci

The meristematic perithecial primordia located all along the periphery of the pulvinate stroma show at their centre a single broadly club-shaped deeply staining specialized cell. This cell is vertical in position and has a multinucleate status which is separated from its basal stalk cell by a septum and represents the ascogonium (Figs. 4A.1, 4B.1). The stalk cell bears a narrowly cylindrical but also multinucleate branch which develops close to the ascogonium and lies closely appressed over the ascogonium. This is the antheridium (Figs. 4A.1, 4B.1). The two cells become closely appressed and provide the sexual stimulus for spontaneous fertilization of the ascogonium through fusion of the walls of the sexual organs (Figs. 4A.1, 4B.1). Actual migration of the male nuclei into the ascogonium was not observed. The nuclei in the ascogonium now pair among themselves. Four to six nuclei have been noticed in both ascogonium and antheridium. No trichogyne or receptive papilla were observed. The fertilized ascogonium now becomes septate, cells of which give rise to several buds which ultimately develop into septate ascogenous hyphae (Figs. 4A.2, 4B.2). Each cell of these
ascogenous hyphae have a dikaryon brought about and maintained through conjugate division of the original ascogonial nuclei (Figs. 4A.3, 4A.4, 4B.4, 4B.3). The binucleate ascogenous hyphae arising from the ascogonium have a fan-shaped arrangement which accounts for the resulting fasciculate development of asci attained within the ascocarp of this fungus (Figs. 4A.2, 4B.2). The dikaryotic ascogenous hyphae now elongate and give rise to apical 'Crooks' or croziers the penultimate cells of which are invariably dikaryotic, the basal and the apical cells being uninucleate (Figs. 4A.5, 4A.6, 4B.6, 4B.5). The penultimate dikaryotic cell or crozier now grows and becomes the ascus mother cell which directly develops into the ascus, where the dikaryons fuse resulting in karyogamy thus terminating the dikaryophase (Figs. 4A.6 to 4A.8, 4B.5, 4B.8, 4B.7, 4B.9). Lateral buds arise from the basal cells of the hooks repeating the process and the nuclear events through the phenomenon of proliferation and conjugate division of nuclei (Figs. 4A.7, 4A.8, 4B.8, 4B.9). Thus the entire structure of highly branched fasciculate ascogenous system originates from a single basal ascogonium. The entire process and nuclear events leading to the development of ascogenous hyphae and asci obtained in this fungus are depicted in Plate 4A and 4B.

Discussion of Results

The mode of development of ascocarp has essentially the same pattern as reported for Claviceps microcephala and Claviceps purpurea (Vincens 1917, Killian 1919). The stromal
cells in the vicinity of ascogonium separate to form schizogenous cavities ultimately forming a definite perithecial wall, due to the pressure exerted by the developing ascogenous system. The meristematic region towards the periphery show definite polarity through which lysogenic ostiole develops lined with free periphyses. The free hyphae, which are in the nature of true paraphyses originate from the inner-most cells of the perithecial wall and grow inward towards the ascogenous system. These are pushed towards the periphery during the development of the ascogenous system.

Jenkins (1934) studying with Cordyceps agariciformia was of the opinion that the ascocarp wall is formed by the branches from the surrounding hyphae, some of them might have been originated from the ascogonia. Varitchak (1931) though did not describe the formation of ascocarp wall in Cordyceps militaris, his illustrations show that the development may be similar to C. agariciformia. In the 'Xylaria' type of centrum ontogeny recently demonstrated by Rogers (1967) in Hypoxylon fuscum, the origin of the envelop which later becomes the ascocarp wall could not be determined. But he has suggested that it might have originated from the ascogonal coil. In Balansia claviceps the perithecial wall does not arise directly from the ascogenous system but from the original meristematic hyphae which later form the perithecial wall, might have given rise to ascogonium and anthridium. The ascocarp of Balansia claviceps develops in a manner essentially consistent with what
Luttrell (1951) has called 'Xylaria' type of development. Doguet (1960) who made a detailed study of <i>Epichloë typhina</i> reported that it also had the 'Xylaria' type centrum similar to that obtained in species of <i>Claviceps</i> and <i>Cordyceps</i>.

Members of the family <i>Clavicipitaceae</i> essentially resemble <i>Xylaria</i> in the formation of paraphyses but they definitely differ in the fasciculate arrangement of asci which arise in a single basal paraphysate cluster from a plexus at the base of the perithecial cavity. The paraphyses are limited to the sides of the perithecial wall and are evanescent. The perithecial development is essentially ascohymenial. The asci are unitunicate with a cap-like hemispherical apical crown penetrated by a fine pore. The developmental type is thus distinct from the 'Xylaria' type as originally defined by Luttrell (1951) and may, therefore, be designated as 'Claviceps' type quite distinct from the other types defined in the literature.

On the basis of the manner of the development of the ascosarp centrum designated here as "Claviceps" type, presence of unitunicate asci with thickened hemispherical cap penetrated by a fine pore, presence of filiform multi-septate ascospores, the fungus <i>Balansia claviceps</i> is accordingly placed under the family <i>Clavicipitaceae</i> order <i>Clavicipitales</i> of "Pyrenomycetes" of Euascomycetes following in this respect Nannfeldt (1932), Gaumann (1952), Arx and Mueller (1954), Alexopoulos (1962), Dennis (1968) and very recently Rogerson (1970).
Sexuality and origin of asei in Balansa claviceps var. indica.

A1,B1 Photomicrograph and Camera Lucida drawing showing respectively multinucleate condition of the closely appressed ascogonium and antheridium. Note the paired nuclear status. A1 x 4000.

A2,B2 Photomicrograph and Camera Lucida drawing showing production of several asogenous hyphae from the ascogonial cell. A2 x 4000.

A3,A4, Photomicrographs and Camera Lucida drawings showing the binucleate condition of the asogenous hyphae. A3 x 3000, A4 x 5000.

A5,A6, Photomicrographs and Camera Lucida drawings showing formation of croziers and ascus B7. mother cell A5 x 2,500; A6 x 3,000.

A7,A8, Photomicrographs and Camera Lucida drawings showing development of asci through the process of proliferation. A7, A8 x 1500.
The two species of *Cordyceps* so far worked out by Varitchak (1931), and Jenkins (1934) are characterised by the occurrence of autogamous sexuality. Three to five septate ascogonia are reported in both the cases consisting of uni- or binucleate cells. On the other hand parthenogamy has been found to be a characteristic feature of sexuality in the two species of *Epichloe* (Vincens 1917, Gaumann 1927) whereas in two species of *Claviceps*, true gametangy has been reported (Vincens 1917, Killian 1919, Kulkarni 1963) closely similar to the one observed in *Balansia claviceps*. Thus in four genera and species of the family Clavicipitaceae so far studied we encounter three distinct types of sexual phenomenon such as gametangy, autogamy and parthenogamy showing a progressive degeneration of sexuality as described by Gaumann (1952) for members of Sordariaceae. But the progressive decadence of sexuality in the genera of Clavicipitaceae studied so far does not bear such correlation to the probable evolutionary line drawn by Gaumann (1952) for the order Clavicipitaceae based on the morphological development of fructifications as in Sordariaceae and Pyronemaceae. *Claviceps* and *Balansia* considered to be morphologically evolved forms by Gaumann (1952) show true gametangy while species of *Epichloe* the non-evolved types show parthenogamy and the species of *Cordyceps* the other evolved form are characterised by autogamy.

The antheridium appears to be non-functional since actual migration of the male nuclei into the ascogonium was
not observed. The close appression of the antheridium with
the ascogonium provides probably the necessary sexual
stimulus for further development and nuclear events in the
ascogenium, with pairing of the original sister nuclei of
the ascogenium and its further development into ascogenous
hyphae, hook formation and development of the asci from the
binucleate penultimate cell of the hook. This pattern of
sexuality has all the characteristics of the classical
"Pyronema" type except for the presence of non-functional
antheridium being intermediate between the true "gametangy"
and Parthenogamy.
CHAPTER - III

NUCLEAR EVENTS IN THE ASCUS, CHROMOSOME COMPLIMENT AND ASCOSPORE ORGANIZATION AND DEVELOPMENT

The basic knowledge regarding cytological studies in the Ascomycetes is derived from the pioneer investigations of Dangeard (1894-1907), Harper (1895-1905), Maire (1903, 1905), Guillermond (1905-1911), Blackman and Fraser (1905-1911) and others. According to their studies it is known that the Ascomycetes are characterised by the occurrence of a sexual phase in their life-cycle which results in the production of perfect spores, the ascospores, beginning with plasmogamy and terminating with karyogamy with an intervening Dikaryophase. Also their studies have served to show the occurrence of alternation of generation in the Ascomycetes even as in rust fungi. Regarding the concept of nuclear fusions in this class of fungi two theories have been put forward (1) Single fusion theory or "Claussen type" originally put forward by Dangeard (1907) and Claussen (1912) in which a single fusion occurs in the ascus mother cell followed by a single reduction division in the ascus. This type of nuclear fusion has been known to be of common occurrence in the vast majority of the Ascomycetes so far studied and has been supported by Colson (1938), Hirsch (1950), Singleton (1953), Olive (1950, 1953) and many others in recent years. (2) The other theory is "Double fusion" or "Harper type" according to which two fusions occur, one in the ascogonium and the other in the
ascus mother cell followed by two reductional divisions called "Brachymeliosis" which was put forward by Harper (1895) and was supported by subsequent workers like Blackman and Fraser (1906), Fraser (1907, 1908), Cutting (1909) and others.

Recent experimental work based on a more refined cytological microtechnique with better staining methods has revealed that the "double fusion theory" no more holds good for the large number of Ascomycetes so far investigated as is evidenced from the careful work of Olive (1950), Hirsch (1950) and many others.

**Historical Review**

No cytological studies have been so far reported in the genus Balansa. However, Varitchak (1931) and Jenkins (1934) have reported such studies in the allied genus Cordyceps viz. in C. militaris and C. agariciformia respectively. Therefore, such studies into nuclear events and behaviour in the ascus were undertaken in an Indian species of Balansa viz. Balansa claviceps to determine the possible cytological similarities as recently suggested by Rogerson (1970) with those reported in the two species of the allied genus Cordyceps and Claviceps.

Nuclear Events in the Ascus

In short, a general resume of the nuclear events as they occur in the ascus is given at the outset followed by a detailed account of this aspect as such a procedure is likely to give a better picture of the entire phenomenon as a whole.

It has already been stated that the sexual apparatus in B. claviceps consists of specialised gemmae the ascogonium and antheridium located at the centre of the developing ascocarp initial. The antheridium is non-functional. The nuclei in the ascogonium pair among themselves to produce dikaryons through the process of "spontaneous heterokaryosis". Further development takes place as in "Pyronema" pattern with the production of ascogenous hyphae, hooks or crosiers and through conjugate division of the dikaryons and a fasciculate system of proliferation of the ascogenous hyphae. Asci arise from the dikaryotic penultimate cells of the crosiers where karyogamy takes place. The two nuclei making the dikaryons of the
penultimate cell are indistinguishable and take homogenous stain. As the ascus mother cell enlarges, the two nuclei fuse. This is the only diploid stage observed in the life cycle of this fungus. Later, this diploid nucleus undergoes a single reduction division and two mitotic divisions within the ascus resulting in the production of 8 haploid nuclei. The ascospore initiation takes place at this stage. Further division of the haploid nuclei takes place in the spore initials followed by subsequent septation resulting in the formation of 8 filiform ascospores.

**Behaviour of the Diploid Nuclear in the Ascus**

The diploid nucleus so formed occupies a central position in the developing ascus measuring 2-2.5 μ (Figs. 5.1 to 5.3) and undergoes several changes prior to its first division, the most significant being its remarkable size and varying shape, measuring upto 3-5 μ (Figs. 5.5 to 5.9). The nucleus at this stage does not lose its homogeneity and can be seen as a densely staining fuzzy mass of chromatin with an intact nuclear membrane corresponding to the condensed nuclear type reported by Knox-Davis and Dickson (1960). Similar situation has been reported in *Cordyceps militaris* (Varitchak 1927) and in *Hypocrean oestrina var. americana* (Canham 1967) and in *Coniocheta lignaria* (Rogers, 1965). The nucleolus either disappears early, or is masked since it is rarely stained except for what appears to be ascenetic nucleolus (Fig. 5.3). The position of the nucleolus is sometimes represented by
negative image due to non-staining as reported by Finlay (1970) in *Pellicularia colorata* (Fig. 6.4). The typical prophase configuration of chromosomes is not clear at this stage. During prophase two distinct and prominent centrioles emerge out on either pole and remain attached to the nucleus each anchored by single stranded spindle fibres (Figs. 5.10 to 5.14, 6.1 to 6.6). At this stage the nuclear membrane may be intact or disintegrated. The centrioles might remain very close or move apart from the nucleus. Many times one of the centrioles moves apart and the other being still remaining attached to the undivided chromosomes (Figs. 5.11 to 5.14). The spindle is intranuclear in origin anchored by knob-like centrioles at each pole. Sometimes they get separated and with the disintegration of the nuclear membrane the centrioles look extra nuclear so that the closely lying centrioles along with unseparated bivalents give a wrong chromosome configuration of 3 bivalents (Figs. 6.7 to 6.11). At metaphase, two bivalents were clearly observed arranged in a linear fashion each anchored by a distinct centriolar fibril (Figs. 6.14 to 6.16) and sometimes 2-fibered spindle apparatus were seen each fibre anchored by distinct centrioles (Figs. 6.4 to 6.6). Precocious nuclear division is not uncommon. Sometimes, one of the chromosomes of two bivalents gets separated early and moves towards the centriole (Figs. 6.12, 6.13). Figure 6.17 shows the linear chromosome configuration of the well separated 4 chromosomes of the two bivalents along with two prominent centrioles at each end. Many times the orientation of the
bivalents is not linear, it may be perpendicular or slightly oblique anchored by the centrioles at each end (Figs. 7.1 to 7.4).

During anaphase, two non-sister chromosomes move to each pole along with centrioles and constitute the resulting diads (Figs. 7.1 to 7.7). Lagging chromosomes were seen during telophase due to the non-synchronized disjunction of the chromosome pairs (Fig. 7.5). The spindle orientation is either parallel to the main axis or slightly oblique.

During 2nd nuclear division the typical metaphase configurations of the first division were not seen clearly probably due to the short duration. If the spindle orientation during 2nd division is oblique, the resulting 4-nuclei lie in a close proximity to the ascus wall (Figs. 7.9, 7.10). On the other hand, if the spindle orientation is parallel the nuclear arrangement is linear (Figs. 7.8, 7.11, 7.12). The resulting nuclei may be widely separated or may lie in close proximity. Due to the oblique spindle orientation it is probable that the crossing over might take place as reported by Furtudo (1970) in *Sordaria brevicole* (Fig. 7.9).

During the third division the most interesting feature noted was mitotic crossing over of the sister nuclei. One new type of crossing over was noted in addition to the usual type as seen during 2nd division. In this type (Fig. 7.15), which occurred among the two sister nuclei of the monads situated near the distal end of the ascus, due to the unequal length of
the resulting spindles and due to the movement of the nuclei towards one pole, two sister nuclei lie together occupying the 2nd and 3rd position in the ascus and the other two nuclei get widely separated which occupy apical and 4th position respectively. The same ascus shows the other type of crossing over as occurred during second division of the meiosis, between the two sister nuclei. Probability of such rare meiotic spindle overlap and crossing over of nuclei during third division of meiosis has been suggested by Emerson (1966).

Consequently three successive divisions are involved and separation of the homologous chromosomes occurring in the first meiotic divisions with segregation of genetic makers occurring in either of the first two divisions and depending upon the type of spindle overlap and with the segregation of genetic makers, result in all types of ascospore arrangements within the ascus as reported in the case of Neurospora crassa (Olive 1965) and in species of Phyllocladus (Villasse 1969b). Such observations are not possible in the present case since all ascospores are morphologically similar.

Discussion

The most interesting feature noted during meiosis in Balansia claviceps is the presence of prominent persistent centrioles during first division. Centrioles are knob-like in structure. Presence of centrioles have been demonstrated earlier in several Ascomycetes such as Cordyceps agariciformis

During early metaphase when the two centrioles emerge out and lie close to the nucleus the whole configuration resembles like that reported for *Armillaria mellea* (Motta 1969) and for *Catanaria anguillulae* during mitosis (Ichida and Fuller 1968).

Wells (1970) working with *Ascobolus stercorarius* has reported the plaque like centrioles rather than dense globular bodies. Recently the meiotic metaphase configuration with globular centrioles has been reported in species of *Cembobasidium* and *Pelicularia* during somatic mitosis (Finlay 1970).

During metaphase numerous preparations showed bivalents lined up in two rows or in complete linear arrangement and later during anaphase bivalents separate and dyads move to opposite poles along with centrioles to constitute the sister nuclei. Similar 2-fibre arrangement of bivalents has been recently reported in *Fuccinia lobata* during meiosis (Berkson and Britton 1969).

The second and the third divisions were of quick succession. During the 2nd division the spindle orientation was either
oblique or parallel to the main axis of the ascus. Such oblique orientation of the nuclear spindle may give rise to the alternate arrangement of the sister nuclei within the ascus brought through meiotic spindle overlaps and crossing over. During 3rd division the important observations made were the occurrence of a different type of spindle overlap in the same ascus in addition to the normal one resulting in different types of ascospore arrangement. Such types of spindle overlaps have been suggested recently by Emerson (1966).

Ascospore Organization and Development

Ascospore organization in the ascomycetes is known to be brought about through several mechanisms. This has been recently summarized by Reeves (1967). According to him, four mechanisms of ascospore organization have been reported in literature.

1. Gjurasic (1893) reported that the divisions of the nuclei in the ascus were karyokinetiC and that asters were associated with the division process. In the 8-nucleate stage of the ascus he described the foldings of the rays of the aster around the individual nuclei. Harper (1897, 1899, 1900) expanded these observations of nuclear behaviour and designated it as "free cell formation" a unique process that appears to be of common occurrence in the Ascomycetes. A summary of the events leading to the process of "free cell formation" is as follows: (1) in the 8-nucleate ascus each of the haploid nuclei
forms a beak with a persistent central body and astral rays at the tip of the beak; (2) the astral rays swing outwards and downwards and form a thin membrane which cuts out the young spore; (3) the membrane around each spore separates the sporoplasm and included nucleus, leaving the epiplasm in the ascus. With few exceptions, these ideas have been verified and expanded by the majority of subsequent investigators. Dodge (1927) believed that the large abnormal multinucleate spores obtained in some species of Neurospora were the result of multi-linear activity among the astral rays of several nuclei, although the process was essentially the same as that described by Harper (1905) for powdery mildew fungi.

2. A second method of ascospore organization was presented by Dangeard (1907). He was unable to find the astral rays described by Harper. Rather, Dangeard described a sheet of material that spread from the central body of each nucleus and gradually envelopes the spore. The absence of astral rays led Dangeard to modify Harper's original description. Similar observations were made by Faull (1905, 1912). He considered that the spores were delimited through a double membrane system prior to the appearance of the astral rays in the delimitation of the ascospores and not through the mechanism of astral rays.

3. Jones (1925, 1926), Jenkins (1934), Raymond (1934) (for some species), Heim (1952) and Hayman (1964) have described a third mechanism of ascosporogenesis. All of
these mycologists agreed that the cytoplasm was divided into segments around the nuclei without the aid of astral rays or centrioles. Jones (1926) thought that the planes of cleavage were initiated through the appearance of narrow vacuoles, along certain points near the ascus wall. Raymond, Heim and Hayman were unable to determine the mechanism involved in the segmenting of the cytoplasm around the nuclei. However, Heim (1952) thought that the astral rays and centrioles reported by other investigators were either artifacts of fixation and staining or misinterpretations of nuclear division figures.

4. The fourth method of ascospore delimitation, and the most radically different theory presented thus far, was that provided by Mittmann (1932), Andrus and Harter (1933, 1937), Andrus (1936) and Chadefaud (1943, 1960). These investigators held that, prior to the sporogenesis, all the 8 nuclei of the ascus were enveloped in a very thin membrane (the ascus vesicle). Ascospore organization occurred through the construction of this membrane around the individual nuclei. None of these investigations obtained evidence of the role of astral rays and centriole complex described by other microscopists in the delimitation of ascospores. Gwynne-Vanghan and Broadhead (1936) reinvestigated the fungus Ceratostomella fimbriata studied by Andrus and Harter and suggested that the reported "ascus vesicle" was actually a large vacuole. Chadefaud (1960) has demonstrated that the "ascus vesicle" in his material was not a vacuole.
Studies on fine structural aspects of higher Ascomycetes have been made by Ceruti et al. (1964), Moore (1965), Wilsenach and Kessel (1965), Beckett (1966), Delay (1966), Rudolph and Giesy (1966), Schraats (1966, 1967), Bracker and Williams (1966) and Carroll (1966, 1967). These investigators found that prior to ascospore organization a double membrane was formed around the entire mass of cytoplasm in which the 8 nuclei were embedded, the ascospores were delimited from the rest of the cytoplasm of the ascus through progressive constriction of this double-membrane system around the individual nuclei. Reeves (1967) working with Pyronema domesticium with the help of Electron microscope reports that the ascus vesicle surrounding the complement of 8 nuclei is composed of two unit membranes, and that these membranes appear to be the agents by which the ascospores are delimited.

According to Reeves, this double membrane system appeared to be essentially the same as that described in the light microscopic studies by Mittmann (1932), Andrus and Harter (1933, 1937), Andrus (1936), and Chadeaud (1943, 1960).

The writer's observations on ascosporogenesis in Balansia clavipes based on several hematoxylin smear preparations with light microscopy essentially agree with the third mechanism proposed by Jones (1925, 1926), Jenkins (1934), Raymond (1934), Heim (1952) and Hayman (1964).

No 'Ascus vesicle' mechanism composed of two unit membranes as reported by Reeves (1967) was observed at any stage of ascospore organization in the fungus under study. In Balansia
Claviceps the delimitation of ascospore initials commences only after the organization of a full complement of 8-haploid nuclei is completed. This is followed by the appearance of cytoplasmic haloes around each of the encapsulated nuclei separating them from the epiplasm (Fig. 7.16). The fine structure involved in the process of organization of the ascospore has been described by Moore (1965) in case of Cordyceps militaris. He emphasized the role of endoplasmic reticulum in delimiting the ascospore wall. He reports that the ascospore initials are surrounded by an outer matrix membrane separated by a plasma membrane and that the ascospore wall develops between these two membranes with the development of an investing wall over it. At maturity the ascospore wall has been shown to be layered. It is also shown the epiplasm, which is the enucleated ascus cytoplasm left after the ascospore nucleus and other organelles are encapsulated, breaks down as the spore matures. After further mitotic divisions of these nuclei in the spore initials it is shown to be separated by the invagination of the inner nuclear membrane to partition the nucleoplasm into sub-units, termed as "Karyomes" by Moore (1965) and the process is described as "Karyochorosis".

Though such observations are beyond the scope of light microscopy, there is a clear homology in R. claviceps to that described for Cordyceps militaris (Moore 1964). The nuclei in the ascospore initials divide and lie in groups of 2-3, which may be in the nature of karyoms (Fig. 7.17). At this stage
ascospore initials show rapid elongation. The spores become highly vacuolated. The mature spore is thus multiseptate and multinucleate (Fig. 7,18).

Summary and conclusions

1. Nuclear fusion is of *Claussen type* i.e. single-fusion occurs in the ascus mother cell followed by a single reduction division in the ascus.

2. The chromosome number is determined to be \( n = 2 \) haploid, which is the normal complement for the family.

3. Presence of prominent centrioles and non-synchronized disjunction of the chromosomes have been observed.

4. Meiotic crossing over and spindle overlaps resulting in different types of spore arrangements have been demonstrated.

5. Organization of ascospores takes place through the mechanism of cytoplasmic cleavage or "free cell formation" at 8-nucleate stage.

6. Mechanism involved in further development of ascospore initials into ascospores is probably in the nature of "Karyochorasis".

7. The results so obtained are substantiated with a series of photomicrographs.
Plates 5

(Figs. 5.1 - 5.14)

Cytology of *Balansia clavipes*
var. *indica*.

1. Photomicrograph showing diploid nuclei in the asci x 1500.

2. Photomicrograph showing expanded diploid nucleus x 1500.

3. Photomicrograph of diploid nucleus showing eccentric nucleolus. x 1500.

4-9 Photomicrographs of expanded diploid nuclei showing various shapes and sizes x 1500.

10-14 Photomicrographs of Prophase I nuclei showing emergence of globular centrioles at either pole of the nucleus anchored by centriolar fibrill 10 x 850 and 11-14 x 1500.
Plate 6
(Figs. 6.1 - 6.17)

Cytology of Balansia claviceps var. indica.

1-3 Photomicrographs of prophase I nuclei showing prominent globular centrioles and expanding deeply stained undifferentiated chromatin x 1500.

4 Photomicrograph of Prophase I nucleus showing an unstained negative image of the nucleolus indicated by an arrow x 1500.

4 Mid anaphase. Note the centrioles and what (left) appears to be a two fibred spindles x 1500.

6-12 Photomicrograph showing prominent globular centrioles on either side of the unseparated bivalents. Note the overlapping of the 2 bivalents in Fig. 9 indicated by central arrows and upper & bottom arrows indicate the centrioles x 1500.

13. Photomicrograph showing precocious division of metaphase I. One of the chromatids of two bivalents separated and moving towards the centrioles (arrows indicate centrioles) x 1500.

14-16 Photomicrographs showing metaphase I of linear arrangement of two bivalents along with one pair of centrioles on either pole x 1500.

17. Photomicrograph showing metaphase I. Note the 4 chromosomes of two bivalents arranged in a linear fashion (arrows indicate the centrioles) x 1500.
PLATE 6
Plate 7
(Figs. 7.1 - 7.18)

Cytology of *Balania olaviceps* var. *indica*.

1-4 Photomicrographs showing metaphase-anaphase of I division x 1500.

5. Photomicrograph showing telophase I. Note the lagging chromosome x 1500.

6,7. Photomicrographs showing two-nucleate stage of the ascus x 1500.

6. Photomicrograph showing telophase II. Note the parallel orientation of the spindle to the main axis of the ascus x 1500.

9. Photomicrograph showing telophase II. Note the slightly oblique orientation of the spindle to the main axis of the ascus and possibility of spindle overlaps and crossing over of daughter nuclei x 1500.

10. Photomicrograph showing telophase II. Note the oblique orientation of the spindle to the main axis of the ascus x 1500.

11-13 Photomicrographs showing 4-nucleate stage of linear arrangement in the ascus x 1500.

14-15 Photomicrograph showing 8-nucleate stage. Fig. 15 shows rare type of spindle overlap and crossing over of daughter nuclei in the upper region of the ascus, while in the lower region the normal type of spindle overlap and crossing over of daughter nuclei is seen x 1500.

16. Photomicrograph showing ascospore organization and formation of cytoplasmic cleavage and hollow around (sporokinesis) each of the eight nuclei x 1500.

17. Photomicrograph showing further division of nuclei in the ascospore initials. Note the daughter nuclei (karyomes) in groups x 1500.

18. Photomicrograph showing a portion of mature ascospore and its multinucleate condition x 3000.
CHAPTER - IV

STUDIES IN ARTIFICIAL CULTIVATION AND LIFE CYCLE

The form-genus Ephelis Fr. associated with the ascomycetous genus Balansia (Clavicipitales) has been treated as a member of the form-order Sphaeropsidales by Clements and Shear (1931), Bender (1934), and Barnett (1960). On the other hand, Diehl (1930, 1950) considered the conidial fructifications as a sporodochium (F. Tuberculariaceae) except for the presence of basal excipulum, describing it as a "cupulate or patailate structure emerging from hypothallus". He (1950) further referred to it as "either a pycnidium or asccervulus that is erumpent from beneath the surface of the hypothecium". Govindu and Thirumalachar (1961) considered the conidial fructification as irregular sporodochium comparable to the spacial stage of Claviceps. Kamat (1961) placed the form-genus under the form family Tuberculariaceae considering the ephelidial fructification to be a sporodochium, in this respect following Hohnel (1923). Bender (1934) placed it under the form-family Excipulaceae of the order Spharopsidales. These reports were based on the observations of the fruit bodies obtained naturally on the host or under moist chamber conditions (Diehl 1930). These conflicting reports on the nature and structure of the ephelidial fructifications of this form genus and its taxonomic status, thus, needed critical examination and clarification not only through more detailed observations and studies on this fungus as it occurs on the host under natural conditions but also through its behaviour in artificial culture.
Besides, while close association between Ephelis and Balansia states had been observed on the host under natural conditions, no convincing evidence had been presented regarding their genetical relationship through pure culture studies.

**Ephelidial (conidial) Fructifications on the host**

Examination of free hand sections of the ephelidial fructifications obtained from the host in different stages of development revealed that these fructifications were modified sporodochia emerging through a basal cushion-shaped stroma or hypothecium over which the conidiophores and acicular conidia were densely crowded together (Figs. 8.1, 8.2). In some cases variations were also noted in the shape of these fructifications which were patellate or lunate in structure, located over and emerging through an excipulum like stroma. These bodies lacked a true peridium and therefore may be considered as sporodochia emerging through a basal excipulum (Figs. 8.3, 8.4, 8.5). These modifications in the form and structure of the ephelidial fructifications may be due to the nature and moisture content of the host tissue involved, as pointed out by Biehl (1950).

**Germination of Conidia**

The acicular conidia swell considerably in sterile water and water agar just prior to germination and put forth, at the end of 48th hour, 1-5 germ tubes which may be polar or lateral. The number of germ tubes produced by a conidium is determined by the nuclear status of the conidium. The germinating conidia
at this stage become multicellular and cells sometimes becomes bulbous putting forth more than one germ tubes behaving in this respect like ascospores as observed by Diehl (195c). The germ tubes branch or may remain unbranched and produce at their ends a secondary crop of conidia of the original type at the end of 72 hours (Figs. 10.1, 10.2). The conidia so formed secrete a gummy substance which holds them together in masses or in whorls. These primary conidia may germinate in situ and give rise to a secondary or tertiary crop of conidia through budding. The process may be repeated several times until at the end of a week or 10 days, minute specks of yeast-like colonies of the fungus appear in the medium. Such colonies consists of mycelium producing conidiophores and ascicular conidia characteristic of the form-genus *Ephelis*.

**Germination of Ascospores**

The ascospores of the *Balansia* stage obtained from the host germinated readily in sterilized water and water agar, often in situ (while still in the ascus) with the production of one or more germ tubes which soon branch to produce ascicular conidia very similar to those produced by conidia of the ephilidial stage (Figs. 9.1 to 9.4, 9.1A to 9.4A).

**Cultural Behaviour**

On potato dextrose agar the fungus made extremely slow growth producing a colony of 10 mm. at the end of 10 days. The colony was yeast-like, glistening, cream coloured, raised,
and consisted mainly of masses of conidia. Cultures arising from ascospores gave rise to the typical sporodochial bodies similar in morphology and structure to those produced by the conidial cultures. Segments of germinating ascospores sometimes became swollen, as observed by Diehl (1950), putting forth more than one germ tubes. Minute raised stromatic pustules appeared in the medium at the end of 20 days. These pustules slowly turned colour, becoming light orange, spherical or cushion shaped, velvety, fully raised, either isolated or aggregated, finally becoming dark amber in colour with age, attaining full maturity at the end of 30 days (Figs. 11.1, 11.3, 11.4, 11.6, 11.7).

The colony characters of the ephidial stages were studied in the following media: (1) Potato agar, (2) Carrot agar, (3) Rice meal agar, (4) Oat meal agar, (5) Corn meal agar, (6) Sorghum meal agar, (7) Wheat meal agar, (8) Nutrient agar, (9) Coons agar, (10) Glucose peptone agar, (11) Czapek's Dox agar, (12) Browns agar and (13) Richard's agar. The fungus was grown in 30 mm triplicate Petri-dishes containing 15 cc. of the above media and incubated at room temperature (25° - 28° C). The observations recorded after three weeks are given in Table II (Figs. 12.1, 12.2).

The study of the tabulated results does not show much variation in the colony characters and rate of growth of the
**TABLE - II**

Behaviour of the mass culture of *Balansa claviceps* Speg. var. *indica* Ullasa in different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Colour</th>
<th>Topography</th>
<th>Nature</th>
<th>Margin</th>
<th>Diameter of colony (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Potato agar</td>
<td>White</td>
<td>Raised</td>
<td>Myceloid</td>
<td>Entire</td>
<td>6</td>
</tr>
<tr>
<td>2. Carrot agar</td>
<td>Ivory white</td>
<td>Flat</td>
<td>Myceloid</td>
<td>Lobed</td>
<td>5</td>
</tr>
<tr>
<td>3. Rice meal agar</td>
<td>Dull white</td>
<td>Raised</td>
<td>Stromatic</td>
<td>Lobed</td>
<td>4</td>
</tr>
<tr>
<td>4. Oat meal agar</td>
<td>Dull white</td>
<td>Raised</td>
<td>Stromatic</td>
<td>Lobed</td>
<td>3</td>
</tr>
<tr>
<td>5. Corn meal agar</td>
<td>Creamy white</td>
<td>Rugose</td>
<td>Stromatic</td>
<td>Lobed</td>
<td>7</td>
</tr>
<tr>
<td>6. Sorghum meal agar</td>
<td>Dull white</td>
<td>Rugose</td>
<td>Stromatic</td>
<td>Wavy +</td>
<td>Myceloid</td>
</tr>
<tr>
<td>7. Wheat meal agar</td>
<td>Dull white</td>
<td>Raised</td>
<td>Stromatic</td>
<td>Wavy</td>
<td>4</td>
</tr>
<tr>
<td>8. Nutrient agar</td>
<td>Dull white</td>
<td>Rugose</td>
<td>Stromatic</td>
<td>Lobed +</td>
<td>Myceloid</td>
</tr>
<tr>
<td>9. Goons agar</td>
<td>Creamy white</td>
<td>Rugose</td>
<td>Stromatic</td>
<td>Lobed +</td>
<td>Myceloid</td>
</tr>
<tr>
<td>10. Glucose Peptone Agar</td>
<td>Ivory white</td>
<td>Flat</td>
<td>Stromatic</td>
<td>Wavy +</td>
<td>Myceloid</td>
</tr>
<tr>
<td>11. Czapex Dox Agar</td>
<td>Dull white</td>
<td>Flat</td>
<td>Stromatic</td>
<td>Lobes +</td>
<td>Myceloid</td>
</tr>
<tr>
<td>12. Brown's agar</td>
<td>Dull white</td>
<td>Raised</td>
<td>Stromatic</td>
<td>Entire</td>
<td>4</td>
</tr>
<tr>
<td>13. Richard's agar</td>
<td>Dull white</td>
<td>Flat</td>
<td>Stromatic</td>
<td>Entire</td>
<td>6</td>
</tr>
</tbody>
</table>
fungus in different media. The colony is either stromatic or myceloid depending upon the medium (Vide Table II). Sorghum meal agar, Corn meal agar, Nutrient agar, Coons agar, Glucose peptone agar, Czapek Dox agar were found to support fairly good growth.

**Ascigerous Stage in Culture**

The cushion shaped ephelidial pustules turned deep amber to dark brown with age until at the end of 4-6 weeks, the pustules become hardened and appear like sclerotal bodies (Figs. 11.2, 11.5). Sections through such stromatic bodies revealed the formation of ephemeral flask-shaped cavities along the periphery of the basal stroma. These were probably the rudiment of asco carp initials located just below the conidial layer. Such asco carp initials, however, remained abortive and did not develop into mature perithecia even at the end of eight weeks on potato-dextrose agar.

**Development of Conidial Fructification in Artificial Culture**

Stromatic initials develop in 4 week old cultures. The cells of the stroma are pseudoparenchymatous and organization of the fertile layer takes place just below the developing stroma from a deeply staining zone, from where the conidiophores arise in the form of small pockets over the sporogenous layer while still covered by the loose mycelium (Fig.13.12). The conidiophores are exposed and appear in the form of a well-developed sporodochium over the stroma which is completely hollow from inside. At certain places, locules develop deeper
into the stroma in which the conidia are produced in masses and ooze out in the gummy secretion (Fig. 13.13).

**Conidium Ontogeny**

The ascospores and conidia germinate readily putting forth one to many germ tubes, each of which terminates with the production of conidia or branch profusely giving rise to several determinate conidiophores at regular intervals which terminate with the production of conidia, thus establishing tiny colonies.

The growing tip of the conidiophore appears slightly blown out, at first followed by the production of a slight constriction at the base of the developing conidium which is cut off from the main axis by a septum. The conidiophore now starts growth just below the septum pushing the primary conidium aside and giving rise to a secondary conidium in the same manner. This process is repeated several times resulting in the formation of as many as twelve or more conidia in a sympodial manner characteristic of "termino radulospora" type of development as defined by Tubaki (1963) and agrees with the mode of development described by Hughes' (1953) in his Section II. The conidia so produced are persistent, appear like fingers over the axis (Figs. 13.1 to 13.10). The conidia are borne over minute denticulate scars or sterigmata which, however, are easily overlooked. The distance between developing conidia may vary depending upon the rapidity of extension of the growing
point of the conidiophore. It is not uncommon to find primary conidia giving rise to secondary and tertiary crops of conidia through direct budding in the acropetal succession.

**Development of Conidiophores**

The conidiophores are simple when directly produced from the germinating ascospores or conidia or from free mycelium (Fig. 13.12) but show variation in structure and morphology when produced from the stromatic fructifications. The conidiophores in the latter case are thick, septate, branching irregularly or in verticellate manner (Figs. 13.14, 13.15).

**Discussion of results**

No member of the genus *Balansia* (F. Clavicipitaceae) has so far been cultured on artificial media. Diehl (1930) reported development of the ephelidal state on the host under moist chamber conditions but did not obtain the (Balansia) perfect state under this condition, in species of *Balansia* studied by him. It is noteworthy that Diehl (1930) failed to obtain the fungus in pure culture in either state and thought that "the fungus did not lend itself to artificial culture". The non-development of ascoecarp initials into mature perithecia in artificial culture may be attributed to either special nutritional preferences of the fungus or its heterothallic nature. The results obtained and observations made by the writer on the nature, structure and morphology of the ephelidal fructification
of *Balansla claviceps* in host as well as in artificial culture prove conclusively that the conidia of *Ephelis* state of the fungus are not pycnidiospores capable of giving rise to pycnidia, on the basis of which this conidial fungus should find a place under the form-family Tuberculariaceae, form-order Moniliiales thus confirming the observations of Hohnel (1923) and Kamat (1961).

The conidium ontogeny of the *B. claviceps* agrees with the mode of development described by Hughes (1953) in his Section II or group "radulosporae" of Tubaki (1963). Conidia so produced are in the nature of "terminoradulospora*. Conidia may be produced either from single conidiophores arising directly from the germinating ascospores or conidia or from free growing mycelium or even from branched conidiophores arising directly from cupulate or patellate stromatic bodies. The fungus, thus, gives rise to two distinct types of conidiophores but the same type of conidia in either case.

**Summary and Conclusions**

1. The species of *Balansla* inciting "agarbatta" disease of *Cyrtococcum oxyphyllum* Stapf. collected from Coorg Dist. (Mysore State) which on detailed microscopical examination and comparison with *Balansla claviceps* Speg. is treated as a new variety of the type species viz. *Balansla claviceps* var. *indica* var. nov. *Ullasa*.
2. The sexual apparatus consists of specialized ascogonium and antheridium located at the centre of the developing ascocarp initial. The antheridium is non-functional. The nuclei in the ascogonium pair to produce dikaryon through the process of "spontaneous heterokaryosis". Further development takes place as in "Pyronema" pattern with the production of ascogenous hyphae, hooks or croziers and through conjugate division of dikaryon and system of proliferation.

3. Perithecial development is characteristic of Ascohymeniales. The asci are uniloculate and arise in fascicles (and not in wall layers) from the basal hymenium. The developmental type is distinct from the "Xylaria" type as defined by Luttrell (1951) and may be designated as "Claviceps" type, quite distinct from other types defined in literature.

4. There is only a single nuclear fusion in the ascus mother cell followed by only a single reductional division in the ascus which conforms to "single fusion theory" or "Claussen type".

5. The presence of definite centrioles, origin of intranuclear spindle have been reported and the haploid chromosome complement has been determined as n = 2. The occurrence of rare types of spindles overlap which result in different types of arrangements of ascospores within the ascus has been reported for the first time.
6. Ascospore delimitation appears to be initiated through the role of endoplasmic reticulum in this fungus similar in character as suggested for Cordyceps militaris by Moore (1965).

7. Occurrence of Claviceps type of development, origin of the asci in basal fascicles and provided with an apical hyaline cap, Scolecosporic ascospores, origin of paraphyses in wall layers are unique to this group and not so far reported in any member of the Pyrenomycetes (Ascohymeniales) other than the family Clavicipitaceae, which is remarkably homogenous in this respect.

8. The genus Balanisia has been brought under artificial culture for the first time, beginning with ephelidal and ascigerous states thus establishing their genetical relationship and life cycle pattern for this fungus.

9. Studies into the conidial fructifications both in the host and in artificial culture and conidium ontogeny revealed that the form genus Ephelis Fr. is a member of Tuberculariaceae of the order Moniliales.
Plate 8
(Figs. 8.1 - 8.6)

*Balansia claviceps var. indica* sections through the Ephelidial fructifications.

1 & 3. Cushion shaped  x 300

2. Cupulate  x 300

4. Patellate  x 300

5. Lunate  x 300

6. Cross section through the Ephelidial fructification obtained from artificial culture  x 120.
PLATE 8
Plate 9
(Figs. 9.1 - 9.4; 9.1A-9.4A)

Balansia claviceps var. indica.
Ascospore germination.

9.1, 9.1A. Germination of ascospore in situ x 300.

9.2, 9.3, 9.2A, 9.3A. Germination of single ascospores
showing long germ tubes producing conidia x 450.

9.4, 9.4A. Nature of conidiophore and conidia
produced by a germinating ascospore x 700.
Plate 10
(Figs. 9.1 - 9.2)

Balansia claviceps var. indica

1 & 2. Germination of conidia in
water agar with the production
of polar and lateral germ tubes
and secondary conidia.
Plate 11

(Figs. 11.1 - 11.7)

Balansia claviceps var. indica
in artificial culture (Potato, Dextrose Agar)

1,3,4. Conidial fructification in artificial culture.

2. Development of sterile ascostromata.

5. Cross section of sterile ascostromata x 70

6. Young developing colony.

7. Conidial fructifications x 60.
Plate 12
(Figs. 12.1 - 12.2)

_Balansa claviceps var. indica_

in artificial culture.

1. Comparative growth of _Balansa claviceps var. indica_ in different vegetable media.
   Plates 1. Potato agar, 2. Carrot agar,
   3. Rice meal agar, 4. Cotton meal agar,
   5. Corn meal agar, 6. Sorghum meal agar,
   7. Wheat meal agar.

2. Comparative growth of _Balansa claviceps var. indica_ in different synthetic media.
   Plates 1. Nutrient agar, 2. Coons agar,
   3. Glucose-Peptone agar, 4. Czapek Dox agar,
Plate 13
(Figs. 13.1 - 13.15)

*Rulaosia elaviceps* far, *indica*
conidium ontogeny.

1-10. Photomicrographs showing successive stages of conidium development (Terminoradulosores) x 500.

11. Mycelial stage producing conidiophores at regular intervals with persistent conidia held in clusters x 100.

12. Stromatic stage producing conidiophores in a fertile layer organized in the form of pockets x 70.

13. Stromatic locules produced late in the stage of development with conidiophores and conidia x 70.

14 & 15. Nature of conidiophores produced in the stromatic stage.
PART II

STUDIES INTO CRYPTOMYCES MUELLERI ULLASA
CHAPTER - I

TAXONOMY AND DIAGNOSIS OF CRYPTOYMES MUELLERI ULLASA

The genus Cryptoymes was established by Greville in 1826 with Cryptoymes wauchii as type. Later, Hehm (1888) described Cryptoymes maximus based on Rhytisaa maximus Fies (1823). Cryptoymes maximus has been treated as a new combination and has been accepted as type species of the genus Cryptoymes Grev. by subsequent workers like Saccardo (1889), Alcock (1926), Clements and Shear (1934), Arx and Mueller (1954), Ainsworth (1961) and Dennis (1968). Cryptoymes wauchii Grev. the original type of the genus has thus been treated as a synonym of Cryptoymes maximus (Fr.) Rehm.

This fungus has been reported to be parasitic on twigs of various species of Salix in European countries (Alcock, 1926; Arx and Mueller 1954). Though several species have been attributed to this genus (Saccardo 1889) all except the type species have been proved to belong to other genera (Emil Mueller, personal communication). Thus so far the genus was monotypic and has been treated as the type of the family Cryptoymetaceae, order Phacidiatales by Arx and Mueller (1954) and Dennis (1968). Kimbrough (1970), based on centrum studies of Gordon (1966, 1968) on species of Lophodermium, suggested that the subgelatinous and fleshy stroma characteristic of the family Cryptoymetaceae is not unlike those of Hypodermataceae. Clements and Shear (1934) placed the genus Cryptoymes under the family Phacidiaceae of the order Phacidiales.
Since no work has been reported on such fundamental aspects as centrum development, sexuality, origin of asci and cytology in this genus, an intensive investigation was undertaken into this interesting Indian species viz. Cryptomyces muelleri Ullasa with a view to furnish adequate data on the developmental pattern so as to facilitate comparison with the other members of Phacidiales and ultimately to determine the relationship and exact taxonomic position of this genus, the results of which are presented in the following pages.

**Diagnosis and identity of the fungus Cryptomyces Muelleri Ullasa**

Apothecia strictly foliicolous, epiphyllous, intrapodermal in origin, bright, yellowish, dehiscing irregularly at maturity in the form of lobes; asci cylindrical to clavate, unitunicate, iodine negative in reaction, 8-spored, 100-130 x 18-20 μ, ascospores ovate to ellipsoid, hyaline, uniseriate to irregularly biseriate, 16-24 x 7-10 μ; paraphyses cylindrical to clavate, thin, septate, light-brown, in palisade layer, producing a thin epithecial layer above the asci.

Latin diagnosis: Cryptomyces muelleri sp. nov. Ullasa

Apothecia omnino foliicola, epiphylla, origine intrapoderali, vivida, flavida, irregulariter dehiscentia ad maturitatem, in ambitu lobata; asci cylindracei vel cylindraceo-clavati, tenuiter tunicati, octospori, 100-130 x 18-22 μ sporae ovoidea vel ellipsoidae, mono vel irregulariter distichae, 16-24 x 7-10 μ; paraphyses clavatae epicidibus bulbosis, tenuissimi, septatae, brunneolae, efformante serie epithecialia supra ascos

(Figs. 14.1 to 14.4, 15.1 to 15.7).

The fungus under study collected on Salix tetrasperma Roxb. was accommodated into a new taxon on the basis of critical and comparative studies and differential habit as compared with the type species, with the following results.

**TABLE - III**

<table>
<thead>
<tr>
<th>Species</th>
<th>Asci</th>
<th>Ascospores</th>
<th>Habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. maximus</td>
<td>180-250 x</td>
<td>30-88 x</td>
<td>Lignicolous</td>
</tr>
<tr>
<td></td>
<td>25-33 μ</td>
<td>14-17 μ</td>
<td></td>
</tr>
<tr>
<td>Indian sp.</td>
<td>100-130 x</td>
<td>16-24 x</td>
<td>Foliicolous</td>
</tr>
<tr>
<td></td>
<td>18-22 μ</td>
<td>7-10 μ</td>
<td></td>
</tr>
</tbody>
</table>

It is quite clear from the above table that the Indian collection of Cryptomyces is significantly distinct from the type Cryptomyces maximus Rehm described from Europe in respect of dimensions of asci and ascospores besides having strictly foliicolous habit and on the basis of which it merits accommodation in a new taxon.

**Remarks**

Spermogonia were of common occurrence either occurring independently or in close association with the developing...
Ascoecarp. They are lenticular to lecaniar in shape, dark, stromatic, bearing spermatothophores in basal layers and producing numerous spermatia, 300-1500 x 100-150 μ.

Spermatothophores stout, septate, thick walled, dark at the bottom and hyaline at the tip, 8-16 x 2-5 μ. Spermatia globose to rod shaped hyaline, single celled 0.8 - 1.6 x 2 - 3.2 μ.

This species has been established after Dr. Emil Mueller of the Institute of Special Botany, Zurich, Switzerland, in recognition of his outstanding contributions to the Ascomycetes.
Plate 14
(Figs. 14.1. – 14.4)

*Cryptomyces muelleri* Ullasa

**Habit**

1. Photograph of mature ruptured apothecia exposing the central hymenium. Note the elevated marginal lobes.

2. Photograph showing nature of immature and unruptured apothecia. Note the bright colour and close association of the dark stromatic spermogonial bodies.

3. Photograph showing mature but still unruptured single apothecium. Note the preformed line of dehiscence along the veins, and also note the central dark spermogonial bodies.

4. Photograph showing mature and ruptured apothecia.
PLATE 14
Plate 15
(Figs. 15.1 - 15.7)
Cryptomyces muelleri Ullasa

1. Habit.
2. Section through the apothecium.
3. Section through the apothecium and part of spermogonium.
4. Ascospores.
5. Ascus and paraphysis.
6. Photomicrograph showing section through the spermogonium closely associated with the apothecium x 250.
7. Photomicrograph showing a linear spermogonium developed independent of apothecia x 150.
CHAPTER - II
INTERNAL MORPHOLOGY, STRUCTURE AND DEVELOPMENT OF ASCOCARP,
SEXUALITY AND ORIGIN OF ASCI

Introduction

The genus Cryptomyces Grév. was available abundantly in the forests of Coorg (India) and persists from June to October as a folliculous pathogen on Salix tetrasperma. This fungus was especially selected for a detailed study into the internal morphology, the centrum development, the nuclear behaviour and other related phenomena since no work has been so far reported in this fungus in this respect. Besides such studies have vital importance in determining its exact taxonomic position and the trend of evolutionary development of the fungus within this heterogenous group of fungi.

Historical Review

There have been several significant studies and reports on the development of ascocarpic centrum and sexuality in members belonging to the Phaoidiales. According to Killian and Likhite (1924) long chains of approximately 40 ascogonial cells develop from one or more enlarged cells (ascogonial mother cells) in the mature apothecial stroma of Lophodermium hysteroides (Pers.) Sač. The centrum is composed of pseudoparenchymatous cells which distinegrate and give way to the formation of multinucleate paraphyses which develop from specialized spherical cells located in the basal region of this Hysteroideal fruit body and the continued growth of the
paraphyses elevate the upper shield or clypeus thus providing space for the development of the asci. The ascogonial cells located in the basal region give rise to ascogenous hyphae from which asci arise. Jones (1925) in his comprehensive studies on the life history and cytology of *Rhytisma acerinum* (Pers.) Fries., described the development of ascocarp in this fungus as being made up of 3 distinct zones - a central zone of comparatively loosely arranged hyphae with a zone making up the roof and the other at the basal region both made up of compact, hyphal tissue which is pseudoparenchymatous in nature. According to him the central loosely arranged hyphae making up the ascocarpic centrum were in the nature of vegetative mycelium, some of which later grow actively in the upward direction reaching the roof of this developing ascocarp to which these hyphae become fixed. According to him, other hyphae, making up the centrum appeared to abstract several globoid cells from their apices, which accumulate in the region of the roof and later become firmly attached to each other to produce a thick covering over the ascocarp. This process was considered by Jones (1925) in the nature of roof building phenomenon, which continues and gives rise to a thick and densely packed plectanchyme. This process goes on for a considerable time until tissue making up the roof approximates in thickness to the depth of the main apothecial chamber and is completed with the vertical vegetative hyphae giving rise to conical terminal cells by division. These vertical hyphae according to Jones (1925)
were distinguishable from the true paraphyses, which have more or less an erect habit, many of them becoming linked together with the formation of well marked 'H' shaped connections establishing protoplasmic continuity between these hyphae.

Thus the two distinct types of vertical hyphae could be distinguished in the developing ascocarp one of which represented true paraphyses with free ends and the other pseudoparaphyses in nature.

According to Jones (1925) *Rhytisma acerina*um (Pers.) Fr. produced structures resembling acervuli within the black stroma provided with papilla which in turn produced large quantities of rod-shaped minute microconidia (spermatia) extruding through the papilla. Jones (1925) was unable to attribute any definite sexual function to these microconidia, although he observed multinucleate ascogonia bearing multinucleate terminal trichogynes, dissolution of their intervening walls and passage of the trichogonial nuclei down into the basal ascogonia where they pair with the ascogonial nuclei followed by the development of ascogenous hyphae and origin of asci without the intervention of croziers.

Morphological studies by Jones (1935) on *Lophodermium pinnastri* (Schrad ex Fries) Chev. parasitizing *Pinus sylvestris* is by far the most fully illustrated and comprehensive investigation into the microconidial locules (spermogonial) and ascocarpic development. He described two
modes of ascocarpic development in this fungus one in which the ascocarpic development was directly associated with the spermogonial stage and the other in which the ascocarps develop in areas of the needle independent of spermogonia or microconidial locules. He also differentiated these two types of ascocarpic development by their distinctive gynei. In the former case he has suggested that the specialized hyphae or trichogynes produced in the basal layers inside the spermogonial locules make contacts with the spermatia, although actual copulation between spermatia and trichogynes was not observed by him and considered the process as similar to that described for Lichens. He interpreted the ascervulus stage (microconidia) as a spermogonium producing spermatiophores, spermatia and ascogonia bearing trichogynes and development of the apothecial bodies derived from such spermogonia. In the latter case where the apothecia develop independently of spermogonia the ascocarp develops as a result of copulation or anastomosis between mycelia belonging to two sex strains of this fungus, a phenomenon which could be interpreted as heterothallism, resulting in dikaryotic asogenous cells followed by the development of dikaryotic asogenous hyphae the cells of which produce ascii with or without the formation of croziers.

The best illustrated and most comprehensive investigations of ascocarpic centrum ontogeny of species of Hypodermataceae is that of Gordon (1966, 1968) who working with 39 species described three basic types of centrum ontogeny in which the ascal initiation within Type I - centrum occurs in the basal
cells of the pseudoparaphyses through the mechanism of anastomoses, while ascospore initiation within Type II occurs in cells of a plectanymbomatous centrum, with no prior anastomosing in the ascocarp. The Type III centrum of Gordon (1968) is characterised by the occurrence of a layer of hyaline cells in the central region of the primordium which is meristematic in nature and is the seat of all the subsequent structures making up the ascocarpic centrum. The ascospore initiation in this case, takes place in a manner as described for the Type I.

Wehmeyer (1966) reported that fern fronds infected with Cryptomyces pteridis produced pycnidia in association with ascomyces in which the archicarp provided with erect trichogynes are formed in a central meristem of the ascocarpic centrum but which later degenerate. Ascogenous hyphae arise from intact or fragmented strips of the central "meristem". Asci develop from these strips without producing crosiers. Ascocarps mature on overwintering fronds and mature ascospores are produced in early spring. Killian (1915) contrasted the elongate ascogonia of Cryptomyces with those of other Discomycetes. Helicoid ascogonia provided with trichogynes were reported in Coccomyces bisporus (Higgins 1914) and Phacidium repandum Satina (1927). Studies on ascospore development in Rhytisma acerinum were reported by Aragno (1967). Woo and Partridge (1969) reported on the life history and cytology of Rhytisma punctum parasitizing big leaf maple.
Thyr and Shaw (1966) have studied the centrum development in *Hypodermella arcuata* and reported similar types of centrum ontogeny as reported by Gordon (1966). According to them the asci develop from the base of the pseudoparenchymatous stroma at the same level as the base of the pseudoparaphyses. Pairing of the nuclei or dikaryotization according to them apparently occurs before the initiation of ascocarp. This they suggested that it might be brought about through the mechanism of anastomoses between basal cells of uninucleate hyphae. They failed to observe either spermatia or trichogyynes during ascocarp development.

Recently Bellemere (1967) working with developmental pattern and evolution in several members of inoperculate Discomycetes divided them into the following two groups:

1. **Discostromiens**, (2) Paratheciens. Again the Discostromiens are sub-divided into (1) Discostromiens proprement dits and (ii) Discostromiens evolves.

The "Discostromiens proprement dits" group is further divided into following three sections:

A. Les Lenticulaires
B. Non Lenticulaires
C. Les Pseudodiscopodiens

Under his first group "Les Lenticulaires" the following fungi were included and studied by him:

1. *Phaeidiostrum multivalve* (DC) von Hohnel
2. *Therya fuckelii* (Wehm) Kujala
4. *Colpoma juniperi* (Karst) Dennis
5. *Propolis faginea* (Sachrad) Karst.

The results obtained by him in the lenticular Discomycetes are tabulated as given by him.

**TABLE - IV**

**TYPES APOTHECIAUX**

( After Bellacere 1969 )

<table>
<thead>
<tr>
<th>Discostromiens proprement dits</th>
<th>Stromatique</th>
<th>Pseudodiscopodiens</th>
<th>Concepticulaires</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraphysoides</td>
<td>Phacidiostroma:</td>
<td>multivalve</td>
<td>Therrya fucelli</td>
</tr>
<tr>
<td>Paraphyses</td>
<td>Phytiaae:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lenticulaires</td>
<td>Rhytisma aserina: (hypodermales)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Lenticulaires</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to Bellacere (1969) *Phacidiostroma* and *Therrya* are true stromatic forms and possess paraphysoids; *Colpoma*, which is also a stromatic form possesses both paraphysoides and paraphyses; *Rhytisma* according to him possesses only paraphyses. In the genera *Propolis* and *Nemacyclus* the apothecia are concepticular in nature and possess both paraphysoides and paraphyses.
Cryptomyces muellcrl provided excellent material for detailed studies of its fundamental characters in view of its abundant availability in the forests of Coorg in all stages of development. Besides close and constant association of spermogonial locules with the developing asccarps was an interesting character, which needed further study and investigation into its nature and significance in the life cycle of this interesting fungus.

**Morphology and Early Development**

Transactions through infection spots at an early stage of development showed that the entire leaf tissue is invaded and permeated by septate mycelium which may be inter or intra-cellular and uni- or multinucleate (Fig. 16.1). These hyphae later grow upwards and concentrate themselves in the epidermal cells of the leaf tissue and produce pockets of pseudoparenchymatous cells through the process of continuous cell division. The stromatic body so produced increases in size intraepidermally and eventually breaks the epidermal cells in the centre in the process of development (Fig. 16.2). The central region of this intraepidermal pseudoparenchymatous stroma now becomes meristematic and is characterized by the occurrence of loosely arranged or interwoven prosenchymatous hyphal strands making up the central core with pseudoparenchymatous epidermal layers on the top and hypothecium at the bottom producing a palisade like structure (Fig. 16.3). The upper stromatic epidermal layer is continuously pushed up and elevated due to the rapid
intercalary growth of the prosenchymatous hyphal strands making up the centrum (Fig. 16.4). During the process of elongation and intercalary growth the prosenchymatous hyphal strands become thin and assume distinctive structure in the form of intertheccial threads which are attached at both the ends from the very beginning, characteristic of pseudo-paraphyses (Figs. 16.3, 16.4). The apical portion of the pseudoparaphysate hyphae forms the basal layer of the epithecium while the apical layer of cells of the pseudoparaphysate hyphae disintegrate due to lysis (Fig. 16.5). Thus the intertheccial filaments which are pseudoparaphysate in the early stages become apically free at full maturity and appear like true paraphyses (Fig. 16.7). At this stage of development, the ascocarp "centrum" is made up of three distinct regions (1) the thin basal hypophecium made up of hyaline pseudoparenchymatous cells, (2) the central region (the centrum) consisting of a palisade of hyaline apically free pseudoparaphyses and (3) the disintegrating fungal epithecial layer along with the upper epidermal cover consisting of pseudoparenchymatous cells (Fig. 16.6A). The entire origin and development of ascocarp is thus intraglisericular.

The development of young ascocarp is more rapid in the central region and symmetrical. The asci mature earlier in the central region of the hymenium and the interascal filaments become more elongated and thinner in this region giving the developing ascocarp a discoid shape (Figs. 16.5, 16.7, 16.8).
The ascocarp breaks open in the centre with the elevated marginal lobes of ruptured epidermis and epithelium thus completely exposing the hymenium (Figs. 16.5, 16.7, 16.8). The developing ascocarp is at various stages of differentiation towards the periphery with inter-thecial threads and asci at different stages of development. The stroma becomes progressively thinner towards the periphery where it tends to merge with the individual hyphae.

Thus the overall developmental pattern of ascocarp centrum in this fungus is similar to that reported by Jones (1925, 1935), Thyr and Shaw (1966), Gordon (1966, 1968), Bellemere (1967) and Woo and Partridge (1969) in various members of the Phacidiales.

**Sexual Reproduction**

The spermogonia: The spermogonia are minute black, oval to elongate, shining, generally intra epidermal in origin and develop prior to the formation of apothecia, although frequently both spermogonia and apothecia develop simultaneously in close association in the same infection spot (Figs. 14.2, 14.3, 15.6, 17.1 to 17.6). On the other hand many apothecia are also produced quite independent of spermogonia (Figs. 14.1, 14.4). The spermatiophores are dark brown in colour, sclerotioid, with thickwalled pseudoparenchymatous cells, arranged vertically in basal layers which in turn cut off from their ends successively numerous, rod shaped hyaline spermatia. These recepticles were entirely spermogonial in character and show no development of
ascogonia and trichogynes characteristic of asccarps as reported for *Lophodermium hysterioides* by Jones (1935) or for *Coccovyes hiemalis* (Backus 1933, 1934). The entire structure and the subsequent pattern of spermatization closely agree with that reported for *Rhytisma acerinum* by Jones (1925) for *Elainea thirumalachari* by Chiponker (1966) and for *Phyllachora symplocicola* by Jagtap (1968).

The Ascogonia and the Trichogynes: Microtome sections revealed the development of several multinucleate ascgonia provided with long flexuous trichogynes occurring within the asccarp primordium (Figs. 18.3, 18.4). Such trichogynes were seen to emerge through the ostioles of the ascgonial locules rupturing the epidermis in the process and standing out well above the epidermis of the host (Figs. 18.1, 18.2). The actual copulation of the spermatia with the protruding trichogynes (well known as "spermatia-trichogyne copulation") were observed in rare cases. Several nuclei were observed in the process of migration along the entire length of the trichogynes which was strongly suggestive of active process of spermatization which resulted in the dikaryotic status of the basal ascgonia (Figs. 18.3, 18.4). Such dikaryotic ascgonia give rise to several binucleate septate ascogenous hyphae the cells of which are directly converted into asci without the formation of croziers (Figs. 18.4, 18.5). On the other hand numerous apothecia were found to develop in the absence of "spermatia trichogyne copulation" in a manner...
described by Jones (1935) for Lophodermium pinastri. Such development could be explained on the basis of multiple infections by heterothallic strains of this fungus resulting in hyphal fusions (somatogamous copulation) ultimately resulting in dikaryotic ascogenous cells and ascogenous hyphae originating from such cells. Such hyphal fusions with attendant migration of nuclei and formation of dikaryotic ascogenous cells were observed by the writer in several of his preparations at the base of the pseudoparaphyses in a manner as reported by Gordon (1966) in Type I and Gordon (1966) in Type III (Fig. 16.6R). However, in no case the dikaryotic basal cells of the pseudoparaphyses directly gave rise to asci without the development of ascogenous hyphae.

Thus the entire sexual phenomenon in this fungus in which the asci originate as a result of "spermatid trichogynic copulation" on the one hand or hyphal fusions (somatogamous copulation) on the other hand has essentially the same pattern as described by previous workers like Brooks (1916), Higgins (1920, 1929), Brandtiff (1936), Dodge (1932), Drayton (1932, 1934), Jenkins (1930, 1938, 1939), Wolf (1943), Luttrell (1951, 1960), Shoemaker (1955), Jagtap (1969), Chiplonkar (1969), Ananthanarayanan (1970) and others for numerous species of Ascomycetes.

Summary

The above studies into and observations on the developmental pattern of the ascocarp in Cryptomyces muelleri Ell.
revealed that it is essentially a member of a stromatic Discomycetes characterised by unitunicate asci. The results so obtained during this study are summarised in brief.

1. The fungus produces bright crustaceous infection spots with uniloculate stroma which is intra-epidermal in origin.

2. The developmental pattern of the asccocarp conforms to the "pleospora type" as defined by Luttrell (1951) for ascolocular forms.

3. The interascocarp filaments are vertical hyphae have an intercalary growth are attached at both ends and complete their development long before the origin of asci behaving in this respect like pseudoparaphyses.

4. The asci are unitunicate without an apical apparatus and grow between the pseudoparaphyses.

5. The asccocarp development is typical of Type I described by Gordon (1966) for Lophodermium juniperinum (Fr.) de Not.

6. In the early stages the asccocarp is angiocarpic becoming hemiangiocarpic in the later stages as described by Corner (1929) for apothecial fungi.

7. The pattern of sexual reproduction is in the nature of spermatization with 'spermatia trichogyne copulation' in general and in rare cases this function is taken over by somatogamous copulation.
8. The ascii develop directly from the dikaryotic cells of the ascogenous hyphae without the intervention of "crosiers" or "hooks".

9. The interascal filaments are pseudoparaphysate in the early stage and paraphysate in the later stage with free ends.

10. The upper shield or pseudoepithecium is brought about through the process of the lysis of the apical stromatic cells thus exposing the hymenial layer.
Plate 16
(Figs. 16.1 - 16.8)

_Cryptomyces muelleri_ Ullas

Development of ascocarp

1. Section through the infection spot in the early stage showing intra- and intercellular nature of the mycelium and the aggregation of mycelia in the epidermal cells of the leaf tissue x 450.

2. Photomicrograph of a section showing the intracuticular nature of the young, developing ascocarp and its pseudoparenchymatous cells x 350.

3,4. Photomicrographs showing cross section of intracuticular ascocarp development and elongation of pseudoparaphyses. Note the attachment of intercellular threads at both the ends x 400, x 300.

5,6. Photomicrographs of sections through the developing ascocarps showing pseudoparaphyses and young asci. Note the ruptured epithecium and lysis of the apical region of the pseudoparaphyses x 400.

6B. Photomicrograph of a section through the ascocarp showing nature of pseudoparaphyses and the hyphal fusion at the base of pseudoparaphyses and nuclear status x 800.

7,8. Photomicrographs of sections through matured and ruptured ascocarps showing central hymenium and elevated marginal lobes consisting of host epidermis and epithecium x 250.
PLATE 16
Plate 17
(Figs. 17.1 - 17.6)

Cryptomyces muelleri Ullas

Spermogonia

1, 2. Photomicrographs showing sections through the linear spermogonia closely associated with the apothecia x 150.

3. Photomicrograph of a section through the spermogonium developing simultaneously above the ascomyx x 400.

4, 5, 6. Photomicrographs showing sections through the spermogonia developed independent of apothecia x 150, x 400, x 400.
Plate 18
(Figs. 18.1 - 18.5)

Cryptomyces muelleri Ullasa

Ascogonia and Trichogynes

1,2. Photomicrographs of sections showing ascocarp initials provided with basal ascogonia and long trichogynes protruding through the papillate ostioles rupturing the host epidermis. Note the (in Fig. 2) developing ascocarp below the papillate ascogonium x 400.

3. Photomicrograph of a section showing trichogyne and its multi-nucleate status x 650.

4. Photomicrograph of a section showing multi-nucleate condition of the long trichogynes. Note the septate and binucleate condition of the ascogenous hyphal cells developed in the ascocarp initials below the trichogynes x 650.

5. Photomicrograph of a section showing mature and origin of asci. Note the development of asci directly from the cells of the ascogenous hyphae without the intervention of croziers x 600
CHAPTER - III

NUCLEAR BEHAVIOUR, CHROMOSOME COMPLIMENT AND ORGANIZATION OF ASCOSPORES

Little is known about the cytology and mode of ascogenesis in members of this group i.e. Phasidiales except for the work of Jones (1925, 1935) in Lophodermium pinastri and Rhytisma acerinum and Woo and Partridge (1969) in Rhytisma punctum.

Nuclear events in the Ascus

The general trend of the nuclear events as they occur in the ascus of this fungus is indicated briefly to be followed by a detailed account on this phase which, it is hoped, will help in the proper understanding of the entire behaviour of the diploid nucleus and related nuclear events.

It has been already established that the sexual reproduction in this fungus is in the nature of spermatization or where the apothecia develop independent of spermogonia it is presumed to be somatogamous copulation leading to the dikaryotization of basal cells of the ascocarp initial followed by the production of dikaryotic ascogenous hyphae. The ascogenous hyphae are septate each cell of which contains conjugate nuclei (Figs.19.1, 19.2). This is followed by karyogamy in the dikaryotic cells of the ascogenous hyphae and the asci develop directly from such diploid cells without the intervention of crosiers (Fig.19.3). This is the only diploid stage observed in the entire life cycle.
of this fungus. The diploid nucleus in the ascus now undergoes three divisions beginning with a reduction division followed by two mitotic divisions resulting in the formation of 8-nuclei. The ascospore initials are recognized at 8-nucleate stage. Rarely a further mitotic division of the nuclei has been observed in the ascospore initials resulting in binucleate status of some of the ascospores.

**Behaviour of the Diploid Nucleus in the Ascus**

The two nuclei of the binucleate ascogenous cell fuse and the cell then elongates to form the ascus. As the ascus enlarges the diploid nucleus moves above and occupies a central position within the ascus. At this stage it measures 2 - 2.5 μ in diam. The fusion nucleus and the chromosomes making it increase greatly in size during early prophase, measuring upto 7 μ in diam. The maximum length of the chromosomes is attained at the leptotene of the meiotic phase. During this time the nucleolus is also observed to increase greatly in size (Figs. 19.5 to 19.7). At pachytene the chromosomes elongate and appear in the form of threads (Fig. 19.7). The nucleolus is large and deeply staining. During these stages, the chromosomes are not distinct enough to be counted. Following pachytene the bivalents contract into diplotene. Diplotene chromosomes show "Chiasmata" or "Crossing Over" phenomenon. The nucleolus usually disappears at this stage (Figs. 19.8, 19.9).

As the meiosis continued, the chromosomes contract. The maximum contraction is reached at Metaphase I, where they appear
as small dot-like bodies and five bivalents could be clearly counted (Fig. 19.10). Sometimes, the five bivalents are seen as five pairs of separating but still attached univalents at late metaphase or at early anaphase due to the occurrence of precocious division and non-synchronized disjunction of chromatids (Fig. 19.11). Two rod-shaped centrioles were observed at late anaphase or early telophase located at each pole of the spindle (Fig. 19.12). Immediately after this stage the meiotic diads constitute the two nucleate stage of the ascus (Fig. 20.1).

The resulting two nuclei occupy the upper position of the ascus little above the central region (Figs. 20.1, 20.2). It is apparent from the position of the two nuclei in the ascus that the spindle orientation is slightly oblique to the main axis of the ascus (Fig. 20.1). The resulting two nuclei may lie one below the other in oblique manner or they may be side by side in a parallel manner (Figs. 20.1, 20.2). If the two nuclei are obliquely oriented one below the other the resulting four nuclei will also have a linear position obliquely oriented in the ascus lying one below the other (Fig. 20.4) and later after the 3rd division giving rise to uniseriate arrangement of the eight ascospores in the ascus (Fig. 20.8). On the other hand if the meiotic diads lie parallel side by side (Fig. 20.2), they result in four nucleate stage lying two, below the other two (Fig. 20.3) and ultimately resulting in the irregular (Fig. 20.5) or biseriate (Fig. 20.7) arrangement of the ascospores in the ascus.
The second and the third divisional stages were not seen clearly. Beaked nuclei were frequently observed at 2, 4 and 8 nucleate stages (Figs. 20.2, 20.4, 20.5, 20.6) as reported in the case of Stephemia shanori (Ucker 1967), Neurospora dodgi (Nelson and Backus, 1968), Hypoxylon fusum (Rogers 1965), Rhytisma punctum (Woo and Partridge 1969), Ascobolus stercorarius (Wells, 1970). Similar beaked nuclear stages have been figured by Jones (1925, 1926, 1935) in Rhytisma acerinum, Ophiobolus graminis and in Lophodermium pinnastri.

**Ascospore Organization**

After the completion of the 8-nucleate stage cytoplasm cleavages appear around each spore initial (Figs. 20.5, 20.6). Light microscopic studies did not reveal any details of the actual mechanism followed in ascosporogenesis, though at early stages, condensation of cytoplasm followed by cytoplasmic cleavages were seen around each nuclei which help in organization of ascospores as reported by Jones (1925, 1926), Jenkins (1934), Heim (1952), and Hayman (1964). Such observations were considered by Reeves (1967) to be 'post facto' who working with *Pyronema domoticum* demonstrated through electron microscopy the organization of an ascus vesicle, composed of two unit membranes surrounding the 8-nuclei prior to ascospore delimitation. According to Reeves (1967) "ascus vesicle" composed of two membranes was responsible for initiating ascosporogenesis and ultimate organization of the ascospores in *Pyronema domoticum*. However, such observations are beyond the light microscopic studies.
The mature spore is elliptical to spindle shaped and is uninucleate, smooth walled and hyaline (Fig. 20.6). Occasionally two nucleate ascospores were also seen each of the two nuclei being situated at the opposite poles of the ascospore (Fig. 20.9).

The ascospores occupy less space in the large ascus and each spore is delimited by a protoplasmic pseudoseptum in the ascus (Fig. 20.8).

Summary and Conclusions

1. There is only a single nuclear fusion in the dikaryotic ascogenous cell followed by only a single reduction division in the ascus which conforms to single fusion theory or 'clausen type.'

2. Disjunction of the chromatids is precocious and non synchronized.

3. The chromosome complement has been determined as n=5 haploid.

4. The spore delimitation takes place at 8-nucleate stage through the mechanism of "free cell formation".

5. The 'ascus vesicle' mechanism described by Reeves (1967) was not noticed in this fungus.

6. The results have been substantiated by a series of photomicrographs.
Plate 19
(Figs. 19.1 - 19.12)

Cryptomyces muelleri Ullasè

Cytology

1, 2. Photomicrographs showing dikaryotic cells of the ascogenous hyphae x 1000.

3. Photomicrograph showing diploid cell of the ascogenous hyphae developing directly into ascus without the intervention of croziers x 100.

4. Photomicrograph of young asci showing diploid conditions x 1000.

5-7. Photomicrographs showing pachytene stages of the Prophase I. Note the thread-like chromosomes and prominent nucleoli x 2000.

8, 9. Photomicrographs showing diplétene stage of Prophase I. Note the absence of nucleolus and crossing over, contraction of the individual chromosomes x 1000.

10. Photomicrograph showing Metaphase I. Note the five bivalents in the form of five dots. x 1000.

11. Photomicrograph showing late metaphase or early anaphase. Note the precocious division x 1000.

12. Photomicrograph showing late anaphase or early telophase. Arrow indicates the centrioles at either pole x 1000.
Plate 20
(Figs. 20.1 - 20.9)

Cryptomyces muelleri Ullasa

Cytology.

1. Photomicrograph showing two nucleate stage of the ascus. Note the linear but slightly oblique orientation of the nuclei to the main axis of the ascus x 1000.

2. Photomicrograph showing two nucleate stage of the ascus. Note the parallel orientation and beaked conditions of the nuclei x 1000.

3. Photomicrograph showing four-nucleate stage of the ascus and position of the nuclei situated two below the other two x 1000.

4. Photomicrograph of four-nucleate stage of the ascus showing oblique orientation, beaked conditions and linear arrangement of nuclei in the ascus x 1000.

5. Photomicrograph showing eight-nucleate stage of the ascus. Note the beaked conditions of some of the nuclei x 1000.

6,7. Photomicrographs showing eight-nucleate stages of ascii. Note the biserial arrangement and ascospore initiation through cytoplasmic cleavage x 1000.

8. Photomicrograph of mature ascus showing uniseriate arrangement of eight ascospores and protoplasmic pseudo-septation around each spore x 800.

9. Photomicrograph showing binucleate condition of the ascospore x 11000.
PART III

TAXONOMIC STUDIES IN SOME INDIAN ASCOMYCETES
INTRODUCTION

The ascomycetous fungi with richness of their pattern, morphology and highly heterogenous nature comprising pathogens as well as saprogener occur on different parts of host plants and are widely distributed all over the world and are recognised through their chief characters i.e. the asci, which in higher forms are borne in various types of fructifications like oleistothecla, perithecia, apothecia, pseudoathecia, thyrothecia, discothecia, hysterothecia etc. Although large number of species have been enumerated from different parts of India in the form of regional lists, diagnostic descriptions are available in comparatively few species. The main contributions being of Uppal, Patel and Kamat (1935), Uppal, Patel and Bhide (1949), Sanwal (1953), Ramakrishnan (1953, 1956), Thind and his co-workers (1957-1970), Chona, Munjal and Bajaj (1958), Viswanathan (1959), Beta (1960), Govindu and Thirumalachar (1960), Bose (1961, 1962), Kapoor and Gill (1961), Tilak (1958, 1969), Ananthanarayan (1964), Bose and Mueller (1964, 1965, 1967), Patil and Thirumalachar (1965), Patwardhan (1966), Ramachandra Rao (1966), Seshadri (1967), Wuthappa (1967), Chiponkar (1969), Pande (1969), Anahosur (1969, 1970), Korf and Warnitch (1971) and others.

During his mycological survey carried out at the forests of Coorg (Mysore State) and in and around Poona (Maharashtra) the
writer collected several Ascomycetes, a detailed study into the diagnosis and determination of which revealed that some needed taxonomic revision, some were found to be new species, some in the nature of new host records and new reports to India based on critical comparative studies with and examination of authentic herbarium material wherever necessary.

The Coorg Forests of Mysore State, India situated at an altitude of 4,000 ft. above sea level with an average annual rainfall of 150 inches are rich in different types of evergreen vegetation and thus provide favourable conditions for the development of various fungi and therefore provided an excellent opportunity to the writer to study the prevailing fungus flora with special reference to the Ascomycetes. These forests have not been much exploited by the Indian Mycologists except for the recent brief mycological survey carried out by Ramakrishnan (1956), Muthappa (1967), Anahosur (1969) and Rangaswami et al. (1970). The writer, therefore, undertook a more detailed and comprehensive survey of these forests during the period of 1967-1970, with the object of studying the fungus flora of this locality with periodical visits to these forests in different seasons of the year, and collected several Ascomycetes and Deuteromycetes. Hand sections of the fungus collections with different stages of development were employed by the writer to study their morphology, structure and 'centrum' characters, which were helpful in determination and identification of this highly heterogenous group of fungi.
A brief account of the diagnostic characters of the Ascomycetes of Coorg and some collected from in and around Poona based on their Camera Lucida drawings drawn from the hand sections have been presented in this part with photomicrographs wherever necessary. The genera are arranged in alphabetical order.

1. Achorella Theiss. & Syd.


F. Venturiaceae

O. Dothioroles

Sub-Class Ascoloculares

The genus Achorella was established by Theissen and Sydow in 1915 with Achorella ambethyla (Rehm) Theiss. & Syd. as type based on Dothidiella ambethyla Rehm. The genus is characterized by dothidiaceous stroma provided with hyaline to sub-hyaline, 2-celled ascospores. On comparative studies, writer's collection was identified as Achorella plectroniae T.S. & K. Harm., which is described here.

Achorella plectroniae T.S. & K. Harmr. (Fig. 21.1)


Stromata dark, erumpent, amphigenous, isolated or crowded, sub-epidermal in origin. Locules separate, half immersed in a stroma, with dark wall, oval to globose, ostiolate, 200 x 150 μ in diam. Asci clavate, hyaline, paraphysate, with a round apex, and with a short narrow foot, 70-90 x 14-18 μ. Ascospores 8,
fusoid, tapering towards the ends, one septate, light yellowish to hyaline 40-50 x 3-4 µ.

**Habit:** Infecting the living leaves of *Canthium dicoccum* (= *Plactroniae didymae*) collected at Coorg District of Mysore State, in October 1968, M.A.C.S. Herb. No. 1173.

**Remarks:** An undescribed species of the Sphaeropsidaceae genus *Hemidothis* Sydow. was closely associated with the fungus as originally reported by Ramkrishnan and Ramkrishnan (1948), although many times without its association with the ascigerous state. A collection made from Mahabaleshwar revealed that no ascigerous state was present in the entire collection.

Comparative studies between *Hemidothis* of Ramkrishnan's collection HCIG No.19790 and *Phlyctaena canthicola* Seshadri, M.A.C.S. Herb. No.241 revealed that they are identical and the new combination needed is proposed as follows:

**Hemidothis canthicola** (Seshadri) Comb. nov.

**Basionym:** *Phlyctaena canthicola* Seshadri Mycopath et Mycol Appl. 30 : 181 (1966)

**Holotype:** M.A.C.S. Herb. No. 241.

**Remarks:** *Achorella plactroniae* is a new addition to the fungal flora of Coorg District.


F. Hemiphacidiaceae
C. Felotiales,
Sub-class Ascochyumeniales.
An apothecial tar spot fungus parasitizing leaves of Ficus tjakela Burm. was collected by the writer from Coorg forests, Mysore State, India with the following characteristics. Apothecia thin, sub-cuticular, with little basal stroma, becoming erumpent at maturity and dehiscing irregularly resulting in marginal cuticular lobes or central cleft exposing the central hymenium, epithecium lacking, asci in basal layers, eight spored, unitunicate, thick-walled, paraphysate, ascospores globose, dark brown, single celled.

These characters are typically hemipachidiaceous and have not been duplicated in any of the known genera belonging to this family and hence the writer's collection is presented as a new genus with A. indica as the type species.

Arxonia Kamat & Ullasa Gen. Nov. (Figs. 21.2 to 21.5)

Apothecia thin, subcuticular, erumpent, dehiscos irregularly with marginal cuticular lobes or circularly with a central cleft. Ascii unitunicate, thick-walled, hyaline, cylindrical, persistent in parallel hymenial layer, octosporous. Ascospores single celled, globose, dark brown, thick walled, uniseriate to bisericate. Paraphyses thin, filiform, clavate, septate and branching.

Type species Arxonia indica.

Arxonia indica Kamat & Ullasa sp. nov.

Infection spots dark, scattered or crowded along with the midrib, circular sometimes becoming irregular at maturity,
epiphyllus 0.5-3.5 mm in diameter. Stroma monoapothecial. Thin subcuticular in origin becoming erumpent, dehiscing irregularly at maturity resulting in marginal lobes or circularly with a central host cleft. 600-1200 x 130-160 µ.

Asci narrow, cylindrical to clavate, unitunicate, thick-walled, hyaline, persistent, paraphysate, in parallel hymenial layer, octosporous 100-120 x 8-12 µ. Ascospores single, globose, dark brown, thick-walled uniseriate, 5-8 µ in diameter. Paraphyses thin, filiform, clavate, septate, often branching, epidermal layer lacking.

Holotype: On living leaves of Ficus takela Burm. Nallur, Coorg (India) collected on 15-6-1960, M.A.C.S. Herb. No.1166 (Type).

Discussion: The fungus is closely allied and comparable to the apothecial genera Eupropolella Hoehn., Pseudopeziza Flk., Leptotrochila Karst. and Criella Sacc. The genus Eupropolella has septate ascospores while the other two are characterised by hyaline ascospores. The position with regard to the genus Criella Sacc. is on a different footing. The genus Criella was established by Saccardo for an apothecial fungus affecting Symplocus sp. but has subsequently been rejected by von Hohm el as no type material of this fungus was available. The name, therefore, becomes invalid. (Personal communication from Dr. J.A. von Arx). Thus the Coorg collection differs from all the four above genera in having single celled, dark coloured ascospores. These characters have not been duplicated in any other known hemiphasidiaceous genera. The writer's collection,
therefore, would need accommodation as a new genus. Reid
and Cain (1962) described a new genus *Nothophacidium* infecting
*Abies baalsiae*, based on *Phacidium abietinellum* Beattie which
possesses similar type of asci and ascospores but differs in
having typically cupulate apothecia with well defined
exciplum. This fungus is considered as a member of
Hemisphaeriaceae since it has very poorly defined basal stratum
and completely lacks epithelial tissue, and excipulum as
defined by Korf (1962).

3. *Bagniella* Speg. Fungi Argent., 111, 22 1880

F. Myriangiales

G. Myriangiales

Sub-Class Ascoloculares

The genus *Bagniella* was founded by Spagazini (1880) with
*Bagniella australis* Speg. as the type, collected on *Acacia
bonariensis* Gill. Arx & Mueller (1954) have placed this genus
in Botryosphaeriaceae of Dothiales, where as Luttrell (1955)
is of the opinion that it occupies an intermediate place between
Myriangiales and Dothiales. The writer's observations show
that in *Bagniella*, the asci develop amongst pseudoparenchymatous
cells which are persistent and take the appearance of inter-
thecial tissues. The asci may be considered as in the nature of
"uniascal locules" as in the genus *Myriangium* in which they are
scattered unlike in *Bagniella* which shows parallel
arrangement of asci with the developmental pattern of ascocarp
similar to "Eisinoe" type and hence the genus may be placed
under Myriangiales.
Tilak (1963), Seshadri (1967), Ramachandra Rao (1966b, 1967a) have described several species of this genus from India.

The writer's collection of Baganiella has the following characters. This fungus was saprophytic on barks of Mangifera indica and was found to agree with Baganiella mangiferae in all respects.

Baganiella mangiferae Tilak & Rao (Fig. 21.6)

Ascospora black, circular to irregular, erumpent, multi-loculate, up to 1.1 mm. long, with 3-5 compartments and non-ostiolate. Asci cylinbro-clavatus, representing a locule, bitunicate, pedicellate in palisade layers, octospores 88-100 x 18-24 μ. Ascospenes fusoid to cylindrical, thick-walled, hyaline to sub-hyaline, irregularly biseriate, 1-celled, 16-25 x 8-12 μ. Only inter-tissueal tissues are present in between 2 asci i.e. locules.


Remarks: No species of Baganiella has been reported from Coorg District, Mysore State, India.


Syll. Fung. 8 : 738 (1889)
F. Parmulariaceae
O. Dothiorales
Sub-Class Ascoloculares
The genus *Cocconia* was established by Saccardo (1889) with *Cocconia placenta* as type from Ceylon infecting living leaves of *Symplcuas spicata* Boxb. The writer's collection was identified as similar to the type species a brief description of which is given below since it was not previously reported among Indian Fungi.

*Cocconia placenta* (Berk. & Br. ) Sacc. (Fig. 21.7)


Infection spots circular, dark, amphigenous, scutellum radiate, ascostroma attached at the centre and sometimes radially at the other places, 1-6 mm. in diameter and 250-300 μ in thickness.

Locules linear and radiate, 800-1000 x 300-400 μ. Ascii club-shaped bitunicate, parallel in basal layers, pseudoparaphyses present, 80-85 x 30-35 μ. Ascospores 8, roughly distichous, dark-brown, 2-celled, equally septate, deeply constricted at the septum both the cells are round to slightly oval and often get separated 30-38 x 15-17 μ.

Habit : On living leaves of *Symplcuas spicata* Boxb. collected at Mercara, Coorg, Mysore State, 15th May 1969, M.A.C.S.Herb. No. 1175.

Remarks : This is a new generic record to India.

   F. Diaporthaceae
   O. Sphaeriales
   Sub-Class Ascohymeniales
A leaf spotting fungus infecting the living leaves of *Celastrus paniculata* Wild was collected from Khandala (Maharashtra State) with the following characters:

Perithecia, innate, single, non-stromatic placed diagonally or horizontally with long oblique neck with the papillate ostiole in the leaf mesophyll. Ostiole periphysate, non-scyphate. Ascii in wall layers, gelatinizing at maturity, provided with thick apical canal and apparatus. Ascospores hyaline, spindle shaped unequally 2-celled with the septum at the distal end.

Discussion: A critical perusal of literature revealed that the present fungus has some resemblance with the following discomycetous genera (1) *Muelleromyces*, Kamat and Anahosur, (2) *Pseudothia* Theiss. & S.d. (3) *Anisomyces* Theiss. & Syd., (4) *Savulescua* Petr., (5) *Plagiostigma* Syd., (6) *Plagiostoma* Fuck., (7) *Plagiostomella* Fohn. The first three genera differ from the present fungus in having dark coloured ascospores. The genus *Savulescua* has equally two celled hyaline ascospores. *Plagiostoma* and *Plagiostomella* also have hyaline 2-celled ascospores but differ from the present fungus by the fact that their ascii gelatinise from the perithecial wall at an early stage and lie free in the perithecial cavity and further their ascospores are septate either in the middle or towards the lower end.

The present fungus has some similarities with the genus *Plagiostigma*. Ascospores are hyaline in both the genera but are septate at the middle or towards the lower end in *Plagiostigma* whereas in the present collection it is towards the upper end.
The ascospores in *Plagiostigma* are also provided with hyaline appendages. The other important character which distinguishes it from *Plagiostigma* is the presence of dark perithecial wall. From the discussion it is clear that these characters are not duplicated in any of the known diaporthaceous Ascomycetes. Therefore the present collection is accommodated in a new taxon named after Dr. Chadefaud, Director of the Institute of Mycology, Paris (France) for his outstanding contributions to our knowledge of Ascomycetes.

*Chadefaudia Kamat & Ullas* Gen. Nov. (In Press) (Figs. 22.1 to 22.2)

Perithecia non-stromatic, separate, innate, lying diagonally or horizontally with a long neck provided with an ostiole, brown, non-clypeate. Asci unitunicate, with a thick apical canal and apparatus, gelatinizing at maturity, octosporous, aparaphysate. Ascospores thin walled, hyaline, unequally 2-celled, septum at the distal end.

**Type species** *Chadefaudia indica*

*Chadefaudia indica* Kamat & Ullas sp. nov.

Infection spots brown, circular, amphigenous 0.5-1.0 mm. in diameter. Perithecia brown, non-clypeate, separate, oval to oblong, innate, lying diagonally or horizontally in the leaf tissue, provided with a long neck, ostiolate, ostiole periphysate, 130-160 x 100-130 μ. Perithecial cells brown made of 2-3 layers of polygonal cells of 8-12 μ. Neck 100-130 μ long. Ascii cylindrical, thick-walled, with prominent shining apical...
apparatus and an apical canal, unitunicate, pedicellate, gelatinizing at maturity, octosporous, hyaline, non-paraphysate, 60-80 x 8-13 µ. Ascospores oblong hyaline, unequally 2-celled, septum at the distal end, non-constricted at the septum, rounded at the apex, uniseriate or irregularly distichous 10-13 x 5-6 µ.

Habit: Infecting living leaves of Celastrus paniculata.

6. Daldinia de Not.
F. Xylariaceae
O. Sphaeriales
Sub-Class Ascohyphales

The genus Daldinia founded by Cesati and de Notaris in 1847 with Daldinia concentrica as type. Xylariaceous fungus is characterized by bulbous stroma provided with perithecia all along its outer surface. In section this fungus shows characteristic concentric rings.

Writer's collection completely agrees with the type species in all respects, a brief description of which is presented here.

Daldinia concentrica (Bolt) Ces. et de Not. (Fig. 22.3)

Stroma grayish to brown, 2-10 mm. in diameter, bulbous or globose, Perithecia obpyriform, long, ostiolate, 500-700 x 300-400 µ. Asci unitunicate, hyaline, cylindrical with a long stipe, paraphysate in wall layers, 80-120 x 10-14 µ. Ascospores
dark, oval to ellipsoidal, thick-walled, single celled, uniseriate 16-20 x 8-10 μ.


Remarks : This is a new addition to the fungal flora of Coorg District.


F. Diaporthaceae,
C. Sphaeriales
Sub-Class *Ascochromenales.

A leaf spotting fungus parasitizing leaves of *Eugenia jambolana* Lamb producing cankerous spots was collected by the writer with the following characters.

Perithecia non-stromatic, with oblique neck provided with slightly protruding ostioles. Asci octosporous, unitunicate, paraphysate, provided with apical apparatus, pedicel gelatinizing at maturity. Ascospores brown and equally 2-celled. The fungus was closely associated with another pycnidial fungus viz. *Mycohyphallage* Sutton in the same infection spots.

Critical perusal of the literature revealed that this fungus has received different treatments by previous workers. Ramakrishnan et al. (1953) reported it as *Didymosphaeria jambolana* collected by them from Madras on *Syzygium cumini*.
characterized by brown coloured 2-celled ascospores. Nanthanarayanan (1963) described an ascomycete collected on the same host as *Plagiostigme deodaricr* based on his collections made from Mhabeleshwar (Maharashtra), which he described as having 2-celled but hyaline to subhyaline ascospores. Ullas (1970) proposed a new combination for this fungus as *Plagiostigme lambolana* after critical examination of the type materials. Writer's critical comparative studies of several collections of this fungus revealed that the previous workers apparently dealt with the same fungus at different stages of development and maturity. This fungus produces equally 2-celled dark brown ascospores as against unequally 2-celled hyaline ascospores as reported for *Plagiostigme* Syd. Since it produces dark brown equally 2-celled ascospores its present treatment under the genus *Plagiostigme* Syd. appears to be incorrect. The genus *Plagiostigme* Syd. is characterised by having hyaline, unequally 2-celled ascospores with an apical appendage at either end (Clements and Shear 1931, Mueller and rx, 1962, Petrak, 1965).

On the other hand this fungus has some resemblance to the allied *Pseudothidiosporous* disporothacous Ascomycetes like *Anisomyces* Theiss. & Syd. *Muelleromyces* Kamat & Anahosur, *Pseudothidiosporous* Theiss. & Syd. But it differs from *Pseudothidiosporous* and *Anisomyces* in having equally two celled ascospores and its non-stromatic nature. *Muelleromyces* which is devoid of stroma differs from it in having vertical ostioles and unequally 2-celled ascospores.
The present fungus resembles the genus Savulescua Petrak in inciting cankerous leaf spots and in having equally two-celled ascospores but differs in having non-stromatic perithecia, 8-spored asci and brown ascospores.

Thus the present fungus is quite distinct from any of the known members of the family Diaporthaceae and justifies its accommodation as a new genus.

Deshpandella Kamat & Ullasa Gen. Nov. (Figs. 23.1 to 23.4)

Perithecia non-stromatic, globose, produced in groups, innate, with long oblique or curved ostioles, periphysate with indistinct clypeus. Asci unitunicate, with thick apical apparatus provided with an apical canal, pedicellate, pedicels gelatinizing at maturity, octosporous, paraphysate, ascospores thin-walled, brown, equally 2-celled.

Type Species Deshpandella jambolana


Basionym : Didymosphaeria jambolana Ramskr. et al.


Holotype : On Syzygium cumini HCIO No. 20148

Synonym : Plagiostigma deodikari ‘namt.


Plagiostigma jambolana (Ramskr. et al.) Ullasa

The ascomycetous genus here in newly erected is named after Dr. R.S. Deshpande, Professor and Head of the Dept. of Mycology and Plant Pathology, College of Agriculture, Bharwar and now Deputy Director of Agriculture (Research), University of Agricultural Sciences, Bangalore at Mudigere, Chikamagalore District, Mysore State.

Deshpandella jambolana (Ramkr. et al.) Comb. Nov.

Infection spots epiphyllous, cankerous, raised, scattered often aggregated, waxy 0.5-1.0 cm. Ascoecyta ovoid, non-stromatic, separate produced in groups of 2-3 provided with a curved neck, ostiolate, ostiole paraphysate and indistinctly clypeate, 200-450 μ in diam. Ascii unitunicate, paraphysate, cylindrical, with a short pedicel, 8-spored provided with a prominent ascospore apparatus and apical pore, gelatinize at maturity, 150-180 x 17-18 μ. Ascospores dark brown, thin-walled, equally 2-cellul, deeply constricted at the septum, with round ends, spindle shaped, 25.5 - 26.0 x 13 - 15.5 μ.

Remarks: A pycnidial fungus Mycohyphallae Sutton was closely associated with this fungus (Figs. 23.5 to 23.6).

8. Didymosphaeria Fuckel Sym. Myc. 140 (1869)

P. Pleosporaceae
Q. Pleosporales

Sub-Class Neoascomycetes

The genus Didymosphaeria was established by Fuckel in 1869. The Lectotype is D. futilla (Berk. and Broo) Hehn. Clements and Shear (1931) have included this genus under Sphaeriales
whereas Mueller and Arx (1962) placed it in Pseudosphaerales, Luttrell (1955) has included it under Didymosphaeriaceae of Pleosporales. About 300 species of this genus are distributed all over the world as saprophytes and parasites.

In India the main contributions being those of Ramakrishnan et al. (1953), Muthappa (1967) and Tilak (1967).

The writer's collection mycoparasitic in nature on a Phyllachoraceous fungus was identified as Didymosphaeria cocconiae von Arx on the basis of nature, habit and dimensions which is described here.

Didymosphaeria cocconiae von Arx (Fig. 22.4)
Sydowia 12: 401 (1958)


Remarks: This fungus has been earlier reported from Ceylon and Philippines as a mycoparasite on Cocconia spercaria.
parasitizing Artocarpus integrifolia L. This is the first report of its occurrence from India.

9. Hypoxylon Bull. ex Fr.
   Champ. France 1, 168 (1791)-
   Summa vig Sound 363 (1849)
   F. Xylariaceae
   C. Sphaeriales
   Sub-Class Ascomycetes

   The genus Hypoxylon was established by Bulliard (1791) and subsequently revised by Fries in 1849. The type species is Hypoxylon cookeanum Bull. Several species have been reported from different parts, the main contributions being those of Ellis and Everhart (1887), Miller (1930, 1932, 1957, 1961). Rogers (1967) has studied the developmental pattern of the ascocarps in H. fuscum and considered it to be of "Xylaria" type.

   Recently the conidial stage of Hypoxylon has been described by Greenhagh and Chester (1968), Chester and Greenhagh (1964) and Jong and Rogers (1968) as Isaria, Peniculosporium, Nodulosporium or Acrostaphylus all belonging to Moniliales.

   The writer's collection has been identified as Hypoxylon archeri Berk. a brief description of which is presented here.

Hypoxylon archeri Berk. Fl. Tasm. 2:260 (1860) (Fig. 22.5)

   Stroma dark, grey, aggregate, each stroma contains 6-12 perithecia. Perithecia globose to sub-globose, ostiolate, with a thin wall, in a thick hard, carbonaceous stroma, 200-400 x 150-250 μ. Asci unitunicate, cylindrical to clavate, in wall
layers with a long stipe, paraphysate, 150-200 x 4-6 μ.
Ascospores ellipsoid, dark brown, thick walled, uniseriate, 1-celled provided with an elongate germ slit, 10-12 x 4-6 μ.


Remarks: Hypoxylon archeri is a new addition to the Indian fungi.

10. Lophomerum Ouellette & Magasi

Mycologia 58 : 275-280 (1966)

F. Hypodermataceae

O. Thacidiales

Sub-Class Ascohyphiales

The new genus Lophomerum was established by Ouellette and Magasi (1966) for those apothecial fungi which have septate filiform ascospores retaining the original genus Lophodermium for those members having unicellular filiform ascospores. Tilak (1959) described two species of Lophodermium of which one species viz. L. agharkarii has been transferred to this new genus Lophomerum by Darker (1967).

Lophomerum kamati sp. nov. (Figs. 22.6 to 22.7)

Apothecia hysteroid, dark, epiphyllous erumpent, aggregate, fleshy, opening by a longitudinal slit 80-90 x 40-44 μ. Asci numerous, cylindrical in palisade layers, paraphysate, unitunicate, rounded at the apex 78-84 x 4-6 μ. Paraphyses thin,
filiform, hyaline, septate as long as or even longer than asci. Ascospores 8, hyaline, needle shaped, uniform in width, 3-5 septate, 75-80 x 1-1.5 μ.


Remarks: Maranta arundinacea L. is a new host for this fungus.

11. Muellerosmyces Kamat and Anahosur, Apoud Anahosur

Experientia 24 : 849 (1968)
F. Diaporthaceae
O. Sphaeriales
Sub-Class Ascomycotinales

The new genus Muellerosmyces was established by Kamat and Anahosur for a diaporthaceous didymosporous Ascomycete infecting living leaves of Syzygium cumuni (L.) Skeels with Muellerosmyces indica as type.

Later the infected leaves of S. cumuni were collected by the writer, which on microscopic examination revealed the presence of two closely associated fungi (Figs. 24.1 to 24.6). One was identified as Muellerosmyces indica Kamat & Anahosur (1968) (Fig. 24.6), the second one showed close similarities with the pycnidial fungus Kamatella indica Anahosur (1969) (Figs. 25.3, 25.4, 25.6). During an attempt to identify these fungi on Syzygium cumuni, however, reports of two previously described pycnidial fungi were encountered, namely Botryodiplodia variispora (Died.) Zambatakis (Zambatakis 1954), and
Diploidea longipedicellata T.S. & K. Ramakr. (Ramakrishnan and Ramakrishnan 1950) infecting the same host genus. Subsequent studies proved that although these two fungi had received different treatments, by previous workers (Diedicke 1916, Zambatakis 1954, Ramakrishnan and Ramakrishnan 1950, Anahosur 1968, 1969) and were described under different names, essentially the same fungi were involved. Hence the critical comparative study of the four type materials was undertaken together with the recently collected material.

Material examined

1. Diploidea varispora Died. in H. & P. Sydow & F.J. Butler (1916), Fig 251. Host: Syzygium cumini (L.) Skeels (= Eugenia jambolana Lam.) Holotype: Butler No. 1775 at HCl, New Delhi, India.


Observations and discussion

Except in the case of Diplodia longipedicellata infecting Syzygium montana where only the pycnidial state was observed the other three showed close association of pycnidial and ascigerous states in the same infection spots. Diedicke (1916) in describing Diplodia variispora clearly illustrated (in his Fig. 1, p.196) two types of spores which was the basis for the specific epithet "variispora". From comparative studies, however, it was quite clear that of the two types of spores noted by Diedicke, the first type was, without doubt, ascospores of Muelleromyces and the second type of spores represented pycnidiospores of Kamaratella. It was, therefore, suspected that the ascospores of Muelleromyces and pycnidiospores of Kamaratella were mixed up in the material examined by Diedicke (1916), probably because of the evanacent nature of the asci and ascospores of the former, and close association of these two fungi. Thus Diedicke believed that the two types of spores which are morphologically similar belonged to a single fungus and accordingly included both under Diplodia variispora mistaking the 2-celled brown ascospores for pycnidiospores of Diplodia (Figs. 24.2 to 24.4). Since, however, it is now discovered that the fungus was in reality mixture of ascospores of Muelleromyces (Figs. 24.5 to 24.6) and in part the pycnidiospores of Kamaratella (Figs. 25.3, 25.4, 25.6), these two fungi described under here single name needs segregation into their proper taxonomic places leading to a nomenclatural revision.

First segregate of Diplodia variispora: This includes the fungus with the first type of spores described by Diedicke (1916). This
fungus is an ascomycete identified as *Muelleromyces indicus* and not a species of *Diplodia*. However, according to the International Code of Botanical Nomenclature (Article No.10, 59 vide Langouw et al. 1956; Rogers 1948; Korf personal communication), since the specific epithet "variispora" was based on the material (Butler No.1775) with "perfect stage" (ascosposes) this epithet gains priority over the epithet "indicus". Accordingly the following combination is proposed and a brief description is presented.

*Muelleromyces variispora* (Died.) Ullas Comb. Nov. (Figs. 24.1 to 24.3, 24.5, 24.6)

Basionym: *Diplodia variispora* Died (1916)

Synonym: *Botryodiplodia variispora* (Died) Zambatakis (1954)

*Muelleromyces indicus* Kamat and Anahosur in Anahosur (1965)

Infection spots black, circular in the beginning, becoming irregular later, epiphyllous, 1-1.5 x 0.5 mm. Perithecia non-stromatic, separate, innate, with a well developed beak and highly developed clypeus, ostiolute, globose to conical, 210-260 μ diam. Perithecial wall light brown, 2-3 layers thick, 8-12 μ in diam. Clypeus black, highly developed, made up of thick walled cells, 20-30 μ thick. Beak 100-130 μ long. Asci cylindrical, thick walled, highly thickened at the apex, with apical canal, bulged at the base, unitunicate, in wall layers, with short pedicels gelatinizing at maturity and lying free in the perithecial cavity, octosporous, aseparophyse, 145.5-170 x 16-20 μ. Ascospores oblong, dark brown at the tips and sub-hyaline in the centre, unequally 2-celled, non-constricted at
the septum, rounded at the ends, uniiseriate, to bisieriate, 16-20 x 4-8 μ. Paraphyses lacking, ostiolar periphyses abundant, filiform, slender, hyaline and facing upwards.


Second segregate of Diplodia variispora: This includes the fungus with the second type of spores described by Diedicke (1916). This fungus agrees with the type material of Kamatella indica and Diplodia longipedicellata. Since, however, the two species of Diplodia were described earlier, the specific epithet of Kamatella loses its validity. The choice of epithet for the new combination now would be either "variispora" or "longipedicellata" since, however, the epithet "variispora" is preoccupied for the earlier combination of Muelleromyces and since the genetic connection between the two closely associated fungi has not been proved, the epithet "variispora" is not available leaving the epithet "longipedicellata" as a valid and available epithet for the present fungus. Accordingly the new combination is proposed. The original description of Kamatella Anahosur (1969) also needs amendment, since the type material of this fungus presents several significant characters not reported earlier.

Emend Ullasaa (Figs.25.3, 25.4, 25.6)

Pyochidia ovoid to globoid, non-stromatic, innate, distinctly ostiolar, ostiole elliptical, non-papillate, conidiophores simple, non-septate, hyaline, thin, cylindrical, in wall layers, conidia
dark-brown, unequally 2-celled, napiform, with 2 circular hyaline zones on either side of the upper cell. Conidia develop singly through direct transformation of the apical portion of the conidiophore as "gangliospores".


Synonym: Kamatella indica Anahosur (1969)

A collection of Kamatella made on Syzygium caryophyllaeum from Coorg by the writer was found not associated with the ascigerous state Muelleromyces Kamat and Anahosur. The pycnidiospores differed slightly from the original species in having long peg like sub-cell with a rounded end. It produces silvery gray infection spots on the leaves (Figs. 25.5, 25.7, 25.8). This fungus is described here as a new variety of Kamatella longipedicellata on the basis of long sub-cellular characters besides being collected on a new host.

Kamatella longipedicellata (T.S. & K. Ramakr.) Ullasa var. longisubcellularis Ullasa var. nov.

Infection spots grayish, amphiogenous, isolated or aggregated, circular, 0.5-7 mm. Pycnidia epiphyllous, immersed, ovoid, numerous in infection spots, ostiolate, clypeate, non-stromatic 150-200 x 50-100 μ. Conidiophores simple, hyaline, non-septate, cylindrical produced from the inner cells of the pycnidial wall 5-10 x 1-2 μ. Pycnidiospores napiform, dark brown, unequally 2-celled, with 2 hyaline central zones in the upper cell, lower cell peg like with rounded end 14-18 x 12-13 μ.
Habit: Infecting living leaves of *S. caryophyllaceum* collected at Coorg, October 1968, M.A.C. & Herb. No. 1240 (Type).


F. **Hypodermataceae**

O. **Phacidiales**

Sub-Class **Ascohymeniales**.

The genus **Naemacyclus** was proposed by Fuckel (1873) for an hypodermataceous apothecial fungus causing needle blight of *Pinus* sp. It was monotypic genus when it was proposed. Korf (1962) doubts that there may be still one more good species of this genus on species of *Pinus*.

Korf (1962) erected the family **Hemiphalacidiaceae** for a number of inoperculate discomycetes which cause needle blights or snow blights of conifers, and form orange to yellow-brown, occasionally greenish brown, fleshy apothecia within or beneath the host epidermis or hypodermis. The ascomata are simple with a poorly differentiated stratum giving rise to asci and paraphyses, with scarcely any marginal excipular tissue and usually lacking covering layer of the fungus growth. However, the two doubtful genera **Naemacyclus** and **Lophophacidium** were provisionally included in his new family.

However, writer's critical study of the two collections made on non-coniferous hosts revealed that it has a clearly defined stroma comprising epiteium above the hymenium and below the host tissue a characteristic feature of the members of
Hypodermataceae, on the basis of which the fungus is considered to be a member of Hypodermataceae.

i. *Naemocyclus korfall* sp. nov. (Figs. 26.1, 26.2)

Apothecia strictly foliicolous, epiphyllous, intra-epidermal in origin, bright coloured, dehiscing irregularly at maturity in the form of lobes, 400-500 x 120-150 μ. Asci cylindrical to clavate, uniloculate in basal layers, 8-spored 85-100 x 7-9 μ. Ascospores filiform, thin, septate, hyaline, 60-65 x 1.5-2 μ. Paraphyses cylindrical to clavate, slender, septate, light brown, epithecium indistinct.


ii. *Naemocyclus arxii* sp. nov. (Figs. 26.3, 26.4)

Apothecia strictly foliicolous, epiphyllous, intra-epidermal in origin, bright, dehiscing irregularly at maturity in the form of lobes, 1000-1400 x 200-240 μ. Asci cylindrical to clavate, uniloculate, 8-spored, in basal layers, 90-120 x 8-10 μ. Ascospores filiform, thin, septate, hyaline, 80-90 x 8-10 μ. Paraphyses cylindrical to clavate, slender, septate, light brown, epithecium indistinct.


F. Parmulariaceae

O. Dothiorales

Sub-Class Ascoloculaires

The genus *Hysterostomella* was established by Spézazzini with *Hysterostomella gaurentiana* as type. The genus is characterised by dark crust-like superficial stromata attached to the host epidermis at regular intervals. Locules are borne in these superficial crusts at regular intervals. This genus is included under the family Parmulariaceae of the order Dothiorales by Mueller & Arx (1962).

Writer's collection made from Coorg District of Mysore State agrees with *Hysterostomella orbiculata* which is described here.

*Hysterostomella orbiculata* (Syd.) Arx. in Mueller & Arx (Fig. 26.5) Kryptogamenflora Schweiz 11: 63 (1962).

Stroma epiphyllous, dark, dull, measure 2-6 mm in diameter, attached to the leaf tissue through stromal placenta-like structures. Locules cup-shaped, wall pseudoparenchymatous, epithecium present, measure 200-300 x 200-400 μ. Asci clavate, hyaline, in basal layer, bitunicate, pseudoparaphysate, 85-110 x 24-26 μ. Ascospores sub-hyaline to brown in colour, spindle shaped, 2-celled, somewhat equally septate, constricted at the septum, biseriate, 26-30 x 10-12 μ. Pseudoparaphyses present forming epithecium at early stage of development.

Habit: Collected on living leaves of *Ixora* sp. from Coorg Dist. Mysore State, October 1968, W.A.C.S. Herb. No. 1308.
Remarks: Hysterostomella is a new generic record to India. 
Ixia is a new host for this fungus.


F. Xylariaceae

0. Sphaeriales

Sub-Class Ascohyphalales

The genus *Penzigia* was established by Saccardo (1888) for a Xylarioid fungus having white internal stromal tissue characteristic of *Xylaria* but featuring stromata of hypoxylariaid form. Miller (1934) considered *Penzigia* in the above sense as intermediate between *Xylaria* and *Hypoxylon*. Martin (1967, 1968) does not recognize the genus and distributed species of *Penzigia* among the two genera viz. *Xylaria* and *Hypoxylon*. This genus is accepted here as valid sensu Miller following in this respect Jong & Rogers (1970).

The writer's collection was identified as *Penzigia placenta* Fetch which was originally reported from Ceylon on dead trunks of *Hevea* species. This is the second species of the genus recorded from India, the first being that of *Penzigia capparidii* (Mund. & Ahmad) Ahmad (1948). A brief description of *Penzigia placenta* is given here.

*Penzigia placenta* Fetch Ann. Roy. Bot. Gard. Piredeniya 9:142 (1924) (Fig. 26.6)

Ascostromata inhabiting the dry twigs of *Artocarpus integrifolia* L. develop subcortically and emerge out rupturing
the bark. The stromata are grayish, dark, rectangular to somewhat irregular in shape with a flat surface, sessile with a broad basal attachment. Perithecia embedded in hard stroma, globose, ostiolate, arranged in a parallel layer 600-800 μ. Ascii cylindrical to clavate, unitunicate, pedicellate with thickened apex provided with an apical canal and pore, octosporous 200-220 x 6-8 μ. Ascospores ellipsoid, dark-brown, thick-walled, uniseriate, 8-10 x 4-6 μ, paraphyses and periphryses abundant, filiform, slender and hyaline.


F. Pleosporaceae
O. Pleosporales
Sub-Class Ascoloculares

The genus Parodiella was established by Spegazzini in 1880 with Parodiella perisporioides (Berl. & Curt.) Speg. as the type species. Clements and Shear (1931) treated this genus as a member of the Sphaeriales, whereas Hansford (1946) has placed it under Parodiellinaceae of Myriangiales on the basis of uniloculate nature of ascostroma.

The developmental studies of a species of Parodiella, carried out recently by Tendulkar (1969) have proved the affinity of this genus to Pleosporaceae as the development is of "Pleospora" type and the pseudotheiosium together with other centrum characters
fit in with the concept of Pleosporaceae of Pleosporales as defined by Luttrell (1965a, 1965b).

Mueller and Arx (1962) have merged all the previously reported species into the type i.e. *Parodiella perlsporioides* in view of its remarkable host specificity and morphological characters being similar. Tilek (1963) made a comparative study of the five collections on different legume hosts from Bombay (India) and considered them as formae speciales of the type species following Mueller and Arx (1962). Those parasitizing host plants other than legumes and assigned to the genus *Parodiella* are according to Mueller and Arx (1962) species belonging to other genera like *Gibbera* and *Scolinessa*.

On microscopic examination of the writer’s collection, it was revealed that the stomata were multi-loculate, rarely uniloculate, on the basis of which this collection is being described as a form of *Parodiella perlsporioides* (Berk. & Curt.) Speg. with which it agrees in all essential morphological characters and dimensions, except for its multiloculate nature.

*Parodiella perlsporioides* (Berk. et Curt.) Speg. f. multiloculatae

(Figs. 27.1, 27.2)

Fruit bodies epiphyllous, scattered foliicolous, Ascosporangia generally multi-loculate (8-10), rarely uniloculate, originating sub-cutaneously. Locules globose non-ostiolate, black, 187-312 x 187-327 μ in diameter, multiloculate stroma measure 377-608 μ. in width. Walls 3-4 cells in thickness, cells thick-walled, angular, brown to black. Asci in parallel
layers, cylindrical to clavate, bitunicate, thickened at the apex, pedicellate, 8-spored, 93-124 x 15-23 μ. Pseudoparaphyses numerous, thread like, cellular. Ascospores 8 to an ascus, biseriate, elongated to somewhat spindle shaped, equally septate, constricted at the septum, dark-brown, indistinctly striate, 26-66 x 7-10 μ.

Habit: on living leaves of Crotolaria striata var. acutifolia L. collected at Coorg, India, 15th June 1967, M.A.C. Herb. No. 493 (Type).

Germination studies (Figs. 27.3 to 27.8)

It is noteworthy that all the species, without exception have been collected on legume hosts. This host parasite relationship is a unique feature of this genus, the nature of which has not been fully understood. Tilak (1963) made a comparative study of five collections and considered these as formae specialae of type species, following in this respect. Mueller & Arx (1962) pending further investigations based on cultural and cross-inoculation studies. No conidial state has been recorded for this fungus except for the report by Petrak (1934) of Scleroparodia leguminosarum Petrak. These considerations induced the writer to undertake trials on the germination of ascospores of this genus with the ultimate object of obtaining these fungi in culture for a more detailed study into the life cycles and conidial state if any and its biology. Critical examination of the literature revealed no report on ascospore germination in this genus.

Pre-treatment of ascospores with freezing, desiccation and thawing and chemical treatments with HCl, H2SO4 and D-xylose as
suggested by Emerson (1948) failed to induce germination. The writer, however, succeeded in obtaining germination of the ascospores with the special technique described below.

Fresh leaves of Alysiaarpus sp. infected with Parodiella perisporioides collected from Mahabaleshwar (1372 m. altitude) in October 1968 were used in these studies. The material was dried at room temperature (25 - 27°C). The ascospores were scraped on to a clean glass slide with a sterile blade and teased between two slides in sterile water to separate out the asci and ascospores. The spore suspension made in sterile water was then treated with traces of oxy-tetracyclin hydrochloride to prevent bacterial contamination. The suspension was gently spread out over a sterile glass slide previously coated with egg albumin to obtain a uniform smear of the spores. The slide was then allowed to dry in air and immersed in 0.2% solution of potassium permanganate for one minute and placed in inverted position on a U-shaped thick glass rod in a petri dish containing sterile water. Care was taken to keep sufficient space between the surface of the water below and the slide above. A wet cotton swab was placed over the back of the slide with the upper lid of the dish partly open to facilitate ready condensation of water. This arrangement provided favourable conditions for germination of ascospores.

Observations were made periodically over a period of 72 hours. Ascospores begin to germinate at the end of 2 hours with the production of single germ tubes either from one or both the
cells. No predetermined germ pores were observed. The germ tubes originate from the thinner subhyaline or translucent central region of the cell and emerge by breaking through the dark thick cell wall. It is simple or branched, hyaline and septate. Each cell is capable of giving rise to a single germ tube. None of the germ tubes produced appressoria as recently reported by Ullas (1969) in species of Phyllachora. Ascospores still in the ascus were also found to germinate in situ. Germination was as high as 50% with the above technique. There was no tendency on the part of the germinating spores to produce colonies in agar media, behaving this respect like species of Phyllachora as reported by Ullas (1969).

16. Polystigma DC. Flore France 5 : 164 (1815)

F. Polystigmataceae
G. Sphaeriales
Sub-Class Ascohymeniales

The genus Polystigma was established by Decandolle (1815) with Polystigma rubrum (Fr.) DC as type parasitizing leaves of Prunus domestica L. Clements and Shear (1931) and Lessey (1950) have treated this genus as a member of Hypocreaceae on the basis of bright coloured perithecial wall. The presence of apically free paraphyses with light coloured perithecia and presence of paraphyses in this genus shows its affinity to Sphaeriales as suggested by Arx and Mueller (1954) who have placed this genus under Polystigmataceae. A significant character of Hypocreaceous fungus is the presence of "apical paraphyses" which are attached
at the top of the ascocarp with free ends at the bottom as defined by Luttrell (1965) and reported by Hanlin (1965) in Hypocreopsis schwelinitii.

A collection of an Ascomycete made by the writer on the leaves of Embelia viridiflora Scheff from Mahabaleshwar was identified as a species of Polystigma DC with bright perithecia embedded in orange red raised infection spots. Critical examination and comparative studies revealed that writer's collection agreed with Phyllachora mahabaleshwarensis Ananthanarayanan (1964) and later revised as Physalosporina anamalainen var. mahabaleshwarensis by Chiplonkar (1969) in all respects, all of which parasitize the same host viz. Embelia viridiflora. This was further confirmed through examination of type materials of Phyllachora mahabaleshwarensis obtained from N.A.C.S. Herb. No.156. The writer's collection is, therefore, treated as a new combination.

Polystigma mahabaleshwarensis (Ananth.)Ullasa comb. nov. (Fig.27,9)

Physalosporina anamalainen var. mahabaleshwarensis

Infection spots amphigenous, scattered, orange brown, waxy, 1 to 5 mm. in diameter. Perithecia flask shaped, narrowly ostiolate, 6-8 per infection spot, with bright wall, 345-475 x 194-275 μ. Ascii paraphysate, 8 spored, thin walled, cylindrical, unitunicate, rounded at the apex, narrow at the base provided with disc shaped apical apparatus, 172-202 x 8.5-13 μ. Paraphyses
and paraphyses abundant, filiform slender, hyaline, septate.
Ascospores one-celled, hyaline, thin-walled, elliptical, usually uniseriate, rarely irregularly arranged, 13-16 x 5.5-8.5 μ.

Remarks: Physalospora anamalaisensis Ramkr. T.S. & K. (1954) (HCL No. 18821) infecting Embelia ribes, Burn has been identified as Plectosphaera embelia (Yeasts) von Arx (1954). This fungus was found always associated with an unidentified pycnidial fungus which was later accommodated in a new genus Ramakrishnanella Ramat and Ullas in Ullas (1970).

17. Phyllachora (Pers. ex Fr.) Nke. in Pohl. Symb. Myc. 216 (1869)
F. Phyllachoraceae
G. Sphaeriales
Sub-Class Ascomycotina

The genus Phyllachora was established by Nitschke with Phyllachora graminis as type in "Symboleae Mycologicae" by Fuckel (1869). The genus was originally referred to as Sphaeria by Persoon (1796). Fries recognized this genus but later (1916) changed it to Dothidea. Theissen and Sydow (1912) included the genus in the order Dothidiales in their monographic studies on the assumption that the ascomycarp was a stromatic locule and not a true perithecium. Orton (1924) in his critical studies on Phyllachora graminis stressed the Sphaeriacous nature of the ascomycarp and placed the family Phyllachoraceae under the stromatic Sphaeriales on the basis of true perithecial walls, predetermined ostiole, and apically free paraphyses. Thus studies of Orton (1924), Miller (1941, 1949) and Petrak (1924)
fully justify the removal of this genus from the family
Dothidiaceae and its accommodation under Sphaeriales. The
recent developmental studies on Phyllachora simplicicola by
Jagtap (1967) also report its Sphaeriacious nature which support
the above position.

According to Parberry (1967) the total number of world
descriptions is about 1023 including homonyms. In India so far about
100 species have been reported including homonyms and synonyms,
the main contributions are those of Tilak (1959), Ananthanarayan

The writer collected 11 species of Phyllachora, the brief
description of which are narrated here with some interesting
observations.

(1) Phyllachora sjerekari H. & P. Sydow. (Fig. 28.1)
Ann. Mycol. 10 : 408 (1912)

Infection spots amorphous, black, more or less circular,
shining, scattered, stroma pluriperithelial (6-8) 0.5-1 mm.
Ascocarps deeply embedded in the host tissue, flask-shaped,
periphyses lining the ostiolar region, 180-300 μ. Asci in wall
layers, slender, numerous, cylindrical, eight-spored, unitunicate,
apical apparatus lacking 90.3-105 x 8-12 μ. Paraphyses and
periphyses numerous, filiform, hyaline, slender. Ascospores
biseriate, hyaline, thin walled, single celled, cylindrical,
smooth-walled, 20-28 x 4-6 μ.

Habit: Incites tar spots on living leaves, stems and petioles of
Tylaphora dalzellii Hook collected at Siddapur and Kabinakadu,
Remarks: This fungus is interesting since it infects stems and petioles besides leaves unlike many species of Phyllachora. This species is a new addition to the fungal flora of Coorg District.

(ii) Phyllachora cynodontis (Sacc.) Niessl. (Fig. 28.2)

Not. Pyren 54, (1882)

Stromata dark, minute, shining, amphigenous, isolated or aggregated, 0.2-0.5 mm. in diam. Perithecia pyriform to globoid, 2-4 per stroma, ostiolate, asci 60-80 x 12-14 μm, unitunicate, hyaline, paraphysate, octosporous, ascospores monostichous or distichous, hyaline, ovoid to globular 8-14 x 4-6 μm. Appressoria, sessile or clavate, dark brown.


Remarks: This is a new report for Coorg Flora.

(iii) Phyllachora dalbergiae Niessl. (Fig. 26.3)

Hedwigia 20 : 99 (1881)

Infection spots scattered, rarely aggregated, epiphyllous, black, shining, irregular in outline. Perithecia ovoid, amphigenous, stroma monoperithecial, ostiolate, 200-275 x 150-225 μm. Asci lanceolate to clavate, 8-spored, thin-walled, unitunicate with obtuse apex, non-pedicellate, parallel, in basal layers, 64-80 x 14-16 μm. Ascospores distichous, oval to oblong, single celled, hyaline, thin walled 8-20 x 4-6 μm. Paraphyses and periphyseis numerous.

Remarks: This fungus also infects young shoots, stems, petioles and pods. This is reported for the first time from Coorg District.

(iv) *Phyllachora diospyrosae* Ullasa (Fig. 28.4)

*Indian Phytopath* 22: 75 (1969)

Stromata black, irregular, dispersed, erumpent, epiphyllous, scattered with a necrotic hollow, 1-3 perithecia in a stroma; perithecia immersed, subglobose, 300 x 400 μ; asci cylindrical, stipitate, paraphysate, 100-160 x 6.5-9 μ; ascospores monosporous, 1-celled, hyaline, oblong, with a little constriction in the middle 8-9 x 6-7 μ.


Remarks: Since there is no report of *Phyllachora* species on this host family, this has been described as a new species.

(v) *Phyllachora ficus-gibbosae* Ullasa (Fig. 28.5)

*Indian Phytopath* 22: 76 (1969)

Stroma black, round, erumpent, epiphyllous, sub-cuticular, scattered, cushion-like, shining, 0.5-6.0 mm multiloculate, 2-6 perithecia in a stroma; perithecia ostiolate, bowl shaped, 156-390 x 312-596 μ. Asci cylindrical, sessile, octosporous,
paraphysate, unitunicate, apical apparatus lacking, 87.4-114.0 x 10.4-20.9 μ. Ascospores uniseriate, hyaline, oblong, 12.3-19.0 x 5.7-9.5 μ.

Spermogonial stromata 2-6 loculate, distinct, spermogonia sub-globose, spermatia numerous, spirally curved.

Habit: On living leaves of Ficus gibbosae var. parasitica collected at Coorg District, Mysore State, 15th October 1967, M.A.C.S. Herb. No. 538 (Type).

Remarks: A comparative study revealed that out of nearly 16 species of Phyllachora affecting species of Ficus, the writer's collection had some agreement with P. repens (Corda) Sacc. On closer and critical study, however, the Coorg collection was found to be distinct in respect of habit on host, cylindrical sessile asci with uniseriate arrangement of ascospores, besides having been collected on a new host, on the basis of which it is described as a new species.

(vi) Phyllachora gudalurensis T.S. & K. Ramakr. (Fig.28.6)


Spots indistinct or light brown in colour, amphigenous, stromata amphigenous, black, shining, multiloculate. Asci cylindrical to elevate, paraphysate, unitunicate, hyaline, 80-120 x 6-8 μ. Ascospores 8, uniseriate, hyaline, citriform to oval, single celled, 10-14 x 4-7 μ.

Remarks: This species of *Phyllachora* is characterised by alternate arrangement of the ascospores of citriform and oval ascospores produced in the same ascus in addition to the other types of arrangements reported by the writer as in case of *Phyllachora ixorae* in this species (Ullass, 1969) (Figs. 29.2H-K, 29.9).

(vii) *Phyllachora ixorae* Theiss et Syd. (Fig. 29.1)

*Ann. Mycol.* 13, 553 (1915)

Infection spots epiphyllous, mostly circular, prominent, black, scattered, waxy, shining and raised, cushion shaped, 3-3.5 mm in diam. Perithecia flask shaped, innate, ostiolate, 300-380 x 240-280 μ. Asci numerous in basal layers, cylindrical, 8-spored, unitunicate with a short pedicel, apical apparatus lacking, 64.5-84.2 x 6-10.5 μ. Paraphyses and Periphyses numerous, filiform to hair like, hyaline. Ascospores uniseriate, elongate, oblong to oval with both the ends rounded arranged end to end, hyaline, single celled, 8.6-12.9 x 4.3-5.3 μ.


Remarks: The writer (1969) has reported interesting types of ascospore arrangements in this fungus. This fungus produces 2 types of ascospores, the oblong and the oval which are generally arranged alternately, while the alternate arrangement of the two types of ascospores within an ascus was of common occurrence; other type of spore arrangement with different sequence were also noticed. It was interesting to note that irrespective of the position of the
ascospores within the ascus, of the 8 ascospores within the ascus four were always of one type and four of the other. This interesting phenomenon has not been so far reported in literature in this fungus genus (Figs. 29.2A-G, 29.3, 29.4-29.8).

(viii) Phyllachora ischaemi Syd.  (Fig. 29.10)

Ann. Mycol. 13 : 40 (1915)

Stroma shining black, aggregated or isolated amphigenous, perithecia bowl-shaped to pyriform, ostiolate, 2-4 per stroma, 200-250 x 300-350 μ. Asci cyllhdral to saccate, pedicellate, unitunicate, paraphysate, octosporous 60-100 x 10-12 μ. Ascospores mono- or distichous, hyaline, oval to ovoid, rarely ellipsoid 10-20 x 5-10 μ, appressoria, ovoid to obclavate, often having sigmoid axis, sessile.

Habit : Inciting tar spots on living leaves of Ischaemum sp. collected at Kalasubai, near Nasik, Maharashtra, in March 1969, M.A.C.S. Herb. No. 1315.

Remarks : This fungus is a new report to Maharashtra.

(ix) Phyllachora lacrimiformis Ullasa  (Figs.29.11,30.1,30.2)


Stromata black, round, erumpent, amphigenous, scattered, shining, 0.25 x 1.00 mm., 1-2 perithecia in a stroma; perithecia ostiolate, immersed, flask-shaped, 150 x 250 μ, asci saccate, briefly stipitate, paraphysate, 58-68 x 6-8 μ. Ascospores strictly distichous, lacrimiform in shape, 18-22 x 2.5-3.5 μ.

Habit : Infecting living leaves of Crytococcus oxyphylum Stapf.,
collected at Cottebetta, Coorg, Mysore State, M.A.C.S. Herb. No. 695 (Type).

Remarks: In this particular species the ascospores were strictly distichous which is an exception to the graminicolous species of *Phyllachora*. According to Parberry (1967) some species e.g. *P. graminis*, *P. punctum* always exhibit monostichous arrangement, but no graminicolous species is known in which spores are always distichous or inordinate. In case of *P. stenospora* parasitizing *Cytocomcum trigonum* A. Camus, the ascospores are ellipsoid, often with a slightly sigmoid axis giving a lacriform shape while the spores in the writer's collection are typically lacriform in shape with rhomboidal edges with double the size of those of *Phyllachora stenospora* (B. & Br.) Sacc. besides being collected on a new host.

(x) *Phyllachora pennisitina* Syd. (Figs. 30.3-30.6)  
Stroma epiphyllous, black, scattered, shining, raised, cushion like, multiloculate (1 to 4), 0.25-0.5 x 0.5-2.0 mm. perithecium bowl shaped, ostiolate, 93.6-249.6 x 187.2-45.6 μ. Aeci cylindrical to clavate, stipitate, 8-spored, copiously paraphyses, 57-95 x 123-19 μ. Ascospores uniseriate, subglobose to globose, hyaline, 9.5-10.4 x 9.5-10.4 μ, spermogonia lenticular 31.2 x 124.8 μ. Spermata single celled, hyaline, linear.

Habit: Incites tar spots on living leaves of *Pennisetum hohenakerti* Hochst. collected at Coorg District, Mysore State, 15th October 1967, M.A.C.S. Herb. No. 539.
Remarks: This agrees in ascus and spore measurements with *P. pennisetina* Syd. except for the uniseriate arrangement of the ascospores. *Pennisetum hohenakeri* is a new host for this fungus. Association of spermogonia with developing ascocarp, is reported for the first time in this species. Besides, the fungus is a new report to India.

An interesting observation made by the writer in this species was the mode of germination and nature of appresoria produced by the ascospores, which gave out short germ tubes with amoeboid appresoria at their ends (Fig. 30.6).

(xi) *Phyllachora platyelliptica* Parbery (Fig. 30.7)


Stromata dark, shining, 1.5-2 x 0.5-1 mm., scattered, raised on the dorsal surface of the host leaf and not so on ventral surface. Each stroma has a distinct dorsal as well as ventral clypeus with 3-6 perithecia, upper clypeus thicker. Perithecia, pyriform to ovoid opening through ventral side 100-160 x 80-100 μ. Asci cylindrical, briefly stipitate, 82-100 x 10-16 μ. Paraphysate, unitunicate, octosporous, ascospores mono- or distichous, narrow or slightly ellipsoid, some are flat sided or slightly bent, 14-21 x 5-7 μ.

Habit: On living leaves of *Theaeda* sp. collected at Nallur, Coorg Dist., Mysore State, M.A.C.S. Herb. No. 1312.

Remarks: This fungus has been reported by Parberry (1967) from India (Wynand) on *Anthistiria* sp. This is the first report of this fungus on *Theaeda* sp. from India.
18. *Plectosphaera* Theiss. (Fig.36.4)


F. *Polystigmataceae*

G. *Sphaeriales*

Sub-Class *Ascochymeniales*

The members of this group are considered as intermediate between *Phyllachora* and *Polystigma*. The genus was established by Theissen (1916) with *P. bersanae* as type. Arx and Mueller (1954) have described 20 species of this genus. The fungus described here is obtained from *HCIO* reported as *Physalospora* *anamalsiensis* by T.S. & K. Ramakrishnan (1950) infecting *Emelia ribes* Burm. which on comparative studies was found to be

*Plectosphaera embelia* (Yeates) Arx and Mueller which is presented here and *P. anamalsiensis* T.S. & K. Ramakrishnan is treated as synonym.

*Plectosphaera embelia* (Yeates) Arx & Mueller

*Kryptogamenflora 11 203* (1954)

Synonym: *Physalospora anamalsiensis* T.S. & K. Ramakrishnan

Proc. Ind. Acad. Sci. B, **32**: 69 (1950)

Infection spots epiphyllous, necrotic, indefinite, slightly raised, perithecia punctiform, brown, innate, crowded in the spot, globose, ostiolate, clypeate 200 x 300 μμ. Asci hyaline, cylindrical, pedicellate, paraphysate, paraphyses, thin, filiform, unitunicate, octosporous, 80-100 x 10-12 μμ. Ascospores hyaline, elliptical to oblong, one celled provided with a gelatinous covering, 10-12 x 7-10 μμ.


*Giorn Bot. Ital.* 1, 334, (1844)

F. Xylariaceae

O. Sphaeriales

Sub-Class Ascomycetidae.

The genus *Rosellinia* was established by *Denotarls* (1847) with *R. aquila* (Fr.) Den. as type. *Clements* and *Shear* (1931) have placed this genus under the family Sphaeriales, whereas *Arx* and *Mueller* (1954) have placed it under Xylariaceae of Sphaeriales. *Luttrell* (1955) and *Martin* (1961) placed the genus under Xylariaceae. More than 200 species are known from all parts of the world most of them being saprophytes on barks and stems of various host plants. The ascospores in this genus are ellipsoid, coloured, 1-celled and because of these characters of the ascospores *Vincens* (1921), *Wehmeyer* (1926) and *Miller* (1928) suggested that this belongs to Xylariaceae.

The characters of the writer's collection closely agree with the type species which is presented here.

*Rosellinia aquila* (Fr.) Den.  (Fig. 31.1)

Stroma black, erumpent, globose, aggregated 0.5-0.9 x 0.6-0.9 mm. Perithecia globose to subsphaerical, usually single but rarely 2 in each stroma, black, ostiolate, 300-400 μ in diam. Asci clavate, unitunicate, pedicellate, in wall layers, octosporous, apex
thickened, 120–150 x 8–10 μ. Ascospores ellipsoidal to lenticular, dark-brown, 1-celled, uniseriate, thick-walled, 18–22 x 5–7 μ. Paraphyses and periphyses abundant, filiform, and hyaline.


Remarks: This fungus is a new addition to fungi of India.

20. Seynesia Saccardo Syll. Fung. 2, 668 (1883)

F. Amphiasphaeriaceae
O. Sphaeriales
Sub-Class Ascohymeniales.

The genus Seynesia was founded by Saccardo (1883) for a sphaeraceous fungus with thick clypeate perithecia. The fungus is characterised by sub-sphaerical perithecia having paraphysate asci. Ascospores are 2-celled olavaceous brown with papillate ends. Since this is a new generic record to India it is described here.

Seynesia erumpens (Berk. & Curt.) (Figs. 31.2, 31.3)

Perithecia lignicolous, erumpent, ostiolate, clypeate, amphiasphaerical, with little basal stroma, 500–900 x 100–200 μ. Asci cylindrical to clavate, unitunicate, pedicellate, paraphysate, octosporous, 250–300 x 7–10 μ. Ascospores 8, uniseriate, olavaceous brown, two celled, septate in the middle, with a typical curved papillate ends, 22–36 x 6–8 μ.

Remarks: Calamus rotang L. is a new host for this fungus. Besides it is a new generic record to India.


F. Phyllachoraceae
O. Sphaeriales
Sub-Cl. Ascomycotiales

The genus Stigmacora was established by Theissen and Sydow in 1914 for a phyllachoraceous fungus characterized by having dark stroma and 2-celled hyaline ascospores, with Stigmacora controversa (Starb) Theiss. & Syd. as type. Mueller and Arx (1962) have described five species of this genus. This genus, like Parodiella infects various species of Leguminosae. This genus has been treated as a member of the family Polystigmataceae of the order Sphaeriales by Mueller and Arx (1962).

Writer made two collections of this fungus infecting the living leaves of Albizzia procera Benth. and Acacia initia Wild. which were identified as Stigmacora deightonii (Syd.) von Arx. Endodothella kanarensis Ramakr. & Sund. (1952) infecting living leaves of Albizzia odoratissima Benth. obtained from HCIO, New Delhi, No. 19815 was found to be the same which is treated here as a synonym of Stigmacora deightonii, von Arx.

The genus Endodothella Theiss. & Syd. was established by Theissen and Sydow (1915) for an ascomycete considering the
ascospores to be 2-celled on the basis of mistaken identity due to the densely staining band as septum in the equatorial region of the ascospores. This fungus was, therefore, reduced to synonymy with genus Phyllachora Nke. (von Arx 1958, Mueller & Arx 1962).

*Stigmochora deightonii* (Syd.) von Arx in Mueller & Arx (Figs. 31.4, 31.5)
Kryptogamenflora Schweiz 11 (2) : 662 (1962)

Synonym: *Phaeothelie kamarensis* T.S. Ramakr. & Sund.

Stroma dark, shining, erumpent, aggregated or isolated.
Perithecia, oval to globose 2-4 in a stroma, olypeate, oatiolate.
Asci hyaline, uaitunicate, paraphysate, shortly pedicellate, in basal layers 90-100 x 18-22 μ. Ascospores spindle shaped, hyaline, septate in the middle, tapering towards the ends.
Biseriate or uniseriate, 20-28 x 6-8 μ in diameter.


Remarks: *Acacia initia* Willd is a new host for this fungus. Closely associated with this fungus are other Phyllachoraceous fungi identified as *Pseudothis coccodes* (Lev.) Theiss. & Syd. an *Ascomycete* and *Lasmania globulifera* (Teb.) Hohnel a pycnidial fungus.

   - Beyer Fl. 2, 566 (1789)

F. Xylariaceae
O. Sphaeriales
Sub-Class Ascohymeniales
The type of the genus is *Xylaria hypoxylon* (L. ex Fr.) Grev. Dennis (1958) adopted the name *Xylosphaeria* Dumortier since this name predates the name *Xylaria* (Hill) Schranck and as such later homonym of *Xylosphaeria*. However, Grayholm and Mueller (1965) have proposed conservation of the name *Xylaria* as against *Xylosphaeria* Dumortier. The name *Xylosphaeria* has been adopted by Joly (1968) and Thind and Waritch (1969), while Morgan Jones and Lim (1968), Mukerjee et al. (1969) adopted the name *Xylaria*. Writer adopts the name *Xylaria* following Grayholm and Mueller (1965) and others.

1. *Xylaria anisopleura* Mont. Sacc. Syll. Fung. 1: 323 (Fig. 31.6)

Stroma erect, black, gregarious in clusters, obovate to sub-globose, varying from stipitate to sessile, simple, apex rounded up to 2 cm in total height. Fertile region up to 1 cm. Perithecium globose to sub-globose, immersed or slightly protruding, ostioles papillate, black, stipe up to 0.2-0.5 cm., cylindrical. Asci 8-spored, cylindrical, apex obtuse, tapering below into a narrow stalk, 180-250 x 10-13 μ. Ascospores uniseriate, smooth, inequilateral, ellipsoid, dark brown, guttulate, 18-24 x 8-10 μ. Paraphyses and periphyses numerous, slender, hyaline, filiform.


Remarks: This is a new addition to the Fungi of Maharashtra.
11) *Xylaria botrys* Pat.  
*Suoc. Syl. Fung. 9: 534 (1891)*

Stromata black, stipitate, simple, botriose, 400-500 x 500-600 mm.
Perithecia oval to globose, slightly protruding above the stroma.
Ostiolate, ostiole papillate, 150-180 x 6-8 µ. Asci hyaline, paraphysate,
in wall layers, cylindrical, stipitate, octosporous, 200-220 x 10-12 µ.
Ascospores dark, unicellular oval to ellipsoidal, uniseriate, smooth,
20-25 x 8-10 µ.

**Habit**: Saprophytic on dead twigs of *Artocarpus integrifolia* L.

**Remarks**: Fungus is a new record to the Flora of Coorg.

iii) *Xylaria coorgiana* sp. nov.  
*Suoc. Syl. Fung. 9: 534 (1891)*

Stroma grayish black, stipitate to sessile, perithecia 3-5 in a stroma, protruding, ostiolate, ostiole papillate, 1.5 x 2.5 mm.,
stipe up to 1-1.5 cm. Perithecia globose to sub-globose, with a hyaline internal wall, ostiolate, ostiole papillate, 280-320 x 180-220 µ. Asci cylindrical, hyaline, stipitate, paraphysate, in wall layers, 80-120 x 8-10 µ. Ascospores, ellipsoidal, unicellular, dark-brown, guttulate, uniseriate to inordinate, 10-12 x 6-8 µ.

**Habit**: Collected on decaying leaves of *Syzygium cumini* (L) Skeels at Coorg District, Mysore State, in June 1968, M.A.C.S. Herb. No. 1321 (Type).

iv) *Xylaria dealbata* Berk & Curt.  
*Suoc. Syl. Fung. 1: 323, (1882)*

Stroma clavate, branching, 2 to 3 stroma in a stalk, grayish-
black, surface rough with a linear fissures, stipitate, hard, 2-5 cm in height and 0.5-1 cm in breadth. Perithecia oval to globose, ostiolate, ostiole papillate, 400-600 x 500-700 μ. Asci elevate to cylindrical, paraphysate, stipitate, hyaline, octosporous, 100-120 x 8-10 μ. Ascospores dark brown, single celled, oval to inequilateral, uniseriate, 12-15 x 6-8 μ.

**Habit:** Saprophytic on *Bambusa* sp. collected at Coorg, Mysore State, in October 1968. M.A.O.K. Herb. No.1322.

Xylaria digitata (Linn.) Grev.  
(Fig. 32.4)  
Sacc. Syll. Fung. 1 : 326 (1882)

Stromata oval to cylindrical 0.5-1 cm. in height, with an apical projection, dark, sessile to shortly stipitate, perithecia, globose to sub-globose, ostiolate, ostiole papillate, 300-400 x 300-450 μ. Asci hyaline, cylindrical, paraphysate, octosporous, stipitate in 1-2 layers, 100-120 x 6-8 μ. Ascospores oval to oblong, dark brown, smooth, unicellular, uniseriate, 8-10 x 4-6 μ.

**Habit:** On dead twigs of *Ficus glomerata* Roxb. collected at Coorg, Mysore State, in June 1968, M.A.C.S. Herb. No.1323.

**Remarks:** This is a new addition to the fungal flora of Coorg District.

Xylaria obovata Berk.  
(Fig. 32.5, 32.6)  
Sacc. Syll. Fung. 1 : 317 (1882)

Stromata separate, globose, carbonaceous, completely dark,
with thick and short pedicel, 0.5 x 1 cm. in diam. Perithecia globose to sub-globose, ostiolate, non-papillate, 300-400 x 400-600 μ. Asci simple, clavate, unitunicate, stipitate, paraphysate, octosporous, in wall layers, 90-110 x 5-7 μ. Ascospores dark brown, inequilateral to oval, guttulate, unicellular, uniseriate or inordinate, 8-10 x 4-6 μ.

Habit: On dead trunks of Albizia odoratissima Benth. collected at Coorg district, Mysore State, in October 1968, M.A.C.S. Herb. No. 1324.

Remarks: This is a new addition to the fungal flora of Coorg, Mysore State.

SUMMARY

The writer's taxonomic studies include 36 species of Ascomycetes belonging to 22 genera which are summarised as follows:

I. New genera established


II. New species established

1. Lophonera kamati Sp. Nov. Ullasa

7. *Xylaria coorgiana* Sp. Nov. Ullasa

**III. New generic records to India**

1. *Cocconia placenta* (Berk. & Exr.) Sacc.
2. *Seynesia erumpens* (Berk. & Curt.) Petr.
3. *Haemocylus karthii, N. arxii*

**IV. New Records of Ascomycetes to India.**

1. *Didymosphaeria cocooniae* var. Arx
2. *Hypoxylon archeri* Berk.
5. *Rosellinia aquila* (Fr.) Dén.

**V. New Combinations proposed**


**VI. Synonyms**

2. *Endodothella kanarensis* Ramakr. T.S. and Sund. have been found to be facultative synonyms of *Plectosphaera embelia* (Yeats) von Arx and *Stigmochora deightenii* (Syd.) von Arx respectively.
VII. Rest of the Ascomycetes described were not reported earlier from the respective localities from where they were collected viz. Coorg District of Mysore State and in and around Poona, Maharashtra State.

VIII. Besides, studies on ascospore germination in *Parodiella perisporioides* and studies on ascospore arrangement in species of *Phyllechora* have been carried out.
Fig. 1. Achorella plectroniae T.S. & K. Ramakr. 
and Hemidothis canthicola (Seshadri) Ullasa comb. nov.
A. Habit, B. Section through the fruit bodies showing ascocarps and pycnidia, C. Ascii, D. Ascospores,
E. Section through the ascocarp, F. Section through the pycnidium = Hemidothis canthicola (Seshadri) Ullasa comb. nov.
G-I. Conidiophores and conidia

Figs. 2-5. Arxonia indica Kamat & Ullasa Gen. et. sp. nov.

Fig. 6. Bagnisiella mangiferae Tilak & Rao.
A. Habit, B. Section through ascostromata,
C. Ascii, D. Ascospores.

Fig. 7. Cocconia placenta (Berk. & Br.) Saoc.
A. Habit, B. Section through the ascocarp,
C. Ascii, D. Ascospores.
Plate 22

Figs. 1-2. Chadefaudia indica Kamat and Ullasa Gen. et.sp.nov.

1. A. Habit, B(1-2) Section through the peritheciun and the associated pycnidium, C. Asci, D. Ascospores, E. Development of conidiophores and conidia, F. Conidia.

2. Photomicrograph of an ascus showing shining apical apparatus and unequally 2-celled ascospores.

Fig. 3. Daldinia concentrica (Bolt) Ces.et. de Not.
A. Ascocarp, B. Peritheciun, C. Asci, D. Ascospores.

Fig. 4. Didymosphaeria cocconia v. Arx
A. Habit, B. Section through ascocarp, C. Asci, D. Ascospores.

Fig. 5. Hypoxylon archeri Berk.
A. Habit, B. Section through the ascocarp, C. Ascospores, D. Asci.

Figs. 6-7. Lophomerum kamati Ullasa sp. nov.

6. A. Habit, B. Section through the apothecium, C. Asci and paraphyses, D. Ascospores.

7. Photomicrograph showing the section of an apothecium.
Plate 23

Figs. 1-4. *Deshpadorea jambolana* (Ramakr.T.S.,Sriv.,Sund.)

1-2. Photomicrographs of sections through the infection spots showing diagonally placed asccarps and evanescent nature of the asci.

3. Asci and ascospores. Note the dark brown equally 2-celled ascospores.


Fig. 5. Photomicrograph showing section through the pycnidium of *Mycophallage congesta*.

Fig. 6. Conidia of *Mycophallage congesta*. 

1. Photograph showing habit.

2–3. Photomicrographs showing association of pycnidium and peritheciun of *Kamatella longipedicellata* and *Muelleromyces variisporus* respectively from Coorg collection.

4. Photomicrograph of a thin spore crust showing mixture of pycnidiospores and ascospores of *Kamatella longipedicellata* and *Muelleromyces variisporus* from Coorg collection.

5. Photomicrograph showing ascospores of *Muelleromyces variisporus*.

6. Photomicrograph showing asci of *Muelleromyces variisporus*. 
Plate 28

Figs. 1-8. Muelleromyces and Kamatella

1. Photomicrographs of the ascospores of Muelleromyces variisporus and Kamatella longipedicellata in a lesion named Diplodia variispora by Diedicke (Butler No. 1775 Type).


3. Photomicrograph showing mode of conidium ontogeny and conidiophore development in K. longipedicellata. Arrow indicates the developing conidiophore and conidium.

4. Photomicrograph showing clypeate ostiole of the pycnidium of Kamatella longipedicellata.

5. Photomicrograph showing conidia of Kamatella longipedicellata var. longisubcellularis var. nov.

6. Photomicrograph showing uniform nature of conidia of Kamatella longipedicellata.

7. Kamatella longipedicellata var. longisubcellularis

8. Habit of Kamatella longipedicellata var. longisubcellularis var. nov.
Plate 26

Figs. 1 & 2. 

*Naeacyclus korfii* Ullasa sp. nov.

1. A. Habit, B. Section through the ascocarp, C. Asci and Paraphyses, D. Ascospores.

2. Photomicrograph showing section through an ascocarp.

Figs. 3 & 4. *

*Naeacyclus arxii* Ullasa sp. nov.

3. A. Habit, B. Section through the ascocarp, C. Asci and Paraphyses, D. Ascospores.

4. Photomicrograph showing section through an ascocarp.

Fig. 5. 

*Histerostomella arbicularia* (Syd.)v.Arx

A. Habit, B. Section through the ascocarp, C. Asci, D. Ascospores.

Fig. 6. 

*Pennigia placenta* Petch.

A. Habit, B. Section through the peritheciurn, C. Asci, D. Ascospores.
Plate 27

Figs. 1 & 2. **Parodiella perisporioides** (Berk. et Curt) Speg. f. *multiloculata* Ullasa
1. Section showing uniloculate nature of ascostroma and asci.
2. Sections through the multiloculate ascostromata.

Figs. 3-8. Ascospore germination in *Parodiella perisporioides*
3-5. Photomicrographs showing ascospore germination in situ.
6,7. Photomicrographs showing individual ascospore germination.
8. Camera Lucida Drawing showing different types of ascospore germination in *P. perisporioides*.

Fig. 9. **Polystigma mahadevawarensis** (Amant) Ullasa Comb. Nov.
A. Habit, B. Section through the Ascocarps, C. Asci, D. Ascospores.
Fig. 1. Phyllachora ajrekari H. & P. Sydow.
   A. Habit, B. Section through the ascocarp,
   C. Ascus, D. Ascospores.

Fig. 2. Phyllachora cynodontis (Sacc.) Niessl.
   A. Habit, B. Section through the ascocarp,
   C. Ascus, D. Ascospores and germinated
   ascospores having appressoria.

Fig. 3. Phyllachora dalbergiae Niessl.
   A. Habit, B. Section through the perithecium,
   C. Ascii, D. Ascospores.

Fig. 4. Phyllachora diospyrosae Ullasas sp. nov.
   A. Section through the perithecium,
   B. Ascii, C. Ascospores, D. Habit.

Fig. 5. Phyllachora ficus-gibbosae Ullasa sp. nov.
   A. Section through the ascocarp,
   B. Ascus, C. Ascospores, D. Habit.

Fig. 6. Phyllachora gudalurensis T.S. & K. Ramakr.
   A. Habit, B. Section through the infection
   spot, C. Ascii, D. Ascospores.
Plate 29.

Fig. 1. *Phyllachora ixorae* Theiss. & Syd.
A. Habit,  B. Section through the perithecia,
C. Asci,  D. Ascospores.

Figs. 2 A–G, Different types of ascospore arrangements
3, 4–8. in the asci of *Phyllachora ixorae*.

Figs. 2 H–K, Different types of ascospore arrangements
9. in the asci of *Phyllachora gudalurensis*.

Fig. 10. *Phyllachora ischaemi* Syd.
A. Habit,  B. Section through the perithecia,
C. Asci,  D. Ascospores.

Fig. 11. *Phyllachora lacrimiformis* Ullas sp. nov.
A. Section through the perithecia,
B. Ascus,  C. Ascospores,  D. Habit.
Plate 30

Figs. 1-2. *Phyllachora lacrimiformis* Ullasa sp. nov.

1. Photomicrograph showing section through the perithecia.

2. Photomicrograph showing asci.

Figs. 3-6. *Phyllachora pennisitina* Syd.

3. A. Section through the perithecia,
   B. Ascus,  C. Ascospores,  D. Habit,
   E. Germinating ascospores with appressoria,  F. Spermatiophore,
   G. Spermata,  H. Spermogonium.

4. Photomicrograph showing section through the asccarp.

5. Photomicrograph showing section through the closely associated spermogonium.

6. Germination of ascospores showing amoeboid appressoria.

Fig. 7. *Phyllachora platyelleptica* Parbery

B. Section through the perithecia.

C. Ascii, D. Ascospores.
Plate 31

Fig. 1. *Rosellinia aquila* (Fr.) Den.
   A. Habit, B. Section through the ascoecarp, C. Asci, D. Ascospores.

Fig. 2. *Seynesia erumpens* (Berk. & Curt.) Petr.
   A. Habit, B. Section through the perithecia, C. Asci, D. Ascospores.

Fig. 3. Photomicrograph showing asci and ascospores of *Seynesia erumpens*.

Figs. 4-5. *Stigmoehora deightonii* (Syd.) von Arx.
   4. On *Albizia procera* Benth.
   5. On *Acacia initia* Willd.
   A. Habit, B. Section through the ascoecarp, C. Asci, D. Ascospores.

Fig. 6. *Xylaria anisoploera* Mont.
   A. Habit, B. Section through the peritheciurn, C. Asci, D. Ascospores, E. Section through the ascoecarp showing position of perithecia (Diagrammatic).
Plate 32

Fig. 1. Xylaria coorgiana Ullassa sp. nov.
A. Habit, B. Section through the ascocarp, C. Ascus, D. Ascospores.

Fig. 2. Xylaria botrys Pat.
A. Habit, B. Section through the perithecium, C. Asci, D. Ascospores, E. Section through the ascocarp (Diagrammatic)

Fig. 3. Xylaria dealbata Berk. et Curt.
A. Ascocarps, B. Section through the perithecium, C. Asci, D. Ascospores, E. Section through the ascocarps (Diagrammatic)

Fig. 4. Xylaria digitata (Linn.) Grev.
A. Habit, B. Section through the perithecium, C. Asci, D. Ascospores, E. Section through the ascocarp (Diagrammatic).

Figs. 5-6. Xylaria obovata Berk.
A. Habit, B. Section through the perithecium, C. Asci, D. Ascospores, E. Section through the ascocarp (Diagrammatic)
6. Photograph showing ascocarps.
PART IV

TAXONOMIC AND ONTOGENIC STUDIES IN SOME INDIAN FUNGI IMPERFECTI
CHAPTER I

TAXONOMY AND CONIDIIUM ONTOGENY IN SOME SPECIES OF INDIAN FUNGI IMPERFECTI

Introduction

During his mycological survey carried out at the forests of Coorg, Mysore State and in and around Poona, Maharashtra State for Ascomycetes the writer also collected some Hyphomycetes and Sphaeropsidaceous fungi, a detailed study into the diagnosis and determination of which are presented in the following Chapter of this Part. Chapter I pertains to the taxonomic and ontogenetic account of six Hyphomycetes and two Sphaeropsidaceous fungi and Chapter II deals with the comparative ontogenetic study of four sphaeropsidaceous fungi constantly associated with some Ascomycetes.

Sporulating structures were first used as a taxonomic criterion by Saccardo in the "Sylloge Fungorum". Saccardo divided spores into many types based on morphological characteristics such as shape, septation and colour. He was, however, not concerned with the mode of conidial production and it was Vuillemin (1910, 1911, 1912) who stimulated interest in the mode of spore development rather than the characteristics of the spores themselves in the taxonomic arrangement of these fungi. He drew attention to the difficulties involved in classifying different kinds of spores of Fungi Imperfecti under the single term conidium and recognized two basic spore types: the thallospores and the conidium verum. Vuillemin's proposals were reviewed
in detail and clarified by Mason (1933) who introduced additional categories, and Longerson and Vanbreuseghem (1932) following Vuillemin and Kason used the term "Thallospore" to include arthrosapores, blastospores, chlamydospores, dictyospores and aleuriospores and the term "Conidiospore" (conidium verum) to include pycnosapores terminus spores and phialospores (Meristem spores). Moreau (1932, 1953) was first to extend Vuillemin's concept on conidial ontogeny from the Hypocreales to other Imperfect Fungi. In 'formes de propagation', he discussed several types of spore and aleuriospore production was recognized in Discosia, Pestalotia and Sirdium. A year later, Hughes (1953) advanced criteria for differentiating modes of conidial development in the Hypocreales. He divided the various modes of spore production on the basis of conidial and conidiophore ontogeny, into the following eight types:

Section Ia, Blastospores produced in chains.

Section Ib, Blastospores: the botryose solitary Blastospore as in Botrytis and the botryose blastospores as in Conatoebotrya, Neartagonium in which the spores are in chains.

Section II, The Terminus spores as in Ramularia or Heterosporium and the botryose terminus spore as in Arthrobotrya.

Section III, Chlamydospores, solitary as in Bactridium, or successively produced on annellospores as in Scopulariopsis.

Section IV, Phialospores, in basipetal succession, as in Phialophora or on polyphialides as in Catenularia.
Section V, Meristem arthrospores produced in true basipetal chains due to the meristematic growth of the sporophore, as in Erysiphe.

Section VI, Prospores, as in Helminthosporium

Section VII, Arthrospores, as in Geotrichum

Section VIII, Spores on basiaxic conidiophores.

Hughes (1970) further stressed the use of such spore ontogeny in Uredinales as well, as a possible criterion of classification and divided mode of spore production in Uredinales into four sections.

Goose (1956) discussed Hughes's scheme and listed and redefined the spore terms and Tubaki (1958) somewhat amplified Hughes's scheme by dividing each sections III, IV and VII into two or three sub-sections and recognised a new section IX, for Trichotheccium type spores. Subramanian (1962) believed that several more categories were needed to classify the Indian and other tropical fungi. He, therefore, proposed the following six morphological categories of the spores based on their ontogeny:

1. the blastospore,
2. the ginglymospore,
3. the phialospore,
4. the porospora,
5. the arthrospore,
6. the meristem arthrospore.

He also foresaw a seventh category, the spiculospore, which is formed at the tip of a pointed structure, as in Mirmutella and Akanthomyces. Tubaki (1963) extending his previous work, discussed spore and sporophore ontogeny and proposed six divisions in Hyphomycetes. He defined three new ones, in addition
to the foregoing spore types: 'terminoladulospore', as in Beauveria, 'meristem aleuriospore' as in Trichothecium, and 'pleuroradulospore' as in Aureobasidium. In addition, he considered that the method by which a conidiurn functions as a growing point should be taken as a differential character.

Recently Kendrick, Cole and Bhat (1968), Cole and Kendrick (1968, 1969a, 1969b, 1969c), Kendrick and Cole (1968, 1969) have analysed the conidium and conidiophore ontogeny in the following members of Hyphomycetes: Conotobotryum apiculatum, Basinetospora rubra, Beauveria globulifera, Curvularia inaequalis, Trichothecium roseum, Phialophora lagerthurgii, Penicillum cyclophilum, Thielaviopsis sp., Scopulariopsis brevicollis, Oidiocendron truncatum, Geotrichium candidum, Sporangium purpurascens with the help of time lapse photomicrographic technique developed by Cole et al. (1969). They have proposed subdivisions of Hughe's group V into Sections VA and VB. Section VA comprises those forms with an extended meristematic region and a presumed organic continuity allowing translocation of food to and through the gradually maturing conidia. Section VB comprises conidial state of Monascus ruber and Trichothecium roseum with its meristematic region restricted to the production of one conidium at a time and its basipetal chains of originally isolated conidia. Works of Kendrick and his co-workers have greatly added to the knowledge of different types of conidiochore and conidium development.

Recently Hartman (1971) has used Teflon membrane slide culture technique for phase microscope observations which facilitate observations of nuclear status in new growing mycelia as well.
In the following pages, an account of diagnostic features, taxonomy and conidium ontogeny of some of the writer's collections of Fungi-Imperfecti is presented with detailed description of individual species.

1. **Cladosporiella** Deighton Mycol. Papers 101: 34 (1965)
   
   F. D ematiaceae
   O. Moniliales

   This Cladosporiaceous new genus was established by Deighton (1965) for a mycoparasite infecting colonies of **Cercospora** with **Cladosporiella cercosporicola** as type. It differs from **Cladosporium** in its long narrow pluriseptate conidia and from "**Stenella**" and "**Biharla**" in being entirely smooth. Its mycoparasitic habit is also distinctive.

   Writer reported an unidentified species of **Cladosporium** infecting telial sori of **Puccinia sodmii** P. Henn affecting **Polygonum chinensii** L. (Ullasa, 1968). This has been later described as **Cladosporiella urdinis** by Deighton (1969). A brief description of writer's collection is presented here.

   **Cladosporiella urdinis** Deighton (Figs. 33.1 to 33.4)

   Colonies olaceous green, dense, lying above and around the telial sori 0.5 to 1 cm in diameter. Mycelium composed of pale to sub-hyaline olaceous green, smooth, septate, branched hyphae, extending into the surrounding leaf tissue, above and inside the telial sori the hyphae aggregate to form stromata of pseudo-parenchymatous cells upto 20-60 x 30-90 μ deep. These stromata
produce numerous slender erect or weavy conidiophores measuring 50–250 x 2–5 μ, in diam. Conidia pale olivaceous, fusiform, straight or curved, thin-walled, smooth, 0–2 septate, non-constricted at the septum, catenulate in chains, 12.5–35 x 3–6 μ. Detached conidia frequently proliferate and produce secondary conidia.

Development of conidia: Conidia develop as blastospores from the conidiophores, young conidia are blown out from the tips of the conidiophores. Septa are laid down at maturity. 2–4 conidia are seen in chains produced in accropetal succession. Such type of conidia are termed as "Blastospores" and included underHughes's Section IA (Hughes 1953) or "Blastosporae" of Tubaki (1963).


2. Epicoccum Link Mycol. Obs. 2: 32 (1816)
   F. Dematiaceae,
   O. Moniliales

Epicoccum nigrum Link (Figs. 33.5 to 33.6)

This fungus was isolated from the dead berries of grapes and the morphology and the development of conidiophore and conidia were studied in artificial culture.

Young colonies on P.D.A. are whitish in appearance. As they grow older produce concentric rings of deep yellowish orange sporogenous layers.
The mycelium is hyaline, septate, isolated or intertwining with numerous anastomoses. Individual hyphae measure 4 - 6 µ in thickness, while intertwining hyphal strands measure 15-30 µ in diameter. These anastomosing hyphae produce conidiophore directly from the surface as blown out ends. The tips of these conidiophores are directly converted into conidium. 1-3 septation may be laid down in the conidiophore or it may be non-septate, 4-30 x 6-15 µ in diameter. Conidia are dark coloured, multi-septate, separated from the conidiophore by the disintegration of the cells of the conidiophore, conidia measure 15 - 25 µ in diameter.

Development of conidium: This type of conidium development where the conidiophore bears solitary apical thick-walled and coloured conidium and thereby terminating the growth of the conidiophore has been included under his Section III by Hughes (1953). Such type of conidium development has been reported in case of Trichocladium opacum, Coniosporium paradoxum and in Sporidemminum larvatum by Hughes (1953). Similar type of conidium has been referred as solitary auroiospores by Tubaki (1963) under his group "Aleuriosporae". This type of spore development is also consistent with the gangliospore type of development as defined by Subramanian (1962, 1970).


3. Cercoosporidium Earle Muhlenberia 1: 16 (1901)
   F. Dematiaceae
   0. Moniliiales
Cercosporidium deightoni sp. nov. (Figs. 34.1 to 34.3)

Colonies, hyphophyllous, irregular, separate, coalescing, brownish in colour, velvety, 1-8 x 1-3 μ in diam. Conidiophores simple, unbranched, non-septate, erect or bent, yellowish to pale brown, densely crowded on a stroma developed sub-epidermally 20-40 x 4-7 μ. Conidia acrogenous, porospores produced as blown out ends from inside the conidiophores, napiform to obclavate, 1-4 septate, conidial scars not prominently thickened, lower cell brown in colour, pale above with attenuated obtuse apex, 15-40 x 6-8 μ.


Conidium development: The conidium development is typical of "porospore" of Tubaki (1963) or as in Hughes' Section VI. The conidium develops from inside the conidiophore as a blown out end through a minute pore on the conidiophore singly; such conidia are produced on lateral sides of the conidiophore successively leaving several conidial scars.

Remarks: Cercospora mundulae Sacc. & Syd. (1904) on Mundulae suberosa possesses much paler conidia and measures upto 70 μ in length and 1-2 septate.


F. Dematiaceae
O. Moniliales
Caaptoneris albizzia (Petch) Mason in Hansford (Fig. 34.4)

Infection spots hypophyllous, black, circular, scattered, rarely aggregated 0.5 mm in diam. Basal stroma lacking, conidio-phores simple unbranched, non-geniculate, non-septate, stout, brown, densely crowded in a tuft, emerging through the stromata, with 6-8 conidial scars at the tip, 25-40 x 8-10 μ. Conidia acrogenous, produced singly as blastospores, obclavate, slightly bent in the apical region, 3-4 septate tapering towards the apex, uniformly brown, finely verrucose, 40-60 x 10-12 μ.


Conidium development: Conidia develop as blastospores in a manner described for Virgiella atra and Dactylosporium macropus in his section II by Hughes (1953) or 'Blastospores' of Tubaki (1963).

Remarks: The type material of Prathlgada indica Muthappa (M.A.C.S. Herb. No. 273) was examined and found to agree with Camptomeris albizzia and hence is treated as synonym.

5. Protostroma Batista Revista de Biologia 1: 109 (1957)

F. Sphaeropsidaceae
G. Sphaeropsidales

The genus Protostroma was established by Batista in 1957 with Protostroma hyphaeneae as type. Writer's collection infecting graminaceous host is described here as a new species.
Protostroma graminicola sp. nov. (Fig. 34.5)

Stromata dark, carbonaceous, shining, epiphyllous, 0.1-0.2 mm in diam. Intra-epidermal in development. Pycnidia develop inside the stroma. 2-10 in a stroma, many times coalesce to form large locules. Locules measure 20-100 x 20-30 μ in diameter. Conidiophores are short, borne from the inner cells of the pycnostroma, hyaline, slightly tapering towards the apex, 6-8 x 2-4 μ in diameter, in wall layers. Conidia globose to oval, ellipsoidal, hyaline, smooth walled, single celled, 6-8 x 3-5 μ.

Habit: On living leaves of Themeda sp. collected at Khandala in June 1968, M.A.C.S. Herb. No. 1241 (Type).

Development of Conidium: The tip of the conidiophore develops and produces a single conidium in the beginning, followed by the successive development of conidia as phialospores. Conidiophores act as phialides and produce a succession of conidia. An indistinct collerette is seen at the point where the first conidium was cut off.

Hughes (1953) included fungi producing phialospores under his Section IV, and Tubaki (1963) grouped these types of fungi having phialides, under his group "Phialosporae".

Remarks: The genus Protostroma is a new generic record to India.


F. Dematiaceae

O. Moniliaceae

Spiropes gauricola (Stev) Ciferi (Fig. 34.6)
Colonies effused dark, blackish brown, to black, hairy, mycelium superficial, pale olavaceous brown, smooth, septate, 2-4 μ thick. Conidiophores arising singly or in groups as lateral branches on the hyphae, erect, sterile lower part is straight and flexuous upper fertile part is zigzag. Septate, brown to dark brown, pale near the apex, with numerous well defined conidial scars, upto 150-200 μ long, 6-8 μ thick. Conidia formed singly as blown out ends, at the apex of the conidiophores, and the tips of the new growing points which develop alternately below the previous terminal conidium, broadly fusiform, pale to dark brown, smooth, 3-4 septate, 25-35 x 10-12 μ in diameter.

Habit: Overgrowing the colonies of Meliola sp. on Musa indica. Collected at Coorg, October 1968, M.A.C.S.Herb. No. 1238.

Conidium development: The type of conidium development is termed as "blastosporic" by Hughes (1953) and fungi producing such spores have been included under his Section II. Tubaki (1963) included fungi producing this type of conidia under his group "Terminoradulosporae".

7. Stigmina Sacc. Michelia 2: 22 (1881)

F. Dematiaceae
O. Moniliales

The genus Stigmina was established by Saccardo in 1881 with Stigmina platani (Fkl.) Sacc. as type. Ellis (1959, 1963) has extensively worked on this fungus and has provided keys for the identification of several species. Writer's collection made at
Coorg was identified as a species of *Stigmata* and is described here.

*Stigmata kamati* sp. nov. (Figs. 35.1 to 35.8)

Stroma hypophyllous, punctiform, in the beginning, then circular, dark, mycelium immersed in the substratum. Composed of branched septate hyphae, hyaline to sub-hyaline, smooth walled 2-3 μ in thickness. Stroma develop sub-epidermally rupturing the epidermis, exposing the developing conidiophores, light brown, pseudoparenchymatous, 25-70 μ wide, 30-50 μ in height. Conidiophores arising from the upper cells of the stroma, straight, cylindrical, dark brown, slightly paler at the apex, always roughly and irregularly warted, 20-100 x 3-6 μ in diam. with successive 3-8 anallations (proliferation). Conidia formed singly at the apex of each conidiophore, which proliferates through the scar after the first conidium has fallen and forms another conidium at a higher level, straight, cylindrical, oblong, slightly broader at the base, rounded at the ends, dark brown, verrucose, 1-2 septate, 16-30 x 5-7 μ in diameter.

Habit: On living leaves of *Syzygium caryophyllum*. Collected at Coorg, 15th October 1968, M.A.C.S. Herb. No. 1239 (Type).

Conidium ontogeny: First a single conidium develops at the apex of the conidiophore and subsequent conidial development is attained through its proliferation through the conidial scar left by the previous conidium. Successive repetition of this proliferation
results in the development of successive conidia accompanied
by a successive increase in the length of the conidiophore,
thus leaving several annelations. Fungi having this type of
conidium development are included by Hughes (1953) under his
Section III and by Tubaki (1963) in his group "Aleuriospora".

Remarks: Since there is no report of this fungus on the host
family it is described as new species. The fungus is closely
associated with another sphaeropsidaceous fungus viz. Kamatella.

8. Ramakrishnanella Kamat and Ullasa in Ullasa


F. Sphaeropsidaceae
O. Sphaeropsidales

Comparative studies between Phyllachora mahabaleshwarensis
Ananth. (Ananthanarayanan 1964) parasitizing Embelia viridiflora
Scheff. collected by the writer in January 1969 from Mahabaleshwar,
Poona and the type material of Physalospora anamalaiensis T.S. & K.
Raman. on Embelia ribes Burm. obtained from HClO, New Delhi,
No. 18821, revealed the close association of a pycnidial fungus
with Physalospora anamalaiensis on Embelia ribes. Although this
pycnidial fungus and its close association with P. anamalaiensis
was reported by Ramakrishnan and Ramakrishnan (1950), it has not
been assigned to any known genus of the Sphaeropsidales. Critical
study of the fungus was undertaken and a detailed diagnosis is
presented.
Ramakrishnanella Kamat and Ullas Gen. Nov. (Figs. 36.4, 37.4, 38.5, 38.9)

F. Sphaeropsidaceae

O. Sphaeropsidales

Class Funzi Imperfecti

Pycnidia distinct, innate, immersed deep in the mesophyll tissue, isolated, non-stromatous, globose, ostiolate, non-clypeate, ostiole non-papillate, pycnidial wall in 2-4 layers of irregular hyaline cells. Conidiophores simple, non-septate, hyaline, clavate in wall layers. Conidia (gangliospores) dark brown, oblong, zonate, produced singly by the transformation of apical portion of the conidiophore. Type species Ramakrishnanella indica.

Ramakrishnanella indica sp. nov.

Infection spots brown, follicolous, somewhat erumpent, indefinite, 5 x 8 mm. Pycnidia epiphyllous, punctiform, developing deep in the mesophyll tissue, sphaerical to globose, isolated, non-stromatous, 100-140 x 80-120 μm, ostiolate, ostiole non-papillate, non-clypeate, 15-20 μm in diameter. Pycnidial wall in 2-4 layers of irregular hyaline cells measuring 2-6 μm. Conidiophores in wall layers, simple, hyaline, non-septate, clavate, narrow at the base, broader at the top, 25-30 x 3-5 μm. Conidia (gangliospores) are produced by the transformation of the apical portion of the conidiophore, solitary, oblong, dark-brown, 1-celled, broader at the base, slightly narrow at the tip with an equatorial hyaline band, 12-14 x 8-9 μm.
Habit: Infecting living leaves of *Emblica ribes* Burm. Leg. 

Discussion: *Ramakrishnanella indica* shows some similarities with the following fungi: *Poropeltis davillae* P. Henn. var. *mediofasciata* Batista and Herrera (1964), *Lasenla macrosperma* Batista and Silva (1964), *Manginula leucospermi* Batista and Maia in Batista et al. (1936), *Asterostomella veronicae* (Desm.) Arn. (Batista and Ciferri, 1959), *Asterostomopsis ghanaensis* Batista and Maia in Batista and Ciferri (1959) and with the conidial state of *Phaeochorella parinarii* (P. Henn.) Theiss. & Syd. as described by Swart (1965). In all of these fungi the conidia are ellipsoidal to oblong and have an equatorial hyaline band closely resembling the fungus under study. However, these fungi are known to produce their conidia in a pycnoastroma and not in a true pycnidium. These characters clearly distinguish the writer's fungus from any of the known genera of the Sphaeropsidales.

The most noteworthy characters of *Ramakrishnanella* distinguishing it from any of the known pycnidial fungi are the non-stromatic nature of the pycnidia, the clavate non-septate conidiophores, the conidia with an equatorial hyaline band and an exogenous mode of production through direct transformation of the apical portion of the conidiophores. These characters have not been duplicated in any of the known form genera belonging to the Sphaeropsidales and therefore, justify the establishment of
the new genus. *Ramakrishnanella* is named in honour of Dr. T.S. Ramakrishnan and Dr. K.Ramakrishnan in recognition of their pioneer contributions in the field of Indian Mycology and Plant Pathology.
Plate 33

Figs. 1-4. *Cladosporiella uridinae* Deighton

1. Habit, 2. Photomicrograph showing infection through the germ pore of the upper cell of the teliospore.

3. A. Section through the *Polygonum chinense* leaf with the telium affected by hyperparasite. B. Section showing sub-epidermal stroma of hyperparasite with conidiophores, C. Conidiophores and conidia, D-E. Infection through the germ-pores, F. Infection through the pedicel, G. Direct infection of the teliospore.

4. Photomicrograph showing infection through the teliospore pedicel.

Figs. 5-6. *Epiconium nigrum* Link.

5. Photomicrograph showing anastomosed hyphae producing numerous conidiophores.

6. Photomicrograph showing mode of formation of young conidia through the direct transformation of the conidiophore tips.
Plate 34

Figs. 1-3. *Cercosporidium deightonii* Ullasa sp. nov.

1. Photomicrograph showing conidiophores and conidia.
2. Photomicrograph showing conidia.
3. A. Habit, B. Section through the infection spot, C. Conidium ontogeny, D. Conidia.

Fig. 4. *Camptomeris albissia* Syd.

A. Habit, B,C. Conidiophores, D. Conidia.

Fig. 5. *Protostroma graminicola* Ullasa sp. nov.

A. Habit, B. Section through the pycnostroma, C. Developing conidiophores and conidia, D. Conidia.

Fig. 6. *Spiroplaga gauricolor* Ciferi

A. Conidiophore showing zigzag nature with prominent conidial scars and developing conidia in some of their tips, B. Conidia.
Plate 35

Figs. 1-8. Stigmella kamatii Ullasa sp. nov.

1. Habit,

2. Photomicrograph of section through a young developing sub-epidermal colony,

3. Photomicrographs showing conidiophores producing young conidia at their tips. Note the annellations and proliferations through the old conidial smear.

4. Photomicrograph showing separation of matured conidia from the conidiophores.

5-7. Photomicrographs showing nature of conidiophores. Note the annellations and bifurcations.

6. Conidia.
CHAPTER II

COMPARATIVE CONIDIUM ONTOGENIC STUDIES IN SOME Sphaeropsidaceous INDIAN FUNGI

During his routine mycological collections from Coorg forests, Mysore State, India, the writer made several collections of interesting Ascomycetes having diaporthaceous affinity constantly associated with Sphaeropsidaceous fungi producing deciduous versicolor conidia singly over the conidiophores. He has also observed some type materials of similar nature from different herbaria while making comparative studies.

Critical studies on conidium ontogeny were undertaken in respect of the following four Sphaeropsidaceous fungi, the results of which are presented here.

Materials studied:


2. Lasmenia globulifera (Nab.) Hohnel associated with Pseudothis cocodes Syd. on Acasia initia.

3. Mycohypallage congesta (Berk. & Br.) Sutton associated with Plagiostigme deodikari Anant. on Syzygium cumini.


Materials and Methods: The four foliicolous fungi obtained at different stages of development were hand sectioned and observed in distilled water or with lactophenol stained with cotton blue.

Observations and results:

1. Kamatella longipedicellata

The association between the ascigerous and conidial state is always very close and constant. The conidiophores develop as blown out ends from triangular cells of the inner wall layer of the pyenidiu. The conidiophores are very thin at the base with a swollen apex which enlarges into a conidium like "gangliospore". As the conidium matures it turns dark brown and becomes unequally septate. Two circular hyaline zones develop on the flat sides of the upper cell and probably act as germ pores. No evidence of proliferation of the conidiophores was observed. A single conidium develops from each conidiophore (Figs. 36.1, 37.1, 38.1, 38.6).
2. Lasmenia globulifera

The stromatic pycnidia of this fungus are constantly associated with a diaporthaceous ascomycete with a phyllachoraceous stroma. The fungus is very similar to *Kamatella* in its conidial characters except that the pycnidiospores are one celled. The mode of development of conidiophore has a pattern very similar to that of the previous fungus in the formation of first conidium. The conidiophores are produced from the inner cells of the pycnostroma with their tips enlarging into conidia. At maturity the pycnidium develops an ostiole. Although the mode of conidium ontogeny, agrees in general with the "*anelliospore*" mode of development in the initial spore formation it differs in that outer wall of the conidiophore splits irregularly at maturity forming a colletette as shown by Sutton in species of *Malenconium* (1969). After the first conidium is cut off, the succession of conidial development may take place through the proliferation of conidiophores and the conidia in such cases are in the nature of anelliospores (Figs. 36.2, 37.2, 38.2, 38.3).

3. Mycohyphellae congesta

This Sphaeropsidaceous fungus is closely associated with an Ascomycete *Plagiostigma deodikari*. Although the mode of conidium ontogeny was studied by Sutton (1963), writer noted several important aspects not previously reported. The irregular pycnidium has a poorly defined wall, which consists of prosenchymatous interwoven hyphae developing in the host mesophyll. The pycnidium
enlarges lysogenically dissolving host cells and in the process develops an lysogenic ostiole. The conidiophores develop from the loosely interwoven hyphal cells of the inner prosenchymatous wall layers. The conidiophores are simple and generally unbranched but are occasionally branched and septate. They are initially spear like, the apical end of the conidiophores turns into an appendage. The 2-5 additional appendages develop basipetally and alternately. At the same time the central portion of the conidiophore which is destined to become conidium enlarges and becomes broader at the apical portion, bearing appendages and with a narrow base where a septum develops separating it from the conidiophore. Thus the conidium development is intercalary and ultimately turns dark brown and becomes septate. Protoplasmic continuity with the appendages has not been seen at this stage and is separated from the mother conidiophores through the development of a septum (Figs. 36.3, 37.3, 38.4, 38.8).

4. Ramakrishnanella indica

This pycnidal fungus occurs in close association with an Ascomycete, Physalospora anamolaisensis. The wall of the pycnidium consists of pseudoparenchymatous cells and the conidiophores develop from the cells of the inner layer of the pycnidial wall in the form of blown out structures which are elevated from the very beginning with their upper middle portion swollen. The enlargement of the conidiophore is uniform at all stages of development. The formation of the conidium is by the direct
transformation of the uniformly enlarged conidiophore unlike as in "gangliospores" type of development where the apical portion of the conidiophore enlarges and differentiates into conidium. At maturity the conidium becomes thick walled, turns dark with a hyaline zone in the centre of the conidium.

At maturity the conidium becomes thick-walled, turns dark with a hyaline zone in the centre of the conidium. No septum was found to develop separating the conidium from the mother conidiophore. The base of the conidium acts as a septum and breaks from the conidiophore. The conidiophore at this stage appears like a phialide with a wide opening. This process of conidium ontogeny has been mistaken for endogenous development by Ramakrishnan T.S. & K.(1950)(Figs. 36.4, 37.4, 38.5, 38.9).

Discussion: The pattern of conidium ontogeny studied in above mentioned four fungi is basically different.

In case of Kamazella the conidiophores and conidium development is essentially similar to the "gangliospore" type of development. Such type of conidium development has been reported by Sutton (1964, 1968) in Aristatoa, and in Kellermania and Scolecosporiella; by Sutton and Sellar (1966) in Tosisporiopsis and by Subramanian (1953) in Petromycetes, and basically these agree with that of Hughes' Section II or III (Hughes 1953), or of Tubaki's "Aleurospores", Tubaki (1943) where the conidia are in the nature of solitary aleuriospores. Conidia are not produced in succession and thus no annexations are noticed on their conidiophores.

In Lasmania after the first conidium is separated a distinct colleratte is seen and successive conidia are produced from the
proliferations of the conidiophores through the older conidiophore. This type of conidium development has been reported by Sutton and Sandhu (1969) in species of Cryptosporiopsis, Phoma and Malanconium. This type of conidium development agrees with that of Hughes' Section III and group "Aleuriospore" of Tubaki.

In Mycohyphallage the conidiophore does not develop from the pseudoparenchymatous cells of the wall as reported by Sutton (1983), but arises from the hyphal cells of the loose prosenchymatous wall of the pycnidium. The conidiophore is spear like the tip of which develops into an appendage and the central portion of the conidiophore develop into conidium proper and thus the mode of conidium development is intercalary, and sub-apical and similar to chlamydosporophore formation.

The mode of conidium development in Ramakrishnanella is through the direct transformation of the apical portion of the conidiophore without the intervention of a septum which has been described as "gangliospore" type of development by the writer earlier (Ullasa 1970). The protoplasmic contents aggregate near the tip of the conidiophore. As the conidiophore matures a hyaline zone develops separating the conidium proper. No septum is laid down, except for the development of the basal portion of the conidial wall. The conidium except for the basal portion consists of the wall of the conidiophore proper. Thus the nature of formation and separation of conidium may be termed as "arthrosporic" in nature and distinct from "gangliospore" type of development. The comparative conidium ontogeny in 4 Sphaeropsidaceous fungi are diagrammatically represented in Figs. 38.6 to 38.10.
Plate 36

Fig. 1. A. Section through the infection spot showing association of *Ramatella longipedicellata* (T.-S. & K. Ramakr.) Ullasa and *Muelleromyces variisporus* (Died.) Ullasa.

B. Ascus and ascospores,

C. Developing conidiophores and conidia.

Fig. 2. A. Section through the infection spot showing association of *Lasmenia globulifera* (Rab.) Hohnel, and *Pseudothis cocodes* Syd.

B. Ascus and ascospores, C. Conidiophores and conidia.

Fig. 3. A. Section through the ascocarp showing association of *Mycohyphalge congesta* (Berk. & Br.) Sutton and *Plagiostigma deodikari* Anant.

B. Ascus and ascospores,

C. A portion of conidiophore with attached conidium.

Fig. 4. A. Section through the infection spot showing association of *Ramakrishnamella indica* Ullasa and *Physalospora annamalaiensis* T.S. & K. Ramakr.

B. Ascus and ascospores,

C. Conidiophores and conidia.
Plate 37

Fig. 1. *Kamatella longipedicellata* (T.S. & K. Ramakr.) Ullasa.

A. Habit, B. Section through the pycnidium, Note the clypeus and definite ostiole, C. Conidiophores and Conidia at different stages of development.

Fig. 2. *Lasmenia globulifera* (Rab.) Hohnel.

A. Habit, B. Section through the Pycnostroma, C. Development of conidiophore and conidia, D. Conidia.

Fig. 3. *Mycobyphellage congesta* (Ferk. & Br.) Sutton

A. Habit, B. Section through the pycnidium, C. Development of conidiophores and conidia, D. Conidia.

Fig. 4. *Ramakrishmanella indica* Ullasa

A. Habit, B. Section through the pycnidium, C. Conidiophores and conidia at different stages of development, D. Matured conidia.
Fig. 1. Photomicrograph showing developing conidiophores and conidia in *Kamatella longipedicellata* (arrow indicates the young developing conidiophore and conidium).

Fig. 2. Photomicrograph showing developing conidiophores and conidia (arrows indicate the young developing conidiophore and conidium) of *L. globulifera*.

Fig. 3. Photomicrograph showing proliferated conidiophore through the old conidiophore producing conidium (arrow indicates the collarette of old conidiophore) of *L. globulifera*.

Fig. 4. Photomicrograph of a portion of pycnidium of *Mycobryophallace congregata* showing conidiophores and conidia at different stages of development.

Fig. 5. Photomicrograph of a portion of a pycnidium of *Ramakrishnanella indica* showing conidiophores and conidia at different stages of development.

Figs. 6-10. Diagrammatic representation of conidiophore and conidium development in *Kamatella indica*, *Lasmenia globulifera*, *Mycobryophallace congregata* and *Ramakrishnanella indica* respectively. Fig. 10 indicates 'gangliospore' type of conidium development in *Drechslera* (after Subramanian, 1970).
PART V

GENERAL DISCUSSION
PART V

GENERAL DISCUSSION

I. Studies into Balansia claviceps Spag.

The work presented in the foregoing pages in Balansia claviceps var. indica in respect of developmental pattern of ascoecarp, centrum characters, mode of sexual reproduction, origin of asci and nuclear events, and chromosome complement prove the occurrence of remarkable uniformities within this group and close relationship with the allied genera Cordyceps, Claviceps and Epichloe.

Luttrell (1951) considered this group of Ascomycetes as a distinct unit in having "Claviceps" type of asci and "Xylaria" type of development and included it under Xylariales (Sphaeriales) as a separate family. However, he further suggested that this group differs from "Xylaria" type of development in having fasciculate arrangement of asci and their origin in clusters instead of in wall layers and further thought that on this basis there may be some justification for placing this family in a separate order, as originally suggested by Nannfeldt (1932). This group has been recognized as a separate order by Gaumann (1952), Arx and Mueller (1954), Dennis (1968) and recently by Rogerson (1970). The intensive investigations carried out by the writer in respect of several fundamental characters of this fungus together with those of previous workers in other allied genera like Cordyceps, Claviceps and Epichloe have proved the highly homogenous character and specialized position of this family as a well knit
The rare type of "spindle overlap" and crossing over of nuclei as observed by the writer during the third nuclear division in *Balansia claviceps* var. *indica* explain the abnormal arrangement of ascospores in the disordered ascus patterns which are, however, generally of rare occurrence and could be interpreted on the basis of abnormal nuclear and
spindle orientation leading to the nuclear displacements which have been already summarised by Emerson (1966).

Besides, the fungus Balansia has been brought in artificial culture for the first time and the life cycle pattern determined on the basis of conidial as well as ascigerous (perfect) states thus convincingly proving the true genetic relationship between the Ascigerous (Balansia) state and its conidial (Ephelidial) state, Ephelis.

II. Studies into Cryptomyces muelleri Ullasa

On the basis of foregoing account on developmental pattern of ascocarp, its structure, nature and ontogeny of inter ascal filaments and mode of sexual phenomenon and the sequence of events followed during ascocarpic development by this fungus the following aspects need consideration:

1. Developmental pattern of ascocarp centrum,
2. Ontogeny and nature of interascal filaments,
3. Mechanism of sex and
4. Taxonomic position and relationship of this fungus within the order Phacidiales.

1. Developmental pattern of ascocarp centrum:

The overall developmental pattern of ascocarp centrum in this fungus has very close similarities to that reported by Jones (1926, 1935) in Lophodermium pinnastri and Rhytisma acerinum, by Thyr and Shaw (1966) in Hypodermella arcuata, by
Gordon (1966, 1968) in several species of *Lophodermium*, Woo and Partridge (1969) in *Rhytisma punctatum* and by Bellemere (1967) for lenticular apothecial genera all of which are members of the apothecial order Phacidiales, which according to Dennis (1968), is a heterogenous group comprising of both ascogenous and ascolocular members as defined by Nannfeldt (1932), Miller (1940) and Luttrell (1951). Similar type of ascocarpic centrum has been reported in members of the Patellariaceae (O. Hysteriales) which are characterised by bitunicate asci and forcible ejection of the ascospores which are the main characters of the series Ascoloculares. The ascocarp in the latter series has been described as "DISCOTHICUM" by Korf (1962) and such apothecial fungi with bitunicate asci have been designated by Luttrell (1955) as "Bitunicate discomycetes" to differentiate them from the true unitunicate Discomycetes. A discussion on this aspect of study and evolutionary line of development has been presented by Muthappa (1967a, 1967b), Seshadri (1967), Seshadri and Muthappa (1969), Anahosur (1969a, 1969b, 1969c) and recently by Kamat and Anahosur (1971) for members belonging to the family Patellariaceae. On the other hand, Bellemere (1967) working with *Lacanidion atratum* and Muthappa (1970) with *Tryblidiella olavispora* (F. Patellariaceae) found these two fungi to occupy an intermediate position between the two series the Ascoloculares and the Ascochy Utilities, in respect of developmental pattern of ascocarp, its centrum characters, and nature and origin of asci and interascal filaments.
On the other hand, similar type of ascomycetic development has been reported for members of the Pyrenomycetes by Kowalski (1965) who working with *Didymosphaera sadassavani* a unitunicate form, reports the occurrence of pseudoparaphyses in a true perithecium. Thus the developmental pattern of the ascomarp, centrum characters, the nature and ontogeny of the interascal filaments and above all the occurrence of spermatization and nature of sexual process in this fungus are characteristic which appear to be intermediate between the two main large subclasses of Ascomycetes viz. the Loculoascomycetes and Euroascomycetes.

2. **Ontogeny and nature of interascal filaments**

The vertical hyphae in the present fungus have a distinctive ontogeny and intercalary mode of growth besides playing important role in the enlargement and development of ascocarp. Although these filaments are pseudoparaphysate in early stages as defined by Luttrell (1965) having an intercalary mode of development and completing their development long before the origin of asci, in the final analysis these hyphae appear as true paraphyses becoming free at the apical region and producing a distinct layer of pseudoepithecium above, through a lysogenic process thus having all the characteristics in this respect of members of the Discomycetes.

3. **Mechanism of Sex**

The pattern of sexuality in this fungus has both evolutionary as well as retrogressive line of development ranging from true spermatization to somatogamous copulation thus combining the characters of both Ascohymeniales and Ascoloculares.
It is thus clear that the fungus Cryptomyces combines both ascolocular and ascohyphomerial characters referred to above. The position thus appears to be extremely conflicting and would seem to do away with the distinction between the two large subclasses of Ascomycetes, the Ascoloculares and Ascohymeniales. Such conflicting results must, however, be considered as only apparent in view of occurrence of several overlapping and intergraded forms within the two large subclasses, the Ascohymeniales and the Ascoloculares, during the process of evolution and where all types of combination of characters are encountered in respect of developmental type, ascocarp centrum, tunicate nature of asci and their apical apparatus, nature and origin of interascal filaments, and mode of sexuality as already reported by previous workers in both Ascoloculares as well as Ascohymeniales.

A detailed report on this aspect of evolution within the class Ascomycetes has been made by Chedfau (1960) and recently by Kamat and Anahosur (1971).

4. Taxonomic position and relationship of this fungus within the order Phacidiales.

The apothecial order Phacidiales is related to the Helotiales through several intergraded forms (Korf, 1962). Several mycologists have recognised this group by the presence of stroma, its uniloculate character, typically rupturing along straight or several longitudinal lines and producing dark black crusts over the host surface. This order is considered by Dennis (1968)
to be heterogenous in character belonging to both ascohymenial and ascolocular forms. Nannfeldt (1932) treated this taxon as a family of Helotiales. Bessey (1950) and Wolf and Wolf (1947) followed Nannfeldt. On the other hand several workers like Terrier (1942), Fitzpatrick and Korf (1959), Gaumann (1964) and Dennis (1968) recognised it as a separate order. Terrier (1942) considered this group as heterogenous consisting of both ascohymenial and ascolocular forms. Fitzpatrick and Korf (1959) followed Terrier (1942) in recognising the three families, Hypodermataceae, Rhytismaceae and Phacidiaeae. von Arx and Mueller (1954) recognised the family Cryptomycetaceae. Recently Kinbrough (1970) divided the order into three families viz. Hypodermataceae, Rhytismaceae and Phacidiaeae following in this respect Terrier (1942) and Fitzpatrick and Korf (1959) and considered the family Cryptomycetaceae synonymous with Hypodermataceae. The forms included under the family Hypodermataceae are characterised by the production of dark crustaceous folicular infection spots, stromatic in nature, uniloculate in character with dark stromatic basal as well as covering layers exposing the hymenial part through irregular rupture along one or more lines.

On the basis of detailed studies into the developmental pattern of the asocarp, presence of stroma, centrum characters, nature and origin of intertheelial threads, unitunicate and non-amaloid nature of asci and above all its habit on the host in forming the bright folicular crusts rupturing through irregular wear and tear along one or more lines, the fungus Cryptomyces muelleri Ullasa should find a place under the family Hypodermataceae, Order Phacidiales.
III. Taxonomic studies in some Indian Ascomycetes

Besides the above fundamental aspects of investigations into the two Ascomycetes, the writer made a large collection of ascomycetous fungi from Coorg forests (Mysore State) and in and around Poona (Maharashtra State) and made a detailed comparative study of these fungi resulting in the description of three new genera viz.

1) *Arxonia* Kamat and Ullasa (F. Hemiphalidiales)
2) *Chadefaudia* Kamat and Ullasa (F. Diaporthaceae)
3) *Deshpandella* Kamat and Ullasa (F. Diaporthaceae)

The nomenclatural status of the closely associated genera *Muelleromyces* (Diaporthaceae) and *Kamatella* (Sphaeropsidaceae) affecting *Syzygium cumini* has been determined. In accordance with the code of Botanical Nomenclature new combinations are proposed as *Muelleromyces variisporus* (Died.) comb. nov. for ascigerous fungus and *Kamatella longipesicellata* (T.S. & K. Ramakr.) comb. nov. for pycnidial fungus. The new combination *Polystigma mahabaleshwarensis* (Anant.) comb. nov. is proposed for *Phyllachora mahabaleshwarensis* Anant. since the type material of this species did not possess any characters of the genus *Phyllachora* but completely agreed with the genus *Polystigma*.

The two species of Ascomycetes viz. *Physalospora anamalensis* T.S. & K. Ramakr. and *Endodonthella kanarensis* T.S. Ramakr. and Sund. were determined to be facultative synonyms of *Plectosphaera embelia* (Yeats) von Arx and *Stigmochora deightonii* (Syd.) von Arx respectively.
Cocconia placenta (Berk. & Br.) Saec., Seynesia erumpens (Berk. & Curt.) Pat. and Naemocyclus korfii, N. arxii are new generic records to India. Besides several species of Ascomycetes have been described some of which are new to science and some are new reports to India.

IV. Taxonomic and ontogenetic studies in some Fungi Imperfecti

In addition to the taxonomic studies into some of the Indian Ascomycetes, writer also collected some species of Fungi Imperfecti and their taxonomic and ontogenetic studies were carried out.

A new genus of the Sphaeropsidales viz. Ramakrishnanella indica Kamat and Ullasa has been established after Dr. T. S. Ramakrishnan and Dr. K. Ramakrishnan and the following three new species have been described viz.

1) Cereosporidium deightonii Ullasa sp. nov.
2) Stigmina kamati Ullasa sp. nov.,
3) Protostroma graminicola Ullasa sp. nov.

The species Prathigada indica Muthappa has been determined as a synonym with Camptomeris albizzia (Petch) Masson with which it agreed in all respects. Besides a brief account of nature and mode of spore production (conidium ontogeny) has been determined and described in each case. The conidia are in the nature of 'Blastospores' in Cladosporiella urdinis and in Camptomeris albizziae; 'gangliospores' or in the nature of
'solitary aleuriospores' in Epicoccum nigrum; 'porospores' in Cercosporidium deightoni; and 'phialospores' in Protostroma graminicola.

Comparative conidium ontogenic studies have been carried out in four sphaeropsidaceous fungi closely associated with Ascomycetes viz. 1) Ramakrishnanella indica, 2) Kamatella longipedicellata, 3) Lasmenia globulifera, 4) Mycohyphallage congesta.

The mode of conidiophore and conidium development is typical of 'gangliospore' type of development in Ramakrishnanella indica and in Kamatella longipedicellata but the spore delimitation and separation appears to be 'arthrosporic' involving no constriction at the base of the conidium in Ramakrishnanella. The conidiophores are 'annellophoric' in Lasmenia globulifera and have a distinct collarette when the conidium is cut off and succession of conidia are formed through the proliferation of old conidiophores. The nature and production of conidia in Mycohyphallage congesta is intercalary and subapical and similar to chlamydosporale formation and the appendages are in the nature of modified apical region of the conidiophores proper.
PART VI

RESEARCH PUBLICATIONS
During the course of his research work writer has published the following research papers and some are awaiting publication.


(with V.C. Rao)
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