STUDIES IN SOME INDIAN ASCOMYCETES

A Thesis

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MAHARASHTRA KRISHI VIDYAPEETH

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[AGRICULTURE]

IN

MYCOLOGY AND PLANT PATHOLOGY

By

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Type material of all new species has been deposited at the M.A.C.S.Herbarium under appropriate accession number and will be also deposited at the Herb. Orientalis, New Delhi, India and C.M.I., Kew, England.

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

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EXPLANATION OF PLATE 1

Tryblidaria maharashtrensis

Fig.1.1. Habit.

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GENERAL INTRODUCTION

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As the Ascomycetes with richness of their pattern and morphology of the ascocarp represent an extremely heterogenous group of fungi, the taxonomist is often confronted with the intricate problem in proper arrangement of the various groups comprising them with a phylogenetic relationship. Lindau (1897) in Engler and Prantel (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, consistency, habitat, manner of opening and other structural characteristics. He recognised three types of ascocarps while proposing his classification viz. 1. Cleistothecium : the ascocarp is completely closed and the asci and ascospores are released by the disintegration of the ascocarp wall. 2. Perithecium : Flask shaped ascocarp with a true perithecial wall and a true ostiole for the release of asci and ascospores. 3. Apothecium : Cup or disc shaped ascocarp in which the asci 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.

Considerable work has since been done in the recent years on cytology, sexuality, developmental pattern of the ascocarp centrum, nature of the ascus wall, the manner of ascus dehiscence and the presence or absence of interthecial threads their nature and origin which are of fundamental importance in the proper understanding and rearrangement of this complex group

of fungi. These investigations have greatly contributed to the discovery of several fundamental aspects in the internal structures, development, cytology, sexuality and the nature of ascus which after all represent the fundamental organs of the Ascomycetes which would eventually lead to the phylogenetic system of classification in place of the present artificial groupings. The present trend is to recognise in this class two distinct series viz. Ascoloculares and Ascohymeniales of Nannfeldt (1932), Pseudothecium and Perithecium of Miller (1949), and Bitunicatae and Unitunicatae of Luttrell (1951), based on the fundamental characters such as the origin and development of the ascocarp 'centrum', arrangement of asci their structure and dehiscence, the presence of paraphyses, pseudoparaphyses, interthecial tissues and apical paraphyses, their nature and origin. Various workers viz. Von Hohnel (1907-1923), Arnaud (1918-1925), Orton (1924-1944), Petrak (1907-1923), Nannfeldt (1932), Hansford (1946), Wehmeyer (1948-1954), Miller (1949), Chadefaud (1942-1965), Luttrell (1951-1965), Munk (1953), Arx and Muller (1954), Holm (1958), Martin (1961), Muller and Arx (1962), Korf (1954-1962) and many others have variously contributed to these fundamental aspects, which would eventually help in formulating a phylogenetic system of classification in place of artificial groupings thus shifting emphasis on the more fundamental criteria derived from the study of the ascocarp 'centrum' as originally proposed by Nannfeldt (1932) and subsequently emphasised by Miller (1949).

In India the Ascomycetes have received some attention in respect of their taxonomy in the form of enumeration of species and preparation of regional lists. Unfortunately very little attention has been paid to the sphere of developmental pattern of the ascocarp centrum, sexual reproduction, conidial stages, nuclear behaviour and other related phenomena. The recent Indian contributions in this field of investigations of Ascomycetes are those of Tilak (1959), Ananthanarayanan (1964), Kalani (1965), Patwardhan (1966), Jagtap (1967), Seshadri (1967), Muthappa (1967) on some of the typically tropical species of the genera Parodiella, Rosenscheldiella, Cyclotheca, Pseudopeziza, Elsinoe, Phyllactinia, Tryblidiella, Mycosphaerella and Lembosina. As the position is still unsatisfactory in relation to tropical species which have received scant attention from this point of view, it was proposed to undertake an intensive investigation into some of the commonly occurring typically tropical Indian Ascomycetes, viz. species of Tryblidaria (Sacc.) Rehm., Lecanidion Endl. and Tryblidiella Sacc. all of which belong to an obscure group, the Hysteriales.

These three genera were specifically selected for these investigations as no attempt has been made in the past to study these genera in respect of their fundamental aspects with a view to determine their exact taxonomic position since these three genera were treated as true Discomycetes by previous workers. Especially the genus Tryblidiella is often confused with the allied genus Hysterium and therefore the investigations into the

fundamental aspects of this genus were undertaken which would help to determine its true taxonomic position as this genus has been treated under Ascohymeniales by some workers and Ascoloculares by others.

A large number of collections of Ascomycetes were also made at the forests of Coorg (Mysore State, India) and in the vicinity of Poona (Maharashtra, India) during the period of this research project and the various genera so collected were studied critically in respect of their morphology and taxonomy.

These taxonomic studies often revealed close associations of conidial fungi and even spermogonial bodies with the ascigerous stages in nature, the exact significance and nature of which needed further examination and study through cultural studies with a view to establish the true life cycle pattern followed by these fungi. Life-cycle studies were therefore undertaken into some of the commonly occurring species of the genera Bagnisiella Speg. and Didymosphaeria Fckl. under artificial culture

The research work carried out by the writer for the past three years is presented in the following parts :

Part I : deals with Taxonomy, Internal morphology, structure and development of ascocarp, sexual reproduction and origin of asci, ascus dehiscence, nuclear behaviour, chromosome complement and ascospore formation in three members of the family Patellariaceae (O. Hysteriales).

Part II : deals with the life cycle studies of

1) Bagnisiella acaciae Anahosur.

2) Bagnisiella australis Spag.

3) Didymosphaeria saprophytica Anahosur.

in artificial culture.

Part III : is based on the taxonomic studies into some Indian Ascomycetes collected from Coorg and Maharashtra.

Part IV : General Discussion.

Part V : Research Publications.

Materials and Methods

The genus Tryblidaria occurring abundantly as a saprophyte on the dead branches of Lantana camara in the Law College hills of Poona (Maharashtra, India) was collected during September 1967, which persists during all seasons of the year. The genus Lecanidion occurring saprophytically on the dead branches of Caesalpinnia pulcherrima in the Law College Hills of Poona was collected during August 1968 which is also found throughout the year. The genus Tryblidiella was collected as a saprophyte on the dead branches of Scutia indica at Coorg forests (Mysore State, India) which was also found to persist throughout the year as observed by the writer during his frequent visits. These fungi were fixed in various fixatives for the cytological and developmental studies. On the spot, fixation was also made in field at different intervals to secure adequate stages of

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) Carnoy'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 Carnoy'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 the materials fixed in Cornoy's fluid and F.A.A. in case of Tryblidaria and Lecanidion and in F.A.A. in case of Tryblidiella.

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

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

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

iii) 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, paradichloro-benzene 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 paradichlorobenzene and methyl alcohol were found to give 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), Johanson (1940) and Purvis et al (1966) which is 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 (1945), 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) Mordant and destainers :

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

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

As the Squash techniques did not yield satisfactory results for the study of nuclear behaviour and its divisional stages, the study was made entirely with the microtome sections stained with Heidenhein's hematoxylin.

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

Special treatments :

1) Melzier's reagent :

Iodine	.....	1 gm.
Potassium iodide	....	1 gm.
Distilled water	....	100 cc.

used for determining the tunicate nature of the ascus wall.

2) As the ascocarps of Tryblidiella are carbonaceous in consistency, 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 1 month. Satisfactory results were obtained. This method was followed throughout the studies.

Sectioning : Sections at 5-10 μ were cut with Rotary microtome during cool hours for the nuclear studies and developmental studies. Hand sections of the materials were employed for the study of morphological characters of various collections of fungi for the purpose of taxonomic studies.

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PART - I

STUDIES INTO TAXONOMY, INTERNAL MORPHOLOGY, STRUCTURE AND DEVELOPMENT OF ASCOCARP, SEXUALITY, NUCLEAR BEHAVIOUR, CHROMOSOME COMPLEMENT OF THE THREE MEMBERS OF THE FAMILY : PATELLARIACEAE.

SECTION - A

STUDIES INTO TRYBLIDARIA MAHARASHTRENSIS ANAHOSUR

CHAPTER 1

TAXONOMY OF TRYBLIDARIA MAHARASHTRENSIS ANAHOSUR

Introduction.

The genus Tryblidaria (Sacc.)Rehm. was established by Saccardo in 1889 as a sub-genus and was later revised by Rehm (1904). The type species is Tryblidaria fenestratum (C. & E.) Rehm. (Syn. Blitrydium fenestratum C. & E.). Regarding the authority of this genus Clements and Shear (1931), Muller (1957) and others have quoted as Tryblidaria Sacc. But according to the code of International Botanical Nomenclature Article No.49, 1956 Tryblidaria (Sacc.)Rehm. is suggested in "Dictionary of Fungi" and the writer has adopted the same throughout his studies.

Saccardo (1889, 1899) places this genus under Patellariaceae of the order Pezizales based on the gross morphological characters. Clements and Shear (1931) also follow this treatment.

Nannfeldt (1932) has included this genus under Patellariaceae with affinities to Discomycete Lichens.

Luttrell (1955) considers the genera like Lecanidion, Johansonia and Tryblidiella and others having discoid ascocarps and bitunicate asci as "bitunicate discomycetes". which according to him should find a place under Hysteriales of Loculoascomycetes.

Muller and Arx (1962) place the genera Eutryblidiella and Rhytidhysterium in the order Dothiorales of Ascoloculare series. Martin (1961) recognises two families in order Hysteriales on the basis of manner of opening and shape of the ascocarp viz.

1. Hysteriaceae - characterised by elongated ascocarps with narrow cleft. 2. Patellariaceae - characterised by discoid ascocarps with wide cleft.

Luttrell (1965a) follows Martin (1961) in this respect.

As this genus is of rare occurrence, little is known about its exact taxonomic position. All this confusion regarding the taxonomic position of this genus is due to the lack of ontogenic and developmental studies of the ascocarp centrum in this genus which would greatly help to clear the taxonomic position and its inter-relationship within the Ascomycetes.

Thus there was an urgent need for a detailed study into the developmental pattern of the ascocarp centrum, nature and mode of origin of interthecial threads, and asci, pattern of sexuality, the manner of ascus dehiscence, nuclear behaviour and other related phenomena in this rare genus before any definite conclusions could be drawn regarding its exact taxonomic status. An intensive investigation was, therefore, undertaken into the fungus Tryblidaria maharashtrensis Anahosur growing saprophytically on the dried twigs of Lantana camara L. the results of which are presented in the following pages.

Description and diagnosis of the fungus (Figs.1.1 to 1.6)

Tryblidaria maharashtrensis Anahosur

(Sydowia 1969 in Press).

Discothecia soft to leathery, scattered to aggregated, black with pinkish tinge, margin raised, central portion bulged in the

early stages and depressed later, discoid, upto 2 mm. in diameter. Locules cup-like, closed in the beginning and open circularly before ascospore discharge, unilocular, rarely aggregated, 0.8-2 mm. broad and 0.25-0.4 mm. high. Asci clavate, bitunicate, pedicellate, uniformly 8-spored, in basal layers, arising amongst the interthecial threads, 140-180 x 38-46 µ. Ascospores oblong to ellipsoid, typically muriform, arranged biseriately in the ascus, yellow to light brown in colour, 36-42 x 14-20 µ. Interthecial threads hyaline, septate, slender, profusely branched in the apical region. The tips of the interthecial threads together with the remnent of the stroma at the top represent 'Epithecium like' structure which disintegrates at maturity.

Habit : Saprophytic on the dead twigs of Lantana camara L. (Verbenaceae) collected by the writer at the Law College Hills, Poona (Maharashtra) India during February 1967. M.A.C.S.Herb. No. 685.

Remarks : As this fungus was collected on a new host, a comparison was made with the type species with the following results :

Table 1

Comparison between species of Tryblidaria (Sacc.) Rehm.

Species	Ascocarp	Asci	Ascospores
T.fenestratum Rehm (type)	Black, discoid	Subclavate, 4-8 spored	45-50 µ long.
T.Maharashtrensis Ambekar	Black with pinkish tinge, discoid	Clavate, uniformly 8-spored.	36-42 x 14-20 µ.

It is clear from the above table that the writer's collection i.e. Tryblidaria maharashtrensis is significantly distinct from the type in having uniformly 8-spored asci, smaller spores and also collected on an entirely new host, and hence has been accommodated into a new taxon. The Latin diagnosis has been provided elsewhere.

CHAPTER 2

INTERNAL MORPHOLOGY, STRUCTURE AND DEVELOPMENT OF ASCOCARP, SEXUALITY AND ORIGIN OF ASCI AND ASCUS DEHISCENCE.

The genus Tryblidaria (Sacc.)Rehm. which has very limited distribution was abundantly available at the Law College,Hills in the vicinity of Poona (Maharashtra) India and persists throughout the year saprophytically on the dead twigs of Lantana camara L. This genus was specially selected for a detailed study into the internal morphology, the development of the ascocarp centrum, nuclear behaviour and other related phenomena since no work of this type had been previously reported. Also its easy availability and typically tropical nature induced the writer to undertake an intensive investigation into the above mentioned aspects of this fungus.

Historical Review

So far no work is reported in this genus in respect of developmental pattern of the ascocarp centrum, nuclear behaviour, sexuality and other related phenomena. However, Rogg (1943), Luttrell (1953) and recently Seshadri and Muthappa (1969) have worked out in detail the developmental pattern in the allied fungi like Hysterographium fraxini, Glonium stellatum and Tryblidiella rufula respectively all of which have been classified under Hysteriales.

The mode of sexual reproduction in this group as far as is known is of retrogressive type. However, Rogg (1943) and

Luttrell (1953) have reported the presence of ascogonia in the young ascocarps of Hysterographium fraxini and Glonium stellatum respectively. Zogg (1948) also has observed the copulation between somatic hyphae and ascogonia which probably results in plasmogamy. Gaumann (1950) defines this type of sexuality as loss of Gametangy. In Tryblidiella rufula, sexual reproduction is brought about through somatogamy according to Seshadri (1967) and Muthappa (1967).

Morphology and early development :

The fungus develops saprophytically on the dead twigs of Lantana camara L. The ascospores discharged from the older ascocarps happen to lodge on the same as well as close by dried twigs and germinate under favourable conditions producing as many germ tubes as the number of cells in the spores. It was observed under the laboratory conditions that the ascospores germinated in 12-14 hours on solidified water-agar medium putting out 8-10 germ tubes originating from all the cells ultimately producing a small colony within 48 hours (Fig. 2.1).

The germ tubes of the germinated spores probably enter the host and a hyaline intra-cellular mycelium is formed which ramifies in all the cells as well as below the epidermis(Fig.2.2). The mycelial mat formed in the xylem vessels (Fig.2.3) turn brown and later dark-brown forming a small spherical stromatic body within the xylem vessels just below the epidermis (Fig.2.4). This small stromatic body resulting through the aggregation of the brown mycelium is the ascocarp initial which due to its

further expansion exerts pressure as a result of which the wall of the xylem vessels and the epidermis are ruptured resulting in the exposure of this ascocarp initial thus assuming an erumpent nature (Fig. 2.5). The further development of the fungus takes place external to the surface of the host.

The terminology employed here for describing the various parts of the ascocarp are those adopted by Corner (1929) and Korf (1962).

All observations on the developmental pattern of the ascocarp 'centrum' and other phenomena are based on fixed microtome sections.

Transections through young developing ascocarp revealed that the fruit body was entirely made up of dark-brown thick-walled, pseudoparenchymatic cells representing the cortex. The young ascocarp was completely closed at this stage with an irregular margin. No sex organs were found responsible for the initiation of the ascocarp indicating its purely vegetative nature and origin as against the true Discomycetes such as Helvella crispa Fr. (Carruthers 1911), H. elastica Bull (McCubbin 1910), Morchella conica Pers. and M. esculenta (L) Pers.(Gries 1950) whose sex organs are first differentiated around which the ascocarp development takes place. Further development takes place through the differentiation of the young developing ascocarp into two distinct parts (Fig. 3.1).

1. The outer thick-black layer made up of thick-walled dark brown pseudoparenchymatous cells representing the wall layer of the ascocarp.

2. The inner central region (Centrum) comprising of thin-walled, hyaline, deeply staining pseudoparenchymatous to prosenchymatous polygonal cells, which is considered to be the predetermined cavity for the future developing asci and other associated structures. At this stage also no specialised sex organs were observed as reported in the allied fungi Hysterographium fraxini and Glonium stellatum but showed similarity to Tryblidiella rufula.

Later it was observed that the "centrum" develops vertical hyphal cells which appear to originate in the apical region of the ascostroma and are arranged one below the other showing downwards growth (Fig. 3.3). These hyphal cells are attached at top indicating their origin from the top of the ascostroma and are uniformly uninucleate. These vertical cells extend upwards as well as downwards through intercalary growth thus bringing about the vertical growth of the ascocarp by pushing up the apical stromatic part. In the initial stages these vertical hyphal cells lie at different angles but the typical vertical arrangement of these cells becomes apparent as the ascocarp develops further and reaches advanced stage (Fig. 3.3).

At this stage, thread-like hyphae develop at the bottom and periphery of the ascocarp which help in bringing about the horizontal as well as marginal distension of the ascocarp similar

to the mechanism described by Corner (1929) and Seshadri and Muthappa (1969) (Fig. 3.3).

In the advanced stages of ascocarp development, these hyphal cells appear thread-like due to their characteristic vertical growth (Fig.4.1). These are termed as "vertical hyphae" in the following account and are found to be continuous from the top to the bottom of the ascocarp and merge into the lower hypothecial layer. The "vertical hyphae" are thicker at the peripheral region of the ascocarp and thinner in the central region (Fig.4.2). This may be due to the rapid vertical growth of the ascocarp which in turn is the result of successive divisions of these vertical cells and pressure created by the adjoining developing cells.

The ascocarp develops both horizontally as well as vertically thus bringing about the distention of the lateral walls of the ascocarp giving the developing ascocarp a discoid shape. However the "vertical hyphae" are still attached at the top and merged with the hypothecium at the bottom (Fig. 4.3). Branching of these vertical hyphae at their apical region was also noticed frequently at this stage (Fig.4.3). At later stages following the origin of asci profusely branched "vertical hyphae" were visible. It was also observed that the layer of stromatic cells at the top is thin and tears off easily during sectioning the ascocarps (Fig. 4.2). This upper thin layer is made up of tips of the branched vertical hyphae entangled with the disintegrated stromatic cells appears like an epithecial layer over the hymeniu

Thus the whole locule is filled with these "vertical hyphae" still attached at the top and merged in the hypothecial region at the bottom (Fig. 5.1). The topmost layers of stromatic cells together with the intermingled tips of the branched vertical hyphae appear like an epithecium over the developing asci still covering the hymenium similar to Tryblidiella rufula.

Gradually young asci begin to develop and grow vertically amongst these "vertical hyphae" which complete their development long before the development of asci (Fig.5.2, 5.3, 6.1 to 6.3) in a manner to be described under sexuality.

These branched vertical hyphae intermingle with the remnents of the stromatic cells of the ascocarp at the top layer resulting in the formation of a thin layer of epithecium-like structure at the final stage (Fig.7.1) without the features and ontogeny of the true epithecium produced in members of the Discomycetes belonging to Asechymeniales and is similar to the structure described for Tryblidiella rufula as reported by Seshadri and Muthappa (1969), and Hypodermella arcuata (Thyr. and Shaw, 1968).

No definite cleft was observed in this fungus as in Tryblidiella rufula and other members of Hysteriales (Figs.7.1 & 7.2). Before the discharge of asci, the upper 'epithecium like' structure is torn out indicating the circular opening along margin of the discoid ascocarp (Figs.7.3 & 7.4).

Thus overall developmental pattern of the ascocarp centrum is similar to that of Hysterographium fraxini , Glonium stellatum

and Tryblidiella rufula defined as "Gloniun stellatum" type by Luttrell (1953) with typical bitunicate asci and "vertical hyphae".

Sexual Reproduction

No specialised sex organs have been observed in the young developing ascocarp as noted in the case of Hysterographium fraxini and Gloniun stellatum. The earliest signs of origin of asci is the presence of deeply staining uninucleate hyphae in the hypothecial layer (Fig.8.1). Two uninucleate hyphal strands lying close by copulate dissolving the intervening walls in the manner similar to cell fusions observed in rust fungi and is similar to 'somatogamy' reported in Tryblidiella rufula by Seshadri and Muthappa (1969) (Figs.8.2 & 8.20). This somatogamous copulation results in the formation of a dikaryotic cell which has the tendency to branch further and the dikaryons divide conjugately thus resulting in the production of dikaryotic ascogenous cells through proliferation (Figs. 8.3 to 8.6 & 8.21 to 8.22). These dikaryotic cells proliferate vertically as well as horizontally in the hypothecial region (Figs. 8.7 to 8.13 & 8.23 to 8.26). Such phenomena of proliferation of ascogenous cells has been previously reported in Dothidea collecta, Trichometasphaeria taurica (Luttrell 1951a, 1964) and recently by Seshadri (1967) in Mycosphaerella mysorensis. The two nuclei making the dikaryons in the dikaryotic ascogenous cells fuse to produce diploid nuclei which are double the size (3.5 - 4.0 μ) of the two fusing nuclei (1.2 - 1.9 μ) (Figs.8.12, 8.13 & 8.25, 8.26).

This is followed by the vertical growth of the diploid cell directly into an ascus without the intervention of croziers (Figs. 8.14 to 8.19 & 8.27 to 8.28).

Thus the sexual phenomenon in this fungus can be considered to be of a degenerate type, as encountered in lower Ascomycetes and is similar to 'somatogamy' as reported in Tryblidiella rufula except in the proliferation of dikaryotic ascogenous cells immediately. "Somatogamy" has been reported in many members of the true Discomycetes of Ascohymeniales series as well. In Rhytisma acerinum (Pers.) Fr. the mode of sexuality is in the nature of parthenogamous copulation (Jones 1925) and in Ascobolus carbonarius, it is spermatization (Dodge 1912). However several members of this group (Hysteriales) like Hysterographium fraxini, Glonium stellatum the exact manner of dikaryotization has not been definitely determined although ascogonia have been reported. In Glonium stellatum microconidial (Spermogonial) chambers have been observed but their definite role in the dikaryotization has not been determined (Luttrell 1955). In Tryblidiella rufula the sexual reproduction takes place through 'somatogamy' (Seshadri and Muthappa 1960).

Description of the mature ascocarp (Fig.9.1)

In the young ascocarp the upper stromatic layer is bulged and the ascocarp appears spherical. But the well developed ascocarp, appears discoid. The upper thin stromatic layer resulting from the intermingling of the tips of the branched vertical hyphae and the

remnants of stromatic cells appears like an Epithecium-like structure which is torn out and disintegrates at the later stages just before the ascus dehiscence, thus exhibiting its Hemiangiocarpous nature imparting it the appearance of a cup-fungus. A well developed ascocarp comprises of the following different parts.

1. Epithecium :

This structure is heterogenous in nature and is a thin layer formed by the intermingling of the tips of the branched vertical hyphae and the remnants of the stromatic cells at the top. It lacks the characteristics of a true "epithecium" commonly observed in the Discomycetes (Asechymeniales). The structure varies in thickness varying from 12-18 μ and is very uneven and made up of little pockets. The origin and composition and nature of this structure are similar to that reported in Hypodermella sulcigena by Thyr and Shaw (1966) and Tryblidiella rufula by Seshadri and Muthappa (1969).

2. Vertical hyphae :

These hyphae make their appearance very early in the young ascocarp long before the development of asci. They originate from the stromatic cells lining the top of the ascostroma and show tendency to extend downwards becoming attached at the top and bottom before the development of asci. Actually their tips are merged within the hypothecial layer at the bottom. Their growth is intercalary. They are sparsely branched before the development.

of asci but branch profusely in the well matured ascocarp indicating their active growth even after the origin of asci. The vertical growth of these hyphae pushes away the top layer of the ascocarp which consequently becomes thin in the mature ascocarp. The cells of the vertical hyphae become vacuolated at the later stages when ascocarp attains its fullest development.

3. Hymenium :

It is a fertile layer comprising of clavate bitunicate asci arranged in basal layers interspersed with branched vertical hyphae.

4. Asci :

They are bitunicate, clavate, pedicellate with apical projection, arranged in basal layer, intermingled with vertical hyphae and originate long after the vertical hyphae.

5. Ascospores :

Oblong to ellipsoid, light brown, typically muriform, arranged biseriately in the ascus.

6. Hypothecium :

A thick layer of loosely interwoven hyaline hyphae just below the hymenium measuring upto 12.0 μ thickness and is the place where sexual differentiation takes place resulting in the origin of numerous dikaryotic ascogenous cells.

7. Exeipulum :

It is made up of pseudoparenchymatous and prosenchymatous cells. According to Korf (1962) and also recently adopted by Muthappa (1967a) the exeipulum comprises of two parts.

a) Ectal exeipulum : It is made up of thick-walled dark brown pseudoparenchymatous cells and provides protection to the ascocarp centrum.

b) Medullary exeipulum : It is made up of hyaline to sub-hyaline prosenchymatous cells and its function is to support the hymenium.

Ascus dehiscence and ascospore discharge (Figs.9.2 & 9.3)

It was Butler (1939) who first described an unique method of ascus dehiscence viz. "Endoascus" type in Leucaidion stratum belonging to this group of fungi. Originally Fringscheim (1958) had described a similar mode of ascus dehiscence in Sphaeria scirpii which was commonly known as "Sphaeria scirpii type" of ascus dehiscence. In the present fungus the ascus dehiscence is similar to that of Leucaidion stratum (Butler 1939) and Tryblidiella rufula (Muthappa 1967a). Under the influence of pressure the "ectoascus" ruptures with simultaneous elongation of the endoascus. The ectoascus ruptures at the tip or at the centre and forms a collar-like structure in the centre or at the base around the endoascus similar to be phenomenon obtained in Dothidea colleata (Luttrell 1951). The ascospores are liberated through the projection i.e. pore present at the tip of the

endoascus successively. The mechanism of ascus dehiscence as stressed by Butler (1939) and others is of great value in determining the broad taxonomic status of the fungus as it gives a clue to the tunicate nature of the ascus.

Discussion of Results.

The above studies into and observations on the developmental pattern of the ascocarp in *Tryblidaria maharashtrensis* Anahosur (F. Patellariaceae) revealed that it is essentially a loculo-ascomycete with bitunicate asci and "interthecial threads". The results so obtained during this study are summarized in brief :

1. The ascocarp develops as a single locule on the outer surface of the host without any prior sexual stimulus and lacks a definite opening in the early stages.

2. The centrum of the young developing ascocarp is made up of thin-walled hyaline prosenchymatic cells in the beginning later followed by the development of "vertical hyphae" which fill the locule and appear continuous from the top to the bottom of the ascocarp. These "vertical hyphae" were observed to originate in the apical region of the ascostroma from the stromatic cells lining the top of the ascocarp, and merging at the bottom with the hypothecial layer. Branching of the vertical hyphae was observed before as well as after the origin of asci. The "vertical hyphae" appear continuous from top to the bottom and complete their development long before the origin of asci which later grow among them,

typical of the "Gloeium stellatum pattern" of ascocarp centrum as defined by Luttrell (1953).

3. The manner of growth of the ascocarp in the early stages is similar to 'Hemiangiocarpic' type as described by Corner (1929) for true Discomycetes (Ascohymeniales series) except for the absence of ascogonia.

4. The function of sexual reproduction is taken over by somatic hyphal cells in the hypothecial layer and is similar to "somatogi

5. The dikaryotic cells proliferate vertically as well as horizontally resulting in the production of numerous dikaryotic ascogenous cells along the hypothecial layer followed by karyogamy.

6. The asci grow vertically directly from the diploid cells among the 'vertical hyphae' without intervention of crosiers and are arranged in the basal layers of the ascocarp in a parallel manner.

7. The horizontal and marginal growth of the ascocarp brings about the distention of the two ectal excipula, thus imparting a discoid shape to the ascocarp.

8. The actively growing "vertical hyphae" push away the upper thick layer of stromatic cells and together with the tips of the branched vertical hyphae form an "epithecium-like" structure over the hymenium.

9. The thin layer i.e. "epithecium like" structure is thrown out before the discharge of ascospores thus exposing the hymenium typical of "Hemiangiocarpic" form of ascocarp.

10. Ascus dehiscence is "endoascus type" similar to that obtained in Lecanidion atratum and Tryblidiella rufula.

The pattern of the development of the ascocarp centrum agrees closely in respect of the presence of bitunicate asci and "vertical hyphae" to Gloniun stellatum, Hysterographium fraxini and Tryblidiella rufula with a "Gloniun type" of centrum but differs from the former two in having no ascogonia and from all the three in having no predetermined cleft.

It has been already mentioned earlier that this genus was included under Pezizales, belonging to Discomycetes of Ascohymeniales series mainly on the basis of gross morphological characters without any ontogenic studies in respect of developmental pattern of the ascocarp "centrum" which has gained vital taxonomic importance in the modern system of classification of this heterogeneous group of fungi. Miller (1928,1949) for the first time suggested the rearrangement of this heterogenous group mainly based on the "ascocarp centrum" as the chief character and this concept was later emphasized and elaborated by Nannfeldt (1932), Wehmeyer (1926, 1955) and Luttrell (1951, 1955, 1965a).

The ascocarp in this genus develops heimangiocarpously and is closed till the time the ascospores are discharged at which stage the "epithecium like" structure is torn off giving the ascocarp a discoid shape with a circular opening. The present developmental studies carried out by the writer conclusively prove this cup fungus to be definitely ascoloculare in its affinities in all respects.

According to Luttrell (1965) the distinctive vertical paraphyses-like hyphae developing in the centrum of Loculoascomycetes prior to the formation of asci are properly termed as "pseudoparaphyses". The other type described in the literature as "paraphysoides" are homologous to "pseudoparaphyses".

The 'vertical hyphae' or 'interthecial threads' referred to in this treatment combine characters of 'pseudoparaphyses' on the one hand and true 'paraphyses' on the other hand in respect of their ontogeny, origin and nature.

Recently, Kowalski (1965) defines 3 types of sterile threads in the Ascomycetes.

1. Paraphyses similar to those defined by Luttrell (1965).
2. Paraphysoides which are attached at the top as well as bottom of the ascocarp from the beginning.
3. Pseudoparaphyses which originate from the top and become free at the bottom.

The 4th type described by Luttrell (1965) is termed as Apical paraphyses which originate at the top and become free at the bottom. This type is characteristic of members of the Hypocreales and "Neetria" type of development and is homologous to the "Pseudoparaphyse" of Kowalski (1966).

Subsequently Kowalski (1966) working with Preussia funiculata has described the hyphae which are attached at the top and bottom of the ascocarp from the very beginning as "Paraphysoides". But

according to Luttrell's (1965) definition the "paraphysoides" and "pseudoparaphyses" of Kowalski (1965, 1966) appear to be the same as they originate and complete their development long before the origin of the asci.

In view of their nature, origin, their essentially intercalary growth and their origin long before the development of asci and above all their free and branched apices, the 'vertical hyphae' obtained in Tryblidaria maharashtrensis are closely similar in respect of their nature and development to those described by Dodge (1937), Chesters (1938), Jones (1926), Wehmeyer (1955), and recently by Kowalski (1965), Luttrell (1953, 1964), Gordon (1966, 1968), Corlett (1967), Kennedy and Stewart (1967), Seshadri and Muthappa (1969). Although Gordon (1966, 1968) has worked on true Discomycetes like Lophodermium juniperinum and L. nitens belonging to Asccohymeniales series characterised by unitunicate asci, the interthecial threads have been described by him as "pseudoparaphyses" on the basis of their ontogeny, development and origin. The apical branching of the 'vertical hyphae' in the present fungus was very common and such branching of the interthecial threads has been reported in Apiosporina collinsii (Kennedy & Stewart 1967) and in Tryblidiella rufula (Seshadri & Muthappa 1969). The tips of the 'vertical hyphae' become free when the epithecium-like structure disintegrates during ascus dehiscence or by damage. Accordingly on the basis of the writer's studies and observations, the 'vertical hyphae' obtained in Tryblidaria maharashtrensis Anahosur may be

termed as "Intercalary paraphyses", distinct from any of the four types of intertheecial threads defined by Luttrell (1955) and recently by Kowalski (1965).

The upper "epithecium-like structure resulting from the intermingling of the bulbous tips of the branched intertheecial threads with the remnants of the upper stromatic cells, is not a true epithecium so commonly obtained in true Discomycetes but has a heterogenous origin, composition and nature and is similar to the structure obtained in Hypodermella arcuata and Tryblidiella rufula. Hence it may be termed as "Epistroma" as described by Seshadri and Muthappa (1969) in the case of Tryblidiella rufula.

The mode of sexual reproduction is similar to the one observed in Tryblidiella rufula and may be termed as "somatogamy".

Although the gross morphological characters of this fungus indicate its affinities to apothecial fungi, the presence of bitunicate asci, the developmental pattern of the ascocarp centrum, the presence of apically free and branched intertheecial threads which are the important features of the Ascoloculare series, essentially support its place under the Ascoloculare series. Luttrell (1955) has aptly termed such fungi with cup-shaped ascocarp characterized by the above characters as 'Bitunicate Discomycetes". Earlier literature shows that such "Bitunicate Discomycetes" were placed in the Family Patellariaceae of the order Pezizales in inoperculate series, a few of which were

suspected to be allied to Lichens. As a matter of fact a few members of Patellariaceae have been actually transferred to Lecidiaceae of Cyclocarpinae of Lichens (Butler 1939). Butler was of the opinion that the members now included under Patellariaceae are heterogeneous and indicate their relationships to Phacidiaceae, Tryblidiaceae, Hysteriaceae as well as Lecidiaceae in respect of the character of the ascocarp, asci and ascospores. However, he was of the opinion that the genera like Tryblidiella and others which have an endoascous type of ascus dehiscence are more closely related to the genus Leconidion (F. Patellariaceae).

Luttrell (1955) was of the opinion that the genera of Patellariaceae, the Bitunicate Discomycetes, such as Tryblidiella, Leconidion, Johanssonia, and others ultimately should find a place under Hysteriales of Ascoloculare series. Muller (1957) was of the opinion that the genus Tryblidaria having bitunicate asci is similar to Dothiora Fr. and may be placed under Patellariaceae of Dothiorales of Ascoloculare series. Martin (1961) recognised two families in Hysteriales (1) Hysteriaceae, characterised by elongated ascocarps with narrow cleft. (2) Patellariaceae with discoid ascocarps and wide cleft. According to him Tryblidaria, should find a place in Patellariaceae.

Korf (1962) has pointed out that such fungi which develop their fructifications reminiscent of an apothecium having bitunicate asci are not true Discomycetes but are regarded as

EXPLANATION OF PLATE 2

Trybliderie maharashtrensis

Fig. 2.1. Photomicrograph of the germinated ascospore obtained from water agar medium.

Fig. 2.2. Photomicrograph showing the intra-cellular mycelium in the xylem vessel.

Fig. 2.3. Photomicrograph showing the intra-cellular mycelium aggregated in the xylem vessel.

Fig. 2.4. Ascocarp initial in the xylem vessel.

Fig. 2.5. Photomicrograph showing emerging ascocarp initial by breaking the xylem vessel exhibiting crumpled nature.

EXPLANATION OF PLATE 3

Tylidiella subcarnosula

Fig. 3.1. Photomicrograph of the young ascocarp showing outer black thick-walled pseudo-parenchymatic cells and the central region of hyaline cells.

Fig. 3.2. Photomicrograph of young developing ascocarp showing deeply stained "Vertical Cells" (the apical paraphyses initials) in the apical region.

Fig. 3.3. Photomicrograph of developing ascocarp showing further elongation of vertical cells and horizontal growth of the ascocarp.

EXPLANATION OF PLATE 4

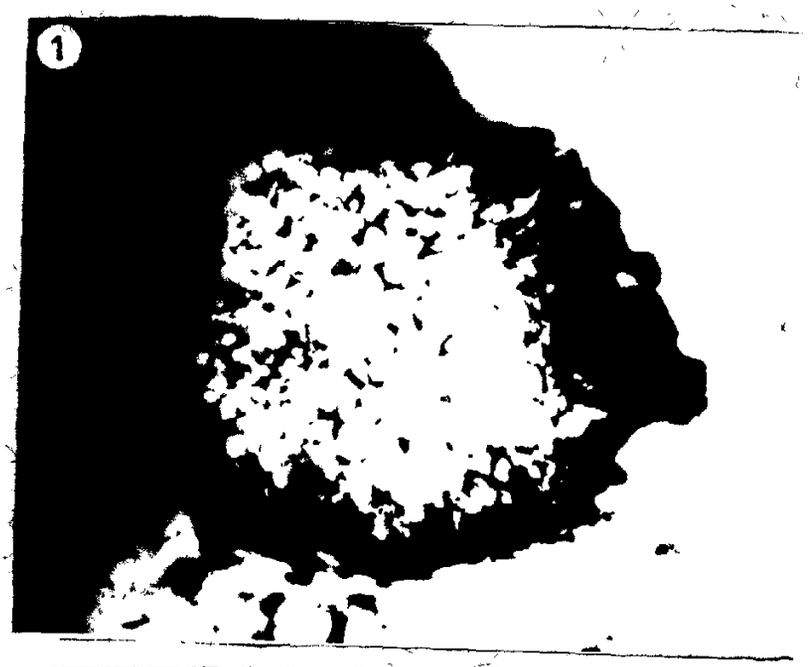
Tryblidaria sabarashtrensis

Fig. 4.1. Photomicrograph of the developing ascocarp showing further elongation of vertical cells which have become hyphae-like.

Fig. 4.2. Photomicrograph of the ascocarp showing thread-like "vertical hyphae" in the centre and thick cells in the periphery. Note the ruptured epistroma.

Fig. 4.3. Photomicrograph showing the hypothecial region (deeply stained hyphal strands at the base) and the tips of the "vertical hyphae" at the bottom merge with this layer. Note the sparsely branched vertical hyphae.

PLATE 3



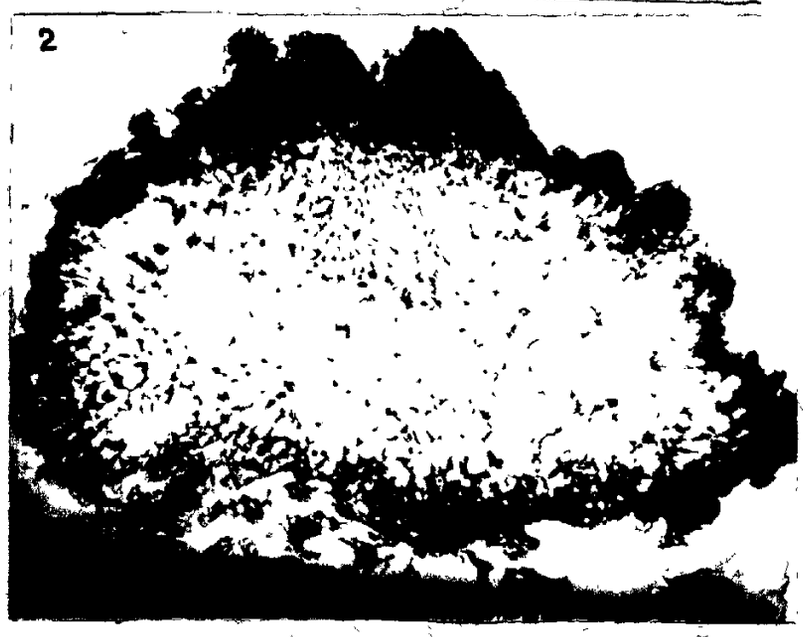
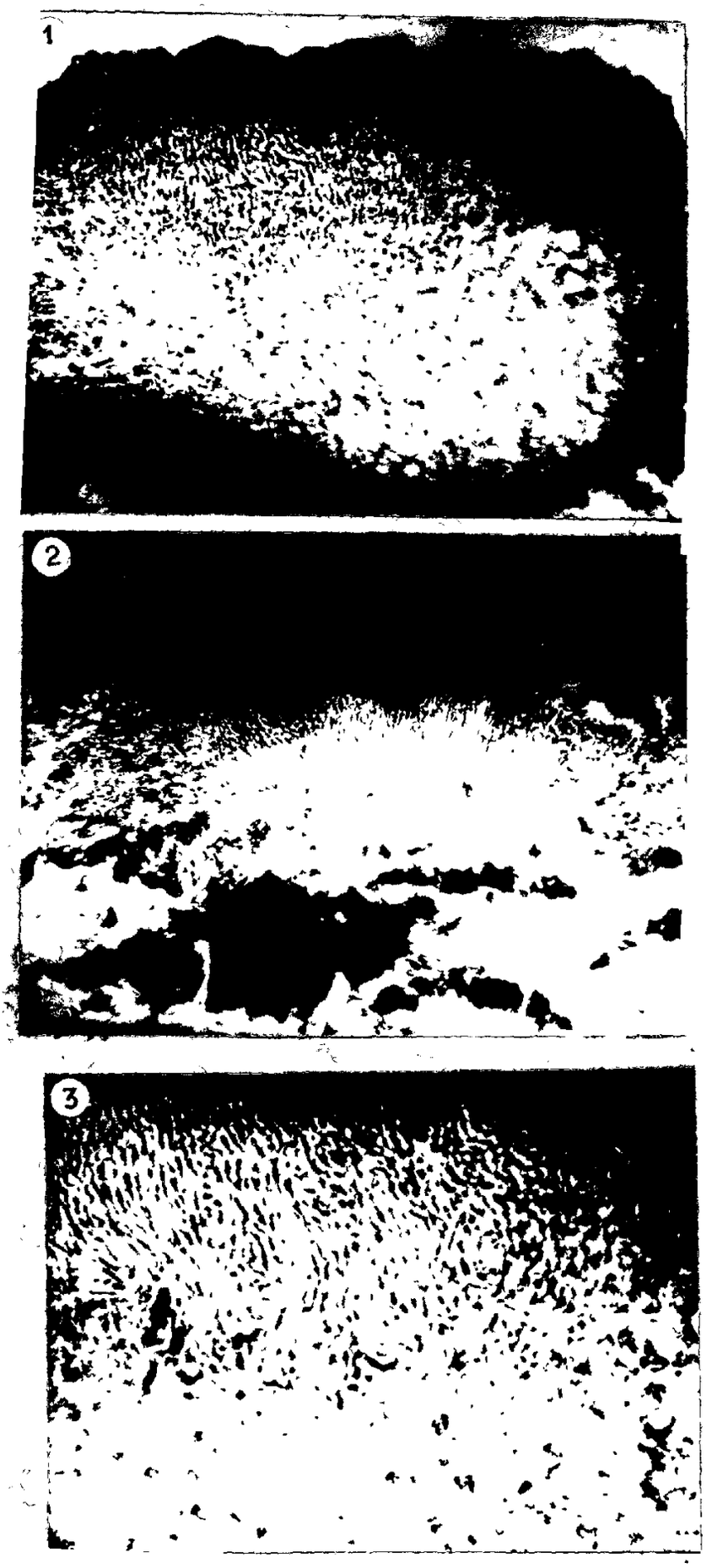


PLATE 4



EXPLANATION OF PLATE 5

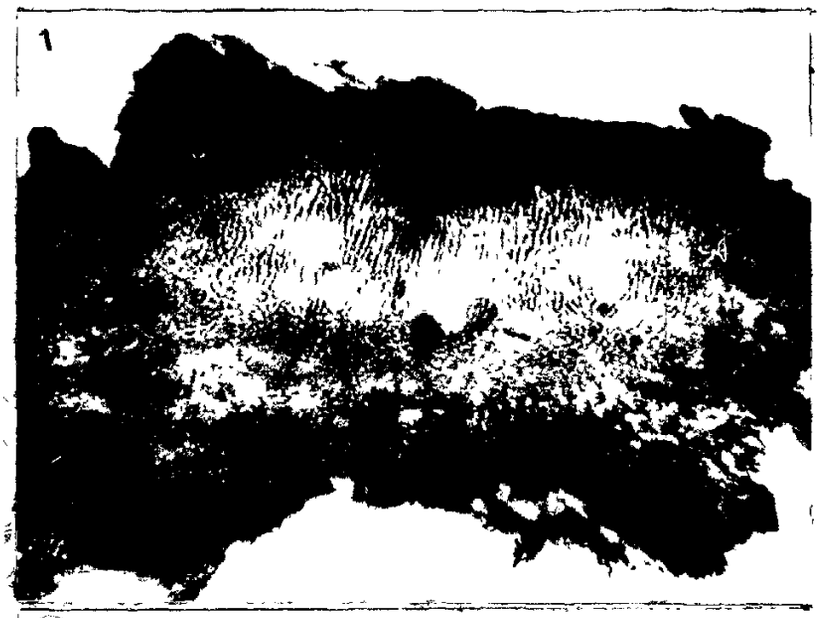
Tryblidiella rhabdosphaerensis

Fig. 5.1. Photomicrograph showing the ascocarp filled with "vertical hyphae" which are continuous from roof to the hypothecial layer in the bottom.

Fig. 5.2. Photomicrograph of the ascocarp showing the 'vertical hyphae' branched at their apices as well as the originating asci amongst "vertical hyphae".

Fig. 5.3. Enlarged view of Fig. 5.2.

PLATE 5



EXPLANATION OF PLATE 6

Tryblidaria maharashtrensis

Fig. 6.1. Photomicrograph of the mesocarp showing deeply stained hypothecial layer and the originating asci amongst 'vertical hyphae'.

Fig. 6.2. Photomicrograph showing further growth of asci amongst the 'vertical hyphae'. Note the ruptured epistroma during sectioning.

Fig. 6.3. Photomicrograph showing well-developed hypothecium and young asci with diploid nucleus.


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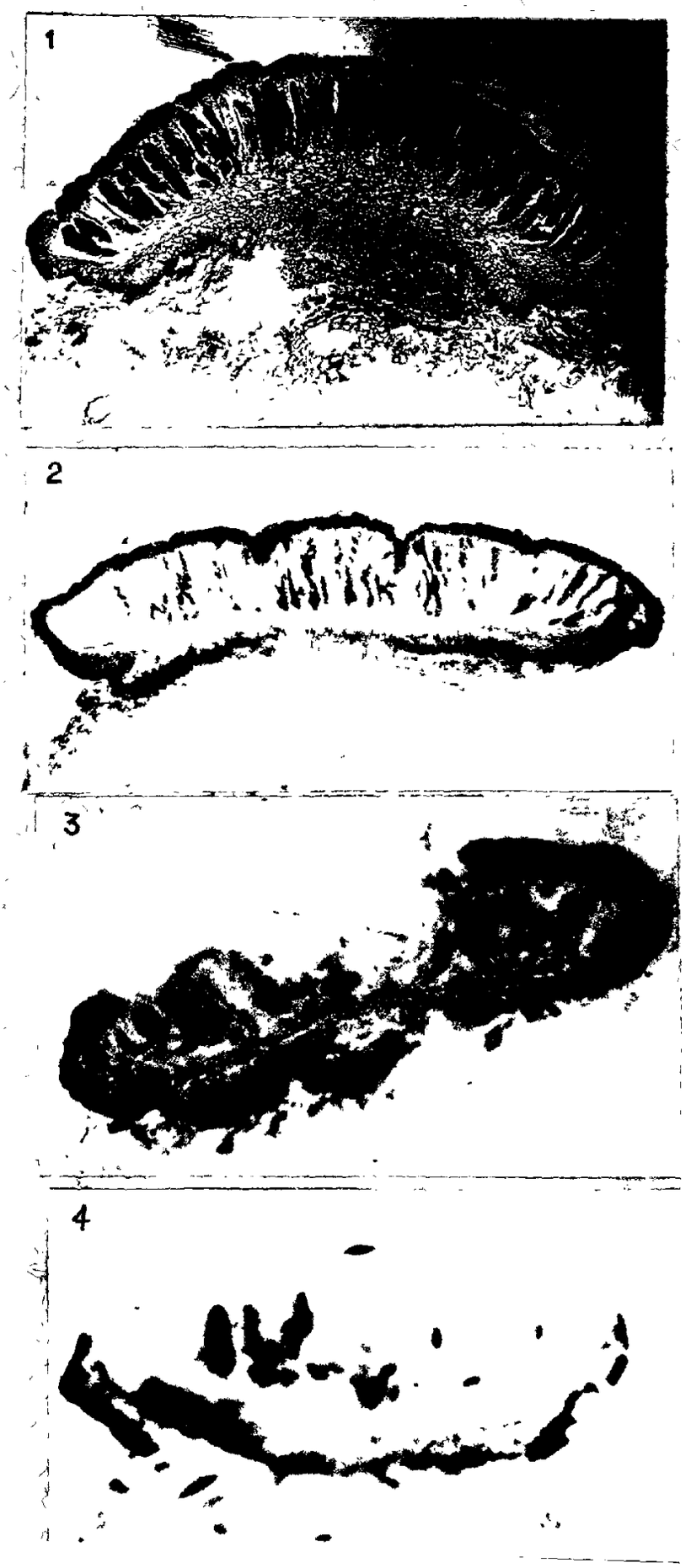
PLATE 7



EXPLANATION OF PLATE 8

Rhizoctonia solani

Fig. 8.1. Uninucleate hyphal strands.

Figs. 8.2 & 8.3 Somatogamous copulation and resulted dikaryotic cell.

Figs. 8.4 & 8.5. Proliferation of dikaryotic cells.

Fig. 8.6. Conjugation division.

Figs. 8.7 to 8.11. Dikaryotic cells.

Fig. 8.12. Karyogamy.

Fig. 8.13. Proliferated dikaryotic cells showing karyogamy.

Figs. 8.14 to 8.18. Various stages of growing asci

Figs. 8.20 & 8.21 Photomicrographs of Figs. 8.2 & 8.3.

Fig. 8.22. Photomicrograph of Figs. 8.4 & 8.5.

Fig. 8.23. Photomicrograph of Fig. 8.7.

Fig. 8.24. Photomicrograph of Fig. 8.10 & 8.11.

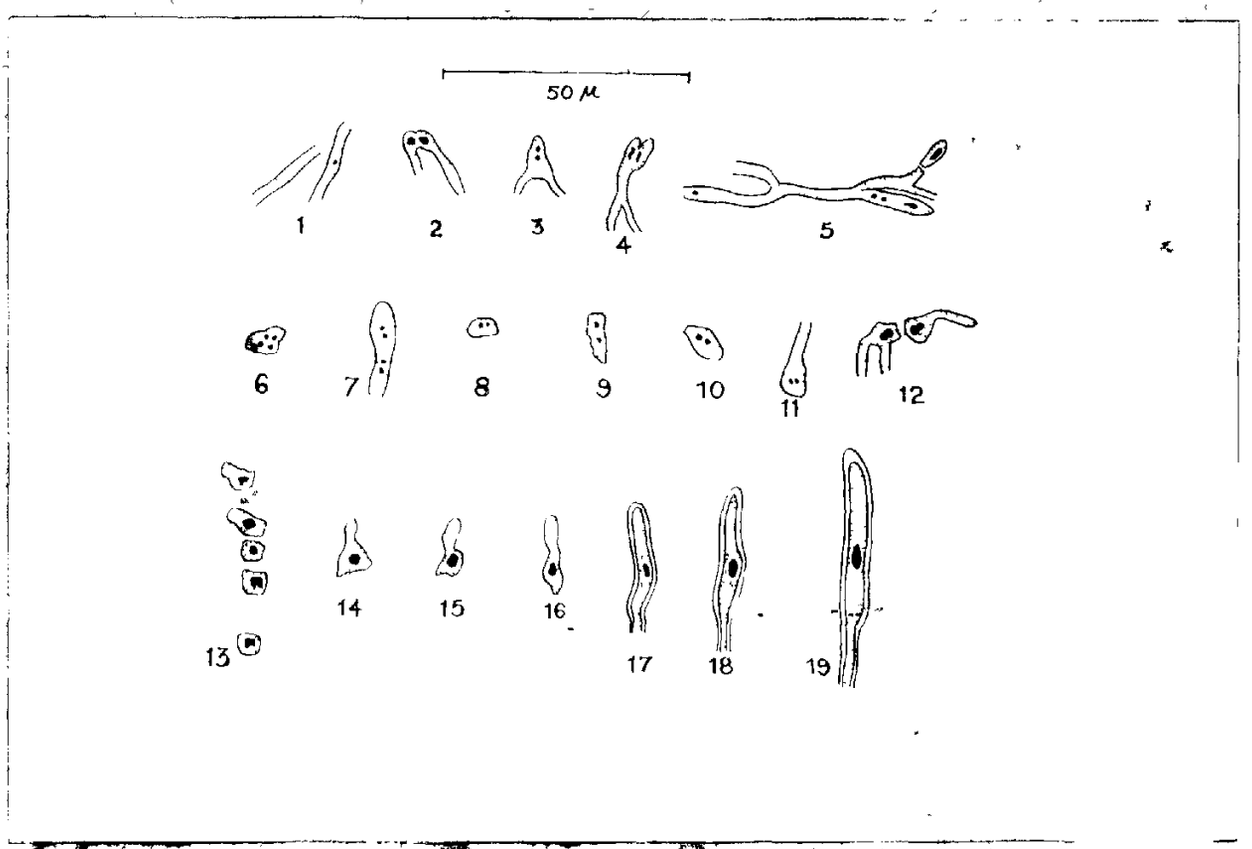
Fig. 8.25. Photomicrograph of Fig. 8.12.

Fig. 8.26. Photomicrograph of Fig. 8.13.

Fig. 8.27. Photomicrograph of Fig. 8.15

Fig. 8.28. Photomicrograph of Figs. 8.16 to 8.19.

PLATE 8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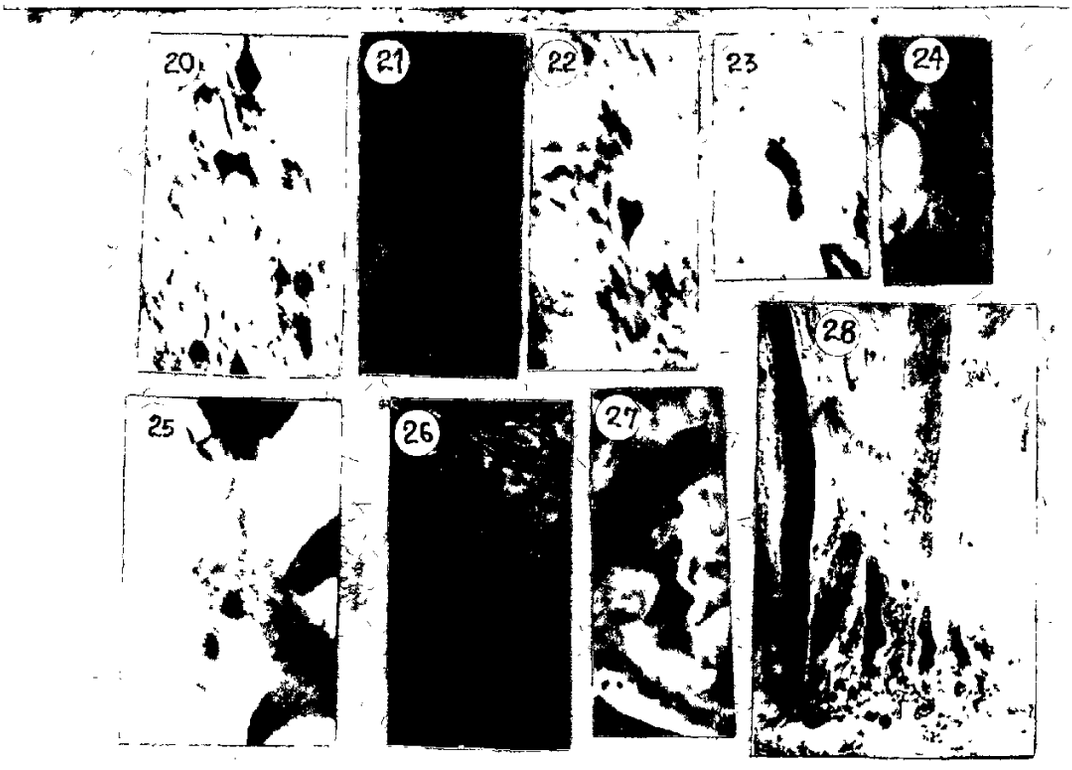
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EXPLANATION OF PLATE 9

Tryblidaria maharashtrensis

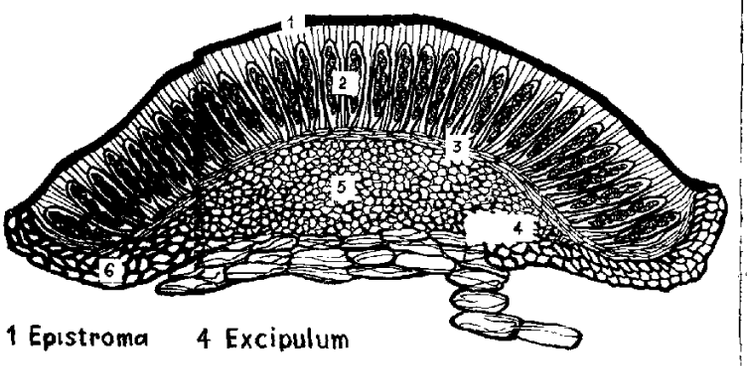
Fig. 9.1. Photograph showing different parts of a well-developed ascocarp.

Fig. 9.2. Discharge of ascospores into the elongated endoascus still containing only one spore.

Fig. 9.3. Ascus dehiscence showing elongated endoascus and ruptured 'ectoascus'.

PLATE 9

1



1 Epistroma 4 Excipulum

2 Hymenium 5 Medullary excipulum

3 Hypothecium 6 Ectal excipulum

2

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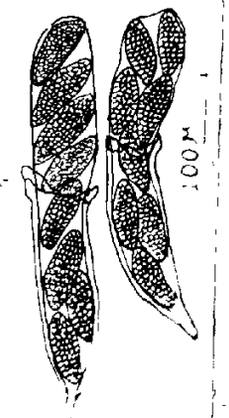


CHAPTER 3

NUCLEAR BEHAVIOUR, CHROMOSOME COMPLEMENT AND ASCOSPORE ORGANIZATION

The basic knowledge regarding cytological studies in the Ascomycetes is derived from the pioneer investigations of Dangeard (1894-1907), Harper (1895-1905), Maire (1903-1907), 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 ph 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 of "Claussen type" - originally put forward by Dangeard (1907) and Claussen (1912) in which a single fusion occur 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 and has been supported by Colsen (1938), Hirsch (1950), Singleton (1953), Olive (1950, 1953) and many others in recent years.

The other theory is "Double fusion" or "Harper type" according to which 2 fusions occur, one in the ascogonium and the other in the ascus mother cell followed by 2 reductional divisions

called "Brachymeiosis" which was put forward by Harper (1895) and was supported by subsequent workers like Blackman & Fraser (1906), Fraser (1906-1907), Fraser & Welsford (1906), 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

The importance of cytological studies in Ascomycetes was brought out through the pioneer investigation of Harper (1895, 1897 and 1905), Dangeard (1894) who employed such studies for determining the nuclear life-cycles and organisation of nuclear structures in Powdery mildew fungi. Later Dodge (1927), Drayton (1934), Gwynn Vaughan (1934, 1937), Colson (1924, 1938), Olive (1949, 1950, 1953, 1956), Singleton (1953), and recently Kowalski (1964, 1965, 1966, 1968), Rogers (1967), Stowell and Backus (1967), Ueker (1967) and many others have contributed voluminous literature to this field of study.

In India, Tilak (1959), Ananthanarayanan (1964), and recently Seshadri (1967), Muthappa (1967) and Chiplonkar (1969), Pande(1969) and others have contributed to this field of study working with tropical Ascomycetous genera like Phyllachora, Parodiella, Rosenscheldiella, Elsinoe, Microcyclus, Myxosphaerella, Lembosina and others.

No cytological studies have been so far reported in the genus Tryblidaria. However, Seshadri (1967) and Muthappa (1967) have reported such studies in Tryblidiella rufula. The work of Zogg (1948) and Luttrell (1953) on Hysterographium fraxini and Glonium stellatum respectively, pertains to developmental aspect with no reports on cytological studies. Therefore the Indian species Tryblidaria maharashtrensis Anahosur was specially selected for such an investigation with special reference to studies into nuclear events in the ascus, a detailed account of which is presented in the following pages.

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 on this aspect as such a procedure is likely to give a better picture of the entire phenomena as a whole.

It has been already stated in a previous chapter that sexual reproduction in this fungus is brought about through somatogamous copulation leading to the production of numerous dikaryotic ascogenous cells where karyogamy takes place and asci develop directly from the diploid cells without the intervention of croziers. This is the only diploidstage observed in the life-cycle of this fungus similar to the allied fungus Tryblidiella rufula. Later the diploid nucleus undergoes a single reduction division and 2 mitotic divisions resulting in the production of 8 haploid nuclei. The ascospore initiation takes place at this

stage through the mechanism of condensation of cytoplasm around the haploid nuclei. Further divisions of the haploid nucleus take place in the spore initials followed by subsequent septation resulting in the formation of 8 muriform asco-spores with uninucleate cells. The terminology used in these pages to describe the cytological events is the one employed by Singleton (1953). The I and II divisions together represent meiotic phase, and I phase being reductional and the subsequent phases are mitotic and are indicated as Division III etc.

Behaviour of the Diploid nucleus in the Ascus

Dikaryotic ascogenous cells multiply by proliferation and the two nuclei making up the dikaryon fuse resulting in a diploid nucleus.

Division I : Generally the diploid nucleus occupies a central position in the developing ascus and rarely in the apical region. This diploid nucleus appears as a homogenous mass (Figs.10.1 & 10.2, 11.1 & 11.2) measuring 2-2.5 u as in Dothidea colleata Luttrell (1951a), Parodiella perisporoides (Tilak, 1959), Rosenscheldiella eugeniae Ammathanarayanam (1964), Tryblidiella rufula and Myeosphaerella mysorensis (Seshadri, 1967). The shape of the diploid nucleus also varies from spherical, triangular to ovoid (Figs. 10.3 to 10.10 & 11.3 to 11.7). Just prior to the 1st division the nucleus expands considerably in size measuring upto 5.5 - 6.0 μ a phenomenon of common occurrence in the majority

of the Ascomycetes so far studied. Beaked nuclei were also observed and probably the beak represents the position of the nucleolus in the diploid nucleus (Figs. 10.11 & 11.8). The presence of beaked nuclei has been reported by Seshadri (1967) and Muthappa (1967) in Tryblidiella rufula.

Prophase I :

As the whole chromatin structures took heavy stain, it was not possible to observe the earlier stages of nuclear divisions (Figs. 10.12, 1013 & 11.9). However, it is presumed that synapsis follows condensation of paired chromosomes indicating the pairs to be homologous. Such conditions has been observed in Neurospora crassa (McClintock, 1945), Rosenscheldiella eugenies(Ananthanarayanan, 1964), Hypomyces solani f. cucurbitae (A.S. El. Alni 1956), in Tryblidiella rufula and Mycosphaerella mycerensis by Seshadri (1967). However, at Pachytene, 3 pairs of chromosomes were observed accompanied by a prominent nucleolus (Figs.10.14 & 11.10). The chromosomes were thick at this stage indicating their double nature. Diplotene was visible with 3 pairs of chromosomes one at each end of the nucleolus and the 3rd pair lying free or attached to one of the 2 pairs (Figs.10.15 & 10.16). At this stage the size of the nucleolus is comparatively reduced.

Metaphase I : The nucleolus completely disappeared and 3 pairs of chromosomes were clearly visible in the equatorial plate (Figs. 10.17 & 11.11).

In Anaphase I 3 chromosomes pass to each pole (Figs. 10.18 to 10.20 & 11.12 to 11.13).

In Telophase I a bridge or cytokinesis is formed (Figs. 10.21 to 10.22 & 11.14 to 11.15) measuring 2.5-3.0 μ in length as reported in Sphaerostibe aurentifolia and Pothidea collecta Luttrell (1944, 1951), Rosenscheldiella eugeniae Ananthanarayanan (1964), Tryblidiella rufula and Hysosphaerella mysorensis Seshadri (1967). At the end of Telophase I 2 daughter nuclei are organised around the chromosomes (Figs. 10.23, 1024 & 11.16, 11.17).

Division II : Two haploid nuclei resulting from the reduction division are smaller in size than the Diploid nucleus (Figs.10.25, 10.26 & 11.18, 11.19). They undergo a period of rest before proceeding to the next division.

Prophase II : This phase was indicated by the increase in size of the 2 haploid nuclei and slightly irregular shape (Figs.10.27, and 11.20).

Metaphase II : At this phase the 2 haploid nuclei undergo the 1st mitotic or equational division, which however, does not synchronise, the upper nucleus séarbs division earlier than the lower one (Figs. 10.28 & 11.21). At the end of Telophase II (Fig.10.29) 4 haploid nuclei are seen (Figs.10.30, 10.31, 10.32 & 11.22, 12.1). The division is slightly oblique to the longitudinal axis of the ascus.

Division III : The 4 haploid nuclei so formed proceed immediately with the next division (Figs.10.33 & 13.2) and the Prophase III was indicated by the enlarged size of the nuclei with chromatin threads liberating from the nuclei which are arranged either parallel to the ascus wall or obliquely (Figs. 10.34 and 13.3). The divisions do not synchronize.

Anaphase III : was observed (Figs.10.35 & 13.4). At the end of Telophase III (Figs. 10.36, 1037 & 13.5) 8 haploid nuclei are formed which are arranged oblique to the ascus wall (Figs.10.38 to 12.43 & 13.6 to 13.9). These nuclei further undergo a period of rest and then proceed to the next division.

Further divisions take place within the spore initials and are mitotic in nature.

Division IV : The nuclei in the ascospore initials (Figs.12.44 to 12.49 & 12.54, 13.10 to 13.15) elongate and undergo further mitotic division resulting in the production of 2-nucleate condition. A septum is formed resulting in the formation of 2-celled spores, each cell with a single nucleus (Figs.12.50, 12.55).

Division V : This division synchronizes. At the end of this the 2 haploid nuclei undergo further division resulting in 4-nuclei (Figs. 12.51,12.52 & 12.56 to 12.59 & 13.16, 13.17 & 13.19 to 13.21) and with further septation the spores become 4-celled, each cell with a single nucleus.

Organization of Ascospores in the Asems

Ascospore organization in the Ascomycetes is known to be brought about through several mechanisms, sometimes of a specialized nature. Harper (1900, 1905) first stressed the importance of astral rays in the delimitation of the ascospores in the powdery mildew fungi. Later his observations were confirmed by McClintock (1945), Singleton (1953), Coleon (1938), Fare (1953) and Damle (1960) and others. All these workers report the radiation of the astral rays from the centriole prior to the delimitation of the ascospore initials. Jones (1926) and Jenkins (1934) working with Ophiobolus graminis Sacc. and Cordyceps agaricoformis observed no cleavage planes in the cytoplasm until after the eight ascospore initials are formed. At the last division i.e. Division III, the cytoplasm showed signs of condensation near the spindles. No evidence of astral rays initiating the phenomenon of cleavage in the cytoplasm was observed. The organization of ascospore initials in the above two fungi appeared to be initiated by the cytoplasmic condensation around the nuclei followed by the formation of a series of vacuoles coalescing along the periphery of the spore initials thus bringing about cytoplasmic cleavage in the delimitation of ascospores.

Bagchee (1925) has reported a different mechanism leading to the delimitation of ascospores in case of Pustularia bolarioides Rams. According to him the appearance of delicate

chromatin network of beaded structures around the nuclei prior to the organisation of ascospores is responsible for the delimitation of ascospores. Such a mechanism has been reported in Paradiella perisporoides and Phyllachora actinodophora (Tilak, 1959) and Phyllachinia yarwoodii Patwardhan (1966) and Phyllachora symplocicola (Jagtap, 1967).

Recently Reeves Jr. (1967) working with Pyrenopeziza domesticum reports an entirely different type of mechanism of ascospore delimitation with the help of Electron microscopic observations. According to him the ascus vesicle composed of two unit membranes surround the 8 haploid nuclei in the ascus earlier to the delimitation of ascospores. This structure appears to be the agent through which the ascospores are delimited. Actually during the process following the complete organisation of 8 nuclei, there is a progressive unfolding and constriction of the the ascus vesicle around the individual nuclei, thus bringing about separation of the ascospore nuclei and spore plasm from the ascus cytoplasm. The matrix membranes form a cuplike structure as they fold around the spore plasm and thus help in the organisation of ascospores.

In the present fungus i.e. Tryblidaria maharashtrensis Anahosur the delimitation of ascospore initials commences only after the organisation of 8 haploid nuclei is completed. The condensation of the cytoplasm around the nuclei was noted and

numerous small vacuoles were observed which coalesced with each other thus bringing about cytoplasmic cleavage (Figs.12.42, 12.43 & 13.8 & 13.9) around the nuclei which initiated the delimitation of ascospore initials similar to the phenomena reported in Ophiobolus graminis Jones (1926), Cordyceps agariciformis Jenkins (1934), Lembosina aulographoides Muthappa (1967) and Mycosphaerella mysorensis Seshadri (1967) (Figs.12.44 & 12.45, 13.10 & 13.11).

Summary and Conclusions

1. Sexual reproduction is in the nature of somatogamous copulation and the asci develop directly from the diploid cells without croziers or hooks.
2. 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".
3. The chromosome complement has been determined as n = 3 haploid.
4. Nuclear divisions in the ascus generally do not synchronize.
5. At the 8-nucleate stage the spore delimitation takes place through cytoplasmic cleavage brought about by condensation of cytoplasm and vacuolation. First transverse septum is formed at 16-nucleate stage. Further divisions of the nuclei and septa formation result in the formation of 6-8 celled ascospores, each cell with a single haploid nucleus. Vertical septa are laid down after this stage.
6. The results have been substantiated by a series of Camera Lucida drawings as well as photomicrographs.

EXPLANATION OF PLATE 10

Trybliderie maharashtrensis

Figs.10.1 & 10.2. Young asci with diploid nucleus.

Figs.10.3 to 10.10 Different shapes of diploid nuclei at different positions in the ascus.

Fig. 10.11 Beaked nucleus.

Figs.10.12 & 10.13. Prophase I.

Fig. 10.14. Pachytene with nucleolus at the bottom and 3 pairs of chromosomes.

Fig. 10.15. Diplotene showing 3 pairs of chromosomes and small nucleolus, one pair at the bottom and two at the top.

Fig. 10.16. Diplotene showing 2 pairs of chromosomes at the top attached to the nucleolus and one pair at the bottom.

Fig. 10.17. Metaphase I showing 3 pairs of Chromosomes.

Figs.10.18,10.19 & 10.20. Anaphase I.

Figs.10.21 & 10.22. Telophase I.

Fig. 10.23 Endof of Telophase I.

Figs.10.24 to 10.26. 2 nucleate stage.

Fig. 10.27. Phophase II

Fig. 10.28 Metaphase II

Fig. 10.29 Telophase II.

Fig. 10.30. End of Telophase II.

Figs.10.31 to 10.33 4 Nucleate stage.

Fig. 10.34 Prophase III

Fig. 10.35. Anaphase III

Figs.10.36 & 10.37. Telophase III.

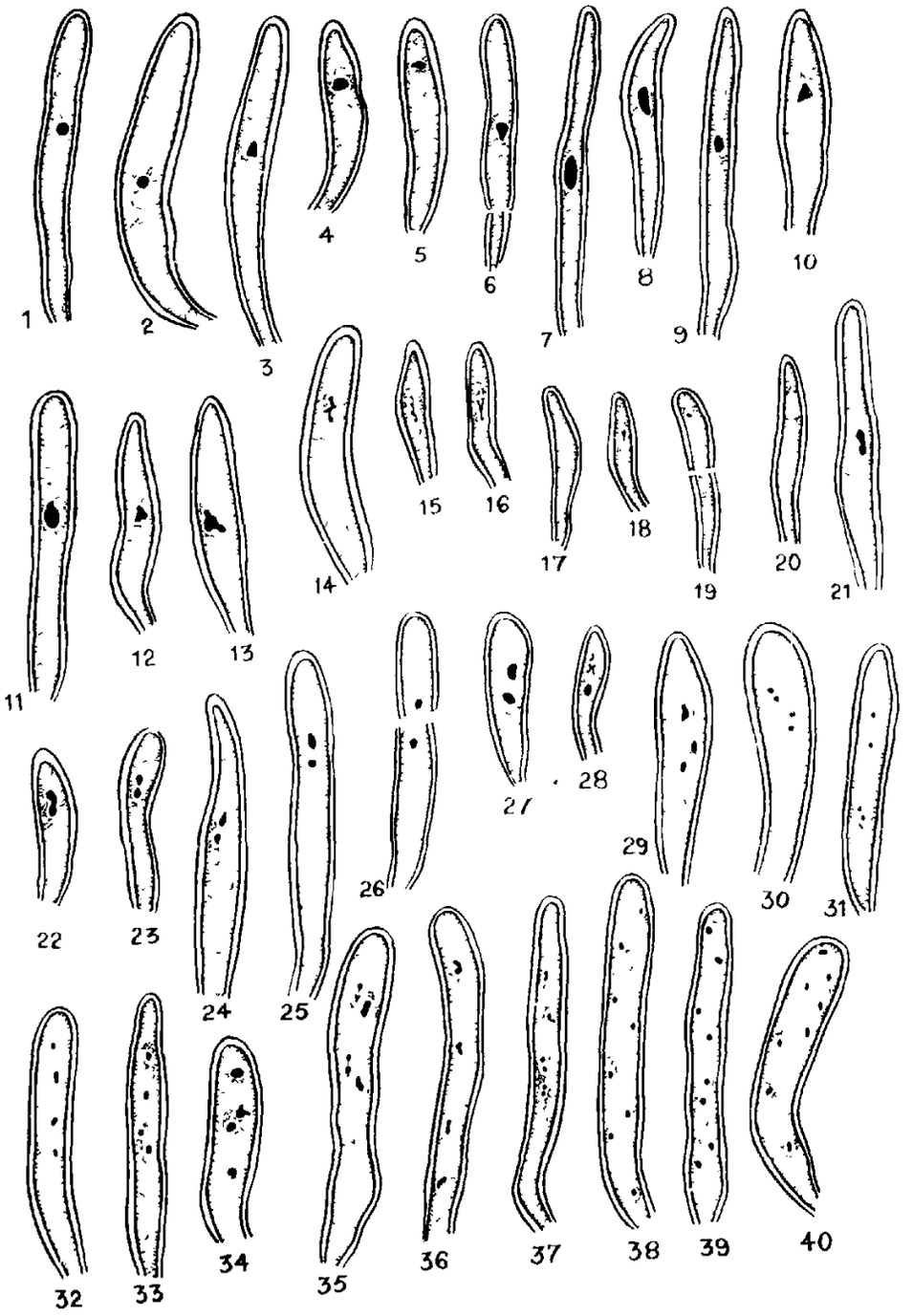
Figs.10.38 to 8 nucleate stage.

EXPLANATION OF PLATE 11

Tryblidaria maharashtrensis

Fig. 11.1. Photomicrograph of Fig. 10.1.
Fig. 11.2. " of Fig. 10.2.
Fig. 11.3. " of Fig. 10.3.
Fig. 11.4. " of Fig. 10.4.
Fig. 11.5. " of Fig. 10.6.
Fig. 11.6. " of Fig. 10.7.
Fig. 11.7. " of Fig. 10.8.
Fig. 11.8. " of Fig. 10.11
Fig. 11.9. " of Fig. 10.13
Fig. 11.10 " of Fig. 10.14.
Fig. 11.11 " of Fig. 10.17.
Fig. 11.12 " of Fig. 10.18.
Fig. 11.13. " of Fig. 10.20.
Fig. 11.14 " of Fig. 10.21.
Fig. 11.15 " of Fig. 10.22.
Fig. 11.16 " of Fig. 10.23.
Fig. 11.17 " of Fig. 10.24.
Fig. 11.18. " of Fig. 10.25.
Fig. 11.19. " of Fig. 10.26.
Fig. 11.20. " of Fig. 10.27.
Fig. 11.21. " of Fig. 10.28.
Fig. 11.22. " of Fig. 10.30.

PLATE 10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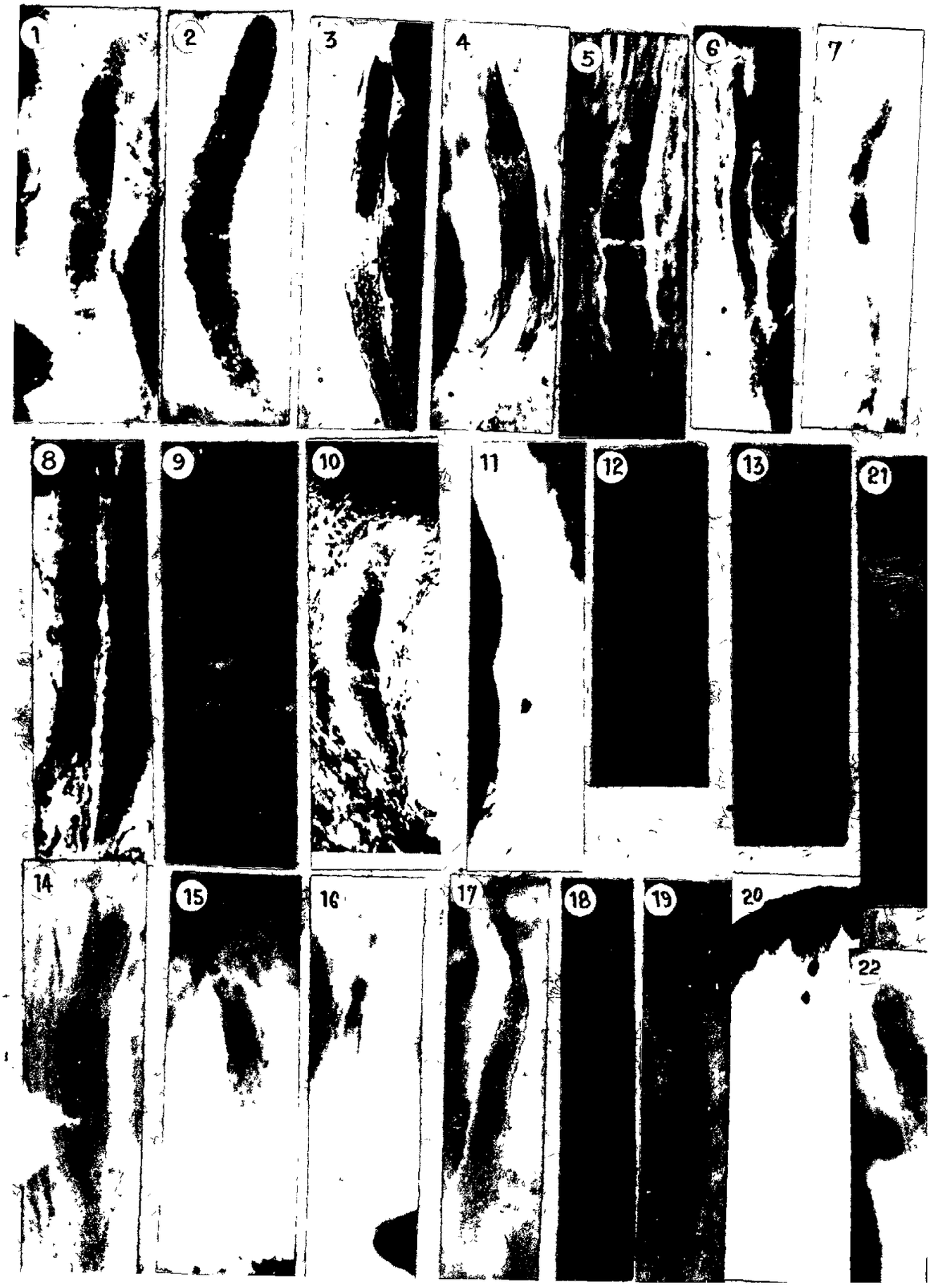
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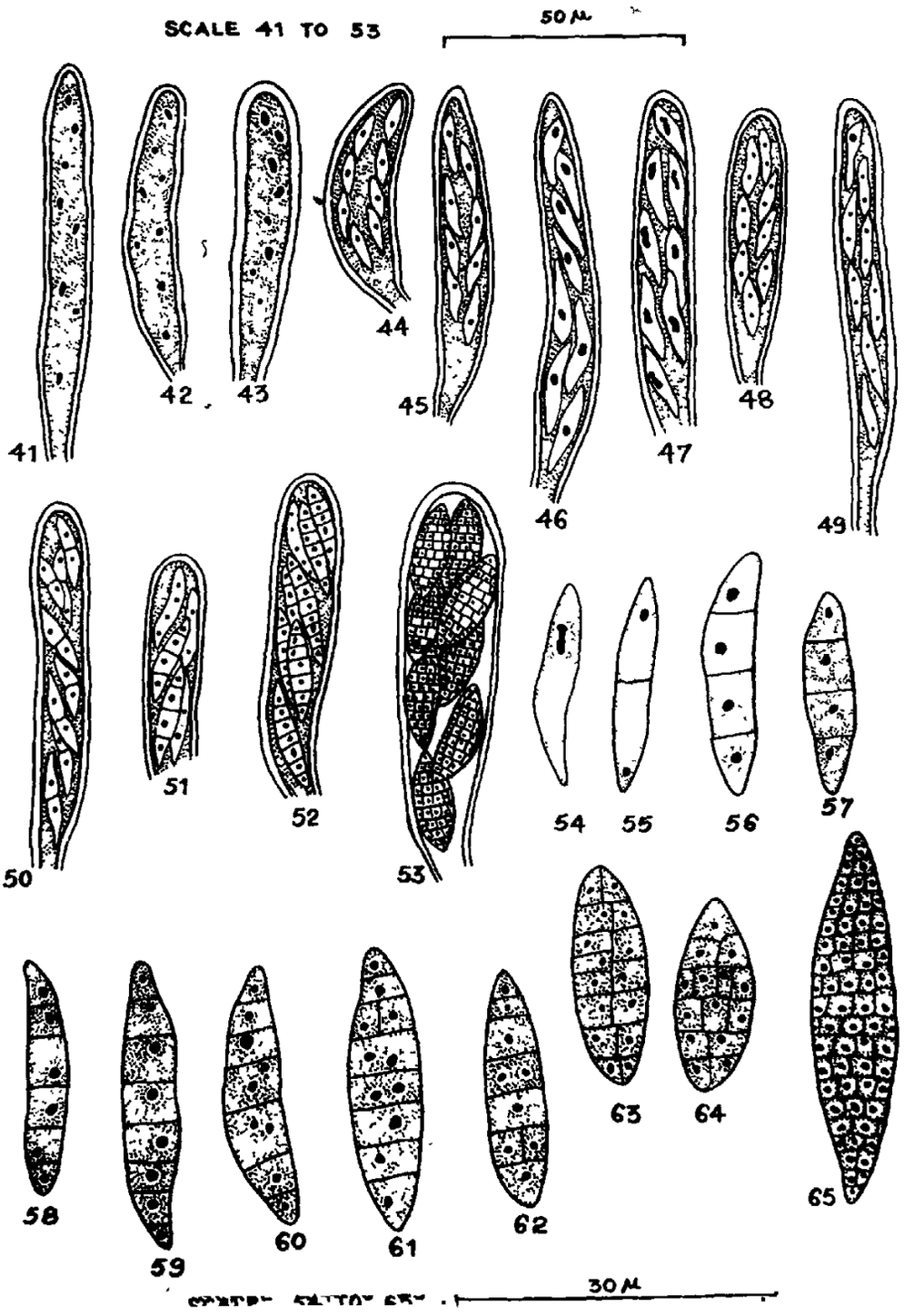
EXPLANATION OF PLATE 12

Tryblidaria maharashtrensis

Figs. 12.41 & 12.42	8-nucleate stage.
Fig. 12.43	Ascospore initiation.
Figs. 12.44 to 12.46.	Uninucleate ascospores.
Figs. 12.47 & 12.54.	Division of the nucleus in the ascospore initials.
Figs. 12.48 to 12.50, and 12.55.	2-nucleate ascospores.
Figs. 12.51, 12.56 & 12.57.	4-nucleate ascospores.
Figs. 12.52, 12.58 & 12.59.	8-nucleate ascospores.
Figs. 12.53, 12.60 to 12.65.	Muriform ascospores.

PLATE 12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4. Asci :

These are bitunicate, octosporous, clavate, arise in basal layers, interspersed with vertical hyphae, and are produced directly from the diplodized cells.

5. Ascospores :

These are hyaline, biseriate to irregularly biseriate, 6-8 celled, oblong, highly vacuolated, non-constricted at septa.

6. Hypothecium :

A thin layer of interwoven hyaline monokaryotic hyphal strands just below the hymenium and is the place where dikaryotic cells appear.

7. Excipulum :

It is the covering of the ascocarp made up of pseudoparenchymatic and prosenchymatic cells. According to Korf (1962) and Muthappa (1967a) two types of excipula can be distinguished in this fungus.

a. Ectal excipulum : It is the outer covering of the ascocarp made up of thick-walled dark brown pseudoparenchymatous cells.

b. Medullary excipulum : This forms the inner portion below the hypothecium and is made up of the thin-walled hyaline to subhyaline prosenchymatous cells.

Ascus dehiscence and ascospore discharge (Fig.15.1 E)

Under the influence of pressure the ectoascus ruptures and simultaneously the endoascus considerably elongates. The ascospores

are liberated successively from the tip of the endoascus. The mechanism of ascus dehiscence as stressed by Butler (1939) and others is of utmost importance in determining the tunicate nature of the ascus, which in turn has important taxonomic value.

Results and Discussion

The above study and observations pertaining to the developmental pattern of the ascocarp centrum of Lecanidion caesalpinii Anahosur revealed its affinities exclusively towards the Loculoascomycetes with bitunicate asci and vertical hyphae (may be termed as Pseudoparaphyses according to Luttrell, 1965) as defined by Luttrell, 1951, 1955, 1965b). A brief summary of the results so obtained are presented below:

1. The ascocarp is a single locule, erumpent and attains its fullest development outside the host with a wide cleft.

2. The centrum of the young ascocarp comprises of thin walled delicate hyaline pseudoparenchymatous cells followed later by the formation of 'vertical hyphae' which originate by the elongation and division of the deeply staining cells present in the centrum in the central region as well as the top. These hyphae appear long before the development of asci and are attached at the top and bottom of the ascocarp. They have an intercalary growth.

3. The manner of development of the ascocarp in the earlier stages is similar to the type described by Corner (1929) for hemiangiocarpic Discomycetes and to Tryblidiella rufula by Seshadri and Muthappa (1969).

4. The mode of sexual reproduction is of retrogressive type and is in the nature of "spontaneous heterokaryosis" type.

5. The asci arise directly from the diploid cells without the intervention of croziers or hooks amongst the "vertical hyphae" and develop long after the development of "Vertical hyphae" and are arranged in basal layers.

6. The cleft of the ascocarp is formed by the lateral distension and disintegration of the outer stromatic layers during the development of the fruiting body, which in the final stage appears discoid with a wide opening similar to Tryblidiella rufula.

7. The 'epistroma' or 'modified epithecium' is a thin layer at the top of the ascocarp resulting by the intermingling of the bulbous tips of the branched vertical hyphae with the disintegrated upper stromatic cells and is heterogenous in nature, composition and origin.

8. Ascus dehiscence is similar to that of Lecanidion stratum Butler (1939) and Tryblidiella rufula Muthappa (1967a) and is termed as "Endoascus type".

The pattern of the development of the ascocarp centrum agrees with that of Glonium stellatum, Hysterographium fraxini and Tryblidiella rufula but differs from the former two in having no ascogonia, with 'vertical hyphae' amongst which bitunicate asci develop later exhibiting the typical "Glonium" type of ascocarp centrum.

It has already been discussed earlier that this genus was placed under different orders belonging to Ascohymeniales series due to the lack of ontological studies in respect of the developmental pattern of the ascocarp 'centum' characters which have gained vital importance in the modern concept of the taxonomic treatment of this heterogenous group of fungi. Firstly it was Miller (1928, 1949) who suggested the rearrangement of this heterogenous group into a more homogenous system taking into consideration the "ascocarp centrum" as the chief character and was later emphasized by Nannfeldt (1932), Dehmeyer (1926-1955) and Luttrell (1951, 1955, 1965a).

The present developmental studies carried out by the writer proved that this 'cup-fungus' is definitely an ascoloculare in its affinities both in respect of "vertical hyphae".

According to Luttrell (1965) the distinct vertical paraphyses-like hyphae developing in the centrum of Loculoascomycetes prior to the formation of asci are properly termed "pseudoparaphyses" and the "paraphysoides" of other workers are homologous to "pseudoparaphyses". Kowalski (1965) defines three types of sterile threads 1. Paraphyses, similar to Luttrell (1965) 2. Paraphysoides - are those hyphae which are attached at the top and bottom of the ascocarp from the beginning. 3. Pseudoparaphyses are those which grow from top to the bottom with free tips. Subsequently Kowalski (1966) adopted "paraphysoides" for designating those hyphae which were attached at the top and bottom of the ascocarp from the beginning as in case of Preussia

funiculata. But according to Luttrell (1965) the "paraphysoides" and "pseudoparaphyses" of Kowalski (1965, 1966) are synonyms.

In view of their nature, elongation by intercalary growth, and origin long before the development of asci, the "vertical hyphae" observed in Lecanidion caesalpinii are closely similar to those observed by Dodge (1937), Chesters (1938), Jones (1926), Luttrell (1953,1964), Kowalski (1965), Corlett (1967), Kennedy and Stewart (1967), Routien (1956) and recently by Seshadri and Muthappa (1969) and also similar to those described by Gordon (1966 and 1968). Although some of the fungi worked out by the above mentioned workers belong to Ascohymeniales series with unitunicate asci, the 'vertical hyphae' have been described as "Pseudoparaphyses" because of their nature, structure and origin. On the basis of writer's studies and observations, and their essentially intercalary growth and their free tips, the 'vertical hyphae' obtained in Lecanidion caesalpinii may be described as 'Intercalary paraphyses'. The upper 'epithecium-like' structure formed by the intermingled tips of the branched intercalary paraphyses together with the disintegrated upper stromatic cells is heterogenous in composition, structure and origin thus lacks the characteristics of a true epithecium obtained in the true Discomycetes and is similar to the structure of Tryblidiella rufula and may be termed as "Epistroma".

The sexual reproduction is of retrogressive type and is similar to that reported for Parodiella paraguensis by Tendulkar (1969) and may be described as "Spontaneous heterokaryosis".

As the gross morphological characters of the ascocarp of Lecanidion caesalpinii indicate its Discomycetous affinities but the presence of bitunicate asci, nature of the development of ascocarp, origin and nature of 'vertical hyphae' would place the genus under Loculoascomycetes. Earlier literature shows that such cuplike fungi with bitunicate asci were placed under Patellariaceae.

Luttrell (1965a) recognized two families under Hysteriales: Hysteriaceae and Patellariaceae and according to him the genus Lecanidion should go under Patellariaceae of Hysteriales. Muller and Arx (1962) place the genus Lecanidion in Patellariaceae of Dothiorales of Ascoloculare series.

The investigations carried out by the writer into Lecanidion caesalpinii Anahosur have shown that the ascocarp of this fungus develops and opens in a manner similar to that interpreted by Korf (1962) and is therefore termed as "DISCOTHECIUM".

On the basis of this detailed study into the developmental pattern of the ascocarp, its centrum characters, nature and origin of interthecial threads, bitunicate nature of asci and their endoascus type of ascus dehiscence and above all the highly heterogenous nature of the episteroma, the fungus Lecanidion caesalpinii Anahosur should find a place under the Family Patellariaceae, Order Hysteriales (Dothiorales of Muller & Arx, 1962).

EXPLANATION OF PLATE 16

Lecanidion caesalpinii

Fig. 16.1. Photograph showing the germinated ascospores obtained from water-agar medium under laboratory conditions.

Fig. 16.2. Photomicrograph showing the aggregated mycelium turning to brown, below the host epidermis.

Fig. 16.3. Photomicrograph showing the ascocarp initial (Spherical stromatic body) below the epidermis.

PLATE 16

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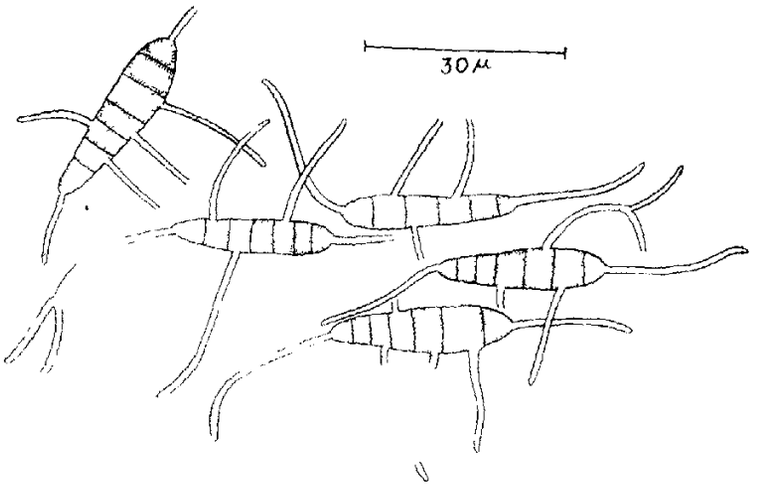

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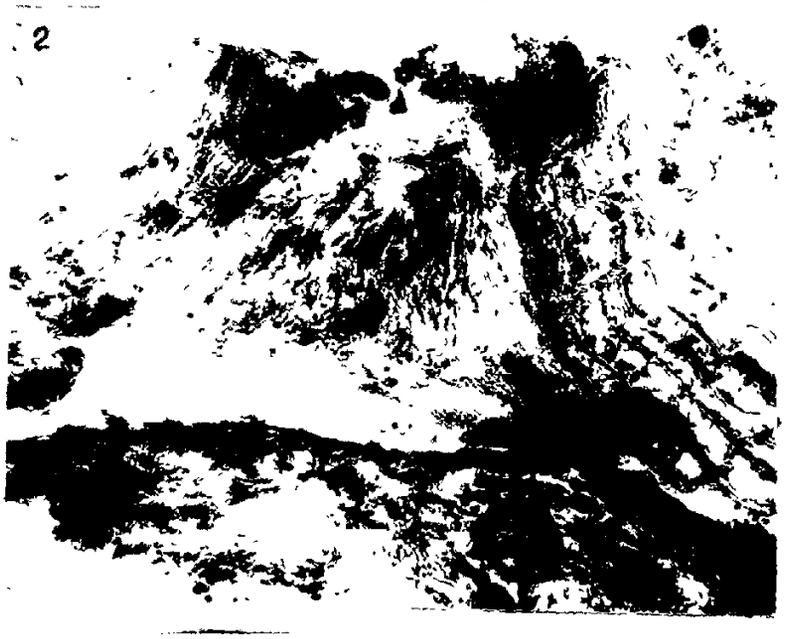

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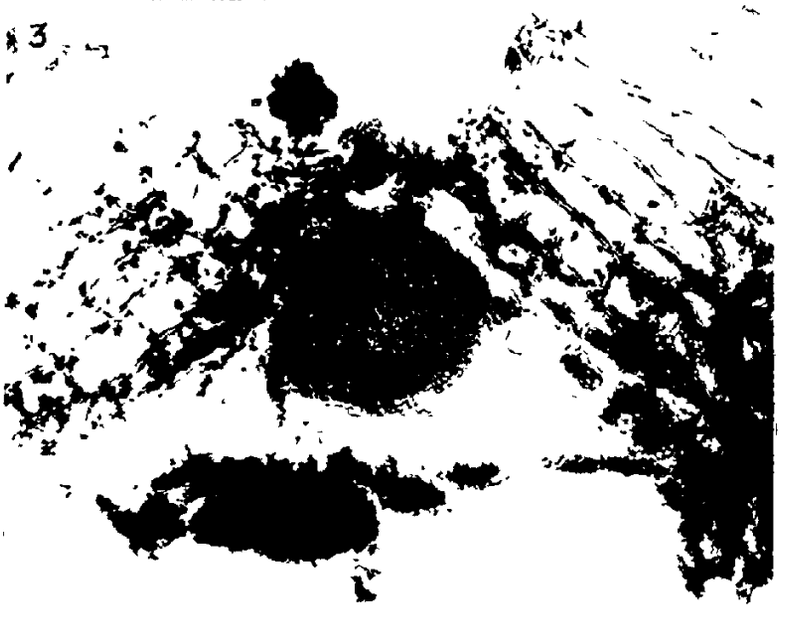

EXPLANATION OF PLATE 17

Lecanidion caesalpinii

Fig. 17.1. Photomicrograph showing the young ascocarp made up of black thick-walled pseudoparenchymatic cells and its erumpent nature.

Fig. 17.2. Photomicrograph showing the young ascocarp made up of thin-walled hyaline cells in the central region and outer thick-walled pseudoparenchymatic cells.

Fig. 17.3. Photomicrograph showing irregularly arranged 'vertical cells' (stained heavily) in the central regions and note the elongating vertical cells at the periphery towards top and bottom.

PLATE 18

EXPLANATION OF PLATE 19

Lecanidion caesalpinii

Fig. 19.1. Photomicrograph showing the 'Vertical hyphae' continuous from roof to the bottom of the ascocarp. Note the uninucleate condition of the vertical hyphal cells.

Fig. 19.2. Photomicrograph showing the further growth of 'vertical hyphae' and the differentiated hypothecium (deeply stained cells) at the bottom.

Fig. 19.3. Photomicrograph showing the further growth of the 'Vertical Hyphae' and the ascocarp.

PLATE 17

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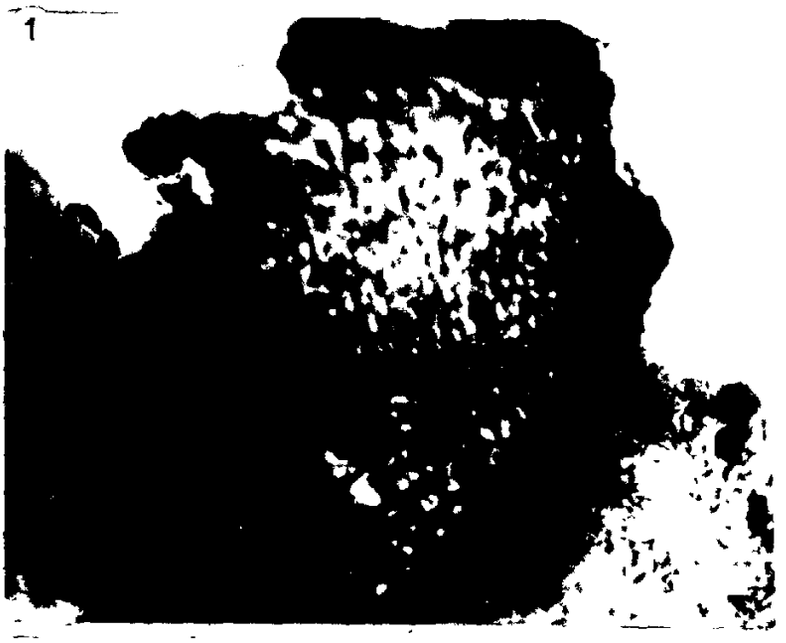

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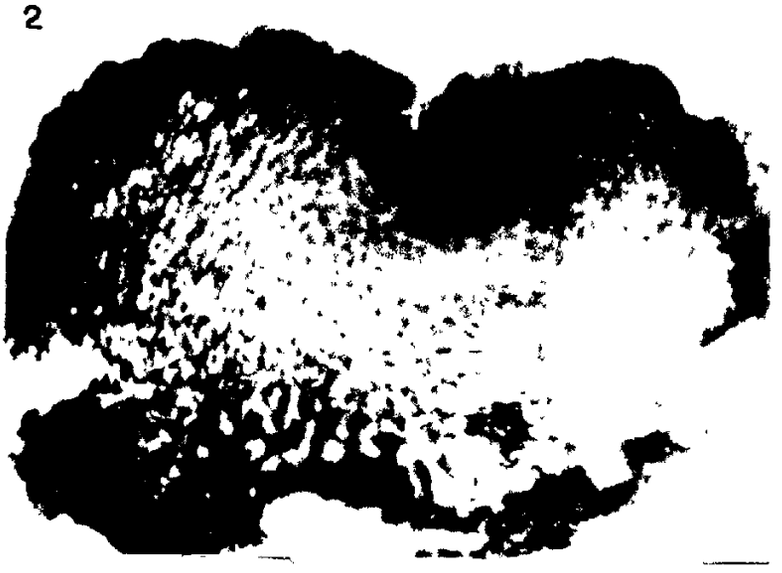

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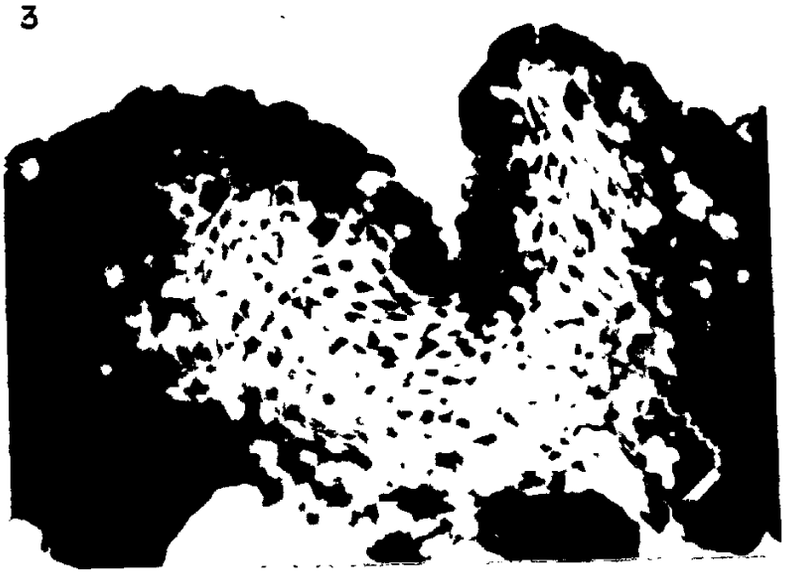

EXPLANATION OF PLATE 18

Lecanidion caesalpinii

Fig. 18.1. Photomicrograph showing the further elongated 'vertical cells' in the central region.

Figs.18.2 & 18.3. Photomicrograph showing 'Vertical cells' becoming hyphae-like and touching the roof and the bottom of the ascocarp. Note the deeply stained cells at the base and along the inner periphery which bring about the horizontal growth of the ascocarp.

PLATE 19

EXPLANATION OF PLATE 20

Lecanidion caesalpinii

Fig. 20.1. Photomicrograph showing the branching of the 'vertical hyphae' at their apical region and a thin layer of epistroma at the top.

Figs. 20.2 & 20.3. Photomicrograph showing the originating asci amongst the 'Vertical hyphae'.

PLATE 20

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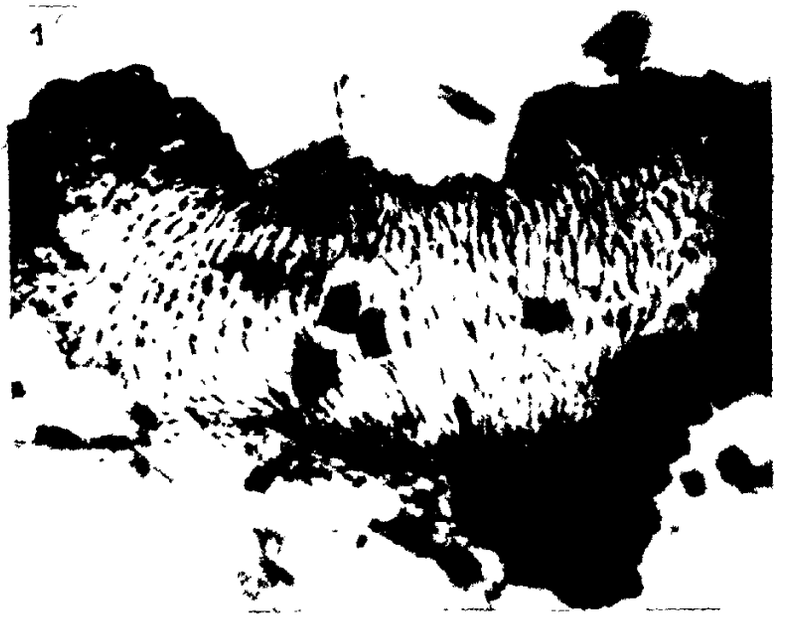

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EXPLANATION OF PLATE 21

Lecanidion caesalpinii

Fig. 21.1. Photomicrograph showing the profused branching of "Vertical Hyphae".

Fig. 21.2. Photomicrograph showing prominently vacuolated cells of the 'Vertical hyphae' and their branching. Note the epistroma.

Figs.21.2 & 21.3. Photomicrograph showing small pockets of epistroma over the hymenium formed by the union of tips of branched 'Vertical Hyphae' together with the disintegrated stromatic cells.

PLATE 21

EXPLANATION OF PLATE 32

Leucanidion caesalpinii

Fig. 22.1. Photomicrograph of the ascocarp showing the developing asci of different stages. Note the erumpent nature of the ascocarp.

PLATE 22

CHAPTER 3

NUCLEAR BEHAVIOUR, CHROMOSOME COMPLEMENT AND ORGANIZATION OF ASCOSPORES

Little is known about the cytology of the members of this group i.e. Hysteriales except for the recent work of Seshadri (1967) and Muthappa) on Tryblidiella rufula obtained from different hosts. The work done on the related fungi Hysterographium fraxini Zogg (1943) and Glonium stellatum Luttrell (1953) pertains to the developmental aspect with no cytological studies.

Nuclear events in the ascus

In short, the general trend of the nuclear events as they occur in the ascus of this fungus to be followed by detailed account on this phase, has been given here for the proper understanding of the nuclear behaviour at a stretch as a whole.

It is already discussed that the sexual reproduction is in the nature of "Spontaneous heterokaryosis" type resulting in the production of dikaryotic ascogenous cells where Karyogamy takes place and the asci develop directly from the diploid cells without the intervention of croziers. This is the only diploid stage observed in the life-cycle of this fungus. Later this diploid nucleus in the ascus undergoes a reduction division followed by two mitotic divisions resulting in the formation of 8 nuclei. The ascospore initials are recognised at 8-nucleate stage and further mitotic divisions of the nucleus in the spore initials result in the production of 6-8-celled spores, each cell with a single

nucleus. The terminology used in these pages to describe the cytological events is the one employed by Singleton (1953). The I and II divisions together represent Meiotic phase. The division I being the reductional and the subsequent divisions are mitotic and are indicated by Division III etc.

Behaviour of the Diploid nucleus in the Ascus

The ascus directly develops from the diploid cell. The outer wall of the ascus develops first later followed by nuclear divisions within. It was rather difficult to trace the development of the two walls of asci in their earlier stages of development.

Division I :

Usually the diploid nucleus is situated in the central position of the ascus and sometimes in the apical region. At this stage the diploid nucleus is homogenous in appearance and measures 1.8-2.4 μ (Figs.23.13 to 23.23 & 24.13 to 24.20). The shape of the nucleus also varies from spherical to ovoid (Figs.23.15 to 23.19 & 24.15 to 24.16). Just prior to the division the diploid nucleus expands considerably attaining an irregular shape measuring upto 4.5 μ (Figs.23.20 to 23.23 & 24.17 to 24.20). At this stage a highly reticulate mass of chromosomes can be observed accompanied by a prominent nucleolus (Figs.23.24,23.25 & 24.21).

Prophase I : The earlier divisions were rather not recognisable since the whole chromatin mass took deep stain.

No clear Pachytene was visible. However, the Diplotene was clearly observed with a prominent nucleolus and 3 pairs of chromosomes. Usually 2 pairs were seen attached to the nucleolus and the other was free (Figs.23.26,23.27 & 24.22, 24.23).

In Prometaphase the size of the nucleolus was considerably reduced and the chromosomes were also condensed in length (Fig. 23.28).

In Metaphase I: The nucleolus completely disappears and 3 pairs of chromosomes are clearly visible in the equatorial plate (Fig.23.29).

In Anaphase I : Three chromosomes pass to each pole (Figs. 23.30 & 24.24).

In Telophase I : A bridge was formed connecting the two organising haploid nuclei (Figs.23.31,23.32 & 24.25 & 24.26), measuring 1.8-2.5 μ as reported in Sphaerostilbe aurentifolia, Dothidea collecta Luttrell (1944 & 1951), Rosenscheldiella eugeniae Ananthanarayanan (1964) and Seshadri (1967) in the allied fungus Tryblidiella rufula. At the end of Telophase I two daughter nuclei are organised around the chromosomes at both the poles.

Division II :

The two haploid nuclei resulted after the first division are smaller in size (0.9-1.2 μ) than original diploid nucleus (Figs.23.33 to 23.35 & 24.27, 24.28). These two nuclei undergo a period of rest before undergoing the next division.

Metaphase II was observed (Figs.23.36 to 23.38 & 26.1 to 26.3)

However non-synthronization of the divisions of the two nuclei were very frequently noted. 4 haploid nuclei were formed which undergo third division after a period of rest (Figs.23.40,, 25.41 to 25.44 & 26.5 to 26.8).

Division III :

The orientation of the nuclei in this division is either parallel or slightly oblique to the longitudinal axis of the ascus. The division is oblique to the ascus wall. At the end of Telophase III (Figs.25.45 to 25.49 & 26.9,26.10) eight haploid nuclei are organized (Figs.25.50 to 25.54 & 26.11, 26.15). These nuclei undergo a periodof rest and then enter into the Division IV. Further Divisions IV and V and VI take place in the spores resulting in the formation of 6-8-nucleate spores, one nucleus in each cell.

It is quite clear from the foregoing account of the nuclear events occurring in the ascus of Lecanidion caesalpinii Anahosur that of the six divisions, the first one is always reductional and others are mitotic divisions.

On the basis of the above studies and observations, the haploid number of chromosomes in Lecanidion caesalpinii is determined as n = 3 and diploid number 2n = 6. In the allied fungus Tryblidiella rufula, the haploid number of chromosome is reported to be n = 2 (Seshadri 1967).

EXPLANATION OF PLATE 24

Lecanidion caesalpinii

Fig. 24.1. Photomicrograph of Figs.23.1 & 23.2.

Fig. 24.2. Photomicrograph of enlarged vegetative nucleus.

Fig. 24.3. Photomicrograph showing monokaryotic and Dikaryotic hyphal strands.

Figs.24.4 & 24.5. Photomicrograph of Figs.23.3 & 23.4.

Fig. 24.6. Photomicrograph of Fig. 23.5.

Fig. 24.7. Photomicrograph of Figs.23.6 & 23.7.

Figs.24.8 & 24.9. Photomicrographs of Figs.23.8 & 23.9.

Figs.24.10. Diploid nucleus in the ascus mother cell.

Figs.24.11 & 24.12. Photomicrograph of Figs.23.10 & 23.11.

Fig. 24.13. Photomicrograph of Fig. 23.13.

Fig. 24.14. Photomicrograph of Fig. 23.14.

Fig. 24.15. " of Fig. 23.15.

Fig. 24.16. " of Fig. 23.18

Figs.24.17 & 24.18. Photomicrographs of Figs.23.20 & 23.21.

Figs.24.19 & 24.20 " of Figs.23.22 & 23.23

Fig. 24.21. Photomicrograph of Fig.23.24.

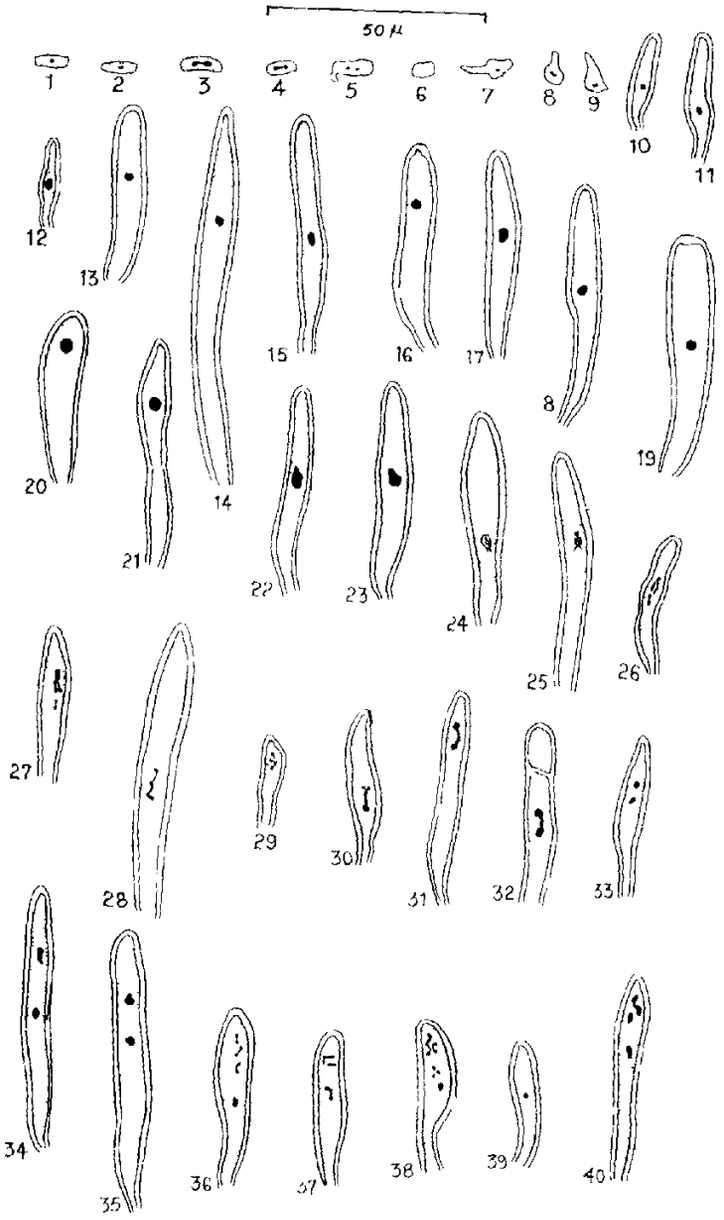
Figs.24.22 & 24.23. Photomicrographs of Figs.23.27 & 23.28.

Fig. 24.24. Photomicrograph of Fig.23.30.

Figs.24.25 & 24.26 Photomicrographs of Figs.23.31 & 23.32.

Figs.24.27 & 24.28. Photomicrographs of Figs.23.33 & 23.35.

PLATE 23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Nuclear movement in the ascus

It is wellknown that the orientation of the nuclear spindle determines the manner of arrangement of ascospores in the ascus. In this fungus the first division is parallel to the longitudinal axis of the ascus and II and III are oblique, and the eight nuclei are arranged oblique to the ascus wall indicating the biseriate formation of ascospores.

Organization of ascospores in the ascus

In Lecanidion caesalpinii Anahosur the delimitation begins at 8-nucleate stage through condensation of cytoplasm and completed after the Division VI. Small vacuoles were observed in the cytoplasm around the nuclei which coalesce and bring about the cytoplasmic cleavage and delimitation of ascospores (Figs.25.53, 25.54 & 25.55; 26.14 to 26.16), similar to the mechanism reported in Ophiobolus graminis, Cordyceps agariciformis, Myeosphaerella mysorensis and Lembosina aulographoides by Jones (1926), Jenkins (1934), Seshadri (1967) and Muthappa (1967) respectively. A thin membrane was formed around each spore initials which later develop into well developed ascospores.

The septation of the spores was studied in detail. The nucleus situated at one end of the spore initial undergoes division IV (Figs.25.61 & 27.1) resulting in the formation of binucleate spores (Figs.25.56, 25.57, 25.62 & 26.17). A septum is laid down between 2 nuclei in each spore thus completing the

formation of two-celled ascospores. fter the Division V 4 nuclei are formed two in each cell. Vacuoles are prominently visible which bring about the cytoplasmic cleavage and two more septa are formed thus leaving each cell with a single nucleus. The 2 nuclei are situated at the tips and the other two nuclei near the middle septum, and thus each cell is left with a single nucleus (Figs. 25.58,25.63,25.64 & 26.18,27.2). During Division VI probably one or two of the four nuclei may not divide resulting in the formation of 6-8 nucleate spores (Figs.25.59,25.60,25.65 to 25.68 & 26.19 to 26.20, 27.3 to 27.7). Vacuoles are prominently visible.Each nucleus is surrounded by a big vacuole representing a cell (Figs.25.55, 25.66 & 27.4, 27.5). Septa are laid down, each cell with a nucleus situated either near the septum or the side wall of the spore thus resulting in the formation of ascospores with 6-8 cells each with a single nucleus surrounded by a big vacuole (Figs.25.67, 25.68 & 27.6, 27.7). Well developed spores are 6-8 celled, each cell with a single nucleus surrounded by big vacuole and the spores are hyaline and arranged biseriate to irregularly biseriate in the ascus (Figs.25.59, 25.60 & 26.19 to 26.21).

Summary

1. Sexual reproduction is in the nature of "Spontaneous heterokaryotis" type and the asci develop directly from the ascus mother cells without the croziers.

2. There is only one nuclear fusion occurring in the ascus mother cell, is followed by a single reduction division similar to "Claussen type".

3. The chromosome complement has been determined to be n = 3 haploid.

4. Nuclear divisions in the ascus seldom synchronize.

5. The organization of ascospores is brought about by the mechanism of cytoplasmic cleavage through condensation of cytoplasm and vacuolation. First septum is laid down at 16-nucleate stage and further septa after the subsequent mitotic divisions resulting in the formation of 6-8-celled ascospores, each cell having a big vacuole and a single nucleus.

6. The results obtained in the above studies have been substantiated by means of photomicrographs as well as Camera Lucida drawings.

EXPLANATION OF PLATE 23

Lecanidion caesalpinii

Figs.23.1 & 23.2. Uninucleate hyphal cells.

Figs.23.3 & 23.4. Division of the vegetative nucleus (Telophase).

Figs.23.5 to 23.7. Dikaryotic ascogenous cells.

Figs. 23.8 & 23.9. Karyogamy

Figs. 23.10 & 23.11. Young asci originating by the elongation of the diplodized cells.

Figs. 23.12 to 23.21 Diploid nucleus in the ascus.

Figs. 23.22 & 23.23. Prophase I.

Figs. 23.24 & 23.25. Pachytene.

Figs. 23.26 & 23.27. Diplotene

Fig. 23.28 Prometaphase.

Fig. 23.29 Metaphase I.

Figs.23.30 Late Anaphase I.

Figs.23.31 & 23.32. Telophase I.

Figs.23.33 to 23.35. 2-nucleate state.

Figs.23.36 to 23.38. Metaphase II

Fig. 23.39 Anaphase II

Fig. 23.40 Telophase II

PLATE 24

EXPLANATION OF PLATE 25

Lecanidion caesalpinii

Figs. 25.41 to 25.44. 4-nucleate stage.

Figs. 25.45 to 25.47. Telophase III.

Figs. 25.48 to 25.49. End of Telophase III.

Figs. 25.50 to 25.54. 8-nucleate stage. Note the vacuoles in Figs.25.53 & Fig.25.54.

Fig. 25.55. Ascospore initials.

Figs. 25.56 & 25.57. 2-nucleate ascospores.

Fig. 25.58. 4-nucleate ascospores.

Figs. 25.59 & 25.60. 6-8-nucleate ascospores.

Figs 25.61. Division of the nucleus in the young ascospore.

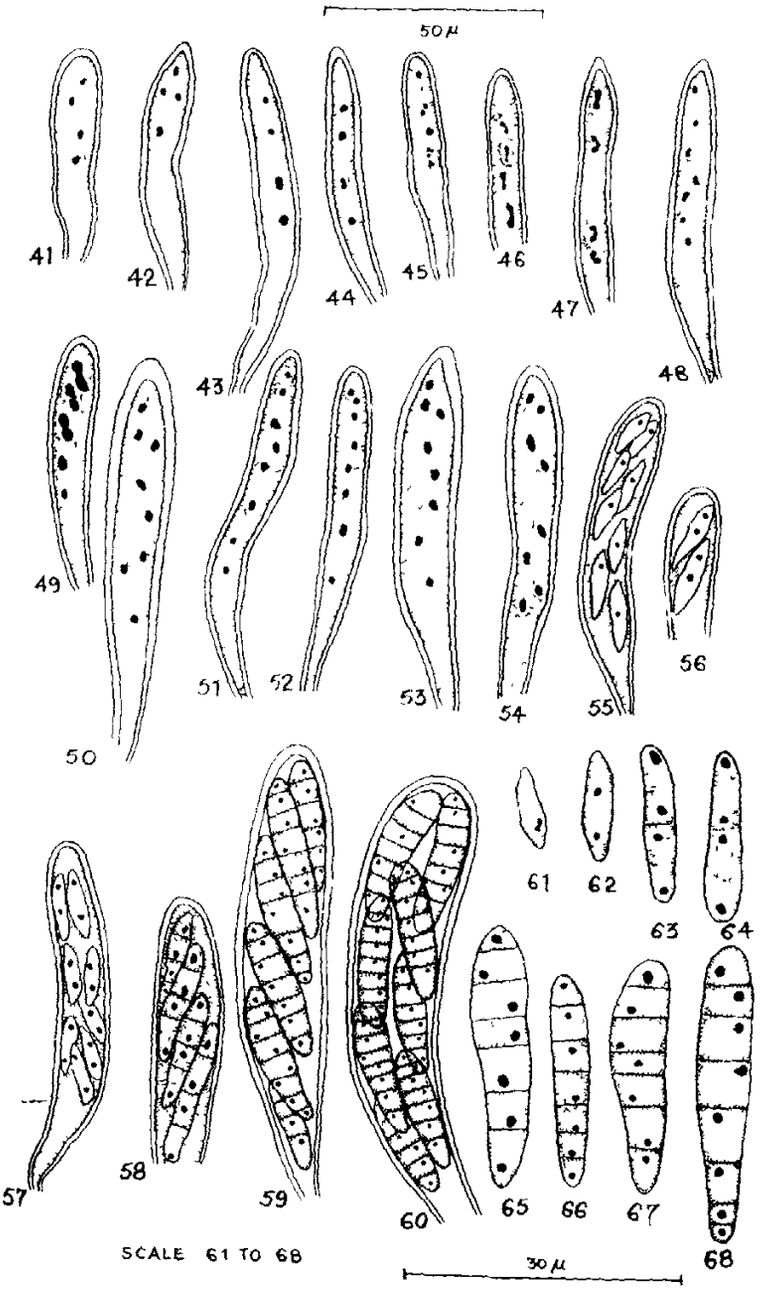
Fig. 25.62. 2-nucleate ascospore.

Figs. 25.63 & 25.64. 4-nucleate ascospores. Note the vacuoles and septa.

Figs. 25.65 to 25.67. 7-nucleate ascospores.

Fig. 25.68. 8-nucleate ascospore.

PLATE 25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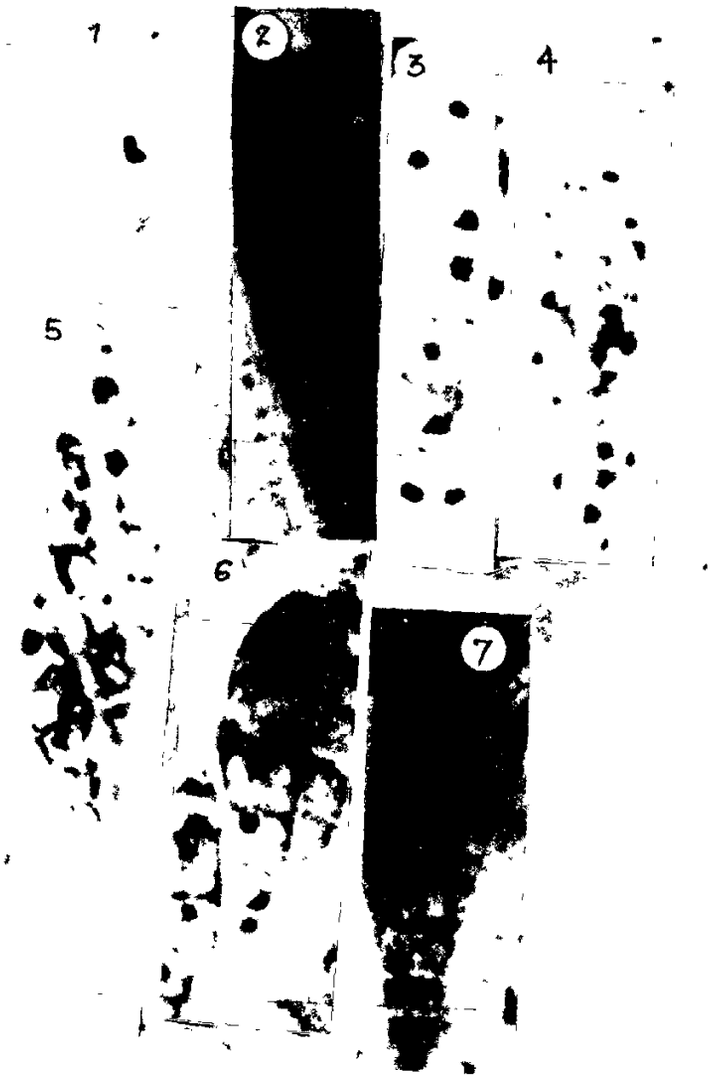
EXPLANATION OF PLATE 26

Lecanidion caesalpinii

Figs.26.1, 26.2 & 26.3.	Photomicrographs of Figs.23.36, 23.37 & 23.38 respectively.
Fig.26.4.	Photomicrograph of Fig. 23.39.
Fig.26.5.	" of Fig. 25.42
Fig.26.6.	" of Fig. 23.40
Fig.26.7.	" of Fig. 25.41
Fig.26.8.	" of Fig. 25.43
Fig.26.9.	" of Fig. 25.47
Fig.26.10.	End of Telophase III.
Figs.26.11,26.12, 26.13,26.14, 26.15	Photomicrographs of Figs.25.50, 25.51,25.52, 25.53,25.54 respectively.
Fig. 26.16.	Photomicrograph of Fig.25.55.
Fig. 26.17.	" of Fig.25.57
Fig. 26.18.	" of Fig.25.58
Figs.26.19 & 26.20.	Photomicrographs of Figs.25.59 & 25.60.
Fig. 26.21.	Well developed ascospores in the ascus.

PLATE 26

PLATE 27



SECTION - C

STUDIES INTO THYBLIDIELLA INDICA ANAHOSUR

CHAPTER 1

TAXONOMY OF TRYBLIDIELLA INDICA ANAMOSUP

Introduction

The genus Tryblidiella was founded by Saccardo (1883) for a "discomycetous" fungus with Tryblidiella rufula (Spreng) Sacc. (Syn. Hysterium rufulum Spreng.) as the type species; later this genus was reduced to a sub-genus Tryblidium by Ellis and Everhart (1892). Further Rehm (1904) divided the genus Tryblidiella into two sub-genera viz. Eutryblidiella characterised by 2-celled brown ascospores and Rhytidhysterium with X-celled ascospores. Muller & Arx (1962) have recognized Eutryblidiella for 1-septate ascospores provided with germ pores and Rhytidhysterium for 2 or more septate ascospores lacking germ pores.

Petrak (1957) has pleaded for the usage of Rhytidhysterium Rehm. treating Tryblidiella Sacc. as its synonym and according to him R. rufulum (Spreng.) Petrak (Syn: Tryblidiella rufula (Spreng) Sacc.) is the type species. However, in the recent years the name Tryblidiella Sacc. is employed by Luttrell (1955), Muthappa (1967). Seshadri & Muthappa (1969) and others. Hence the writer also has adopted the name Tryblidiella Sacc. in the present account of his study.

Originally this genus was described by Sprengel as Hysterium rufulum indicating the confusion regarding the identification of this genus. Recently species of Tryblidiella have been wrongly described as Hysterium by Tilak (1963) and Tilak and Ramachandra Rao (1966).

This confusion had aroused because of the treatment this genus has received from different taxonomists who had placed it under different families and under different orders mainly based on the gross morphological characters. The morphological characters of this genus show its affinities to Discomycetes belonging to Ascohymeniales series but the presence of bitunicate asci show closer affinity to Loculoascomycetes. Butler (1939) and Muller (1954) have confirmed the presence of bitunicate asci in the family Patellariaceae in which the fungi with bitunicate asci produced in cup-like fruiting bodies were included previously as seen in the literature.

Clements and Shear (1931) have included the genus Tryblidiella under Hysteriaceae of Phacidiales and Dermataceae of Pezizales purely based on the morphological characters. It is included in Hysteriaceae when characterised by the presence of coriaceous or subcoriaceous apothecia with a narrow slit and in Dermataceae when it has elliptic to oblong apothecia opening by a wide cleft, thus making the position still more confusing regarding its exact taxonomic position and nomenclature of this genus. Nannfeldt (1932) treats this genus under Patellariaceae with affinities to Discomycete Lichens.

Luttrell (1955) considers the genera Tryblidiella, Lecanidion and Johansonia characterised by discoid ascocarps with longitudinal slit and bitunicate asci as "Bitunicate Discomycetes" which according to him should find ultimately a place under Hysteriales of Loculoascomycetes. Muller and Arx (1962) place the genera Eutryblidiella and Rhytidhysterium in Patellariaceae of Dothiorales.

Martin (1961) (Vide Dictionary of Fungi 1961) has recognised two families under Hysteriales viz. Hysteriaceae: characterised by elongated ascocarps having narrow cleft, Patellariaceae: characterised by discoid ascocarps with wide cleft. This is followed by Luttrell (1965a).

Recently Muthappa (1967,1967a) and Seshaeri(1967) have pleaded for the inclusion of this genus in the Patellariaceae (O. Hysteriales) based on their developmental studies carried out on Tryblidiella rufula.

This confusion regarding the identification and taxonomic position of this genus was due to the lack of enough studies into the developmental pattern of the ascocarp 'centrum', But the recent work of Seshadri and Muthappa (1969) on Tryblidiella rufula has greatly helped to determine the taxonomic position of this genus. Therefore in a view to bring about still more clear idea about the correct nomenclature, diagnosis and its exact taxonomic position, the writer carried out an intensive investigations into the developmental pattern of the ascocarp centrum, nature and origin of sterile threads, nuclear behaviour, sexual reproduction and ascus dehiscence in one more species of this fungus genus viz. Tryblidiella indica,a new species described and collected by the writer on dead branches of Scutia indica Brongn.(Rhamnaceae) as a saprophyte at the Coorg Forests, India. The following account relates to such a study carried out by the writer during this period.

Description and diagnosis of the fungus (Figs.28.1 & 28.2)

Tryblidiella indica Anahosur
Sydowia 1969 (in Press).

Ascocarps black, carbonaceous, scattered, erumpent, uniloculate, discoid to slightly elongated, 1.8-3 mm. long, with a moderate sized long slit which opens at later stages. Locule stromatic, cupulate, lips curved, joining the upper stromatic layer formed by the union of the tips of the sterile threads and disintegrated upper stromatic cells, upto 1 mm. broad and 0.8 mm. high.

Asci bitunicate, pedicellate, cylindrical, more or less arranged in wall-layers, 200-220 x 18-20 µ.

Ascospores dark-brown, elliptical, uniformly 3-septate, end cells slightly tapering at the tips, constricted at septa, uniseriate, 30-32 x 12-15 µ, Sterile threads slender, hyaline, septate unbranched at the apex, persistent, intermingled with asci, the tips uniting with the disintegrated stromatic cells at the apex giving the appearance of an epithecium-like structure.

On the dead twigs of Scutia indica Brongn.(Rhamnaceae) collected by the writer at Coorg Forests (Mysore State) India, during October 1966. M.A.C.S.Herb.No.540.

Remarks : Vorhees (1939) recognises only two species of Tryblidiella viz. T. rufula (Spreng.)Sacc. and T. fusca Rehm. based on : 1. Striations on the lips of ascocarp, 2. thickness of ascocarp lip, 3. Colour of the hymenium and 4. Colour of the ascocarp.

Muthappa (1967a) studied 10 collections of Tryblidiella rufula obtained from 10 different hosts and Scutia indica is one of the hosts on which the writer also collected a species of Tryblidiella. Hence a comparison was made between the writer's collection and Tryblidiella rufula (Table 1) with the following results:

Table 1

Comparison between species of Tryblidiella occurring on Scutia indica

Sr. No.	Species	Ascocarp	Hymenium	Asci	Ascospores
1.	T.rufula(Spreng.) Sacc. (c.f.Muthappa, 1967a)	Discoid to sub-discoid. Lips striated with wide cleft 1-2.5 x 0.5 mm.	Brick-red	160-177 x 10-12 µ	22-28 x 9-10 µ
2.	T.indica Anahosur	Discoid to elongated, lips not striated. Cleft narrow 1.5-3 mm.long.	Ash coloured to slightly black.	200-220 x 18-20 µ	30-32 x 12-14 µ

Interthecial threads in T. rufula are branched or bifurcated at the anex, but in T. indica they are simple.

It is clear from the above table that the writer's collection differs from T. rufula in having :

1. Discoid to elongate ascocarps, with narrow cleft, non-striated lips, black and carbonaceous in consistency.
2. Hymenium is ash coloured to slightly black.

PLATE 28

2

CHAPTER 2

INTERNAL MORPHOLOGY, STRUCTURE AND DEVELOPMENT OF THE ASCOCARP, SEXUAL REPRODUCTION AND ORIGIN OF ASCI AND ASCOSPORE DISCHARGE

Introduction

Tryblidiella indica Anahosur is available abundantly in the forests of Coorg (India) and persists throughout the year saprophytically on the twigs of Scutia indica. This fungus was especially selected for a study into the internal morphology, the centrum development and nuclear behaviour and other related phenomena because of its certain distinctive characters from T. rufula with a view to bring about still more clear understanding about the developmental pattern of the ascocarp 'centrum' and its exact taxonomic position of the genus Tryblidiella Sacc.

Historical review

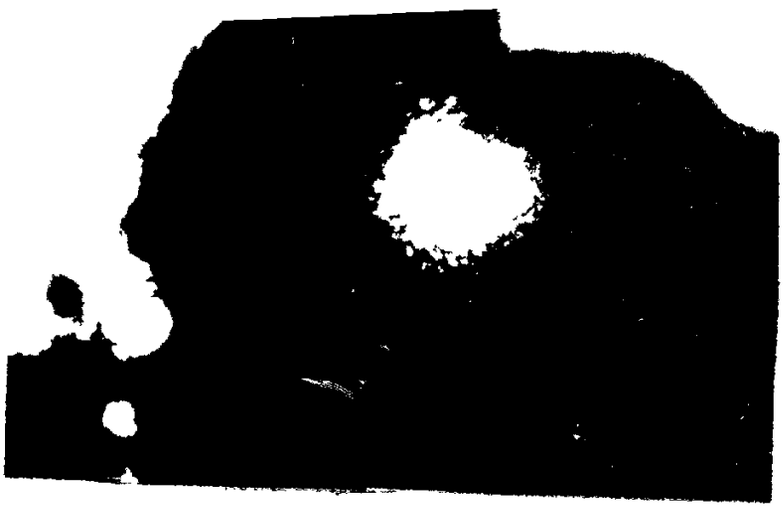
The recent work of Seshadri (1967) and Muthappa (1967) on Tryblidiella rufula is the only one reported in this genus. Zogg (1943) and Luttrell (1953) have worked out the developmental pattern of the ascocarps in the allied fungi like Hysterographium fraxini and Glonium stellatum respectively both belonging to the family Hysteriaceae. All these have been included in Hysteriales (Dothiorales of Muller and Arx 1962).

The mode of sexual reproduction as far as known in this group is of retrogressive type. Seshadri (1967) and Muthappa (1967) have reported the sexuality to be 'somatogamy' in T. rufula. However, Zogg (1943) and Luttrell (1953) in Hysterographium fraxini and

wall made up of thick-walled dark-brown pseudoparenchymatic cells and the central region made up of thin walled, delicate, hyaline prosenchymatous cells and was considered to be a predetermined cavity for the developingsterile threads and asci within (Fig. 30.1). No specialised sex organs were observed at this stage as they were also observed in case of Hysterographium fraxini and Glonium stellatum. Also no sex organs were found responsible for the initiation of the ascocarp but usually in Discomycetes belonging to Ascohymeniales the ascogonium is the first to appear during the process of development as reported in Morchella esculanta (L.) Pers. and M. conica Pers.(Gries 1940), Helvella elastica Bull. (McCubbin 1910), and H. crispa Fr. (Carruthers 1911).

Further development of the ascocarp was indicated by the origin of the deeply staining cells arranged one above the other filling the central region (Fig.30.2). However, these vertically arranged cells appear to be continuous from top to the bottom of the ascocarp behaving like "pseudoparaphyses" (Luttrell, 1965) or paraphysoides (Kowalski, 1965, 1966), Characteristic of the "Glonium"type of ascocarp centrum. As these vertically arranged cells were continuous from top to the bottom of the ascocarp even at the younger stages, it was rather difficult to trace their exact mode of origin (Fig.30.3).

In advanced stages of ascocarp development, these vertical cells appear clearly to be continuous from top to the bottom of the ascocarp. These vertical cells designated as 'vertical hyphae'



EXPLANATION OF PLATE 29

Tryblidiella indica.

Fig. 29.1. Photomicrograph showing an ascospore lodged on the host.

Fig. 29.2. Photograph showing germinated ascospores from water-agar medium under the laboratory conditions.

Fig. 29.3. Photomicrograph of an young ascocarp consisting of Pseudoparenchymatic cells showing erumpent nature.

Sexual reproduction and origin of asci

No specialised sex organs were observed either before the initiation of the ascocarp or in the central region of the young developing ascocarp, similar to Tryblidiella rufula (Seshadri & Muthappa 1969). But Luttrell (1953) and Zogg (1943) have reported the presence of ascogonia in the allied fungi like Glonium stellatum and Hysterographium fraxini respectively. The earliest signs of the development of asci are represented by the presence of uninucleate deeply stained hyphal cells present in the hypothecial region (Figs.34.1 & 34.24). Two uninucleate hyphae lying close-by fuse dissolving the intervening wall in a manner similar to 'cell fusions' in rust fungi (Figs.34.2 to 34.11 & 34.25 & 34.27). This plasmogamous copulation results in the formation of a spherical cell wherein the two haploid nuclei fuse to produce the diploid nucleus which is almost double the size of the two fusing haploid nuclei (Figs.34.12 to 34.13 & 34.28). Further the diploid cell i.e. the ascus mother cell directly grows out vertically into ascus without indication or intervention of croziers or hooks (Figs.34.14 to 34.23 & 34.29 to 34.31). These young asci grow amongst the 'vertical hyphae' which have originated long before the origin of asci.

Thus the sexual reproduction in Tryblidiella indica is of "retrogressive type" similar to the one reported in Tryblidiella rufula and accordingly may be termed as "Somatogamous copulation". In Rhytisma acerinum (Pers.)Fr., the sexuality is in the nature of "Parthenogamous copulation" Jones (1925) while in Ascobolus

and composition, lacking the characteristics of 'true epithecium' found in Discomycetes of Ascohymeniales series and is similar to the one described by Seshadri and Muthappa (1969) and Thyr and Shaw (1966) in Tryblidiella rufula and Hypodermella arcuata respectively.

2. 'Vertical hyphae' :

These hyphae appear very early in the developing ascocarps and are found to be attached at the top and hypothecial region in the bottom long before the development of asci. Their further growth is probably through intercalary growth. Later the tips of these "vertical hyphae" become free at the apex which together with the disintegrated cells of top most layer of cortical cells represent an "epithecium-like" structure. The vertical hyphae are neither bifurcated nor branched at the apex as against bifurcated to branched vertical hyphae of Tryblidiella rufula (Seshadri 1967).

3. Hymenium :

A fertile layer comprising of cylindrical asci which are arranged more or less in the wall-layers of the cavity interspersed amongst the 'vertical hyphae' which are continuous from top to the hypothecial region in the bottom and always the asci originate after the origin of these 'vertical hyphae', a chief character of pleospora type of 'centrum'.

4. Asci :

They are bitunicate, cylindrical, pedicellate, more or less lining the entire cavity, produced directly from the diploid cells without the intervention of croziers or hooks.

PLATE 33

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EXPLANATION OF PLATE 34

Tryblidiella indica

Fig.34.1. Uninucleate hyphal strands.

Figs.34.2 to 34.9 Different stages of somatogamous copulation between hyphal strands.

Figs.34.10 & 34.11 Dikaryotic stage.

Figs.34.12 & 34.13 Karyogamy

Figs.34.14 to 34.23 Different stages of growth of asci.

Fig. 34.24 Photomicrograph of Fig. 34.1.

Fig. 34.25 Photomicrograph of Fig. 34.3.

Fig. 34.26 Photomicrograph of Fig. 34.5

Fig. 34.27 Photomicrograph of Fig. 34.10.

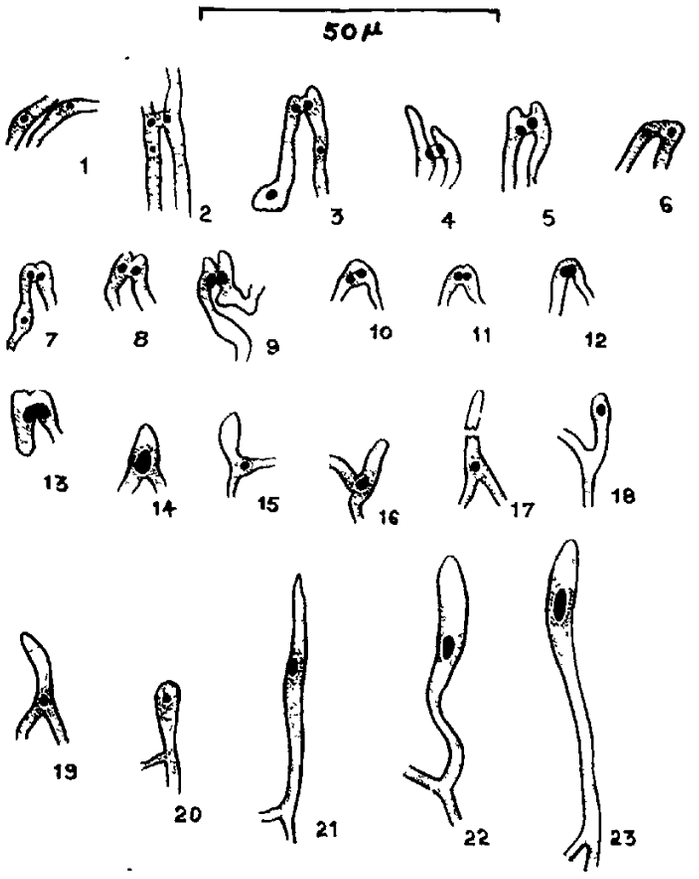
Fig. 34.28 Photomicrograph of Fig. 34.13.

Fig. 34.29 Photomicrograph of Fig. 34.16.

Fig. 34.30 Photomicrograph of Fig. 34.20

Fig. 34.31 Photomicrograph of Fig. 34.21.

PLATE 34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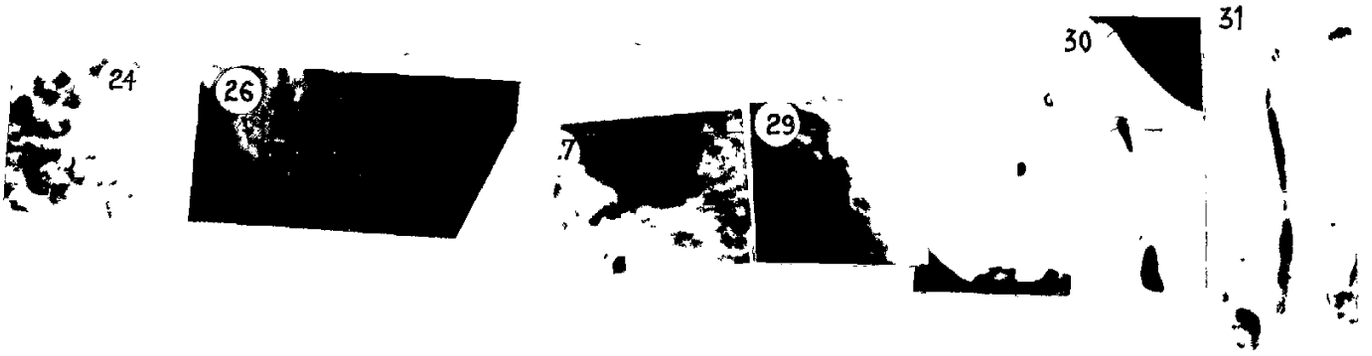
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EXPLANATION OF PLATE 35

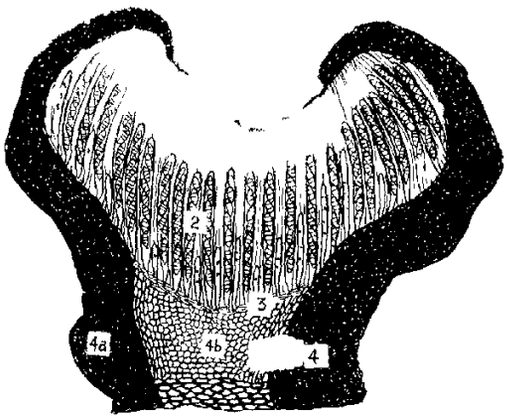
Tryblidiella indica

Fig. 35.1. Photograph showing the different parts of a well developed Discothecium.

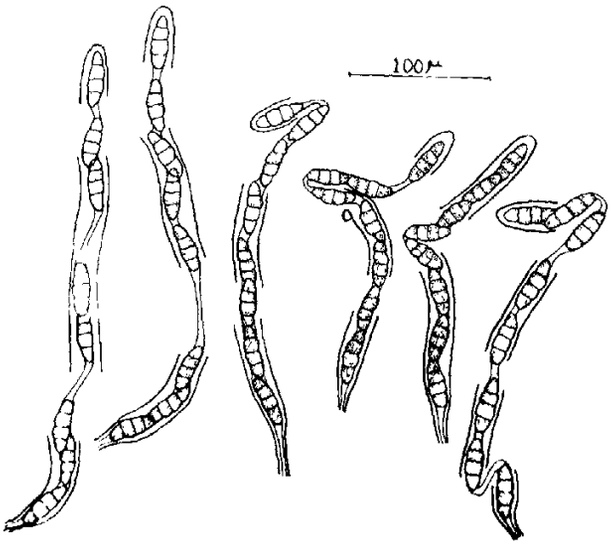
Fig. 35.2. Photograph showing the dehiscence of asci as well as their bitunicate nature.

PLATE 35

1



1 EPISTROMA
2 HYMENIUM
3 HYPOTHECIUM
4 EXCIPULUM
4a ECTAL
4b MEDULLARY



CHAPTER 3

NUCLEAR BEHAVIOUR, CHROMOSOME COMPLEMENT AND ASCOSPORE ORGANIZATION

In brief, the general trend of the nuclear events in the ascus are given here for the proper understanding of the nuclear behaviour as a whole.

It has already been pointed out that the mode of sexual reproduction is sometogamy, leading to the formation of diploid cell where karyogamy takes place. This ascus mother cell develops directly into an ascus. This is the only diploid stage in the life-cycle of this fungus. Later on this diploid nucleus undergoes a reduction division resulting in the formation of 2 nuclei. These two nuclei again undergo 4 mitotic divisions leading to the production of 32 nuclei in the ascus. The ascospores are organised at 16-nucleate stage.

The terminology of Singleton (1953) has been adopted here to describe the cytological process. The division I and II together constitute meiotic phase, Division I being the reductional and the subsequent divisions are mitotic and are denoted as Division III and so on.

Behaviour of the Diploid nucleus in the ascus

As already seen, the diploid nucleus is resulted by the fusion of two nuclei in the ascus mother cell which directly grows out vertically into an ascus. The outer wall of the ascus develops first followed by the nuclear divisions later in it. In early stages of development of the ascus, it was rather difficult to determine the exact manner of the origin of the two-walls of the ascus.

Division I :

The diploid nucleus is situated usually at the centre or slightly towards the tapering part of the ascus (Figs.36.1 to 36.15 & 37.1 to 37.11). Prior to the division the diploid nucleus is homogenous in appearance, varying in shape viz. spherical, conical, ovoid, rectangular, and expands considerably insize, upto 6.5 μ (Figs. 36.4 to 36.12 & 37.2 to 37.9).

Prophase I : Due to heavy staining of the chromatin material, it was not possible to observe earlier divisions (Figs.36.13 to 36.15 & 37.10 to 37.11). Pachytene was not clearly observed. However, Diplotene was seen clearly with a prominent nucleolus and two pairs of chromomeres which were lying one at each end of the nucleolus and sometimes both in chain one of them being attached to the nucleolus. No centrioles were seen as they were already visible in Tryblidiella rufula Seshadri (1967)(Figs.36.16 to 36.19 & 37.12 to 37.14).

In Pro-metaphase the size of the nucleolus was considerably reduced and the 2 pairs of chromosomes were approximately lying, in the equatorial plane (Figs.36.20 & 37.15). In Metaphase I the nucleolus was completely disappeared and 2 pairs of chromosomes are clearly visible in the equatorial plane (Figs.36.21 to 36.23 and 37.16, 37.17).

In Anaphase I two chromomeres move to opposite poles(Figs. 36.24, 36.25).

Telophase I : is represented by a bridge or cytokinensis measuring 1.8-2.4 μ in length (Figs.36.26, 36.27 & 37.18), as reported in case of Sphaerostilbe aurentifolia and Dothidea collecta (Luttrell, 1944, 1951), Rosenscheldiella eugeniae Ananthanarayanan (1964), and in Tryblidiella rufula by Seshadri (1967). At the end of Telophase I (Figs.36.28 & 37.19) two daughter nuclei are organised around the 2-chromosomes at both poles (Figs.36.29 to 36.31 & 37.20, 37.21).

Division II : The size of the two haploid nuclei is smaller than diploid nuclei and vary from 1-1.5 μ. These nuclei undergo a period of rest and expand in size prior to the further divisions (Figs.36.32, 36.33 & 37.22).

Metaphase II was observed (Figs.36.34, 36.35 & 37.23). There was no synchronization of the division similar to Tryblidiella rufula Seshadri (1967). The division is usually parallel to the longitudinal axis of the ascus. At the end of Telophase II (Figs.38.36 & 37.24) 4 nuclei were organized and are arranged parallel to the ascus wall (Figs.38.37 & 37.25).

Division III : This division was slightly oblique to the ascus wall Prior to the division the four nuclei were bigger in size and slightly irregular in shape (Figs.38.38 & 37.26). Metaphase III was observed and non-synthronization of the division was visible (Figs.38.39 & 39.1). At the end of Telophase III (Figs.38.40,38.41 & 39.2, 39.3) eight nuclei were organised which later were arranged parallel to longitudinal axis of the ascus (Figs.38.42 & 39.4).

Division IV : The 8-nucleate so formed further undergo division without any resting period (Figs.38.43, 38.44 & 39.5,39.6). No condensation of the cytoplasm and vacuole formation were noticed as against the condensation and presence of vacuoles around nuclei in Tryblidiella rufula Seshadri (1967) and Muthappa (1967). At the end of Telophase IV (Fig.38.45), 16 nuclei are formed which are arranged parallel to the longitudinal axis of the ascus (Figs.38.46, ,38.47 & 39.7 to 39.9).

Division V : This division takes place in the ascospore and because of the pigmentation of the spore, it was not clearly visible.

Thus at the end of Division V eight ascospores each with 4-cells are formed.

From the above study and observations on Tryblidiella indica it is clear that out of 5 divisions, the Division I is always reductional division and remaining four divisions are equational divisions.

On the basis of these studies the chromosome number in Tryblidiella indica Anahosur is determined as n = 2 haploid. No synchronisation of the divisions was observed throughout the nuclear divisions in the ascus.

Nuclear movement in the ascus

Usually the plane of spindles in the ascus determines the arrangement of the ascospores in the ascus. In Tryblidiella indica

the spindles are parallel to the long axis of the ascus during I and II divisions but are slightly oblique during Division III and IV indicating the obliquely uniseriate arrangement of the ascospores.

Organization of Ascospores in the Ascus

In the present fungus the ascospore delimitation was recognizable at 16-nucleate stage and the similar type is reported by Kowalski (1965) in Preussia typharum. No condensation of the cytoplasm and formation of vacuoles at 8-nucleate stage were recognizable. Cleavage of the ascus cytoplasm was brought about resulting in formation of segments each containing 2 nuclei (Figs.38.48 & 39.10). Further vacuoles were seen around the nuclei which coalesce and bring about the cytoplasmic cleavage around the nuclei resulting in the delimitation of ascospores (Figs.38.46, 38.47 & 38.44 & 39.7, 39.8), a mechanism similar to the one reported in the vast majority of the Ascomycetes. A septum is formed transversely and 2-celled ascospores are formed (Figs.38.50 & 39.11, 39.12). Later a thin subhyaline membrane is formed around each spore becoming darker in the later stages (Figs.38.51 to 38.53 & 39.13, 39.14). Further division of these two nuclei and by the formation of 2 more septa in each spore four celled ascospores are formed, each cell with a single nucleus derived from one of the eight nuclei present in the ascus. Later the ascospore wall thickens and becomes dark brown (Fig.39.15).

Summary and conclusions.

1. Sexual reproduction is somatogamous and the asci are directly produced from the diploid cell without the intervention of hooks or croziers.

2. 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.

3. The chromosome number is determined to be n = 2 haploid.

4. Nuclear divisions in the ascus usually do not synchronize.

5. The organization of ascospores takes place through the mechanism of cytoplasmic cleavage brought about by the condensation of cytoplasm around the nuclei followed by the the formation of vacuoles at the periphery of the nuclei. First septum is laid down at 16-nucleate stage and further 2 septa are laid down after the last division i.e. Division V which takes place in the spore.

6. The results so obtained are substantiated with photo-micrographs as well as Camera Lucida drawings.

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* Originals not seen.

EXPLANATION OF PLATE 36

Tryblidiella indica

Figs.36.1 to 36.3. Young asci with diploid nucleus.

Figs.36.4 to 36.12. Different shapes of diploid nuclei at different positions in the ascus.

Figs.36.13 to 36.15 Prophase I.

Figs.36.16 to 36.19 Diplotene, showing 2 pairs of chromosomes and nucleolus.

Fig. 36.20. Pro-metaphase.

Figs.36.21 to 36.23 Metaphase I.

Figs.36.24 & 36.25 Anaphase I

Figs.36.26 & 36.27 Telophase I.

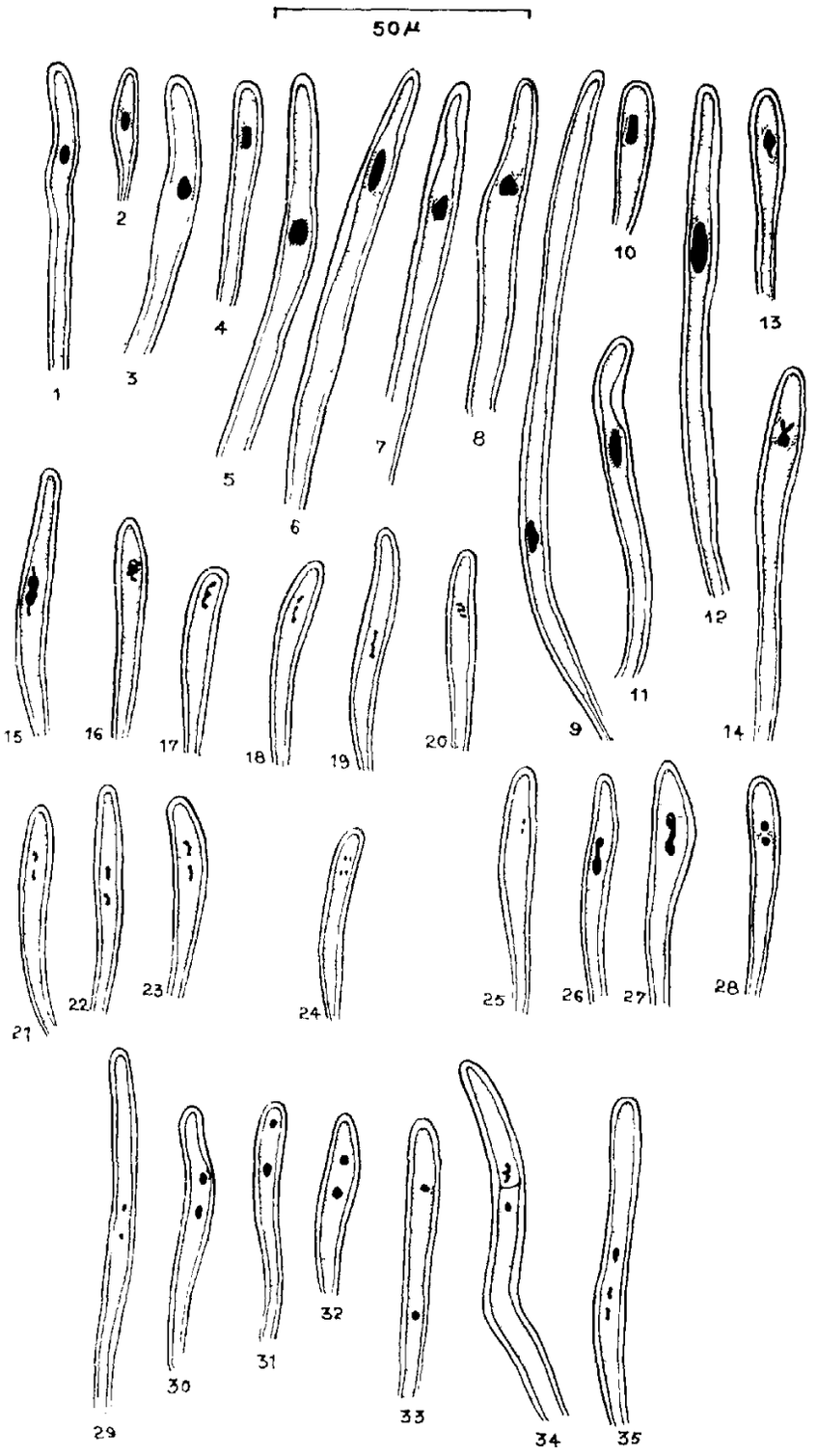
Fig. 36.28 End of Telophase I.

Figs.36.29 to 36.31. 2 nucleate stage

Figs.36.32 & 36.33 Prophase II

Figs.36.34 & 36.35. Metaphase II.

PLATE 36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EXPLANATION OF PLATE 37

Tryblidiella indica

Fig. 37.1. Photomicrograph of Fig. 36.1.

Fig. 37.2. Photomicrograph of Fig. 36.4.

Fig. 37.3. Photomicrograph of Fig. 36.5.

Fig. 37.4. Photomicrograph of Fig. 36.6

Fig. 37.5. Photomicrograph of Fig. 36.8.

Fig. 37.6. Photomicrograph of Fig. 36.9.

Fig. 37.7. Photomicrograph of Fig. 36.11.

Fig. 37.8. Photomicrograph of Fig. 36.13.

Fig. 37.9. Photomicrograph of Fig. 36.12

Figs.37.10 & 37.11. Photomicrographs of Figs.36.14 & 36.15.

Figs.37.12, 37.13 & 37.14. Photomicrographs of Figs.36.16,36.17 & 36.19 respectively.

Fig. 37.15. Pro-metaphase.

Figs.37.16 & 37.17. Photomicrographs of Figs.36.22 & 36.23.

Fig. 37.18 Photomicrograph of Fig. 36.26.

Figs.37.19,37.20 & 37.21. Photomicrographs of Figs.36.28,36.30 & 36.31 respectively.

Fig. 37.22 Photomicrograph of Fig. 36.32.

Fig. 37.23. Photomicrograph of Fig. 36.35.

Fig. 37.24. Photomicrograph of Fig. 36.36

Figs.37.25 & 37.26. Photomicrograph of Figs. 36.37 & 36.38.

EXPLANATION OF PLATE 38

Tryblidiella indica

Fig. 38.36. Telophase II.

Fig. 38.37. 4 nucleate stage.

Fig. 38.38. Prophase III.

Fig. 38.39. Metaphase III.

Fig. 38.40. Telophase III.

Fig. 38.41. End of Telophase III.

Fig. 38.42. 8 nucleate stage

Figs.38.43 & 38.44. Above nuclei dividing.

Fig. 38.45. Telophase IV.

Figs.38.46 & 38.47. 16-nucleate stages. Note the vacuoles.

Fig. 38.48. Portion of the 16-nucleate state showing each cleaved segment with 2-nuclei.

Fig. 38.49. Ascospore initials resulted by the coalescing of vacuoles.

Fig. 38.50. 2-nucleate spores. Note the vacuoles.

Figs.38.51 & 38.52. A thin membrane is formed around each spore.

Fig. 38.53. Enlarged nuclei in the young ascospores prior to further division.

EXPLANATION OF PLATE 39

Tryblidiella indica

Fig. 39.1. Photomicrograph of Fig. 38.39.

Figs.39.2 & 39.3. Photomicrographs of Figs.38.40 & 38.41.

Figs.39.4, 39.5 & 39.6. Photomicrographs of Figs.38.42,38.43 & 38.44 respectively.

Fig. 39.7. Photomicrograph of Figs.38.46 & 38.47.

Figs.39.8 & 39.9 16-nucleate stages.

Fig. 39.10 Photomicrograph of Fig. 38.48.

Fig. 39.11. Photomicrograph of Fig. 38.50.

Fig. 39.12. Photomicrograph of 2-nucleate ascospores.

Figs.39.13 & 39.14. Photomicrographs of Figs.38.51 & 38.52.

Fig. 39.15. Well-developed 4-celled ascospores in the ascus.

PLATE 38

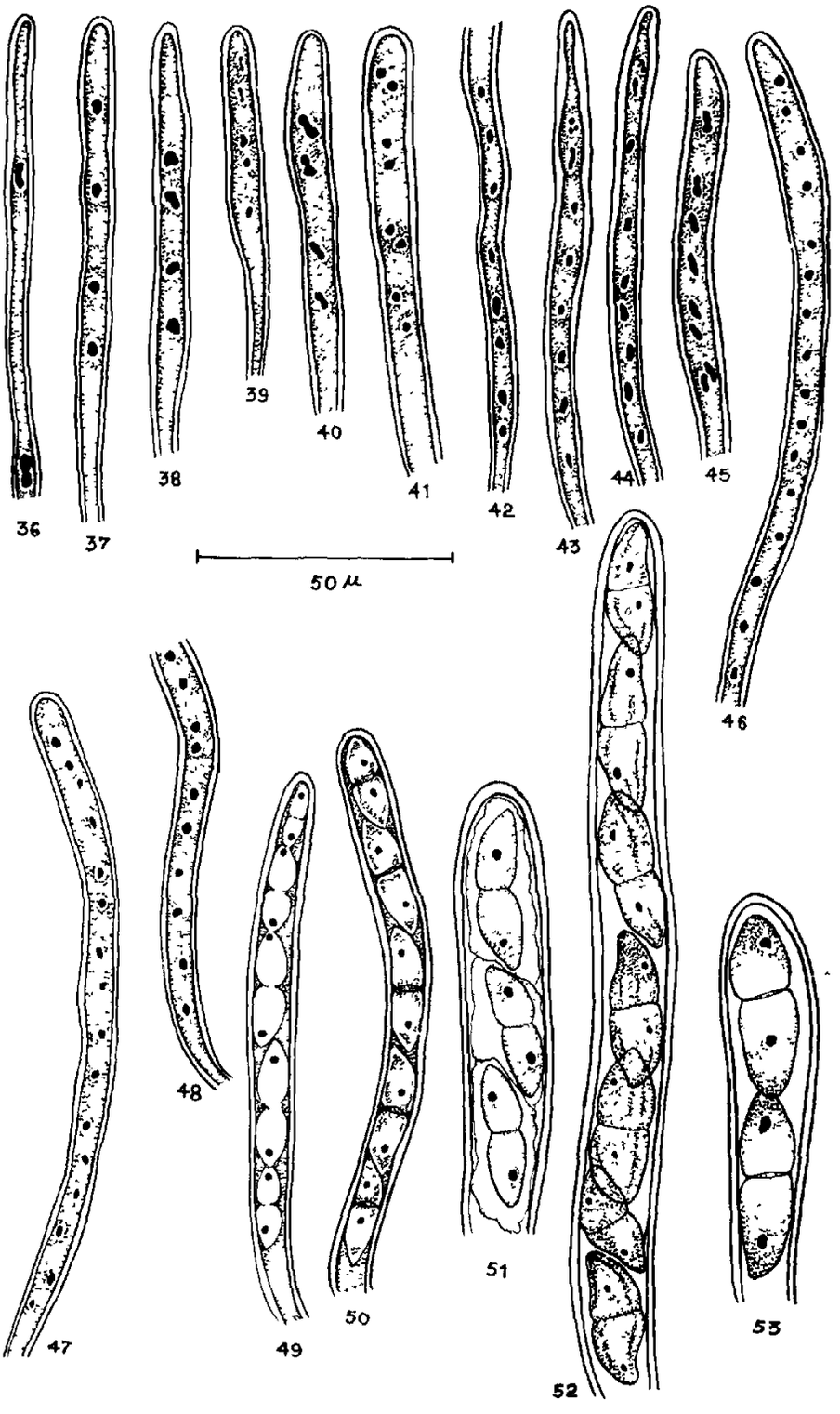

PLATE 37

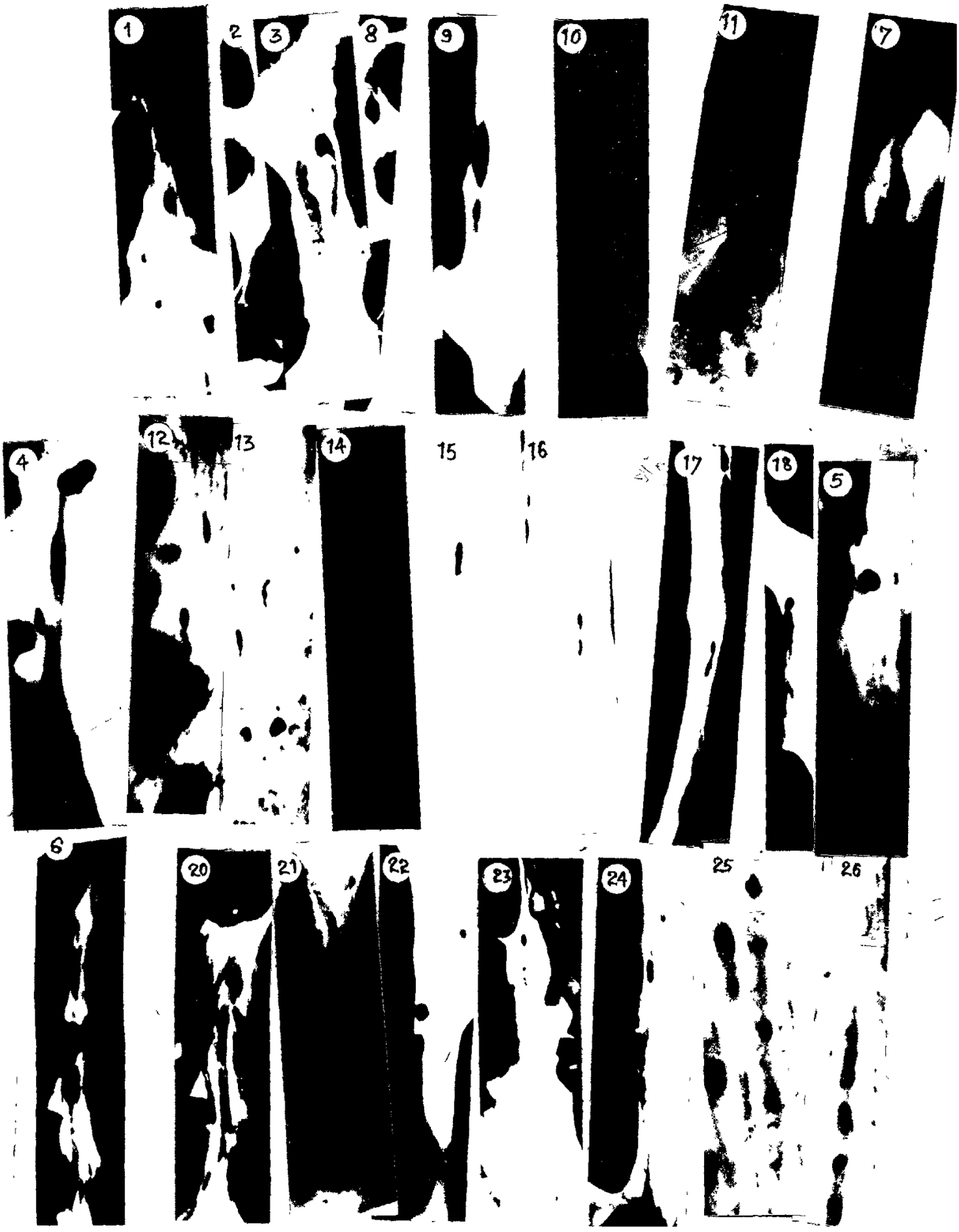

PLATE 39

PART - II

STUDIES INTO LIFE-CYCLES OF SOME INDIAN ASCOMYCETES

STUDIES INTO LIFE-CYCLES OF SOME INDIAN ASCOMYCETES

Introduction

In the course of his studies on Indian Ascomycetes carried out for a period of 3 years (1966-1968), the writer often noted close and constant association of conidial fungi with ascigerous states in nature. Similar associations have been reported by many previous workers, the latest reports being those of Viswanathan (1959), Tilak (1959,1968), Arnold and Russell(1960), Ananthanarayanan (1964), Patwardhan (1966a) and many others. Such associations may not necessarily indicate their true genetical relationship, the nature of which can only be determined through pure culture studies and cross-inoculation experiments. Such studies become imperitive particularly since these ascomycetes are mostly encountered in their ascigerous states in nature and are often widely separated from their conidial phases if any, both in time as well as stages of hosts. In many of the parasitic species the asexual stage alone occurs on the living host while the sexual phase is formed only on the dead tissues. Besides, further complications arise due to a single ascomycetous genus like Mycosphaerella Johanson having several conidial states belonging to different groups of Deuteromycetes depending upon the species such as species of Cercospora, Ramularia, Cladosporium belonging to Moniliales and Ascochyta, Septoria, Phyllosticta belonging to Sphaeropsidales. Therefore it is essential to carry out cultural studies for determining the true genetic relationship between the associated conidial fungus and the ascigerous state, which often occur in association on a host. The importance of

such cultural studies has been emphasized through the pioneer works of Klebahn (1905) and Higgins (1920) supplemented later by those of Lohman (1932), Shear (1933), Wolf (1935), Wehmeyer (1940), Ayers (1941), Webster (1955), Muller & Corbaz (1956), Barnett (1957), Buxton (1959), Luttrell (1963), Chesters and Greenhalgh (1964) and recently by Rogers (1966), Muthappa (1967), Pai (1968), Booth (1968), Jang and Rogers (1968), Funk (1968), Srinivasan and Thirumalachar (1969) and many others.

During the course of his studies on Indian Ascomycetes, the writer often observed the association of conidial fungi with the ascigerous phases in the following three fungi :

1. Bagnisiella acaciae Anahosur was found in association with Haplosporella subhyalinae Anahosur occurring on Acacia arabica,
2. Bagnisiella australis Speg. was found in association with Haplosporella cosmopolitus Muthappa occurring on Lantana camara L.
3. Didymosphaeria saprophytica Anahosur found in association with Diplodia and Haplosporella spp. occurring on Lantana camara L.

The close and constant association of the above conidial fungi with ascigerous states evoked interest and needed further study in respect of their possible genetic relationship. Therefore pure cultural studies were undertaken in to these ascomycetous fungi with a view to determine their true relationship with the respective

conidial fungi and the possible life-cycle pattern followed by them, the results of which are presented in this part.

Materials and Methods

Individual ascocarps were picked out from freshly collected infected twigs with the help of a sterilised needle and placed over a sterilised slide in drops of water containing Terramycin to prevent bacterial contamination and the ascocarps were teased out with the help of another sterilised glass-slide. A dilute suspension of ascospores was obtained by adding terramycin water which was then spread over the surface of the solidified water-agar medium in the Petri-dish. Even distribution of the ascospores was secured by gently rolling the Petri-dish. The germinated ascospores were marked out by a glass-marking pencil on the corresponding lower lid of the Petri-plate under the low power objective of the Microscope and then transferred to P.D.A. plates under asceptic conditions with the help of sterilised needle and single ascospore colonies were thus obtained for further observation. All work was carried out at the room temperature (25-27° C) with diurnal light.

I. Life-Cycle of Bagnisiella acaciae Anahosur.

The writer's collection of Bagnisiella acaciae Anahosur (1969) on Acacia arabica Willd (Leguminosae) occurring abundantly in the vicinity of Poona (Maharashtra) India, was found to be closely and constantly associated with a Sphaeropidaceous fungus,

identified as a species of Haplosporella Speg. characterised by subhyaline to light-brown cylindrical conidia borne in multi-loculate pycnostromata. This pycnidial fungus has been accommodated as a new species i.e. Haplosporella subhyalinae Anahosur on the basis of comparative studies and the unusually subhyaline nature of the pycnidiospores, the Latin diagnosis of which is given elsewhere. Studies into cultural behaviour were, therefore, undertaken to determine the true nature of this association with Haplosporella state and life-cycle pattern followed by the Ascomycete.

Materials and Methods :

From the freshly collected infected twigs of Acacia arabica the single ascospore and single conidial colonies were obtained for further observations according to the technique described under Materials and Methods. The ascospores readily yielded to germination on water-agar medium putting forward a single germtube in 8-10 hours. The conidia also germinated in 10-12 hours on water-agar medium putting forth a single germtube from each pycnidiospore.

Conidial State (Figs.40.2, 40.3 & 40.4) :

The single germinated ascospores made good growth on P.D.A. resulting in a colony of 62 mm. in 6 days. The colonies were aerial, white, flat with entire margin for the first three days and then turned to buff with dull-white aerial mycelium all over the colony. At the end of 6 days small hyphal knots were observed

over the entire surface of the colony. These pustules gradually turned dark-green to black after a week (Fig.40.1). Microscopic examination of sections through these dark-green stromatic bodies revealed the development of multiloculate pycno-stromata characteristic of the form-genus Haplosporella, which matured at the end of two weeks, a brief description of which is given below :

Pycnostromata dark green to black, spherical to irregular, multiloculate, upto 2.5 mm. Locules spherical to elongated, upto 12 in each stroma, monostiolate, 160-256 x 200-352 µ. Conidiophores short, cylindrical, simple, in wall-layers, 4-6 x 1-2 µ. Pycnidiospores cylindrical, straight to slightly curved, rounded at both ends, 1-celled, subhyaline to light brown, 20-24 x 4-6 µ. Sterile threads lacking.

A comparison between the Haplosporella state obtained in culture (P.D.A.) and the one on the host in nature showed complete agreement in respect of morphological characters, structure and dimensions as given in Table I.

Table I

Comparison between the Haplosporella state obtained in culture (P.D.A.) and from host

Source	Pycno-stroma	Locule	Conidio-phores	Conidia	Sterile threads
Host *Acacia arabica* Willd.	Upto 1.7 mm.	140-180x160-210 µ (upto 9)	4-6 x 2 µ	18-22 x 4-6 µ	Lacking
Culture (P.D.A.)	Upto 2.5 mm.	160-256x200-352 µ (upto 12)	4-6 x 1-2 µ	20-24 x 4-6 µ	Lacking

These single ascospore isolation experiments were repeated several times and in every case the same conidial state i.e. Haplosporella subhyalinae Anahosur was obtained in culture which agreed with the one produced on the host under natural conditions.

Micro-conidial State (Figs. 40.5, 41.1 & 41.2) :

Amongst these dark-green to black stromatic bodies produced in culture were found scattered minute spherical papillate stromatic bodies releasing white ooze over their mouths which on microscopic examination were found to be in the nature of micro-conidial locules filled with masses of minute microconidia. Such microconidial locules were, however, not observed on the host in nature. A brief description of the microconidial state is given here :

Stromata spherical, upto 800 u diam., multiloculate, dull white. Locules spherical, upto 11 in each stroma, ostiolate, 50-80 μ diam. Conidiophores cylindrical, branching, in wall-layers, hyaline, 10-14 x 2 μ. Microconidia cylindrical, hyaline, 4-6 μ long.

These microconidia were found to be incapable of germination and appeared to be in the nature of true spermatia, the exact function of which is not known and needs further study.

Ascigerous State (Figs. 41.3 & 41.4):

Observations of the four-weeks old single ascospore colonies revealed that following the production of the conidial state i.e. Haplosporella subhyalinae, the stromatic bodies also produced

immature ascocarps in close association with the conidial state. These stromatic bodies were carbonaceous, hard, spherical to cushion shaped, black in colour (Fig.40.1). Hand sections through such hard stromatic bodies revealed the development of discoid to cushion shaped but immature ascocarps made up of 3-5 locules arranged in parallel lines but separated by stromatic tissue. These immature ascocarps consisted of an upper layer of thin-walled hyaline cells and a basal layer of thick-walled dark pseudoparenchymatous cells typical of the structure of the immature ascocarps obtained on the host i.e. Acacia arabica in nature. The ascocarp initials showed no tendency for further development and remained sterile. The entire stromatic structure consisted of immature ascocarps placed on the top layers and the conidial state i.e. Haplosporella state at the basal layers in close association with each other (Fig. 41.4).

Efforts to induce the production of mature ascocarps in culture failed inspite of stimulatory treatments like freezing, temperatures, light etc. and the use of different media like carrot agar, cornmeal agar, Richard's agar, Brown's agar and M_2 medium. These experiments were repeated in the reverse direction, beginning with single spore cultures of the pycnidial fungus H. subhyalinae obtained from Acacia arabica which invariably gave rise to the immature ascocarp initials in culture (P.D.A.) similar in structure to the ones produced in single ascospore cultures, followed by the production of conidial state, i.e.

Haplosporella state as well as Microconidial state thus confirming the life-cycle pattern of this ascomycetous fungus.

Discussion and Conclusions

This is the first report on the artificial culture of this myriangiaceous genus Bagnisiella Speg. i.e. Bagnisiella acaciae Anahosur. These studies into the behaviour and the life-cycle pattern of this Ascomycete have helped to prove the true genetical relationship between this Ascomycete and the pycnidial fungus Haplosporella subhyalinae Anahosur, through pure culture experiments

The non-maturation of the ascocarps in culture may be attributed to lack of certain specific growth substances in the medium or genetical factors, the nature of which needs further study. The fungus is homothallic in sex compatibility.

It was also interesting to notice that the single ascospore cultures of Bagnisiella acaciae Anahosur obtained from a different host i.e. Glyricidia sp. collected in the vicinity of Poona (Maharashtra) India, invariably gave rise to the conidial state i.e. Haplosporella subhyalinae, microconidial state leading to the formation of the immature ascocarps thus confirming the life-cycle pattern of this Ascomycete characterised by its constant production of conidial state in the form-genus Haplosporella.

II. Life Cycle of Bagnisiella australis Speg.

Bagnisiella australis Speg. occurring abundantly as a saprophyte on Lantana camara L. in the vicinity of Poona(Maharashtra

India was collected by the writer during different seasons. Microscopic observations through the fungus lesions revealed the close association of Sphaeropsidaceous fungus later identified as a species of Haplosporella with the Ascomycete in the same infection spots. Such associations which were found to be a constant feature of this collection on the host under natural conditions stimulated interest and needed further studies into the exact nature of such associations through cultural studies with a view to establish the relationship, if any, of this Ascomycete with the conidial fungus (Species of Haplosporella).

Materials and Methods :

The ascospores of D. australis obtained from the freshly collected infected twigs of Lantana Camara yielded to germination in 8-10 hours on water-agar putting forward a single germ tube from each spore; single ascospore colonies were obtained on P.D.A. from such single germinated ascospores according to the technique described under materials and methods.

Conidial State (Figs. 42.2, 42.3 & 42.4) :

The germinated ascospores made good growth on P.D.A. resulting in a colony of 71 mm. at the end of 6 days. The colonies were aerial, dull-white for the first three days and turned to dark-green with dull-white aerial mycelium all over the colony. At

the end of 5 days small hyphal knots were observed all over the surface of the colony which gradually turned dark-green to black. Black oozings were noticed over the surface of these stromatic bodies at the end of 10-12 days which consisted of masses of dark-brown 1-celled conidia (Fig.42.1). Microscopic examination of sections through such dark-green to black stromatic bodies obtained from culture revealed the development of the pycnidial fungus, a species of Haplosporella, a brief description of which is given below :

Pycnostromata spherical to clavate, often proliferating at the apex, dark-green to black, multiloculate, upto 2.5 mm. Locules spherical to elongated, ostiolate, 88-320 μ diam., 8-12 in each stroma. Conidiophores simple, short, cylindrical, in wall-layers, hyaline, 6-8 x 2 μ. Pycnidiospores ovoid to oblong, dark-brown, thick-walled, 1-celled, 18-22 x 8-10 μ. Sterile threads abundant, filiform and hyaline.

The Haplosporella state obtained in culture (P.D.A.) was compared with the Haplosporella state obtained from the host and was found to agree in respect of morphological characters, structure and dimensions as given in the Table II.

Table II

Comparison between Haplosporella species obtained from host and in culture (P.D.A.)

Source	Pycno-stromata	Locule	Conidio-phores	Conidia
Host (Lantana camara)	Upto 1.9 mm.	100-380 µ (3-6)	6-8 x 2 µ	16-20 x 10-12 µ
Culture (P.D.A.).	Upto 2.5 mm.	88-320 µ (8-12)	6-8 x 2 µ	18-22 x 8-10 µ

Remarks: The pycnidial fungus was identified as Haplosporella cosmopolitus Muthappa on the basis of comparative studies as described by Muthappa (1967).

Ascigerous state (Fig. 42.5) :

The stromatic structures in culture turned to dark green and hard in consistency with age at the end of four weeks and were found to produce discoid to cushion-shaped ascocarp initials similar in structure to those produced by the myriangiaceous genus Bagnisiella Speg. on host characterised by upper layers of thin-walled hyaline cells and basal layers of thick-walled dark brown pseudoparenchymatous cells, typical of the immature ascocarps obtained from the host produced under natural conditions. The ascocarp initials remained immature and did not give rise to mature ascocarps.

Efforts to obtain mature ascocarps in culture failed in spite of stimulatory treatments like freezing temperatures, light etc. and the use of different media like carrot agar, cornmeal agar, Richard's agar, M_2-medium.

These experiments were repeated in the reverse direction, beginning with the single conidial cultures of the Haplosporella state obtained from the host i.e. Lantana camara which invariably yielded immature ascocarps of the Bagnisiella state proving the true relationship of the Haplosporella (conidial) state with the Bagnisiella (Ascigerous) state.

The following media were also employed in addition to P.D.A. (Fig.43.1) on which the conidial fungus (Haplosporella cosmopolitus) was successfully grown. A brief description of colony characters, stromatic bodies etc. is given here.

1. Potato-Dextrose-Agar (P.D.A.) :

Colonies dark-green in the centre and dull-white in the margin with aerial mycelium all over the colony, reaching a diameter of 71 mm. at the end of 5 days. The pycnidial initials in the beginning were in the form of hyphal knots which matured at the end of 10 days indicated by black oozing over their surface. The stromatic bodies gradually turned to dark-green to black, spherical to clavate, profusely proliferating at the apex and their production was profuse.

2. Cornmeal Agar :

Colonies dark-green in the centre, growth flat, reaching a diameter of 74 mm. at the end of 5 days, with white aerial mycelium in the centre. Pycnostromatic bodies abundant, scattered all over the colony with black oozing over their surface, proliferating profusely at the apex, spherical to clavate at maturity (Figs. 43.2 & 43.3).

3. Carrot Agar :

Colonies dark-green in the centre and buff in the margin with white aerial mycelium in the centre, reaching 60 mm. at the end of 5 days. On 6th day pycnidial initiation was noticed in the form of small hyphal knots starting from the margin. On 10th day these pycnidial bodies were with black oozing over their surface indicating their matured condition, spherical to clavate; pycnidial production was moderate.

4. Peas Agar :

Colonies cottony white with rich white aerial mycelium in the centre reaching a colony diameter of 33 mm. at the end of 5 days. Matured pycnostromatic bodies were obtained at the end of 12 days indicated by the black oozing over their surface. These pycnidial bodies were aggregated only in the centre and their production was poor.

5. Potato agar :

Growth scanty for the first 5 days and reached a diameter of 40 mm. at the end of 10th day. Pycnidial bodies scattered in the margin were found to be matured at the end of 15th day indicated by the black oozing over their surface and their production was poor.

Hand sections of the pycnostromatic bodies obtained from the above media revealed the presence of the conidial state in all cases, i.e. H. cosmopolitus similar to the one obtained from

single ascospore cultures in respect of morphological characters and dimensions. On further observations of the 4-weeks old cultures, it was noticed that only in P.D.A., cornmeal and carrot agar the stromatic bodies consisted of immature ascocarps of Bagnisiella australis, quite similar in morphological characters to the ones obtained in culture (P.D.A.) obtained from single ascospore cultures, thus confirming the true genetic relationship of Haplosporella cosmopolitus Muthappa with Bagnisiella australis Speg. and the life-cycle pattern. None of the media employed above, however, induced the production of mature ascocarps.

Discussion and conclusions

The myriangiaceous fungus Bagnisiella Speg. has been proved to produce a conidial stete in the form-genus Haplosporella identified as H. cosmopolitus Muthappa, on the basis of not only natural associations obtained on host but also through pure culture studies. It is interesting to note that the writer's studies on another species of Bagnisiella Speg. i.e. B. acaciae Anahosur occurring on Acacia arabica described earlier has revealed the similar pattern of life cycle, the conidial state here being identified as Haplosporella subhyalinae Anahosur. The non-maturity of the ascocarps in culture may be attributed to lack of certain specific nutrients in the medium or genetic factors as was the case with B. acaciae described earlier.

The ascigerous fungus Bagnisiella belonging to Myriangiaceae thus shows remarkable uniformity in respect of its conidial state in the form-genus Haplosporella belonging to Sphaeropsidaceae.

Such uniformity in life cycle pattern has been previously noted in several Ascomycetes viz. Glomerella - Colletotrichum, Powdery mildew fungi (Erysiphaceae), Claviceps -Sphacelia, Balansia - Ephelis Complex. On the other hand, the position is more complex in the ascigerous genus Mycosphaerella, where several conidial fungi are involved such as Cercospora, Ramularia, Ascochyta, Septoria and Phyllosticta in its life cycle depending upon the species involved.

III. Life Cycle of Didymosphaeria saprophytica Ambhosur

Didymosphaeria saprophytica described by the writer (1969) collected on Lantana camara L. occurring abundantly in the vicinity of Poona (Maharashtra) India was found to be constantly and closely associated with two Sphaeropsidaceous fungi i.e. species of Haplosporella Speg. and Diplodia Fr. in nature.Cultural studies were, therefore, undertaken into this Ascomycete with a view to study its behaviour and life-cycle pattern with special reference to the possible relationship of the two conidial fungi which were,

found to be so closely associated with this ascomycete on the host in nature.

Materials and Methods :

The ascospores germinated readily on water-agar medium in 16-18 hours giving out a single germ tube from one of the two cells. In no case both cells of an ascospore germinated. Single ascospore colonies were obtained according to the technique described previously under Materials and Methods.

Conidial State (Figs.44.2 & 44.3) :

The ascospores made good growth on P.D.A. resulting in a colony of 56 mm. at the end of 9 days. The colonies were aerial, white for the first four days and gradually turned to dark-green with white scanty aerial mycelium all over the colony. At the end of 10 days the single ascospore cultures produced small hyphal knots all over the surface of the colony which on microscopic examination were found to be in the nature of pycnidial initials (Fig.44.1). These pycnidial initials matured at the end of 18-20 days and produced well developed pycnidia with 2-celled dark-brown pycnidiospores on the basis of which this fungus was identified as a species of the form-genus Diplodia Fr. having the following description.

Pycnidia globose, ostiolate, dark-brown, separate, 200-280 x 260-360 µ. Conidiophores, cylindrical, simple, in wall-layers,

hyaline, 4-6 x 1-2 μ. Pycnidiospores dark-brown, double walled, equally 2-celled, oblong, 16-20 x 4 μ.

These single ascospore experiments were repeated several times and in every case the same species of Diplodia was produced in culture. None of the numerous single ascospore colonies produced Haplosporella stage in culture although this fungus was invariably found associated with the ascomycete on the host in nature.

Ascigerous State (Figs.44.1, 44.4, 45.1 to 45.5) :

Further observations on the single ascospore colonies revealed that following the production of Diplodia stage, minute cupulate pustules developed in the deeper layers of these colonies by the end of 3 weeks. Microscopic examination of these pustules revealed that they consisted of ascocarp initials filled with thin hyaline filamentous structures continuous from the apex to the bottom of the ascocarp among which were produced numerous young asci. These cupulate pustules developed into mature ascocarps at the end of 4 weeks with a 'centrum' consisting of pseudoparaphyses, clavate bitunicate asci provided with long pedicels and spindle-shaped, equally 2-celled, dark-brown ascospores characteristic of the genus Didymosphaeria Fckl., a brief description of which is given below:

Ascostromata (Pseudothecia) uniloculate, globose, ostiolate 210-400 μ. Asci clavate, bitunicate, in wall-layers, octosporous, pedicellate, 96-108 x 8-12 μ. Ascospores dark-brown, equally 2-ce biseriate, thick-walled, 14-18 x 4-8 μ. Pseudoparaphyses abundant filiform, slender and hyaline.

The ascocarps produced in culture (P.D.A.) agreed with those obtained from the host in nature in all essential respects including morphological characters, structure and dimensions as given in Table III.

Table III

Comparison between Didymosphaeria saprophytica Anahosur obtained in culture (P.D.A.) and from the host

Source	Ascostromata	Asci	Ascospores
Host *Lantana camara* L.	280-390 μ diam.	90-100x8-10 μ	16-18x4-8 μ
Culture (P.D.A.)	210-400 μ diam.	96-108x8-12 μ	14-18x4-8 μ

These experiments were repeated in the reverse direction beginning with the single spore cultures of the pycnidial fungus (*Diplodia* sp.) obtained from the host which invariably gave rise to the ascigerous phase in culture (P.D.A.) characteristic of *Didymosphaeria saprophytica* thus confirming the true genetic relationship of this ascomycete with the pycnidial state (*Diplodia* sp.) and its life-cycle pattern as outlined in the above experiments.

Discussion and Conclusions

This is the first report on the cultural behaviour of this Ascomycete and offers conclusive evidence on the life-cycle pattern and the conidial phase of this fungus being a species of the form-genus *Diplodia* Fr. The close association of *Haplosporella* stage with this Ascomycete on the host under natural conditions is thus of accidental character without any genetic relationship. The production of ascigerous state by single ascospore cultures proves this fungus to be homothallic in its sexual compatibility.

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* Original not seen.

EXPLANATION OF PLATE 40

Bagnisiella acaciae Anahosur

Fig.40. 1. Photograph showing the fructifications of **Bagnisiella acaciae** in P.D.A.

Conidial State
(**Haplosporella subhyalinae** Anahosur)

Fig.40. 2. Section of the Pycnostromatic body showing multiloculate habit.

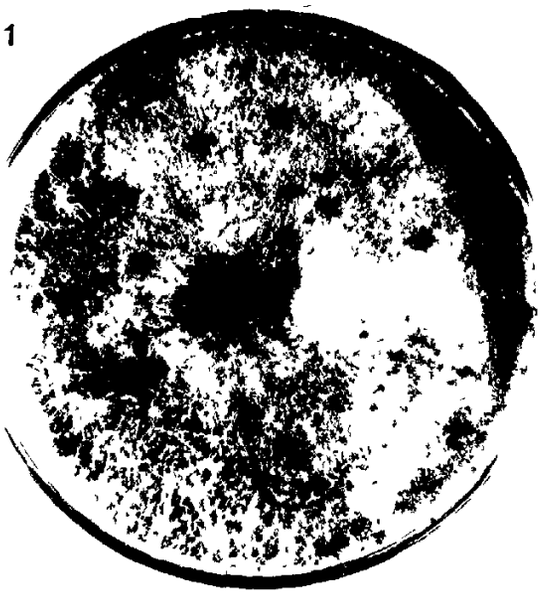
Fig.40. 3. Individual locule enlarged.

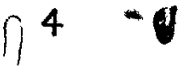
Fig.40. 4. Conidia.

Micro-conidial State

Fig.40. 5. Section of the stroma showing multiloculate nature.

PLATE 40





EXPLANATION OF PLATE 41

Bagnisiella acaciae Anahosur

Fig.41. 1. Individual Micro-Pycnidial locule enlarged.

Fig.41. 2. Conidiophores and Micro-conidia.

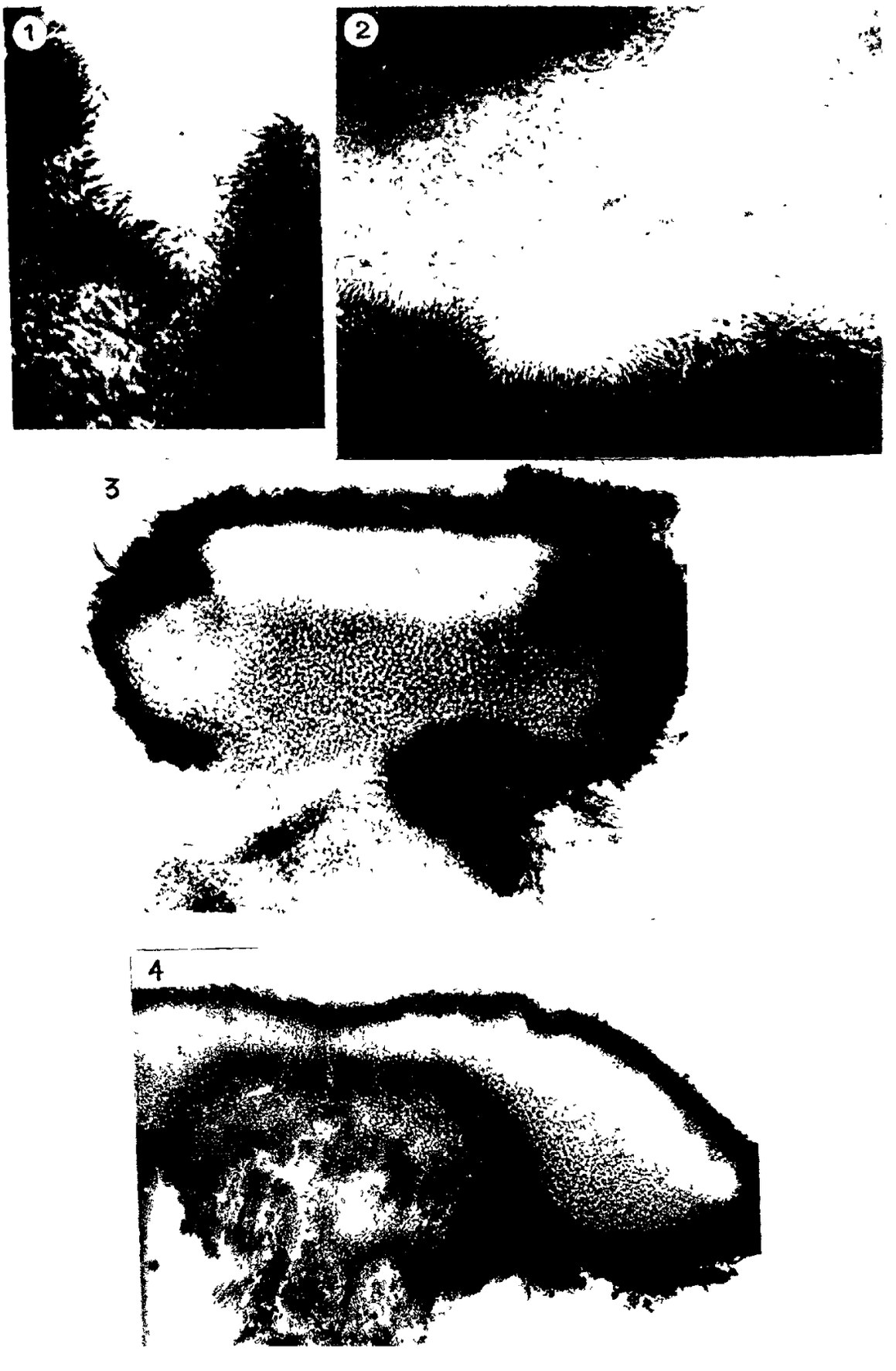
Ascigerous State

(Bagnisiella acaciae Anahosur)

Fig.41.3. Section of immature ascocarp of **Bagnisiella acaciae** (P.D.A.).

Fig.41.4. Section of the stromatic body with upper layer of ascigerous state (**Bagnisiella acaciae** Anahosur) and basal layer of Pycnidial locules (**Haplosporella subhyalinae** Anahosur).

PLATE 41



EXPLANATION OF PLATE 42

Bagnisiella australis Speg.

Fig.42. 1. Photograph showing stromatic bodies obtained from single ascospore of *B. australis* in P.D.A.

Conidial State
(**Haplosporella cosmopolitus** Muthappa)

Fig.42. 2. Section of the Pycnostromatic body showing multiloculate nature.

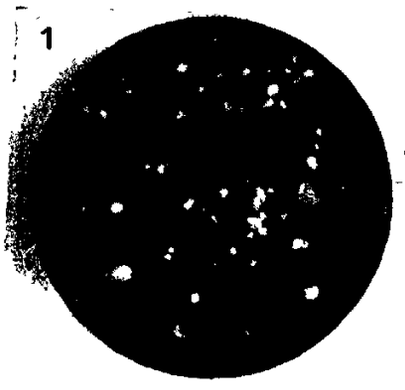
Fig.42. 3. Individual loeule enlarged showing sterile threads.

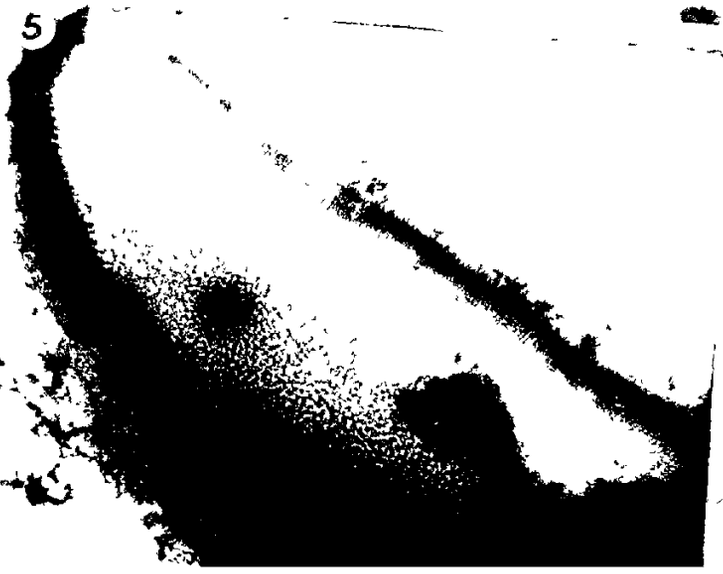
Fig.42. 4. Conidia.

Ascigerous State
(**Bagnisiella australis** Speg.)

Fig.42. 5. Section of an immature ascostroma obtained from P.D.A.

PLATE 42





EXPLANATION OF PLATE 43

Haplosporella cosmopolitus

The conidial State of Bagnisiella australis Speg.

Behaviour in aritifical culture

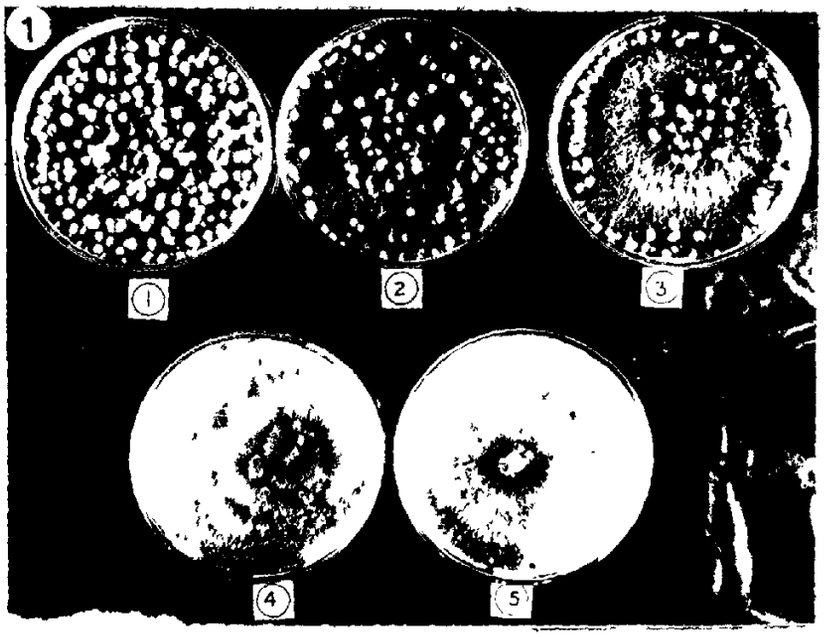
Fig.43.1. Production of stromatic bodies in different media from single conidium.

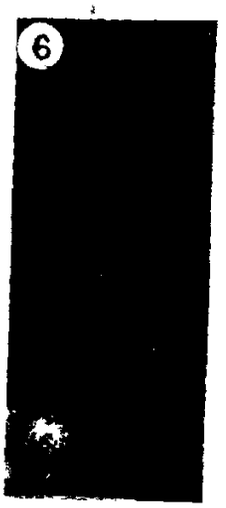
(1) Cornmeal Agar, (2) Potato Dextrose Agar,
(3) Carrot Agar, (4) Peas Agar,
(5) Potato Agar.

Fig.43.2. Photograph showing the proliferation of the stromatic bodies.

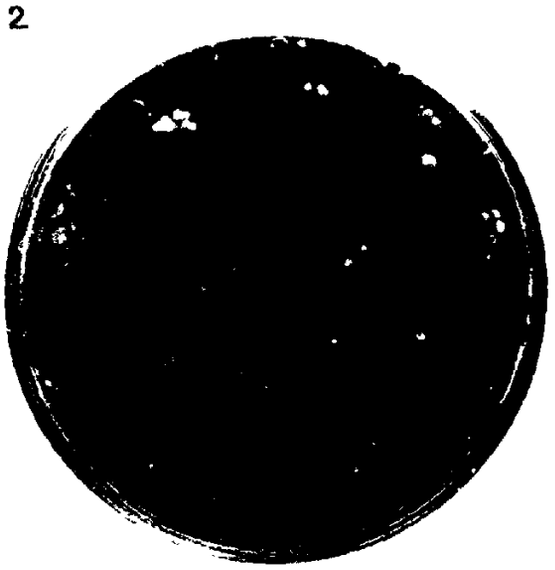
Fig.43.3. Individual proliferated stromata.

PLATE 43





2



EXPLANATION OF PLATE 44

Didymosphaeria saprophytica Anahosur

Fig. 44.1. Photograph showing the fructifications in P.D.A.

Conidial State
(*Diplodia* sp.)

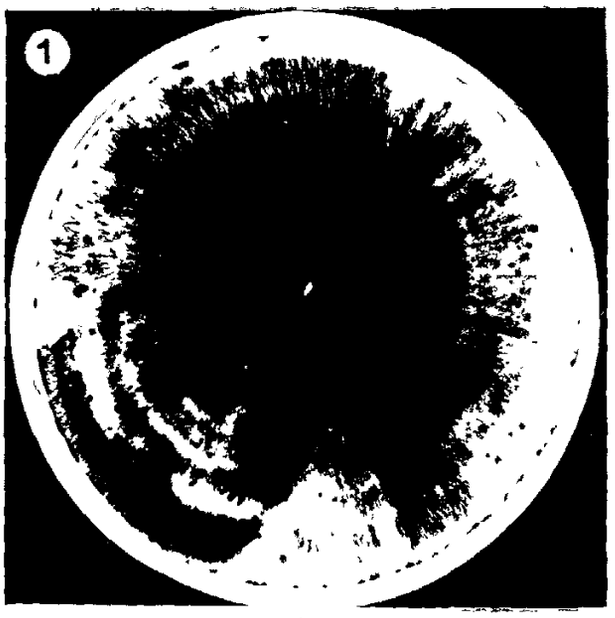
Fig. 44.2. Pycnidium.

Fig. 44.3. Conidia.

Ascigerous State
(*Didymosphaeria saprophytica*)

Fig. 44.4. Section through the stromatic body showing uniloculate nature of the ascocarp.

PLATE 44



EXPLANATION OF PLATE 45

Didymosphaeria saprophytica Anahosur

Ascigerous state contd. (in culture)

Fig.45.1. Individual locule of the ascocarp enlarged showing Pseudoparaphyses, Asci and Ascospores.

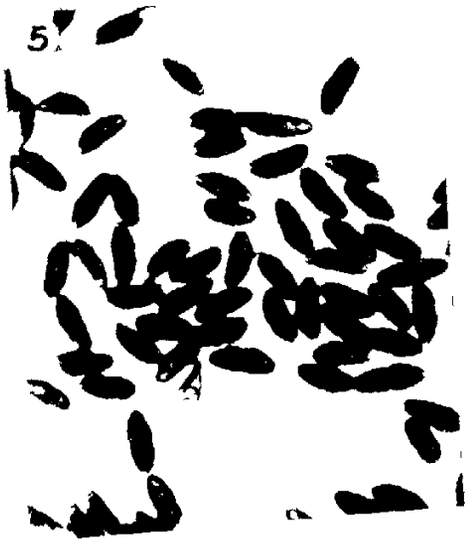
Fig.45.2. Asci released from ascocarp, under pressure.

Fig.45.3. Asci with long pedicels.

Fig.45.4. Biseriate arrangement of ascospores in the ascus.

Fig.45.5. Ascospores.

PLATE 45



PART - III

TAXONOMIC STUDIES IN SOME INDIAN ASCOMYCETES

TAXONOMIC STUDIES IN SOME INDIAN ASCOMYCETES

The ascomyceteous fungi with richness of their pattern, morphology and highly heterogenous nature comprising pathogens as well as Saprogens occur on different parts of host plants and are widely distributed all over the world and are recognized through their chief characters i.e. the asci, which in higher forms are borne in various types of fructifications like perithecia, cleistothecia, apothecia, pseudothecia, 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 Bhide (1949), Sanwal (1953), Ramakrishnan (1953, 1956), Thind & Batra (1957), Chona, Munjal and Bajaj (1958), Viswanathan (1959), Batra (1960), Govindu and Thirumalachar (1960), Bose (1961, 1962), Kapoor and Gill (1961), Tilak (1958-1969), Ananthanarayanan (1964), Bose and Muller (1964, 1965, 1967), Kaloni (1965), Patil and Thirumalachar (1965), Patwardhan (1966), Ramchandra Rao (1966), Seshadri (1967), Muthappa (1967), Chiplonkar (1969) and Pande (1969).

During his mycological survey carried out at the forests of Coorg (Mysore State) and in and around Poona (Maharashtra) the writer collected 52 Ascomycetes, a detailed study into the diagnosis and determination of which revealed that 24 were new to science, 25 were in the nature of new host records and 3 new reports to India. the Taxonomic studies comprise of two sections. Section A - pertains to the Ascomycetes of Coorg and Section B - to Ascomycetes of Maharashtra.

SECTION - A

ASCOMYCETES OF COORG (MYSORE STATE)

INDIA

SECTION A

ASCOMYCETES OF COORG (MYSORE STATE), INDIA

INTRODUCTION.

The Coorg forests of Mysore State, India situated at an altitude of 4000 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 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) and Muthappa (1967). The writer, therefore, undertook a more detailed and comprehensive survey of these forests during the period 1966-1968, 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 some 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.

Repeated visits to Coorg forests resulted in the collection of 44 Ascomycetes comprising 31 different genera of which 21 species were determined as new species on the basis of critical and

comparative studies and host relationship, 20 are new host records and 2 new reports to India. An interesting Ascomycete parasitizing leaves of Eugenia jambolana Lam.(F.Myrtaceae) has been assigned to a new genus "Muelleromyces" Kamat & Anahosur, a new member of Diaporthaceae, named in the honour of Dr.E.Muller of Zurich. Among Deuteromycetes, a Sphaeropsidaceous fungus parasitizing leaves of Eugenia jambolana Lam. has been assigned to a new form-genus "Kamatella" Anahosur, named in honour of my Research Guide, Prof. M.N.Kamat.

A brief account of the diagnostic characters of all the Ascomycetes of Coorg based on their camera lucida drawings drawn from the hand sections of fungus materials has been presented in this section with photomicrographs wherever necessary. The genera are arranged in alphabetical order.

1. Apiospora Sacc.

F. Amphisphaeriaceae. O. Sphaeriales.

Sub-Class : Ascohymeniales.

Introduction:

The genus Apiospora was established by Saccardo (1875) with Apiospora montagnei Sacc. as the type. Clements and Shear (1931) and Muller and Arx (1962) have placed this genus in the family Amphisphaeriaceae of the order Sphaeriales. This genus was represented in India by 3 species till 1962, all of which are reported to be saprophytic in habit. The writer's collection of Apiospora on two different hosts were identified at C.M.I.,Kew,England as A.montagnei and A. curvispora,brief descriptions of which are given here.

a. Apiospora curvispora (Speg)Behm. (Fig.46.1)

Muller & Arx 1962, p.683.

Infection spots black, shining, aggregated and sometimes coalesce to produce long streaks. Perithecia isolated, globose, clypeate, ostiolate, 150-180 µ diam. Asci clavate, unitunicate, pedicellate, octosporous, in basal layers, 110-136 x 12-16 µ. Ascospores unequally 2-celled, hyaline, biseriate, oblong, 14-18 x 4 µ.

Habit : Saprophytic on the twigs of Oxytenanthera sp.(f.Graminae) collected during October 1966. M.A.C.S.Herb. No.499.

Remarks : This is a new host record for this fungus.

b. Apiospora montagnei Sacc. (Fig.46.2).

Syll.Fung. 1:201, 1882.

Infection spots linear, black, coalesce forming streaks, erumpent, 0.3-0.8 mm. long. Perithecia isolated to aggregated, clypeate, innate, becoming erumpent at maturity, globose, ostiolate, 140-200 µ diam. Asci clavate, paraphysate, unitunicate, pedicellate in basal layers, octosporous, 80-120 x 14-18 µ. Ascospores oblong, slightly curved at the end, unequally 2-celled, biseriate, hyaline, 26-34 x 4-6 µ. Paraphyses and periphyses abundant, slender, filiform and hyaline.

Habit : Saprophytic on the twigs of Bambusa sp.(F. Graminae) collected during October 1966, M.A.C.S.Herb. No.500.

Remarks : Bambusa sp. is a new host record to this fungus.

2. Botryosphaeria Ces. deNot.

F. Botryosphaeriaceae. O. Dothideales.

Sub-Class Loculeascomycetes.

The genus Botryosphaeria was founded by Cesati and deNotaris (1863). The type species is Botryosphaeria ribis Gross. & Dug. Further the genus was emended by Shear in 1924. Most of the species are saprophytic on the twigs of various host plants and very few species are reported from India. Botryosphaeria hysteroidea F. & E. is a parasite on the leaves of Peraloe dayi reported by Ellis and Everhart (1895). Theissen (1916) has contributed few species and placed the genus under Dothideales. Luttrell (1965a) follows Theissen (1916) while Arx & Muller (1954) treat this genus as a member of the order Dothiorales. The writer's collections of Botryosphaeria on three different hosts were identified as similar to Botryosphaeria dothidea, a brief description of which is given below.

a. Botryosphaeria dothidea (Moug. ex Fr.)Ces. deNot.(Figs.46.3 to 46.5)

Arx & Muller 1954, p. 37.

Ascostromata black, innate, becoming erumpent, multiloculate, upto 4 locules in each stroma, upto 0.8 mm. long. Locules globose, ostiolate, 140-190 µ diam. Asci clavate, bitunicate, in wall-layers, pedicellate, octosporous, 70-92 x 20-26 µ. Ascospores spindle-shaped, biseriate, hyaline, with 2-3 vacuoles, 14-18 x 4-6 µ. Interthecial tissues present.

Habit: Saprophytic on the twigs of Rosa sp.(F.Rosaceae)M.A.C.S.Herb. No. 504, Cassia occidentalis Naves (F.Leguminosae) M.A.C.S.Herb.No. 505 and Haeptaplureum venulosum Sm.(F.Araliaceae) M.A.C.S.Herb. No.

Remarks : The three hosts are new host records and the fungus is an addition to fungi of India.

b. Botryosphaeria laricis (Wehm) Comb.nov. Arx & Muller.(Fig.46.6)

Arx & Muller 1954, p. 42.

Ascostromata black, innate, becoming erumpent, 200-600 μ long. Locules globose, ostiolate, 1-3 in each stroma, 140-250 μ diam. Asci clavate, bitunicate, pedicellate, octosporous, in basal layers, provided with apical pore, 120-152 x 24-28 μ. Ascospores spindle-shaped, dark brown with a subhyaline band across the spore, rounded ends, biseriate, 26-32 x 8-12 μ. Interthecial tissues present.

Habit : Saprophytic on the twigs of Alseodephne semicarpifolia Nees. (F. Lauraceae) collected during October 1967.

Remarks: The writer's collection of Botryosphaeria was found to agree with B. laricis in all respects except being a new host record The fungus is an addition to Fungi of India not so far reported from India.

3. Diatrype Fr.

F. Diatrypaceae. O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Diatrype was established by Fries (1849) with Diatrype disciformis (Hofm.) Fr. as the type. The species of this genus are mostly found as saprophytes on stems, and barks of various host plants. Clements and Shear (1931) and Arx & Muller (1954) have included it under the Family Diatrypaceae (Allantosphaeriaceae of Bessey 1950) of Sphaeriales whereas Luttrell(1955) and Martin (1961) consider it as a member of Xylariales.

In India 7 species are reported, the recent contributions being those of Tilak (1964), Ramachandra Rao (1966, 1966a). The writer collected on 7 different hosts which on detailed comparative and critical study were found to agree with Diatrype disciformis in some cases and D. amorphae in others. A brief account of both the species is presented here.

a. Diatrype amorphae Saval & Sandu (Figs.47.1, 47.2 & 47.3).
Hedwigia 25 : 177, 1935.

Stroma black, erumpent, 0.8-1.7 mm. long and 0.6-1.0 mm. high. Perithecia black, conical, ostiolate, beaked, upto 5 in each stroma, 280-400 μ broad and 680-980 μ high. Asci cylindro-clavate, thickened at the apex, unitunicate, in wall-layers, paraphysate, octosporous, 74-94 x 6-8 μ. Ascospores allantoid, subhyaline, biseriate, 1-celled, 6-8 x 2 μ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit : Saprophytic on the twigs of Acacia sp. M.A.C.S.Herb.No.524, Cassia occidentalis Naves M.A.C.S.Herb.No.523 and Erythrina indica Roll M.A.C.S.Herb.No.522, collected during October 1967.

Remarks : The writer's collection of Diatrype on the three hosts of the family Leguminosae on comparison with Diatrype amorphea occurring on Leguminoseous hosts was found to be similar to D. amorphae.

Remarks : The three hosts are thus new host records for this fungus.

b. Diatrype disciformis (Hofm.) Fr.(Figs.47.4 to 47.6 & 48.1.)
Syll. Fung. 1 : 191, 1882.

Stroma black, erumpent, 0.8-2.1 mm. long and 0.6-0.8 mm. high.

Perithecia black, conical to globose, beaked, ostiolate, upto 6 in each stroma, 210-410 μ broad and 450-800 μ high. Asci clavate, unitunicate, thick-walled, thickened at the apex, with apical canal and pore, arranged in wall-layers, paraphysate, 74-104 x 8-10 μ. Ascospores, allantoid, subhyaline, biseriate, eight in each ascus, 1-celled, 5-8 x 2 μ. Paraphyses and periphyses abundent, filiform, slender and hyaline.

Habit : Saprophytic on the twigs of Eugenia jambolana Lam. (F.Myrtaceae) M.A.C.S.Herb.No.525, Grevillea robusta A.Cunn (F.Proteaceae) M.A.C.S.Herb.No.528, Lantana camara L (F.Verbenaceae) M.A.C.S.Herb.No.529 and Scutia indica Brongn. (F.Rhamnaceae)M.A.C.S. Herb.No.527 collected during October 1967.

Remarks : The writer's collection of D.disciformis has thus a fairly wide host range, the four hosts being in the nature of new host records for this fungus.

4. Diatrypella deNot.

F. Diatrypaceae O. Sphaeriales.

Sub-Class Ascohymeniales.

DeNotaris (1863) established the genus Diatrypella with the type species Diatrypella verucaeformis (Ehrb.) Nke. Clements and Shear (1931), Arx and Muller (1954) have indicated this genus under Sphaeriales whereas Luttrell (1955) and Martin (1961) consider it to belong to the order Xylariales. This genus was not represented in India till 1962, when Tilak (1966a), Muthappa (1967) and Ramachandra Rao (1964) contributed a few species of this genus. The species of

this genus are mostly saprophytes occurring on dead stems and bark. The writer's collections of Diatrypella on four hosts were found to agree with Diatrypella verrucaeformis, a brief description of which is presented here.

Diatrypella verrucaeformis (Ehrb.) Nke. (Figs.48.2 to 48.5). Syll. Fung. 1 : 200, 1882.

Stroma black, erumpent, 1-2 mm. broad, 0.5-0.9 mm. high, erumpent. Perithecia globose to conical, beaked, osticlate, upto six in each stroma, 260-390 µ broad and 600-780 µ high. Asci clavate, unitunicate, thick-walled, in wall-layers, paraphysate, pedicellate, multisporate, with apical canal and pore, 100-120 x 8-10 µ. Ascospores allantoid, subhyaline, crowded in the ascus, 1-celled, 6-8 x 2 µ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit: Saprophytic on the twigs of Ficus glomerata Hort. M.A.C.S. Herb.No.532, Galphimia glauca Hort.ex Bantl. (F.Malphigiaceae) M.A.C.S.Herb.No.533, Glyricidia sp.(F.Leguminosae) M.A.C.S.Herb.No. 530 and Scutia indica Brongn (F.Rhamnaceae) M.A.C.S.Herb.No.531 collected during October 1968.

Remarks: The four hosts constitute new host records for this fungus. In addition to these hosts, this fungus has been reported on Citrus cinensis Pers. (Rutaceae) by Muthappa (1967) thus exhibiting its highly cosmppolitan nature.

5. Gnomonia Ces. & deNot.

F. Diaporthaceae. O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Gnomonia was established by Cesati and deNotaris (1861) with Gnomonia setacea (Pers. ex Fr.) Ces.& deNot. as the type species. Clements & Shear (1931) have included this genus in Sphaeriaceae of Sphaeriales whereas Muller & Arx (1962) place it under Diaporthaceae of Diaporthales. Members of Gnomonia are parasitic as well as saprophytic on leaves, stems and other portions of vascular plants. Many species are parasitic and produce conidia on the living tissues and the ascigerous state at a later stage. Gnomonia venata (Sacc. & Speg.) Kleb. parasitic on leaves and stems of Sycamore or "Plane tree" has its conidial stage belonging to the form genera Gloeosporium, Sporonema, and Fusicoccum. Gnomonia sanwalii Muller is a saprophyte on the twigs of Rosa moschata Benth.

In India, this genus was represented till 1962 by about five species, mainly contributed by Muller (1957) who collected them from the Himalayan region. The writer's collection of Gnomonia is new to science with the following diagnostic characters.

Gnomonia grewiae sp. nov. (Fig. 48.6)
Anahosur 1969 Sydowia (in Press).

Infection spots ellipsoid to spherical, mostly marginal, isolated, dark-brown, 2-3 mm. Perithecia dark brown, epiphyllous, aggregated, innate, globose, with a long beak, projecting outside the host, 180-220 x 100-126 μ. Beak cylindrical, ostiolate, periphysate, 140-160 x 28-32 μ. Asci clavate, unitunicate, pedicellate, pedicel gelatinizing at maturity and releasing asci which

fill the perithecium, apex thickened, with apical apparatus, octosporous, in basal-layers, 42-50 x 8-10 μ. Ascospores oblong, hyaline, unequally 2-celled, rounded at the ends, biseriate, 8-10 x 2-4 μ. Paraphyses lacking. Periphyses abundant and hyaline.

Habit: Incites necrotic patches on the living leaves of Grewia pilosa Roxb.(Tiliaceae) collected during February 1968,M.A.C.S.Herb. No.619 (Type).

Remarks : Gnomonia grewiae differs from the type species i.e. G. setacea in having significantly smaller perithecia with quite long beaks, bigger asci and unequally 2-celled oblong ascospores. No conidial state was noted in association with this Ascomycete in nature.

6. Guignardia Viala & Ravaz

F. Dothideaceae O. Dothideales.

Sub-Class Loculoascomycetes.

The genus Guignardia was established by Viala and Ravaz (1892) with G.bidwellii (Ellis) V. & R.(Syn. Sphaerella bidwellii Ellis.) as the type species. Lindau (1897) described Guignardia with 2-celled ascospores. Donald (1911) was of the opinion that the ascospores of G. bidwellii were single-celled with a hyaline swollen portion at one end which may be mucilaginous and help the spores to stick to the leaf. Petrak (1921) has emended the diagnostic characters of the genus. Miller and Thompson (1940) agree with Petrak (1921) in considering G. bidwellii as the type species and characterised by uniloculate stroma (pseudothecium), absence of

beak, fasciculate origin of asci, paraphyses lacking and 1-celled ascospores. Muller (1951) revised the genus. Miller (1949) reports the occurrence of multiloculate ascostromata in this genus even as in the case with Mycosphaerella.

Arx & Muller (1954) have placed this genus in Botryosphaeriaceae of Dothiorales which was again supported by Reusser (1964) who has contributed few species. Luttrell (1955, 1965a) treats this genus as a member of Dothideaceae of Dothideales.

In India this genus is represented by five species till 1962, the recent contributions being those of Rao and Kale (1965). The writer collected two species of Guignardia, a brief account of which is given below :

a. Guignardia bidwellii (Ellis.) V. & R. (Fig. 49.1).

Arx & Muller 1954 p. 44.

Ascostromata black, globose, uniloculate, innate, ostiolate, 100-135 μ diam.;wall-thickness 8-12 μ. Asci clavate, bitunicate, pedicellate, octosporous, in basal layers, 45-55 x 10-14 μ. Ascospores spindle-shaped, biseriate, 1-celled,hyaline, 14-18 x 3-5 μ. Paraphyses, pseudoparaphyses or interthecial tissues lacking

Habit : Causes leaf blight of Callistemon lanceolata Sweet. (Myrtaceae) collected during February 1967. M.A.C.S.Herb.No.506.

Remarks: The writer's collection of Guignardia agreed with the type in all essential morphological characters. This is a new host record for this fungus.

b. Guignardia flecourtiae sp. nov. (Fig.49.2)

Infection spots circular, scattered, erumpent, brown, epiphyllous,upto 0.5 mm. diam. Ascostromata black,multiloculate, upto four locules in each stroma, subepidermal, 356-486.8 μ long. Locules conical with well developed clypeus, 150-180 x 100-120 μ. Asci cylindro-clavate, bitunicate, with apical projection, pedicellate, octosporous, in basal layers 60-80 x 40-48 μ. Ascospores spindle-shaped to cylindrical, with hyaline disc-like gelatinous portion at both ends, hyaline, irregularly arranged, 1-celled, 40.4-48.8 x 12-16 μ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit : Parasitic on the leaves of Flecourtia sepiaria Roxb. (Flecourtiaceae) collected during February 1967, M.A.C.S.Herb.No. 496 (Type).

Remarks : This species is distinguished from the type by the multiloculate character of the ascostromata, bigger asci and quite distinctively characterised bigger ascospores which are arranged irregularly in the ascus. The genus Guignardia although characterised by the formation of uniloculate ascostroma, may rarely produce multiloculate stromata even as in the allied genus Mycosphaerella as stated by Miller (1949).

In the writer's collection, micro-conidial locules were also observed in association with the ascostromata.

7. Herpotrichia Fckl.

F. Pleosporaceae. O. Pleosporales.

Sub-Class Loculoascomycetes.

The genus Herpotrichia was founded by Fuckel (1869) with Herpotrichia rhenana Fckl. (Syn. Herpotrichia rubi Fckl.) as the type species. Luttrell (1955), Martin (1961) are of the opinion that it should be included in Herpotrichiellaceae of Pleosporales whereas Muller and Arx (1962) have placed it under Pleosporaceae of Pleosporales. Bose (1961) is also of the same opinion.

The species of this genus occur as saprophytes on bark and stems of various host plants and very few species are reported from different places and the main contributions being those from Stingis (1913), Saever (1915), Muller (1957), Bose (1961). Bose (1961) has described two new species of Herpotrichia and has revised 10 other species with the provision of a key for the identification of the species.

The writer's collection of Herpotrichia is considered as new to science with the following description.

Herpotrichia indica sp. nov. (Figs. 49.3 & 49.4)

Ascostromata black, membranous, setose, uniloculate, cupulate, ostiolate, erumpent, aggregated, 420-600 μ broad. Wall black, made up of thick-walled pseudoparenchymatic cells, 26-40 μ thick. Setae myceloid, numerous, dark-brown, septate, pointed at the tips, 4-6 μ thick and 200-400 μ long. Asci clavate, pedicellate, bitunicate, rounded at the apex, arranged in wall-layers,

octosporous, 150-170 x 16-18 µ. Ascospores spindle-shaped, subhyaline, equally two celled, slightly constricted at the septum, thick-walled, biseriate to slightly irregular, 36-38 x 4-6 µ. Pseudoparaphyses numerous, filiformis, slender and hyaline.

Habit : Saprophytic on the dead twigs of Duranta plumeri Facq. (Verbenaceae) collected during October 1967, M.A.C.S.Herb. No. 542 (Type).

Remarks : A critical and comparative study carried out between the writer's collection and the related species of Herpotrichia revealed that the writer's collection has some resemblance to H. diffusa and H. schiedermayeriana according to the key given by Bose (1961) but differs from the former in having significantly bigger ascocarps, asci and ascospores and from the latter in having erumpent stroma, bigger asci and strictly 2-celled subhyaline ascospores as against 1-3 septate, light brown ascospores of the latter besides being collected on a new host. Hence it is considered as a new species.

8. Hypoxylon Bull. ex Fr.

F. Xylariaceae. O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Hypoxylon was established by Bulliard in 1791 and subsequently revised by Fries in 1849. The type species is Hypoxylon coccineum Bull. Clements and Shear (1931), Miller (1949), Arx and Muller (1954) have included this genus under Xylariaceae of Sphaeriales whereas Luttrell (1955) and Martin (1961) place it

under Xylariales. According to Miller (1932) Hypoxylon Bull. is synonym of Hypoxylon Fr. and H. coccineum is the synonym of H. coherens (Pers.) Fr. which is the type species. But according to Arx and Muller (1954) and 'Dictionary of Fungi', the authority of this genus is Hypoxylon Bull.ex Fr. and the writer has adopted the same. Several species of this genus are reported from different parts, the main contributions being those of Ellis and Everhart (1887), Miller (1930, 1932, 1957 and 1961). Rogers (1967) has studied the developmental pattern of the ascocarps in H. fuscus and considers it to be of 'Xylaria' type. Recently the conidial stages of Hypoxylon have been described by Greenhalgh and Chesters (1968), Chesters and Greenhalgh (1964) and Jong and Rogers (1968) as belonging to the form-genera Isaria, Geniculosporium, Nodulosporium or Acrostaphyllus all belonging to Moniliales.

In India this genus was represented by about 22 species till 1962. Most of the species occur as saprophytes on stems and barks of various host plants. The two collections made by the writer on two different hosts are saprophytes and were identified as H. rubiginosum var. tropica Miller and H. coherens (Pers)Fr. A brief account of both the species is given below.

a. Hypoxylon coherens (Pers.) Fr. (Fig. 49.5)

Trans. Brit. Mycol.Soc. 15 : 134-154, 1934.

Stroma black, erumpent, aggregated, 1.6-3.8 mm. long. Perithecia globose, ostiolate, with light coloured true perithecial wall, sunken in the stroma, upto eight in each stroma, 280-410 μ diam.

9. Irene Theiss. & Syd.

F. Meliolaceae. O. Meliolales.

Sub-Class Ascohymeniales.

The genus Irene was established by Theissen and Sydow (1917) with Irene inermis (K. & C.) Theiss. & Syd.(Syn.Meliola inermis K. & C.) as the type species. Clements and Shear (1931) have treated this genus as a member of perisporiaceae of perisporiales. Hansford (1946) treats this as a member of Meliolaceae of Myriangiales. Luttrell (1955) and Martin (1961) have treated this genus as a member of Meliolaceae of Meliolales. Theissen & Sydow (1917) have distinguished 19 genera in this family of which Irene with many hundreds of species are very abundant in the tropics and few in temperate regions. Toro (1925), Stevens & Tehon (1926) have described and revised many species of Irene and provided a key for the identification of species. This genus is not represented in India.

The writer's collection of Irene was compared with the type and other related species and was found to be new to science, a brief description of which is presented here.

Irene indica sp. nov. (Fig. 50.1)

Anahosur,1969, Sydowia (in Press).

Colonies black, dense, circular, hypophyllous, 1.5-3.4 mm. diam. Mycelium dark-brown, septate, superficial, hyphopodiate, 5-7 μ broad. Hyphopodia capitate, 2-celled, dark brown, alternate to irregular, 14-18 x 10-16 μ. Perithecia superficial,globose,

setose, dark-brown, thick-walled, non-ostiolate, made up of thick-walled pseudoparenchymatic cells, 110-135 µ diam. Perithecial setae dark-brown, septate, tapering at the apex, upto 100 u long. Asci clavate to cylindrical, in wall-layers, hyaline, unitunicate, bi-to tri-sporate, pedicellate, 60-72 x 31.4-41.4 µ. Ascospores elliptical, 4-septate, constricted at septa, thick-walled, dark-brown, arranged irregularly, 44-46 x 12-16 µ. Paraphyses and periphyses abundent, filiform, slender and hyaline.

Habit : Parasitic on the leaves of Amoora rohituka Wight. & Arn. (F. Meliaceae) collected during October 1967, M.A.C.S.Herb.No.620 (Type).

Remarks : The writer's collection of Irene was compared and found to be different from the type i.e. I. inermis in having significantly smaller perithecia as well as ascospores, besides being collected from an unreported host and host family and is therefore described as new to science. This genus is a new generic record to Indian Fungi.

A species of the form genus Exosporium Link was found in close and constant association with this Ascomycete and was found to be similar to Exosporium teliae Link. The conidiophores were observed to be directly borne on the dark-brown hyphopodiate mycelium of I. indica. This type of association of these two fungi on the host constitutes the first report although associations of some Deuteromycetes belonging to the form genera Neamosphaeria, Isaria, Arthrobotryum, Cephalosporium, Spegazzinia etc. with species of

Meliola have been reported by Hansford (1946) and Stevens (1918) who describe such associated Deuteromycetes as hyperparasites on the respective Ascomycetes.

10. Lecanidion Endl.

F.Patellariaceae O. Hysteriales.

Sub-Class Loculoascomycetes.

The genus Lecanidion (Syn. Patellaria Fr. and Patellaria Hedw. emend Sacc.) was established by Endlicher (1830). The type species is Lecanidion atratum (Hedw.) Endl. (Syn. Patellaria atrata Fr.). There is some confusion on the taxonomy of the genera Lecanidion Endl. and Patellaria Fr. The name Patellaria was first used by Frhart (1789) to describe a Lichen i.e. Lichen upsaliensis. Later Fries (1822) used the name Patellaria for a fungus genus. According to the International Code of Botanical Nimenclature Article 64 (1956), the name Patellaria Fr. becomes invalid as the name was already preoccupied. Therefore Endlicher (1830) proposed the name Lecanidion for Patellaria Fr. including only one species Lecanidion atratum which therefore is the type. Butler (1939, 1940) has pleaded for the invalidity of Patellaria Fr and proposed the usage of Lecanidion Endl.which was later followed by Luttrell (1955) and Muller & Arx (1962). Therefore the writer has adopted Lecanidion Endl. as the valid name for the genus in place of Patellaria Fr.

Clements and Shear (1930) have placed this genus under Patellariaceae of True Discomycetes. Muller & Arx (1962) treat the genus as a member of Dothiorales.

In India Mundkur & Ahmed (1946), Ramachandra Rao (1967), Tilak & Srinivasan (1969), have described several species of Patellaria Fr. over looking the correct nomenclature and taxonomy of the fungus as suggested by Butler (1940).

The writer's collection of Lecanidion a saprophyte, is considered as a new species with the following description.

Lecanidion gorgicii Anahosur sp.nov. (Fig.50.2)

Discothecia black, discoid to cupulate, with wide opening, erumpent, uniloculate, aggregated, sessile, 896-1190 x 180-200 µ. Asci clavate, bitunicate, pedicellate, in basal layers, flat at the apex, octosporous, 62-71.4 x 12-16 µ. Ascospores ellipsoid, uniformly 8-celled, hyaline, irregularly biseriate, rounded at the tips, 29.4-32.4 x 3-5 µ. Pseudoparaphyses hyaline, slender, septate, branched at the apex.

Habit : Saprophytic on the twigs of Grevillea robusta A.Cunn. (Proteaceae) collected during October 1967, M.A.C.S.Herb.No.543 (Type).

Remarks : Butler (1939, 1940), Mundkur and Ahmed (1946), Ramchandra Rao (1967), Tilak & Srinivasan (1969) have described the ascocarps of this genus as an apothecium, interthecial threads as paraphyses and remnents of the apical stroma as the epithecium. The recent investigations carried out by Muthappa (1967) and Seshadri & Muthappa (1969) on a closely allied fungus Tryblidiella rufula (Spreng.) Sacc. belonging to the family Patellariaceae prove very

conclusively that the ascocarp in this family is in the nature of a 'Discothecium' as originally described by Korf (1962) and interthecial threads continuous from top to the bottom with '*Glonium*' type of development and the stroma at the top of the ascocarp as a pseudo-epithecium made up of remnents of the stromatic cells together with the tips of the interthecial threads. On the basis of these characters, the writer is of the opinion that the genus *Lecanidion*, a bitunicate Discomycete with similar characters should find its place under family Patellariaceeae order Hysteriales.

11. *Lembosia* Lev.

F. Microthyriaceae. O. Hemisphaeriales.

Sub-Class Loculoascomycetes.

The genus *Lembosia* was established by Leveille (1845) with *L.tenella* Lev. as the type. Theissen and Sydow (1917) have placed this genus under Microthyriaceae of Hemisphaeriales which system is followed by Bessey (1952), and recently by Luttrell (1965a), Muller and Arx (1962) have placed the family under Pseudosphaeriales. Hansford (1946) has included this genus in Asterinaceae fam.nov. under Microthyriales. Many species of *Lembosia* have been reported from different parts and Theissen (1913a) has reviewed and described several species for which he has provided a key which is valuable in identification of the species.

Till 1962 only two species were reported from India. Recently Bose & Muller (1964) and Pande (1969) have contributed some

b. Lembosia inaequalis sp. nov. (Fig. 50.4).

Anahosur 1969, Nova Hedwigia (in Press).

Infection spots circular, epiphyllous, crustaceous, black, with multiloculate ascocarps, 3-6 mm. diam. Mycelium superficial, dark-brown, septate, radiate, 4-7 μ broad. Thyrothecia, black, carbonaceous, linear, epiphyllous, superficial, hypostroma lacking, crack opens longitudinally, 500-800 x 170-250 μ. Asci clavate, bitunicate, in basal-layers, parallel, sessile, octosporous, 75.4-91.4 x 24-30.8 μ. Ascospores dark-brown, unequally two-celled cylindrical, irregularly arranged, 17.4-23.8 x 6-10 μ. Pseudo-paraphyses abundant, slender, septate, with bulbous apices, hyaline attached to the epistroma in the early stages becoming free at the tips at maturity.

Habit : Incites leaf spots on the living leaves of Plectronia rheedi Bedd. (Rubiaceae) collected during October 1966,M.A.C.S.Herb No.454 (Type).

Remarks : The distinguishing characters of L. inaequalis from the type are bigger ascocarps and asci and much smaller ascospores which are unequally two-celled with a new host record.

12. Leptosphaeria Ces. & deNot.

F. Pleosporaceae. O. Pleosporales.

Sub-Class Loculoascomycetes.

The genus Leptosphaeria was established by Cesati and deNotaris (1863) with Leptosphaeria doliolum (Pers.)Ces. & deNot. as the type. Clements and Shear (1931) have placed this genus unde Sphaeriales. The genus is characterised by the presence of

uniloculate ascostromata with pseudoparaphyses continuous from top to bottom in between which bitunicate asci arise. This character justifies accommodation of this fungus under Pleosporales as proposed by Luttrell (1955) and Martin (1961). Muller (1950) has described about 50 species of this genus.

15 species were reported from India till 1962 comprising parasites as well as saprophytes. L. oryzina, L. capparidicola Mundk. & Ahmd. are saprophytes occurring on dead leaves and palea of Oryza sativa and on dead branches of Capparis aphylla respectively. Mundkur and Ahmed (1935) studied the life cycle pattern of L. salvinii Catt. and obtained the conidial stage Helminthosporium sigmoideum Cav. in culture. Webster (1955) has reported Hendersonia typhae as the conidial state of L. typharum. Recently Lucas (1967) has described various conidial stages of British species of Leptosphaeria. The writer collected two species of Leptosphaeria one being parasitic on leaves and the other being on stems, the descriptions of which are given below.

a. Leptosphaeria corgicii sp. nov. (Fig.50.5)
Anahosur 1969, J. Biol. Sci., Bombay (in Press).

Infection spots linear, black, shining, erumpent, aggregated, 1.2-3 x 0.2-0.4 mm. Ascostromata subepidermal, uniloculate, with brown wall, globose to conical, aggregated, 120-160 μ broad, 150-170 μ high. Asci clavate, bitunicate, pedicellate, in basal-layers, octosporous, numerous, 100-118.4 x 20-24 μ. Ascospores fusoid, with prominent as globules, 5-6 celled, rarely 7-celled, tapering at the ends, brown, constricted at septa, 35-39 x 4-7 μ. Pseudoparaphyses numerous, filiform, slender, simple and hyaline.

Habit : Parasitic on the stems of Plerei aquilina collected during February 1967, M.A.C.S.Herb.No. 537 (Type).

Remarks : The distinguishing characters of this species from the type are lignicolous habit, significantly smaller clavate asci and x-celled ascospores which are arranged biseriately in the ascus. In India it is the first parasitic species of Leptosphaeria reported on stems.

b. Leptosphaeria lobeliae sp. nov. (Fig.50.6)

Anahosur 1969, J.Biol.Sci.Bombay (in Press).

Infection spots circular, coalescing in the later stages forming necrotic patches, containing black, round fruiting bodies, 0.5-2.5 x 0.5 mm. Ascostromata black, globose, soft, innate, uniloculate, ostiolate, thick-walled, made up of pseudoparenchymatic cells, 150-210 µ diam. Asci clavate, bitunicate, in basal-layers, pedicellate, rounded at the apex, octosporous, 70-85 x 12-16 µ. Ascospores fusoid, biseriate, curved to straight, 3-5 septate, non-constricted, at septa, sub-hyaline, biseriate, 29-33 x 6-8 µ. Pseudoparaphyses numerous, filiform, slender, simple and hyaline.

Habit : Parasitic on the leaves of Lobelia excelsa Bonpl. (Campanulaceae) collected during February 1967, M.A.C.S.Herb.No. 535 (Type).

Remarks: The distinguishing characters of this species from the type are small locules, small clavate asci and bigger ascospores which are arranged biseriately in the ascus. Also it is collected on an hitherto unreported host.

13. Meliola Fr.

F. Meliolaceae. O. Meliolales.

Sub-Class Ascohymeniales.

The genus Meliola was established by Fries in 1825. The type species is M. nidulans (Schw.) Cke. Clements and Shear (1931) have placed this genus under Perisporiaceae of Perisporiales. Hansford (1946) has treated this genus as a member of Meliolaceae of Myriangiales on the basis of its uniloculate nature of ascocarp irrespective of the nature of asci. Luttrell (1955) and Martin (1961) have treated it as a member of Meliolaceae of Meliolales on the basis of dark setose superficial mycelium bearing perithecia. In this family Theissen and Sydow (1917) have distinguished 19 genera of which Meliola with many hundreds of species are very abundant in the tropics and rare in temperate zones. Stevens (1927,1928) has published a monograph of this genus which is indispensable for identifying species of this genus.

In India, this genus was represented by about 65 species till 1962, the main contributions being those of Hansford and Thirumalachar (1948) and recently by Bose and Muller (1967), Kapoor (1967).

The writer's collection of Meliola was compared and found to resemble Meliola maesicola Hans. & Stevens. in all essential morphological characters and dimensions, a brief description of which is presented here since it is collected on a new host.

Meliola maesicola Hans. et. Stevens. (Fig.51.1).
Jour.Linn. Soc.Lond. 37 : 27: 1937.

Colonies black, dense, hypophyllous, scattered, 2-4 mm. in diam. Mycelium dark-brown, septate, superficial, setose, hyphopodiate, 5-7 µ broad. Hyphopodia clavate, opposite, 2-celled, dark-brown, 14-18 x 6-8 µ. Perithecia globose, dark-brown, non-ostiolate, superficial, separate, 90-110 µ diam. Asci clavate, unitunicate, pedicellate, bi- to quadrisporate, hyaline,60-82 x 20-24 µ. Ascospores ellipsoid, uniformly 4-septate, constricted at septa, rounded at the tips, dark-brown, 40-44 x 8-10 µ. Paraphyses filiform, slender and hyaline.

Habit : Parasitic on the leaves of Maesa indica Wall(Myrsinaceae) collected during October 1967, M.A.C.S.Herb.No. 572.

Remarks : Maesa indica is a new host record for this fungus. It was interesting to note that a conidial fungus belonging to the form genus Exosporium Link was found in constant and close association with this ascomycete and was diagnosed as Exosporium teliae Link. Stevens (1918) and Hansford (1946), have described association of several species of Deuteromycetes with the species of Meliola and consider these Deuteromycetes as hyperparasites. In case of writer's collection, the conidiophores of E.teliae were observed to be directly borne on the brown setose mycelium of Meliola maesicola. The true relationship of this association needs further inoculation studies.

14. Meliolina Syd.

F. Meliolaceae. O. Meliolales

Sub-Class Ascohymeniales.

Sydow (1914) founded this genus with Meliolina cladotricha (Lev.) Syd. as the type species. This genus differs from Meliola in having nonhyphopidiate mycelium. Even though Hansford (1946) has placed this genus under parodiellinae of Myriangiales, its proper place is under Meliolaceae of Meliolales as suggested by Luttrell (1955), Martin (1961) and Clements and Shear (1931).

In India only one species viz. Meliolina arborescens Syd. was recorded till 1962. The recent contributions being of Kapoor (1967) and Ponnappa (1967).

The writer's collection of Meliolina was identified as M. mollis (Berk. & Br.) Hohn.,a brief description of which is given here.

Meliolina mollis (Berk. & Br.) Hohn. (Fig.51.2)

Colonies dense, black, scattered, circular, hypophyllous, rarely am higenous, superficial,2-5 mm. diam. Mycelium dark-brown, septate, non-hyphopodiate, setose, 5-7 μ broad. Perithecia separate, superficial, setose, globose to cup-shaped, black, stromatic, 180-250 μ diam. Setae black, branched 180-250 μ long. Asci clavate, unitunicate, pedicellate, in basal-layers, 120-140 x 20-24 μ. Ascospores dark brown, thick-walled, uniformly 3-septate, slightly constricted at septa, cylindrical, biseriate to irregular, 66-74 x 8-12 μ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit : Parasitic on the leaves of Syzigium jambolanum DC. (Myrtaceae) collected during October 1966, M.A.C.S.Herb.No.501.

Remarks: The host is a new record for this species.

15. Microcyclus Sacc.
F.Dothideaceae; O.Dothideales; Sub-Class Loculoascomycetes.

The genus Microcyclus was established by Saccardo (1904) for a Dothideaceous fungus. The type species is Microcyclus angolensis Sacc. & Syd. Clements and Shear (1931) have placed this genus under Dothideaceae of Dothideales whereas Muller & Arx (1962) have placed it under Myeosphaerellaceae of Pseudosphaeriales The recent developmental studies of the ascocarps of Microcyclus indicus Tilak carried out by Chiplonkar (1969) have exhibited its affinities to Dothideaceae having 'Dothidea' type of development and justifies its accommodation under Dothideaceae of Dothideales as suggested by Luttrell (1955, 1965a).

In India this genus is represented by only two species viz. M. phoebes reported by Ramakrishnan (1956) collected on the fallen leaves of Phoebe paniculata from Coorg (India), and M. indicus Tilak reported by Tilak (1958) parasitic on the leaves of Actinodaphne hookeri Meisson. The writer's collection of Microcyclus was found to agree with M. indicus Tilak, a brief description of which is presented here.

Microcyclus indicus Tilak (Figs. 51.3 & 51.4)
Tilak, S.T.(1958) Sydowia Ser.II 12: 197-199.

Stroma black, circular to irregular, aggregated, hypophyllous,

multiloculate, upto 5 locules in each stroma, superficial with hypostroma, 256-480 μ broad. Locules globose, ostiolate, 80-126 x 80-112 μ. Asci bitunicate, clavate, pedicellate, in fascicles, octosporous, 48-68 x 10-14 μ. Ascospores oblong, unequally 2-celled, biseriate, hyaline, non-constricted at the septa, 12-16 x 2-4 μ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit : Parasitic on the leaves of Actinodaphne hookeri Meissen. (Lauraceae) collected at Coorg (India) during October 1966, M.A.C.S.Herb.No.502.

Remarks: It was interesting to note that microscopic examination of the sections of the ascostromata revealed the presence of spermogonial chambers within the hypostroma of the ascigerous state and the exact nature and the relationship of the association could be ascertained through a detailed study into the development of the fungus which are in progress. Such associations in Phyllachora simplocicola Seshadri have been proved to be helpful in effecting spermatization (Jagtap, 1968). Tilak(1958) who originally reported this species was unable to obtain association in his collections made from Maharashtra. A brief description of spermogonia and spermatia is presented here.

Spermogonia globose to conical, black, stromatic, closely associated with the ascocarps, innate, 120-160 x 70-95 μ. Spermatiophores cylindrical, in wall-layers, hyaline, 6-8 x 2 μ. Spermatial bodies ellipsoid to cylindrical, 1-celled, hyaline, filling the cavity, 2 μ long.

16. Muelleromyces Kamat & Anahosur

Anahosur 1968, Experientia 24: 849.

F. Diaporthaceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

A tarspot fungus parasitizing leaves of Eugenia jambolana Lam. was collected by the writer with the following characters:

Perithecia non-stromatic with highly developed clypeus. Asci octosporous, unitunicate, aparaphysate, provided with apical apparatus, pedicel gelatinizing at maturity. Ascospores brown and unequally 2-celled.

These characters are typically sphaeraceous with Diaporthaceous affinities. A critical study of the fungus at different stages of development revealed that these characters had not been previously duplicated in any of the known genera of Diaporthaceae to which this fungus clearly belongs on the basis of which it is assigned to a new genus with M. indicus as the type species.

Muelleromyces Kamat & Anahosur Gen. nov.

Perithecia non-stromatic, separate, innate, prominently beaked, with a highly developed clypeus, ostiolate. Asci unitunicate, with thick apical canal, pedicel gelatinizing at maturity, aparaphysate, octosporous. Ascospores dark-brown, unequally 2-celled. Paraphyses lacking and periphyses abundant.

Discussion: A careful search of literature showed that an Ascomycete Didymosphaeria jambolana had been described by Ramakrishnan et.al. (1953) parasitizing Eugenia jambolana. The

octosporous, aparaphysate, 145.5-170.8 x 16-20 μ. Ascospores oblong, dark brown at the tips and subhyaline in the centre, unequally 2-celled (upper 12-14 x 7-8 μ lower 4-6 x 4-5 μ), non-constricted at the septum, rounded at the ends, uniseriate, 16-20 x 4-8 μ. Paraphyses lacking, periphyses abundant, filiform, slender hyaline and facing upwards.

Habit: Parasitic on the living leaves of Eugenia jambolana Lam. (Myrtaceae) collected during February 1967, M.A.C.S.Herb.No. 494 (Type).

Remarks : A Sphaeropsidaceous fungus assigned to a new genus Kamatella Anahosur Gen. nov. with K.indica Anahosur as the type, was closely and constantly found in association with the above Ascomycete (Fig.51.6). The true relationship of such association needs cultural studies and cross inoculation experiments. M.A.C.S. Herb. No.495 (Type).

17. Mycomicrothelia Keissler.

F. Pleosporaceae; O. Pleosporales

Sub-Class Loculoascomycetes.

The genus Mycomicrothelia was established by Keissler (1937) with Mycomicrothelia atomaria (DC. ex Merat) Meissler (Syn. Verrucaria atomaria DC.)as the type species. Muller and Arx (1962) have placed this genus under Pleosporaceae of Pseudosphaeriales.

In India this genus is represented by only one species viz. M. palmarum, recently reported by Chaudhari and Rao (1963). The writer's collection of Mycomicrothelia has the following characters

Mycomicrothelia indica Ranabour sp.nov. (Fig.52.1)

Ascostromata black, soft, subepidermal, becoming erumpent, uniloculate, ostiolate, conical, with highly developed clypeus, 310-400 μ broad and 110-124 μ high. Asci clavate, pedicellate, bitunicate, in wall-layers, octosporous, 50-65 x 12-16 μ. Ascospores ellipsoid to ovoid, dark-brown, unequally 2-celled, constricted at the septum, rounded at the ends, biseriate,16-20 x 4-6 μ. Pseudoparaphyses abundant, filiform, slender and hyaline.

Habit : Incites tar spots on the living stems of Randia dumetorum Lam. (Rubiaceae) collected during October 1967, M.A.C.S.Herb. No. 498 (Type).

Remarks : The writer's collection of Mycomicrothelia differs from M. atomaria and M. macularis in having significantly bigger ascostroma, asci and ascospores which are unequally 2-celled with a lignicolous habit. This is the second species of this genus reported from India.

18. Mycosphaerella Johanson

F. Dothideaceae; O. Dothideales.

Sub-Class Loculoascomycetes.

The genus Mycosphaerella was established by Johanson in 1884. The type species is Mycosphaerella ribis (Fkl.) Lind. (Syn. Sphaerella ribis Fkl.). Lindau (1897)and Bansford (1946) have placed this genus under Sphaeriales. Gaumann (1950), Muller & Arx (1962), have placed it under Mycosphaerellaceae of Pseudosphaeriales. The recent developmental studies of

Mycosphaerella mysorensis Seshadri carried out by Seshadri (1967) have proved its affinity to Dothideaceae on the basis of 'Dothidea' type of development defined by Luttrell (1951).

The genus Mycosphaerella is comprised of over 1000 species, many of which are pathogens of great economic importance. The conidial forms are of several types belonging to form genera Cercospora, Ramularia, Cladosporium of Moniliales and Ascochyta, Septoria, Phoma and Phyllosticta of Sphaeropsidales. Klebahn (1918) has used the types of conidial forms as a basis of seggregating the genus into several sub-genera as Ramularisphaerell Septorisphaerella, Cercosphaerella etc.

In India about 50 species have been reported till 1962, recent contributions being of Viswanathan (1958), Viswanathan & Tilak (1960) and Patwardhan (1966a).

The writer collected 4 species on four different hosts three being leaf parasites and one saprophyte, brief description of which is presented here.

a. Mycosphaerella agrostystachea Anahosur sp. nov. (Fig.52.2)

Anahosur 1969, J.Biol. Sci.Bombay (in Press).

Ascostromata black, round, aggregated, innate, strictly uniloculate, globose, ostiolate, 110-140 x 160-180 µ. Wall of the locule black, made up of thick-walled pseudoparenchymatic cells, 18-26 µ, thick. Asci clavate, with truncate base, bitunicate, aparaphysate, in fascicles, pedicellate, octosporous 59.4-70.8 x

10-14 μ. Ascospores oblong,unequally 2-celled, irregularly biseriate, non-constricted at the septum, hyaline, 26-32 x 2-4 μ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit : Parasitic on the leaves of Agrostystachis indica Dansal (Euphorbiaceae) collected during February 1967, M.A.C.S.Herb.No. 536 (Type).

Remarks: The significant features of this species are bigger locules, asci and ascospores as compared to the type species with a new host record.

b. Mycosphaerella bombycina Viswanathan (Fig.52.3).

Ascostromata black, globose, unilocculate, amphigenous, innate, ostiolate, 55-85 μ diam. Asci bitunicate, in fascicles, clavate withtruncate base, subsessile, octosporous, aparaphysate, 25-35 x 4-8 μ. Ascospores oblong, unequally 2-celled, non-constricted at the septum, biseriate, hyaline, 6-8 x 2-3 μ. Paraphyses, pseudo-paraphyses or interthecial tissues lacking.

Habit : Parasitic on the leaves of Callistemon lanceolata Sweet. (Myrtaceae) collected during October 1966, M.A.C.S.Herb.No.545.

Remarks: Callistemon lanceolata is an additional new host record for this species.

c. Mycosphaerella multiloculata Anahosur sp.nov.(Fig.52.4)
Anahosur 1969, J.Biol.Sci.Bombay (in Press).

Ascostromata black, carbonaceous, multiloculate, aggregated in circular necrotic spot, hypophyllous, innate, 500-800 u long.

Locules globose, ostiolate, upto 10 in each stroma, made up of thick-walled cells, 70-88 x 40-55 µ. Asci clavate with truncate base, bitunicate, sessile, aparaphysate, in fascicles, octosporous, 42.4-55 x 10-16 µ. Ascospores oblong, unequally 2-celled, non-constricted at the septum, biseriate, hyaline, 12-15 x 2 µ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit: Parasitic on the leaves of Memecylon umbellatum Presl. (Melastomataceae) collected during October 1966,M.A.C.S.Herb.No. 534 (Type).

Remarks : The genus Mycosphaerella is generally characterised by uniloculate ascostroma although multiloculate species have been encountered as in case of M. effigurata, M. tassiana, M. typhae. The writer's collection differs from type in having multiloculate ascostroma and smaller asci and ascospores.

d. Mycosphaerella sodiaroana Petrak. (Fig.52.5)

Petrak,1950, Sydowia 4:504.

Ascostromata black, uniloculate, innate, hypophyllous, globose 70-100 µ diam. Asci clavate with truncate base, subsessile, in fascicles, bitunicate, octosporous, aparaphysate 36-48 x 8-12 µ. Ascospores oblong, unequally 2-celled, hyaline, irregularly biseriate, non-constricted at the septum, 10-14 x 2-3 µ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit: Saprophytic on the fallen leaves of Agrostystachis indica Dalzal (Euphorbiaceae) collected during February 1967,M.A.C.S.Herb. No.544.

Remarks: The writer's collection of Mycosphaerella was compared and found to agree with M. sodiaroana in all essential morphological characters and dimensions. The host is a new record for this species.

19. Parodiella Speg.

F. Pleosporaceae; O. Pleosporales.

Sub-Class Loculoascomycetes.

The genus Parodiella was established by Spegazzini in 1880 with Parodiella perisporioides (Berk. & Curt.)Speg. as the type species. Clements and Shear (1931) treated this genus as a member of 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 pseudothecium-like perithecium together with other centrum characters fit in with the concept of Pleosporaceae of Pleosporales as defined by Luttrell (1955, 1965a).

Muller and Arx (1962) have merged all the previously reported species into the type i.e. Parodiella perisporioides in view of its remarkable host specificity and morphological characters being similar not much varying in dimensions. Sydow (1917), von Hohnel (1918), Petrak (1947) and von Arx (1952) have contributed several species to this genus. From India Butler & Bisby (1931), Uppal, Patel & Bhide (1949), Payak (1953), Tilak (1959), Ullasa (1969)

have contributed several species all on hosts of Leguminosae. Saccardo (1899, 1902, 1905, 1926), however, has listed few species parasitizing hosts of other families like Myrtaceae, Malphigiaceae, Solanaceae and Gentinaceae. Spegazzini (1884) while describing Parodiella paraguensis has reported the presence of ostiolate or papillated ascocarps in this genus. Uppal, Patel & Bhide (1949) have described a new species Parodiella smithiae in which they report the presence of true paraphyses and non-pedicellate nature of asci. Recently Ullasa (1969) has described a multiloculate Parodiella i.e. P. perisporioides var. multiloculata. The writer's collection was compared and found to agree with P. perisporioides, a brief description of which is given here.

Parodiella perisporioides (Berk.& Curt.) Speg. (Fig.52.6)

Ascostromata black, epiphyllous, spherical, uniloculate, non-ostiolate, 210-250 µ diam. Asci clavate, pedicellate, bitunicate, in basal-layers, octosporous, 84-102 x 16-22 µ. Ascospores spindle-shaped, striated, dark-brown, biseriate, 24-28 x 8-10 µ. Pseudoparaphyses filiform, slender, simple and hyaline.

Habit: Parasitic on the leaves of Desmodium sp. (Leguminosae) collected during October 1966.

20. Phomatospora Sacc.
F. Sphaeriaceae; O. Sphaeriales.
Sub-Class Ascohymeniales.

The genus Phomatospora was established by Saccardo in 1874 with Phomatospora berkeleyi Sacc. as the type species. Clements &

21. Phyllachora (Pers. ex. Fr.) Nke. in Fckl.

F. Phyllachoraceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Phyllachora was established by Nitschke with Phyllachora graminis as the type and was described in the book "Symboleae Mycologicae" published by Fuckel (1869). The genus was originally referred to as Sphaeria Pers. by Persoon (1796). Fries recognised this genus but later (1918) changed it to Dothidea. Theissen and Sydow (1918) include the genus in the order Dothideales in their monographic studies on the assumption that the ascocarp was a stromatic locule and not a true perithecium Orton (1924) in his critical studies on Phyllachora graminis stressed the sphaeraceous nature of the ascocarp. Miller (1941, 1949) with further studies on this genus confirmed its Sphaeraceous nature and placed the family Phyllachoraceae under 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 Dothideaceae and its accommodation under Sphaeriales. The recent developmental studies on Phyllachora simplocicola by Jagtap (1967) also report its Sphaeraceous nature which support the above position.

According to Parberry (1967) the total number of world species 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, Ananthanarayanan, Seshadri & Ullasa, 1959, 1964, 1967 and 1969a respectively. The writer

collected 2 species of Phyllachora, the brief descriptions of which are narrated here.

a. Phyllachora eugeniae sp.nov. (Fig.53.2)

Anahosur 1969, Sydowia (in Press)

Stromata black, shining, carbonaceous, raised, cushion-like, epiphyllous, rarely amphigenous, scattered to aggregated, circular to irregular, sub-cuticular, multiloculate, 20 x 10 mm. Perithecia globose, black, stromatic, ostiolate, with clypeus, 300-500 µ diam. Ascic clavate, in wall layers, unitunicate, pedicellate, with apical canal and pore, 150-180 x 10-14 µ. Ascospores ovoid, uniseriate, 1-celled, hyaline, 14-18 x 4-6 µ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit: Parasitic on the leaves of Eugenia jambolana Lam.(Myrtaceae) collected during October 1966, M.A.C.S.Herb.No.453 (Type).

Remarks : Phyllachora eugeniae differs from the only reported species on Eugenia jambolana i.e. Phyllachora ambigua Syd. in having significantly bigger stroma, longer clavate asci and uniseriately arranged ovoid ascospores.

b. Phyllachora ficus-asperrimae sp. nov. (Fig.53.3).

Anahosur 1969, Sydowia (in Press).

Stromata black, shining, carbonaceous, amphigenous, multiloculate, subepidermal, circular to irregular, scattered, upto 2 mm. long, and 0.8 mm. broad. Perithecia black, stromatic, flask-shaped, with clypeus, ostiolate, upto 6 in each stroma,

220-310 x 300-452.4 μ. Asci cylindrical, with short pedicels, thin-walled, unitunicate, octosporous, arranged in wall-layers, 90-112.4 x 10-14 μ. Ascospores cylindrical, uniseriate, 1-celled, hyaline 14-18 x 6-8 μ. Paraphyses and periphyses numerous, filiform, slender and hyaline.

Habit : Parasitic on the leaves of Ficus asperrima Roxb. (Urticaceae) collected during October 1966, M.A.C.S.Herb.No.452 (Type).

Remarks : Many species of Phyllachora are known to parasitise the host genus Ficus. The writer's collection has resemblance to P. poonensis Seshadri and F. ficus-hispidae Seshadri but differs from both species in having multiperithecial stroma, cylindrical asci and uniseriate cylindrical ascospores.

It was interesting to note that spermogonial chambers were found in close association with the developing ascocarps of this Ascomycete and such association in Phyllachora simplocicola has been proved to be helpful in effecting 'Spermatization' as already reported by Jagtap (1968).

22. Polystigma DC

F. Polystigmataceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Polystigma was established by Decandolle (1815) with Polystigma rubrum (Fr.) DC parasitic on the leaves of Prunus domestica L. Clements and Shear (1931) and Bessey (1950)

have treated this genus as a member of Hypocreaceae on the basis of bright coloured perithecial walls. The presence of apically free paraphyses with light coloured perithecia and presence of periphyses in this genus shows its affinity to sphaeriales as suggested by Arx & Muller (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 Hypocrea schweinitzii.

In India this genus was represented by two species till 1962 i.e. P. ochraceum (Wahlenb) Sacc. on Prunus padus reported by Cooke (1878) and P. rubrum reported by Vasudeva (1960) on Almond.

The writer's collection of Polystigma is considered as a new taxon with the following diagnosis.

Polystigma eugeniae sp. nov. (Fig.53.4)
Anahosur 1969, Sydowia (in Press).

Perithecia stromatic, light coloured, innate, separate, ostiolate, with well developed clypeus, globose, 250-300 µ diam. Asci clavate, pedicellate, unitunicate, thick-walled, in basal-layers, octosporous, paraphysate, 160-180 x 16-18 µ. Ascospores cylindrical with prominent oil globules, slight constriction in the centre, uniseriate, hyaline, 1-celled, 16-20 x 8-10 µ. Paraphyses and periphyses slender, filiform, abundant and hyaline.

arranged in a line, parallel, ostiole projecting outside, 600-800 μ diam. Asci cylindro-clavatis, unitunicate, in wall-layers, pedicellate, apex thick, with apical caudal and pore, octosporous, 200-220 x 6-8 μ. Ascospores ellipsoid, dark-brown, thick-walled, uniseriate, 8-10 x 4-6 μ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit: Saprophytic on the twigs of Artocarpus integrifolia L. (Urticaceae) collected during October 1966, M.A.C.S.Herb.No.571.

Remarks : This is a new host record for this fungus.

Recently Morgan Jones and Lim (1968) have stressed the need to restrict the genus Poronia only to some fungi which are coprophilous in habit as previously recommended by Dennis (1957, 1958a, c.f. Jones and Lim 1968). This type of distinction at generic level seems to be artificial.

24. Pseudothis Theiss. & Syd.

F. Sphaeriaceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Pseudothis was established by Theissen and Sydow (1914) with Pseudothis coccodes (Lev.) Theiss. & Syd. as type. Clements and Shear (1931) have placed this genus under Sphaeriaceae whereas Muller & Arx (1962) treat it as a member of Diaporthaceae (O. Sphaeriales).

In India no species of this genus have been reported so far. The writer's collection was identified as Pseudothis coccodes, a brief description of which is given here.

with R. osbeckia (Berk. et Broom) Theiss. & Syd. as the type. No species of this genus have been reported from India. The writer's collection was found to agree with the type species of R. osbeckia. A brief description is presented here.

Rehmidothis osbeckia (Berk. & Broom) Theiss. & Syd.(Fig.54.1)

Stroma black, shining, amphigenous, Perithecia globose to conical, ostiolate, 2-4 in each stroma, 210-320 μ diam. Asci clavate, unitunicate, subsessile, octosporous, in basal layers, 55-65 x 10-12 μ. Ascospores oblong, unequally 2-celled, hyaline to sub-hyaline, 12-16 x 4 μ. Paraphyses lacking. Periphyses present.

Habit : Parasitic on leaves of Osbeckia octondra Blume (Melastomaceae) collected during October 1967.

Remarks : This is a new record to India.

26. Rosellinia DeNot

F. Xylariaceae, O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Rosellinia was established by DeNotaris (1847) with Rosellinia aquila (Fr.) DEN. Clements and Shear (1931) have placed this genus under the family Sphaeriaceae whereas Arx & Muller (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 world most of them being saprophytes on bark and stem of various

host plants. Several species are dangerous parasites viz. R. necatrix (Hax) Berl. on the roots and underground parts of grape (Vitis). The ascospores in this genus are ellipsoidal, coloured, 1-celled and because of the characters of the ascospores Vincens (1921), Wehmeyer (1926) and Miller (1928) suggested that this belongs to Xylariaceae.

In India 12 species of this genus were recorded till 1962. The description of the writer's collection considered as new to science is as follows :

Rosellinia punicii sp. nov. (Fig.54.3)
Anahosur 1969, Sydowia (in Press).

Stroma black, erumpent, globose, aggregated, 0.5-0.9 x 0.6-0.8 mm. perithecia globose to sphaerical, usually single but rarely 2 in each stroma, black, ostiolate, 316-411.8 μ diam. Asci clavate, unitunicate, pedicellate, in wall-layers, octosporous, apex thickened, with apical apparatus, 120.8-151.4 x 8-10 μ. Ascospores ellipsoidal to lenticular, dark-brown, 1-celled, uniseriate, thick-walled, 16-18 x 6-8 μ. Paraphyses and periphyses abundant, filiform and hyaline.

Habit: Saprophytic on the twigs of Punica grandiflora Hort.ex-Steud. (Lythraceae) collected during October 1967, M.A.C.S.Herb. No. 622 (Type).

Remarks : The erumpent nature of perithecia which are bigger and characteristically ellipsoidal spores with hyaline streak over the

spore body are the distinguishing characters of this species in addition to its multiloculate nature which is a rare character. Tulasne (1853) has reported that 2-3 perithecia coalesce in a common stroma in R. aquila (vide Greenhalgh & Chesters 1967).

27. Rosenscheldiella Theiss. & Syd.

F. Dothideaceae; O. Dothideales.

Sub-Class Loculoascomycetes.

The genus Rosenscheldiella was established by Theissen and Sydow (1915) for a Dothideaceous fungus with Rosenscheldiella styracis (P.Henn.) Theiss. & Syd. as the type, parasitic on Styrax sp. Clements and Shear (1931) and Hansford (1946) have placed this genus under Sphaeriaceae. Muller & Arx (1962) have placed it under Dothiorales.

In India this genus is represented by two species. viz. R. eugeniae Petch, reported by Ananthanarayanan (1962) and R. cinnamomii reported by Muthappa (1967). The developmental studies of ascocarps of R. eugeniae carried out by Ananthanarayanan (1964) have proved its place under Dothideaceae as also suggested by Luttrell (1955, 1965a). The writer's collection of Rosenscheldiella is considered as a new species with the following description.

Rosenscheldiella indica sp. nov. (Fig. 54.3)

Ascostromata black, hypophyllous, superstomatal, scattered to aggregated, circular to irregular, multiloculate, 0.5-0.8 mm. Locules globose to sub-globose, nonostiolate, upto eight in each

stroma, surrounded by thick layers of pseudoparenchymatic dark brown cells, 110-150 μ diam. Asci clavate, with truncate base, bitunicate, aparaphysate, in fascicles, pedicellate, octosporous, 40-50 x 12-14 μ. Ascospores oblong, unequally 2-celled, biseriate, with prominent oil globules, hyaline, 17-19 x 4-6 μ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit : Parasitic on the leaves of Mesua ferea Linn. f. (Guttiferae) collected during October 1967, M.A.C.S.Herb.No.541 (Type).

Remarks : The distinguishing characters of R.indica are super-stomatal nature of ascostroma which is multiloculate and unequally two-celled ascospores. This constitutes the third species reported from India.

28. Stictis Pers.

F. Stictidaceae; O. Phacidiales.

Sub-Class Ascohymeniales.

The genus Stictis was established by Persoon (1796) with S. radiata (L.) Pers. as the type. Clements and Shear (1931) and Miller (1949) placed this genus under Stictidaceae of Phacidiales.

The species of this genus mostly occur as saprophytes on barks and stems of various host plants. In India no species were reported till 1967, Muthappa (1967) the first time reported S. radiata saprophytic on Citrus cinensis Pers. (Rutaceae).

The writer's collection was compared and found to agree with S. radiata, a brief description of which is presented here.

Stictis radiata (L.) Pers. (Fig. 54.4)

Apothecia cup-shaped, scattered to aggregated, slate to ash coloured, erumpent. Asci cylindrical, pedicellate, octosporous, paraphysate, unitunicate, 180-200 x 10-12 μ. Ascospores filiform, septate, hyaline 130-148 x 2 μ; paraphyses abundant and hyaline, form true epithecium at the top.

Habit : Saprophytic on the twigs of Duranta plumeri Facq. (Verbenaceae) collected during October 1967). M.A.C.S.Herb.No.566.

Remarks: This is a new host record for this species.

29. Tryblidiella Sacc.

F. Patellariaceae; O. Hysteriales.

Sub-Class Loculoascomycetes.

The genus Tryblidiella was established by Saccardo (1863) with Tryblidiella rufula (Spreng.)Sacc. as the type. The developmental studies of T. rufula carried out by Muthappa (1967) and Seshadri and Muthappa (1969) have conclusively proved the nature of ascocarp to be a 'Discothecium' and the genus should find its place under Patellariaceae of Hysteriales as suggested by Muthappa (1967a). Muller & Arx (1962) have placed it under Patellariaceae of Dothiorales.

In India Seshadri (1965), Tilak (1963), Tilak and Ramachandra Rao (1966), have described the same fungus genus under Hysterium which needs thorough re-examination and revision.

The writer's collection was compared and identified as T. rufula, a brief description of the species is presented here.

Tryblidiella rufula (Sprong)Sacc. (Fig.54.5)
Syll. Fung. 2 : 757, 1883.

Discothecia discoid, leathery, with a wide cleft, lobed, with brick-red hymenium, 1-2 mm. long, Asci cylindrical, bitunicate, pedicellate, in basal-layers, octosporous 130-150 x 10-12 μ. Ascospores elliptical, 4-celled, constricted at septa, uniseriate, dark-brown 24-28 x 8-10 μ. Interthecial threads septate, hyaline, bifurcated at the tip.

Habit : Saprophytic on the twigs of Alseodephne semicarpifolia Nees (Lauraceae) collected during February 1968, M.A.C.S.Herb.No.566.

Remarks: This is a new host record for this fungus.

30. Vestergrenia Rehm.

F. Dothideaceae; O. Dothideales.

Sub-Class Loculoascomycetes.

The genus Vestergrenia was established by Rehm (1901) with Vestergrenia nervisequia Rehm. as the type. Arx & Muller (1954) treat this genus as a member of Botryosphaeriaceae of Dothiorales.

The genus is characterised by uni- to multiloculate ascostroma with bitunicate asci arising in fascicles and absence of sterile threads and tissues may justify the accommodation of this genus in Dothideaceae of Dothideales, according to the concept of Luttrell (1951, 1955, 1965a).

In India this genus was represented by 6 species till 1962 and the contributions are of Muller (1957) collected from Himalaya and Ramakrishnan & Subramaniam (1952) who had described 2 species as Physalospora which have since been transferred to Vestergrenia by Arx & Muller (1954).

The writer has collected 2 species on two different hosts and the brief descriptions of both are presented here.

a. Vestergrenia indica sp. nov. (Fig.55.1)

Anahosur 1969, Nova Hedwigia (in Press).

Infection spots dark brown, epiphyllous, circular, scattered, upto 1 mm. diam. Ascostromata globose, separate, strictly uniloculate, innate, non-ostiolate, with well developed clypeus, 282.4-321.4 μ diam. Clypeus black, carbonaceous, broad, 180-210 x 72-95 μ. Asci clavate, octosporous, bitunicate, in fascicles, pedicellate, with apical projection, 98.4-210.4 x 26-32 μ. Ascospores oblong, biseriate, 1-celled, hyaline, 22-28 x 6-8 μ. Paraphyses, pseudoparaphyses or interthecial tissues lacking.

Habit : Parasitic on the leaves of Haeptaplureum venulosum Sm. (Araliaceae) collected during October 1966, M.A.C.S.Herb.No.497, (Type).

Remarks : A Sphaeropsidaceous fungus was found in association with this Ascomycete and was identified as a species of Sphaeropsis, a brief description of which is provided here.

Pycnidia separate, black, innate, with well developed clypeus, 200-250 μ diam. Conidiophores cylindrical, simple, non-septate, hyaline, 8-12 μ long. Conidia brown, 1-celled, oblong 28-32 μ long and 2-3 μ broad.

b. Vestergrenia kamatii sp. nov. (Figs.55.2 & 55.3)

Anahosur 1969, Nova Hedwigia (in Press).

Ascostromata, black, shining, carbonaceous, globose, epiphyllous, aggregated in a circular infection spot, strictly uniloculate, innate, globose, stromatic, with a well developed clypeus, 144-210 μ diam. Clypeus black, carbonaceous, 144-198 x 48-58 μ. Asci clavate, bitunicate, in fascicles, with apical projection, and long pedicel, 72.4-161.4 x 22-30 μ. Ascospores oblong, biseriate, 1-celled, hyaline 19-24 x 4-6 μ. Paraphyses, pseudoparaphyses and interthecial tissues lacking.

Habit : Parasitic on the leaves of Glochidion lanceolarium Voight. (Euphorbiaceae) collected during October 1966, M.A.C.S.Herb.No. 456 (Type).

This species is named in the honour of my Research Guide Prof. M.N.Kamat, Head of the Department of Mycology and Plant Pathology, M.A.C.S. Poona (India), in recognition of his outstanding contributions to the field of Mycology and Plant Pathology in India.

Remarks: A Sphaeropsidaceous fungus, diagnosed as a species of Sphaeropsis was found in association with this Ascomycete, a brief description of which is presented here.

Pycnidia separate, innate, with well-developed clypeus, nonostiolate, globose to cylindrical, 70-100 μ diam. Conidiophores short, cylindrical, in wall-layers, hyaline 2-4 x 1 μ. Conidia oblong, brown at maturity, 1-celled, 10-18 x 4 μ.

Spermogonial bodies were also found in association with the developing ascocarps, the relationship and function of which need a detailed study.

31. Xylaria Hill ex. Grev.

F. Xylariaceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Xylaria was established by Hill (1789) and revised by Grevialle (1824). The type species is Xylaria hypoxylon (L. ex Fr.) Grev. Dennis (1958) adopted the name Xylosphaeria Dumortier since this name predates the name Xylaria Hill ex Grev. and the latter is a homonym of Xylosphaeria. However, Grayholm and Muller (1965; vide Jones & Lim,1968) have proposed conservation of the name Xylaria Hill ex Grev. as against Xylosphaeria Dumortier and hence the name Xylaria Hill ex Grev. is adopted here as used by Morgan Jones and Lim (1968) although recently Joly (1968) treated the species of Xylaria as the species of Xylosphaeria.

Ellis and Everhart (1887) have reported many species of Xylaria and other contributors being Miller (1942), Miller and

Neilson (1957) and recently Morgan Jones and Lim (1968). Most of the species of Xylaria are saprobes.

Clements and Shear (1931), Arx and Muller (1954) have placed it under Xylariaceae of Sphaeriales whereas Luttrell (1955) and Martin (1961) have treated it as a member of Xylariaceae of Xylariales.

In India about 45 species of this genus have been recorded till 1962. The writer collected two species of Xylaria and the description of the species are narrated here.

a. Xylaria azadirachti sp. nov. (Figs. 55.4 & 55.5).
Anahosur 1969, Sydowia (in Press).

Stroma dark-brown, spherical to capitate, borne singly at the tip of stipes, 1.1-2.0 mm. diam. Stipes dark brown, simple flexuous, cord-like, 2-4 mm. long. Perithecia globose, ostiolate, arranged along the periphery of the stroma, ostiole facing upwards, 350-460 µ. diam. Asci cylindrical, pedicellate, in wall-layers, octosporous, unitunicate, papillate, 180-200 x 5-7 µ. Ascospores dark-brown, 1-celled, thick-walled, with a prominent oil globule, fusoid to inaequilaterally, ellipsoid, obliquely uniseriate, 16-18 x 4-6 µ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit: Saprophytic on the seeds of Azadirachta indica A.Juss. (Meliaceae) collected during October 1967, M.A.C.S. Herb.No.623(Type)

Remarks: The distinguishing characters of this species are the small stromatic heads borne singly over long cord-like simple stipes, much bigger perithecia, asci and ascospores.

b. Xylaria polymorpha (Fr.) Grev. (Fig. 55.6).

Stroma dark-brown, clavate to cylindrical, simple to branched, 1.8-3.2 x 0.4-0.7 mm. Perithecia globose, ostiolate, arranged along the periphery of the stroma, often coalescing, 500-684 μ diam. Asci cylindrical, papillate, unitunicate, octosporous, pedicellate, in wall-layers, 200-240 x 10-12 μ. Ascospores dark-brown, 1-celled, with a prominent oil globule, obliquely uniseriate, inaequilaterally ellipsoid to fusoid, 22-26 x 6-8 μ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit : Saprophytic on the twigs of Erythrina indica Zoll. (Leguminosae) during October 1967, it was collected. M.A.C.S. Herb. No. 567.

Remarks : Erythrina indica is a new host record for this species.

EXPLANATION OF PLATE 46

Fig. 46.1. *Apiospora curvispora*

A. Habit, B. Section through infection spot.

C. Asci. D. Ascospores.

Fig. 46.2. *Apiospora montagnei*

A. Habit. B. Section through infection spot.

C. Asci. D. Ascospores.

Figs. 46.3, 46.4 & 46.5. *Botryosphaeria dothidea*

A. Habit. B. Section through Ascostromata.

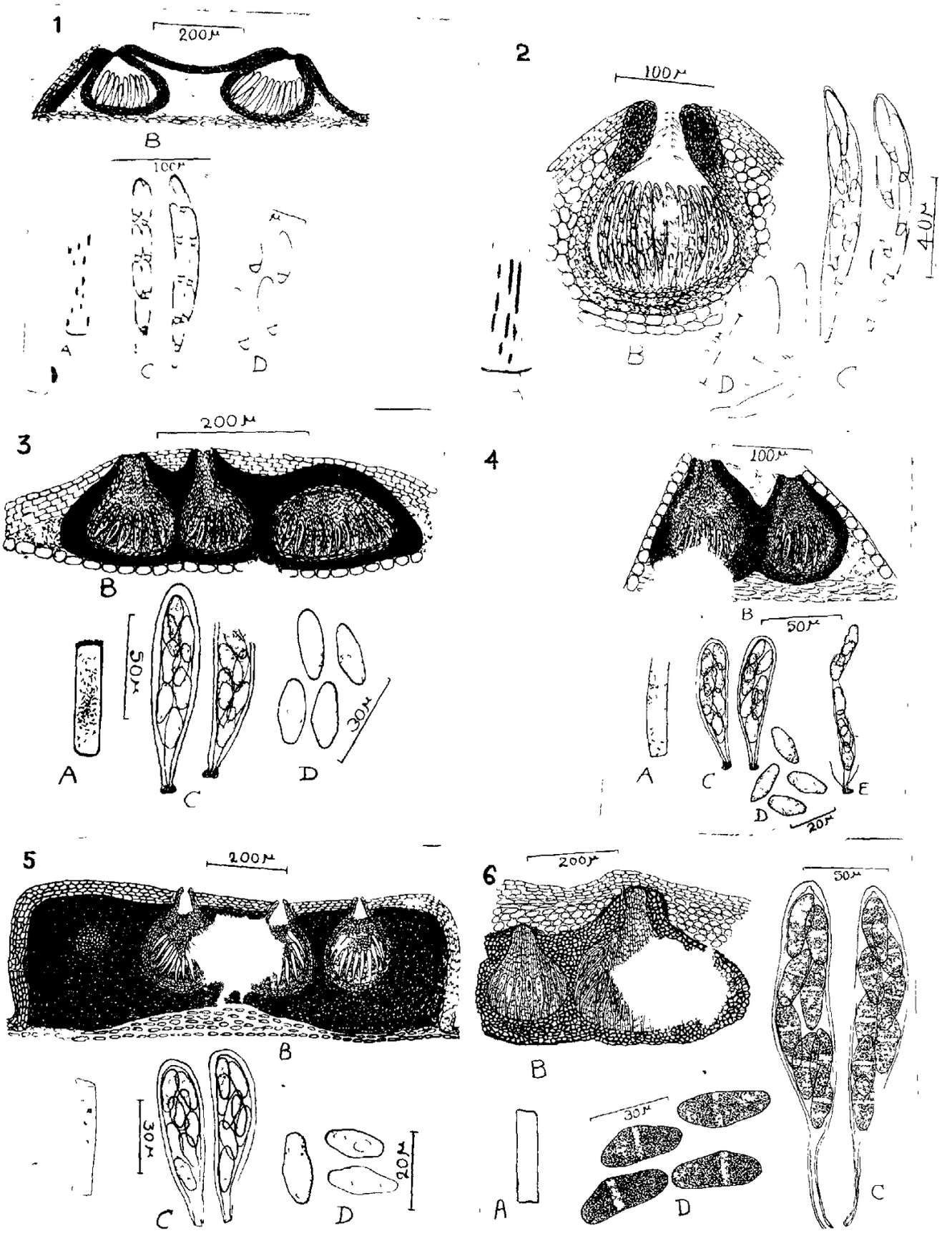
C. Asci, D. Ascospores, E. Ascus dehiscence.

Fig. 46.6. *Botryosphaeria laricis*

A. Habit, B. Section through Ascostroma,

C. Asci, D. Ascospores.

PLATE 46


1
200μ
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A
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D
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D
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40μ
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SECTION - B

ASCOMYCETES OF MAHARASHTRA (INDIA)

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INTRODUCTION.

During his mycological survey in and around Poona(Maharashtra) (India), the writer collected 8 ascomycetous funri, a detailed diagnosis and determination of which revealed that some were new species and others either new host records or new records to India. Upto 1967 about 260 Ascomycetes are reported from Maharashtra (Kamat et al. 1969).

Brief description of the eight Ascomycetes collected by the writer are presented in this Section.

Description of the species

1. Bagnisiella Speg.

F. Myriangiaceae; O. Myriangiales.

Sub-Class Loculoascomycetes.

The genus Bagnisiella was founded by Spegazzini (1880) with Bagnisiella australis Speg.as the type, collected on Acacia bonariensis Gill. Arx & Muller (1954) have placed this genus in Botryosphaeriaceae of Dothiorales whereas Luttrell (1955) is of the opinion that it occupies an intermediate place between Myriangiales and Dothideales. The writer's observations show that in Bagnisiella, the asci develop amongst pseudoparenchymatous cells which are persistent and take the appearance of 'interthecial 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 Bagnisiella which shows parallel arrangement of asci with the developmental pattern of ascocarp similar to "Klasinae" type and hence the genus may be placed under Myriangiales.

Tilak (1963), Seshadri (1967), Ramachandra Rao (1966b, 1967a) have contributed several species to this genus from India.

The writer's collection of Bagnisiella Speg. have the following characters.

a. Bagnisiella acaciae sp.nov. (Figs. 56.1 & 56.3).
Anahosur 1969, Sydowia (in Press)

Ascostromata black, elongated, erumpent, circular to rectangular, shrunken in the centre, multiloculate, upper portion fertile and lower portion sterile, with 4-7 compartments, non-ostiolate, 1-1.5 x 1-1.2 mm. Asci cylindro-clavatis, bitunicate, sessile, represent a locule, with apical projection, in basal layers, parallel, 84-96 x 30-40 µ. Ascospores cylindrical, thick-walled, hyaline to sub-hyaline, irregularly biseriate, 1-celled, 28-38 x 8-10 µ. Only interthecial tissues are present between asci i.e. locules.

Habit : Saprophytic on the twigs of Acacia arebica Willd. collected at Poona during December 1967, M.A.C.S.Herb.No.680 (Type), and on Glyricidia sp. M.A.C.S.Herb.No.681.

Remarks : B. acaciae differs from the type in having bigger stromata, broad sessile asci and bigger ascospores.

Conidial state : A conidial fungus belonging to Sphaeropsidales was found in association with this ascomycete which was diagnosed as a species of Haplosporella Speg. Their true relationship has been ascertained through pure culture studies (Vide Part II).

The conidial fungus is accommodated as a new taxon on the basis of comparative studies and the unusual nature of sub-hyaline to yellow cylindrical conidia and other characters. A brief description of which is presented here.

Haplosporella subhyalinae sp. nov. (Fig.56.2)

Pycnostroma circular to elongated, black, erumpent, multi-loculate, upper part fertile, lower sterile, erumpent, 0.8-1.2 x 0.6-1.0 mm. Loculas globose to rectangular, non-ostiolate, upto 6 in each stroma, 150-280 μ diam. Conidiophores short cylindrical, hyaline, in wall-layers, 4-6 x 2 μ. Conidia cylindrical, sub-hyaline, numerous, 1-celled, 28-32 x 6-8 μ.

b. Hagnisiella australis Speg. (Fig.56.4)

Ann.Mycol. 13 : 651, 1915.

Ascostroma black, circular to rectangular, erumpent, multi-loculate, upto 1.1 mm. long, with 3-5 compartments and non-ostiolate. Asci cylindro-clavatis, representing a locule, bituni-cate, pedicellate, in palisade layer, octosporous, 98-110 x 16-20 μ. Ascospores fusoid to cylindrical, thick-walled, hyaline to sub-hyaline, irregularly biseriate, 1-celled, 26-30 x 4-7 μ. Only interthecial tissues are present in between 2 asci i.e.locules

Habit: Saprophytic on the twigs of Lantana camara L.(Verbenaceae) collected during December 1967, M.A.C.S.Herb. No.682.

Remarks : This is a new host record for this fungus.

A conidial fungus belonging to Sphaeropsidales was found in close association with this Ascomycete and was diagnosed as Haplosporella cosmopolitus Muthappa (1967) and cultural studies carried out by the writer and reported in Part II of this Thesis have proved their true genetical relationship.

2. Calospora Sacc.

F. Sphaeriaceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Calospora was established by Saccardo in 1883. The type species is Calospora platenoides (Pers.) Neissl. Clements Shear (1931) have placed this genus under Sphaeriaceae of Sphaeriales on the basis of beaked perithecia in a stroma, cylindro-clavatis asci with paraphyses and hyalo-phragmoid ascospores.

In India till 1962 this genus was not represented by any species until recently Ramachandra Rao (1966), Tilak (1966), Tilak & Ramchandra Rao (1966) have contributed few species. The writer's collection of Calospora has the following characters.

Calospora lantanae sp. nov. (Fig.56.5)

Anahosur 1969, Sydowia (in Press).

Perithecia stromatic, separate, innate, beaked, ostiolate, globose to conical, 328-568 μ broad and 400-660 μ high. Beak

short, projecting outside the host, 50-60 x 30-34 µ. Asci cylindrical, thick-walled, unitunicate, pedicellate, in wall-layers, with apical apparatus, octosporous, paraphysate, 80-96 x 4-6 µ. Ascospores fusoid, hyaline, 3-septate, constricted at septa, uniseriate, 12-16 x 3-4 µ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit : Saprophytic on the twigs of Lantana camara L. (Verbenaceae) collected during August 1968, M.A.C.S.Herb.No. 683 (Type).

Remarks: The distinguishing characters of Calospora lantanae are the separate stromatic perithecia, short beak, cylindrical narrow asci and smaller ascospores which are arranged uniseriately as compared to the type species.

3. Didymosphaeria Fckl.

F. Pleosporaceae; O. Pleosporales.

Sub-Class Loculoascomycetes.

The genus Didymosphaeria was established by Fuckel in 1869. The type species is Didymosphaeria futilis (Berk et Broom.)Rehm. (Syn. D. epidermidis (Fr.) Fckl.). Clements and Shear (1931) have included this genus under Sphaeriales whereas Muller and Arx (1962) in Pseudosphaeriales. 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 is having the following characters.

Didymosphaeria saprophytica sp. nov. (Fig.56.6)

Ascostromata, black, aggregated, innate, globose, becoming erumpent, uniloculate, ostiolate, 280-390 µ. Asci clavate, pedicellate, with apical projection, bitunicate, in wall-layers, octosporous, 92-100 x 8-10 µ. Ascospores spindle shaped, dark-brown, thick-walled, equally 2-celled, biseriate, 16-22 x 6-8 µ. Pseudoparaphyses abundant, filiform, slender and hyaline.

Habit : Saprophytic on the twigs of Lantana camara L.(Verbenaceae) collected at Poona (India) during September 1967, M.A.C.S.Herb. No.673 (Type).

Remarks : D. saprophytica was compared with D. lantanae Muthappa as both occur on the same host and was found to be distinct from it in having significantly bigger ascostroma, asci and ascospores and in the nature of habit.

4. Glomerella Schrenk. & Spauld.

F. Sphaeriaceae; O. Sphaeriales.

Sub-Class Ascohymeniales.

The genus Glomerella was established by Schrenk and Spaulding in 1903 with Glomerella cingulata (Atkins.) S. & S. as the type species. Clements and Shear (1931) have included this genus in Sphaeriaceae of Sphaeriales whereas Arx & Muller (1954)

Habit : Saprophytic on the twigs of Dolichos lablab var.typicus Prain (Leguminosae) collected during October 1968.

Remarks: This host is a new host record for this Ascomycete.

5. Sphaerotheca Lev.

F. Erysiphaceae; O. Erysiphales.

Sub-Class Ascohymeniales.

The genus Sphaerotheca was established by Leveillae in 1851 with Sphaerotheca pannasa (Wallr.) Lev. Clements & Shear (1931) have placed this genus under perisporiaceae of perisporales whereas Luttrell (1955) and Martin (1961) treat this genus as a member of Erysiphaceae of Erysiphales.

The order Perisporiales and family Perisporiaceae of some authors are based upon the genus Perisporium Fr. In view of the fact that the type species of this genus P. gramineum Fr. has been shown to be not belonging to this order as customarily limited.It is necessary to base the order upon the recognised genus whose connection with the order is beyond doubt. Following the proposal of Gwyne Vaughan, the name Erysiphales based upon the genus Erysiphe has been selected further by most of the authors (vide Bessey,1950).

Homma (1937) has reviewed a monographic study into the Erysiphaceae of Japan and has given a key for the identification of the species. The monographic studies of Salmon (1900) are of monumental type in this group of Ascomycete. Yarwood (1957) has reviewed the literature dealing with this family.

In India about five species of this genus are reported till 1962, recent contributions of Patwardhan (1966) on Erysiphaceae of Maharashtra (India) are worth mentioning here.

The writer's collection of Sphaerotheca agreed with S. fuliginea as defined by Homma (1937), since it is collected on a new host, a brief description of the species is given here.

Sphaerotheca fuliginea (Schlt.)Pollacci (Fig.57.2)

Infection spots snowy white, circular to irregular, and coalescing each other, mostly on leaves, amphigenous. Mycelium broad, superficial, creeping and peristent. Conidia 1-celled, hyaline, ellipsoid to cylindrical, produced in long chains 22-28 x 12-16 µ. Perithecia scattered, dark-brown, globose to spherical, 76.5-93.5 µ diam. Appendages variable in number (5-13), brown at the base, and subhyaline at the tip, usually simple, rarely branching. Wall cells polygonal, dark-brown, thick-walled 20-55 x 12-36 µ. Ascus broadly ovate to subglobose, sessile, octosporous, unitunicate, thick-walled, 68-76 x 55-60 µ. Ascospores subglobose to cylindrical, hyaline, 1-celled, irregularly arranged, 17-21 x 10-12 µ.

Habit : Parasitic on the living leaves of Phaseolus radiatus L. (Leguminosae) collected at Sinhagad near Poona (India) during December 1966, M.A.C.S.Herb.No.334.

Remarks: It is a new record to India.

Muller (1954) have included it as a member of Valsaceae of Diaporthales.

The species of this genus are mostly saprophytes and their conidial state belongs to the form genus Cytospora. In India only 4 species were reported till 1962. The main contributions being those of Sydow and Butler (1911), Padwick (1945).

The writer's collection was compared and found to agree with Valsa ceratophora. As it is collected on an unreported host, a brief description of the species is given here.

Valsa ceratophora Tul. (Fig.57.4)
Syll. Fung. 1 : 108, 1882.

Perithecia, black, innate, separate, globose, clustered, with prominent beaks projecting outside the host. Beak cylindrical ostiolate, periphysate, upto 1.2 mm. long. Asci clavate, pedicellate, in wall-layers, paraphysate, octosporous, unitunicate, 34-42 x 6-8 μ. Ascospores allantoid, biseriate, 1-celled, hyaline 6-8 x 2 μ. Paraphyses and periphyses abundant, filiform, slender and hyaline.

Habit : Saprophytic on the twigs of Caesalpinia pulcherrima Sw. (Leguminosae) Collected during October 1968, M.A.C.S.Herb.No. 687.

Remarks: This is a new host record for this fungus.

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* Originals not seen.

EXPLANATION OF PLATE 57

Fig. 57.1. *Glomerella cingulata* and *Colletotrichum gloeosporioides*

A..Habit, B. Acervulus, C. Conidiophores with conidia, D. Conidia.E.Perithecium, F. Asci, G. Ascospores.

Fig. 57.2. *Sphaerotheca fuliginea*

A. Habit, B. Ascocarp, C. Cells of the ascocarp wall, D. Ascus, E. Ascospores, F. Conidiophores with chains of conidia, G. Conidia.

Fig. 57.3. *Tryblidaria maharashtrensis*

Photomicrograph showing the section of the ascocarp and the hypostroma.

Fig. 57.4. *Valsa ceratophora*

A. Habit, B. Section of the infection spot. C. Asci, D. Ascospores.

GENERAL DISCUSSION

Species	Chromosome number Haploid	Diploid	Authority
	ASCOLOCULARES (contd.)		
Mycosphaerella mysorensis	3	6	Seshadri, 1967
Parodiella perisporioides	3	6	Tilak, 1959
Cyclotheca kamatii	3	6	Ananthanarayanan, 1964.
Rosenscheldiella eugeniae	4	8	"
Lembosina aulographoides	3	6	Muthappa, 1967
Tryblidiella rufula	2	4	Seshadri, 1967
Elsinoe kamatii	4	8	Kalani, 1965
Elsinoe thirumalacharii	3	6	Chiplonkar, 1969
Microcyclus indicus	[illegible]	4	[illegible]ar, 1969

It can be seen from this statement, that, in general the chromosome number is significantly larger in fungi belonging to Ascohymeniales (4-16 haploid) than in fungi belonging to Ascoloculares where the chromosome complement appears to be not only much more uniform in number, but significantly smaller, varying from 2 to 4 haploid. According to Olive (1953) the chromosome number in fungi ranges from 2 to 28 haploid, with species having a complement of less than 10, predominating.

Recently Carr and Olive (1958) have suggested the use of chromosome morphology coupled with chromosomal events during divisional stages in correlation with the ascus development,