1974

The Morphology and Cytology of Preussia vulgare (Corda) Cain

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THE MORPHOLOGY AND CYTOLOGY OF PRUSSIA VULGARE (CORDA) CAIN

(TITLE)

BY

Marcial Antonio Pastor-Corrales

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science in Botany

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1974
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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ACKNOWLEDGEMENTS

The author wishes to express his most sincere and deepest gratitude to Dr. Wesley C. Whiteside for his suggestion of the study. He graciously provided the author with the fungus culture, materials to conduct the research, and with several papers. Only his valuable guidance, criticism, and supervision made this study possible. Mr. L. Crofutt furnished the agar and petri plates; Dr. S. Becker gave valid suggestions and contributed with the microtechnique materials; Dr. G. Gray generously provided the film; Dr. W. Scott contributed with several papers and Dr. C. B. Arzeni with his cheerful encouragement. The writer wishes to acknowledge his gratitude to all.

INTRODUCTION

The present study was undertaken to elucidate the cytology and morphology of Preussia vulgare (Corda) Cain. The genus Preussia, an ascomycete fungus, was erected and published by Fuckel in 1866. The type species is Preussia funiculata (Preuss) Fuckel, a species originally described by Preuss in 1851 as Perisporium funiculatum. Preussia vulgare (Corda) Cain was first described by Corda in 1838 as Perisporium vulgare. Cain (1961), in the first modern study of Preussia, revised this genus and discussed its taxonomic position. He emphasized that the genus Preussia was distinct from Perisporium, a genus first published by Fries in 1825. Cain (1961) concluded that Perisporium is not a valid name for those species of Ascomycetes forming a non-ostiolate ascocarp with the same pattern of development as that in the Loculoascomycetes. He transferred the species of Perisporium to the genus Preussia, which he treated in the family Spormiaceae of the order Pleosporales, subclass Loculoascomycetes.

The genus Preussia is a representative of the ascostromatic Ascomycetes, a group comprising the subclass Loculoascomycetes, this subclass referring to the ascocarp having the bitunicate

asci produced in locules. Cain (1961) recognized twelve species of Preussia. In addition to Preussia funiculata (Preuss) Fuckel, three of these are species of Perisporium, which he transferred to Preussia. These are Perisporium punctata (Auessw.) Sacc. (= Preussia punctata (Auersw.) Cain), Perisporium typharum Sacc. (= Preussia typharum (Sacc.) Cain), and Perisporium vulgare Corda (= Preussia vulgare (Corda) Cain). One species was transferred from each of the following genera: Sporormia (Preussia fleischhakii (Auersw.) Cain, formerly Sporormia fleischhakii Auersw. = Fleischhakia laevis Auersw.), Muellerella (Preussia nigra Routien) Cain, formerly Muellerella nigra Routien), Pycnidiophora (Preussia dispersa (Clum) Cain, formerly Pycnidiophora dispersa Clum), Anixiopsis (Preussia multispora (Saito and Minoura) Cain, formerly Anixiopsis multispora Saito and Minoura = Pseudoerotium multisporum (Saito and Minoura) Stolk), and Thielavia (Preussia indica (Chattop. and Das Gupta) Cain, formerly Thielavia indica Chattop. and Das Gupta = Pseudoritium indicum (Chattop. and Das Gupta) Chattopadhay). Three species Cain described as new: Preussia isomera Cain, Preussia terricola Cain and Preussia purpurea Cain. Clum (1955) created the genus Pycnidiophora for a genus with a single species P. dispersa which he tentatively placed in the family Eurotiaceae (= Aspergillaceae) in the Plectomycetes. Stolk (1955) studied Pseudoerotium multisporum and placed this species in the Eurotiaceae. Cain (1961) transferred both taxa to the genus

Preussia claiming that the 32 spores in the mature ascus are disarticulated segments of four-celled ascospores. Kowalski (1964), who studied the cytology of Pycnidiophora dispersa, rejected Cain's placement of this species in the genus Preussia on the basis that a succession of nuclear divisions yields are ascus with 32 free nuclei, each of which becomes an uninucleate spore. Thompson and Backus (1966) corroborated the findings of Clum (1955) and Kowalski (1964). Thompson and Backus in the first cytological study of Pseudoerotium multisporium found that the ascocarp development of this species is essentially identical with that of Pycnidiophora dispersa, and they concluded that both organisms are plectomyceteous fungi of the family Eurotiaceae. They proposed that Pseudoerotium multisporium be transferred to the genus Pycnidiophora (P. multispora (Saito and Minoura) Thompson and Backus). Maciejowska and Williams (1963) added an additional species, Preussia multilocularis Maciejowska and Williams, for an isolate having multiloculate non-ostiolate ascostromata. Boylan (1970) described a new species, Preussia flanaganii Boylan, for an isolate that has the ability to form uniloculate or multiloculate ascostroma.

Most of the species of Preussia are coprophilous, living in various kinds of herbivore dung (Kowalski, 1965, 1966, 1968 and Parker 1971) although some species have been isolated from soil (Routien (1956), Dennis (1968), Maciejowska and

Williams (1963) and Boylan (1970). The fruit-body, regarded as an ascostroma, grows on the surface of the substratum, scattered or gregarious. The ascocarp is cleistocarpous and varies from globose to subglobose, usually smooth, shining and glabrous, dark brown or black in color. The ascocarp consist of stromatic tissue with no special wall around the ascocarp centrum such as the perithecial wall of the stromatic Pyrenomycetes. The centrum is merely a cavity within the stroma in which the asci are located. The stroma is generally uniloculate, except in Preussia multilocularis and abnormally in certain of the other species. The species of Preussia that have been subject to developmental studies have been found to have the Pleospora type of centrum, with the locule formed by the growth of pseudoparaphysis among which asci develop. Kowalski (1965) describes pseudoparaphysis as originating at the top of the locule and growing downward. In the species of Preussia that have been studied, the sterile threads are present at the early stages of the development of the ascostroma but with maturity they become largely evanescent. The ascus in Preussia is broadly clavate with a long slender stalk and arranged in the ascostroma in an irregular fashion. The asci has eight dark-colored spores, each with three transverse septa dividing the spore into four cells that break apart readily. The ascus is bitunicate with the characteristic thickened wall consisting of the endoascus and the exoascus. At maturity the ascus wall is fragile and

evanescent, with the ascospores being liberated from the ascocarp by the desintegration of the ascostromatic wall or by mechanical breakage. The ascus lacks special means of dehiscence characteristic of the other Loculoascomycetes, in which the exoascus ruptures at the apex, thus allowing the endoascus to expand, forming a long cylindrical sac with the ascospores forcefully discharged through an elastic pore in the endoascus.

Preussia vulgare (Corda) Cain, a coprophilous species (Parker, 1970) was first described by Corda in 1838 as Perisporium vulgare in his Iconis fungorum. Cain in 1961 transferred this species to the genus Preussia. The ascostroma in Preussia vulgare is about 160-200 u in diameter and with a wall four to five cells thick, gregarious, superficial, globose, smooth, black and shining. The ascus is broad, clavate, about 37.5-55.5 x 15-20 u with a short stipe measuring about 7-9.5 u in length. The ascus is eight-spored, with ascospores 30 x 4.5 u three septate, easily fracturing into four cellular segments of equal size (7.5 x 4.5 u) Brown, cylindrical, lying longitudinally parallel within the ascus. Parallel to the long axis of the spore cell is a germinal slit. Pseudoparaphyses are present in the young ascocarp and are filiform, septate and interspersed with the young asci, but disappearing with maturity of the ascocarp.

In 1897 Lindau in his treatment of the Pyrenomycetes, which he regarded as an order of the Ascomycetes, divided the group into four suborders: Perisporales, Hypocreales, Dothideales, and

Sphaeriales. According to Munk (1953) Lindau's system of classification of the Pyrenomycetes follows closely that used by Elias Fries (1823), and later by G. Winter (1887). Ellis and Everhart (1892), in their classic taxonomic work "The North American Pyrenomycetes", adopted the systematic arrangement of Winter as the basis for their classification. This system was based principally on macroscopic characteristics such as the color of the perithecial walls and the stroma, presence or absence of a stroma, and the position of the ascocarp on the substratum. The classification adopted by Lindau (1897) has been followed in its general outlines by most mycologists. However, it was early recognized that many fungi included in the Pyrenomycetes do not form perithecia. In 1823 Fries recognized a different type of ascocarp, referred today by recent mycologists as an ascostroma, in which the asci are formed in perithecium-like cavities or locules in a stroma, the multiloculate type of ascostroma. Fuckel (1869), in the genus Pyrenophora Fries, was apparently the first to recognize the simple type of ascostroma with a single locule. He established the family Dothideceae to include both types of ascostromata. Miller (1828) gave renewed emphasis to the distinction between the ascostromatic pyrenomycetes and those forming true perithecia. Clements and Shear (1931) in the "Genera of Fungi" included the ascostromatic forms in one order, the Dothideales, although they left out several genera that subsequently have been recognized

as ascostromatic. Later Nannfeldt (1932) expanded this concept and proposed the name Ascoloculares for this ascostromatic group, which he recognized as a distinct series of the subclass Euanscomycetes, co-ordinate with the Plectascales and the Ascohymeniales, the latter a group he established to include both the Discomycetes and the Pyrenomycetes with true perithecia. Furthermore, Nannfeldt contributed immensely to the understanding of the ascostromatic group with his association of the thick-walled bitunicate ascus with the Ascoloculares and the ability of fungi of this group to form parenchymatous tissues in the ascocarp, and sometimes also in the ascospores and conidia, resulting in the muriform type of spore. Miller (1949) in his "Revision of the Classification of the Ascomycetes with Special Emphasis on the Pyrenomycetes", failed to adopt Nannfeldt's proposal to remove the ascostromatic forms from the series Pyrenomycetes. Luttrell (1955), on the other hand, placed primary emphasis on whether the ascus was unitunicate or bitunicate and divided the subclass Euascomycetes into two series, the Bitunicatae and the Unitunicatae, the latter being further subdivided into four subseries, the Laboulbeniomycetes and the traditional Plectomycetes, Pyrenomycetes and Discomycetes. In 1965 Luttrell elevated the series Bitunicatae to a subclass, which he called the Loculoascomycetes, while retaining the subclass Euascomycetes for those with unitunicate asci.

Early mycologists such as Ellis and Everhart (1892) and

Lindau (1897) followed Winter's (1887) classification of the genus Preussia along with the powdery mildews in the Erysiphales, sometimes called the Perisporales, an order containing those Pyrenomycetes with ascocarps cleistocarpous. Gwynne Vaughan (1922, 1927) followed this classification of cleistocarpous Pyrenomycetes, although she did not consider the genus Preussia (= Perisporium) in her study. Miller (1928) who emphazised the significance of the ascostromatic Pyrenomycetes, separated the Pyrenomycetes into two series: (1) the ascostromatic forms, which included the Perisporoceae, and (2) the true Pyrenomycetes with asci born in a perithecium. Clements and Shear (1931) included the ascostromatic forms in the order Dothideales, but they regarded the Perisporales as an order of fungi having non-ostiolate perithecia that lacked paraphyses. In this order they considered Perisporium Fries and Preussia Fuckel under different families. Perisporium was included as a member of the family Perisporoceae with a single species in the genus, P. vulgare Corda. They regarded Preussia as a member of the family Eurotiaceae, again with a single species, P. funiculata Fuckel. The powdery mildews comprised one of the seven families of this order.

Nannfeldt (1932) was the first modern mycologist to separate Perisporium from the Erysiphales. He treated the ascostromatic group, which he coined the Ascoloculares, as a series of the subclass Euascomycetes. In this series Nannfeldt considered four

orders, one of which was the Pseudosphaeriales, and in this order under the family Pleosporaceae he included Perisporium as a form related to Pleospora, but not clearly belonging to the family Pleosporaceae. It should be emphasized that Nannfeldt was the first to recognize the genus Preussia (= Perisporium) as ascostromatic with bitunicate asci, as the genus is regarded by modern mycologists. The proposal of Nannfeldt (1932) was not readily accepted by subsequent authors and instead they followed the more traditional classification in which Preussia (= Perisporium) was regarded as a Pyrenomycete. However, Gaumann (1952), recognizing the ascostromatic group as a distinct series, adopted the classification of Nannfeldt, the Ascoloculares, and in this series he placed the Perisporales. One of the families in this order was the Perisporiaceae, with Perisporium as one of the genera. Bessey (1950) did not consider this genus on his work. Alexopolous (1952, 1962), proceeding along with the traditional classification, also did not consider this genus in the two editions of his textbook. Ainsworth and Bisby (1954), in their dictionary of fungi, considered Preussia and Perisporium as two separate genera of the family Erysiphaceae. Luttrell (1955), in his significant review of the morphology and classification of ascomycetes, failed to consider Preussia (= Perisporium). Munk (1957), following Nannfeldt (1932), recognized two groups of Pyrenomycetes, the Ascohymeniales and the Ascoloculares. In the later group he treated Perisphorium as a member of the

family Sporormiaceae. Cain (1961) in the most important modern work dealing with the genus, proposed that Perisporium is not a valid name for P. vulgare Corda and similar species and, therefore, transferred three species of the genus Perisporium to Preussia, a genus he placed in the family Sporormiaceae. Dennis (1968), adopting the modern criteria of classification, acknowledged two main groups of Ascomycetes, the Euascomycetes and the Loculoascomycetes. He employed the traditional generic name of Perisporium rather than Preussia for the only genus listed in the family Perisporiaceae, one of the seven families he treated in the order Pleosporales.

LITERATURE REVIEW ON MORPHOLOGY

Despite the fact that the genus Preussia was erected in 1886 by Fuckel, the first significant work on the morphology of this genus was not published until 1938. Beatus (1938), a German mycologist, studied the development of the ascocarp of Perisporium funiculatum Preuss (= Preussia funiculata (Preuss) Fuckel). Beatus found that the ascocarp in Perisporium funiculatum was initiated by the fusion of two uninucleate hyphal end cells which he called the antheridium and the oogonium, giving rise to the binucleate condition of the ascogonium. Adjacent hyphae, in concurrence with the stalk cell of the ascogonium, form the ascocarp wall. He stated that the binucleate ascogonium was always present; however, the further development of it was not mentioned. Beatus also described true paraphyses for this species, and he did not report the presence of croziers. His observations indicated the haploid chromosome number for this species to be four.

Routien (1956) studied the development of a new species, Muellerella nigra Routien (= Preussia nigra (Routien) Cain), an organism he isolated from soil and considered to have bitunicate asci produced in a pseudothecium. He described the

formation of the pseudothecium from a spherical swollen unicleate cell along a hypha, usually near the tip, and he referred to this structure as the pseudothecium primordium, which presumably developed into a mature ascocarp. He also mentioned the presence of branching pseudoparaphyses in the fruiting bodies. Any structures resembling ascogonia, trichogynes and antheridia were not observed; however, he found darkly staining cells that appear to form first in a basal hymenial layer and then, by prolific crozier development, to extend the hymenial layer up and around the interior of the pseudothecium. Although he thought that these structures may be ascogenous cells, he did not see the manner of their formation, nor did he have enough evidence to support his speculation.

Maciejowska and Williams (1963) published the first morphological work devoted to a multiloculate species of Preussia. They carried out a study on Preussia multilocularis Maciejowska and Williams, a new species named by these workers for an isolate obtained from greenhouse soil. It should be noted that this species differs from the generic description of Preussia by Cain (1961), who considered the genus to be essentially uniloculate. They considered the number of locules per ascostroma to be an important taxonomic criterion in the genus Preussia. According to these investigators, the formation of the ascostroma is initiated by a hyphal cell, apparently uninucleate, which forms irregular aggregations of cells that frequently

anastomose. At points the hyphae coil around each other, forming clumps which will give rise to the ascostroma, with several adjacent cells participating in the process. These authors also reported the presence of small, deeply staining cells from which asci arose. The first cells observed were binucleate. However, they did not regard them as ascogonia, for they failed to report the presence of ascogonial cells. The presence of numerous pseudoparaphysis was mentioned, and the bitunicate condition of the ascus was noted.

Kowalski (1964) reported the development and cytology of Pycnidiophora dispersa Clum, a genus created by Clum (1955) but transferred to Preussia by Cain (1961). On the basis of his study, Kowalski considered Cain's interpretation as incorrect. This is corroborated by a later study by Thompson and Backus (1966) of this species and of Pycnidiophora multispora (Saito and Minoura) Thompson and Backus, a fungus originally named as Pseudoeurotium multisporium (Saito and Minoura) Stalk by Stalk but transferred to the genus Preussia (P. multispora (Saito and Minoura) Cain) by Cain. Kowalski described the ascocarp development in Pycnidiophora dispersa as initiated from a single enlarged, uninucleate cell which increased in size and became multinucleate. This cell also divides in a single plane at right angles to the hypha, forming a chain seven or eight cells in length. Divisions proceeded along this chain in all planes until a spherical stroma was produced. The cells became mainly uninucleate, since nuclear

divisions did not keep pace with cellular divisions. He also reported the presence of dark-staining multinucleate cells that differentiated from the center of the ascostroma and which he designated as "fertile cells". These, according to Kowalski, give rise to ascogenous hypha. Since at least 100 of these cells differentiated in the centrum, he did not consider it correct to refer to them as ascogonia. No sterile threads were observed in the ascocarp. A hymenial layer did not develop, but rather asci formed completely throughout the ascocarp in little fascicles. According to the observations of both Kowalski (1964) and Thompson and Backus (1966), the asci are truly 32-spored with each spore uninucleate and never attached to one another. Homothalism, as well as a haploid chromosome number of 6, is reported for this species by Kowalski.

The following year Kowalski (1965) published his second investigation in the genus with his cytomorphological study of Preussia typharum. According to his study, the ascocarp of this homothallic species originates in an intercalary manner, with the earliest stages observed consisting of three cells, apparently uninucleate. These cells divide to form a chain that may reach ten cells in length, around which divisions proceed in all planes until a spherical stroma is formed. As development proceeds, a few scattered multinucleate cells appear in the center of the stroma. Kowalski referred to these cells, which sometimes contained up to seven nuclei, as the

ascogonial cells. The presence of paraphysoids, sterile threads attached at both the top and bottom of the centrum, was also reported. The ascogonial cells eventually gave rise to a large mass of ascogenous hyphae, from which croziers, and subsequently asci, develop upward into the spaces between the paraphysoids, forming of a distinct hymenium. Kowalski also observed the presence of centrioles associated with the nuclei. Spore formation is initiated at the stage when the ascus contains sixteen nuclei, according to Kowalski. The haploid chromosome number was reported as 11.

In his third detailed study of the genus Preussia, Kowalski (1966) published an investigation on the morphology and cytology of Preussia funiculata (Preuss) Fuckel, the type species for the genus Preussia. This species was reported as homothallic. According to Kowalski, the nuclei of the vegetative mycelium appear to divide in a way very similar to mitosis of higher plants, except that no typical methaphase was observed. In addition, another method of division consists of elongated nuclei splitting longitudinally. The ascocarp development was initiated from an enlarged and swollen intercalary hyphal cell that is multinucleate. Internal divisions take place within this primordium but the nuclear divisions do not keep pace with cellular divisions; thus the cells become mainly uninucleate, although occasionally binucleate. The ascostromatic primordium becomes round in shape and pseudoparenchymatous. The outer layer

differentiates to form the wall, which at maturity attains a thickness of two or three cells. At approximately the same time multinucleate cells arise throughout the centrum. These are the ascogonial cells which in time gave rise to an extensive ascogenous system, consisting of large, multinucleate, lobate cells, that form asci by means of croziers. Kowalski (1966) also reported that at approximately the same time of ascogonial cell formation, paraphysoids begin to grow. At maturity the ascocarp contains a distinct hymenium. The reported haploid chromosome number of Preussia funiculata is 12, and spore formation is initiated at the 8-nucleate condition of the ascus.

Two years later Kowalski (1968) undertook his fourth investigation of the genus Preussia, in his publication, of the morphology and cytology of Preussia isomera Cain, a homothallic species. Nuclear division in the vegetative mycelium appears to be identical to that of Preussia funiculata (Preuss) Fuckel, with the longitudinal splitting of elongated nuclei occurring, in addition to divisions resembling mitosis in higher plants. Likewise, ascocarp development in Preussia isomera is also almost identical to that of Preussia funiculata. The major difference is that some of the cells of the centrum of Preussia isomera become differentiated, producing pseudoparenchymatous cells that are binucleate and slightly larger than the other cells of the ascostroma. These are the "fertile cells" that in time give rise to asci by typical crozier formation. Cells

lining the locule begin to grow and give rise to sterile threads which have absolutely no orientation but simply grow throughout the centrum. A typical hymenium is lacking, for the asci radiate out in all directions from a central region. The reported haploid chromosome number is 8 and the spore formation is initiated at the eight-nucleate condition of the ascus.

In his publication of the cytology and development of Preussia flanaganii Boylan, Boylan (1970) described the life history of this homothallic organism. The ascocarp development is the result of hyphal anastomoses between short lateral branches, giving rise to uninucleate cells that swell and fuse with other similar cells, followed by proliferation of cells at the fusion site. The ascocarps are uniloculate or multiloculate depending on the number of groups of deeply stained cells, these cells being apparently related to the development of asci. If there is one such group of cells, it is centrally located and the ascocarp is uniloculate, but if there are two or more groups they are usually off-center and the ascocarp is multiloculate. The asci develops by typical crozier formation. As the ascocarp grows sterile threads arise from all sides of the centrum and grow inwards. Boylan did not determine whether these hyphae were pseudoparaphysis, paraphysisis or paraphysoids, for he believed they may differ from all of these. The reported chromosome number is 14.

MATERIALS AND METHODS

The culture of Preussia vulgare was isolated by W. C. Whiteside from horse manure collected in a pasture at Tallahassee, Florida, in the autumn of 1957. The identification to species was carried out by Alan Parker through the use of Cain's (1961) monograph of the genus Preussia.

Standard sterile procedures were followed throughout this investigation. Oatmeal agar was the most satisfactory medium utilized. It was prepared by sterilizing about 15 to 20 oatmeal flakes in a petri dish, over which was poured approximately one centimeter of 1.5% water agar. The depth of the agar was not of noticeable importance. Greater depths were preferred to avoid the drying out of the media. The agar plates were inoculated with small pieces of agar bearing vegetative mycelium and ascocarps. Potato Dextrose agar and nutrient agar were also tried. However, on these two media the growth of the vegetative mycelium and the production of ascocarps was not satisfactory.

The cultures were grown at room temperature. The optimum temperature for the production of ascocarps ranged between approximately 70 degrees to 80 degrees F. Higher temperatures

than the optimum seem to disrupt the normal development of the ascocarps and lower temperatures apparently retard the growth of the vegetative mycelium and of the ascocarps. The growth of the vegetative mycelium was apparent in approximately two and half to three days after inoculation. Ascocarp development occurred readily and mature ascocarps were formed within two weeks. The ascocarp initials were apparent within 60 to 72 hours after inoculation. At approximately the same time the vegetative mycelium became visible.

To study the hyphal growth and development of ascocarp initials, an inoculum was placed on sterilized squares of dialyzable membrane deposited over the oatmeal medium. Two and one-half to three days later when the organism had reached the appropriate stage of development, the squares of dialyzable membrane bearing the fungus growth were removed, killed and fixed. Two techniques were used. In one the material was killed and fixed in the Randolph's modification of Navashin fluid (Johansen, 1940) and then aspirated for a few minutes. The material was left in the killing agent for 18 hours, rinsed a few minutes in tap water and placed in 4% freshly prepared iron-alum mordant (4 grams of ferric-ammonium-sulfate in 100 cc of distilled water) for a period of 2 to 3 hours. After rinsing in tap water the material was stained in 0.5% aqueous solution of Heidenhain's iron-alum hematoxylin. Destaining was achieved in 2% iron-alum for 4½ to 5 minutes. The material was then

washed in tap water for 30 minutes and dehydrated for 2 minutes each in 30, 50, 70, and 95% ethyl alcohol and twice in absolute ethyl alcohol, after which it was placed for 5 minutes in a solution composed of half absolute ethyl alcohol and half xylene. Pure xylene served as the clearing agent and Permount as the mounting medium. The fungus growth was then separated from the dialyzable membrane and macerated. The slides were placed on the warming plate to dry for about 8 hours. A shortened variation of this technique was tried, with the results essentially the same. Up to the stage of washing the material for 4½-5 minutes subsequent to destaining with 2% iron-alum, the original technique and the variation of it were identical. However, in the shortened procedure the destained material was not dehydrated. Instead it was macerated and mounted in Moyer's medium (7.5 grams gum arabic, 50 grams choral hydrate, 4 grams glycerine, about 50 ml of water heated to warm), sealed with nail polish and placed in warming plate for about 8 hours. A second technique used for studying the development of the ascocarp initials of Preussia vulgare was one described by Kowalski (1964). In the technique the material was fixed and killed in Newcomer's fluid (Newcomer, 1953), aspirated, and placed in Giemsa stain. The results of this method were not nearly as satisfactory as those obtained with the Navashin killing fluid and hematoxylin stain.

In studying the development and structure of the ascostroma, the fungus was also grown on oatmeal agar. From 5 to 22 days

after inoculation, small blocks of agar approximately 1cm x 0.5cm bearing ascocarps were removed from the petri dish. These were fixed in Randolph's modified Navashin solution, aspirated and left in the killing agent for 18 hours. The blocks were then dehydrated according to the tertiary-butyl-alcohol method (Johansen, 1940). The schedule used is as follows: 2 hours each in grades 1, 2, 3, 4, 5, and 6; overnight in grade 7; 1½ hours each in grades 8 and 9; left again overnight in grade 10; and finally 1½ hours each in grades 11 and 12. Grade 12 has paraffin oil which allows for the gradual infiltration of paraffin. Infiltration was accomplished by pouring the tertiary-butyl-alcohol-paraffin oil of grade 12, containing the agar blocks, into vials three fourths full of solidified paraffin. These were placed in the warming oven at 60 degrees C for 2 to 4 hours until the paraffin melted. The first change of paraffin was made one hour after the blocks settled to the bottom of the vial. The vials were left in the oven overnight, and subsequent changes of paraffin were made before embedding. The material was mounted on wooden blocks and sectioned with a rotary microtome. The most satisfactory thickness was 10 u. The sections of the paraffin ribbon were affixed to the slide with Haupt's adhesive, and the slide placed on the warming plate to dry overnight. The slides were subsequently passed through two changes of xylol, for 5 minutes each, to dissolve the paraffin, followed by 5 minutes in absolute ethyl alcohol and 2 minutes each in 95, 80, 70, 50,

30, and 10% ethyl alcohol and one minute each in 2 changes of distilled water. Staining was accomplished with a modification of the Gram stain or with Heidenhain's iron-alum hematoxylin. For the first method slides were placed in the Gram stain (4cc of aniline oil in 100cc of distilled water, stir and filter; 99cc of filtrate and one gram of crystal violet) for 30 minutes, rinsed in tap water, and transferred for one or two minutes to a solution of iodine-potassium-iodide (one gram of iodine and two grams of potassium-iodide in 300cc of distilled water). This was followed by rinsing the material in tap water, after which destaining was carried out by passing the material through 95% ethyl alcohol, until the violet color was not apparent, and finally in clove oil for 10 seconds. Xylol was used as a clearing agent and permount as the mounting medium. For the Heidenhain's iron-alum-hematoxylin, the material was placed on freshly prepared 4% iron-alum mordant for 2 to 3 hours, followed by rinsing in tap water for 5 minutes each. The slides were then placed in 0.5% aqueous solution of hematoxylin for 2 hours. Following 15 minutes in water, destaining was accomplished in 2% iron-alum for 4½ to 5 minutes. The material was washed in distilled water and dehydrated in an alcohol series through absolute ethyl alcohol at intervals of 2 minutes. Two changes of xylol were used as a clearing agent and Permount as the mounting medium.

In studying the development and cytology of the ascus, the propiono-carmine smear technique was employed. At proper stages

of development, blocks of agar bearing the ascocarps were removed from the petri dish and placed in Carnoy's fluid #1 (1 part of glacial acetic acid, 3 part of absolute ethyl alcohol) and stored for two days in the refrigerator. The material was then transferred to propiono-carmine stain and again stored in the refrigerator for 2 or 3 days. Aided by a dissecting scope and with the use of fine beading needles placed in holders, several ascocarps were separated from the vegetative mycelium and agar and transferred to a fresh drop of propiono-carmine added to the slide. A cover slip was placed over the drop of stain containing the ascocarps, pressure applied and the slide was warmed slowly, avoiding the over-heating of the stain. The cover slip was sealed to the slide, using either nail polish or a fluid containing one part of 45% glacial acetic acid, one part of white Karo syrup, and one part of saturated aqueous suspension of pectin. The latter sealing agent proved to be more desirable. The slides were then stored in the refrigerator for about 12 hours to allow the stain to intensify. Observations were made soon after this 12 hour period since subsequent deterioration of the material occurred.

The observations were made with a Zeiss microscope with a Neofluar oil immersion lens. All the drawings, with their magnifications indicated, were made with the aid of a camera lucida. The photographs were taken with the Yashica Super camera on Panatomic-X film.

OBSERVATIONS

The vegetative mycelium of Preussia vulgare (Corda) Cain is composed of cells 7.5-12.5 u in length and 2.5-3 u in diameter. The majority of the cells are commonly multinucleate (Figs. 1, 2), however, an occasional uninucleate cell was observed. It was not possible to follow the complete nuclear division within the vegetative cells. It appears to resemble mitosis in higher plants. The resting or interphase nuclei are solid and spherical in shape (Fig. 2) and the individual chromosomes are not evident. In prophase the nuclei appear to enlarge in diameter. A typical metaphase as found in higher plants was not observed. Nuclear divisions seems to progress from prophase to anaphase and from here to telophase.

Several single spore transfers were made. Based on the partial evidence obtained, Preussia vulgare appears to be homothallic.

Approximately 48 to 72 hours after inoculation, the ascocarp initial arised from a single enlarged vegetative cell, that is intercalary in position. These swollen cells are approximately 10-17.5 u in length and 5-7.5 u in diameter; 2 or 3 times the size of vegetative cells. In all cases observed these swollen

cells were multinucleate, mostly containing four, five or six nuclei, but swollen cells with two or three nuclei or with a greater number ranging from seven to eleven were not uncommon (Figs. 4, 5, 7, 10). The first cell divisions are internal, each ascocarp initial increasing in number of cells (Figs. 6, 9, 10). Two patterns of cell division within the ascocarp initial were observed. In one pattern, the cell divisions at first are predominantly in one plane at right angles to the hyphae of origin, resulting in a short chain up to 7 or 8 cells in length (Figs. 10, 11, 12, 13). Cell divisions then proceed in all planes along this chain until a spherical stroma is formed. In the second pattern, cell divisions from the beginning are in all planes (Figs. 6, 9, 14, 15). The mass of cells initially has an indefinite shape (Fig. 9), but eventually becomes spherical (Fig. 16). In both patterns, although an occasional binucleate cell was observed, the component cells of the young spherical stroma are mainly uninucleate (Fig. 17), apparently the result of cellular divisions taking place at a faster rate than the nuclear divisions. In the early stages of development, the ascostroma remains relatively stable in size and its component cells are pseudoparenchymatous.

Slightly later stages of development, when the ascocarp was about five days old, were observed in sectioned material. The cells divide rapidly and the ascocarp expands considerably in size (Fig. 18). The formation of the ascocarp wall is

initiated as the outer layer of cells become deep brown and develop thick, darkly pigmented cell walls (Fig. 18). At approximately the same stage of development, some differentiation of the internal tissue becomes apparent. The cells in the center of the ascocarp remain pseudoparenchymatous but become slightly larger and stain more intensely than the other cells of the ascostroma. When first formed these cells in the center of the ascostroma were mainly uninucleate, as were the other cells of the ascostroma; however, when differentiation of internal tissues began, multinucleate cells were observed (Fig. 19). It was not possible to determine the exact origin of these multinucleate cells, although perhaps some of the pseudoparenchymatous cells in the center of the ascocarp enlarge and nuclear divisions take place. Following the terminology of Kowalski (1965, 1966), these cells are regarded as ascogonia. The largest number of nuclei observed in any of the ascogonial cells was four, but the great majority of them were binucleate cells. In time, the ascogonia appear to give rise to ascogenous hyphae consisting of apparently binucleate cells (Fig. 20).

As internal differentiation proceeds, the center of the ascocarp loses its pseudoparenchymatous nature and air spaces are produced (Fig. 21). Coincident with this and with the formation of the ascogenous system, or shortly afterwards, sterile threads are evident. These sterile threads appear to originate from cells lining the locule, that is, from those cells just

peripheral to the darkly stained cells that gave rise to the ascogonia. The threads are unbranched and their cells appear to be multinucleate. These sterile hyphae grow inwards into the centrum and they are found completely around the locule, without a pattern of orientation (Figs. 22, 23). Because of the peculiar form in which these sterile hairs are distributed within the locule, they appear to basically differ from paraphysis, pseudoparaphysis and paraphysoids, as defined by Kowalski (1965) and Boylan (1970). In the present paper, they will remain specifically unnamed. As the ascocarp continues to enlarge, the cells of the ascogenous hyphae form asci. In a mature ascocarp many asci are observed in different stages of development. They mostly grow out from the center of the locule and radiate out in all directions (Fig. 24). A typical hymenium is lacking. At maturity the ascocarp wall is typically 4 or 5 cells thick (Figs. 24, 25), and the sterile hyphae as well as the ascus walls break down, releasing the ascospores from the asci. The mature ascocarp lacks an ostiole and contains only spores, broken into their four component cells which must be released from the ascocarp by the desintegration of the ascocarp wall or by mechanical breakage. The stroma of Preussia vulgare in the great majority of cases is uniloculate (Figs. 24) but abnormally two or three locules form in one stroma (Figs. 26), probably as a result of two ascocarp initials developing in close proximity with one another (Fig. 7).

The ascus formation is preceded by hook-shaped cells with typical cellular and nuclear configuration of a three-celled crozier: a uninucleate, a binucleate penultimate and a uninucleate antepenultimate cell (Figs. 27, 28, 29, 33). It appears that croziers are initiated by binucleate cells of the ascogenous system. However, conjugate division of the two nuclei to form a four-nucleate hooked-cell or the formation of septa delimiting the three-celled crozier were not observed. The croziers appear to behave normally, with the binucleate penultimate cell developing into the ascus (Fig. 29). It appeared that the ultimate cell grows downward and fuses with the antepenultimate cell to form a single binucleate cell, which in turn can form another crozier. This phenomenon is an explanation for the presence of a mass of asci at one point. The penultimate or ascus mother cell, elongates and its two haploid nuclei fuse. As the ascus enlarges, the diploid fusion nucleus increases greatly in size (Fig. 29) and meiosis follows. During late prophase or methaphase of meiosis I the chromosomes are evident as small dots (Figs. 30, 34). It is believed that the haploid chromosomes number of Preussia vulgare is n=6; however, this number is not given with great certainty because the chromosomes tend to remain very close together. On the completion of the second meiotic division the ascus contain four haploid nuclei (Fig. 35) and this is followed by subsequent mitotic divisions. It appears that the spore formation is

initiated at the eight-nucleate condition of the ascus (Fig. 36). This is followed by the occurrence of two simultaneous mitotic divisions producing eight four-nucleate ascospores (Figs. 31, 32). Cross walls are then laid down, dividing each ascospore into four uninucleate spore segments (Fig. 38). One final mitotic division occurs and each cell of the ascospores becomes binucleate. At maturity a thickening and darkening of the spore wall occurs (Fig. 39). The cells of the ascospore are only weakly held together and easily fragment into its four component cellular segments (Fig. 40).

The bitunicate condition of the mature ascus is evident in younger asci (Figs. 35, 36, 37). The mature ascus is broad and clavate, about 37.5-55.5 u x 15-20 u, with a short stipe measuring about 7-9.5 u (Figs. 30, 35, 36). Ascospore discharge was not observed, but the spores were released into the body of the ascocarp, possibly through mechanical breakage of the ascus walls, but more likely by evanescence of the ascus walls.

DISCUSSION

Seven morphological investigations of various species of Preussia have been published since 1938. Five of these works followed Cain's (1961) important monograph on this genus. The similarities and discrepancies between earlier morphological investigations and this present study of Preussia vulgare are here discussed.

The vegetative mycelium of Preussia vulgare is different from that described for Preussia typharum and for Preussia funiculata by Kowalski (1965, 1966), who found two types of hyphae present in the vegetative mycelium of these two species. In both types of hyphae the cells were mainly uninucleate and the ascocarp initial arose from cells with greater diameter. In contrast Preussia isomera (Kowalski, 1968) and Preussia flanaganii (Boylan, 1970) were described as having only one type of vegetative mycelium, with the cells in P. isomera mainly uninucleate or binucleate and in P. flanaganii uninucleate. Preussia vulgare resembles P. isomera and P. flanaganii in having only one type of vegetative mycelium, but differs in that the cells are multinucleate.

The origin and development of the ascocarp initials in

P. vulgare is entirely different from that described by Beatus (1938) for Perisporium funiculatum (= Preussia funiculata). He reported that the ascocarp is initiated by the fusion of two uninucleate vegetative hyphal end cells, which he called the archegonium and the anteridium, giving rise to a binucleate cell, which was the ascocarp initial. However, Kowalski reported for this species (1966) as well as for Preussia isomera (1968) that development is always initiated in an intercalary manner by a swollen multinucleate cell, the same manner of ascocarp initiation that was observed for P. vulgare in this present study. Muellerella nigra (= Preussia nigra) (Routien, 1956) is also described to have this method of ascocarp initiation. The only difference is that the ascocarp initial in this species is uninucleate. This method of ascocarp formation is perhaps the most common in the genus. In P. typharum (Kowalski, 1965) the development of the ascocarp initial is almost identical, but the earliest stage observed consisted of a short chain three cells in length. A different method of ascocarp formation was described by Maciejowska and Williams (1963) for Preussia multilocularis and by Boylan (1970) for P. flanaganii. In these two species, development is initiated by hyphal clumps. It can be safely concluded that ascocarp initiation in the species of Preussia is variable.

In all the published morphological investigations of Preussia, differentiation of the internal tissue of the ascocarp

at rather early stages of development is reported. However, the nature of presence of deeply staining cells or ascogonia is variable. Beatus (1938) reported the presence of ascogonia which always contained paired nuclei for P. funiculatum. On the other hand Routien (1956) apparently did not find any for P. nigra. Binucleate cells were reported by Maciejowska and Williams (1963) for P. multilocularis and by Kowalski (1968) for P. isomera; the latter investigator called them "fertile cells". In Preussia typharum and P. funiculata, Kowalski (1965, 1966) observed multinucleate cells, which he referred to as ascogonia or ascogonia cells, and these same multinucleate cells are present in P. vulgare. For P. flanaganii Boylan (1970) observed the presence of deeply staining cells in the young ascocarp, but did not indicate their nuclear condition.

The nature of the sterile hairs in the ascocarp of Preussia exhibits a great deal of variation. Beatus (1938) reported paraphyses, threads attached at the bottom of the centrum and growing upward, to be present in P. funiculatum. This is the only morphological study of the genus Preussia in which the presence of paraphyses is reported. Kowalski also studied P. funiculata (1966) and reported paraphysoids, or threads attached at both ends, for this species, as well as for P. typharum (1965). Routien (1956) described pseudoparaphyses, threads attached at the top of the ascocarp and free below, in P. nigra, as did Maciejowska and Williams (1963) for P. multilocularis. It should

be noted that Kowalski (1965) suggested that the threads in the photographs of P. funiculatum by Beatus are pseudoparaphyses, rather than the paraphysoids. Kowalski reported for this species, which may mean that Kowalski and Beatus perhaps were not studying the same organism. Kowalski also suggested, judging by the photographs, that P. multilocularis and P. nigra have paraphysoids and not pseudoparaphysis ad had been reported. The sterile hairs of P. vulgare resemble those of P. isomera (Kowalski, 1968) and of P. flanaganii (Boylan, 1970), having, as described by Kowalski (1968), "absolutely no orientation to their growth but simply meander throughout the locule."

Formation of asci from ascogenous hyphae is rather uniform throughout the genus. The major exception seems to be P. funiculata, in which Kowalski (1966) reports that the ascogonial cells give rise to large lobate multinucleate cells designated as ascogenous cells, which in turn produce binucleate cells. The ascogenous hyphae of P. vulgare seem to resemble the majority of the species that have been studied. Croziers, according to Cain (1961), are present in all species of Preussia; however, it should be noted that Beatus (1938) failed to describe them in his study of P. funiculatum. Only in P. typharum and P. funiculata (Kowalski, 1955, 1966), has a typical hymenium been reported.

Ascus development and ascus cytology is rather uniform in Preussia. Spore formation begins with the eight-nucleate condition

of the ascus in P. vulgare, and this has also been reported in P. funiculata (Kowalski, 1966), P. isomera (Kowalski, 1968) and P. flanaganii (Boylan, 1970). In contrast, it starts at the 16-nucleate condition in P. typharum (Kowalski, 1965). The cells of the ascospores are reported as uninucleate in P. typharum, P. funiculata, and P. flanaganii, and binucleate in P. isomera. Preussia vulgare resembles the latter species in having binucleate cells of the ascospores.

There is great disparity in the chromosome count among species of Preussia. As mentioned previously P. vulgare is believed to have a haploid chromosome number, n=6. Beatus (1938) reported, n=4 for P. funiculatum, while Kowalski (1965, 1966) believed the haploid number to be n=11 for P. typharum and n=12 for P. funiculata. Since P. isomera apparently had a basic chromosome, n=8, Kowalski (1968) believed that, n=4, to be the basic haploid number for Preussia, which will confirm the presence of polyploids. However, considering the great difficulties encountered in making chromosome counts, this contention is questionable.

The bitunicate condition of the ascus has been definitely observed in P. multilocularis (Maciejowska and Williams, 1963) and probably in P. flanaganii (Boylan, 1970). In this present study of Preussia vulgare the bitunicate condition has been demonstrated. However, based on the relative thickness of the ascus wall in P. typharum, P. funiculata, and P. isomera,

it was assumed by Kowalski (1965, 1966, 1968) that these three species had bitunicate asci. Forcible discharge of spores has not been reported in any of the morphological investigations thus far published and apparently does not occur in the genus.

Luttrell (1965) reported that at least four basic developmental types occur in the Loculoascomycetes. The development of the ascocarp of Preussia is regarded to be of the Pleospora type (Maciejowska and Williams, 1963 and Kowalski, 1965), as outlined by Luttrell in 1951. In the Pleospora type of development, the pseudoparaphyses appear in the stroma occupied by the ascogonia. The pressure exerted by the elongation of the pseudoparaphyses creates a flask-shaped locule in the stroma, with the asci subsequently growing up among the pseudoparaphyses. Luttrell's (1951) definition of pseudoparaphyses as hyphae attached at both the top and bottom of the locule is different from that of Kowalski (1965), who used the term paraphysoid to describe this same type of structure. According to detailed morphological studies, some members of the genus Preussia vary in certain details from the description of the Pleospora type of centrum development. Only P. typharum and P. funiculata (Kowalski 1965, 1966) and perhaps P. multilocularis (Maciejowska and Williams, 1963) and P. nigra (Routien, 1956) have paraphysoids as defined by Kowalski. The sterile hairs of P. isomera (Kowalski, 1968), P. flanaganii (Boylan, 1970), as well as those of P. vulgare, can hardly be called paraphysoids.

There is no indication that the enlargement of the sterile hairs creates sufficient pressure to form a locule. It appears that the entire ascostroma enlarges as a unit and the sterile hairs are rather loosely arranged.

Preussia is closely related to Sporormia according to Munk (1957), and Cain (1951), who revised the genus Preussia, and who confirmed this contention. Both genera inhabit the same environment; i.e., they are coprophilous fungi. The early stages of ascocarp development are similar for both. Ascocarp formation beginning with a spherical swollen uninucleate cell is described for Sporormia leporina by Arnold (1928), while Dangeard (1907) showed development to begin from a swollen multinucleate cell in Sporormia intermedia. Both of these types of ascocarp initials are found in Preussia. The presence of pseudoparaphyses, as well as croziers, were reported in S. leporina. According to Cain (1961) Preussia is distinguished from Sporormia by the lack of an ostiole, the size of the ascus, and the more superficial position of the ascocarp.

In conclusion, the principal characteristics of Preussia, such as the ascostroma, the presence of sterile hairs, and the bitunicate condition of the ascus would seem to indicate that the placement of this genus in the family Sporomiaceae, order Pleosporales, is indeed correct.

SUMMARY

Cain (1961) transferred Perisporium vulgare to the genus Preussia, indicating that it is the valid name for this non-ostiolate species of Ascomycetes. He placed Preussia in the family spormiaceae of the Pleosporales, an order in the sub-class Loculoascomycetes. The purpose of this study has been to investigate the cytology and morphology of Preussia vulgare (Corda) Cain and to verify Cain's contention.

The vegetative hyphae of Preussia vulgare consists of cylindrical multinucleate cells. Ascocarp development is similar to that found in sporormia and it is ascostromatic. The stroma is initiated by a multinucleate swollen cell that arises in an intercalary manner. The first cell divisions are internal and predominantly in a single plane, producing a short chain of cells, or in several planes, resulting in a mass of cells with an indefinite shape. In both cases the ascocarp eventually becomes spherical and its component cells uninucleate and pseudoparenchy-matous. Differentiation of the cells in the center of the ascostroma follows, giving rise to multinucleate ascogonial cells. In time they form the ascogenous hyphae consisting of binucleate cells, which form asci by means of typical croziers.

Asci grow out from the center of the ascocarp and radiate in all directions. Coincident with the formation of ascogonia, sterile threads appear and grow into the locule without a pattern of orientation. The bitunicate condition of the ascus was demonstrated and the haploid chromosome number appears to be n=6. Spores appear to form at the eight-nuclei condition of the ascus and at maturity they are phragmospores with four binucleate cell segments. The mature ascocarp lacks an ostiole, contains no hymenium and it is typically uniloculate with a wall four or five cells thick.

The major characteristics of Preussia vulgare described above seem to corroborate Cain's (1961) contention that this species does belong in the family Sporormiaceae, order Pleosporales, subclass Loculoascomycetes.

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Fig. 1 Vegetative hyphae with multinucleate cells. X1600.

Fig. 2 Vegetative hyphae showing nuclei at different stages of mitosis. X1600.

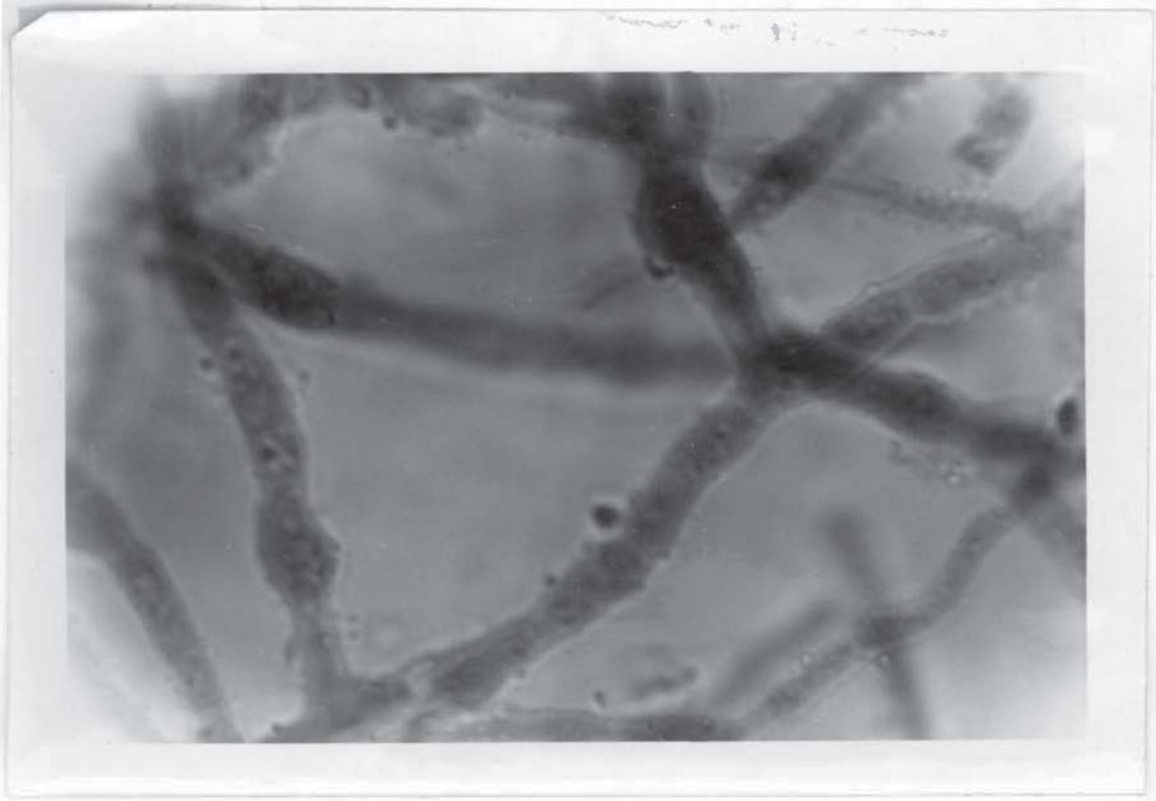

Fig. 1

Fig. 2

Fig. 3 Multinucleate ascocarp initial. X1600.

Fig. 4 Ascocarp initial showing the multinucleate intercalary condition of the cell. X1600.

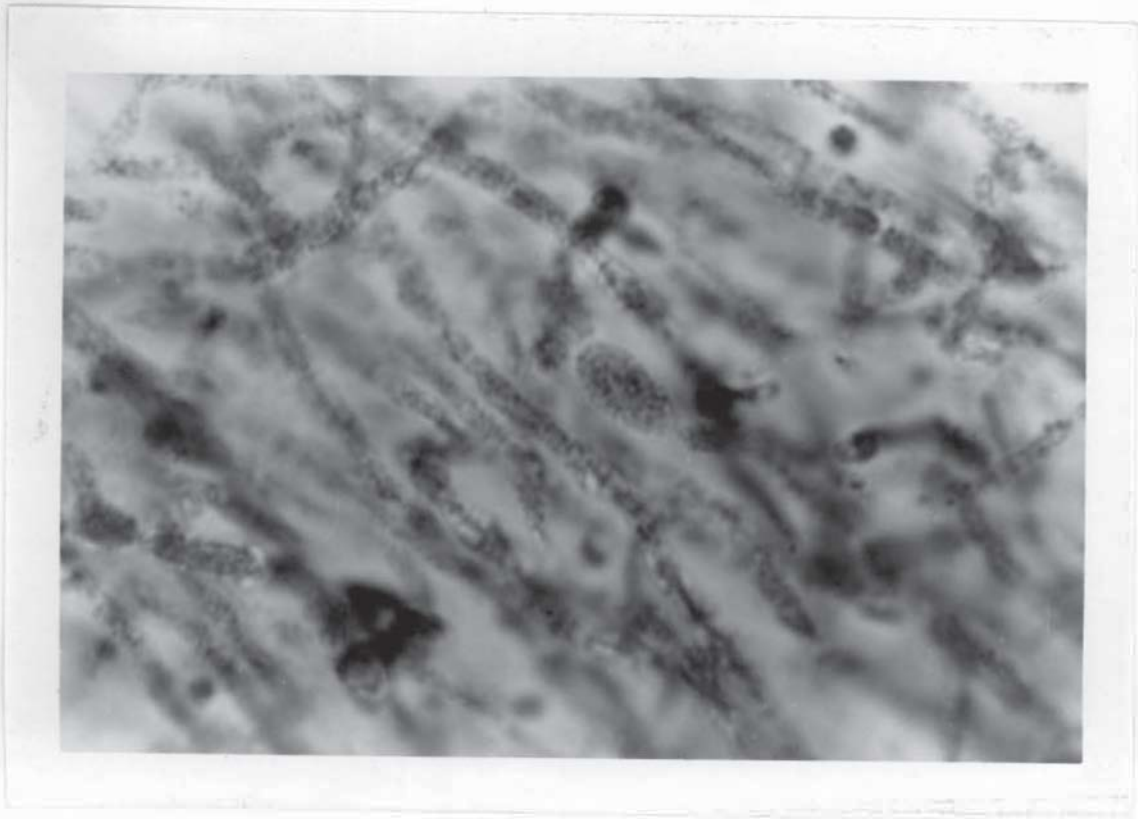

Fig. 3

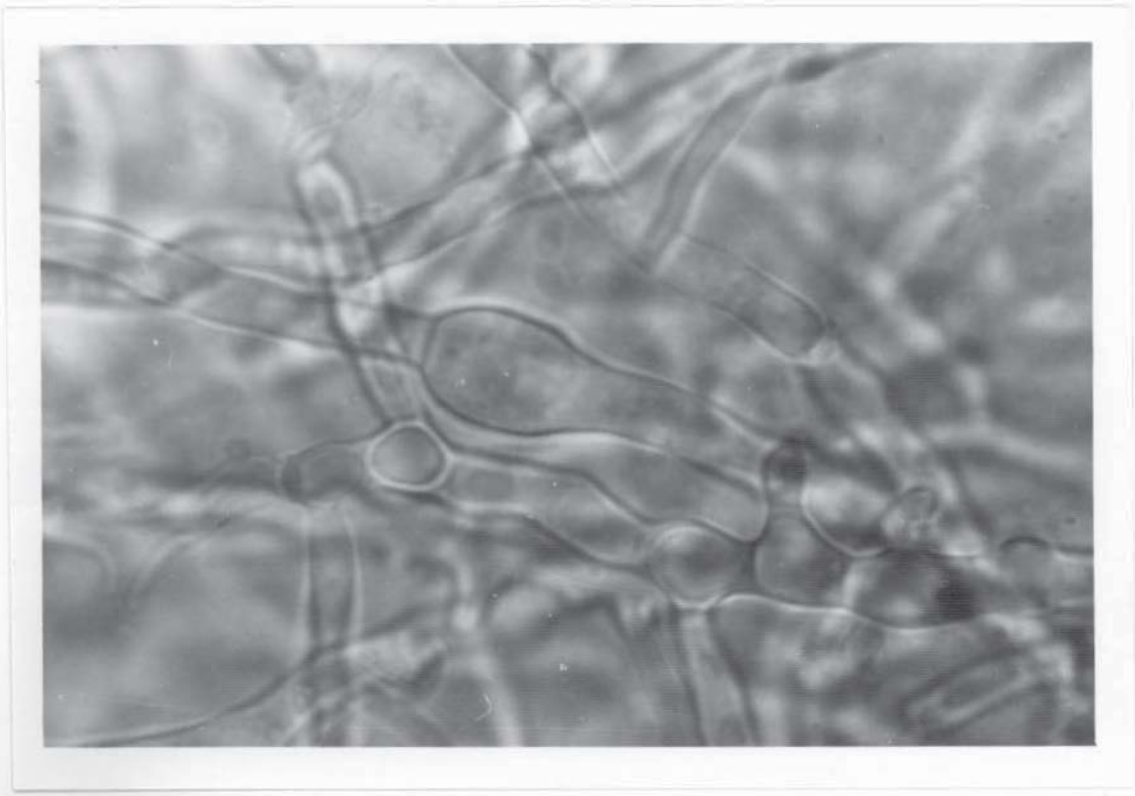

Fig. 4

Fig. 5 Binucleate ascocarp initial. X2000.

Fig. 6 Ascocarp initial dividing internally in several planes. X2000.

Fig. 7 Two ascocarp initials growing in close proximity. X2000.

Fig. 8 Two-celled ascocarp initial. X2000.

Fig. 9 Dividing ascocarp initial with an indefinite shape showing mainly uninucleate cells. X2000.

Fig. 10 Ascocarp initial with internal divisions in one plane. X2000.

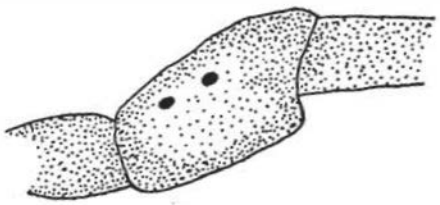

Fig. 5

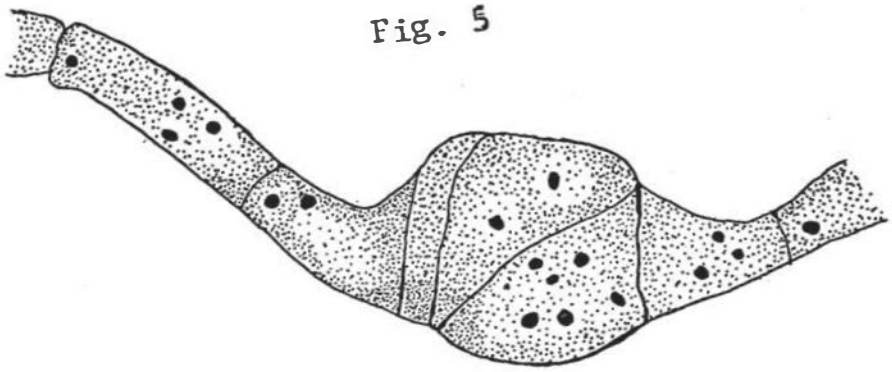

Fig. 6

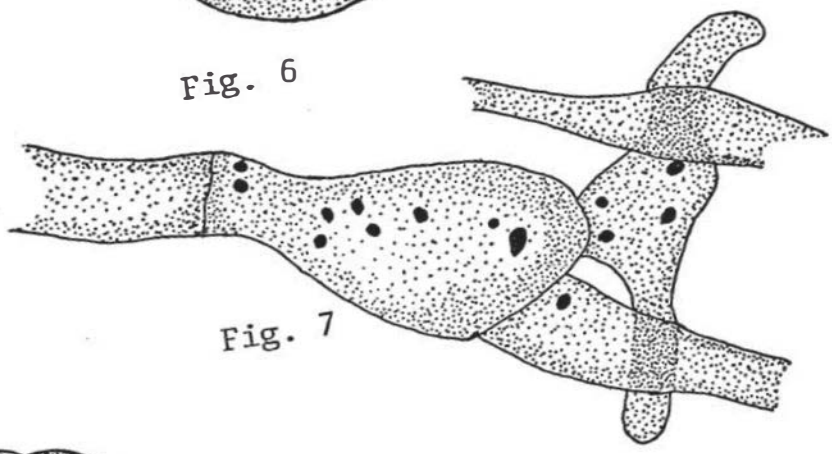

Fig. 7

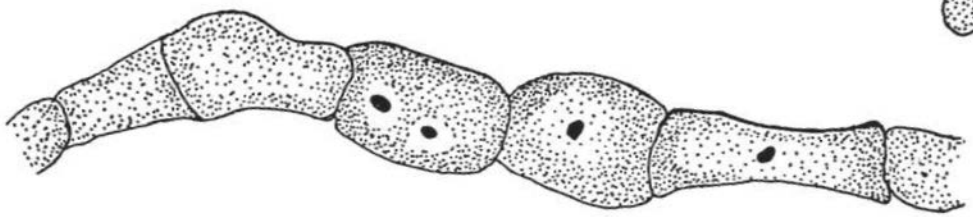

Fig. 8

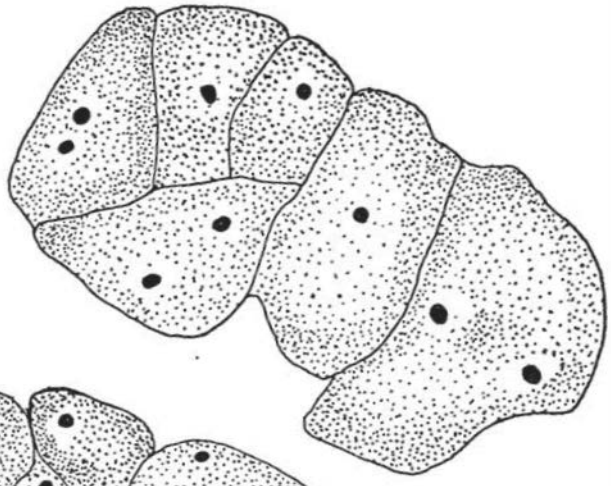

Fig. 9

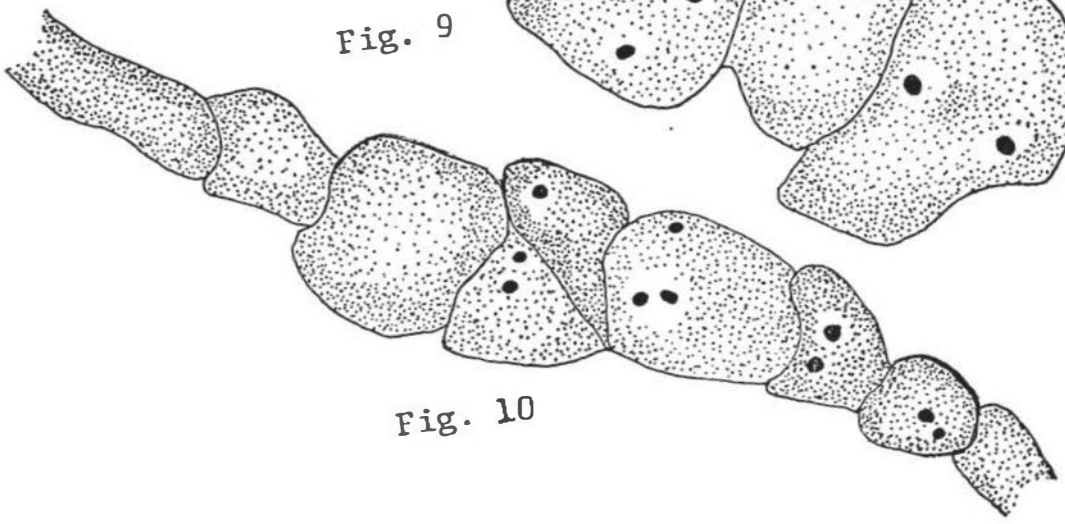

Fig. 10

Fig. 11 Ascocarp initial dividing in one plane. X1600.

Fig. 12 Later stage of ascocarp initial; elongated type. X1600.

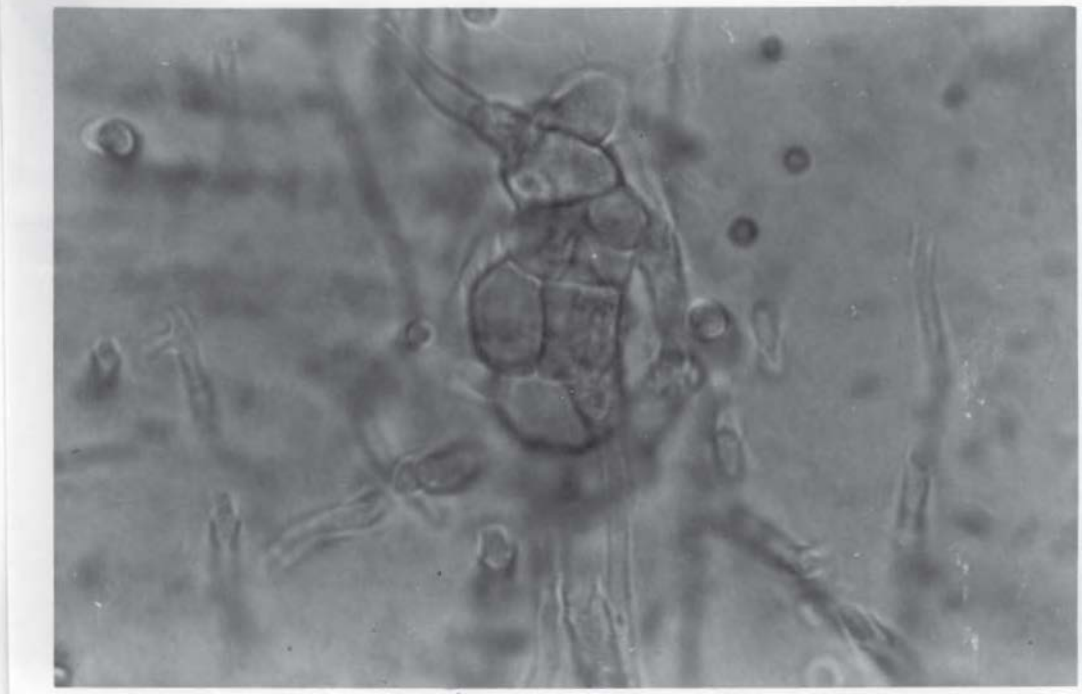

Fig. 11

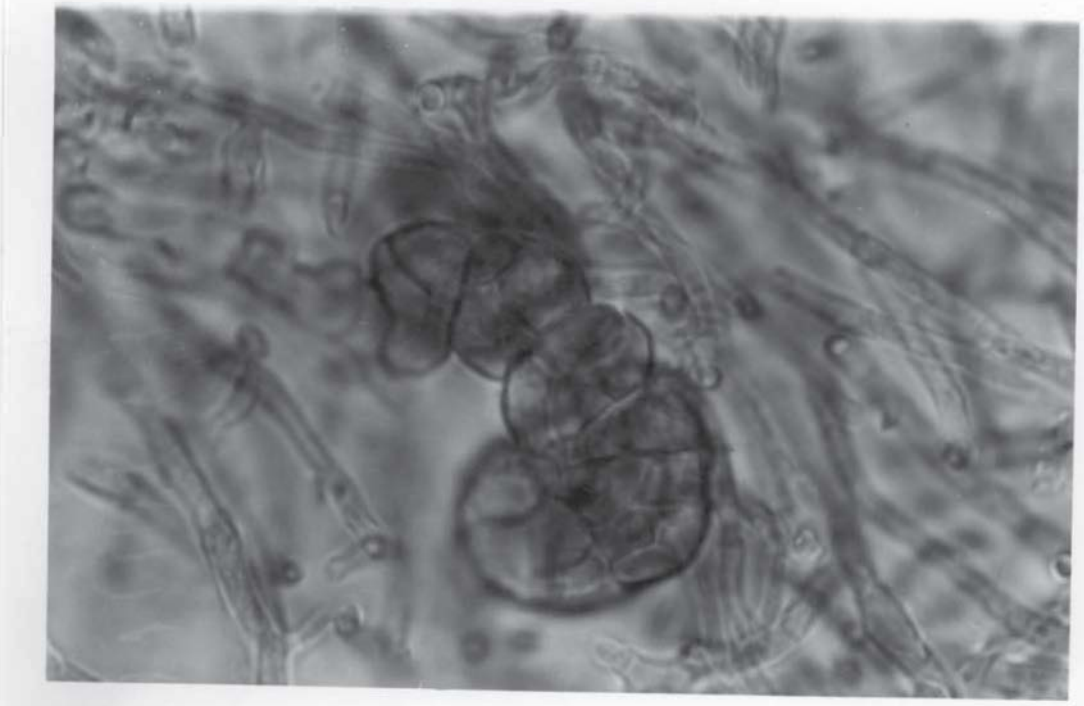

Fig. 12

Fig. 13 Short chain of cells produced by ascocarp initial. X1600.

Fig. 14 Ascocarp initial dividing in several planes. X1600.

Fig. 13

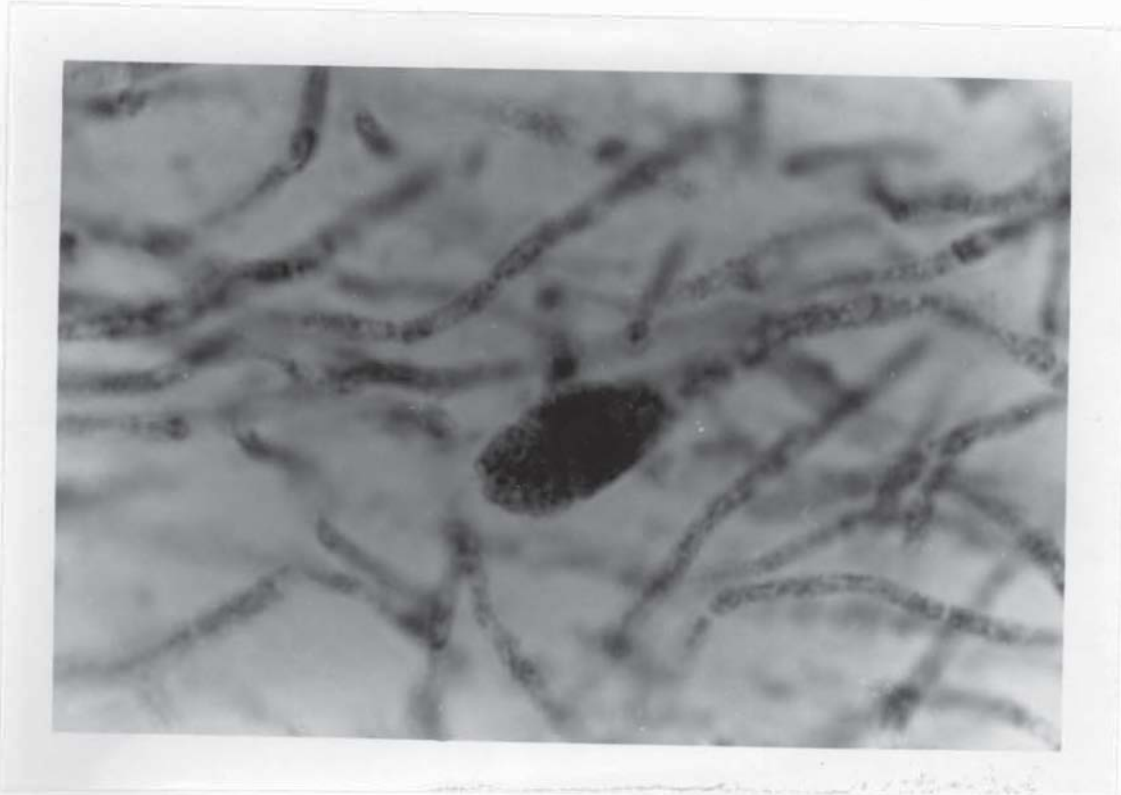

Fig. 14

Fig. 15 Ascocarp initial dividing internally in several planes. X1600.

Fig. 16 Young ascostroma becoming spherical in shape. X1600.

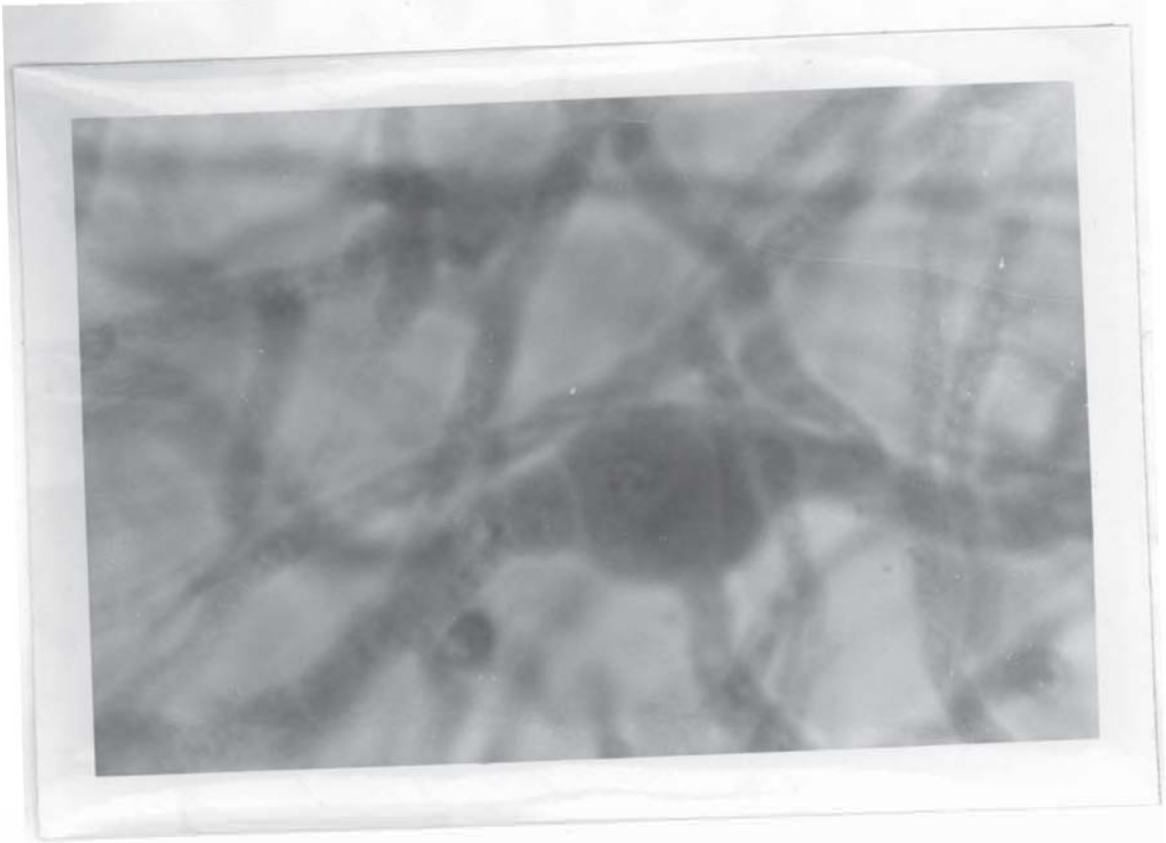

Fig. 15

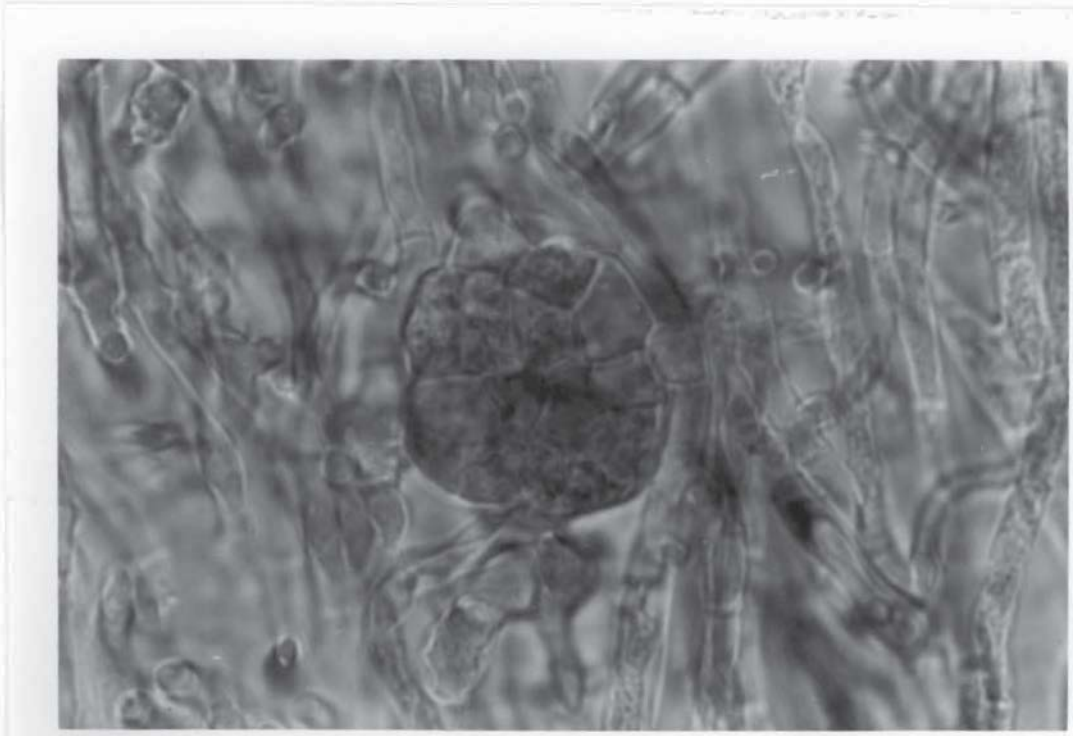

Fig. 16

Fig. 17 Young ascostroma with uninucleate pseudoparenchymatous cells. X1600.

Fig. 18 Young ascostroma with beginning of wall formation. X1600.

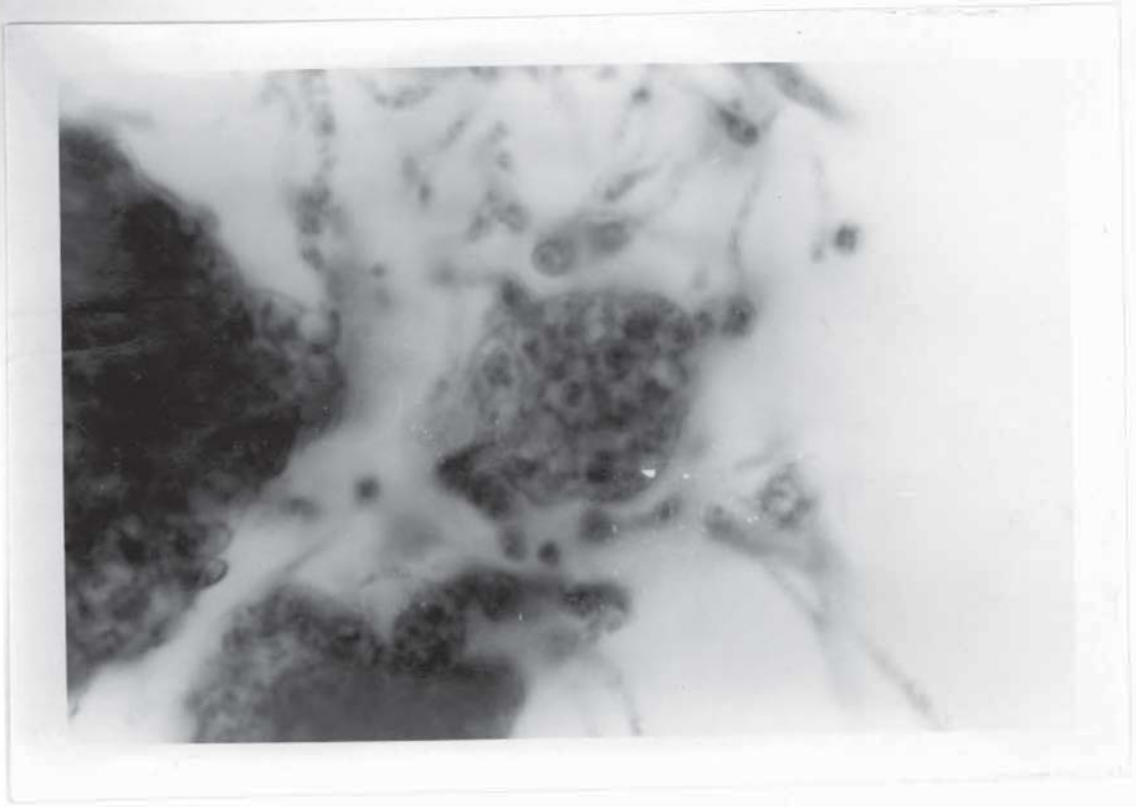

Fig. 17

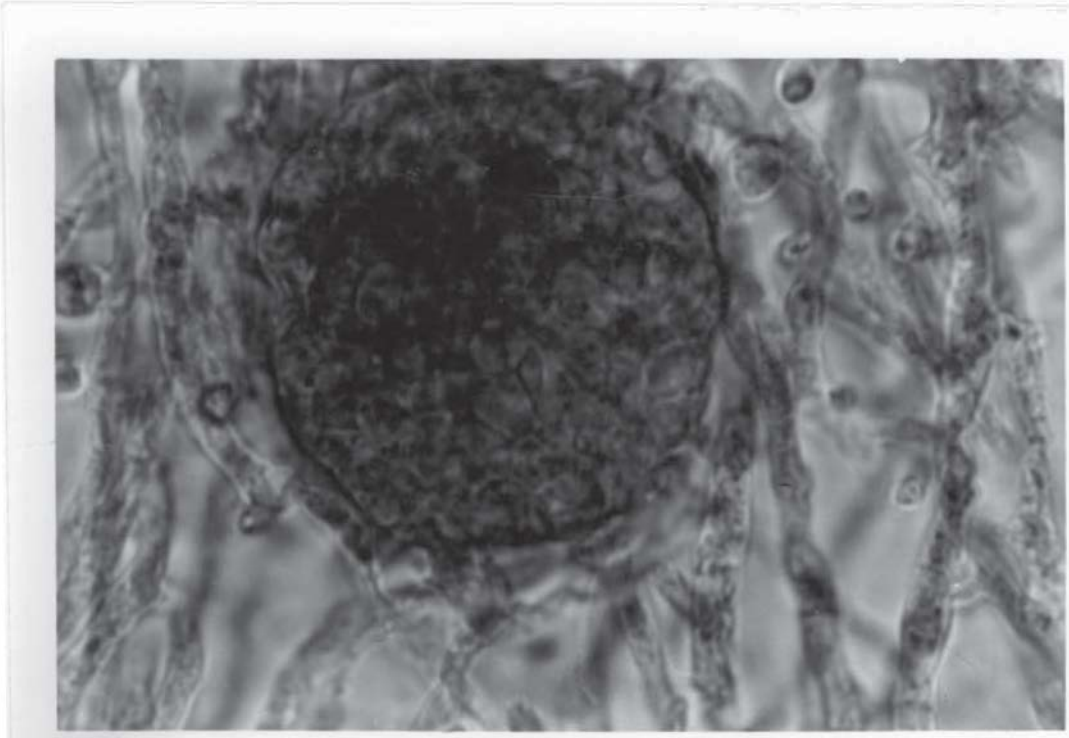

Fig. 18

Fig. 19 Ascostroma with binucleate ascogonia. X640.

Fig. 20 Section through ascostroma with binucleate ascogenous hyphae. X1600.

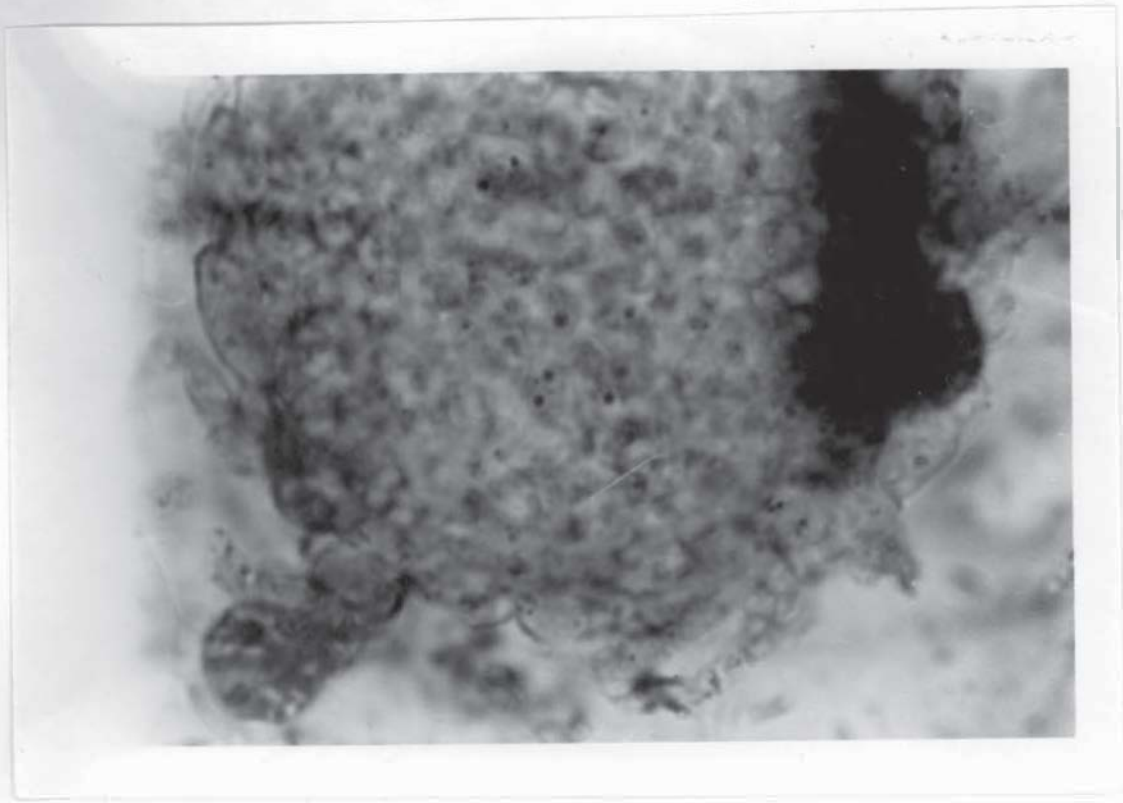

Fig. 19

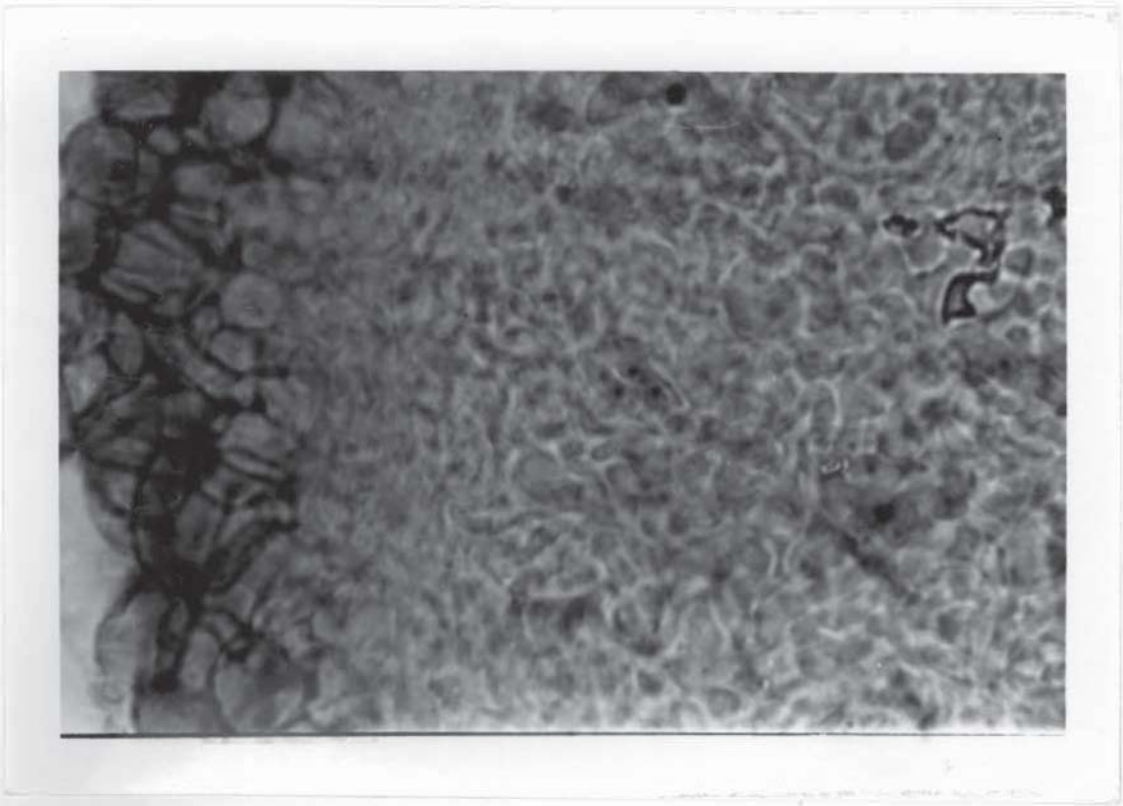

Fig. 20

Fig. 21 Ascostroma losing its pseudoparenchymatous nature producing air spaces. X640.

Fig. 22 Young ascostroma with well developed sterile threads. X1600.

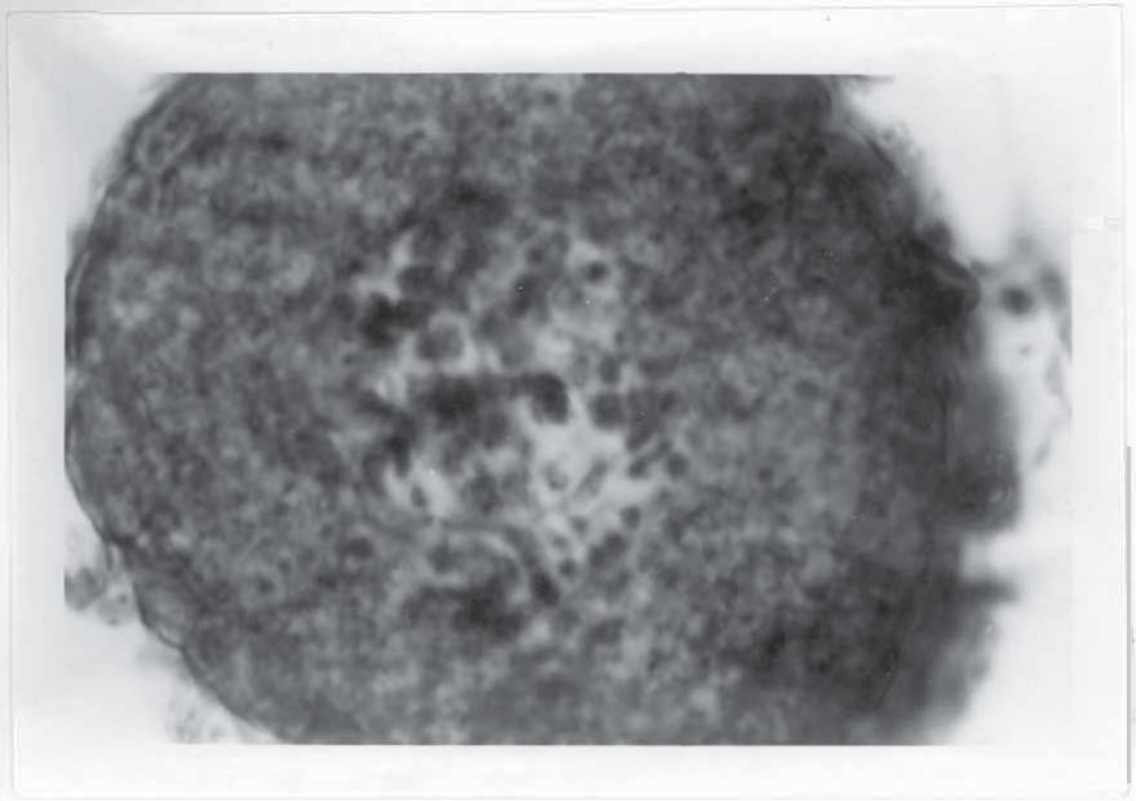

Fig. 21

Fig. 22

Fig. 23 View of entire ascostroma showing sterile threads growing without a pattern of orientation. X400.

Fig. 24 Ascostroma with many asci in different stages of development. X400.

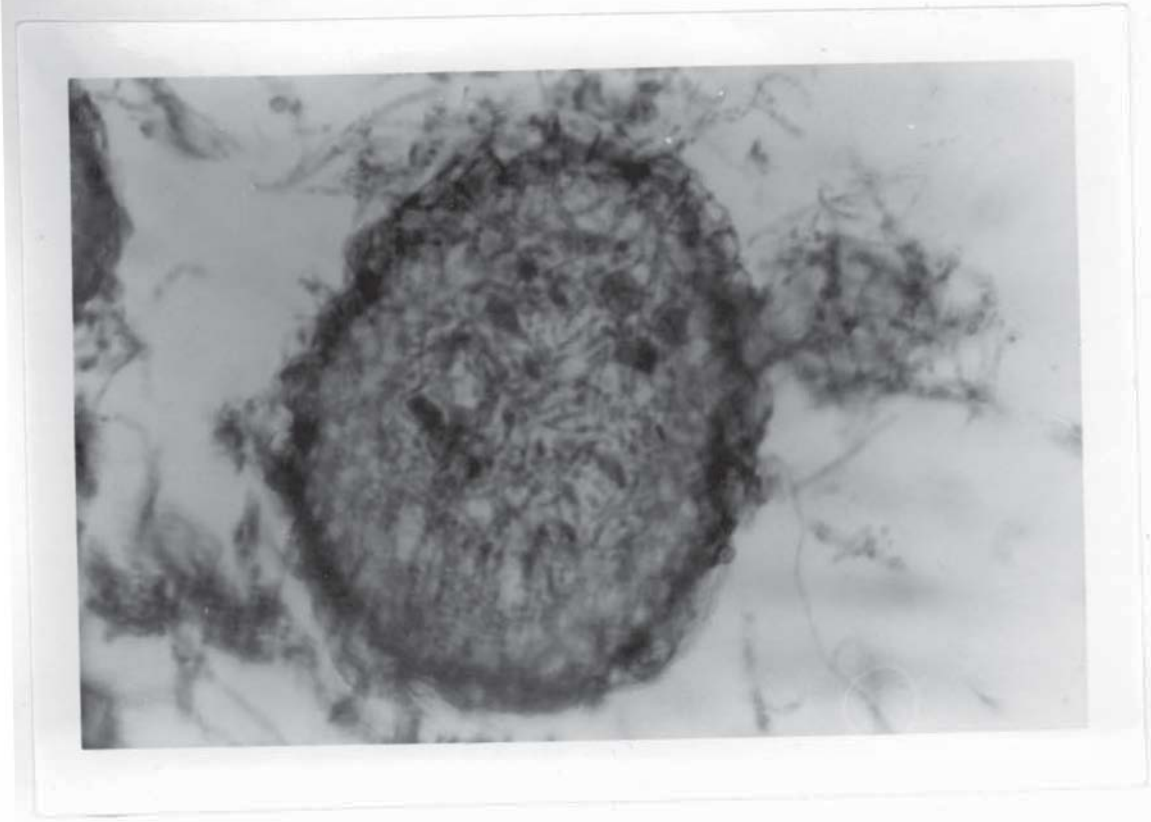

Fig. 23

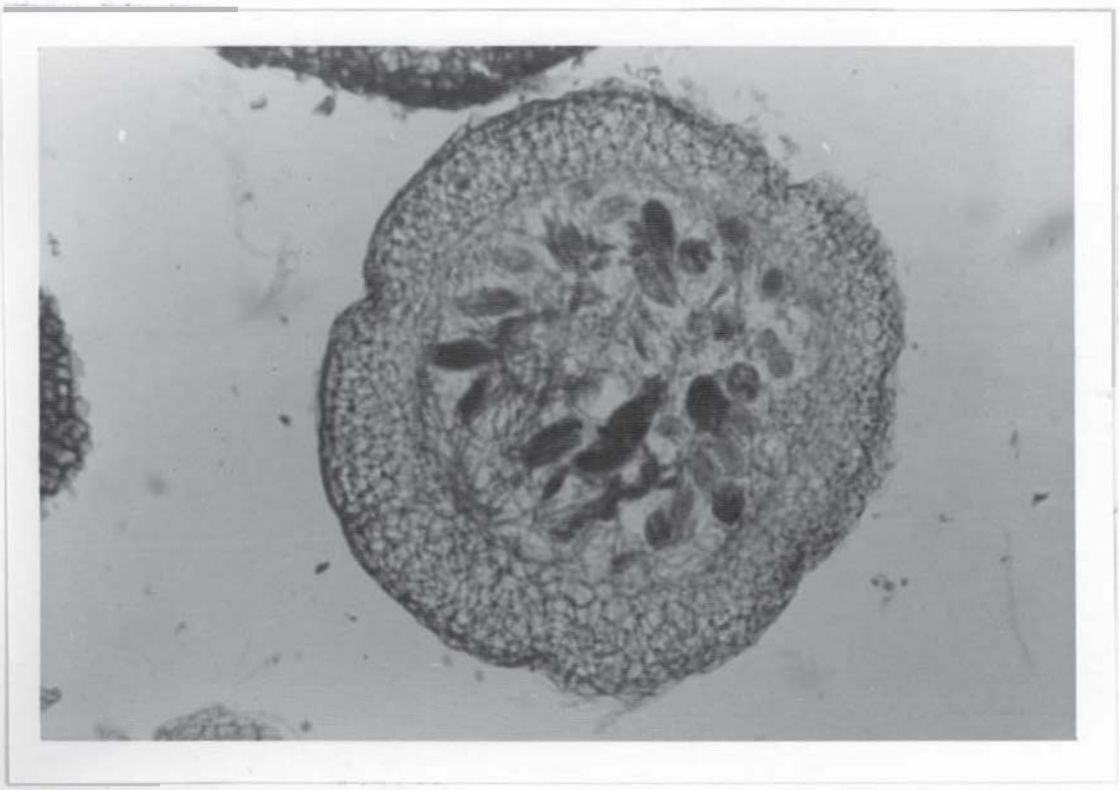

Fig. 24

Fig. 25 Ascostroma with many asci containing mature spores. X640.

Fig. 26 Ascostroma with two locules formed by fusion of two primordia. X400.

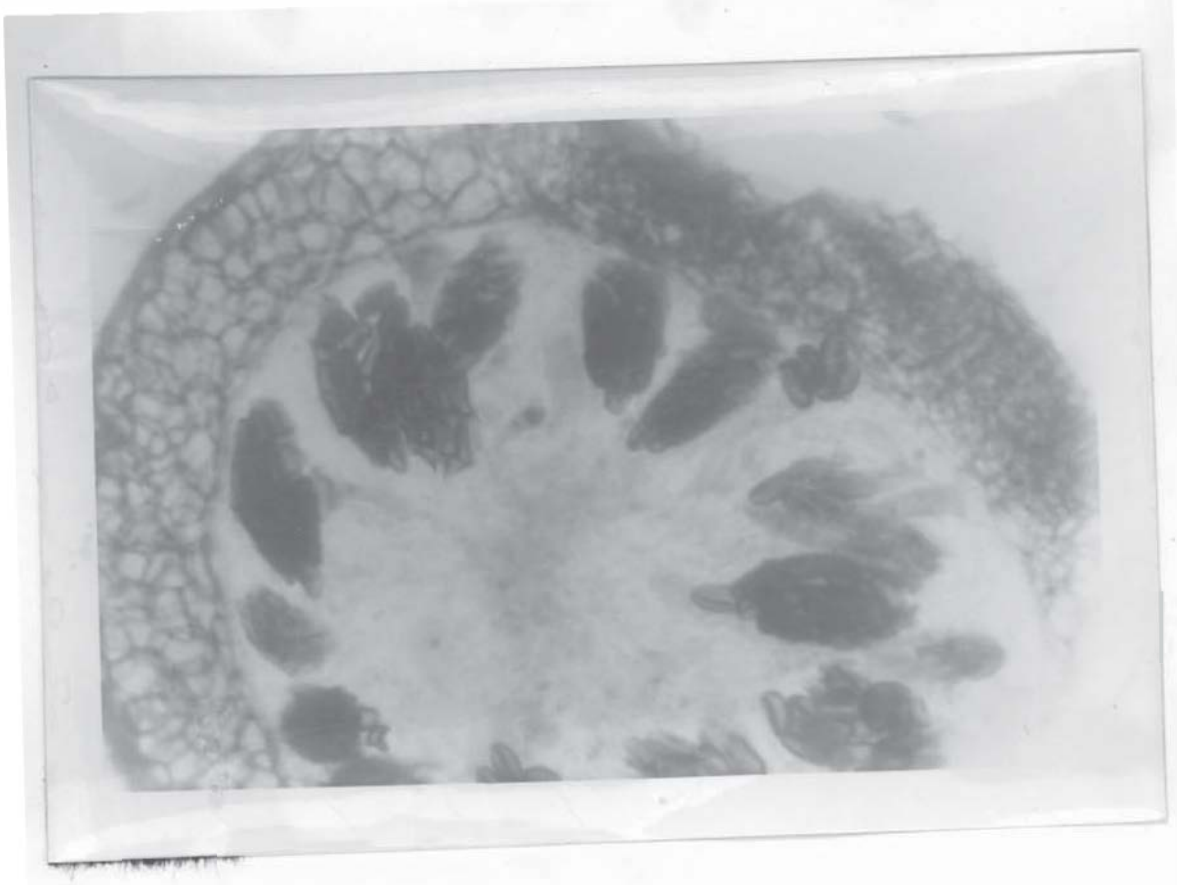

Fig. 25

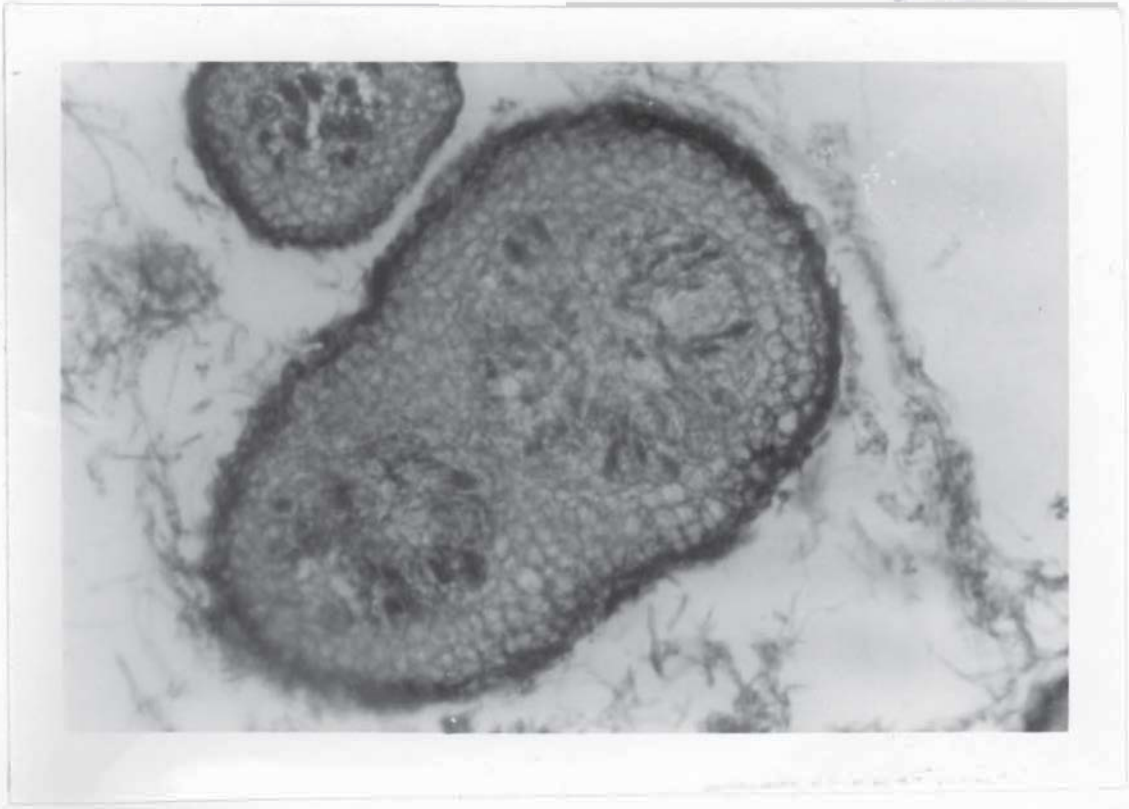

Fig. 26

Fig. 27-28 Four-nucleate crozier after septa formation. X2000.

Fig. 29 Young asci showing two haploid nuclei and fusion nucleus and a binucleate crozier on lower right side. X800.

Fig. 30 Ascus at metaphase I showing six pairs of chromosomes. X800.

Fig. 31 Ascus with eight four-nucleate ascospores at the initiation of spore formation. X800.

Fig. 32 Enlarged four-nucleate ascospore. X2000.

Fig. 27

Fig. 28

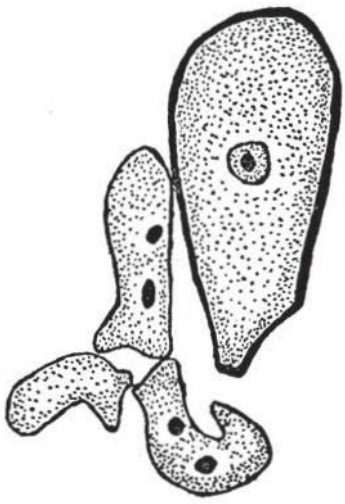

Fig. 29

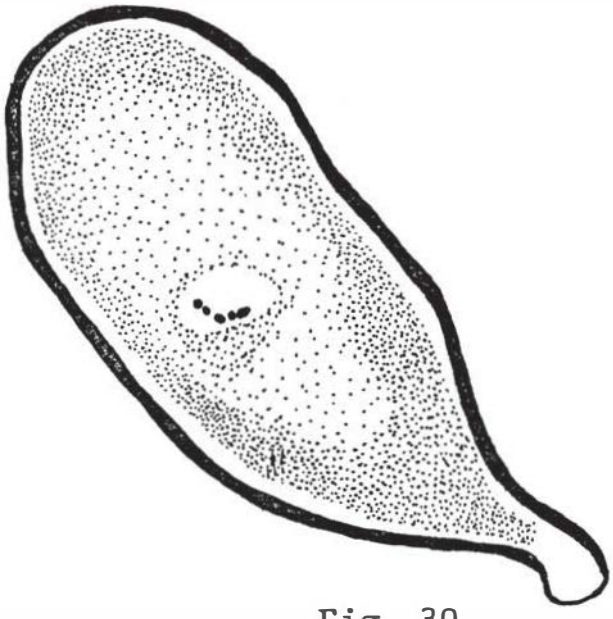

Fig. 30

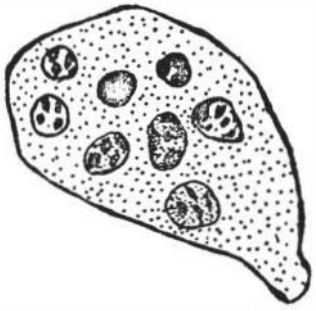

Fig. 31

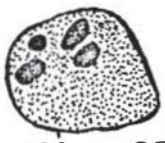

Fig. 32

Fig. 33 Crozier cell. X1600.

Fig. 34 Ascus with six pairs of chromosomes at metaphase I. X1600.

Fig. 33

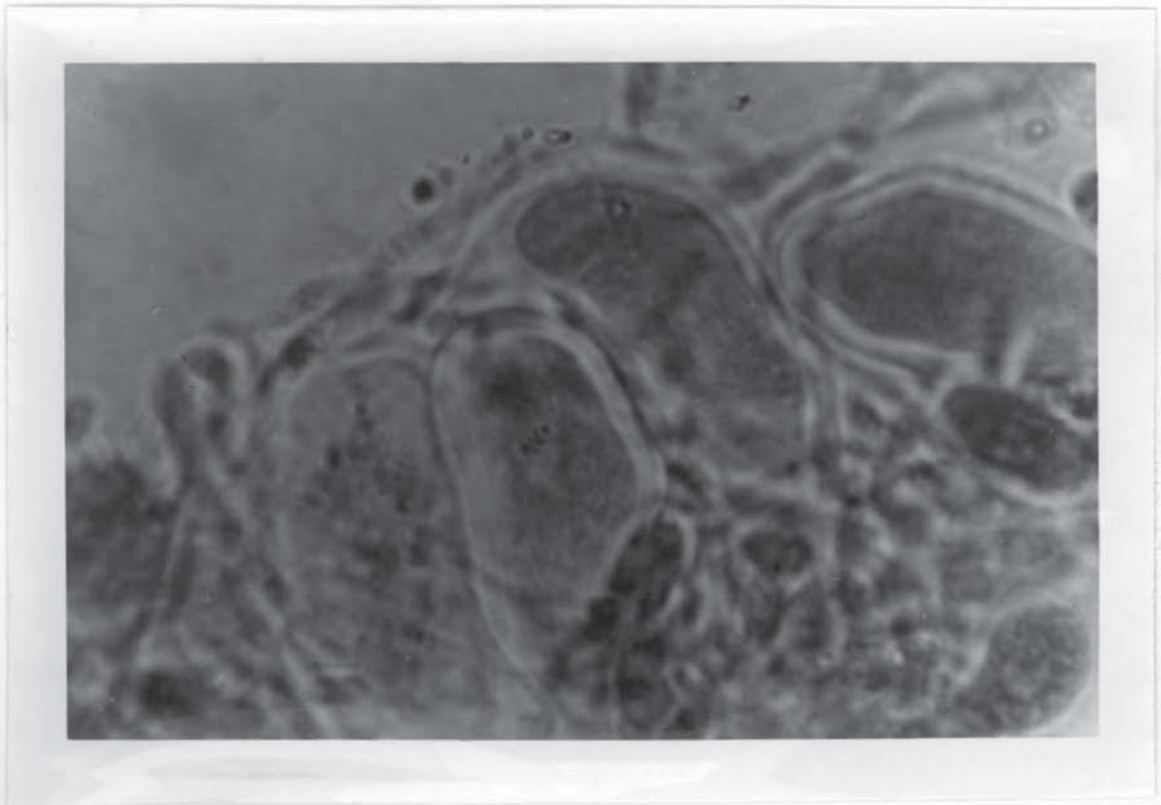

Fig. 34

Fig. 35 Four-nucleate ascus at the completion of Meiosis II. X1600.

Fig. 36 Eight-nucleate ascus at the beginning of spore formation. X1600.

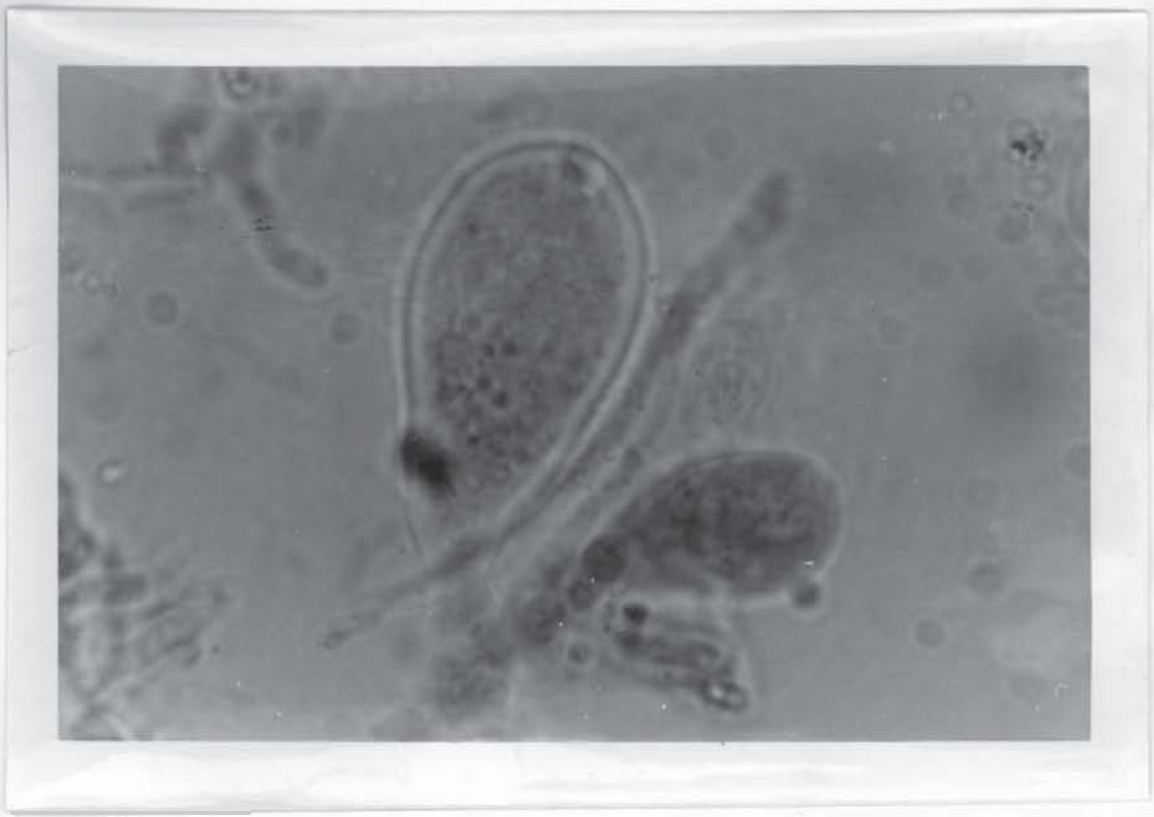

Fig. 35

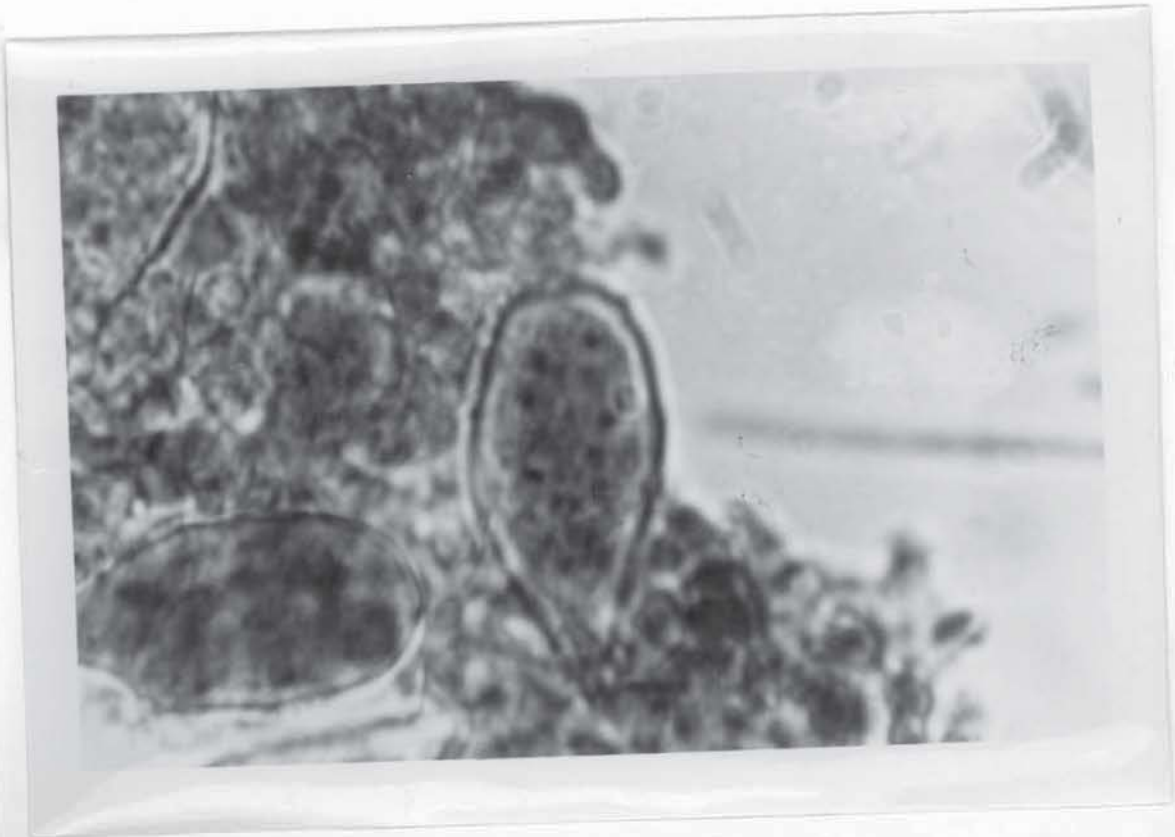

Fig. 36

Fig. 37 Eight-binucleate ascospores. X1600.

Fig. 38 Spores showing cross wall formation and uninucleate cell segments. X1600.

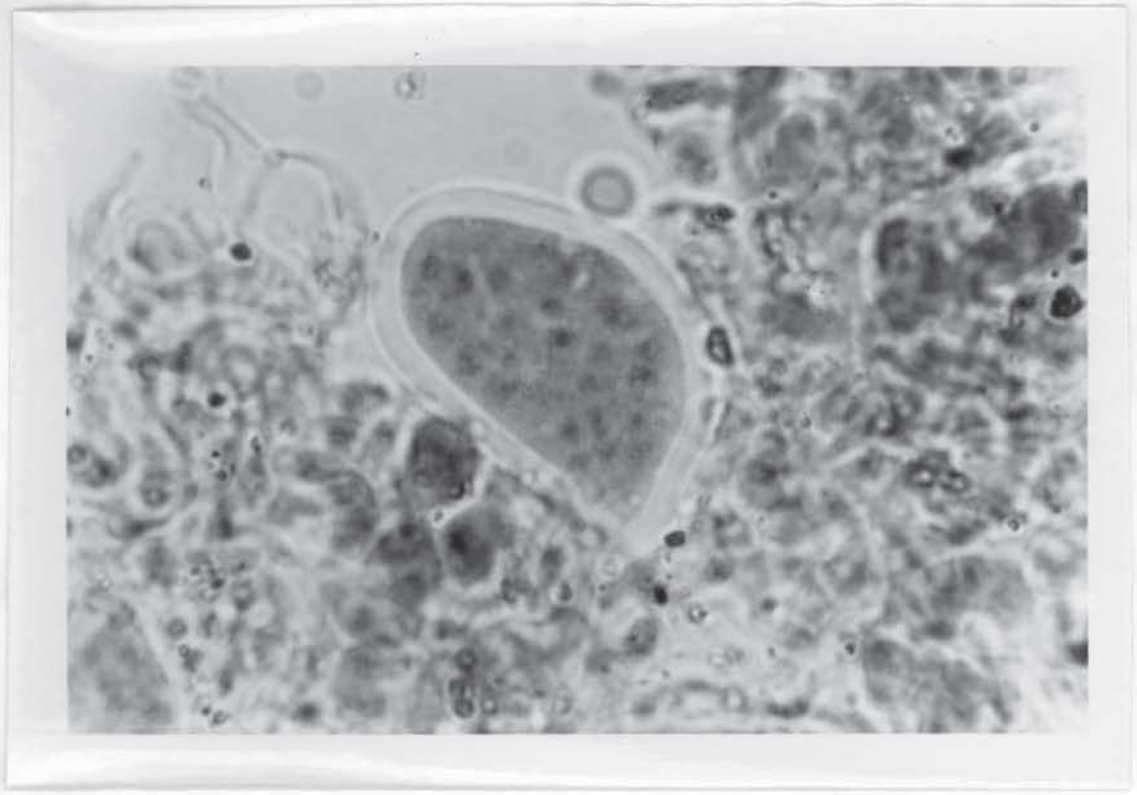

Fig. 37

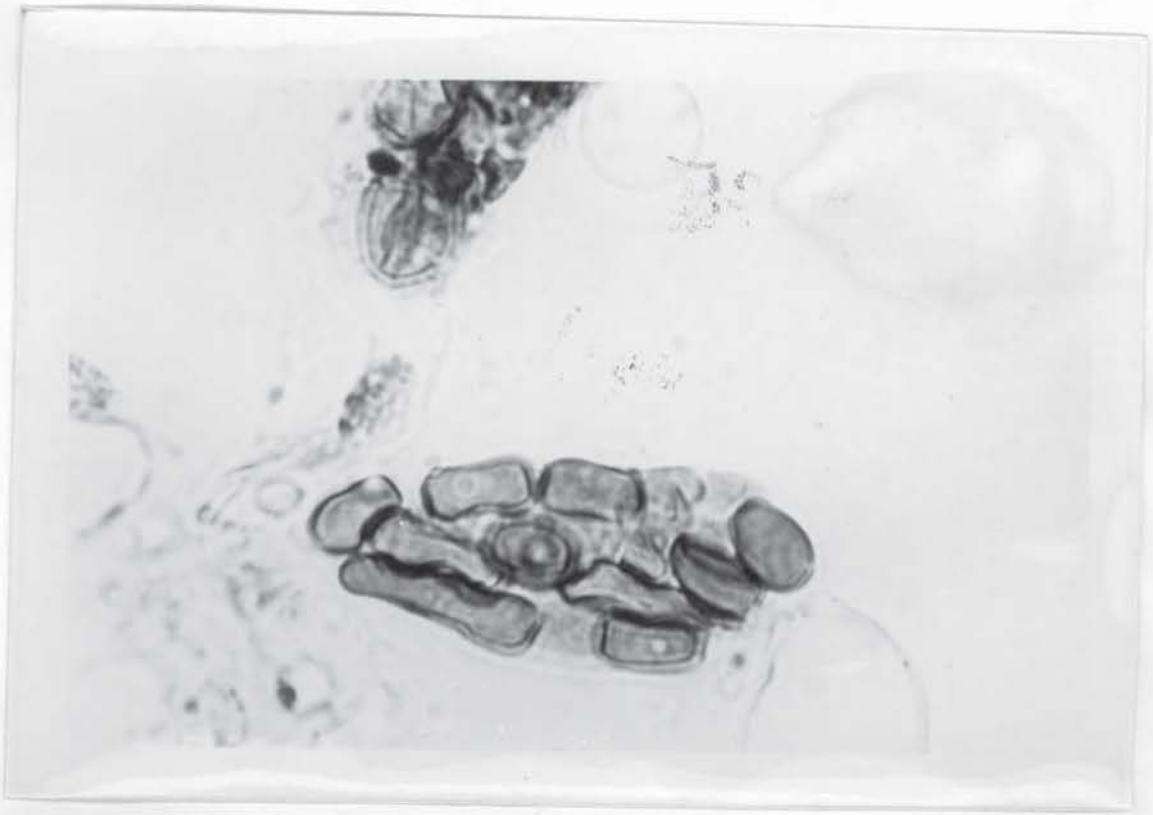

Fig. 38

Fig. 39 Four-celled binucleate ascospores. X1600.

Fig. 40 Fragmented spores showing unicellular segments with germinal slits. X1600.

Fig. 39

Fig. 40