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# CYTOMORPHOLOGY OF THE ASCOCARP OF CHAETOMIUM CRISPATUM FUCKEL

(TITLE)

BY

SHARON LYNNE BROOKMAN

**THESIS**

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# CYTOMORPHOLOGY OF THE ASCOCARP OF *CHAETOMIUM CRISPATUM* FUCKEL

## INTRODUCTION

The Ascomycete genus *Chaetomium* has been the subject of many morphological studies, but relatively few of the numerous species have been investigated. *Chaetomium crispatum* Fuckel is a species that has had only limited attention paid to its morphology. *Chaetomium crispatum* was named in Germany by L. Fuckel in 1870 from material collected on rotting potatoes in his cellar (Ames, 1961). In 1886, H. Zukal undertook a limited morphological study of this fungus. The purpose of this research is to study the early stages of development and the internal structure of the ascocarp, and the ascogenous hyphae of *Chaetomium crispatum* Fuckel, utilizing modern techniques and optical equipment, to clarify the work of Zukal.

*Chaetomium*, a large genus of Ascomycetes, is characterized by having specialized hairs arising from the ostiolar region of the perithecium, in addition to having unspecialized lateral hairs arising from the base and sides of the perithecium. The appearance of the specialized hairs varies from straight to slightly wavy hairs to tightly coiled ones, and these may or may not be branched. Some of these hairs coil clockwise; others coil counterclockwise. The perithecia never occur in a stroma, but are found growing individually,

and the perithecial walls are very thin and become fragile with age. The asci, ranging from club- to cylindrical-shaped, are always evanescent. Although the majority of the species of this genus have 8 spores in each ascus, there is one described species with 4 spores. The ascospores are single-celled and colored, usually olive-brown. A conidial stage has been recognized for only a few species of Chaetomium. All of the species of Chaetomium are saprophytes on paper, fabrics, straw and dung.

Kunze, in 1817, described the genus Chaetomium with Chaetomium globosum as the type species (Chivers, 1915). Zopf (1886) and Palliser (1910) made some of the early contributions to the knowledge of the genus Chaetomium. Bainier (1910) described 22 species and 3 varieties in his monograph of Chaetomium. Chivers (1915) reported that 114 species and 14 varieties of Chaetomium had been described up to that time, but he only recognised 28 species as being valid, reducing the other names to synonymy. Skolko and Groves (1948, 1953), as an outgrowth of their study of seed-borne fungi, monographed the genus Chaetomium and recognised 47 species, 16 of which they had succeeded in isolating from seeds. In his monograph, Ames (1961) gave descriptions of 85 species and listed 11 newly described species which had not been evaluated by him. Since the publication of Ames' monograph, many more new species have been named (Rai and Mukerji, 1962; Rai and Tewari, 1962; Dwivedi, 1963; Saha, 1964; Naplekova and Sergeeva, 1965; Novak, 1966; Massucchetti, 1966; Van Warmelo, 1966; Seth, 1967; 1969a; Meyer and Lamoreau, 1967; Singh, 1968; Mandi,

Rafia, and Pillay, 1968; and Gochenaur, 1969).

The classification of Chaetomium has not been a uniform one. There have been several different interpretations given the genus in the class Ascomycetes. The most frequently used interpretation is to consider Chaetomium as the type genus of the family Chaetomiaceae. This family appears most frequently in the order Sphaeriales, series Pyrenomycetes. Gwynne-Vaughan (1922), Bessey (1950), Martin (1950), and Alexopoulos (1952) support the interpretation of the Chaetomiaceae as a family because of the dark-colored pseudoparenchymatous perithecial wall free from the enclosed asci, which are in a definite hymenial layer. Von Arx and Muller (1954) and Munk (1953) considered Chaetomium in the Melanosporaceae of the Sphaeriales. The Melanosporaceae were defined by them as having clavate asci rounded at the tip with no apical apparatus and the ascus wall becoming gelatinous.

The evanescent nature of the asci leads Mannfeldt (1932) and Miller (1949) to transfer the Chaetomiaceae to the Plectascales. In addition to the evanescent asci, this order is generally described as having a closed ascocarp and irregularly scattered asci in the ascocarp; these latter two characteristics are not applicable to Chaetomium. Gaumann (1928) did not recognize the Chaetomiaceae as a separate family, but instead dealt with the genus in the Sordariaceae, order Sphaeriales. Later he treated it in the Ophiostomataceae of the Plectascales (Gaumann, 1952).

Luttrell (1951) considered the Chaetomiaceae in the Xylariales of the Pyrenomycetes, an order similar in definition to the

Sphaeriales. In his critical analysis of the ascocarp morphology, Luttrell (1951) emphasizes two main structures in the classification of the Pyrenomycetes: (1) the variations in the centrum development and (2) the ascus characteristics.

The variation of the centrum depends upon the place of origin of asci and the sterile tissue, and upon the function and persistence of sterile tissue in the ascocarp.

The ascus structure depends on the form, on the type of apical apparatus, and on the number of walls making up the ascus. He described several types of asci and several types of centra in the Pyrenomycetes. He classifies Chaetomium as having an Ophiostoma-type ascus because in most species it is globoid and is composed of only one thin ascus wall without an apical pore. Luttrell considered the genus as having the Xylaria-type centrum because the paraphyses grow upward into the central part of the perithecium. This upward growth produces pressure on the cells in the central cavity.

The most recent studies have placed the order Chaetomiales in the Chaetomiaceae, an order often treated as Pyrenomycetes. Martin (1961) was the first to use this system of classification. Ames (1961) and Alexopoulos (1962) followed his interpretation.

The general pattern of development of the ascocarp in the Ascomycetes is similar, but details vary. In a typical ascomycete, the vegetative mycelium produces the ascogonium, the cells of which may be uninucleate or multinucleate. The antheridium is formed

from the same or an adjacent filament, and the two organs may be connected by a trichogyne through which the antheridial nuclei migrate into the ascogonium.

The ascocarp which occurs around the sex organs of most Ascomycetes may take several forms, but the walls of ascocarps are formed from the interweaving of somatic hyphae, forming prosenchymatous or pseudoparenchymatous tissue. In some species, the ascocarp may begin development before the sexual organs appear, while in others the development of the ascocarp is delayed until after the sexual organs have formed.

In the ascogonium, the antheridial nuclei pair with the ascogonial nuclei, forming a dikaryotic condition. According to Harper (Bessey, 1950), the paired nuclei fuse, forming diploid nuclei in the ascogonium. However, Claussen (Bessey, 1950) reported that the nuclei did not fuse in the ascogonium - they only paired. Claussen's interpretation has been accepted by most recent mycologists. Numerous ascogenous hyphae appear from the ascogonial surface and the paired nuclei migrate into these hyphae, thus forming dikaryotic cells. In some species, an antheridium is lacking and a vegetative filament or spore-like spermatium will fuse with the ascogonium, providing the male nuclei. In other species, the ascogonium apparently does not fuse with an antheridium or a filament, but instead, the nuclei of the ascogonium pair with each other.

In many Ascomycetes, the dikaryotic cells in the ascogenous hyphae curve to form hook-shaped structures called croziers. The

2 nuclei in the crozier divide simultaneously and 2 septa are formed, leaving 1 nucleus in the tip or ultimate cell, 2 nuclei in the middle or penultimate cell, and 1 in the base or antepenultimate cell. The binucleate penultimate cell will become the ascus and is referred to as the ascus mother cell by Alexopoulos (1962). The nuclei of the penultimate cell fuse, and the resulting fusion nucleus would be, according to Claussen's interpretation, diploid, or, according to Harper's interpretation, tetraploid. The fusion nucleus soon undergoes meiosis, forming 4 nuclei which, according to Claussen, would be haploid, and, according to Harper, would be diploid. Generally this is followed by simultaneous nuclear division, resulting in 8 nuclei in the ascus. Claussen regards this division as mitotic. Harper's interpretation of this third nuclear division is reductional and has been called brachymeiosis (Gwynne-Vaughan, 1922). Both regard the resulting ascospores as being haploid. Some species do not form croziers on the ascogenous hyphae, but the ascospores are produced in a similar way.

## LITERATURE REVIEW ON MORPHOLOGY

The first important morphological treatment of the Chaetomiaceae was a publication by van Tieghen (1875) on the development of the ascocarp in Chaetomium. Although he did not mention the species he treated, he described the ascogonium as a coiled structure from which the asci arose. The walls of the perithecium and the hairs are formed by branched hyphae, which he referred to as the periascogonium. These hyphae arise from the base of the coiled ascogonium and ramify over it. In the following year after studying Chaetomium murorum Corda, Chaetomium indicum Corda, and several other new species, van Tieghen (1876) indicated that when growth on a poor nutritive medium occurred, the walls of the perithecium and the hairs were not formed by the periascogonial hyphae but were formed by the ascogonium itself.

Zopf, in his monograph of Chaetomium (1881), described Chaetomium kunzeanum Zopf, which Chivers (1915) and most subsequent workers have regarded as a synonym of Chaetomium globosum Kunze. Zopf detected some screw-like structures but did not refer to them as the ascogonia that van Tieghen had described. From his observations, Zopf concluded that the initial hyphae coiled around each other, forming the perithecium. Although he did not mention the ascogonium or the periascogonium, he may have observed both of these structures.

From working with Chaetomium kunzeanum Zopf (=Chaetomium globosum Kunze), Eidam (1883) observed a coiled ascogonium, which he referred to as the carpogonium, with hyphal branches arising at the base of the stalked ascogonium or from adjacent hyphae. Zukal (1886) followed Zopf's interpretation by denying the existence of coiled ascogonia in Chaetomium crispatum Fuckel. He described the development of the perithecial wall and the asci as being vegetative outgrowths of specialized hyphae. Following Zopf's investigations and those of Eidam, Oltmanns (1887) reported observations on Chaetomium kunzeanum Zopf (=Chaetomium globosum Kunze) that coincided with those described by van Tieghem and Eidam. He observed some stalked and some sessile coiled ascogonia with the perithecial walls formed by the hyphae at the base of the ascogonia.

There has been considerable morphological work in the twentieth century on the genus Chaetomium. Dangeard (1907) observed that the cells of the vegetative mycelium of Chaetomium spirale Zopf were uninucleate while two nuclei were observed during new branch formation. His report of a coiled ascogonium with associated hyphae forming the walls of the perithecium supported the work of the earlier mycologists on Chaetomium kunzeanum Zopf (=Chaetomium globosum Kunze). Vallory (1911) made a cytological study of Chaetomium kunzeanum Zopf var. chlorinum Mich. (=C. globosum Kunze), reporting that the cells of the ascogonium and the vegetative mycelium were multinucleate. He observed that the nuclei

were frequently paired. From this he concluded that the pairing of nuclei was the result of rapid division, amitotically, and not a sexual process.

Working with coprophilous fungi, Page (1939) isolated several species of Chaetomium and made a limited morphological study on them. He described short, multicellular coiled hyphae forming the walls of the perithecium with hairs arising from the outer layer of the wall. He reported croziers in many of the species he observed, although he did not indicate in which species he had seen them. Paraphyses were present in Chaetomium globosum Kunze, Chaetomium murorum Corda, Chaetomium cochliodes Palliser, Chaetomium bostrychodes Zopf, and Chaetomium carpinum Banier. In some species the ascospores oozed out of the perithecium and formed a rod-like mass at the top, but in Chaetomium globosum he reported the spores formed a mass within the hairs.

Greis (1941) made an extensive investigation of the development of the ascocarp of Chaetomium kunzeanum Zopf (=C. globosum Kunze) and C. bostrychodes Zopf. Since he used sectioned material and slide cultures, his study was more valid than the earlier investigations. According to Greis, the development of the perithecium of Chaetomium kunzeanum begins with the union of the ascogonium by an antheridium or by a vegetative mycelium. He reported that the antheridium contains one or two nuclei which pass into the multinucleate ascogonium. After the antheridial nuclei pair with some of those of the ascogonium, conjugate division occurs, followed by the

formation of crosswalls. Greis indicated that the walls of the perithecium were formed from the primary ascogenous hyphae or directly from the fertilized ascogonium. The ascus was formed without crosiers and the two nuclei in the young ascus united to produce the fusion nucleus. In Chaetomium bostrychodes Zopf, he stated that the development of the perithecium was similar to that in the development of C. kunzeanum, except that in some cases spontaneous nuclear pairing, or autogamy, was substituted for fertilization. From a single ascogonium, he reported several perithecia may be formed in C. kunzeanum while only one perithecium is formed in C. bostrychodes.

A cytological study of Chaetomium globosum Kunze was made by van der Weyen (1954). He utilised the section technique in addition to the carmine smear technique. He was unable to obtain the coiled ascogonia in his sectioned material, but he was able to observe the ascogenous hyphae with the carmine smear technique. He reported that most of the cells of the ascogenous hyphae were uninucleate, and crosiers may be formed on the ends of the hyphae.

In his work with Chaetomium, Whiteside (1957) recognized two patterns of ascogonial development. He made a comparative study of nine species of Chaetomium which had different ascus forms as well as variations in the terminal hairs of the perithecia. In Chaetomium globosum Kunze, C. atterrimum Ellis and Everhart, C. ochraceum Tschudy, C. funicolum Cooke, C. dolichotrichum Ames, C. murorum Corda, and C. pachypodioides Ames, the stalked or

sessile ascogonium is irregularly coiled and the vegetative mycelium lacks hairs. In Chaetomium brasiliense Batista and Pontual and C. aureum Chivers, the ascogonium coils in a symmetrical pattern around the stalk cells and the vegetative mycelium has prominent hairs or seta. Whiteside (1961) also made a morphological study of Chaetomium globosum Kunze and Chaetomium brasiliense Batista and Pontual. He described the ascocarp of Chaetomium globosum as having the basal tuft of asci originating from a conical mass of ascogenous hyphae. These hyphae, he reported, may have originated from the ascogonial coil. Although there were a few scattered binucleate cells, there appeared to be no crozier formation. The cells of the vegetative mycelium were commonly multinucleate with the nuclei occurring in pairs. In Chaetomium brasiliense he indicated that either asci or new croziers arose from the penultimate cell of a crozier. The cells of the vegetative mycelium were also plurinucleate, these nuclei often occurring in pairs.

Corlett (1966) described the development of the ascogonial primordium of Chaetomium trigonosporum (Marchal) Chivers as being similar to the development Whiteside (1961) recorded for Chaetomium globosum Kunze. The only differences were in the nuclear condition of the mycelium. The mycelium of the former was uninucleate, while the mycelium of the latter was plurinucleate. The apex of the ascogenous hyphae developed into the asci directly without forming croziers. Cherepanova (1962), observing the later stages of development of the perithecium, analyzed the variations in the

morphological characteristics of Chaetomium globosum Kunze, C. megalocarpum Banier, and C. murorum Corda.

In 1966 Berkson reported a cytomorphological study he made of the ascogenous hyphae of Chaetomium aureum Chivers, C. murorum Corda, C. caprinum Banier, and C. dolichotrichum Ames. He observed no crosiers prior to ascus formation in C. aureum and C. murorum, but he did see crosiers in C. caprinum and C. dolichotrichum.

Aue and Muller (1967) isolated Chaetomium abuense Lodha, C. uniporum nov. spec., C. gelasinosporum nov. spec., and C. venezuelense Ames from soil obtained in Egypt. The main purpose of their study was the behavior of the germinating ascospores, but they did observe the thallus and the fruiting bodies under different nutrient conditions in addition to illustrating and describing the ascogonia.

Brewer and Duncan (1968) reported that the vegetative hyphal cells of Chaetomium cochliodes Palliser were multinucleate while the ascogenous hyphae were uninucleate. Hook-like structures in the ascogenous hyphae suggesting crosiers were observed, but no typical crosiers were evident. The single nucleus of these hook-like cells in the young ascus divided, followed by nuclear fusion and reduction division.

In a study of Chaetomium bostrychodes Zopf, Range Rao and Mukerji (1968) found no crosiers, the cells of the uninucleate ascogenous hyphae developing directly into asci. The nucleus of the elongated ascus was located in the center and then following meiosis

and mitosis, the ascospores were formed.

From his study of Chaetomium erraticum Ames, Cooke (1969a) described the vegetative hyphae and the ascogonial cells as being uninucleate or binucleate. The sessile ascogonium coils and the hyphae in association with the ascogonial base form the walls of the perithecium and the perithecial hairs. The pseudoparenchyma cells break down and form a central cavity in the centrum. He observed the formation of croziers in the developing ascogenous hyphae. Cooke (1969b), in his morphological study of Chaetomium funicolum Cooke, reported that the cells of the vegetative hyphae were uninucleate while the cells of the coiled ascogonium were uninucleate or binucleate. The ascogenous hyphae developed in the central portion of the centrum and croziers were observed at the tips of the ascogenous hyphae.

The perithecial development of fifteen species of Chaetomium was studied by Seth (1969). Most of the species followed either the pattern of development of Chaetomium globosum or the pattern of Chaetomium brasiliense described by Whiteside (1957). But C. tenuissium Serg., C. torulosum Banier, C. funicola Cooke, and C. quadrangulatum Chivers had a variety of means of coiling of the ascogonium. These he described as having an "intermediate" type in which mycelial hairs may occur in species with the C. globosum pattern of ascogonial development.

Cooke (1970) described the ascogonial coils as being stalked in Chaetomium trilaterale Chivers. He reported the vegetative hyphae were uninucleate and the asci arose from true croziers.

## MATERIALS AND METHODS

The culture of Chaetomium crispatum Fuckel was isolated in January, 1961, from a dung collection obtained in central Illinois. The exact site of the collection was not recorded. Identification was made by Dr. W. C. Whiteside through the use of monographs of the Chaetomiaceae by Chivers (1915), Skolko and Groves (1948, 1953), and Ames (1961). Although this species was named in 1870 by Fuckel, it has been reported relatively few times in North America. According to Chivers (1915), Ames (1961), and Skolko and Groves (1953), collections have been recorded from Massachusetts, South Carolina, Virginia and Tennessee. In 1926, Abbott reported isolating it from soil collected in Louisiana (Gilman, 1957).

Agar plates were inoculated with small blocks of agar containing vegetative mycelium. Growth of the vegetative mycelium was apparent in approximately four days, but no perithecia were produced on malt extract agar, yeast extract agar, tomato juice agar (200 ml tomato juice, 15 gm agar and 800 ml distilled water), and V-8 agar (200 ml V-8 juice, 3 gm calcium carbonate, 15 gm agar and 800 ml distilled water). The mycelial growth was rapid, covering the whole plate on both the tomato juice and V-8 agar. The only medium utilized on which perithecia formed was 1.5% agar

poured over a few sterilized oatmeal flakes. The depth of the agar was of some importance because the organism produced perithecia more readily and more abundantly on agar plates of greater depth. A possible explanation may be that the greater depth of agar results in the fungus being further removed from the food source, or it may be that drying out of the medium is retarded.

The cultures were grown at room temperature as well as in a constant temperature incubator. The optimum temperature for the formation of the ascocarps was found to be approximately 80 degrees to 90 degrees Fahrenheit. Temperatures lower or higher than this optimum seem to inhibit both the vegetative growth and the formation of the perithecia. In most species of the genus, such as Chaetomium globosum and C. bostrychodes, which have been subjected to detailed morphological study, the perithecia are produced rapidly and abundantly on a variety of media. But the development of relatively few perithecia per given surface area and the failure of perithecium formation on many plates indicate that C. crispatum has an erratic development. The length of time for perithecial development varied, but mature perithecia usually formed within two to three weeks.

In demonstrating the ascogenous hyphae, the propiono-carmine smear technique was utilized. The killing and fixing agent employed was Carnoy's fluid (3 parts of absolute ethyl alcohol, 1 part of glacial acetic acid, and 2 to 3 drops of chloroform). The material to be stained by this technique was placed in the Carnoy's fluid for two days and then into a small container of propiono-

carmine stain for three days (Johansen, 1940). Both of these solutions were placed in the refrigerator while the fungus tissue was present. A few perithecia were transferred to a fresh drop of propiono-carmine stain on a microscope slide. With the aid of the dissecting microscope and fine needles, several perithecia were separated from the vegetative mycelium, and agar plus the centra were dissected from the perithecia. A cover slip was placed over the contents, pressure was applied to separate the ascogenous hyphae, and the slide was warmed to intensify the stain. The cover slip was sealed by a fluid containing 1 part of 45% glacial acetic acid, 1 part white Karo syrup, and 1 part saturated aqueous suspension of pectin. Once the slides were made, they were stored in the refrigerator overnight to allow the stain to intensify. Since a breakdown of the material occurred soon after the slides were prepared, it was necessary that observation be made the following day.

Sections cut with a microtome were used for studying the development and structure of the centrum. The Randolph's Modified Navashin and Formalin-Aceto-Alcohol (FAA) fluids (Johansen, 1940) were employed as the killing and fixing agents in this method. Blocks of agar with perithecia were placed in the Navashin fluid, aspirated for an hour, and left in the killing agent for 18 hours. First, the material was rinsed a few minutes in tap water. Then it was dehydrated before being put into the stain. The dehydration schedule was as follows: 2 hours each in 10, 20, 30 and 40% ethyl

alcohol; 2 hours each in 50, 60, 70, 80 and 100% tertiary butyl alcohol (TBA) with eosin added to the 100% TBA; overnight in pure TBA; and 2 hours in pure TBA with paraffin oil. The procedure for material fixed in FAA was similar except that the material was transferred directly from the fixing fluid to 50% alcohol in the dehydration series, since the FAA contains this alcohol percentage. After dehydration, the material was transferred to small vials and infiltrated by a mixture of absolute TBA and paraffin. The vials were placed in the warming oven overnight, and 2 to 3 changes of melted paraffin were made before embedding. The material was mounted on wooden blocks and sectioned with a rotary microtome at a thickness of 10 to 15$\mu$. Haupt's adhesive (Johansen, 1940) was used to affix the paraffin ribbons to microscope slides, which were placed on the warming table to dry overnight. This was followed by placing them in 2 changes of xylene for 5 minutes each to dissolve the paraffin, followed by 5 minutes in absolute ethyl alcohol, 2 minutes each in 85, 70, 50, 30 and 10% ethyl alcohol and 1 minute each in 2 changes of distilled water.

Some material, both that which was fixed in Navashin fluid and in FAA, was stained overnight by Mayer's Hemalum (Johansen, 1940) and rinsed in distilled water until the water was no longer tinted. The fungus tissue was destained in 0.1% hydrochloric acid (1 ml HCl in 1,000 ml $H_2O$). In the other staining procedure, the material was mordanted in a 3% solution of iron alum for 2 hours and rinsed in running water for 15 minutes prior to staining.

Then it was stained overnight in Heidenhain's Hematoxylin (Johansen, 1940), followed by destaining in a 2% solution of iron alum. The Heidenhain's Hematoxylin stain was used on material fixed by both the Navashin fluid and in FAA.

Dehydration of the tissue was according to the following schedule: 5 minutes each in 30, 50, 70, 85% and 2 changes of absolute ethyl alcohol; 1 minute in clove oil to clear the slides; and 1 minute each in 3 changes of xylene. Permount was used to mount the cover slips, and the slides were placed on the warming table to dry.

The method used for observation of ascogonia was a water mount. First, an area on the culture in which perithecia were beginning to form was selected. Small blocks of agar were then removed and placed in a drop of diluted aqueous phloxine solution. The cover slip was placed on the agar and pressure was applied to spread the material. The slides were examined under the medium power objective (X312.5) to locate the widely dispersed ascogonia, after which the individual ascogonia were studied with the oil immersion objective (X1250).

A Zeiss microscope with a Neofluar oil immersion lens was used. The drawings were made with the use of an attached drawing apparatus, and their magnification is indicated.

## OBSERVATIONS

In previous studies of species of Chaetomium, the formation of the ascogonium, the development and structure of the ascus, and the development of the centrum have been given considerable attention. In addition to the perithecial hairs, these features have been among the most important ones used in the characterization and classification of this genus. In this study of Chaetomium crispatum Fuckel, emphasis will be placed on these three characteristics.

### OBSERVATION I: THE ASCOGONIUM

The ascogonium, generally referred to as the female reproduction organ of Ascomycetes, is a specialized hyphal branch that begins to elongate and coil irregularly, forming a tightly coiled sphere (Figures 23 - 27). From the basal cell of the ascogonium arise several hyphae which twist around the ascogonium to form the walls of the perithecium and the specialized hairs (Figures 25 - 27). In the early ascogonium no crosswalls are evident, but after the ascogonium coils several times, crosswalls are formed. Several examples of relatively young ascogonia with hairs present can be observed.

There is a tendency for the ascogonia to occur in clusters, but generally these ascogonia are quite sparse. With the medium power objective (X312.5), two or three ascogonia can often be seen in one field. Some of the ascogonia are close enough to touch each other.

However, at this magnification, most fields show only the branched vegetative hyphae.

## OBSERVATION II: THE ASCUS

Hook-shaped structures that appear to be typical croziers can be observed in the ascogenous hyphae of Chaetomium crispatum (Figures 1 - 12). The penultimate cells are binucleate, while the ultimate and antepenultimate cells are uninucleate. The asci apparently originate from the binucleate penultimate cells.

The mature asci, averaging 68μ x 10μ, are cylindrical with 8 ovoid spores in each ascus, while the average size of the young ascus at the stage when the fusion nucleus is present is 16 ± 2.5μ x 6μ. Many of the ascospores are free in the perithecium, due to the evanescent nature of the walls of the ascus. The size of the mature spores range from 12μ x 4μ to 15μ x 5μ, the average being 14μ x 4.5μ.

## OBSERVATION III: THE CENTRUM

By using the sectioned material, it can be observed that the developing perithecium has a surface layer 3 to 4 cells thick, consisting of relatively small, darkly pigmented cells which are about 1 to 2μ in diameter. From these cells, the coiled terminal hairs and the straight lateral hairs develop. Underlying this is a layer composed of cells 4 to 5μ long which retain considerable stain. The innermost region, 10 to 12 cells thick, consist of large parenchyma-like cells which are thin-walled, highly

vacuolated, and fail to retain much stain because of their vacuolated nature. These large, parenchyma-like cells appear to fill the centrum of the perithecium (Figure 29), but are not clearly evident in perithecia with mature asci. Although a few of these cells can still be observed in perithecia with mature asci, most have disappeared by this time, presumably being crushed by the developing asci. From a wet mount of the perithecial contents, these cells appear to be short filaments 3 to 5 cells long, and often forming short branches. The cells of these filaments are approximately 7 $\mu$ in diameter (Figures 17 - 22) and differ from the paraphyses by having greater diameter and shorter length.

From the middle layer of cells near the ostiole, a layer of periphyses develops and fills the ostiolar region (Figure 29). They originate from the sides of the ostiole. Paraphyses extend up through the centrum. Some of these paraphyses grow from the base of the centrum while others develop from the sides, the paraphyses having their origin from the middle layer of cells. Many of these paraphyses are very long and nearly reach the ostiole, but the ones which originate near the top of the centrum are much shorter and appear to be very numerous. The paraphyses arise prior to the appearance of the asci and are evident even after the ascospores are formed (Figures 28 - 29). The average size of these paraphyses are 53 $\mu$ x 2 $\mu$. In wet mounts, application of pressure to the cover slip causes the cells of the paraphyses to break apart. The asci

from the ascogenous hyphae grow up among the paraphyses into the centrum, but in developing perithecia, the paraphyses are only 3 to 4μ longer than the asci. In mature perithecia, the paraphyses are longer than the asci. The asci and the paraphyses together form a loosely arranged, flat cushion at the base of the centrum.

## DISCUSSION

Chaetomium is a large genus with considerable variation in the gross morphology of the ascocarp, particularly in the form of the terminal perithecial hairs. Studies on the developmental morphology of the perithecium have been carried out on at least thirty species, but the only previous developmental study on the group with hairs coiling counterclockwise and with cylindrical asci was on Chaetomium crispatum made by Zukal in 1886. The history and characteristics of the ascogonium, the paraphyses, the centrum and the ascogenous hyphae of Chaetomium are herewith discussed in further detail.

## THE ASCOGONIUM

The first reference to the coiled nature of the ascogonium of Chaetomium was made by van Tieghan (1875). Although he did not mention the species with which he worked, he reported that the asci had their origin from a coiled ascogonium, while the walls of the perithecium and the perithecial hairs arose from branched hyphae leaving the base of the ascogonium and growing over the surface. He referred to these branched hyphae as perisscogonia. Eidam (1886) described the ascogonial development of C. kunzeanum Zopf (=C. globosum Kunze) as being similar to the development reported by van Tieghan, except that he observed that the branched

hyphae which form the walls of the perithecium may arise from the adjacent hyphal cells as well as from the base cell of the ascogonium. Many other investigators have reported the same coiled nature of the ascogonium through the study of various species of Chaetomium.

Whiteside (1957) was the first to attempt to classify the ascogonia in Chaetomium. He reported two types of ascogonia within the genus. The most frequent type of ascogonium was one which coils about itself in an irregular fashion and was designated as the Chaetomium globosum type. The less frequent type had a prominent ascogonial stalk, with the ascogonial tip coiling about itself and spiraling down the stalk cells in a symmetrical pattern, and was designated as the Chaetomium brasiliense type. Species which appear to have the first pattern of development are C. aterrimum Ellis and Everhart (Whiteside, 1957), C. bostrychodes Zopf (Greis, 1941), C. dolichotrichum Ames (Whiteside, 1957), C. erraticum Ames (Cooke, 1969), C. funicola Cooke (Seth, 1969), C. funicolum Cooke (Cooke, 1969), C. globosum Kunze (=C. kunzeanum Zopf) (Ridam, 1886; Oltmanns, 1887; Vallory, 1911; Greis, 1941, van der Weyen, 1954; Whiteside, 1957), C. ochraceum Tschudy (Whiteside, 1957), C. quadrangulatum Chivers (Seth, 1969), C. spirale Zopf (Dangeard, 1907), C. tenuissium Berg., (Seth, 1969), C. torulosum Benier (Seth, 1969), C. trigonosporum (Marchal) Chivers (Corlett, 1966), and C. trilaterale Chivers (Cooke, 1970). The species which apparently have the second type of ascogonium development are C. aureum Chivers (Whiteside, 1957),

C. brasiliense Batista and Pontual (Whiteside, 1957), and C. uniporum (Awe and Muller, 1967).

Zukal (1887), in his study of the development of the perithecium of Chaetomium crispatum Fuckel, indicated that the perithecium usually originates from the vegetative hyphae, although he did report seeing a few carpogonia (ascogonia) with a screw-like development. His Figure 1, the only illustration that appears to be a young stage, fails to show any coiled structures. In contrast, in this study of Chaetomium crispatum, an ascogonium which coils irregularly around itself several times forming a sphere, is observed (Figures 23 - 27). The ascogonium is the Chaetomium globosum type as described by Whiteside (1957).

## THE PARAPHYSES

In 1941 Greis reported basal paraphyses for both Chaetomium kunzeanum Zopf (=C. globosum Kunze) and C. bostrychodes Zopf. In Chaetomium globosum Kunze, Whiteside (1961) saw septate filaments developed from the inner layer of sterile cells in the perithecium. He regarded them as the paraphyses that Luttrell (1951) reported; however, he emphasized the ephemeral nature of these paraphyses as an explanation for several reports in the literature denying the presence of paraphyses in C. globosum. Whiteside did not observe paraphyses in Chaetomium brasiliense Batista and Pontual.

Corlett (1965) mentioned that there were septate paraphyses in the upper part of the perithecial cavity on C. trigonosporum

(Marchal) Chivers, but indicated that they were absent from the hymenial layer.

Cooke (1969a; 1969b; 1970), in his detailed studies of Chaetomium erraticum Ames, C. funicolum Cooke, and C. trilaterale Chivers, stated that peraphyses are lacking in these three species.

Zukal (1886) observed paraphyses in Chaetomium crispatum which originated from the sides of the perithecial wall and grew upward toward the ostiolar region and mixed with the asci. He felt that their appearance of sloping upward gave the effect that they arose from the same area as the asci. From his drawings (Zukal, Figures 8 - 9), Zukal indicated the paraphyses seem to persist on the sides of the perithecium and among the asci even after the ascospores have formed in the asci. This study reveals that the paraphyses in the earlier stages of C. crispatum are similar to the ones described by Whiteside (1961) for C. globosum, but the paraphyses remain even after the asci are mature, a condition not present in the latter species. However, by the time the asci have completely broken down, the paraphyses have disappeared in Chaetomium crispatum.

## THE CENTRUM

Luttrell (1951) classified the centrum of the genus Chaetomium as the Xylaria type. According to Luttrell, in the Xylaria centrum the perithecium was formed by hyphal branches arising from the stalk cell of the ascogonium or from neighboring hyphae. A cavity in the perithecium was produced by the growth of paraphyses, which lined the base and side of the cavity, thus expanding the perithecium.

The ascogenous hyphae, arising from the ascogonium, grew up from the base and the sides, forming a continuous hymenium of asci generally mixed with paraphyses.

The development of a cavity in the perithecium has been reported for several species of Chaetomium. In his study of Chaetomium globosum Kunze, van der Weyen (1954) recorded a cavity in the perithecium which he interpreted as being formed by the rapid growth of the wall. Oltmanns (1887), and Whiteside (1961) have also described a cavity in this species. Other species in which a perithecial cavity has been reported are Chaetomium brasiliense Batista and Pontual (Whiteside, 1961), C. trigonosporum (Marchal) Chivers (Corlett, 1965), C. funicolum Cooke (Cooke, 1969) and C. trilaterale Chivers (Cooke, 1970). A cavity was also observed in C. erraticum Ames (Cooke, 1969), but the ascogenous hyphae and developing asci were described as pushing up into the centrum tissue before the cavity was formed.

In his paper on Chaetomium crispatum, Zukal indicated in his drawings that a cavity was formed in the perithecium as the paraphyses grew from the sides and base into the center, with the asci growing upward in this cavity toward the ostiole (Zukal, Figures 8 - 11). But in this present study of C. crispatum, a different centrum structure is observed. It appears that the centrum is occupied by highly vacuolated cells which are observed to be present in some perithecia even after the paraphyses have formed, the ascospores have been produced in the asci, and the spores have extruded

through the perithecial ostiole (Figure 29). The presence of these vacuolated cells in the centrum could interfere with the passage of ascospores. One possible explanation may be that canals are present among the cells of the centrum through which the ascospores may pass. Another explanation may be that these cells are loosely arranged and the spores can pass between the cells to the outside. It appears that eventually these vacuolated cells of the centrum disappear, probably breaking down as a result of pressure from the asci and the paraphyses.

The centrum structure makes Chaetomium crispatum distinct from the other species of Chaetomium which have been studied. Of the species that have been previously investigated, only in C. erraticum (Cooke, 1969) have the centrum cells been recorded as persisting for a relatively long period of time, although in this species the perithecial cavity forms before the ascospores are produced. In contrast, in Chaetomium crispatum, the centrum cells are present even after the ascospores are formed. In the closely related genus Chaetomidium, Whiteside (1963) reported that C. fimeti also has a pseudoparaphysatous appearance to the centrum, and the young asci grow up among the sterile cells and finally crush them. This appears to be similar to the centrum of C. crispatum except that paraphyses are not present in Chaetomidium.

## THE ASCOGENOUS HYPHAE

The earlier researchers of Chaetomium did not observe the

ascogenous hyphae and, of course, did not observe croziers. Page (1939) was the first to observe and record croziers in species of Chaetomium, but he did not mention in which species they were present. Since the work of Page, croziers have been reported in several species. Observation of an occasional crozier in the ascogenous hyphae of C. globosum Kunze was reported by van der Weyen (1954), but Whiteside (1961) observed no crozier formation for the same species. However, in the same study, Whiteside reported typical croziers for C. brasiliense Batista and Pontual. Sorgel (1961) indicated that there were no croziers in C. trigonosporum (Marchal) Chivers. Berkson (1966) observed croziers in C. caprinum Banier and C. dolichotrichum Ames but not in C. aureum Chivers and C. murorum Corda. Brewer and Duncan (1968) reported seeing suggestions of croziers for C. cochliodes Palliser, but no true croziers were observed. In their study of C. bostrychodes Zopf, Rango Rao and Mukerji (1968) found no croziers, but reported the ascogenous hyphae developed directly into asci. Cooke recognized croziers in his studies of C. funicolum Cooke (Cooke, 1969b), C. erraticum Ames (Cooke, 1969a), and C. trilaterale Chivers (Cooke, 1970).

In Chaetomium crispatum Zukal reported that the asci grew out of the basal part of the central cone (=centrum), and he made no mention of croziers. He apparently did not utilize a smear technique.

In the present study, the author, by employing the propiono-carmine smear procedure, observes abundant crozier formation on the ascogenous hyphae of this species.

## SUMMARY

Chaetomium crispatum Fuckel forms a coiled ascogonium and, from the basal cells of the ascogonium, specialized hyphae arise and ramify over the ascogonium. Septations form in the hyphae to produce the walls of the perithecium and the hairs which give this genus its name. The walls are made up of two layers of cells, one which has a black pigment, stains dark; and a second layer which consists of larger cells that absorb less stain. From this second layer the paraphyses and the periphyses arise, the former developing very early and not disintegrating as quickly as in other species. The paraphyses from the base of the perithecium elongate and extend up into the centrum, with some nearly reaching the ostiolar region. The asci develop after the appearance of paraphyses and are not as long. The inner layer of the centrum appears to be filled with large vacuolated cells, some of which may remain even after the asci are mature. The asci arise from croziers formed on the ascogenous hyphae.

Some of the work of Zukal (1886) does not agree with this study. He did not see the ascogonium and the specialized hyphae which form the walls of the perithecium, nor did he observe the croziers. He did not record observing the large, highly vacuolated cells that occupy the centrum. But his illustrations do show the

paraphyses, both those in the basal region and those on the side of the perithecium. His illustrations also show that the paraphyses do not break down early, but are present even after the asci are formed. Except for Oltmanns (1887) and Whiteside (1957), Zukal's work has been overlooked in the literature on Chaetomium.

The purpose of this study has been to correct several of Zukal's observations, to clarify other observations, and to give attention to the peculiar morphological features of Chaetomium crispatum. Other species in the group with Chaetomium crispatum with counterclockwise coiled hairs and cylindrical asci, such as C. perpulchrum Ames, C. tortile Bainier, and C. simile Masse and Salmon, might be profitably studied to see if their perithecial morphology is similar.

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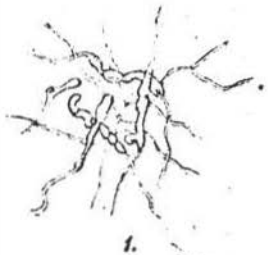
# APPENDIX A

## COPY OF ZUKAL'S ILLUSTRATIONS OF THE MORPHOLOGY OF *CHAETOMIUM CRISPATUM* FUCKEL



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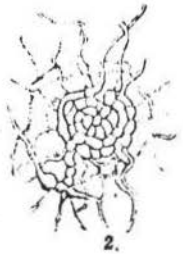

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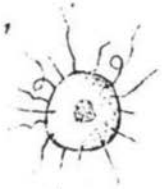

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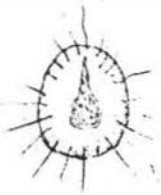

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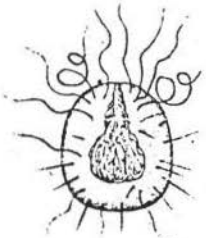

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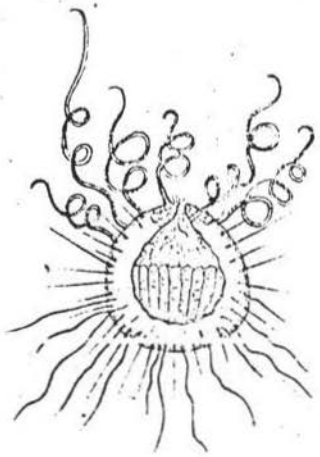

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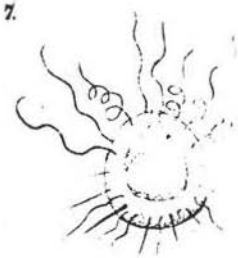

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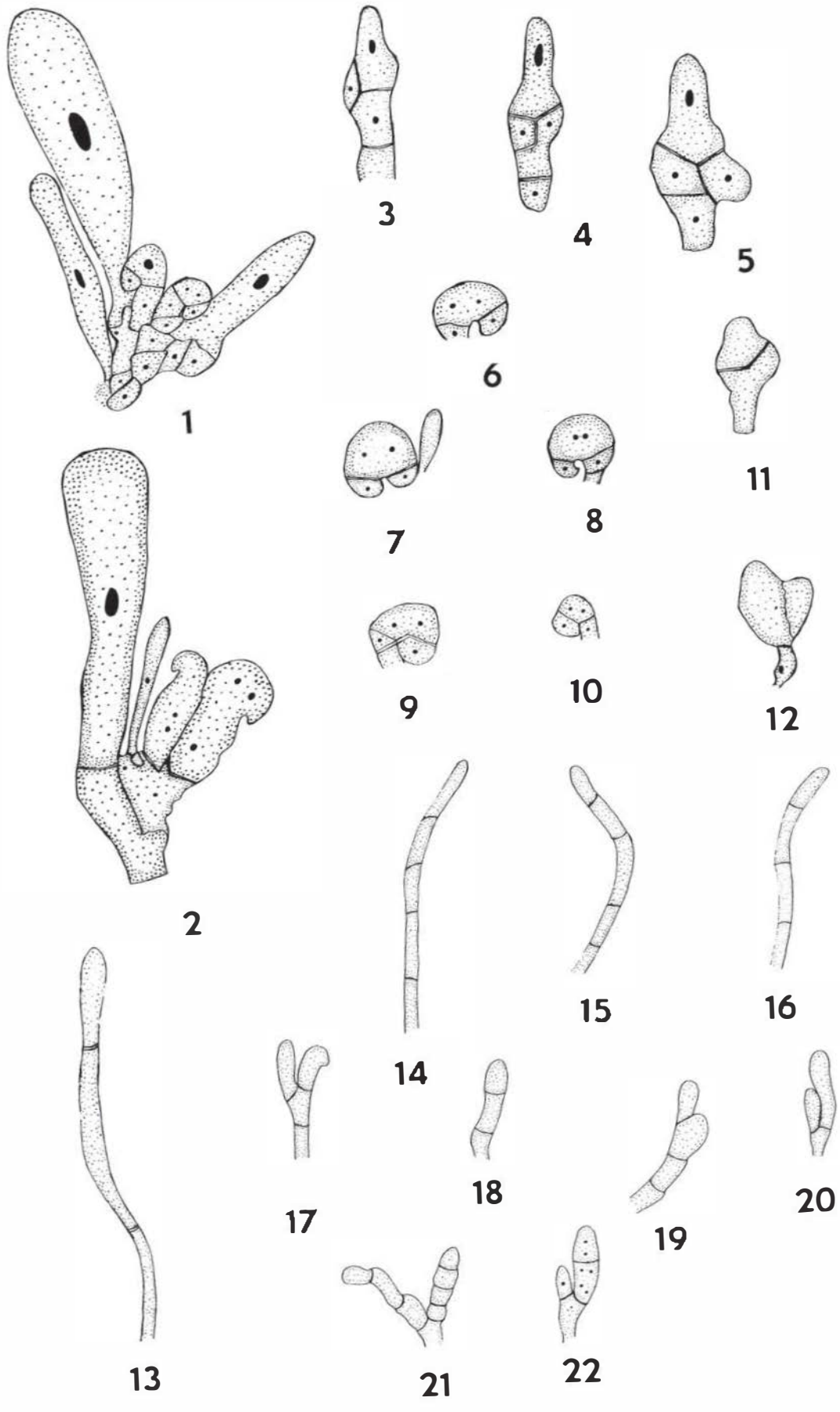
## APPENDIX B

### EXPLANATION OF PLATES

| | |
|---|---|
| Figures 1 - 2 | Ascogenous hyphae. Both figures show young asci and croziers. X750. |
| Figures 3 - 5 | Ascogenous hyphae. These figures show young asci. X750. |
| Figures 6 - 10 | Ascogenous hyphae. These figures show croziers. X750. |
| Figures 11 - 12 | Ascogenous hyphae. These figures show newly formed asci. X750. |
| Figures 13 - 16 | Septate paraphyses. X750. |
| Figures 17 - 22 | Cells in the centrum drawn from a squash mount of a perithecium. X750. |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## EXPLANATION OF PLATES

| | |
|---|---|
| Figures 23 - 24 | Young ascogonia. X750. |
| Figures 25 - 27 | Ascogonia. X750. |
| Figure 28 | Section of young perithecium showing the development of the ascogenous hyphae after the development of the paraphyses. X260. |

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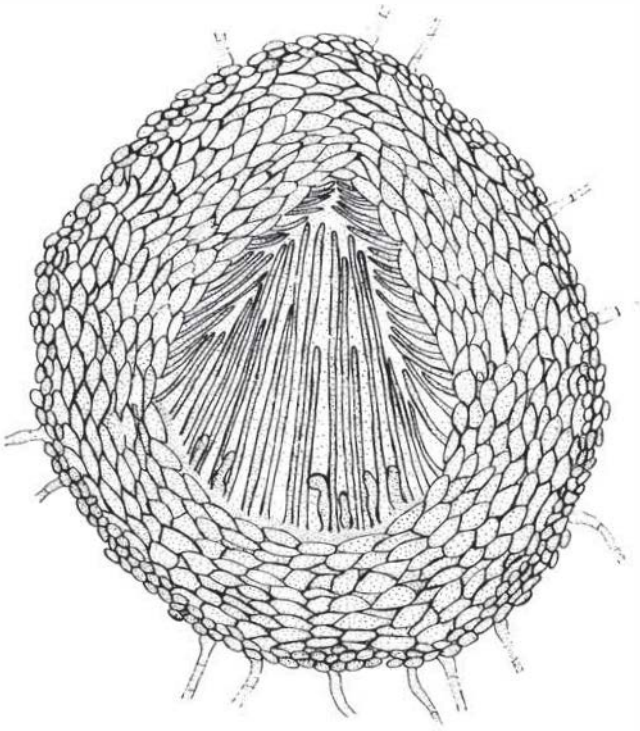

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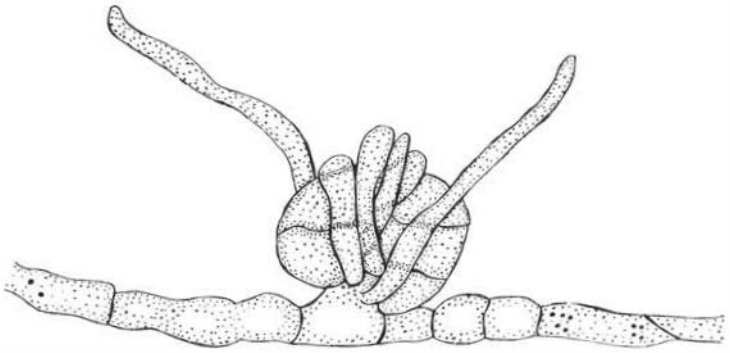

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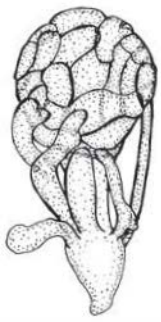

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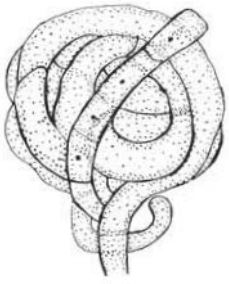

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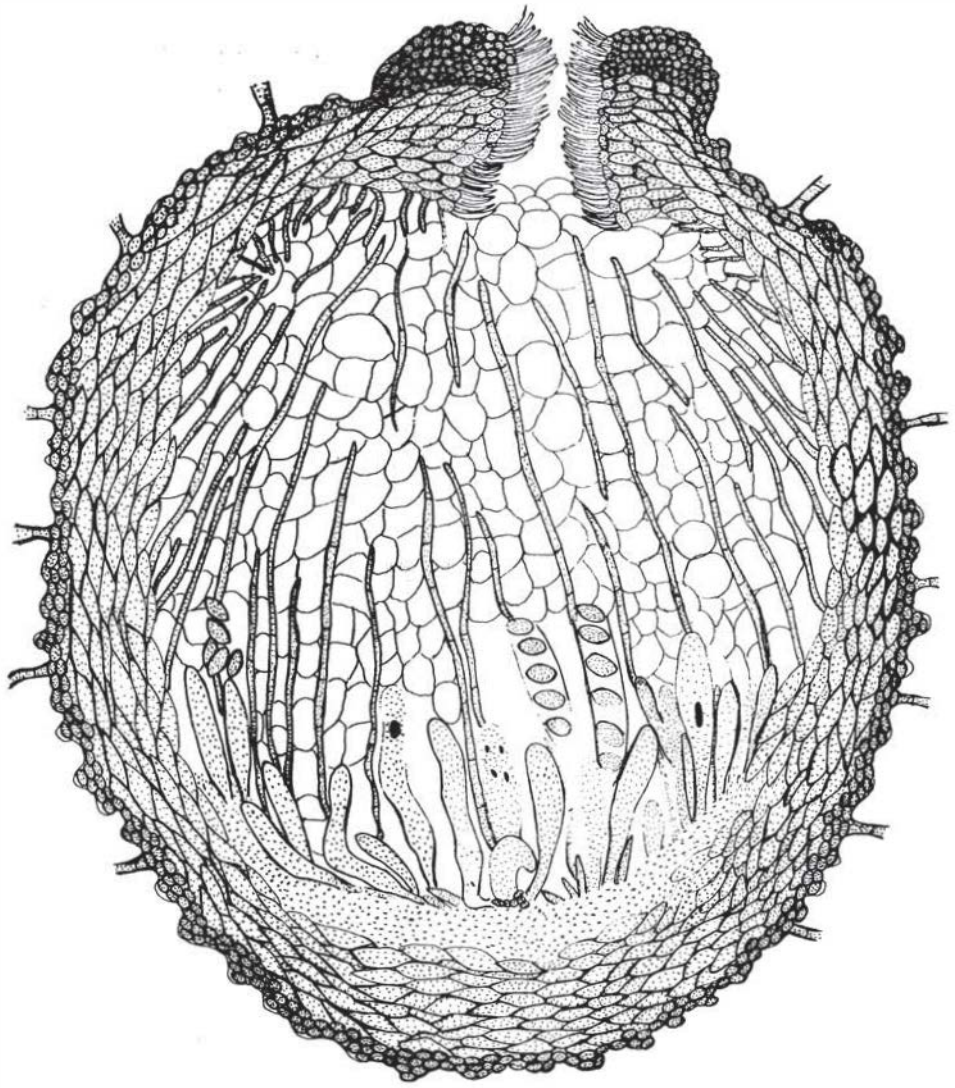
## EXPLANATION OF PLATE

Figure 29 — Section of mature perithecium showing the cells in the centrum and the asci. X216.



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