

MORPHOLOGY, DEVELOPMENT, AND ULTRASTRUCTURE  
OF *Termitaria snyderi* Thaxter

By

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Abstract of Dissertation Presented to the Graduate Council  
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*Termitaria snyderi* Thaxter is an entomophilous fungus growing on the exoskeleton of different species of termites. The fungus is an ectoparasite. Specialized thick-walled haustorial mother cells of the basal layer of the fungal sporodochium send haustoria into the integument of the host. The haustorial peg enters the host through the pore canals of the host cuticle.

The haustoria are lobed, uninucleate, surrounded by a thick wall and separated from the host protoplast by its plasma membrane. Along with usual organelles the haustoria contain certain vacuoles that are lysosomal in nature, and a new organelle, mini-microtubules that are only 80 to 100 Å in diameter and have six sub-units. The mini-microtubules are found in bundles free in the general cytoplasm, between the cisternae of smooth endoplasmic reticulum, between ER cisterna and plasma membrane or between ER cisterna and the nuclear envelope.

The only known means of reproduction is the formation of conidia that are cut off endogenously by the phialides at the

conidiogenous loci into long collarettes in basipetal succession. Phialides are closely clustered into a sporodochium. The conidia are catenate, cylindrical, uninucleate and hyaline.

It is proposed here that the conidia germinate to form a crust-like primary thallus that later, by the development of phialides over it, matures into a sporodochium.

The taxonomic position of *Termitaria* and its relationship with other imperfect fungi are discussed. Within the fungi imperfecti a new family and a new order, Termitariaceae and Termitariales respectively, are proposed to accommodate *Termitaria* and *Mattirolella*. A new species of *Mattirolella* based on the slide of *Termitaria* made by Thaxter is also described.

## CHAPTER I

### THE MORPHOLOGY AND DEVELOPMENT

In 1920 Thaxter described two new fungi growing on the exoskeleton of three species of termites, *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks) and *Nasutitermes costalis* (Holmgren), collected from Washington, D.C., Sardinia, and Granada respectively. He proposed a new genus *Termitaria* with two species, *T. snyderi* and *T. coronata*. Reichensperger (1923) reported a similar fungus growing on the exoskeleton of *Nasutitermes rippertii* (Rambun), *N. arenarius* (Hagen), *Cornitermes cumulans* (Kollar), *Hodotermes thompsoni* (Fuller) and *N. luziae*; the first three were collected from Brazil and the others from the Congo in Africa. He recognized them as *Termitaria thaxteri*. Colla (1929) added *Porotermes quadricollis* (Rambun) and *Neotermes hirtellus* (Silvesteri), both from South America, as hosts of *Termitaria snyderi* Thaxter, and *Nasutitermes corniger* (Motschulsky), *N. guayanae* (Holmgren) and *N. luzonicus* (Oshima) to those of *Termitaria coronata* Thaxter. Pickens (1952) mentioned another species *T. pacedensis* but no details were given.

Thaxter considered the fungus as an external parasite growing on the surface of the host without any indication of actual penetration by the parasite through the integument of the host. He reported that the fungus did not cause any inconvenience to the insect. However, Reichensperger (1923) reported deformation of the infected legs and antennae. Feytaud and Dieuzeide (1927) considered it an internal

parasite, and showed that the adipose tissue was invaded and altered by the mycelium. The cuticle was penetrated by a root-like pedicel and in the process the hypodermis and cuticle were altered, cells disappeared near the break, color became abnormal, and large amoebocytes were found. Heim *et al.* (1951) also thought that this fungus is an internal parasite which enters the body through ingestion or licking, invades the fatty tissues, and finally erupts to the exterior by breaking the exoskeleton of the host.

Thaxter (1920) considered *Termitaria* a Fungus Imperfectus and placed it in family Leptostromaceae, though he noted that its position there was an isolated one. Heim (1972, personal communication) thinks this fungus belongs to Tuberculariales of the Fungi Imperfecti.

In and around Gainesville, Florida, the subterranean termites, *Reticulitermes flavipes* and *R. virginicus* have *Termitaria snyderi* Thaxter growing on their exoskeleton. The rediscovery of *Termitaria* has provided an excellent opportunity to study and resolve many questions about its morphology, development, taxonomy, and mode of nutrition.

#### Materials and Methods

The termites were collected and the studies were done on naturally growing fungus. Different media were tried but the fungus could not be cultured. Methods for the studies are described in detail in the next two chapters.

#### Habit

The fungus grows on any of the body parts - antennae (Fig. 2), legs (Fig. 5), mandibles, head, thorax (Fig. 1), and abdomen (Fig. 3) -

the most common and most prominent infection being that of the abdomen. When growing on an antenna the fungus forms a crust that completely encircles it (Figs. 2, 15). Mature stages were not encountered on antennae.

*Termitaria* does not appear to disturb or irritate the host. Termites with multiple infections have been found to differ little in their activity from normal ones. However, when the legs and antennae are infected certain deformations result. The number of segments in antenna decrease (Fig. 2) and leg becomes swollen (Fig. 5). The abdomen may also swell but it is hard to detect because of its globose nature.

During the process of collection, two very important observations were made. The fungus could be collected year round, and only 6-10% of the members of a colony were found infected. Earlier workers have also reported the scarcity of the fungus in infected colonies (Thaxter, 1920; Reichensperger, 1923).

### Morphology

The mature fungus forms a lenticular to hysterooid sporocarp, spherical in outline or variously elongated depending upon the position of its growth. It is closely appressed to the cuticle of the termite and consists of a many-layered basal pseudoparenchymatous subhymenium from which firmly coherent, simple, parallel conodiophores arise vertically, forming an even hymenial surface (Fig. 7). The whole sporocarp is 70-80  $\mu$  thick, the subhymenium being 18-20  $\mu$  and the hymenium 50-60  $\mu$ . The size of the sporocarp ranges from 375 X 400 - 1000  $\mu$ . A thick-walled area divides the hymenium

into an upper conidium-containing layer and a lower conidium-free one. The upper zone is 22-25  $\mu$  thick and the lower 30-35  $\mu$ .

The peripheral cells are dark and thick walled and form a well defined, sterile rim or excipulum. The margin in contact with the substratum is spreading and only one cell thick at the tip (Fig. 17).

The basal layer is comprised of light-colored cells with dark-colored, thick-walled cells interspersed among them. The structure and arrangement of these cells can be easily understood by looking at a younger stage (Figs. 12, 14, 27). They appear pyriform when viewed from the top, and are often in shiny rows and have a hyaline spot in the center (Fig. 12). They were considered chlamydo spores by Thaxter (1920), Feytaud and Dieuzeide (1927), and Heim (1952). This study has shown that the so-called chlamydo spores are actually haustorial mother cells (for details please see Chapter III) that send penetration pegs into the integument through the cuticle. The hyaline spot in the center in fact marks the position of the penetration peg. The haustorium is lobed, uninucleate, the nuclei lying in the broad base, and has a very thick wall (Figs. 8, 9, 13). The host plasma membrane forms a sheath around the haustorium, thus at no time is the haustorium in direct contact with the host cytoplasm. The haustoria could not be traced beyond the basement membrane, thereby suggesting that the fungus is an ectoparasite.

The haustorial mother cell has been seen both with and without a nucleus, the former situation being rare. It appears as if, after the haustorium has reached a certain size, the nucleus of the

haustorial mother cell migrates to the haustorium. The haustorial mother cell is separated from the cell above by a typical ascomycete septum with Woronin bodies in the vicinity (Fig. 11) and is connected with the haustorium by a narrow neck that passes through the cuticle of the host (Figs. 9, 13).

The subhymenium is comprised of nucleate cells separated from each other by an ascomycete septum (Figs. 16, 18). These nuclei are bounded by a double membrane and have a well defined nucleolus. Mitochondria of different shapes with plate-like cristae are abundant. Endoplasmic reticulum of any kind is scarce. Ribosomes are free in the general cytoplasm. The plasmalemma is elaborated in plasmalemmasomes at various places (Fig. 16). In some sections the cells were found full of glycogen-like bodies (Fig. 18) while in others big globular structures were present inside the subhymenial cells (Fig. 16). The chemical nature of both could not be ascertained but they appear to be some kind of reserve food material. The big globular structures are often interconnected and are full of small light-and dark-colored globular bodies. These also have membranous structures inside that run from one to another globular structure through the connection (Fig. 16).

Closely packed phialides make up the hymenium. Halfway from the base of the phialide there is a conidiogenous locus where the wall is very thick and the conidia are cut off apically into a collarette (details in Chapter II). The conidiogenous loci of all the phialides lie at the same distance from the base creating a distinct thick walled area in the hymenium (Fig. 7). The

phialides are uninucleate, have ribosomes, mitochondria, and rough endoplasmic reticulum. The nuclei and mitochondria both are very elongated. Normally four conidia can be seen inside a mature collarette (Fig. 19). The conidia are  $3.5-4.5 \times 1.5-2 \mu$ , cylindrical, hyaline, thin walled and uninucleate (Figs. 19, 20). The tip of the phialide is very thick and does not appear to have a pore as has been reported earlier (Thaxter, 1920). Discharge of the conidia was not observed nor was any sporulating sporocarp collected. However, when a termite with mature fungus was brought into the laboratory the fungus was found covered with a white mass of spores the next day (Fig. 4). It appears as if the whole tip is blown off the phialide by the force applied by the formation of new conidia at the conidiogenous locus.

The cells of the excipulum are very thick walled, the outer surface as thick as  $2 \mu$ . Though the excipular cells in the hymenium are elongated like conidiophores and are separated from the underlying cells by typical septa as conidiophores are, they are sterile (Fig. 22). The cells of the excipulum are uninucleate, have long mitochondria with plate-like cristae, ribosomes free in the general cytoplasm, cisternae of endoplasmic reticulum and are bounded by a thick wall that is comprised of two layers, the outer dark and the inner light colored (Fig. 21).

The sporocarp is traversed by projecting bristles of the host. The fungus forms a protective filamentous sheath around it (Fig. 10).

The mature sporocarp is a composite structure. The hyphae appear to emerge at different places and then grow towards each

other (Fig. 6). I think that these places mark the position of original primary infection. As far as this study is concerned these have always been found lying in a furrow or the inter-segmental area (Figs. 9, 14) where no sclerotization occurs and the cuticle is very thin. All cells in the basal layer here are haustorial mother cells sending many haustoria all around. Because of its deep lying nature Feytaud and Dieuzeide (1927) called it "pedicule de l'hymenium" and Colla (1929) thought of it as a foot to anchor the fungus. Reichensperger (1923) also noted that the fungus was anchored with knob-like intrusions in the chitinous matrix.

#### Development

Because of the negative response of *Termitaria snyderi* to the media tried for its culture I had to rely completely on field collections for the study of its mode of infection, growth, and development.

It is assumed that the conidia, like the spores of some other entomogenous fungi, become thick walled and highly pigmented before germination. The cuticle of the termite proves to be a barrier to infection. Only the conidia that have come to lie in the thin intersegmental area appear to initiate the infection. Not all of the conidia can find their way to that spot and this perhaps is why such a small percentage of termites are found infected. Reichensperger (1923) has suggested earlier that the infection could occur at the time of molting when the chitin is still soft. This is still a possibility and in certain cases it

may be that the infection does start at that time. But the origin of primary infection in the intersegmental furrow strongly supports my assumption. The integument does not seem to be broken at the time of infection, suggesting that penetration is accomplished by some enzyme action. It is already known that certain bacteria and fungi have an enzyme system that can digest insect cuticle (Richards, 1951).

First, a group of thick walled cells is formed (Figs. 23, 24) and all of them send haustoria into the host. The hyphae originate from this mass of cells and diverge in all directions, keeping in tight lateral contact. The cell at the tip of the hypha is cut off from the rest by an anticlinal wall, and then divides in only one plane, periclinal (Fig. 26), thereby resulting in circumferential as well as radial growth. Thus, the margin of the growing thallus remains only one cell thick (Fig. 25). During the radial growth certain cells next to the cuticle of the host modify and become thick walled haustorial mother cells. It is not clear why one cell turns into a haustorial mother cell while the next one does not. There may be certain weak spots in the cuticle and only the cells opposite them become modified; or perhaps after reaching a certain size the fungus has to send haustoria; or it may be that the growth of the fungus is cyclic like that of certain fungi in agar culture, and haustorial mother cells are formed at the beginning of each new period of growth. Termites, like other arthropods, have minute ducts extending vertically through the procuticle. These are called pore canals and one might speculate that haustorial

passage occurs through them. By observing Fig. 13, where a pore canal and the haustorial path through the cuticle lie in the same vicinity, it can be seen that the haustorial passage appears to be at the original site of a pore canal. Thus, it is possible that only those cells that are opposite pore canals modify into haustorial mother cells. But the diameter of the pore canals is very small compared to that of the haustorial path and it may be suggested that after the initial penetration certain enzymatic activity widens the canal.

The young thallus is represented by Figs. 14 and 27. It forms a circular or variously elongated crust on the host surface. It is parenchymatous and a few cells thick (Fig. 25) depending upon its age. The margin is spreading, only one cell thick, and remains that way even after the thallus has matured into a mature sporocarp (Figs. 17, 26).

The intermediate stages showing the transformation of this crust-like primary thallus into a sporocarp have not been obtained, but it appears likely that after reaching a certain size and thickness the hyphal branches start to grow upward, very closely applied to each other. The tips of these hyphae become thick walled. These are now the conidiophores, which start cutting off phialoconidia apically in collarettes as discussed elsewhere (Chapter II) in detail. The peripheral cells become thick walled, remain sterile and make the excipulum. Thus, the whole thallus turns into a sporocarp.

*Termitaria* spp. were considered external parasites by Thaxter (1920), Reichensperger (1923), Colla (1929) and an internal parasite by Feytaud and Dieuzeide (1927) and Heim (1952). Thaxter did not see any actual penetration but saw hypertrophied cells of the host opposite the dark cells. By looking at his figures it is apparent that he was actually looking at the haustoria and he mistook them for hypertrophied host cells. Reichensperger actually reported that root-like threads emanate from the basal layer into the epithelium and either perforate the cells or encircle them. However, he did not realize the importance of the dark cells and did not regard the penetration pegs as haustoria. It is reported here for the first time that the dark cells are haustorial mother cells and that the fungus is a parasite and probably is nourished by the host. The sections of the sporocarps that were observed by light or electron microscopy did not show the presence of any hyphae in the host, only the haustoria that were uninucleate and did not have any septa, unlike the sporocarp above the cuticle. No haustoria were seen in underlying adipose tissue or anywhere beyond the basement membrane. The channels through which the haustoria go into the integument are very smooth and do not look as if they have been formed by eruption of the fungus emerging to sporulate, which should have been the case if the fungus was an internal parasite. *Termitaria snyderi* is an external fungus that penetrates into the host by haustoria.

The termites with *Termitaria* infection do not look nor behave abnormally. All the knowledge at hand suggests that the infection

is not lethal. But we also know that the infection of antennae and legs results in malformation and deformation. Perhaps at a certain stage of the life cycle the termites are susceptible and maybe the fungus is lethal. The parasitism of *Termitaria snyderi* has been established. The role of this fungus in biological control for termites awaits more extensive field and laboratory research. Rearing of termites in the laboratory is necessary to study the stage at which the termites are most susceptible, how much time it takes for the fungus to sporulate from the time of the germination of the conidia, whether the fungus kills the termites or not, and if it does how it does so.

The geographical distribution is another interesting aspect of the *Termitaria* life cycle. It does not appear to be influenced by climatic or environmental conditions of the area, but corresponds to the geographical distribution of the host and is such that almost all the host genera are infected.

The absence of, or our inability to discover, a sexual phase in the life cycle still leaves the taxonomic position of this unique fungus in doubt.

## PLATE I

- Figures 1-3. Termites showing the habitat of *Termitaria snyderi*.  
1. *Termitaria snyderi* growing on the thorax of the termite (arrow) X15. 2. growing on the antenna of the host (arrow ) X10. 3. growing on the abdomen of the host (arrow) X10.
- Figure 4. Sporulating *Termitaria* X20. Arrow points to the white mass of conidia covering the surface of the whole sporodochium.
- Figure 5. Whole mount of termite legs X20. Compare the infected leg with the uninfected one and notice the swelling and deformation of the former.
- Figure 6. A close-up of the basal layer of the mature sporocarp X100. Arrows indicate the multiple points of origin of infection, making the thallus a composite structure.

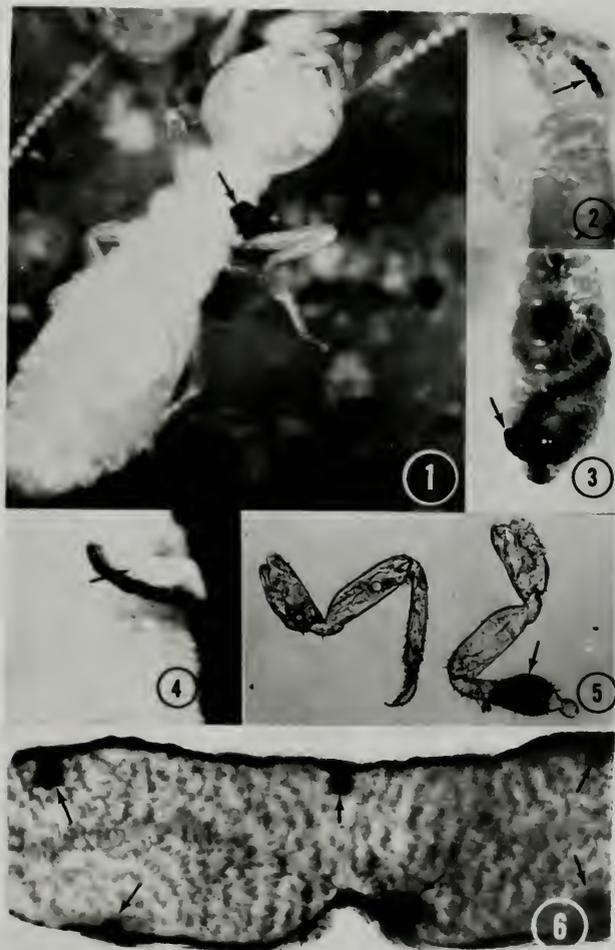


PLATE II

- Figure 7. Cross section of mature sporocarp of *Termitaria snyderi* showing basal layer (BL), subhymenium (SH), hymenium (HY), excipulum (E), and the bristle (B) of the termite cuticle passing through the sporocarp X1,000.
- Figure 8. Plastic section of the sporocarp showing the subhymenium (SH) outside and the haustoria (H) with distinct nuclei (N) inside the host X2,000.
- Figure 9. Plastic section of the sporocarp at the point of the origin of infection showing many thick-walled haustorial mother cells (HMC) and nucleate (N) haustoria (H) X2,000. One of the haustorial mother cells is connected with the underlying haustorium (arrow).
- Figure 10. Cross section of a portion of sporocarp X2,000. Bristle (B) of the termite cuticle passes through the subhymenium (SH), hymenium (HY) and is surrounded by the sterile filament (arrow points to one such filament).

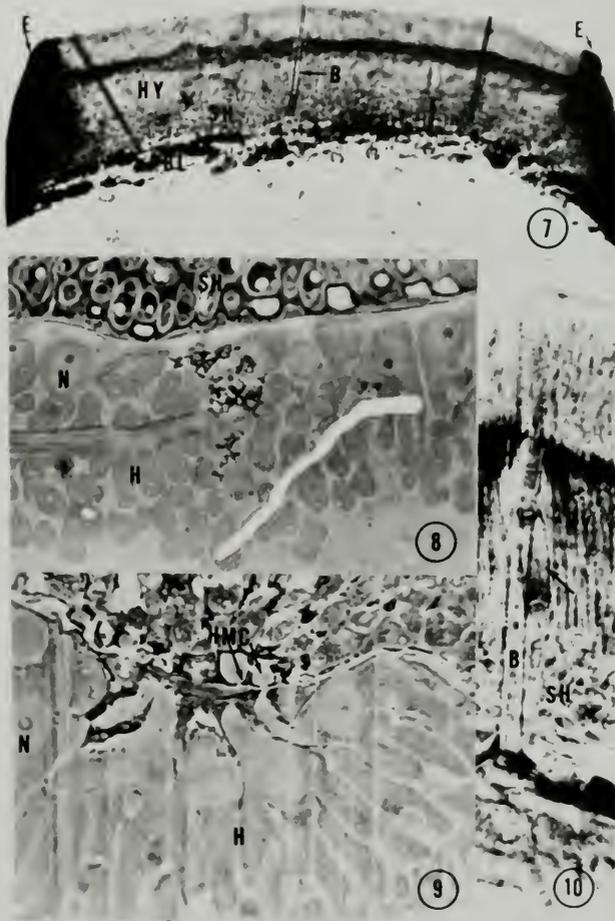


PLATE III

- Figure 11. Haustorial mother cell (HMC) sending a penetration peg into the substrate cuticle (CU) X22,500. The haustorial mother cell has endoplasmic reticulum (ER), mitochondria (M), a nucleus (N) and is separated from the cells of the subhymenium by a typical ascogone septum with Woronin bodies (W) in the vicinity.
- Figure 12. Light micrograph of haustorial mother cells with distinct hyaline spots in their centers X2,000.

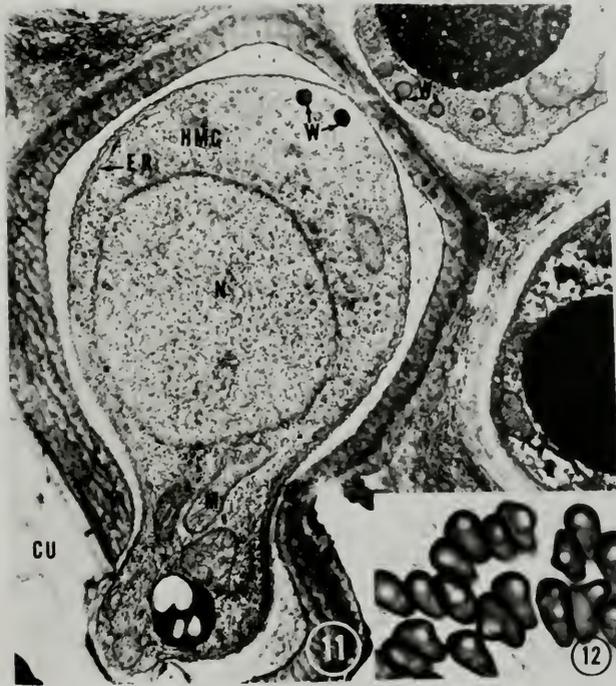


PLATE IV

- Figure 13. A near median section through the neck (n) that connects the haustorial mother cell (HMC) with the haustorium X14,100. The neck appears to pass through a pore canal, since its passage through the cuticle (CU) looks similar to the pore canal (PC). Haustorium has a thick wall (W) and contains a nucleus (N) with a single nucleolus (NU), mitochondria (M), and endoplasmic reticulum (ER).
- Figure 14. Young thallus of *Termitaria* growing on an antennal segment of the termite X200. It appears to originate at the base of the segment and grows upward in many directions. Dark haustorial mother cells (HMC) are very distinct.
- Figure 15. Cross section of the antennal segment showing it surrounded by the fungus crust X250. The bristles (B) of the termite cuticle are passing through the young thallus. Basal layer of the thallus is very dark and prominent.

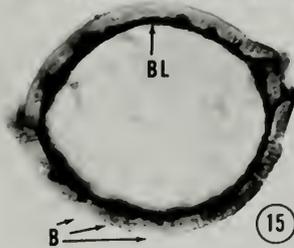
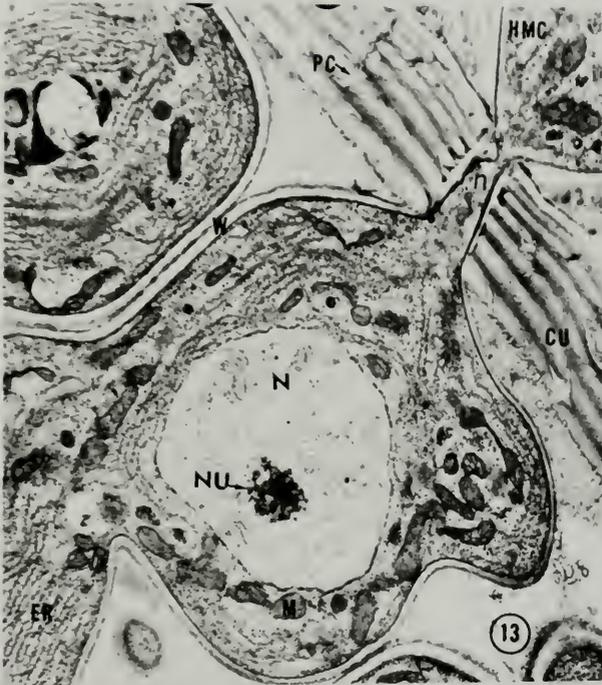


PLATE V

- Figure 16. Subhymenial cells of the sporocarp separated from each other by a typical ascomycete septum having a central pore (arrow head) and Woronin bodies (W) X33,000. Plasmalemma is forming plasmalemmasomes (PL). Subhymenial cells have mitochondria (M) with plate-like cristae, ribosomes in general cytoplasm, and globular structures (GS) that have membranes in them that sometimes run from one globular structure to another (arrow).
- Figure 17. Saggital section through the margin of mature sporocarp X2,000. Note a single cell at the tip.



PLATE VI

Figure 18. Excipular region of the sporocarp showing subhymenial layer and the base of the hymenial excipulum X15,000. The cells are filled with glycogen-like bodies (G). Some of the subhymenial cells have vacuoles (V), globular structures (GS) and one of the cells has a nucleus (N). One subhymenial cell is connected to two sterile hymenial cells of the excipulum with ascomycete septa having central pores (arrows) and Woronin bodies (W).

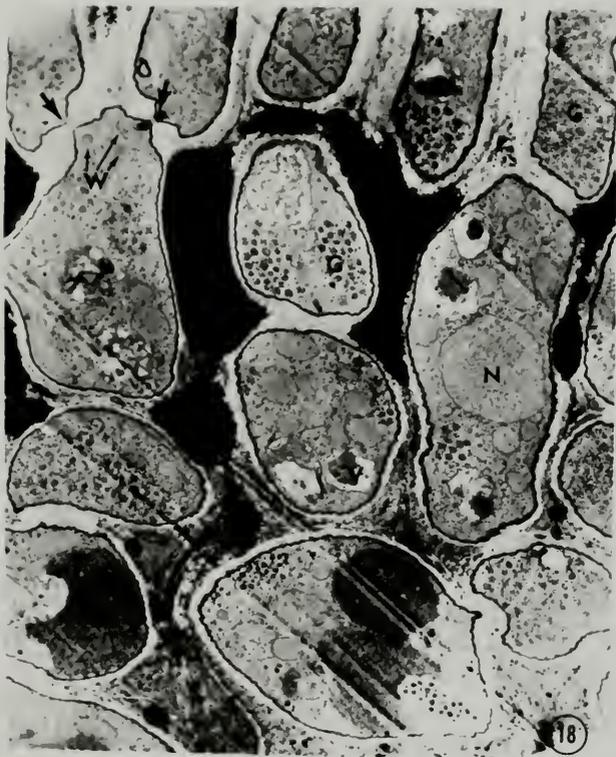


PLATE VII

- Figure 19. This section of the hymenium showing conidiophores (CP) and the excipulum (E) X3,000. The former cut off conidia (C) with nuclei (N) into the collarettes (CO) at the conidiogenous loci (CL).
- Figure 20. Whole mount of conidia X1,800.

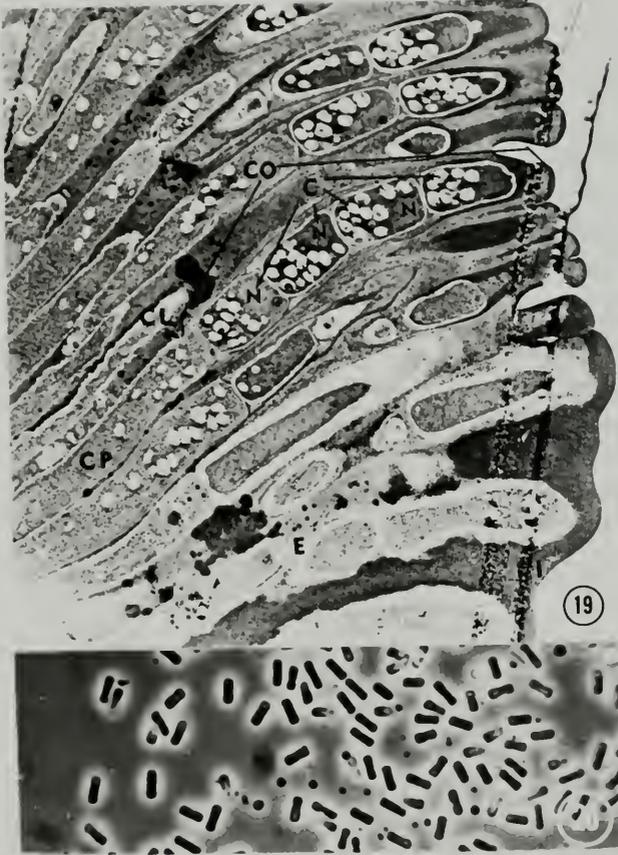
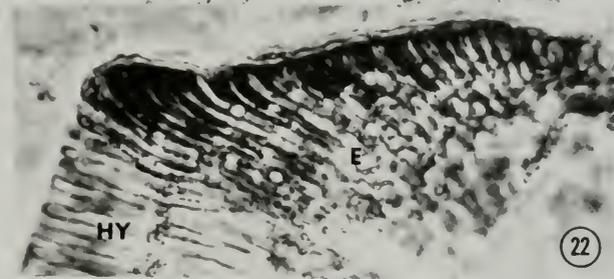


PLATE VIII

- Figure 21. Thin section of the excipular cell of the sporocarp X37,500. The cell is thick walled, has a nucleus (N) with a nucleolus (NU) and mitochondrion (M).
- Figure 22. Cross section of the excipular region of sporocarp showing thick-walled cells in the excipulum (E) and a portion of the hymenium (HY) X2,000.



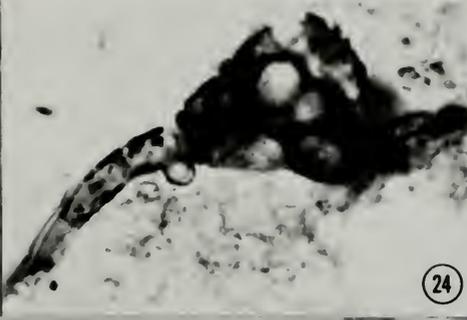
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22

## PLATE IX

- Figure 23. A section of the very young thallus comprised of only a few thick-walled cells growing on the host cuticle (CU) X2,000.
- Figure 24. Whole mount of a very young thallus comprised of thick-walled cells that has just started sending hyphae around itself X2,000.
- Figure 25. Cross section of a young thallus a few cells thick in height growing on the host cuticle (CU) and sending haustoria (H) into the integument from its haustorial mother cells (HMC) X2,000. Spreading margin is only one cell thick (arrow).
- Figure 26. Surface view of the growing margin of the young thallus X2,000.
- Figure 27. Whole mount of a young *Termitaria* thallus viewed from the top X250. Neatly arranged filaments originate from a group of thick-walled cells (arrow) in all directions. Dark haustorial mother cells (HMC) are arranged in rows.



## CHAPTER II

### CONIDIOGENESIS

Hughes (1953) emphasized that "morphologically related imperfect states can be brought together when the precise methods of conidium origin take first place in the delimitation of the major groupings." Since then considerable attention has been focused on the structure of conidiophores and the mode of conidial formation (Tubaki, 1958 and 1963; Subramanian, 1962). Hughes classified the Hypomycetes into eight large sections, each based upon different mechanisms of conidiogenesis. In section IV of his classification he included those Hypomycetes that have "conidia developing in rapidly maturing basipetal series from the apex of a conidiophore which may or may not possess an evident collarette." *Termitaria snyderi* belongs in this section. Hughes restricted the term "phialide" to those unicellular structures which are usually terminal but sometimes intercalary as well, on simple or branched conidiophores. They are oval to subcylindrical to flask shaped or subulate, often with a well differentiated basal swelling and a narrower distal neck, with or without a terminal collarette. From the apex of each phialide develops a basipetal succession of phialaspores without an increase in the length of the phialide itself. This section corresponds to the Phialasporae of Tubaki (1963) and Tuberculariaceae of Subramanian (1962).

Cole and Kendrick (1969) studied the phialides of *Phialophora lagerbergii* (Melin and Nannf.) Conant, *Penicillium corylophilum* Dierckx and *Thielaviopsis paradoxa* (De Seynes) von Höhnelt by time lapse photomicrography. In all three they found a fixed endogenous meristem responsible for conidium formation. The conidiophore ceases elongation once the meristem becomes active and its outer wall is ruptured at the apex by the emerging first conidium or its initial. In each case a portion of the conidiophore wall remains above the meristematic zone and acts as a collarette through which the conidium or its initial protrudes. They defined the phialide as "a sporogenous cell with only one functioning, fixed, endogenous meristem whose position is marked by the deposition of an inner or secondary wall which surrounds each of a basipetal succession of physiologically independent conidia. Spore production incurs no concomitant increase or decrease in the length of the sporogenous cell." At the recent Kananaskis conference on Taxonomy of Fungi Imperfecti (Kendrick, 1971) the phialide was defined as "a conidiogenous cell in which at least the first conidium initial is produced within an apical extension of the cell but is liberated sooner or later by the rupture or dissolution of the upper wall of the parent cell. Thereafter, from a fixed conidiogenous locus a basipetal succession of enteroblastic conidia is produced, each clad in a newly deposited wall to which the wall of the conidiogenous cell does not contribute. Any phialide wall distal to the conidiogenous locus is the collarette. The length of the phialide does not change during the production of succession of conidia."

### Materials and Methods

The subterranean termites *Reticulitermes* spp. bearing typical *Termitaria* lesions were collected from the woods around Gainesville, Florida. The lesions were removed along with the adjoining integument, under 2.5% buffered (Na-Cacodylate pH 7.35) gluteraldehyde and fixed for four hours at 3°C. The material was washed in buffer and post-fixed in buffered 1.5% osmium tetroxide overnight in the refrigerator. After several rinses in water it was dehydrated in a graded series of ethanol and in the end washed in reagent grade acetone. It was infiltrated with graded acetone-plastic mixtures and finally embedded in 100% plastic. Mollenhauer's (1964) plastic mixture #2 was used (62 ml Epon 812, 81 ml Araldite 506, 3-4 ml Dibutyl phthalate; 15 ml of this mixture, 10 ml DDSA and 45 drops of DMP 30 to make the final plastic mixture). For better penetration the material in the mixture was put on a shaker for eight hours, at every change during the graded acetone-plastic mixture series. Bubbles in the plastic were removed in vacuum at 60°C. Polymerization of the plastic was carried out in an oven at 60°C for three days.

The material was stained for two hours in 2% UAc in 70% ethanol during the dehydration and post-stained in 0.5% aqueous UAc for 45 minutes and with lead citrate for 30 minutes. Some grids were stained with methanolic UAc as described by Stempak and Ward (1964).

The sections were cut on a Porter-Blum MT-2 ultramicrotome with a diamond knife and examined with a Hitachi-HU 11 E electron microscope. Some half-micron sections were cut and stained with

AMB (Juniper *et al.*, 1970) for light microscopy. Cryostat sections of the sporocarp were cut without fixation and stained with aniline blue in Hoyer's. Some whole lesions were mounted in Hoyer's and aniline blue, crushed, and observed under the light microscope.

The conidia are cylindrical  $3.5 - 4.5 \mu \times 1.5 - 2 \mu$  (Figs. 30, 31, 32, 41). The conidial wall is composed of two distinct layers, an outer thin electron dense layer and an inner thick electron transparent layer (Figs. 31, 41). Like other fungal spores the conidium surface is smooth. Conidia are uninucleate, the nucleus is bound by a double-membraned nuclear envelope. Usual organelles such as mitochondria, rough endoplasmic reticulum and ribosomes are present (Figs. 31, 43, 48). Many lipid droplets are also present. In certain conidia the droplets look interconnected (Fig. 43). Unusual vacuoles have been seen in some conidia (Fig. 31).

The conidiophores are long, cylindrical, vertically parallel in a sporodochium (Fig. 29). They appear to be fused laterally with each other, giving a honeycomb appearance when viewed from the top. The conidiophores are  $55 - 65 \mu$  long and  $2.0 - 2.5 \mu$  wide, with a very thick and rounded tip (Fig. 40). Less than half way down from the tip is the conidiogenous locus (Fig. 28). The part of the conidiophore is constricted at the locus forming a very short neck (Figs. 34, 37, 43). The conidiophore wall in the region of the collarette consists of two layers, an outer more electron dense, and inner more transparent layer. We could not differentiate the layers in the rest of the conidiophore wall. At the base of the collarette there is a pad of wall material between the outer and

inner layers of the wall (Fig. 47). The apparent thickening in the neck is actually the inner wall layer of the conidiophore that has been sectioned obliquely as is evident by the similarity in electron density. The plasmalemma of the conidiophore is highly convoluted forming paramural bodies (Marchant and Robards, 1968) all along the length of the conidiophore. Sometimes connections can be seen between the vesicle of paramural body and the plasmalemma (Fig. 35).

The conidiophore is separated from the vegetative cell underneath by a septum perforated by a septal pore. There are darkly staining Woronin bodies near the septum (Fig. 42). The Woronin bodies are bounded by a unit-membrane and have a granular matrix. Plasmalemmasomes have been seen projecting into the protoplast of the cell underneath the conidiophore (Fig. 39).

Each conidiophore is uninucleate, has mitochondria, endoplasmic reticulum, ribosomes and vesicles (Figs. 33, 34). The nuclei and mitochondria are very elongated. Each nucleus is delimited by a double membrane and has a conspicuous nucleolus at one end. The nuclei of all the conidiophores are situated halfway from the conidiogenous locus, and we found all of them in interphase stage even though in many cases conidium initials were forming at the loci (Fig. 28).

The conidium initial buds out at the locus into the collarette. The wall of the conidium initial appears to originate within the neck of the conidiophore (Fig. 47). After the conidium initial has reached a certain size and cell organelles have moved into it, a delimiting septum starts growing centripetally near the

conidiogenous locus. The septum has a central electron-transparent layer between two electron dense ones (Fig. 47). At the rim of the growing septum an electron-dense material is present (Figs. 45, 46). The septum appears to become functionally complete by continued centripetal growth (Figs. 44-48). Most of the septum grows at the conidiogenous locus except for a central pore that remains open until the new conidium directly underneath is fully grown (Figs. 36, 38). The thickness of the conidium wall does not increase noticeably during its formation. However, after it has become delimited by the septum an inner electron-light wall layer is deposited that grows in thickness as the conidium matures (Fig. 41). The young conidium is connected with the new initial through a central pore in the septum (Figs. 36, 38, 41). After the septum is complete the electron-light layer of the septum appears to break down, allowing the two halves of the septum to separate and the conidium to secede (Figs. 47, 48). In rare cases we have seen Woronin bodies near the growing septum (Figs. 36, 46, 47). The shape of the young conidium is determined by the tubular collarette and the constricted conidiogenous locus. The young conidium is typically narrow at both ends (Fig. 43) because the septum at both ends originates at the constricted conidiogenous locus.

From the above observations we reach the following conclusions:

1. The conidia are produced in basipetal succession from a fixed conidiogenous locus.
2. The conidia are clad in an entirely new wall not derived from any existing layers of the conidiophore wall.

3. There is no increase in the length of the active conidiophore i.e. from locus to the base of the conidiophore.

#### Conidium Initiation

I am aware of only three reports of the type of Phialoconidiogenesis where the conidia are produced in a tubular collarette. Two of them are of species of *Thielaviopsis*. Cole and Kendrick (1969) have studied phialoconidium ontogeny of *T. paradoxa* (De Seynes) von Höhnelt by time lapse photomicrography, and Del Vecchio *et al.* (1969) of *T. basicola* by light and electron microscopy. The conidiogenesis of an Indian isolate of *T. paradoxa* was studied by Seshadri as reported by Subramanian (1971).

According to Cole and Kendrick (1969) the phialide of *T. paradoxa* is long and cylindrical. One to several conidia differentiate basipetally within the conidiogenous cell. During early stages of differentiation growth of the outer wall of the conidiogenous cell and the conversion of the protoplast into conidia proceeds simultaneously. The center of activity (conidiogenous locus) continues to move downward until the conidiogenous locus is fixed. The phialide stops growing and the continued production of the conidia at the fixed locus exerts a pressure at the tip of phialide resulting in the rupture of the wall thereby releasing the conidia. The cylindrical portion of the phialide beyond the locus is a collarette. The locus is not marked by any constriction or other morphological evidence of the phialide wall. The conidiophore does not elongate after its apex has ruptured. Seshadri's (Subramanian, 1971) work confirmed Cole and Kendrick's

(1969) findings. Moreover, Subramanian (1971) reported the conidia did not appear to be delimited by a process of septation or double septation in the Indian isolate of *T. paradoxa*.

DeI Vecchio *et al.* (1969) described phialides of *T. basicola* enclosed inside "hyphal tubes." They contained endoconidia and were composed of cells which were always devoid of cytoplasmic components. The walls of these cells were never attached to the walls of the endoconidia and were identical to the cell walls of the vegetative hyphae. We think that they misinterpreted the tubular collarette as a hyphal tube. It becomes clear by looking at their Fig. #1 Plate 1 that the conidiogenous locus is deep seated, is not marked by any constriction of the phialide wall, and the conidia are released by the rupture of the phialide tip.

Subramanian (1971), by looking at the reports of Cole and Kendrick (1969), DeI Vecchio *et al.* (1969), and an Indian isolate of the *T. paradoxa*, suggested that the events of conidiogenesis are as follows. The first step in the development of each conidium appears to be the protoplasmic cleavage at the conidiogenous locus within the phialide, followed by development of a totally new wall around the cleaved mass. This process goes on in production of several conidia in basipetal succession.

Our findings agree with those of the above in the presence of tubular collarettes, the wall of the conidia being separate from that of collarettes, the phialide wall not contributing to the wall of the conidia, there being no increase in the length of the phialide after its apex has ruptured, and that the conidia are produced in basipetal succession.

As opposed to the findings of others, the conidiogenous locus in the *Termitaria snyderi* phialide is marked by a constriction in its wall. It appears as if after reaching a certain stage the conidium initial buds out through the thick walled tip of the phialide but the phialide wall is not broken (Fig. 49 a and b). Thus, a conidiogenous locus is established, marked by a constriction. The conidia are produced in basipetal succession from this locus (Figs. 41, 49 c-e). The wall of the conidium appeared to originate at the locus as has also been reported for *Neurospora crassa* by Lowry *et al.* (1967), for *Verticillium albo-atrum* by Buckley *et al.* (1969), for *Penicillium claviforme* by Zachariah and Fitz-James (1967) and for *Metarrhizium anisopliae* by Hamill (1972a). The growth of the wall of the phialide and the formation of conidia proceed simultaneously during early stages. However, we presume that after a while the phialide stops growing and the pressure thus exerted at its tip results in the rupture, releasing the conidia. After that, no increase in the length of the phialide takes place. We were unable to see the formation of the first conidium initial as well as rupture of the phialide, because the fungus does not grow in culture and we could not collect it in those two stages of development. However, Thaxter (1920) has reported that the conidia are released by the rupture of the phialide tip.

The growth of phialides and formation of conidia looks quite synchronized as is apparent in Fig. 28, where the conidiogenous loci of all the phialides appear to be formed at more or less the same level, resulting in a clear cut zone in the sporodochium. All

the phialides are of the same height and most of them have four conidia. This type of synchronous development of adjacent phialides has also been reported by Trinci *et al.* (1968) for *Aspergillus giganteus* Wehmer.

The mode of conidial delimitation is also different from that of *T. paradoxa* and *T. basicola*. It is by double septation. The septum grows centripetally and for quite a while has a central pore which may or may not have Woronin bodies in the vicinity. The septal pore closes by centripetal growth. We have not seen Woronin plugs in *Termitaria* like those reported by Cole and Aldrich (1971) for *Scopulariopsis brevicaulis* (Sacc.) Bain. However, the septum is similar to the septum reported for *S. brevicaulis* by Cole and Aldrich in that it has an electron transparent layer sandwiched between two electron dense layers (Fig. 47). Hamill (1972b) reported a similar type of septum in *Doratomyces nanus* but as the conidiogenesis continued the electron transparent layer underwent a transition and became more electron dense than the other two. Buckley *et al.* (1969, Fig. 12) also show the same construction of the basal conidium septum of *Verticillium albo-atrum*, an electron transparent layer between two electron dense ones.

The dividing septum undergoes lysis along its axis as has also been reported for budding of yeast *Rhodotorula glutinis* by Marchant *et al.* (1967a, Fig. 21).

Thus, phialoconidiogenesis of *Termitaria snyderi* Thaxter differs from that of *Thielaviopsis paradoxa* and *T. basicola* in the following very important details: (1) the conidiogenous locus is fixed in the

beginning; (2) it is marked by a constriction in the phialide; and (3) the conidia are delimited by a double septum.

Fletcher (1971) reported that the conidial chains of three different species of *Penicillium*, *P. clavigerum* Damelius, *P. claviforme* Bainier and *P. corymbiferum* Westling, were enclosed within an electron opaque surface layer that appeared continuous with the surface layer of the phialide wall after gluteraldehyde and OsO<sub>4</sub> fixation. He did not say whether he considered it as a collarette or not.

#### Growth of Conidiophores

Several schemes have been proposed to explain the phenomenon of apical growth. According to Bracker (1967), Grove *et al.* (1970a and 1970b), McClure *et al.* (1968), cytoplasmic vesicles are present to the exclusion of other organelles in the very tip of the hyphae. Grove *et al.* (1970b) reported that the single cisternae Golgi play an important part in the vesicle formation. The vesicles are formed posteriorly, migrate to the apex, fuse with the plasma membrane, and liberate their contents as part of the process of growth. However, Marchant *et al.* (1967a) found two different vesicular systems associated with wall synthesis in regions of active growth and wall synthesis. In the apical region of the hyphae vesicles produced by the endoplasmic reticulum moved to the plasma membrane, fused with it, and were responsible for the primary wall formation. In the older region of the hyphae multivesicular bodies were found that fused with the plasma membrane and gave rise to lomasomes and were apparently associated with secondary wall synthesis. It was

questioned whether all the lomasome-like bodies were homologous in structure and function. Therefore, Marchant *et al.* (1968) proposed later that all the membranous or vesicular structures associated with the plasma membrane be called paramural bodies, that the word lomasome be limited to the structures derived from cytoplasmic vesicles or multivesicular bodies, and that all structures derived from the plasmalemma be called plasmalemmasomes. They also suggested that lomasomes might be involved in the incorporation of wall precursors by transport across the plasmalemma in the same way as the single vesicles originating from ER and Golgi do.

The paramural bodies have also been regarded as artifacts of fixation and their existence in live material and their role in wall synthesis has been questioned. Recently, evidence that the paramural bodies may not be artifacts comes from works of different people. Griffiths (1970) found them in frozen etched replicas of hyphae of *Verticillium dahliae* Kleb. Heath *et al.* (1970) demonstrated their presence by using three types of fixations in the wall of the growing hyphae of *Saprolegnia ferax* (Gruithuisen) Thuret and in the walls of primary spores and their exit papillae in *Dictyochus sterile* Coker. They also used SITS stain which is claimed to bind to specific sites in plasmalemma. After incubation in SITS stain a strong pale blue fluorescence was detected in all hyphae, most intense at their apices, sometimes occurring in diffuse patches, the patches marking the sites of plasmalemmasomes. They also suggested that the plasmalemmasomes are produced when more plasmalemma is produced than is needed to line the cell wall and the plasmalemmasomes may become sequestered in the developing wall.

The paramural bodies have been reported in *Verticillium albo-atrum* at the double septum by Buckley *et al.* (1969), and in *Aspergillus nidulans* in the subapical and basal cytoplasm of growing sterigmata and near their developing septa by Oliver (1972). Multivesicular bodies have been seen in *Metarrhizium anisopliae* by Hammill (1972a) at both sides of growing delimiting septa when wall material was being deposited, which suggests that they may have played a role in its deposition.

The cytoplasmic vesicles have also been reported associated with conidiogenesis. Trinci *et al.* (1968) reported the presence of vesicles in some sections inside phialide primordia. At the time of bud formation in *Rhodotorula glutinis*, Marchant *et al.* (1967a) reported vesicles in the bud at the time of active wall synthesis.

Carroll (1972) found fibrillar material and masses of gray and speckled substances associated with plasmalemmasomes at particular stages of conidiophore and conidium development and near growing septa of *Stemphylium botryosum*. She supported the conclusions of Marchant *et al.* (1968) that plasmalemmasomes may be involved in secondary transformation of wall materials.

In *Termitaria snyderi* Thaxter phialides the paramural bodies are present all along the plasmalemma. However, here the tip of the growing conidium initial is filled with the vesicles (Fig. 10, 16, 18, 19). The vesicles are seen only in the vicinity of the growing delimiting septum and only at that stage. After the septum is complete in the old conidium initial they are not seen. Their location and timing suggest that they may be involved in the synthesis of the septum.

I agree with Heath and Greenwood (1970) that plasmalemmasomes are produced when more plasmalemma is formed than is needed to line the cell wall. I suggest that at the time of conidium production the convoluted plasmalemma stretches and covers the initial and helps in the formation of its first wall. I have not seen any fibrillar or gray or specked substances associated with the plasmalemmasomes.

PLATE X

- Figure 28. Cross section of a portion of the mature sporocarp stained with AMB X2,000. It shows the haustoria (H) beneath the cuticle (CU) and the subhymenium and hymenium above it. The basal layer has many thick walled haustorial mother cells (HMC). The conidiophores (CP) have elongated nuclei (N) and prominent conidiogenous loci (CL) all of which lie at the same distance from the base of the hymenium making a very distinct line across the hymenium.
- Figure 29. A mature sporocarp on the dorsal side of the abdomen of a termite (arrow) X20.
- Figure 30. Whole mount of conidia under phase contrast X2,000. Lipid droplets are distinctly visible.
- Figure 31. Thin section of a conidium inside the collarette showing outer (OL) and inner (IL) layers of conidial wall and lipid droplets (L), vacuole (V) and nucleus inside the conidium X28,000.
- Figure 32. Whole mount of conidia under bright field X2,000.



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PLATE XI

- Figure 33. Thin section through the conidiophores X15,000. Elongated nuclei (N) with nuclear envelope (NE) and nucleolus (NU) are present in the conidiophores. Surface view of the plasmalemma of two conidiophores shows plasmalemmasomes (PL).
- Figure 34. Thin section through the apical region of the conidiophores X15,000. Conidiogenous loci (CL), elongated mitochondria (M) and rough endoplasmic reticulum (ER) are evident.
- Figure 35. Plasma membrane (PM) of the conidiophore with plasmalemmasomes (PL) X88,000. Arrows point to the connections between plasma membrane and the plasmalemmasomes. Cisterna of rough endoplasmic reticulum (ER) is lying close by.

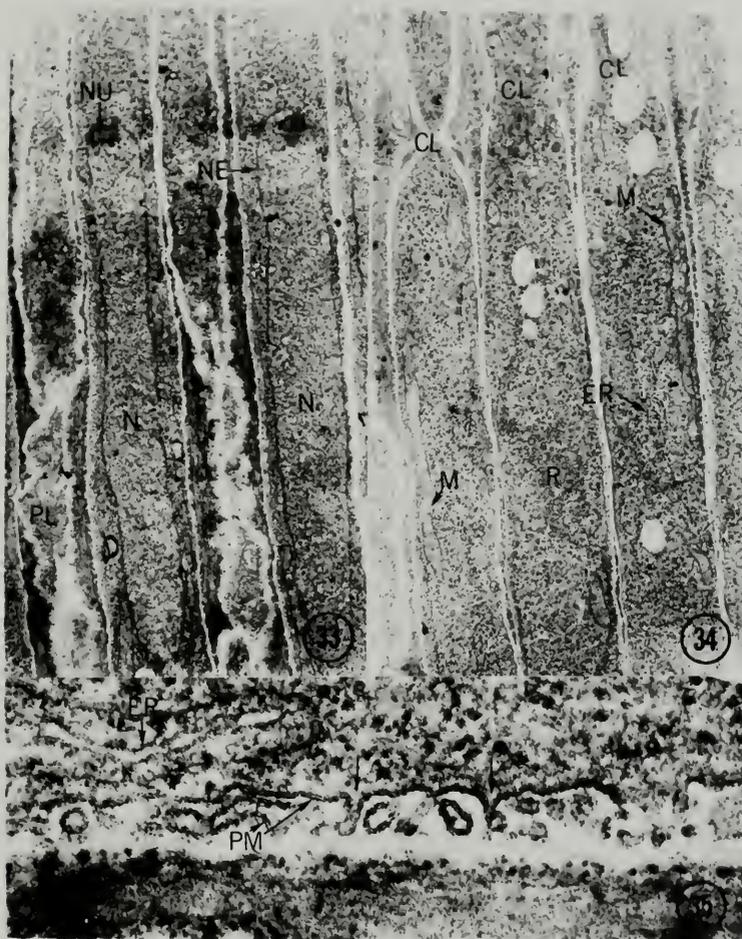
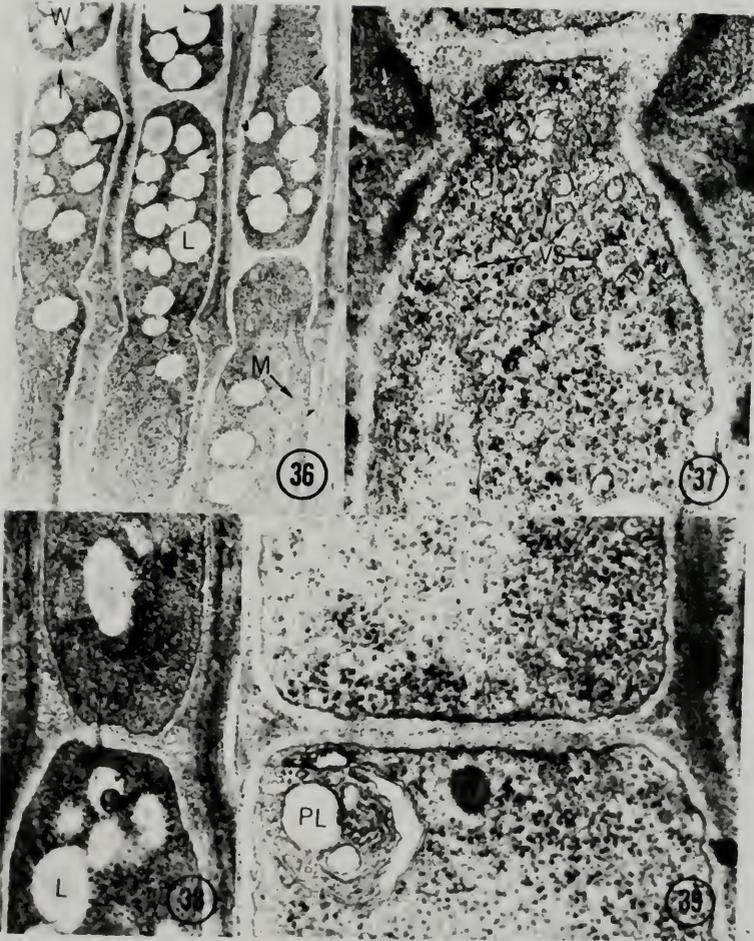


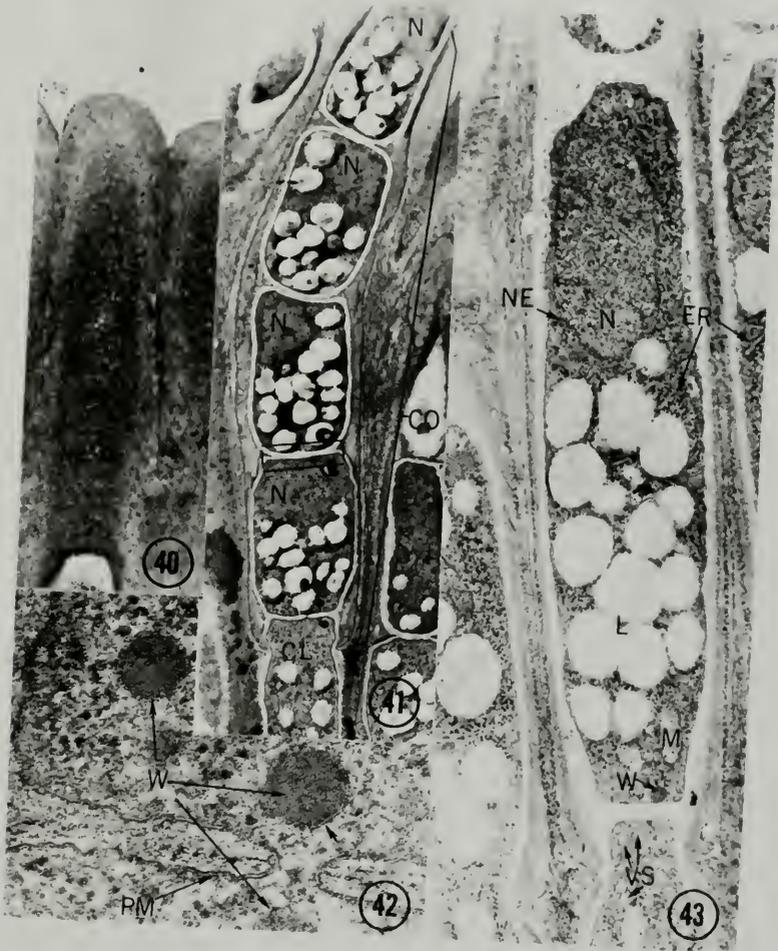
PLATE XII

- Figure 36. Thin median section through the conidiogenous loci showing budding and growing conidia X15,000. Arrow points to the connection between the budding and maturing conidium. Mitochondria (M), lipids (L) and a Woronin body (W) are also there.
- Figure 37. Thin section through the tip of the conidiophore filled with vesicles (VS) X62,000.
- Figure 38. Thin section of young and maturing conidia inside the collarette X40,000. Arrow points to the connection between the two conidia. Note that the conidial wall is separate from the wall of the collarette. Lipids (L) are present inside the conidia.
- Figure 39. Thin section through the septum between the conidiophore and the cell underneath X52,000. A big plasmalemmasome (PL) connected with the plasma membrane is projecting into the cell cytoplasm and a Woronin body is present.



### PLATE XIII

- Figure 40. A near median section through the tip of the collarette X19,200.
- Figure 41. A near median section through the conidiogenous locus (CL) and the collarette (CO) having conidia in different stages of maturity X3,500. The conidia are uninucleate (N).
- Figure 42. A thin section of the septum at the conidiophore base X94,000. Arrow points to the unit membrane around the Woronin body (W). Plasma membrane (PM) is continuous around the edge of the septum towards the pore.
- Figure 43. Thin section through a youngest conidium of the chain X24,500. The conidium has a nucleus (N) bounded by a nuclear envelope (NE), rough endoplasmic reticulum (ER) mitochondria (M) and lipid droplets (L) that are interconnected. Apex of the conidiophore is filled with vesicles (VS). A Woronin body (W) inside the conidium is also present. Note that the young conidium tapers toward both ends.



#### PLATE XIV

Figures 44-48.

Approximately median sections of the conidiophores and the collarettes representing different stages in the formation of double septum and secession of conidia.

44. Young septum originating at the conidigenous locus X28,000. Notice a mitochondrion (M) between conidium and the conidiophore. Lipid droplets (L) and rough endoplasmic reticulum are present in the developing conidium. 45. Later stage in septal growth X30,000. The tip of the conidiophore is filled with vesicles (VS). Arrow points to the electron dense material. 46. Slightly later stage than the previous one in the centripetal growth of the septum X52,800. Arrows point to the electron dense material at the rim of the growing septum. Vesicles (VS), Woronin bodies (W) and lipid droplets (L) are also there. 47. Fully developed double septum with a central pore X40,000. Black arrow points to the electron transparent layer of the septum while the white one indicates the point of conidium wall formation. A granular material is present between the inner (il) and outer (ol) layers of collarette wall. Lipid droplets (L), Woronin bodies (W), and rough endoplasmic reticulum (ER) are also there. 48. Conidial secession X48,000. The arrow points to the place of secession—the electron transparent layer of the double septum.

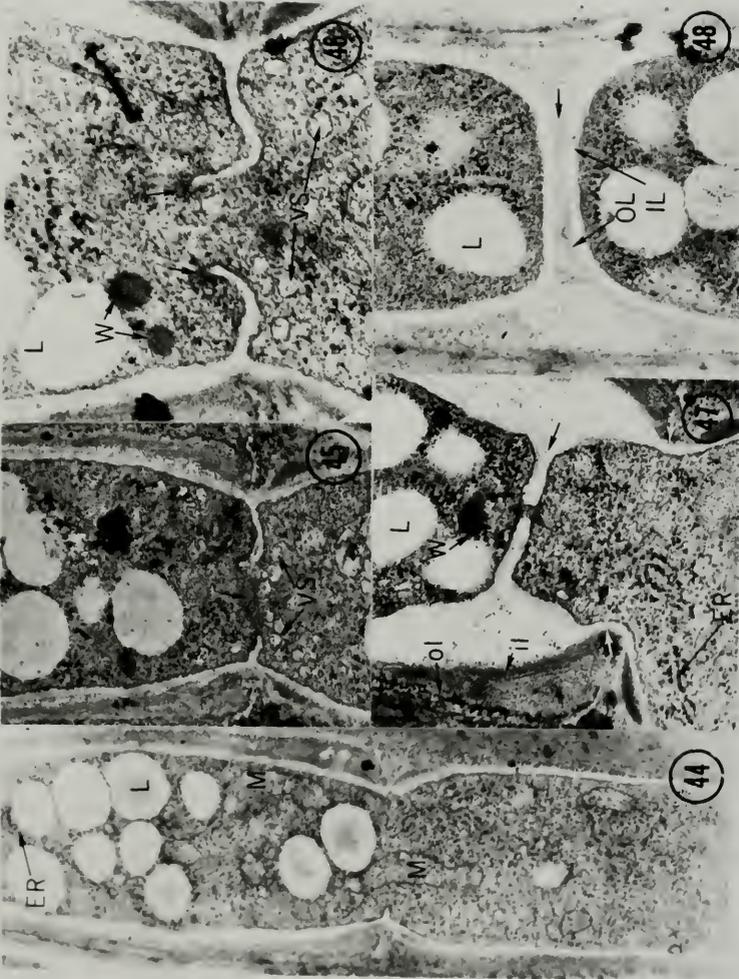
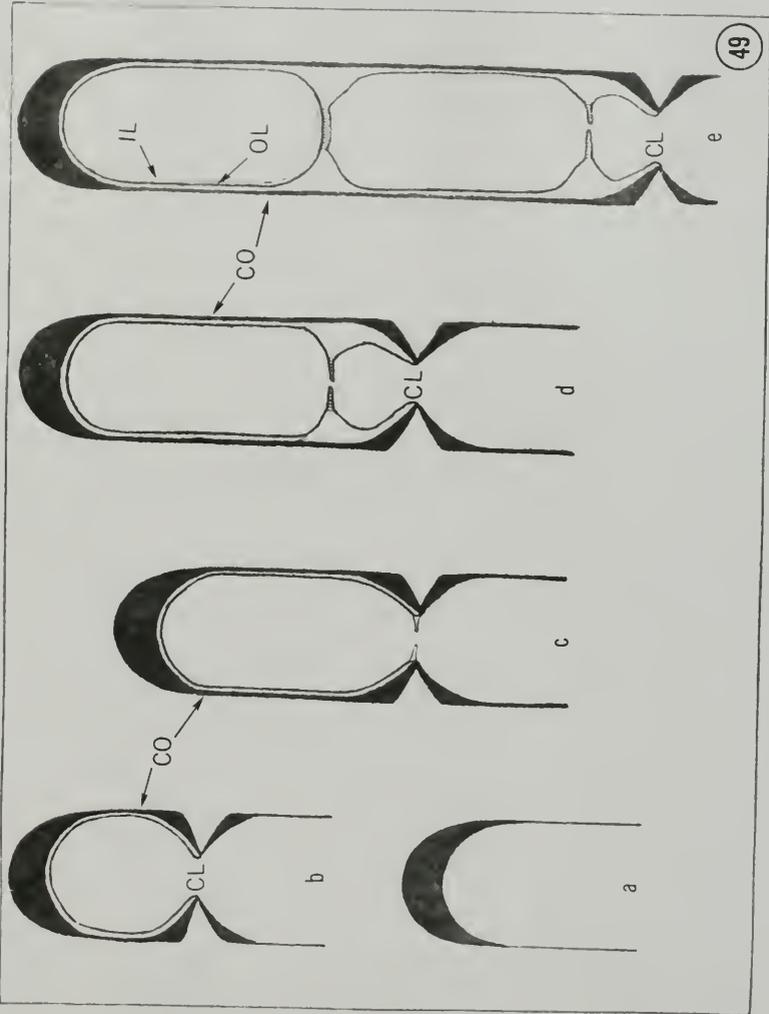


PLATE XV

- Figure 49. Diagrammatic interpretation of sequence of conidiogenesis. a. conidiophore tip. b. budding of first conidium and formation of conidiogenous locus (CL) and the collarette (CO). c. septum initiation at the base of the conidium. d. budding of second conidium, first septum is complete except for a central pore. e. first conidium is seceded from the one underneath, a new inner layer (IL) is being deposited inside the outer one (OL) of the conidium wall, a third conidium is starting to bud out at the conidiogenous locus (CL).



CHAPTER III  
HAUSTORIAL MECHANISM

Studies about fungal haustoria inside the insect hosts are scarce. Richards' work about *Herpomycetes* infecting cockroaches is on the light microscope level. However in recent years a number of workers have published about the haustorial structure inside plant hosts (Hawker, 1964; Bracker, 1967; Ehrlich and Ehrlich, 1971). A generalized concept of the host parasite interface, with a few exceptions, has emerged. A haustorial mother cell, in most cases thick walled, is present. The haustorium and the haustorial mother cell are connected by a narrow neck. The fungal wall is continuous around the neck, an exception being *Albugo candida* haustoria which lack part of the wall around the distal end of the neck (Berlin and Bowen, 1964). Haustoria are uninucleate in all cases except for *Albugo candida*, *Phytophthora infestans*, *P. parasitica*, *Pseudoperonospora cubensis* (Ehrlich and Ehrlich, 1971). The haustoria are surrounded by a sheath (Encapsulation or Zone of Apposition) and are separated from the host protoplast by a membrane that is continuous with the host plasma membrane.

In this study the haustoria of the entomogenous imperfect fungus *Termitaria snyderi* Thaxter infecting the subterranean termites *Reticulitermes* spp. were investigated. The fungus forms lenticular to hysteroiid sporocarps on the exoskeleton of the host

irrespective of the body position and is found closely appressed to the cuticle. The basal layer of the sporocarp is comprised of thin-walled cells interspersed with thicker-walled, bigger, dark-colored cells. These cells act as haustorial mother cells.

#### Materials and Methods

The subterranean termites *Reticulitermes* spp. bearing typical *Termitaria* lesions were collected from the woods around Gainesville, Florida. The lesions were removed along with the adjoining integument under 2.5% buffered (Na-Cacodylate pH 7.35) glutaraldehyde and fixed for 4 hours at 4°C. The material was washed in buffer and post-fixed in buffered 1.5% osmium tetroxide overnight in the refrigerator. After several rinses in water it was dehydrated in a graded series of ethanols and in the end washed in reagent grade acetone. It was infiltrated with graded acetone-plastic mixtures and finally embedded in 100% plastic. Mollenhauer's (1964) plastic mixture #2 (62 ml Epon 812, 81 ml Araldite 506, 3-4 ml Dibutyl phthalate; 15 ml of this mixture, 10 ml DDSA and 45 drops of DMP - 30 make the final plastic mixture) was used. For better penetration the material was put on a shaker for eight hours at every change during the graded acetone-plastic mixture series. Bubbles in the plastic were removed in vacuum at 60°C and the plastic polymerized at 60°C for three days.

The material was stained for two hours in 2% uranyl acetate in 70% ethanol at room temperature during the dehydration and post-stained in 0.5% aqueous uranyl acetate for 45 minutes and with lead nitrate for 30 minutes, on the grids. Some grids were stained with methanolic uranyl acetate as described by Stempak and Ward (1964).

The sections were cut on a Porter-Blum MT-2 ultramicrotome with a diamond knife and examined with a Hitachi-HU 11E electron microscope.

To determine acid phosphatase activity the *Termitaria* lesions were fixed in glutaraldehyde as described above. After fixation the material was rinsed in Na-Cacodylate buffer (7.35 pH) four times, 15 minutes each. Then it was incubated in a modified Gomori's Medium (Barka and Anderson, 1962) at room temperature for 30 minutes at pH 5. After incubation it was washed in Na-Cacodylate buffer at pH 7.35. It was later post-fixed in 2% osmium tetroxide buffered at 7.35 overnight at 4°C. After that it was dehydrated in a graded ethanol series and embedded in an Epon-Araldite mixture as described earlier. The sections were cut, stained and observed in similar manner as for general haustorial study. Control experiments included incubation at pH 7.0, incubation without  $\beta$ -glycerophosphate and incubation without lead nitrate.

Periodic acid silver stain (PAS) (Martino and Zamboni, 1967) was used to test for the presence of polysaccharide. Four different aqueous solutions were made: 2% periodic acid, 3% hexamine, 5% silver nitrate, 5% borax. The staining solution was prepared shortly before use by mixing 23 ml of hexamine, 25 ml of  $\text{AgNO}_3$  and 4 ml of borax. The solution was centrifuged at 2,000 rpm for 30 minutes. Grids containing sections were transferred to the surface of periodic acid solution for 15 minutes at room temperature. Later they were rinsed twice with water and were floated over the staining solution at 60°C for 30 minutes. After staining, the grids were floated individually upon hypo for 30 seconds and

later rinsed in water. Some grids containing sections were stained without prior oxidation by periodic acid.

The haustorial mother cell is very thick walled (Fig. 50, 52, 53). The cell wall is distinctly divided into two zones - the outer dark colored and the inner light colored. The inner light-colored zone may be comprised of many layers. The haustorial mother cell is anucleate, has mitochondria, lipid bodies, vacuoles, cisternae of smooth and rough ER, ribosomes and microbodies. Very rarely some of the haustorial mother cells are uninucleate (Fig. 50). The nucleate haustorial mother cells were not found connected to the haustoria and it is difficult to say whether the haustoria belonging to them were nucleate or not, because serial sectioning could not be done, since sections of this material tear very easily. The mother cell is separated from the adjacent fungal cell by a typical ascomycete septum having Woronin bodies in the vicinity. The mother cell is connected to the haustorium underneath the cuticle through a neck and its inner wall layer is continuous around the neck to the haustorium (Fig. 52, 53). Mitochondria, unique 80 Å microtubules, normal microtubules, and ribosomes were seen in the neck region. The hole in the cuticle through which the neck runs is very smooth. Often at the base of the haustorium there is a collar of some granular material (Fig. 52) in which no membranous structure could be resolved. The electron density of the collar is the same as that of the innermost layer of endocuticle.

The haustorium is a branched structure having its only nucleus in the base. The nucleus has a nucleolus and is surrounded by a

membrane envelope (Fig. 53, 54, 55). Certain membranous structures are present in the nucleoplasm.

The wall of the haustorium is two layered with the outer being darker than the inner layer and being PAS positive (Fig. 53, 62). The inner layer of the wall is continuous with the inner layer of the haustorial mother cell wall.

The haustoria have not been found in direct contact with the host protoplast but are separated from the host protoplast by a membrane that can be traced to the host plasmalemma.

The haustoria displace the epidermal cells and make room for themselves (Fig. 54), thereby resulting in the localized swelling of the body part the fungus is growing on. The fungus evidently does not penetrate beyond the basement membrane of the host integument.

The older haustoria appear to be hanging in the space they created for themselves, and are seen in close contact with the host protoplast only apically (Fig. 54). However, the younger haustoria may be surrounded by the host plasma membrane. In older haustoria the space between the haustorium wall and the membrane separating it from the host is filled with fibrous material (Fig. 56). This material is PAS negative (Fig. 62) suggesting that it is not a polysaccharide. I tried pronase digestion but due to certain technical problems could not reach a conclusion. However, I think that it is proteinaceous in nature. In the young haustoria either there is no such space (Fig. 59) or a very small one. The host cells around the haustoria have the usual organelles:

mitochondria, ribosomes, endoplasmic reticulum, nuclei, microtubules. The most common organelles closest to the haustoria are the microtubules.

The haustoria have mitochondria, microtubules, microbodies, endoplasmic reticulum, lysosomes, vesicles, multivesicular bodies, and nuclei. The haustorial plasmalemma may form plasmalemmasomes projecting into the cytoplasm of the haustorium (Fig. 56).

Autophagic vacuoles have been seen with ER, mitochondria (Fig. 72), and lipid (Fig. 71) inside. The autophagic vacuoles or lysosomes are surrounded by a unit membrane, and are filled with debris and vesicles.

Both smooth and rough endoplasmic reticulum are present. Smooth endoplasmic reticulum is stacked and its cisternae are continuous with those of rough endoplasmic reticulum. Stacks of smooth ER have been observed completely filling the center of the haustorium. Between the cisternae of the stacked smooth endoplasmic reticulum very thin microtubules only 80-100 Å in diameter are present (Fig. 66, 67). From now on these microtubules will be referred to as mini-microtubules, because of their smaller diameter, as distinct from the normal microtubules. Mini-microtubules appear to be thrown out to the periphery of the ER stacks (Fig. 64). In many cases mini-microtubules have been seen in bundles with or without ER associated with them (Fig. 65, 68). They have also been found between ER cisternae and the nuclear envelope (Fig. 61), and smooth ER and plasmalemma of the haustorium (Fig. 60). Single mini-microtubules have not been seen. At higher

magnification these tubules appear to have subunits, most probably six (Fig. 68, 69). Use of Markham *et al.* (1963) image reinforcement technique gives the strongest reinforcement at  $n = 6$ ,  $n$  being the number of equal arcs of a complete circle used in making multiple exposures of the image. The mini-microtubules have been seen running the entire length of the stacked smooth ER cisternae. They appear to be a different organelle than the microtubules with 13 subunits. The stacked smooth ER cuts off vesicles at its periphery (Fig. 67). Acid phosphatase activity has been noticed over the smooth ER stacks (Fig. 70).

The haustoria are packed with mitochondria which have plate-like cristae. Some of the mitochondria have a peculiar shape, very narrow in the middle as if they are undergoing division (Fig. 53, 57). Some very long mitochondria have been observed (Fig. 55). Microbodies have been seen in the vicinity of mitochondria (Fig. 63).

During the study an atypical haustorium was seen. This haustorial mother cell had a large amount of rough endoplasmic reticulum. Towards the periphery of the rough ER stacks, closed spheres of rough ER appeared, with ribosomes inside (Fig. 51). Between the vacuole and the ER, mitochondria could be seen. The cytoplasm looked very diffuse and mitochondria stained differently (Fig. 58). The plasmalemma of the haustorium appeared to be invaginating at many places. The invaginations contained vesicles. Free vesicles and multivesicular bodies were also present in the cytoplasm. Thus, the haustorium had all the organelles that other normal haustoria had except mini-microtubules. This haustorium was separated from the host protoplast by the host plasma membrane.

At the site of original infection, which normally lies deep in the intersegmental area of the host exoskeleton where the cuticle is very thin, a huge number of haustoria are sent by the mother cells. The haustorial neck is rarely visible. There is a dark staining substance between the bases of adjacent haustoria (Fig. 54).

Two entomogenous fungi have been shown sending haustoria into the body of their hosts, *Herpomyces* spp. on cockroaches (Richards and Smith, 1956) and *Stigmatomyces certaophorus* on lesser housefly *Fannia canicularis* (Wisler, 1968). Richards and Smith suggested that the penetration of the host in the case of *Herpomyces* is accomplished by enzymatic activity. The same appears to be the case here. The hole through which the neck of the haustorium runs is very smooth and clean and there appears to be no distortion of laminae of the termite cuticle. This suggests that the cuticle was not penetrated by pressure alone.

From the above observations it becomes clear that the haustoria of the *Termitaria* *guyleri* Thaxter are not very different from the general fungal haustoria (Berlin and Bowen, 1964; Bracker, 1964, 1967, 1968; Coffey *et al.*, 1972; Ehrlich and Ehrlich, 1962, 1963a, 1963b, 1966, 1971; Fraymouth, 1956; Hardwick *et al.*, 1971; Hawker, 1965; Littlefield and Bracker, 1970, 1972; McKeen *et al.*, 1966; Peyton and Bowen, 1963). There is a thick walled haustorial mother cell that is connected with the main body of the haustorium through a neck. The haustorium is packed with mitochondria and endoplasmic reticulum, suggesting a high rate of physiological activity. No direct contact between the host protoplast and the haustorium

exists. There is always a membrane that separates the two. This membrane is continuous with the host plasma membrane.

However, in finer details the haustoria here are very different from all others so far reported. The major difference is due to the structure of the host. The cells of termite epidermis, like other animal cells, do not have rigid cell walls. The haustorial mother cell is not in contact with the host cell at all. The neck in this case passes through the integument of the termite and not the host cell wall. The haustorium does not hang in the cytoplasm of many epidermal cells. Thus the sheath around the haustorium is bounded by the plasmalemma of as many cells as come in contact with it. As reported above, in some cases the host plasma membrane is very close to the haustorium while in others a space exists between the two and in still others no contact between the two can be seen. The latter condition is common in older haustoria. It appears as if with age the haustoria push the epidermal cells aside and make room for themselves. A similar situation has been reported for *Herpomyces* spp., using the light microscope, where the basement membrane of the integument has been seen bulging out into the body cavity (Richards and Smith, 1956).

There is some controversy and confusion concerning the nomenclature for structures associated with haustoria. I do not want to add to this; therefore, I am not proposing any new terms. I use the term haustorial sheath as was suggested by Bracker (1967) and is presently being used by Bracker (1968), Littlefield and Bracker (1970, 1972), and Coffey *et al.* (1972). However, I would

like to re-emphasize that haustoria of *Termitaria snyderi* are not sheathed like other haustoria and that unlike others (Littlefield and Bracker, 1970) the neck is not bounded by an extrahaustorial membrane. The collar is occasionally present at the base of the haustorium but unlike other haustoria does not surround the neck.

A dark-staining ring of wall material occurring midway between proximal and distal ends of the haustorial neck in sections stained with uranyl acetate and poststained with lead citrate has been reported for several rust fungi (Ehrlich and Ehrlich, 1971; Hardwick *et al.*, 1971; Coffey *et al.*, 1972; Littlefield and Bracker, 1972). It has been suggested that the neck ring represent an abrupt transition from the wall of the penetration peg to the wall of the haustorium (Littlefield and Bracker, 1972). I was unable to see any such band in the wall of the haustorial neck.

The wall of the haustorium is continuous all around without any interruptions. Channels extending through the haustorial wall providing physical continuity between the haustorial protoplast and the boundary of the sheath as observed by Ehrlich and Ehrlich in *Puccinia graminis tritici* (1963, 1971) have not been seen.

The haustoria of *Termitaria snyderi* are unique in having microtubules. Similar structures have not been observed in fungi or in any other group of animals or plants. Newcomb (1969) has recently reviewed the literature concerning plant microtubules. Ordinary microtubules are 180-300 Å in diameter. They have an electron lucent core about 100 Å in diameter bounded by an electron opaque cortex or wall about 70 Å thick. They are separated by a space

of about 200 Å or more from each other suggesting that each microtubule may be surrounded by a specialized zone. They appear to be rather rigid unbranched structures usually following a straight path. The wall of plant microtubules consists of thirteen filamentous subunits (Ledbetter and Porter, 1964).

Steer and Newcomb (1969) have reported some 290 Å and 560-660 Å tubules in bean leaf glands. They appear to be different from typical microtubules chemically. The smaller tubules have been observed connected to the endoplasmic reticulum and they first appeared in the perinuclear cytoplasm between the layers of endoplasmic reticulum. Mini-microtubules bear closest resemblance to the tubules of P-protein bodies found in the phloem of the higher plants. Cronshaw and Esau (1967, 1968) observed tubules in the P-protein bodies of the sieve elements of *Cucurbita maxima* and *Nicotiana tabacum*. They called the tubular protein P1-protein. The tubules were 231 Å in diameter in *N. tabacum* and 242 Å in *C. maxima*. They also found fibrillar protein in the P-protein bodies that replaced the tubular protein and assumed that the tubular form becomes reorganized into the fibrillar form. The fibrillar form of the protein was designated as P2-protein. The P1-protein tubules appear to have sub-units and have a central non-staining core. Parthasarathy and Mühlethaler (1969) reported that in *N. tabacum* the protein tubule wall consists of 6 nearly spherical units of 60-70 Å. The mini-microtubules are much smaller in diameter; only 80-100 Å across. They also have a central nonstaining core and peripheral subunits. The number of subunits

is most probably six. Outside the haustorium in the sheath of older haustoria a fibrillar material resembling the P2-protein of the phloem is present. The mini-microtubules are most commonly found between the tubular cisternae of stacked smooth endoplasmic reticulum. They have, however, been seen in bundles in the general cytoplasm also. The cisternae of the stacked smooth ER are continuous with the cisternae of the rough ER. I propose that the proteins are synthesized in the rough ER with the aid of ribosomes and are transferred to the smooth ER. These proteins are polymerized and mini-microtubules are thus formed between the cisternae of the stacked smooth ER. The newly formed mini-microtubules are forced out to the periphery and later into the general cytoplasm. Fibrillar material in the sheath of the haustorium might be the product of depolymerization of mini-microtubule protein, suggesting some kind of exchange between the host and the sheath.

The one atypical haustorium of *Termitaria snyderi* which I observed is very different from those of other fungal parasites. Its protoplast is diffuse. Unlike other sister haustoria it does not have mini-microtubules. It looks as if it is being eaten. The increase in the number of invaginations of the plasmalemma which are unlike plasmalemmasomes may represent some kind of increased activity there. These invaginations may mark some kind of pinocytotic activity. Wheeler *et al.* (1972) have recently reported pinocytosis in root cap cells. Pinocytosis on the host parasite interface has been proposed by Ehrlich and Ehrlich (1963a) and Berlin and Bowen (1964). Other workers have also reported aberrant haustoria

(Berlin and Bowen, 1964; Littlefield and Bracker, 1972). The necrotic haustoria were completely walled off from the host cytoplasm by a material continuous with the host cell wall. In termites there are no cell walls so the aberrant haustorium might be cut off by a physiological barrier that results from the host activity.

The other departure from normal in *Termitaria snyderi* haustoria is the presence of lysosomes that have not been reported so far in other parasites. Autophagic vacuoles with mitochondria, ER, ribosomes and lipid droplets in them are commonly seen. There are other big vacuoles with vesicles in them that exhibit acid-phosphatase activity. The haustoria are packed with multivesicular bodies in the vicinity of the lysosomes. The vesicles inside look similar to the ones cut off on the periphery of the stacks of smooth ER cisternae. Though these multivesicular bodies have not been seen fusing with the lysosomes, they may be carrying lysosomal enzymes. It has already been suggested that the vacuolar system of plants may represent the lysosomes, and that lysosomal enzymes are associated with ER-derived microsomal vesicles (Matile, 1969). It is possible that the haustoria of the *Termitaria snyderi* are getting different kinds of food from the termite than the haustoria inside a plant host thereby making lysosomal activity necessary. By using EM autoradiography Ehrlich and Ehrlich (1970) have observed transfer of  $^{14}C$  from *Puccinia graminis tritici* uredospores to wheat host cell.

The haustoria of *Termitaria snyderi* are the first of its kind studied. More work is needed to ascertain whether this

type of haustorial structure is unique or is shared with other external entomogenous parasites. Further study of the haustoria of this fungus is also required to know more about the nature of mini-microtubules and of parasitism in general.

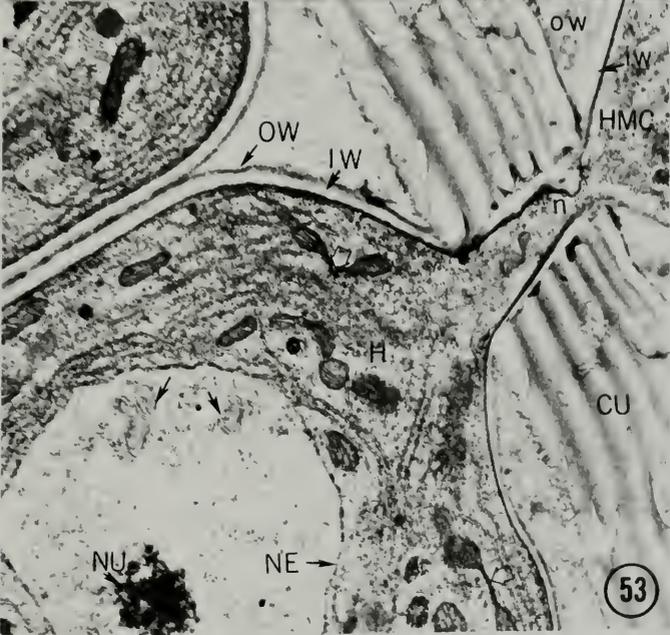
PLATE XVI

- Figure 50. A thin section of old haustorial mother cell X20,000. Notice the many-layered inner wall (iw), smooth endoplasmic reticulum (er), lipid droplets (L), mitochondria (M) and nucleus (N) with membranous structure inside the nucleoplasm (arrow).
- Figure 51. The rough endoplasmic reticulum (ER) of the atypical haustorial mother cell X74,000. Arrows point to the spheres of rough endoplasmic reticulum with ribosomes inside.



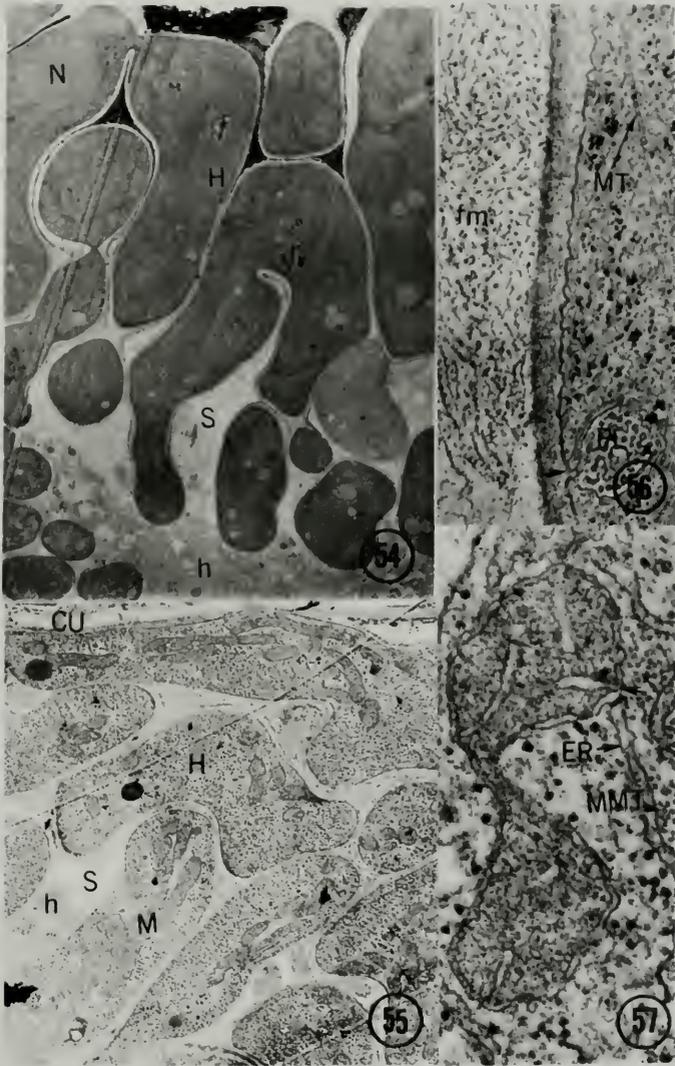
## PLATE XVII

- Figure 52. A median section through the neck of the haustorium (n) X24,500. Notice the thick outer layer (ow) of haustorial mother cell wall and the continuity of the inner layer (iw) of the haustorial mother cell wall with the inner layer (IW) of the wall of the haustorium. Mitochondrion (M), bundle of mini-microtubules (MMT) and microtubules (MT) are present in the neck.
- Figure 53. Near median section through a penetration site X20,750. Haustorial mother cell (HMC) is connected to the haustorium (H) by a neck (n). Outer layer of haustorial mother cell wall (ow) and that of haustorial wall (OW) are dark while the inner layers of both haustorial mother cell wall (IW) and haustorial wall (IW) are electron light and the two appear to be continuous. The hole in the cuticle (CU) through which the neck runs appears to be very smooth. Nucleus (N) has a nucleolus (NU) and is bounded by a double membraned nuclear envelope (NE). Certain membranous structures are present in the nucleoplasm (arrows). Open arrows point to the constricted mitochondria.



### PLATE XVIII

- Figure 54. Thin section of old haustoria (H) at the site of original infection X3,200. Haustoria have lysosomes (L) and nucleus and are separated from the host protoplast (h) by a sheath (S).
- Figure 55. Thin section of haustoria (H) underneath the cuticle (CU) of the termite X7,200. Haustoria are separated from the host protoplast (h) by a sheath (S). Notice the enormous size of the mitochondria (M).
- Figure 56. Thin section of the haustorium X35,750. Plasma membrane is connected (arrow) to a plasmalemmasome (PL) projecting into the haustorial cytoplasm. Outside the haustorium in the sheath there is fibrillar material (fm). A microtubule (MT) is present inside the haustorium cytoplasm.
- Figure 57. Haustorial mitochondrion constricted in the middle X82,000. Arrow points to the connection between the crista and the inner mitochondrial membrane. Notice the nearby mini-microtubules (MMT) and rough endoplasmic reticulum (ER).



## PLATE XIX

- Figure 58. A portion of a typical haustorium with aberrant mitochondria X35,750. Protoplasm of the haustorium looks diffuse. Plasma membrane is invaginated at many places (arrows). The invaginations have vesicles in them. Few vesicles are present in the cytoplasm. Wall of the haustorium (W) has two distinct layers. Host protoplasm (h) is also in picture.
- Figure 59. Host-parasite interface X82,000. The host plasma membrane (hPM) tightly fits against the haustorium wall (W). Compare the diameter of mini-microtubules (MMT) of the haustorium with that of the microtubules (MT) of the host cytoplasm (h).
- Figure 60. Transversely cut mini-microtubules between the plasma membrane (PM) of the haustorium and its endoplasmic reticulum (ER). Wall of the haustorium (W) is very thick.
- Figure 61. Mini-microtubule running (arrow) between nuclear envelope (NE) and the endoplasmic reticulum cisterna.
- Figure 62. Periodic acid silver-stained haustoria X43,750. Haustorial wall (W) is PAS-positive while fibrillar material (fm) in the sheath is PAS-negative.
- Figure 63. Microbody (MB) of the haustorium X54,500. Notice the close association with the endoplasmic reticulum (ER). Mitochondrion (M) is also in vicinity.

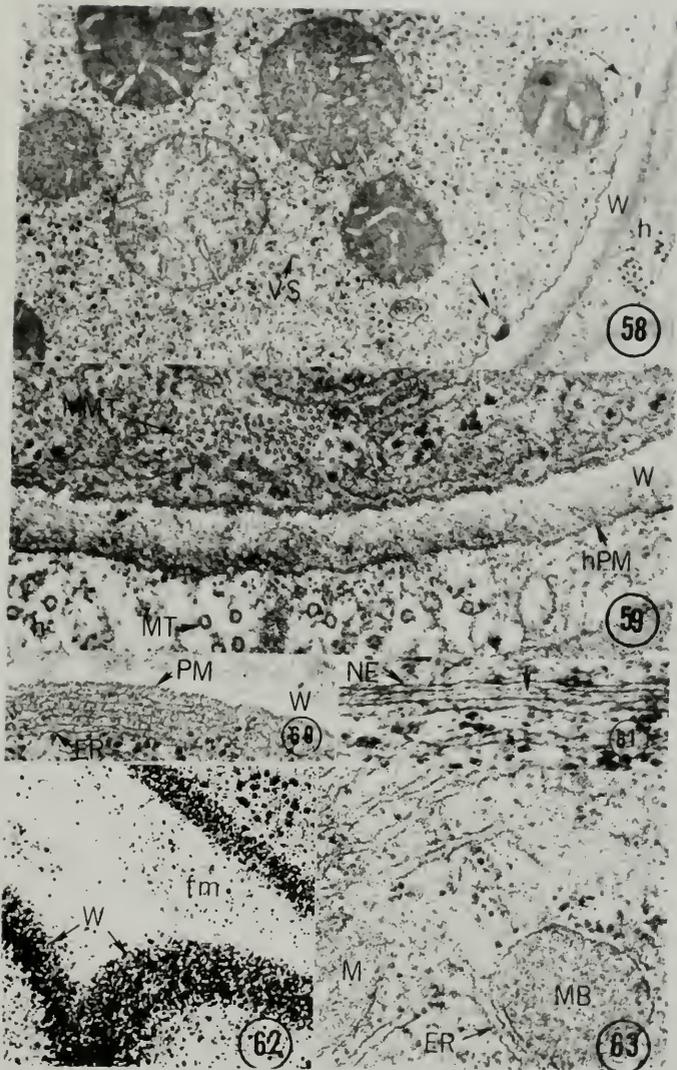


PLATE XX

- Figure 64. Mini-microtubules (MMT) between the cisternae of endoplasmic reticulum (ER) X109,000. The mini-microtubules appear to be produced by endoplasmic reticulum and extruded towards the periphery.
- Figure 65. A bundle of longitudinally cut mini-microtubules X145,000.
- Figure 66. A stack of endoplasmic reticulum with longitudinally cut mini-microtubules between the cisternae (arrows) X54,500.
- Figure 67. A stack of endoplasmic reticulum with transversely cut mini-microtubules between the cisternae X80,000. The endoplasmic reticulum is cutting off vesicles (arrow) at the periphery. Certain vesicles (VS) are present in the cytoplasm.
- Figure 68. A bundle of mini-microtubules cut transversely X356,000.
- Figure 69. Mini-microtubules cut transversely X824,000. Six subunits are clear (arrow) in the wall of the mini-microtubule. Compare the endoplasmic reticulum membrane with the subunits of the wall of mini-microtubules.

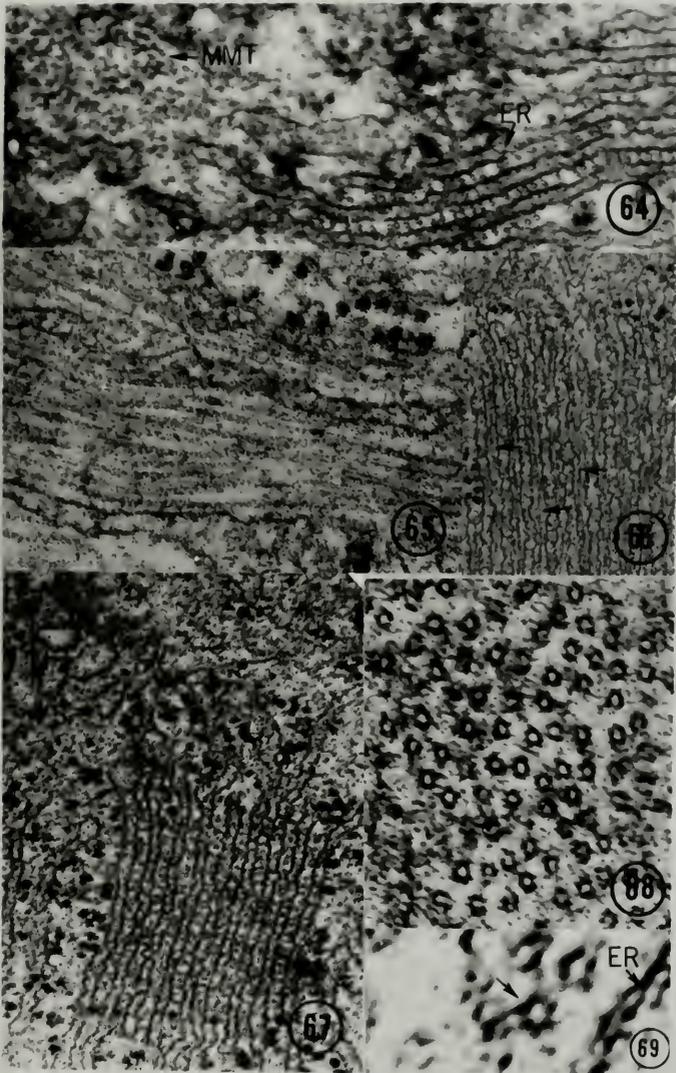
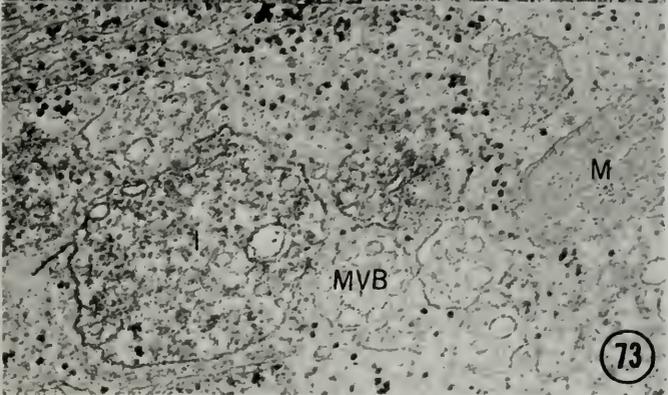
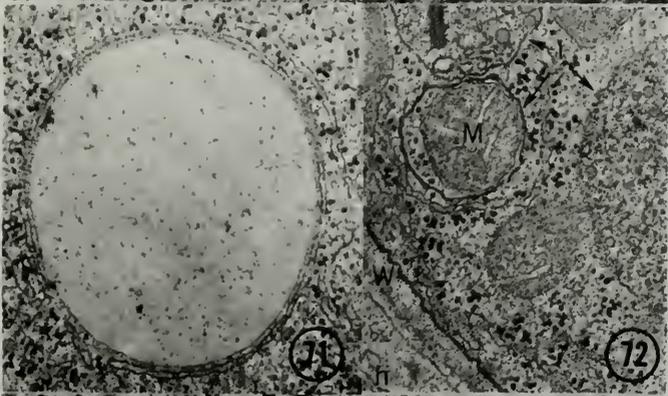
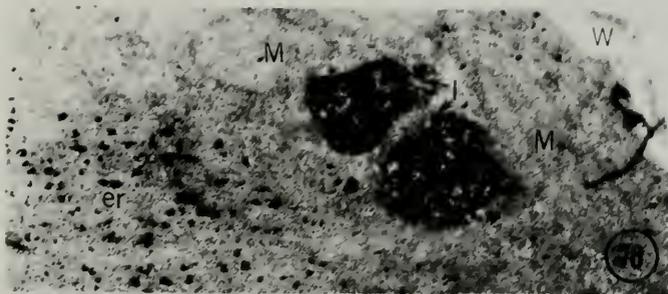


PLATE XXI

- Figure 70. Acid phosphatase activity in the haustorium X56,000. The activity can be seen in the lysosomes (l) and over the stack of smooth endoplasmic reticulum (er) cisternae with mini-microtubules between them. The mitochondria (M) and the haustorial wall (W) show no such activity.
- Figure 71. A lipid droplet being surrounded by endoplasmic reticulum forming autophagic vacuole X51,500.
- Figure 72. Autophagic vacuole (l) with mitochondrion (M) inside X51,500. The wall of the haustorium (W) and the host protoplasm (h) are also seen.
- Figure 73. Haustorium with lysosome (l), mitochondrion (M) and multivesicular bodies (MVB) X53,000. Vesicles can also be seen inside the lysosome.



CHAPTER IV  
TAXONOMIC POSITION

When Thaxter (1920) described *Termitaria* as a new genus of entomophilous fungi on termites, he visualized it as a closely compacted sporodochium and suggested that "its mature condition, however, evidently a Fungus Imperfectus, seems to give it a formal place among the Leptostromaceae." Others (Reichensperger, 1923; Feytaud and Dieuzeide, 1927; Colla, 1929; Heim *et al.*, 1951) also studied this genus but only Colla (1929) and Heim (personal communication) had a different opinion about its taxonomic position. Colla suggested that a new family should be created and Heim thinks it belongs in Tuberculariales of Fungi Imperfecti. My studies (Chapters I, II, III) have shown a number of features of *Termitaria* that were not reported by these authors. These include the ecto-parasitic nature of the fungus, the so-called bhlamydospores being haustorial mother cells in reality, sending haustoria into the host integument, and the phialoconidial nature of the spores. These discoveries necessitate an emendation of the genus.

*Termitaria* Thaxt. emend. Khan

Entomophilous; primary thallus crust-like, stromatic, few cells thick, variously shaped with even margin; haustoria penetrate the integument from specialized thick-walled haustorial mother cells; whole primary thallus matures into a sporodochium of tightly held phialides making

a hymenium over a basal pseudoparenchymatous subhymenium, with a peripheral excipulum of black thick-walled, sterile cells, margins lichenoid, spreading but even; phialides cut off conidia apically in basipetal succession at conidiogenous loci into distinct collarettes; collarettes long containing four or more conidia; conidia catenate,  $3.5 - 4 \mu \times 1.5 - 2 \mu$ , cylindrical, truncate or rounded at ends, hyaline in mass.

Thaxter originally proposed two species, *T. snyderi* and *T. coronata*, on the basis of the length of the phialides, the length of the collarettes and the structure of the tip of the phialide. The phialides and collarettes of *Termitaria coronata* are larger in diameter and length than those of *T. snyderi*. The phialide tip of *T. snyderi* is blunt and that of *T. coronata* has minute pointed prolongations resulting in a minutely echinulate hymenial surface. Later, Reichensperger (1923) described *Termitaria* from the Belgian Congo and Brazil and proposed a new name *T. thaxteri* for the Brazilian specimen "should it turn out to be a new species." Having not examined the type it is impossible for me to know whether it was a new species. Later, Colla (1929) reported the presence of *T. snyderi* and *T. coronata*, the former from Chile and Brazil and the latter from British Guayana, Costa Rica and Phillipines, growing on termites' exoskeletons.

Colla (1929) also described a new genus from British Guayana, parasitizing the integument of the termite *Rhinotermes marginalis* (L.) and proposed a new genus *Mattirolella* with a single species

*M. silvestrii*. *Mattirolella* closely resembles *Termitaria* but its sporodochium has 8 to 10 special cavities containing fertile hyphae surrounded by sterile ones. Colla described *M. silvestrii* as having a star-shaped thallus in young as well as adult stages, the arms being intensely black, carbonaceous and sterile. The mature thallus which is a sporodochium 60-70  $\mu$  in thickness has three distinct regions, a basal 12-20  $\mu$  thick subhymenium, a middle 37-40  $\mu$  thick hymenium, and an apical epihymenium. The basal layer of the pseudoparenchymatous subhymenium is comprised of thick walled cells. A foot is sent into the integument which according to Colla (1929) reacts with maximum hypertrophy of the epidermal cells. Thaxter (1920) reported a similar response to *Termitaria* infection by the epidermal cells of the termite integument. Through my studies I know that what Thaxter thought were hypertrophied epidermal cells actually were haustoria of the *Termitaria*. It can be assumed from Colla's figures that in the case of *Mattirolella* too, those hypertrophied epidermal cells actually were haustoria. Thus, in the basal layer of the subhymenium there are certain specialized haustorial mother cells that send haustoria into the integument. The hymenium is loculate, locules being separated from each other by the columns of sterile hyphae that run from the subhymenium upward, anastomose at the top of the hymenium and form an epihymenium. These locules are full of what Colla called fruiting bodies. She thought they resembled asci. However, she was not sure of this nor was she sure of the phialidic nature of the conidiogenous cells of *Termitaria*. She described them as having spores like that

of *Termitaria* but smaller in size. She also described a dark line across the sporogenous cells at about two-thirds of their length. This dark line resembles the dark line across the hymenium of the *Termitaria* that marks the position of the conidiogenous loci of the phialides. I think that the fruiting bodies were actually phialides and as Colla herself said it was not possible for her to confirm the nature of these sporogenous structures because of their small size. The epihymenium is sterile and makes an arch over the phialides in the locular areas.

During this study I had the good fortune of looking at some of the slides of *Termitaria* made by Thaxter (courtesy Farlow Herbarium). These slides were in very good condition and I found that slide number 32401 of *Termitaria crustosa* is not a *Termitaria* but *Mattirorella*. The fungus was collected from Panama and the slide was made in 1924. The slide has many cross sections of the sporocarp showing subhymenium, hymenium, epihymenium and the sterile hyphae dividing the hymenium into many chambers containing phialides (Fig. 75). There is a basal layer of thick walled cells many of which are haustorial mother cells sending haustoria through the cuticle (Figure 79). Over this there is a pseudoparenchymatous subhymenium 13  $\mu$  in thickness from which arise the phialides arranged in a palisade-like layer making a broad 52  $\mu$  thick hymenium. The hymenium is traversed by sterile hyphae that divide it into chambers. The apices of all the fertile hyphae (phialides) and sterile hyphae are fused with each other and it appears that at maturity the sterile hyphae elongate, as a result of which the thick

tips of the phialides break off (Fig. 78) and become a part of the epihyemenium. The broken ends of the phialides remain free in the cavity under the epihyemenium. It is assumed that the conidia are released in those cavities, put a pressure on the epihyemenium that is broken and flaked off. The epihyemenium is 5  $\mu$  thick and has a reticulate surface (Fig. 80). The space between it and the broken phialide tips is about 8  $\mu$  thick. The conidiogenous loci of all the phialides lie at their bases and each collarette which is approximately as long as the thickness of the hyemenium has 10-12 truncate, hyaline conidia. The conidia are 2.5 - 3.0  $\mu$  X 1.5  $\mu$ , much smaller than that of the *Termitaria* and broader compared to their size (Fig. 74). The whole sporocarp is 85-90  $\mu$  thick.

From the description of the fungus it becomes apparent that this one is slightly different from *M. silvestrii* in having longer phialides, thicker sporocarp, conidiogenous loci lying at the bases of the phialides and that the surface of the epihyemenium is reticulate in this species. Colla's figures also show that the sterile hyphae partitioning the hyemenium into chambers make wider columns in *M. silvestrii*. Thus, it is proposed here that this fungus be regarded as a new species:

*Mattirolella crustosa* sp. nov.

Figs. 74-80.

Entomogenous; thallus maturus sporodochium, densus 85-90  $\mu$ ; hyemenium densum 52  $\mu$  divisum in receptacula numerosa columnis hypharum sterilium latarum paucilocularibus; phialides stricti, loca conidiogenera sunt in fundamentis phialidum, apices crassi

phialidum et apices hypharum sterilium sese conglutinant epihymenium formantes, prolongatio hypharum sterilium phialidium apices frangunt, cavitatem super apices fractos efficientes; conidii endogeniter formati, 10-12 in collaretta singula, truncati, catenati,  $2.8 - 3.0 \times 1.5 \mu$ , hyalini et cylindrati; superficies epihymenii reticulatus.

Entomogenous; mature thallus a sporodochium, 85-90  $\mu$  thick; hymenium 52  $\mu$  thick, divided into many chambers by few cells wide columns of sterile hyphae; phialides tightly held, conidiogenous loci lie at the bases of the phialides, thick tips of phialides and the tips of sterile hyphae fuse with each other forming an epihymenium, elongation of the sterile hyphae break the phialide tips making a cavity over the broken tips, conidia formed endogenously, 10-12 in each collarette, truncate, catenate,  $2.8 - 3.0 \times 1.5 \mu$ , hyaline and cylindrical; the surface of the epihymenium reticulate.

*Mattirolella* and *Termitaria* have many characteristics in common. They both are entomophilous, sending haustoria from specialized thick walled haustorial mother cells into the integument through the cuticle of the host, and the haustoria do not go beyond the epidermis. Both have a foot-like structure which according to this study of *Termitaria snyderi* marks the position of original infection and penetration. Both have a crustose thallus which is stromatic in young stages. In both cases the whole thallus matures into a sporodochium that has a pseudoparenchymatous subhymenium, and a hymenium of phialides. There is an excipulum around the sporodochium in both genera. However, they differ in many respects. All cells

in the basal layer of the thallus are thick walled in *Mattirolella* while in *Termitaria* only a select number are thickened. There is an epihyemium present in *Mattirolella* while in *Termitaria* there is none. The hyemium of *Mattirolella* is divided into many chambers containing phialides separated from each other by the columns of sterile hyphae.

On the basis of major differences between the two, Colla (1929) proposed the genus *Mattirolella* but suggested that it be put in a single family. She did not suggest any name for the family.

The characters of the two genera are so unique that it is very difficult to put them into any recognized group of Fungi Imperfecti. That may be the reason why the taxonomic position of the two is still undetermined.

The imperfect fungi are placed under subdivision Deuteromycotina by Ainsworth (1971). The Deuteromycotina is subdivided as follows:

#### Deutromycotina

Blastomycetes -- yeast and yeast-like forms.

Hyphomycetes -- mycelial forms with or without conidia.

Agonomycetales -- without conidia.

Hyphomycetales -- conidia not on synnemata or sporodochia.

Stilbellales -- synnematous.

Tuberculariales -- sporodochial.

Coelomycetes -- conidia within acervuli or pycnidia.

Sphaeropsidales -- pycnidial.

Melanconiales -- acervulial.

Two other orders of Fungi Imperfecti have been proposed, Peltasterales and Gloeohaustoriales, by Batista and Ciferri (1959) and Heim (1952) respectively, the former for the imperfect states of Microtheriales that have pycnidia with inverted hymenium and the latter for such entomogenous genera as *Antennopsis* and *Chantransiopsis*. Obviously *Mattirolella* and *Termitaria* do not belong to these two orders. They resemble only members of the orders Sphaeropsidales or Tuberculariales because the sporocarp of *Termitaria* is closer to the sporodochium of the latter while that of *Mattirolella* is closer to the stroma having many pycnidia embedded in it like in Sphaeropsidales. The sporodochium is a determinate, pulvinate mass of conidiophores tightly clustered on a stroma and does not have an excipulum of fungal material. The sporocarp of *Termitaria* develops on a stromatic primary thallus, is comprised of tightly held phialides, and has a peripheral excipulum of black thick walled sterile cells. Thus, it is a modified form of sporodochium. The pycnidium is defined as a variously shaped cavity having a pseudoparenchymatous wall that is lined with conidiogenous cells. It may be immersed within a stroma or formed superficially on the surface of the substratum. In *Mattirolella* the hymenial cavities containing phialides can be superficially compared with the pycnidia embedded in a stroma.

As has been discussed earlier *Termitaria* and *Mattirolella* appear to be very closely related but differ in their sporocarp structure. The two on the basis of their general morphology and the structure of their sporocarps should not be put into any of the existing orders. Therefore a new order is proposed here.

Termitariales order nov.

Entomogenous; ectoparasiticus, haustoria in integumentum hospitis penetrans ex cellis matribus crassitunicatis specialibus; haustoria restricta in texto epidermali hospitis; thallus principalis crustosus, stromaticus sine hyphis laxis qui maturat in sporocarpo perobscuro; sporocarpum consistens subhymenio pseudoparenchymate, hymenio phialidibus haesis strictis et cincto excipulo sterili cellarum crassitunicatarum; phialis cylindratus, phialonconidios in ordine basipetalo in locis conidiigeneribus in collarettes apicaliter abscindens; conidii aseptatis, hyalini, cylindrati, catenati, marginibus truncatis aut rotundis.

Entomogenous; ectoparasitic, sending haustoria into the integument of the host from specialized thick walled haustorial mother cells, haustoria limited only to the epidermal tissue of the host; primary thallus crustose, stromatic without any loose hyphae, matures into a very black sporocarp; sporocarp consisting of a pseudoparenchymatous subhymenium, a hymenium of tightly held phialides and surrounded by a sterile excipulum of thick walled cells; phialides cylindrical, cutting off phialoconodia apically in basipetal succession at conidio-  
genous loci into collarettes; conidia aseptate, hyaline, cylindrical, catenate with truncate or round ends.

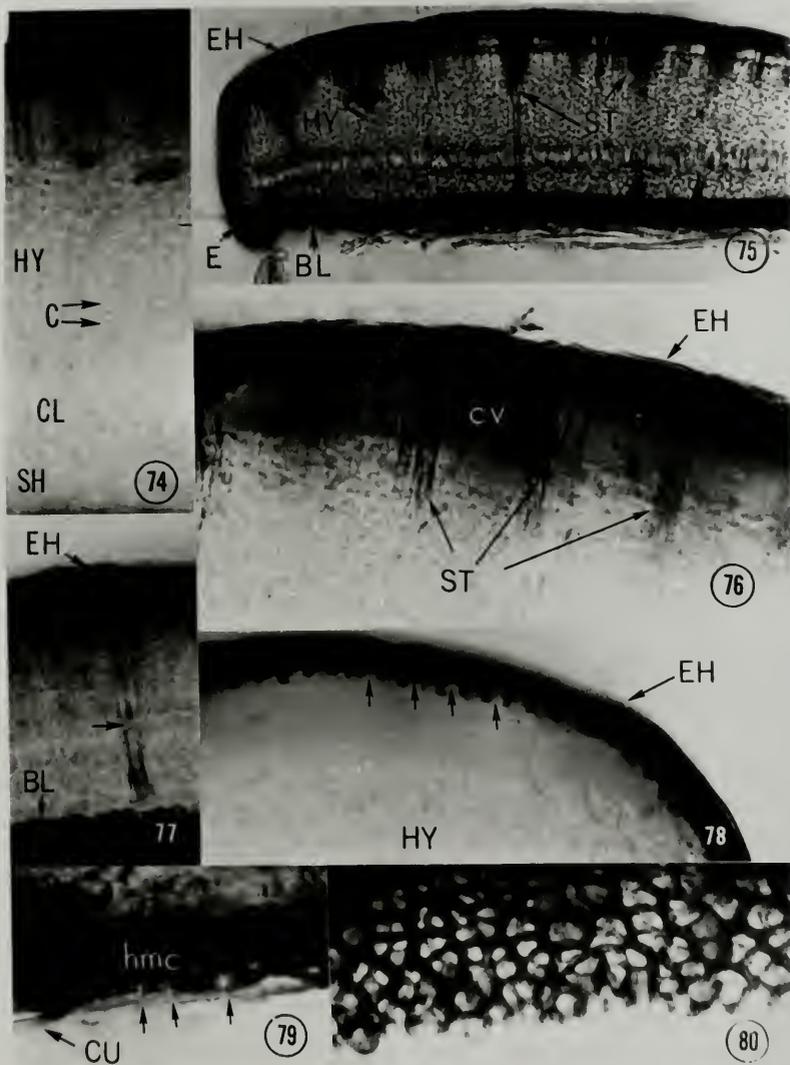
This is a provisional order created for *Termitaria* and *Mattirolella*. Tentatively, a single family Termitariaceae will be included, but when other entomogenous taxa are investigated or perhaps sexual features of these genera are discovered, a complete revision of these taxa will be necessary.

The phialides of *Termitaria* and *Mattirolella* closely resemble those of *Bloxamia truncata* Berk. and Br. (Pirozynski and Morgan-Jones, 1968), *Chalara* (Corda) Rapenh., *Chalaropsis* Peyron and *Thielaviopsis* Went. The phialides of all of them cut off chains of conidia in basipetal succession at conidiogenous loci into the long collarettes. The conidia are cylindrical, aseptate and hyaline or lightly pigmented. *Bloxamia* belongs to Tuberculariales and is saprophytic while the other three belong to Hyphomycetales and can be found as saprophytes. *Thielaviopsis* is a serious pathogen of higher plants. The conidiophores of the synnematiuous fungus *Endosporostilbe* Subram. also show some resemblance to the phialides of *Termitaria* and *Mattirolella*. They are grouped in a single synnema cutting off aseptate, hyaline conidia endogenously at conidiogenous loci in small collarettes. The conidia are extruded in basipetal succession through the open ends of collarettes. The sporocarps of *Termitaria* and *Bloxamia* are alike in many respects. They are black, comprised of tightly held phialides whose conidiogenous loci lie at the same distance from the base of the phialides making a distinct line running across the hymenium.

It can be said that the members of the proposed order closely resemble members of the orders of class Hyphomycetes and therefore it should be placed under the same class next to Tuberculariales.

PLATE XXII

- Figures 74-80. *Mattirolella crustosa* sporocarp.
- Figure 74. A portion of the cross section of the sporocarp showing subhymenium (SH), hymenium (HY), the zone of conidiogenous loci (CL) and the conidia (C) inside the collarettes X1,250.
- Figure 75. Cross section of the sporocarp X250. The sporocarp has three distinct regions, epihymenium (EH), hymenium (HY), and subhymenium (SH). Columns of sterile hyphae (ST) divide the hymenium into many chambers. Conidiogenous loci (CL) at the bases of the phialides make a distinct zone. Basal layer (BL) and the excipulum (E) are also seen.
- Figure 76. Cross section of the sporocarp X1,000. Column of the sterile hyphae (ST) divide the hymenium. Notice the cavities (cv) over the phialides.
- Figure 77. Cross section of the sporocarp X320. A column of sterile hyphae (arrow) is seen all the way through the hymenium running between the epihymenium (EH) and the basal layer (BL).
- Figure 78. Cross section of the sporocarp X1,000. The broken tips of the phialides (arrows) that fuse to help make the epihymenium (EH).
- Figure 79. Cross section of the basal layer of the sporocarp X1,250. Thick walled haustorial mother cells (hmc) send haustoria into the integument of the termite through the channels (arrows) in the host cuticle (CU).
- Figure 80. Surface of the epihymenium X1,000. Note its reticulate nature.



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## BIOGRAPHICAL SKETCH

Saeed-ur-Rehman Khan was born December 25, 1943, in Rampur, India. He attended Hamid Higher Secondary School and Reza Intermediate College in Rampur, where he received his High School and Intermediate Certificates respectively in 1957 and 1960.

He received his Bachelor of Science in Biology Degree in 1962, from Agra University, India. After that he migrated to Pakistan and entered the University of Peshawar. He graduated from the University of Peshawar in 1964 with honors and was awarded the Degree of Master of Science in Botany.

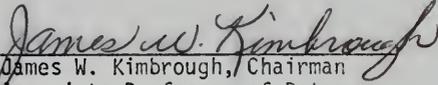
He served as a Lecturer in Botany in various colleges in Northwest Frontier Province of Pakistan from September, 1964 through December, 1971, when he was awarded a Fulbright-Hays Grant to study in the United States.

He entered the University of Florida in January, 1971.

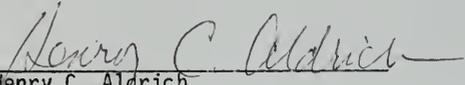
He has been involved with the activities of foreign students in different capacities. At present he is the Chairman of the Council of International Organizations of the University of Florida.

He is a member of the Mycological Society of America, Botanical Society of America, Association of Southeastern Biologists and Omicron Delta Kappa.

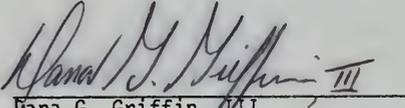
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Associate Professor of Botany

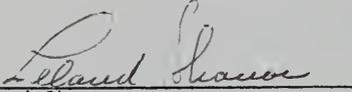
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Associate Professor of Botany

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Dana G. Griffin, III  
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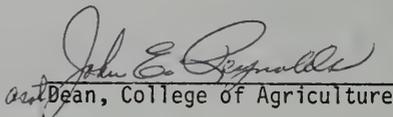


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This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1973



John E. Reynolds  
asst Dean, College of Agriculture

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Dean, Graduate School