

## Bark beetle – fungal symbiosis. II. Fine structure of a basidiomycetous ectosymbiont of the southern pine beetle<sup>1</sup>

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A basidiomycetous yeast is a dimorphic fungal ectosymbiont associated with the southern pine beetle, *Dendroctonus frontalis*. Within the mycangium (a glandular integumental invagination) of the southern pine beetle, the fungus grows in a yeast-like manner, while on some media or in a plant host, it forms mycelial masses. The yeast stage has a lamellate cell wall and forms new cross walls in a manner rather similar to the basidiomycetous yeasts. Like certain ambrosial fungi (*Ascoidea*), the yeast stage is multinucleate. The mycelial stage, initially produced in culture and within the plant host, shows clamp connections and dolipore septa, characteristic of many Basidiomycetes, but its cell wall is not lamellate.

The dolipore septa have septal pore caps and also mirror images of these caps. The septal pore caps (about 400 Å in thickness) have five layers: the two outermost (40 Å) are probably continuous with the 'unit membranes' of the endoplasmic reticulum; the middle layer is similar to the outermost; and the intervening zones (150–170 Å) are homogeneous and of intermediate electron density. The endoplasmic reticulum is both tubular and cisternal, and some portions of the membranes are associated with ribosomes. The multinucleate condition is regarded as a special adaptation for effective colonization; otherwise the ectosymbiont is consistently similar to known basidiomycetous yeasts.

### Introduction

A considerable number of wood-inhabiting insects maintain mutualistic associations with fungi (Buchner 1965; Francke-Grosmann 1967). The fungi apparently contribute to the nourishment of the insects, and the adult insects are the vectors which convey fungal propagules from tree to tree (Norris 1972; French and Roper 1972; Barras 1973). Among the adaptations to allow fungal transport are specialized glandular integumental invaginations on the surface of the insects. These invaginations, termed mycangia (Batra 1963), are selective culture chambers that create a microenvironment within which only the symbiotic species of fungi proliferate (Francke-Grosmann 1967; Barras and Perry 1971, 1972). We have suggested that the mycangial secretions render the microenvironment selective, i.e., suitable for only the mutualistic species (Happ *et al.* 1971).

Many mycangial fungi are pleiomorphic; within the mycangium they grow by budding in a

yeast-like fashion, whereas in the tree or in culture, growth is mycelial. Unfortunately, most of the mycangial fungi are known only from the imperfect state, and thus identification and classification are difficult. However, most of the imperfect 'form genera' have strong resemblance to ascomycetes and appear to be derivatives of *Ceratocystis* or *Monilia* (Francke-Grosmann 1967). Only two genera of insects are known to harbor mycangial fungi with basidiomycetous affinities: *Sirex*, Australian wood wasps, which are associated with the fungus *Amylostereum areolatum* (Gaut 1969); and *Dendroctonus*, North American bark beetles, which are associated with yet-unnamed basidiomycete(s). Basidiomycetous isolates from *Dendroctonus brevicomis* (R593) and *D. frontalis* (SJB 122) have been described recently by Whitney and Cobb (1972) and Barras and Perry (1972), respectively. The present paper is concerned with the basidiomycetous ectosymbiont of *D. frontalis*, the southern pine beetle.

Histological study of the mycangia of *D. frontalis* reveals that the ectosymbiotic fungal propagules are of two very distinct sizes: small (1–2 microns (μ) in diameter) and large (10–15 μ

<sup>1</sup>Part I of this series appeared in *Tissue and Cell*, **3**: 291–306 (1971).

in diameter). Usually each beetle contains only one size of propagule. Occasionally, right and left halves of the mycangium may have differing populations. Exceptionally, a minor contaminant can be isolated (Barras and Perry 1972); however, as a rule, the propagule population is homogeneous: propagules within a single compartment are indistinguishable from one another. Furthermore, smaller propagules consistently yield a *Sporothrix* mycelial stage and larger propagules consistently yield filamentous isolates with clamp connections. Clamp connections indicate basidiomycetous affinities. SJB 122 is one such isolate which grows well as a pure culture. The other fungus (SJB 133) has smaller cells and forms a *Sporothrix* imperfect stage in beetle galleries and on most media but was identified as a variety of *Ceratocystis minor* after protracted growth on potato glucose agar (Barras and Taylor 1973). The present paper describes the ultrastructural cytology of SJB 122 (in vitro) and of the presumed yeast-like phase in the mycangium. In a later paper we will consider the *Ceratocystis* derivative.

On grounds of its apparent affinity, one might compare SJB 122 with the basidiomycetous yeasts *Sporidiobolus*, *Aessosporon*, *Rhodospordium*, *Leucosporidium*, and *Filobasidium* (Kreger-van Rij 1973) and with their probable imperfect relatives *Bullera*, *Sporobolomyces*, *Itersonilia*, *Tilletiopsis*, *Rhodotorula*, *Cryptococcus*, and *Sterigmatomyces* (Kreger-van Rij 1973; Kreger-van Rij and Veenhuis 1971). These yeasts are pleomorphic, exhibiting mycelial or pseudomycelial forms as well as the yeast stage (Lodder 1970; Kreger-van Rij 1973). Most show a lamellar structure in the cell wall of both the budding cells and the hyphae (Kreger-van Rij and Veenhuis 1971). Certain species have clamp connections and (or) dolipore septa (Lodder 1970; Moore and Kreger-van Rij 1972; Kreger-van Rij 1973), while others show ascomycetous septa (Johnson-Reid and Moore 1972; Moore 1972), and at least three genera (*Rhodospordium*, *Aessosporon*, and *Leucosporidium*) show a characteristic mitosis within the bud of the yeast phase (McCully and Robinow, 1972a, 1972b).

#### Materials and Methods

Beetles (*Dendroctonus frontalis* Zimm.) were obtained from naturally infested loblolly pines (*Pinus taeda* L.) at various localities within Louisiana. As the beetles emerged from the logs, they were collected and sexed. Only the females possess an elaborate mycangium as

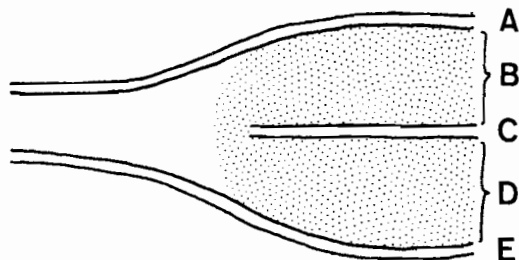


FIG. 1. A diagrammatic interpretation of the wall of the septal pore cap. The outer layers (A and E) appear to be continuous with the membranes of the endoplasmic reticulum.

defined by Batra (1963), whereas males have a pseudomycangium and do not transport SJB 122 (Barras and Perry 1972).

Some females were shipped in refrigerated containers to New York University and Colorado State University, where prothoraces were removed and were fixed for transmission electron microscopy. Tissues were fixed in phosphate- or cacodylate-buffered glutaraldehyde. For the phosphate technique, fixation in 5% glutaraldehyde (0.1 M sodium phosphate buffer, pH 7.4) at 0–4 °C for 2 to 6 h was followed by a 1-h wash in the same buffer with 10% sucrose added (Locke 1966, 1969) and postfixation in 1% osmium tetroxide buffered with phosphate (0.1 M, pH 7.4) and containing 4% sucrose. For the cacodylate technique, tissues were fixed in 2.5% glutaraldehyde (0.05 M cacodylate buffer, pH 7.2, containing 0.15 M sucrose) at 0–4 °C for 2–12 h, washed in several changes of fresh buffer containing 0.5 M sucrose, and postfixated for 1 h in cold veronal acetate buffered 1% osmium tetroxide containing 0.4 M sucrose (Gupta and Smith 1969). For transmission electron microscopy, tissues were dehydrated via an ethanol series and embedded in Epon 812 or Spurr's medium. Thin sections were stained routinely for 20 min with saturated uranyl acetate in ethanol-methanol (equal parts 70% ethanol and absolute methanol) followed by 5 min in lead citrate (Reynolds 1963). Sections were examined and photographed at 50 kV in an RCA EMU 2E or an RCA EMU 3D.

Single-spore pure cultures of the basidiomycetous mycangial fungus were obtained by first surface-sterilizing mycangia and then spreading spores onto water agar as previously reported (Barras and Perry 1972). In the previous study, it was established that large propagules (10–15  $\mu$  diam) observed by phase contrast in the mycangium gave rise to mycelia with clamp connections. For electron microscopy, these large propagules were established on 2.5% malt extract agar and one isolate (SJB 122) was stored at 25 °C for several weeks to obtain good growth. The plates were then flooded with glutaraldehyde in phosphate or cacodylate buffer and small pieces were excised for postfixation in osmium. Processing, dehydration, etc. were as described above for electron microscopy.

#### Observations

##### The Yeast Phase

Within the mycangium, growth of the basidiomycete is by budding or fission. Chains of two or

three cells may remain attached, but the cells are distinct and separated from one another by double walls.

The individual cells are quite large for fungi; often they measure 10–20  $\mu$  in diameter. The larger cells (and perhaps all yeast-phase cells) are multinucleate (Fig. 5). Individual nuclei are ovoid, about 3  $\mu$  in diameter, and frequently contain a prominent nucleolus (Figs. 2, 5). The nucleolus may be oval or ring-shaped (Fig. 5).

The cytoplasmic ground substance is of moderately high electron density and ribosomes are numerous; thus the cells appear very dense and it is rather difficult to see membranous, tubular, or fibrillar elements in the cytoplasm (Figs. 2, 5). Glycogen granules are not uncommon (Fig. 3) and often appear to be clustered around a membrane-bound vesicle with an electron-transparent interior (Fig. 4). Mitochondrial profiles are small, irregular in shape, and contain several parallel cristae (Fig. 2). Fifteen to 50 profiles are visible in each cell.

Ribosome-like granules are present within the mitochondria, both in the interior and along the inner bounding membrane (Fig. 3). Ribosome-like granules are also associated with the outer mitochondrial membrane (Fig. 3). A similar distribution of ribosome-like particles has been previously reported in *Rhodotorula* (Keyhani 1973).

The endomembrane system is difficult to examine because of the high electron density of the cytoplasm. However, a system of narrow cisternae is present beneath the plasma membrane and other more central cisternae can occasionally be seen (Fig. 5).

We have been unable to detect a Golgi region, but it might have been obscured by the high density of the cytoplasm. Several types of vesicles are membrane-bound and are of varying densities. These vesicles might contain polysaccharides or lipids, or they might be lysosome-like regions (Oláh 1973; McKeen 1970; Keyhani 1973). The plasma membrane itself is often infolded slightly to reveal narrow submural spaces (Figs. 6, 7). Paramural bodies (Marchant and Moore 1973) are a regular feature of the yeast cells (Figs. 2, 3, 5, 9). These whorls of parallel electron-dense membranes appear to be continuous with the plasma membrane and therefore are properly termed plasmalemmasomes (Marchant and Roberts 1968; Marchant and Moore 1973).

The cell wall has a distinctly lamellar structure,

as is characteristic of basidiomycetous yeasts (Kreger-van Rij and Veenhuis 1971). Each individual lamella is about 50 m $\mu$  in thickness (Fig. 2) and the outer lamellae are repeatedly sloughed off (Fig. 2), perhaps giving rise to the fibrous outermost material.

Parent cells divide to yield daughter cells (Fig. 7) by a budding (Fig. 6) or fission (Figs. 2, 8, 9). As the plasma membrane invaginates, the layers of the new cross wall are laid down (Figs. 8, 9). The first layer is probably the electron-dense superficial layer (Fig. 2). The lamellae of the new cross wall are continuous with the inner lamellae of the parent cell (Figs. 2, 7, 8, 9). Septum formation continues until the two new daughter cells are separated from one another by complete cross walls but are still enclosed by the outer lamellae of the parental wall (Fig. 7), as in the type B division in Fig. 1 of Moore (1965). The outer lamellae then break to allow the daughters to move apart (Fig. 2).

Plasmalemmasomes (Figs. 2, 9), membrane invaginations (Figs. 6, 8), and vacuoles (Figs. 6, 8, 9) are often associated with presumptive areas of wall formation. No distinct and stable septal structure has been seen in the yeast cells, but an unusual circular structure, resembling the septal pore cap of a dolipore septum, has been seen in a few dividing cells (Fig. 2). The sites of division are marked by scars (Figs. 2, 7).

#### *The Mycelial Phase*

Within the host tree or in culture on Petri plates, the growth of SJB 122 is hyphal, yielding a mycelial mass of intertwined strands with younger and actively growing cells at the edges. The hyphae are usually 2–5 microns in diameter, although smaller aerial branches may be present at the periphery of the mass. Old mycelia are binucleate.

The nuclei are irregular in outline, in contrast to the more spheroid nuclei seen in the yeast phase. The tortuous nuclear envelope frequently blebs outward (Figs. 14, 15) and contains a considerable number of pores (Figs. 10, 14, 15). Nucleoli are compact (Fig. 15) or ring-shaped (Fig. 14), and a membrane-bound 'vesicle' is sometimes found adjacent to a nucleolus (Fig. 14). This membrane-bound structure may be an invagination of the inner membrane of the nuclear envelope and thus be a channel of perinuclear space, as has been described in other systems (Smetana and Busch 1974).

The cytoplasmic ground substance of the hyphal cells is much less dense than that in the yeast-like cells, and ribosomes are considerably more scattered. In the growing hyphae, a few paired endomembrane profiles course through the cytoplasm; often these membranes (or their content?) appear to be rather denser than the plasma membrane itself (Figs. 10, 14). Some cisternae of the endoplasmic reticulum are associated with ribosomes while many are

smooth (Figs. 10, 11, 18). Tubular endoplasmic reticulum is also present in some cells (Fig. 11). Mitochondria are highly irregular in outline and each contains several convoluted cristae in addition to particles which might represent mitochondrial ribosomes. Membrane-bound granular or fibrous material, such as the presumptive polysaccharide reserves seen in the yeast phase, is rare (Fig. 15), and no glycogen particles were observed. However, homogeneous storage vac-

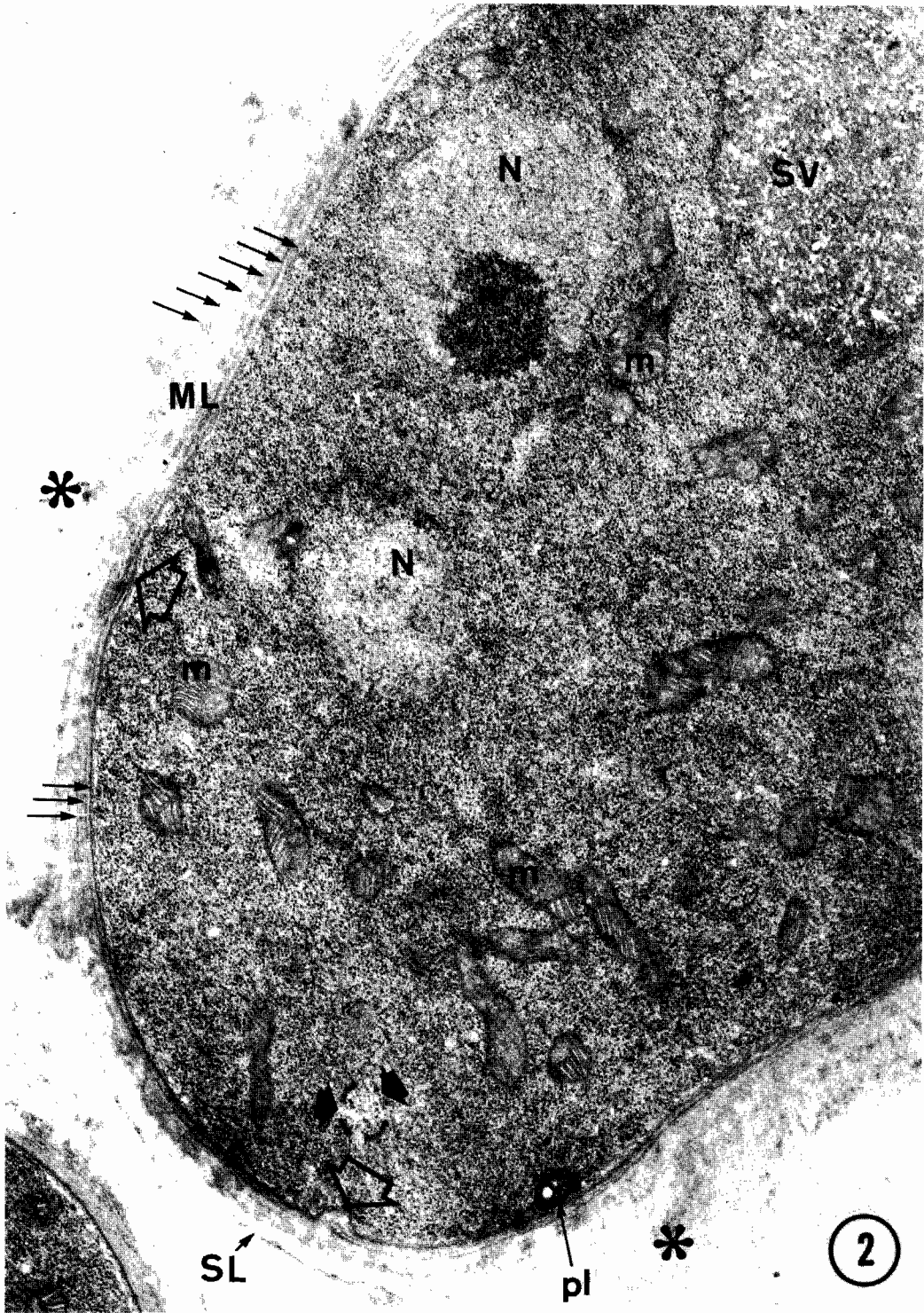
ABBREVIATIONS ON PLATES: C, cross wall; *ds*, dolipore septum; *er*, endoplasmic reticulum; *f*, filamentous coat; *g*, glycogen; *l*, lomasome; *m*, mitochondria; ML, middle lamellae; N, nucleus; *n*, nucleolus; P, parental wall; *pl*, plasmalemmasome; *r*, ribosomes; *rer*, rough endoplasmic reticulum; *sc*, septal pore cap; *ser*, smooth endoplasmic reticulum; SL, superficial layer; SV, storage vacuole; *ter*, tubular endoplasmic reticulum; V, vacuole.

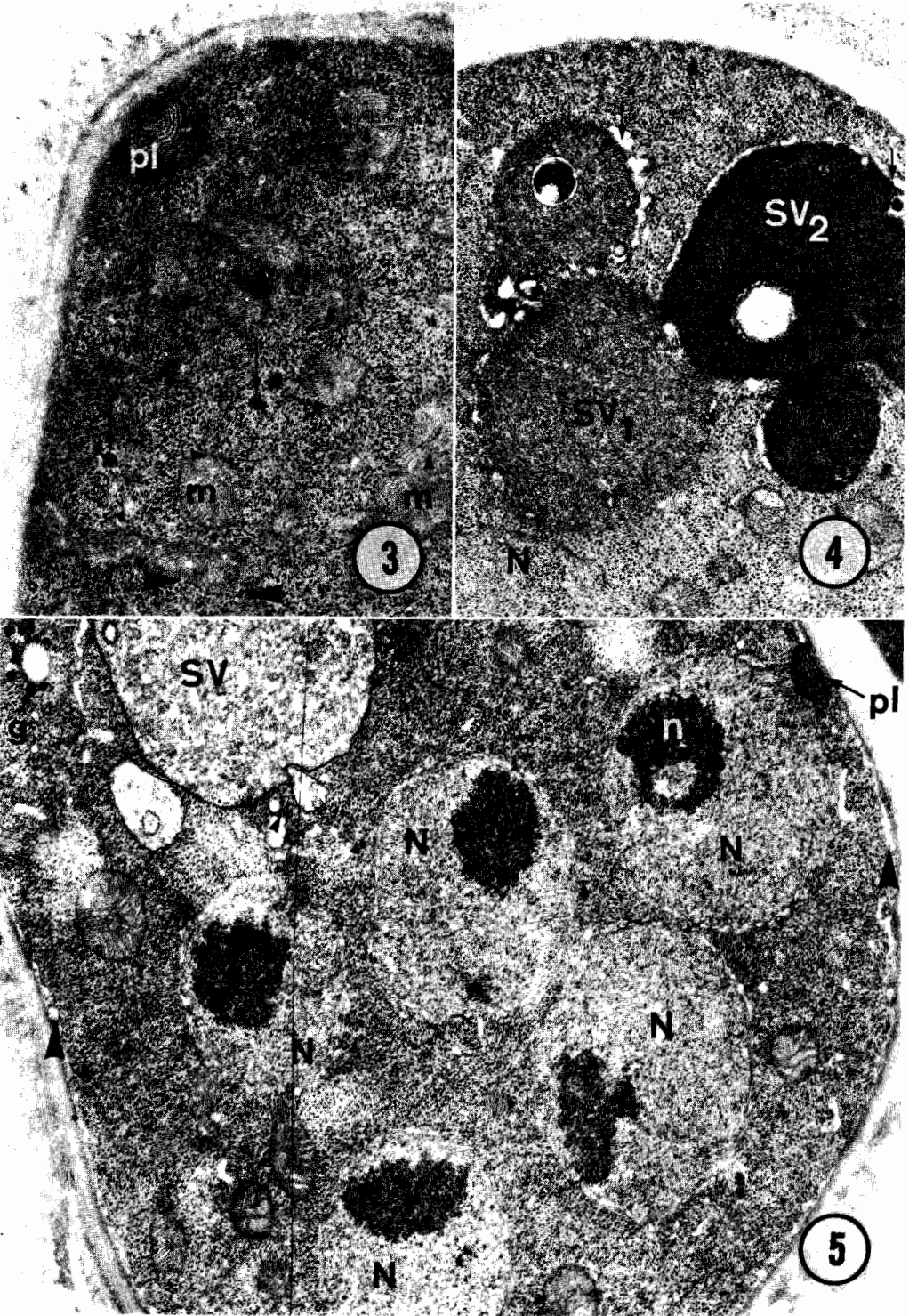
FIG. 2. The yeast stage within the mycangium. The large cell contains at least two nuclei (N) and a storage vacuole (SV) filled with a frothy material. The dense cytoplasm contains many ribosomes and mitochondria (*m*). Most of the cell wall is lamellate: the middle lamellae show a distinct repeat at about 50-m intervals (lower set of parallel arrows). When the cell wall is cut obliquely, the repeat interval appears larger (upper set of parallel arrows). At the lower left is a portion of another cell which has just separated from the major cell in this micrograph. Bud scars are indicated by the hollow arrows; the frayed outermost lamellae of the parental wall are present at the asterisks. A remnant of the superficial layer (SL) is present between the recently separated cells. The circular structure indicated by the short solid arrows resembles a septal cap.  $\times 11\ 600$ .

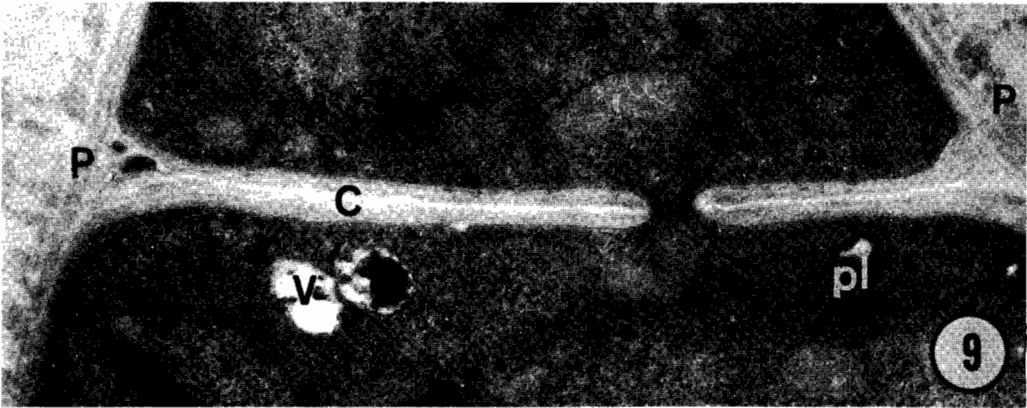
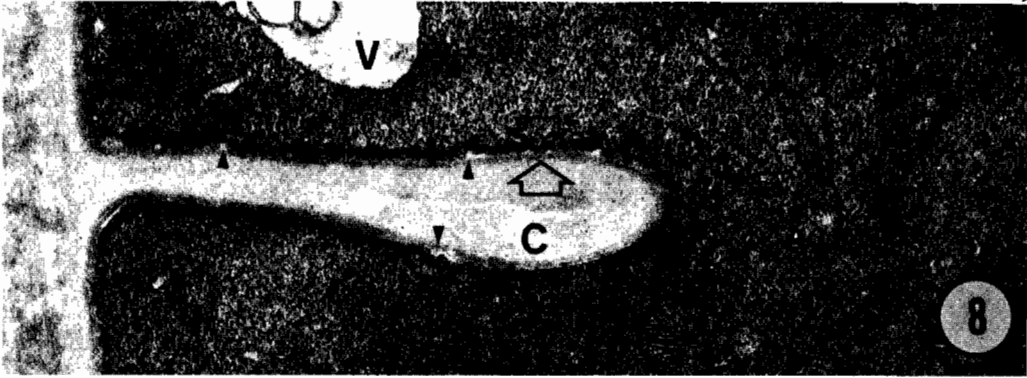
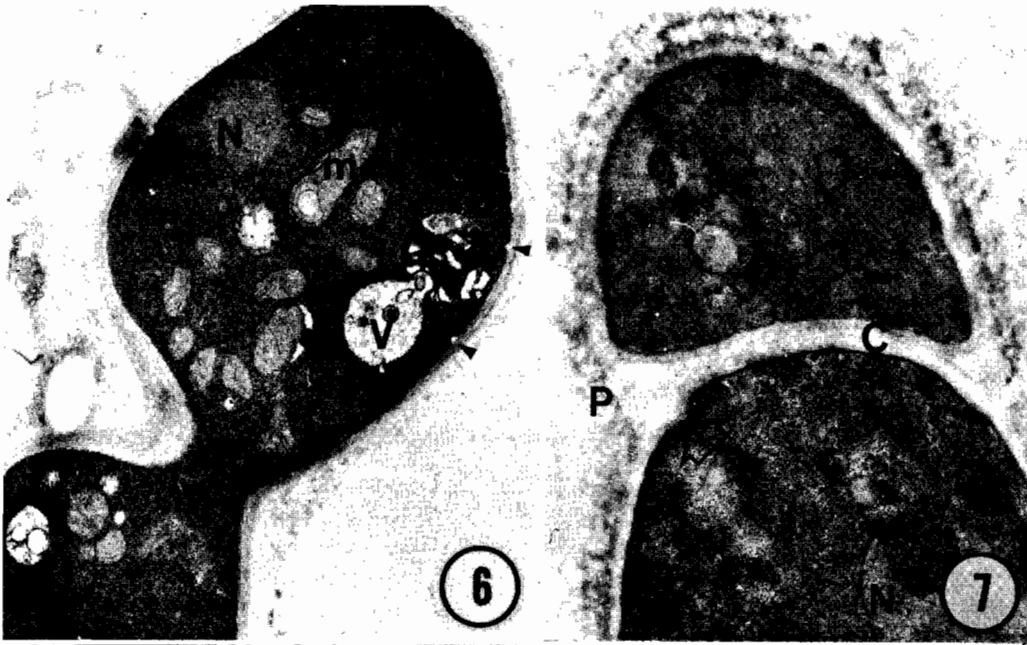
FIG. 3. Cytoplasm of a yeast cell. In addition to mitochondria, ribosomes, and a plasmalemmasome (*pl*), a number of glycogen particles (arrows) are present. A halo of lower electron density appears to surround each glycogen particle. Within the central zones of the mitochondria are ordered arrays of ribosome-like particles (vertical arrowheads), and similar particles are associated with the inner (small horizontal arrowheads) and the outer (between large horizontal arrowheads) boundary membranes.  $\times 23\ 300$ . FIG. 4. Cytoplasm of a yeast cell. The storage vacuoles (SV<sub>1</sub>, SV<sub>2</sub>) are of varying densities (compare also with Figs. 2 and 5) and a cluster of small cisternae surrounds each vacuole.  $\times 15\ 400$ . FIG. 5. A multinucleate yeast cell. The nucleolus at the upper right (*n*) appears to be ring-shaped. Many of the intermembrane spaces are slightly inflated, perhaps as a result of distortion during fixation. Perinuclear spaces can be seen, as well as a lacunar system of flattened cisternae just beneath the plasma membrane (large arrowheads). The glycogen particles (*g*) are clustered around a vesicle.  $\times 16\ 900$ .

FIG. 6. A bud forming. The plasma membrane shows shallow infoldings and ripples (small arrowheads).  $\times 11\ 600$ . FIG. 7. Two daughter cells which are separated by complete cross walls (C) but still linked together by the outer lamellae of the parental wall. Bud scars are indicated by hollow arrows. For a later stage see Fig. 2.  $\times 10\ 800$ . FIG. 8. Cross walls (C) are laid down concurrently with the infolding of the plasma membrane. Note the lack of any plasma membrane infolding 'ahead' (to the right) of the forming cross wall. As in Fig. 6, the plasma membrane is rippled (small arrowheads) and a vacuole is nearby. The innermost lamella of the cross walls (between the hollow arrows) is of higher electron density than the overlying layers.  $\times 20\ 400$ . FIG. 9. Infoldings of plasma membranes and deposition of cross walls from both sides have yielded two daughter cells connected by a narrow neck. In the temporal sequence, this stage lies between that depicted in Fig. 8 and that depicted in Fig. 7.  $\times 23\ 700$ .

FIG. 10. Two cells in the hypha, joined by a dolipore septum (*ds*) with a characteristic septal pore cap, indicated by the large arrowheads in the lower cell. A septal cap is also present in the upper cell. Profiles of the endoplasmic reticulum run parallel to the long axis of the cell and closely approach the plasma membrane (hollow arrows) near the dolipore septum. Numerous pores traverse the nuclear envelope (small arrowheads).  $\times 13\ 200$ . FIG. 11. A hyphal cell. The endoplasmic reticulum appears to be either tubular (*ter*) or cisternal. Some of the cisternae are associated with ribosomes (*rer*) while others appear smooth (*ser*). The storage vacuoles of the hyphal cells (see also Figs. 10 and 12) contain homogeneous contents. Often the storage vacuoles are surrounded by a halo of small vesicles. Numerous irregular vesicles with flocculent contents (asterisks) are scattered throughout the cell. Mitochondria are irregular and contain plate-like cristae.  $\times 27\ 800$ .









uoles of moderate electron density were quite common (Figs. 10, 11, 12) and appear like the lipid granules reported in other fungi.

The plasma membrane of the viable cells is usually closely applied to the cell wall, but in some regions vesiculate lomasomes (Marchant and Robards 1968; Marchant and Moore 1973) are found in the paramural space. Paired membranes of the endoplasmic reticulum often run parallel to the plasma membrane (Figs. 10, 14, 18); similar membrane configurations have been described by Grove *et al.* (1970) in *Pythium*.

The cell wall is not distinctly lamellate as it was in the yeast phase. In viable hyphal branches, very little structure can be seen within the wall (Figs. 10–12, 14). In older and dead cells, fibrous material can be detected (Figs. 13, 16, 17). Dolipore septa, characteristic of certain Basidiomycetes (Moore and McAlear 1962; Bracker 1966), are found between the cells and are associated with fenestrated septal pore caps (Figs. 10, 16, 18). The circular profile in Fig. 16 probably represents a single circular pore. The septal pore cap may have a 'mirror image' (Fig. 18) in some cases. This cap seems to be continuous with membranes of the endoplasmic reticulum (Fig. 18) (compare with Ellis *et al.* (1972)). The cap wall is 350 to 450 Å in thickness: it consists of five distinct layers (Fig. 1). The outermost layers (A and E of Fig. 1) are electron-dense doublets, about 40 Å in thickness, and apparently are continuous with the endoplasmic reticulum. The center layer C is similar to the outermost ones and lies midway between them. Between these thin dense double layers are homogeneous zones (B, D) of 160–180 Å in thickness. Unlike the cap of *Coprinus* (Ellis *et al.* 1972), the homogeneous zones are symmetrical about the central layer. An elaborate tracery of fine filaments is seen in the septa of aging cells (Fig. 16). It is possible that this tracery is always present but is rendered visible only as the wall ages.

### Discussion

Within the mycangium of the adult female southern pine beetle, the yeast-like cells divide rapidly to yield a large mass of independent propagules. Each is surrounded by a thick lamellate wall, and the septa between adjacent cells are entire rather than perforate. In contrast, the hyphal cells communicate via dolipore septa: clearly the need for a coherent tissue is much less

within the mycangium where the selection pressure will favor large numbers of viable propagules, each of which is the potential origin of a new hyphal mass along the gallery inoculated by the female.

Paramural bodies are seen in both yeast and hyphal phases, yet they differ considerably in morphology and probably also in origin. The whorled membranes in the yeast phase suggest 'stored' plasma membrane, and these plasmalemmasomes are associated with invaginations of the rapidly growing cells. The cluster of vesicles that constitute the lomasomes in the hyphae is apparently not continuous with the plasma membrane but perhaps participates in wall formation, a situation analogous to that described by Hughes (1971).

The proper taxonomic classification of the fungus remains enigmatical since portions of the life cycle and critical biochemical and physiological data are lacking. The presence of clamp connections in the hyphae led to its original assignment to the Basidiomycetes (Barras and Perry 1972), and this assignment is confirmed by the distinctive dolipore septa and septal pore caps described in the course of the present investigation.

The dolipore septa argue for affinities with the Tremellales or the Homobasidiomycetes rather than the Uredinales (Coffey *et al.* 1972; Dykstra 1974; Rijo and Sargent 1974). However, Fell (1970) has pointed out the similarity of life cycles in the basidiomycetous yeasts and Ustilaginales.

The fact that the wall of the yeast-like cells is lamellate is quite consistent with basidiomycetous affinities (Kreger-van Rij and Veenhuis 1971). A comparison with the cell walls of *Rhodotorula glutinus* or *Candida muscorum* (as depicted by Kreger-van Rij and Veenhuis (1971)) indicated a detailed resemblance. The outer lamellae are often broken in the thick walls of SJB 122 and they appear to delaminate (Fig. 2) as in *Rhodotorula* (Fig. 1 in Kreger-van Rij and Veenhuis (1971)).

In ascomycetous yeasts, such as *Saccharomyces*, budding occurs by an extension of the parent cell wall, whereas in basidiomycetous yeasts, such as *Rhodotorula*, the bud wall is formed from an entirely new wall laid down within a preexisting one (Marchant and Smith 1967, 1968). Cross wall formation in our ectosymbiont is rather like that of *Rhodotorula*. Further-

more, the bud scar of this ectosymbiont, consisting partly of frayed outer lamellae of the parent cell and partly of new cross wall, is quite similar to the basidiomycetous yeasts *Rhodotorula* (Marchant and Smith 1967) and *Candida muscorum* (Kreger-van Rij and Veenhuis 1971).

An unusual feature of the yeast phase of SJB 122 is its multinucleate condition since most yeasts are uninucleate. We feel that the multinucleate condition merely reflects an adaptation to allow rapid growth when the propagule is freed from the mycangium since cytokinesis and wall formation may occur without requiring an intervening period for deoxyribonucleic acid (DNA) replication and karyokinesis. Multinucleate yeast stages are known in other ectosymbiotic fungi, for example *Ascoidea* (Kreger-van Rij 1973). The high concentration of ribosomes and the density of the 'cytoplasmic ground substance' are consistent with the role of a fungal propagule, which (like an amphibian oocyte) is poised, awaiting only an environmental signal to allow growth and differentiation.

In summation, we feel that the fungus with large propagules within the mycangium of *D. frontalis* shows strong affinities with the Basidiomycetes yet is unusual in being multinucleate.

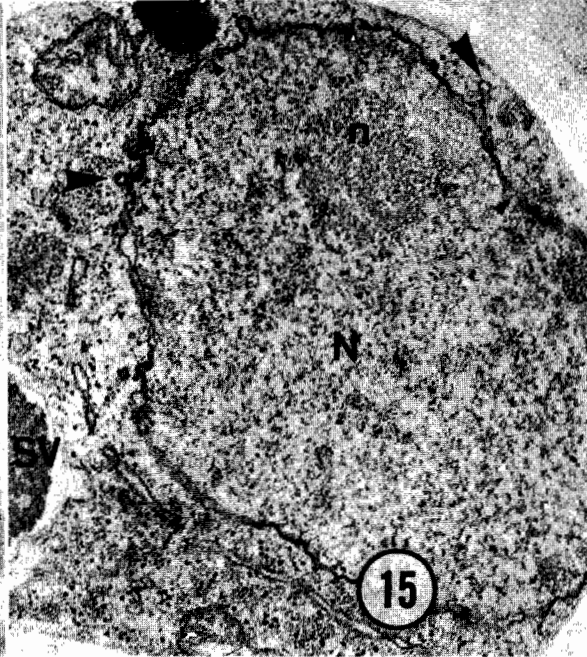
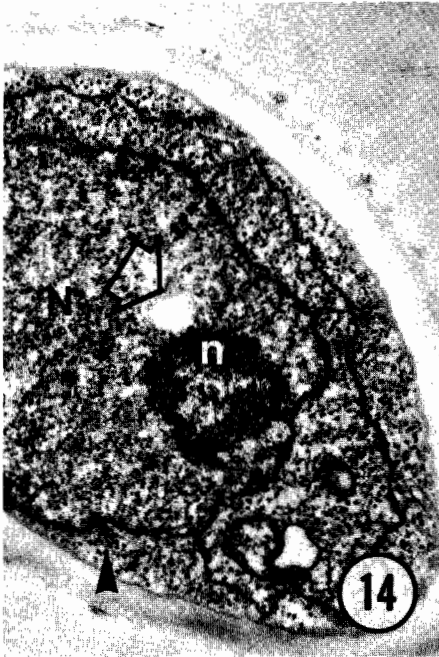
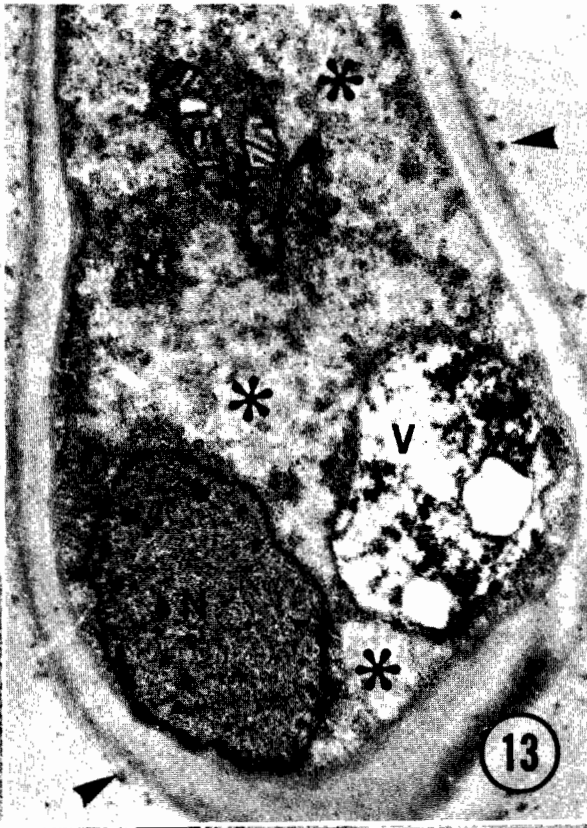
Basidiomycetes are rare as mycangial fungi, perhaps because coevolution of wood-inhabiting insects and Basidiomycetes was not a favorable adaptation or because ascomycetous forms tend to be more effective as colonizers. However, many of the symbiotic imperfect yeasts have not been linked with sexual forms. Thus, the fungal class(es) is not known. The ecology of the presently described heterobasidiomycetous yeasts is poorly known (Fell 1970), and there appears to be some difficulty in aligning the marine forms with the traditionally pathogenic Ustilaginales. A somewhat more natural alignment appears possible with the basidiomycetous forms associated with *Dendroctonus* that inhabit conifers. The ecology and physiology of this interesting fungal ectosymbiont await further study.

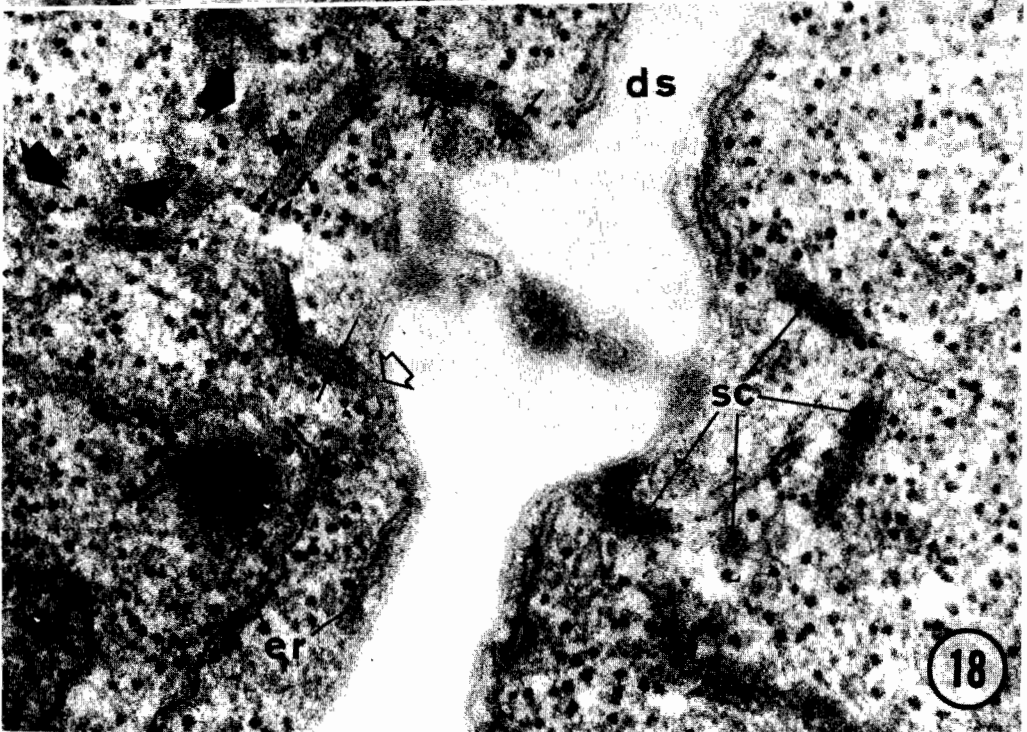
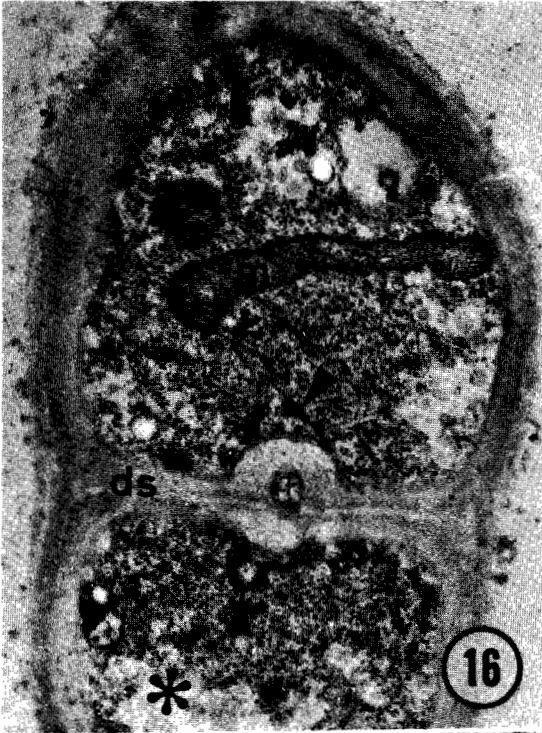
#### Acknowledgments

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FIG. 12. A constriction (between hollow arrows) in the hypha. A heterogeneous collection of cytoplasmic vesicles and vesiculate lomasomes (*l*) is present.  $\times 18\ 000$ . FIG. 13. An aging hyphal cell. Most of the cytoplasm is filled with loose flocculent material (asterisks). The ribosomes (*r*) are clustered together in dense patches along the plasma membrane or around other organelles (mitochondria, nucleus, vacuole). The nuclear content is denser than in younger cells (Figs. 10, 11, 14, 15) and the nuclear envelope contains dense plaques (small arrowheads), which may represent blocked nuclear pores. The cell wall shows a higher affinity for stain than does the wall of younger cells (Figs. 10, 11, 14) and is studded with tufts of dense material (large arrowheads).  $\times 16\ 400$ . FIG. 14. Cross section through a hypha and the nucleus of a young cell. Adjacent to the ring-shaped nucleolus (*n*) is a membrane-bound channel (hollow arrow), which may communicate with the perinuclear space. Nuclear pores are indicated by the small arrowheads and 'blebbing' is indicated by the large arrowhead.  $\times 31\ 300$ . FIG. 15. Hyphal cross section. The nucleus has numerous pores (small arrowheads) and blebs (large arrowheads).  $\times 29\ 300$ .

FIG. 16. A dolipore septum between two aging hyphal cells. As in Fig. 13, the cytoplasm is becoming filled with weakly staining flocculent material (asterisk). The septal pore caps (arrowheads) are poorly organized. As is typical of aging cells, the cell wall is more intensely stained and a fine tracery is seen in the central portion of the dolipore septum.  $\times 20\ 100$ . FIG. 17. The cell wall in the center of the mycelium. The cell is dead; only its electron-dense plasma membrane (arrows) and other electron-dense material persist within the wall. The exterior surface of the wall bears tufts (arrowhead) and a filamentous coat (*f*).  $\times 31\ 700$ . FIG. 18. A dolipore septum between two young cells. Septal pore caps (*sc*) are present in both cells, and within the left cell is a mirror image of the cap (heavy arrows). The wall of the cap consists of five layers: three quite dense lines (small arrowheads) between which lie thicker layers of moderate electron density. When sectioned in a favorable plane (as shown by the pairs of opposed arrows), each of the dense lines appears to be a 'unit membrane.' Continuity with the endoplasmic reticulum is suggested in the region indicated by the hollow arrow.  $\times 86\ 800$ .





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