Conidial state of *Poronia punctata*[1]

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Conidium ontogeny of *Poronia punctata* is described. The conidium wall is continuous with the conidiogenous cell wall, i.e., is holoblastic in origin. Secession of the conidium involves rupture of a highly developed cross wall between conidium and conidiogenous cell.

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Les auteurs décrivent l'ontogénie des conidies de *Poronia punctata*. La paroi de la conidie est en continuité avec la paroi de la cellule conidiogène, c'est-à-dire qu'elle est d'origine holoblastique. La séparation de la conidie fait intervenir la rupture d'une paroi cellulaire transverse fortement développée entre la conidie et la cellule conidiogène. [Traduit par le journal]

Poronia Willd. ex S. F. Gray has been delimited from other xylariaceous genera mainly on the characteristic shape of the stroma and the coprophilous habit (4). A study of conidial *P. oedipus* (Mont.) Mont. indicated that features not previously encountered in other xylariaceous genera might be associated with *Poronia* and thus be taxonomically useful in generic separations in the Xylariaceae (6). A study of *P. punctata* (L. ex Fr.) Fr. was undertaken to extend our information on conidial states of *Poronia*. The more obvious aspects of the conidial state of *P. punctata* were reported by Tulasne and Tulasne (14), Brefeld (1), and Dawson (3). The mechanics of conidium production, however, were not elucidated in these early papers. Recently, Watts (now Russell) (15) completed a preliminary study of conidial *P. punctata*. Her results, along with those of the senior authors, are reported herein.

Materials and Methods

The culture of *Poronia punctata* was given us by Professor George Carroll, University of Oregon. He isolated the fungus from *Bos* dung in Mexico. Voucher material has been deposited in the Washington State University Mycological Herbarium (WSP). Cultures for developmental study were grown on laboratory benches, under prevailing conditions of heat (about 72°F) and light (about 12 h fluorescent) on 2% potato dextrose agar plus 5 g/liter yeast extract (PDYA).

Material to be examined by light microscopy was teased from the stroma and stained with 1% aqueous acid fuchsin or 1% cotton blue in 85% lactic acid. In preparation for transmission electron microscopy stromata were usually fixed in 3.5% glutaraldehyde – 4% formaldehyde in 0.2 *M* cacodylate buffer, pH 7.2, prepared according to Karnovsky (8). All electron microscope figures except those listed below represent material fixed this way. Alternatively, fixation was with 2% glutaraldehyde – H_2O_2 (10) in 0.2 *M* cacodylate buffer, pH 7.2. Figures 9, 10, and 14 represent material fixed in this second way. All fixations were for 4 h at room temperature. After fixation by either procedure, material was washed in 0.2 *M* cacodylate buffer, postfixed for 2 h at room temperature in 2% OsO_4, dehydrated via a graded ethanol series followed by propylene oxide, and finally infiltrated via an ascending series of propylene oxide – Spurr's resin (13). After polymerization, the material was sectioned on a Porter-Blum MT-2B microtome equipped with a diamond knife, stained with uranyl acetate and lead citrate (11), and examined at 50 kV on a Hitachi HS8 electron microscope.

Material to be examined by scanning microscopy was fixed as for transmission microscopy (first fixation procedure), dehydrated via a graded ethanol series, and subsequently through an ethanol – Freon TF series (2). After dehydration, material was subjected to a Bomar SPL-900 critical point apparatus. Material so dried was vacuum-coated with carbon and gold and examined by an ETEC Autoscan scanning electron microscope.

Results

Young nail-shaped stromata of *Poronia punctata* are usually produced on PDYA 10–14 days after mycelial transfers to petri plates. The

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conidial state is evident as a grayish to slightly pinkish bloom on the expanding stromatal discs. Conidia are almost entirely confined to stromata. Transfers that do not form stromata produce very few conidia.

Examination of freehand sections of young stromata show that hyphae of the conidial state are extensions of the ectostromatal hyphae. These hyphae are mostly prostrate, forming a mat on the stromal disc. Each hypha apparently is a potential conidiophore.

Conidia are produced laterally from hyphal cells in no apparent pattern (Figs. 1, 6). A given conidiogenous cell produces one to five, and sometimes more, conidia. Each cell of a hypha appears to be potentially conidiogenous. Sometimes, lengths of hypha can be traced in which every cell is producing or has produced conidia. Other hyphae feature production of conidia by some cells, but not by others.

Hyphae of the conidial state disarticulate by a process that seems similar to the conidial secession process (see later). Entire hyphae fall apart into single cells or short lengths of cells (Figs. 2, 3, 4, 6). Both active and spent conidiogenous cells and cells that have not produced conidia are involved. Hyphal disarticulation can be seen any time that conida are being produced, but is most obvious just before complete cessation of conidial production. At this time the stromal disc is powdery with a mixture of conidia, conidiogenous cells, and other cells. Most of the conidiogenous cells are scarred ghosts almost devoid of cytoplasm, but often with conidia still attached (Figs. 3, 4). Conidia and those disarticulated cells containing cytoplasm germinate readily in distilled water or on solid media (Fig. 5).

Conidiogenesis was observed by the transmission electron microscope. The first evidence of conidiogenesis is a slight papilla or bulge of the wall of a hyphal cell (Fig. 8). Although other ultrastructural features indicative of the commencement of conidiogenesis may have been present, our fixation–staining procedures yielded disappointingly few interpretable cytoplasmic organelles. In any case, the bulge involved the entire hyphal cell wall, which shows an electron transparent inner portion and a much thinner electron-dense outer portion; the outer portion appeared to be two-layered. In addition, the outermost part of the wall is covered with what appears to be a rough sheath. This thin sheath is broken by the growing wall bulge, i.e., incipient conidium (Fig. 9).

The young conidium is irregularly globoid to obovoid with an attenuated isthmus-shaped basal portion (Fig. 10). The wall of the young conidium and the conidiogenous cell are continuous; the conidium develops an outer sheath that is rougher than that of the conidiogenous cell (Fig. 10). A septum develops across the isthmus between conidium and conidiogenous cell (Fig. 11). At first, a cytoplasmic connection apparently exists between conidium and conidiogenous cell through a septal pore. The septal region then becomes very thick and enlarged owing to deposition of electron-transparent material that appears identical with the inner portion of the conidium or conidiogenous cell wall (Figs. 12, 13, 14). It seems likely that both conidium and conidiogenous cell participate in construction of the massive plug.

A conidium secedes when the plug ruptures in the region of the original septum (Figs. 12, 13, 14). The remnants of the plug appear on the conidium as a highly thickened truncate basal scar (Figs. 7, 15) and on the conidiogenous cell as a thickened raised scar (Figs. 6, 11). The thickened scar indicates to us that only a single conidium is ordinarily produced from a given site on a conidiogenous cell. Conidiogenous sites can, however, be exceedingly close together (Fig. 14). Disarticulation of hyphae likewise apparently involves deposition of wall material on both sides of a septum and subsequent rupture in the septal region.

Mature conidia are uninucleate (Fig. 15) and more or less roughened at high magnification (Fig. 7). The degree of roughness corresponds to the development of the outer sheath.

Discussion

The conidial state of *Poronia punctata* resembles that of *P. oedipus* in most respects, but differs chiefly in that conidia of the latter species usually are produced from specialized conidiogenous cells which, in turn, are borne on cells of prostrate hyphae covering the young stromal disc (6). These conidiogenous cells were called sympodulae (6), a term that we now reject in light of newer and more precise definitions (9). The conidiogenous cells of *P. punctata* are those of the prostrate hyphae on the young stromal

cap; no intervening cell types were seen. Thus the conidial state of *P. punctata* seems morphologically simpler than that of *P. oedipus*.

Following the terminology in *Taxonomy of Fungi Imperfecti* (9), conidia of *P. punctata* are blastic and the origin of the conidium wall is holoblastic, i.e., all wall layers of the conidiogenous cell are involved in formation of the conidium wall. Only the outer sheath of the conidiogenous cell wall is thought not to be involved in conidiogenesis.

Conidiogenous cells, at least those behind the growing cells at the hyphal tip and before hyphal disarticulation, can probably be defined as determinate, i.e., conidiogenous cells do not appear to be enlarging or lengthening during conidiogenesis in an intact hypha. The pattern of production of conidia from a given conidiogenous cell appears to be random. After disarticulation, however, conidiogenous cells not devoid of cytoplasm can continue to produce conidia and (or) germ tubes. Thus, in this species every cell of the prostrate hypha is potentially a conidiogenous cell, a propagule, or both. Indeed, following disarticulation and cessation of conidial production, what was formerly called a conidiogenous cell can behave as a conidium and might be definable as a conidium.

The mechanics of conidium production in *Poronia* species seem identical with those that have been found in all investigated fungi that are undoubted members of the family Xylariaceae, i.e., conidia originate blastically and they are produced singly (see 7, for additional discussion and references). Although conidial *P. punctata* is to our knowledge the first imperfect state of a xylariaceous fungus to be investigated with the transmission electron microscope the findings add little new to our concept of conidiogenesis in xylariaceous fungi. The most surprising finding was the massive wall or plug associated with conidium secession. Such a structure is probably associated with conidiogenesis in all xylariaceous fungi and is probably reflected, at the light microscopic level, in the truncate bases of conidia of all xylariaceous fungi observed by us. T. M. Hammill, State University of New York, Oswego, has shown transmission electron micrographs of conidium formation and secession in other types of fungi that produce conidia holoblastically and in a sympodial sequence; his findings are very similar to those reported here (personal communication; 5). Thus, the mechanics of conidiogenesis and conidium secession reported here seem highly correlated with other features of xylariaceous fungi, but are not unique to xylariaceous fungi.

Among xylariaceous genera investigated by us, only *Poronia* shows disarticulating hyphae with both conidiogenous and non-conidiogenous cells that, themselves, can act as propagules. Indeed, we suggest that caution be exercised in the assigning of any fungus to *Poronia* that does not show disarticulating hyphae associated with the conidial state. Moreover, there are fungi now assigned to *Xylaria* that greatly resemble *Poronia* in gross stromal morphology, but which occur on wood. Such fungi should be investigated to determine if their conidial states resemble *Poronia* or *Xylaria*. In our experience *Xylaria* species usually produce a closely packed palisade of upright conidiophores on the stromata and feature a more or less sympodial sequence of conidial production; disarticulating hyphae have not been seen (unpublished data). Limited cytological investigations indicate that *Xylaria* and *Poronia* might likewise be separable on other criteria (see 12 and references therein).

Dawson (3) figured what appear to be trichogynes in *P. punctata*. We were unable to find trichogynous extensions of ascogonia in our isolate of *P. punctata* and assume that conidia function as propagules. We suspect, however, that conidia of this and other xylariaceous fungi arose during evolution as spermatia.

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EXPLANATION OF FIGURES

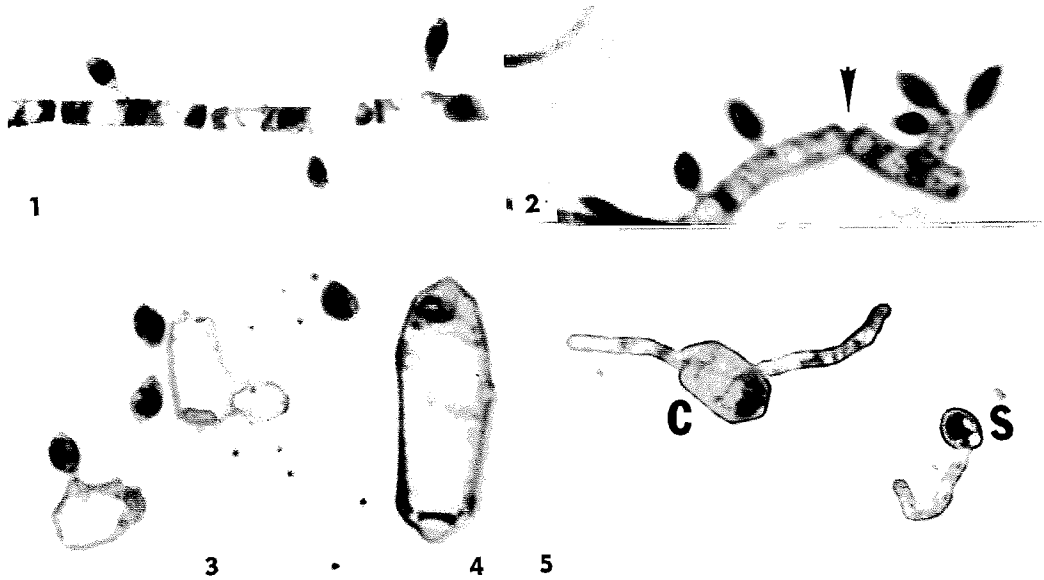
FIGS. 1–8. *Poronia punctata*. Figs. 1–5 by light microscopy; Figs. 6 and 7 by scanning electron microscopy; Fig. 8 by transmission electron microscopy. Figs. 1, 2. Hyphae bearing conidia. Hypha in Fig. 2 beginning to disarticulate (arrow). Fig. 3. Disarticulated conidiogenous cells and conidia. Fig. 4. Conidiogenous cell. Fig. 5. Germinated conidiogenous cell (C) and conidium (S). Fig. 6. Hyphae bearing conidia. Lower hypha shows a conidium and numerous scars indicating former sites of conidium production. Upper hypha is a two-celled fragment that has resulted from hyphal disarticulation. Fig. 7. Conidia. Fig. 8. Conidiogenous cell. Papilla (arrow) is early stage of conidium formation. Figures 1 and 2 from lactic acid – cotton blue mounts; Figs. 3, 4, and 5 from aqueous acid fuchsin mounts; other figures from material prepared as explained in text. Magnifications: Figs. 1 and 5, ca. × 2000; Figs. 2 and 3, ca. × 2200; Fig. 4, ca. × 3300; Fig. 6, ca. × 4700; Fig. 7, ca. × 9000; Fig. 8, ca. × 23 000.

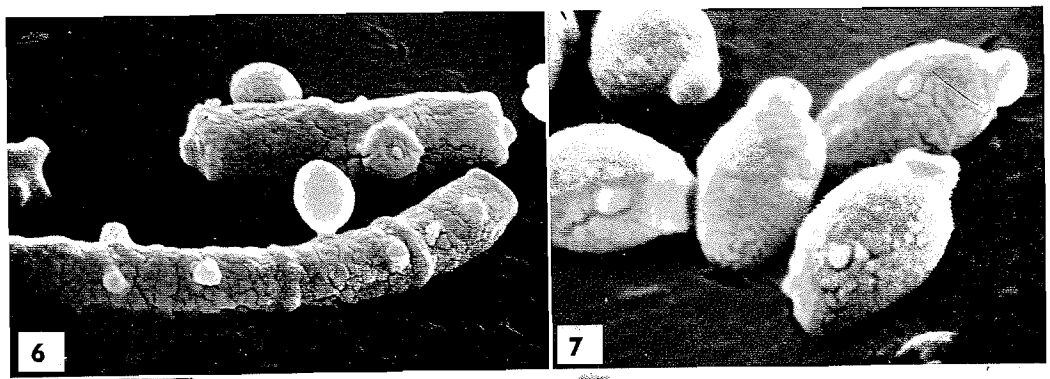
FIGS. 9–12. *Poronia punctata*. Fig. 9. Early stage in conidium production. Note that outer sheath of conidiogenous cell wall has been broken by conidial protuberance (arrow). Fig. 10. Incipient conidium. Note that roughened outer sheath on conidium is not continuous with conidiogenous cell. Fig. 11. Conidiogenous cells. Septum has formed in isthmus between conidiogenous cell and conidium. Small lower cell shows conspicuously thickened wall at apex indicating the former production site of a conidium. Fig. 12. Early stage of conidium secession. The conspicuously thickened cross wall is beginning to rupture in the vicinity of the original septum (arrow). Note narrow septal channel that has apparently been overgrown by wall material. See text for preparation procedures. Magnifications: Fig. 9, ca. × 17 500; Fig. 11, ca. × 18 500; Fig. 12, ca. × 19 000; Fig. 10, ca. × 27 000.

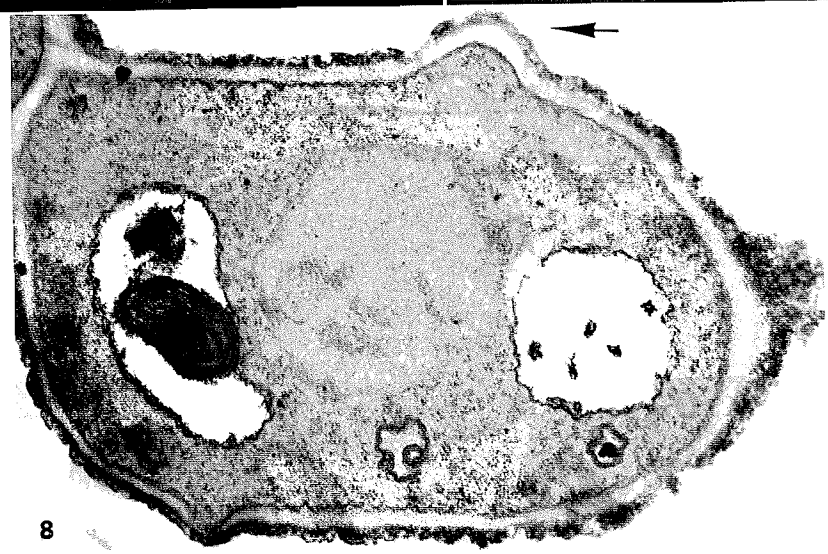
FIGS. 13–15. *Poronia punctata*. Fig. 13. Rupture in massive wall between conidiogenous cell and conidium (arrow). Fig. 14. Conidiogenous cell showing two conidia of about the same age. Fig. 15. Mature conidium. Note plug of heavy wall material at lower truncate end that indicates former site of attachment to conidiogenous cell. See text for preparation procedures. Magnifications: Fig. 14, ca. × 16 500; Fig. 13, ca. × 18 500; Fig. 15, ca. × 28 000.

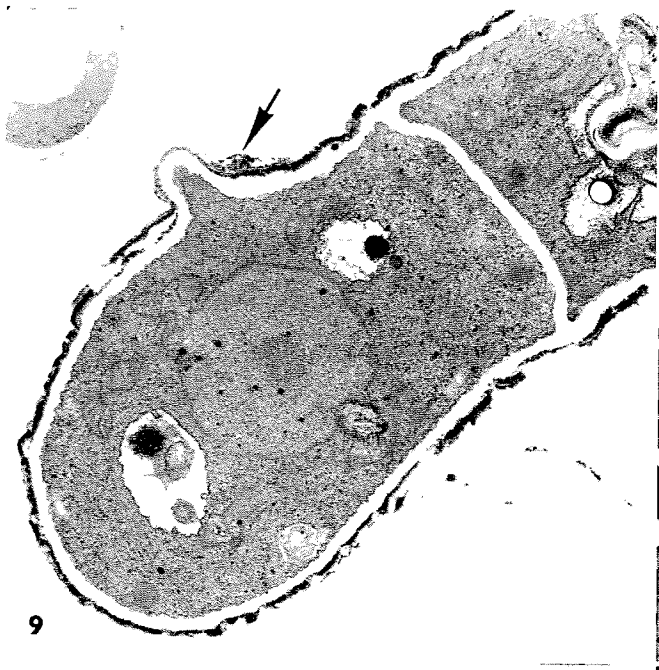
NOTE: Figs. 1–15 follow.

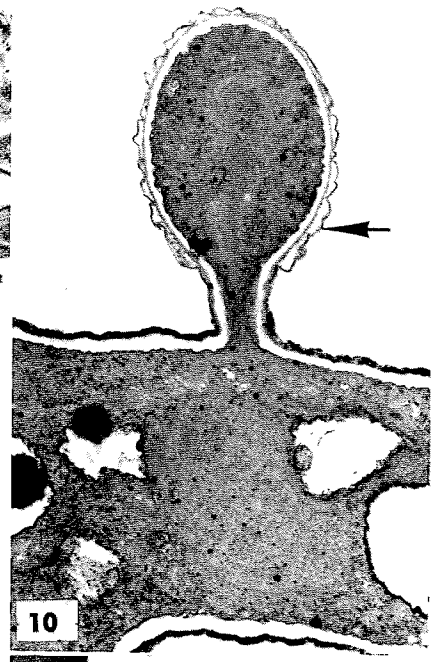
PLATE I

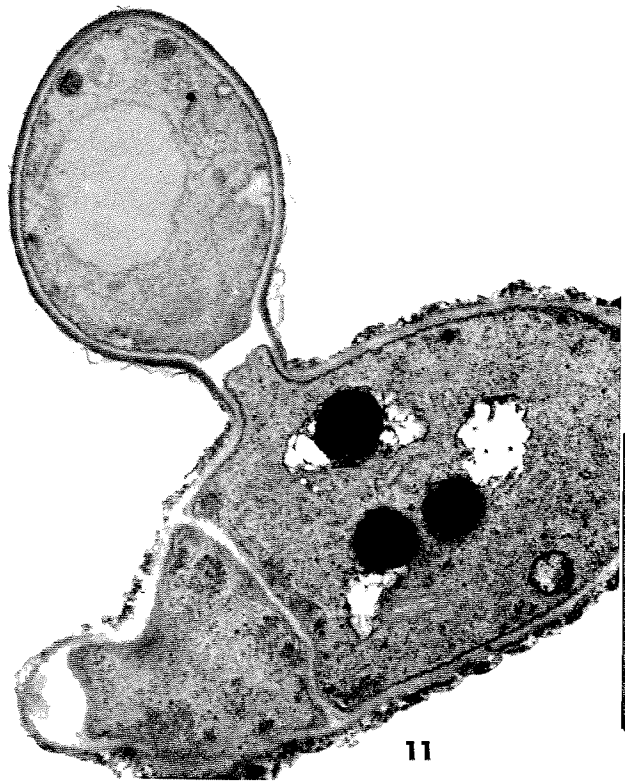


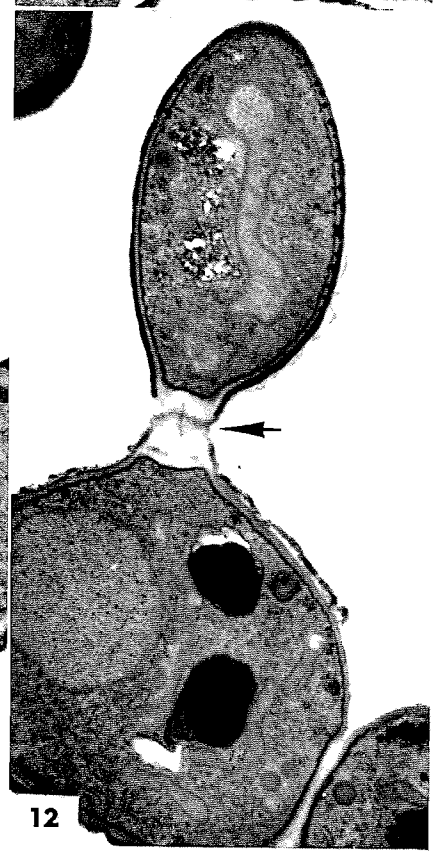


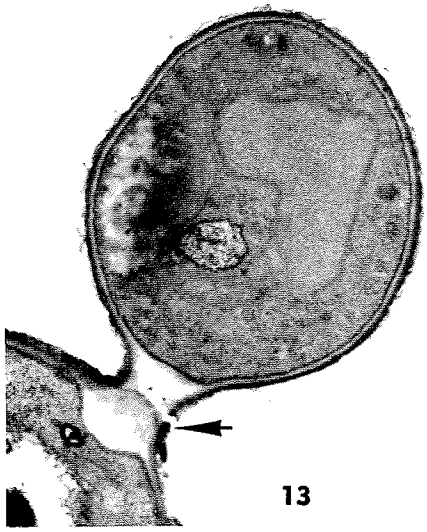












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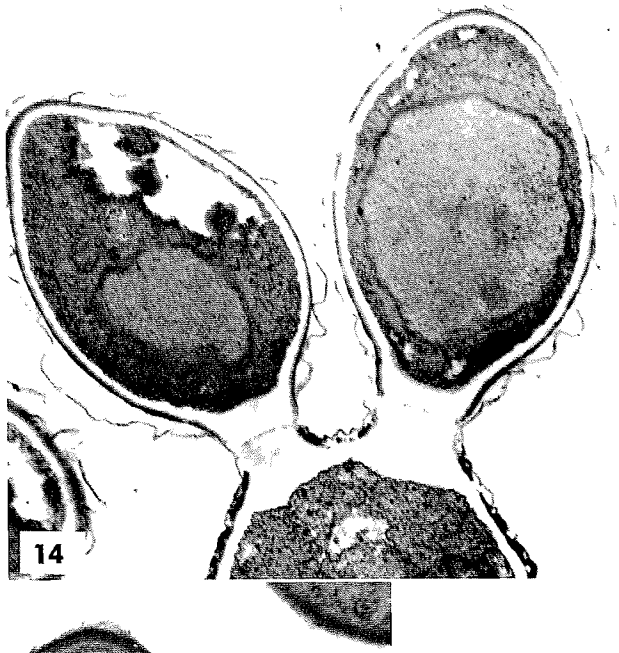

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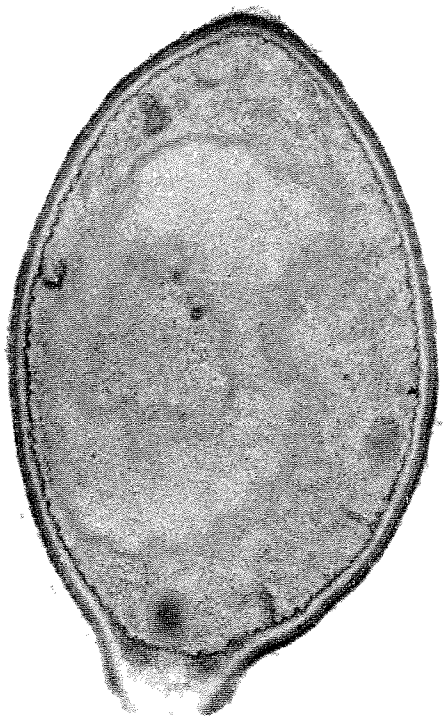

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