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SOME OOMYCETES AND ZYGOMYCETES WITH ASEXUAL ECHINULATE REPRODUCTIVE STRUCTURES

A. J. VAN DER PLAATS-NITERINK, R. A. SAMSON,
J. A. STALPERS, & A. C. M. WEIJMAN

Centraalbureau voor Schimmelcultures, Baarn

(With Plate 18 and two Text-figures)

Fungi producing ornamented asexual structures and belonging to the Oomycetes (*Trachysphaera*) or Zygomycetes (*Azygozygum*, *Mortierella*) are described. They were studied by light and scanning electron microscopy while also mating experiments and carbohydrate analyses were performed. *Azygozygum chlamydosporum* is closely related to *Mortierella indohii* and therefore *Azygozygum* is considered to be a synonym of *Mortierella*. *Mortierella echinosphaera spec. nov.* is also closely related, but no zygotes are known, only ornamented chlamydospores have been observed. Absence of glucuronic acid and fucose and a low glucosamine content in *Trachysphaera fructigena* show that it belongs to the Oomycetes.

INTRODUCTION

A number of Oomycetes and Zygomycetes produce ornamented reproductive structures. In the Oomycetes these structures are generally the sexual state of the fungus, the oogonia; ornamented asexual structures (conidia, chlamydospores) only have been observed in the genus *Trachysphaera* Tabor & Bunting. In the Zygomycetes ornamented chlamydospores (stylospores) are known in the genera *Azygozygum* Chesters and *Mortierella* Coemans. Because the cell wall composition of Oomycetes differs greatly from that of Zygomycetes, cell wall analysis should yield definite taxonomic information.

Tabor & Bunting (1923) described a disease of cocoa and coffee fruit, reminescent of that caused by *Phytophthora faberi* Maubl. The causal agent, *Trachysphaera fructigena*, is characterized by oogonia and amphigynous antheridia, indicating that is is related to *Phytophthora*. *Trachysphaera fructigena* also produces numerous spiny 'conidia' originating from branched stalks which form several vesicles. These vesicles give rise to short projections which may bear either conidia or extend to form another vesicle. Zoosporangia and zoospores are unknown. Tabor & Bunting noticed that when the sex organs are absent, the species may be mistaken for *Muratella* (=*Cunninghamella*).

Azygozygum chlamydosporum Chesters is characterized by the absence of sporangia and the presence of zygospores and spiny chlamydospores, the latter being intercalary

or terminal on erect stalks. Chesters (1933) recognized the affinity with *Mortierella* because the chlamydospores resemble those of *Mortierella polycephala* Coemans. Hesseltine & Ellis (1973) placed the genus provisionally in the Mucoraceae, but also mentioned the Endogonaceae as a possible alternative.

In *Mortierella* similar chlamydospores are known. They have often been confused with one-spored sporangioles, which are borne on sporangiophores with swollen bases, gradually tapering toward the apex (Gams, 1963). Chlamydospores, when terminal, are formed on hyphae of equal diameter over their entire lenght. *Mortierella indohii* Chien is described (Chien *& al.*, 1974) as a species lacking sporangia. In other respects *M. indohii* also closely resembles *Azygozygum chlamydosporum*, but it was placed in *Mortierella* because of the invested zygospores.

The carbohydrate composition of cell walls is a useful criterion for distinguishing larger groups of fungi and can be of importance when the available morphological characters are insufficient. Bartnicki-Garcia (1968, 1970) distinguished eight groups within the fungi, based on overall cell wall composition. *Pythium* and related genera were included in the cellulose-glucan group (Oomycetes), whereas *Mortierella* was placed in the chitosan-chitin group (Mucorales). In addition to glucans, chitosan and chitin, other cell wall carbohydrates may serve as indicators for subdividing fungal taxa. Intact cell analysis, applied to this study, is an abbreviated procedure allowing the prediction of qualitative differences in cell wall composition. This approach has been successfully applied to taxonomic problems with bacteria (Jantzen *& al.*, 1972; Lechevalier & Lechevalier, 1970) and to fungi of the *Ceratocystis-Ophiostoma* group (Weijman & de Hoog, 1975).

METHODS

All strains were grown on cornmeal, 2% malt, soil-extract and potato-carrot agars. In some cases a sucrose-nitrate medium was used (Chesters, 1933). Mating experiments were carried out on 'Bambix' agar which is analogous to Kuhlman's (1972) 'Pablum' agar (Bambix is a commercial baby food, produced by Nutricia, Zoetermeer, The Netherlands), consisting of 12.5 gr Bambix, 15 gr agar and 1 liter distilled water; inocula were placed about 0.5–1 cm apart in Petri dishes and incubated at 15 or 20 °C.

For scanning electron microscopy (SEM) chlamydospores were transferred to squares of double-sided adhesive tape, attached to specimen stubs and air-dried for 24 hours. In other cases small pieces of agar containing mycelium with chlamydospores were fixed in osmium tetroxide, washed in distilled water, dehydrated in an alcohol-series, passed through an amylacetate-series, dried in a Polaron critical point drying apparatus under CO_2 and attached to specimen stubs. The specimens were coated with gold in a sputter coater for two minutes at 1.2 kV. Preparations were examined with a Cambridge Stereoscan microscope at an accelarating voltage of 15 kV.

For carbohydrate analysis the strains were grown on glucose (2%)-peptone (1%)-yeast extract (0.5%) medium in conical flasks for ten days at 25 °C on a rotary shaker, operated at 100 rpm. Carbohydrates released from intact cells by acid hydrolysis were analysed by gas-liquid chromatography (GLC) as their trimethylsilyl ethers as described by Weijman & de Hoog (1975).

Hexosamines were estimated quantitatively by a modification of the Elson-Morgan method, as described by Gatt & Berman (1966), using glucosamine-HCl as a standard. Prior to the analysis samples were hydrolyzed with 2N HCl under N_2 for 12 h. at 110 °C. Measurements were taken with a recording Perkin-Elmer 402 UV-visible spectrophotometer using glass cuvettes.

MATERIAL EXAMINED

Mortierella chlamydospora (Chesters) Plaats-Niterink: CBS 120.34, type strain, *C. G. C. Chesters*, from *Antirrhinum majus* roots, England. — CBS 529.75, I. Blok, from *Saintpaulia* roots, The Netherlands.

Mortierella echinosphaera Plaats-Niterink: CBS 574.75, *J. H. van Emden*, from soil, The Netherlands. — CBS 575.75, type strain, *A. J. van der Plaats-Niterink*, from *Begonia* roots, The Netherlands. — CBS 576.75, *P. S. W. Liu*, from *Citrus mitis*, Malaysia.

Mortierella indohii Chien: CBS 720.71 (—), type strain, *C, Y. Chien*, from animal dung, U.S.A. — CBS 655.70 (—), CBS 666.70 (—), *J. H. van Emden*, from soil, The Netherlands, — CBS 219.72 (—), *J. W. Veenbaas-Rijks*, from soil, The Netherlands. — CBS 220.72 (—), *L. H. Kaastra-Höweler*, from greenhouse soil, The Netherlands. — CBS 331.74 (—), *C. L. de Graaff*, from wheat roots, The Netherlands. — CBS 460.75 (+), *C. Y. Chien*, from animal dung, U.S.A. — CBS 528.75 (—), *W. F. O. Marasas*, from begasse, S. Africa.

Mortierella polycephala Coemans: CBS 649.68, *C. W. Hesseltine.*

Pythium oligandrum Drechsler: CBS 382.34, *C. G. C. Chesters*, from *Viola* spec. roots, England.

Pythium spinosum Sawada: CBS 290.31, *S. F. Ashby*, from *Carica papaya*, S. Africa.

Trachysphaera fructigena Tabor & Bunting: CBS 315.31, type strain, *R. Bunting*, from *Theobroma cacao*, Goldcoast.

RESULTS AND DISCUSSION OF THE CHEMICAL STUDY

The carbohydrate analysis of intact cells of *Trachysphaera* reveals, as in *Pythium spinosum* and *P. oligandrum*, a low hexosamine content and the absence of glucuronic acid and fucose (Table 1; Fig. 1a, 1b). If intact cell samples differ considerably in hexosamine content, this difference would be even more pronounced between purified cell-wall samples. On the other hand, if fucose and glucoronic acid are absent in the intact cells, they may be excluded as important cell wall components. Hexosamine detected in oomycetous fungi is probably not released from chitin (Sietsma *& al.*, 1969), although the absence of chitin in Oomycetes has not been conclusively demonstrated in intact cells (Lin & Aronson, 1970).

The following strains (studied only by GLC) yielded the same sugar pattern as the *Mortierella* strains listed in Table 1: *M. indohii* CBS 665.70, CBS 666.70, CBS 219.72,

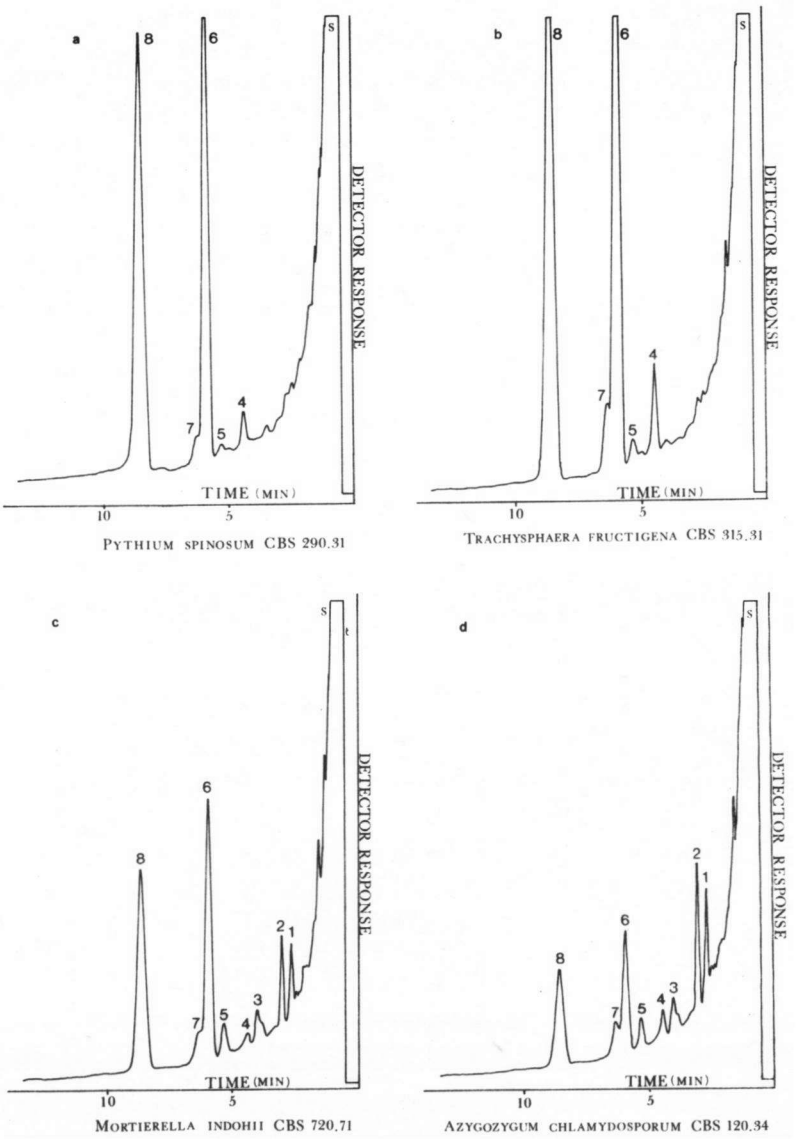

Fig. 1. — Gas chromatograms of carbohydrates (TMS derivatives) released from intact fungal cells by acid hydrolysis. Column temperature: 180°C. Liquid phase: 3% OV–1 coated on Chromosorb W(HP).

S: solvent peak. 1: α-fucose; 2: β-fucose; 3: glucuronolacton; 4: α-mannose; 5: α-galactose; 6: α-glucose; 7: β-mannose and β-galactose; 8: β-glucose.

Table 1. Distribution of hexosamine, fucose and glucuronic acid in some species of *Pythium*, *Trachysphaera*, and *Mortierella*.

strain		hexosamine (%)[1]	fucose[2]	glucuronic acid[2]
Pythium spinosum	CBS 290.31	0.5	—	—
Pythium oligandrum	CBS 382.34	1.0	—	—
Trachysphaera fructigena	CBS 315.31	0.5	—	—
Mortierella chlamydospora	CBS 120.34	6.0	+	+
	CBS 529.75	6.0	+	+
Mortierella indohii	CBS 720.71	5.0	+	+
	CBS 528.75	6.5	+	+

CBS 220.72, *M. echinosphaera* CBS 574.75, CBS 575.75, and *M. polycephala* CBS 649.68. Figures 1c and 1d show characteristic patterns.

Fucose and glucuronic acid polymers are among the common polysaccharides in Zygomycetes (Gooday, 1973). The presence of these components could be demonstrated in all *Mortierella* species studied, but it is not possible to further subdivide these strains on the basis of the chemical data, the gas chromatograms being almost identical. The hexosamine detected in hydrolysates of intact Zygomycete cells is probably derived from chitosan and chitin (Kreger, 1954; Bartnicki-Garcia, 1968).

Descriptions and taxonomic conclusions

(1) Trachysphaera fructigena Tabor & Bunting.—Plate 18e

Colonies on cornmeal and soil-extract-agars submerged with some scanty aerial mycelium bearing clusters of conidia. Hyphae thin-walled, up to 6 μm wide. Oogonia subglobose or pyriform, 25–32 × 24–28 μm, with irregular sac-like outgrowths, varying from short blunt projections to long finger-like processes which may be curved or forked. Antheridia amphigynous, 11–15 × 11–16 μm. Oospores globose, aplerotic, 21–27 μm in diameter, wall 3–4 μm thick. Conidiophores simple or branched, bearing a terminal vesicle or complex of vesicles from which projections arise, reaching up to 30 μm and bearing conidia. Conidia hyaline to yellowish, globose, (24–)32–43(–50) μm in diameter, wall 3–4 μm thick, covered with conical spines up to 3 μm long with blunt, elongated and sometimes slightly curved tips. Daily growth rate: 10 mm at 25 °C.

[1] Average on the basis of intact cell dry weight.
[2] Determined by GLC. Glucuronic acid was analyzed in the lactone form.

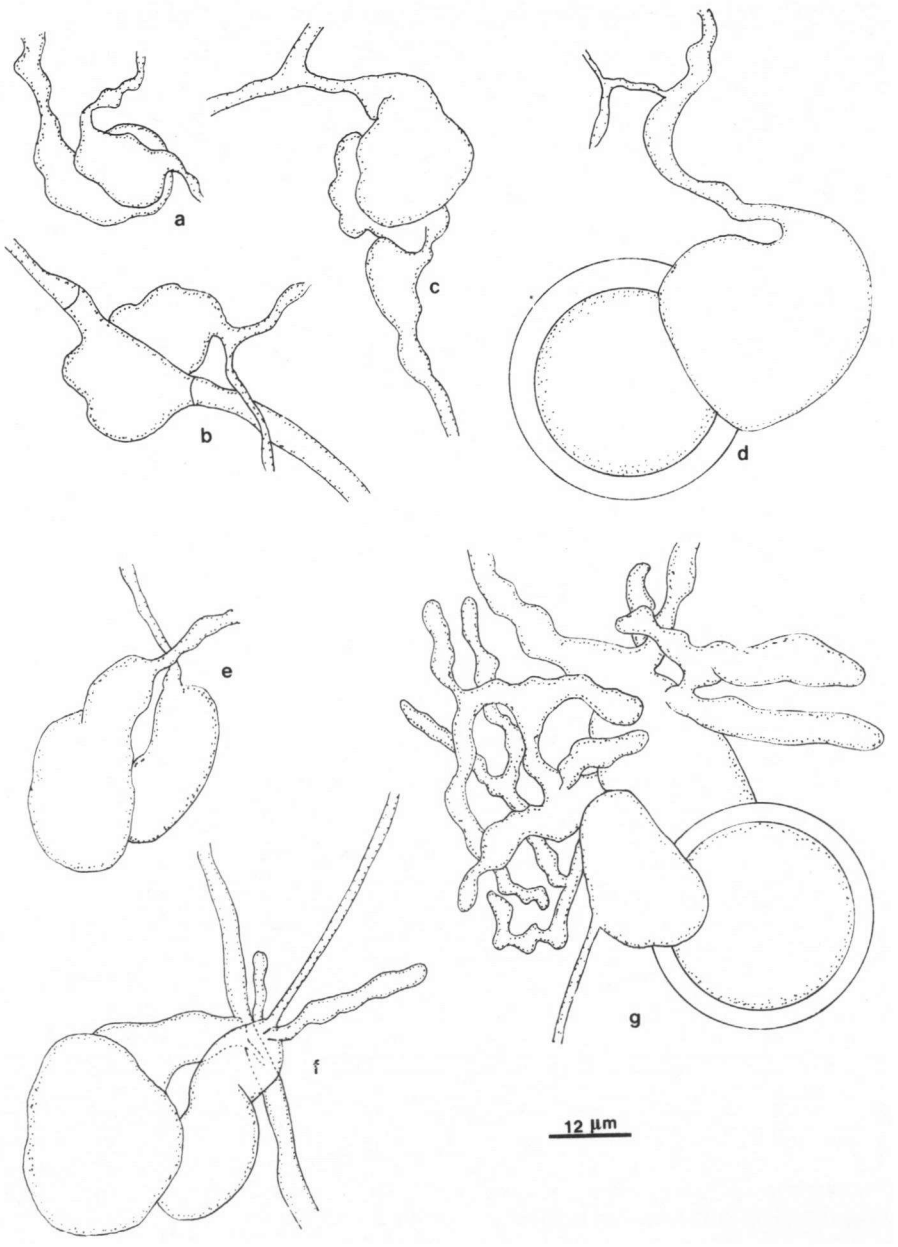

Fig. 2. — Various stages in the development of zygospores. — a–d. *Mortierella chlamydospora*. — e–g. *M. indohii*.

(2) **Mortierella chlamydospora** (Chesters) Plaats-Niterink *comb. nov.*
Plate 18 b, Fig. 2a–d

Azygozygum chlamydosporum Chesters *in* Trans. Br. mycol. Soc. **18**: 213,1933 (basionym).

Colonies on cornmeal agar form a low white aerial mycelium; on soil extract agar only scanty aerial mycelium develops. No or indistinct *Mortierella*-like odour. Hyphae 1–4 μm wide. Sporangia unknown. Chlamydospores terminal and intercalary in the aerial and submerged mycelium on 2–4 μm wide stalks. Chlamydospores globose or elongated when intercalary, with a varying number of spines or sometimes smooth when submerged, (12–)15–26(–28) μm in diameter, wall 1.2 μm thick. Spines cylindrical, about 1 μm wide and up to 4 μm long, with a blunt tip which may be curved. On sucrose-nitrate medium at 20 °C zygospores are formed after one week in single cultures of all strains examined. When mature they are naked, thick-walled, 25–50 μm in diameter, with one inflated suspensor, 25–40 μm in diameter, the other suspensor having disappeared. The initial stage consists of two approaching swollen hyphal tips which later fuse. One suspensor swells until it is nearly the same size as the zygospore which becomes thick-walled. The other suspensor is caducous. In rare cases both zygospore and large suspensor become thick-walled and the thick seperating wall between both cells may be partially or completely absorbed as illustrated by Chesters (1933). Daily growth rate: 9 mm at 25 °C.

(3) **Mortierella echinosphaera** Plaats-Niterink *spec. nov.*—Plate 18 c–d

Coloniae tenues, interdum chlamydosporis farinosae. Mycelium aerium sparsum, albidum. Odor distinctus abest. Hyphae hyalinae, 1–5(–7) μm diametro. Sporangiophora absunt. Chlamydospora normaliter intercalares, interdum terminales, globosae vel elongatae, dense spinulosae, raro glabrae, crassitunicatae, (14–)17–28(–33) μm diametro. Spinulae cylindricae, ad 5 μm longae. Typus: CBS 575.75, e radicibus Begoniae, in Neerlandia.

Colonies on cornmeal agar form a low irregular aerial mycelium, sometimes powdery due to numerous chlamydospores; on soil extract agar submerged, sometimes with some scanty aerial mycelium. No characteristic odour. Hyphae 1–5(–7) μm wide. Chlamydospores usually intercalary, occasionally terminal, in aerial and submerged mycelium, globose to elongated, densely spiny or rarely smooth-walled, (14–)17–28(–33) μm in diameter, wall up to 3 μm thick. Spines cylindrical, sometimes flexuous, with a blunt tip, 1 μm wide and up to 5 μm long. Number of spines variable, all intermediates from smooth to markedly spiny chlamydospores can be found. Sporangia and zygospores unknown. Daily growth rate: 13 mm at 25 °C.

(4) MORTIERELLA INDOHII Chien.—Plate 18a; Fig. 2e–g

Colonies on cornmeal agar with low cottony aerial mycelium, sometimes appearing powdery due to numerous chlamydospores; on soil extract agar aerial mycelium very scanty. Odour characteristic, garlic-like. Hyphae hyaline, 1–4 μm wide. Chlamydospores terminal or sometimes intercalary, originating both from the aerial mycelium as well as from the submerged parts, mostly on simple or sparingly branched stalks with the same diameter as the hyphae, 10–150 μm long, sometimes subterminally swollen. Chlamydospores globose, (11–)14–21(–27) μm in diameter,

occasionally subglobose, limoniform or elongated when intercalary, spiny, rarely smooth, particularly when submerged. Spines cylindrical with a blunt tip, 1 μm wide and up to 3 μm long. On 'Bambix' agar at 15 °C zygospores are formed after one week in the mating line between two compatible strains. Zygospores smooth, globose to subglobose, 24–50 μm in diameter, wall 3–6 μm thick. Suspensors unequal, the larger one developing a mass of strongly branched irregular hyphae at its base. Sporangia unknown. Daily growth rate: 8–10 mm at 25 °C.

The conidia of *Trachysphaera fructigena* show some resemblance to the spores or chlamydospores (the term 'stylospores' is confusing and should be abandoned (Gams, personal communication)) of some Mucorales (e.g. *Cunninghamella*, *Mortierella*), but the sexual state (oogonia and amphigynous antheridia) and the chemical composition are comparable to those of the Oomycetes. The absence of glucuronic acid and fucose and a low hexosamine content in *Trachysphaera fructigena* exclude the possibility of it being related to the Zygomycetes. The genus *Trachysphaera* may be considered to be closely related to *Phytophthora*.

The genus *Mortierella* is characterized by sporangia lacking a columella. Invested zygospores were discovered in the type species *M. polycephala* (Dauphin, 1908). Since 1908 numerous species have been shown to produce naked zygospores with unequal suspensors, mostly after mating (Kuhlman, 1972; Chien *& al.*, 1974). *M. chlamydospora* has typical naked zygospores, but is homothallic. In *M. polycephala* the zygospore-covering hyphae originate, according to Dauphin (1908) from both suspensors; in *M. indohii* only one of the suspensors bears some investing hyphae, so that this species is of an intermediate type. So far there is no reason to subdivide the genus *Mortierella* into one group with naked and one with invested zygospores.

Mortierella indohii, *M. echinosphaera*, and *M. chlamydospora* are distinct but closely related. *Mortierella indohii* differs from the other species by the nature of the chlamydospores which are more often terminal, smaller and more densely spiny while the spines are shorter. *Mortierella indohii* is heterothallic and has a specific aromatic odour which is less distinct or absent in the other species. The sexual state of *M. echinosphaera* is unknown, crossings between the three strains and with *M. indohii* were repeatedly negative. *Mortierella chlamydospora* and *M. echinosphaera* differ not only in their sexual behaviour but also in growth rate, which is significantly faster in *M. echinosphaera*. Like *M. indohii*, *M. chlamydospora*, and *M. echinosphaera* are placed in *Mortierella* on behalf of their zygospores (when present), their overall colony appearance and the resemblance of the chlamydospores to those of other *Mortierella* species, e.g. *M. polycephala*. There is no reason to retain the genus *Azygozygum* as it is considered to be a synonym of *Mortierella*.

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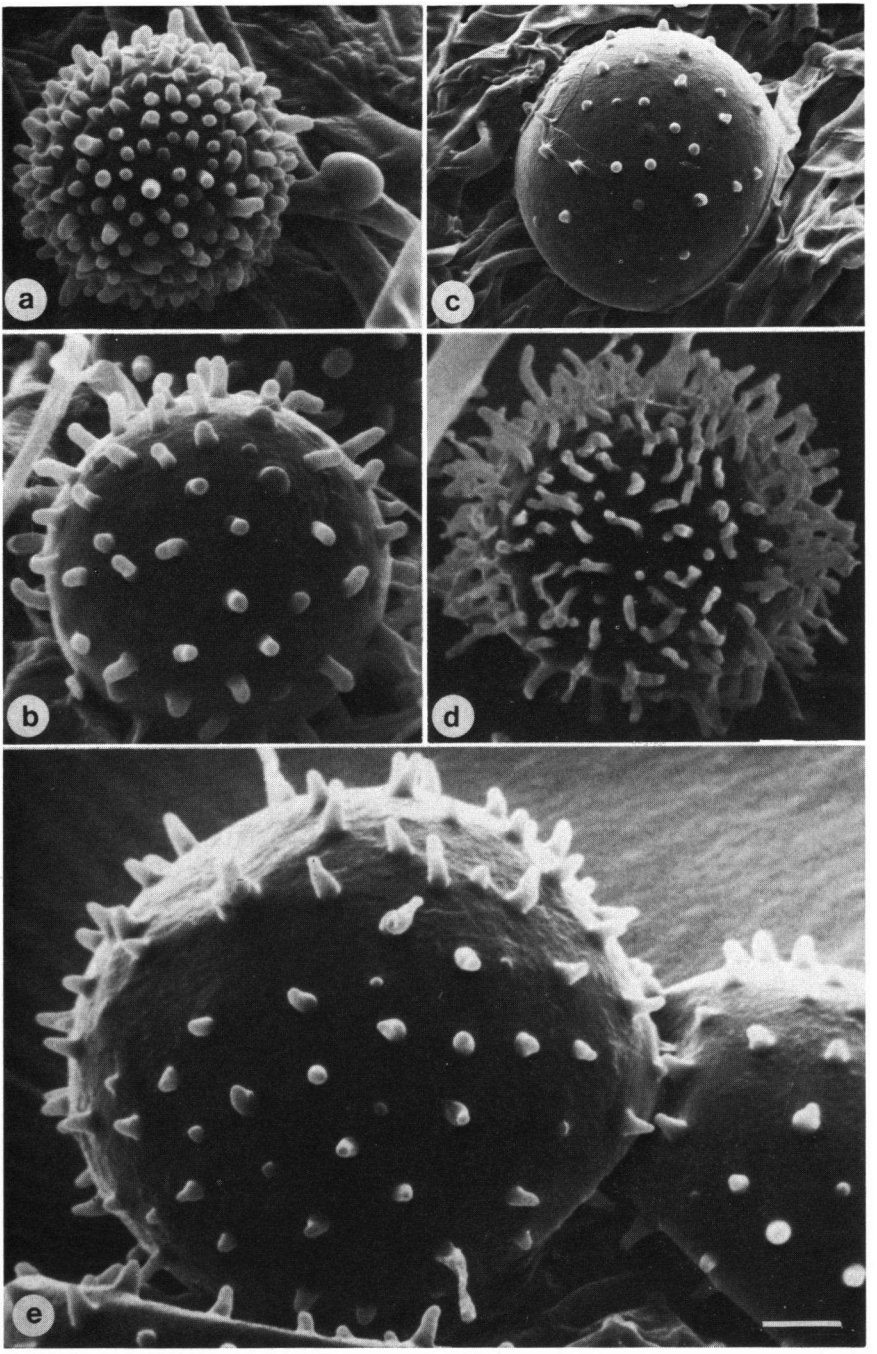
EXPLANATION OF PLATE 18

Fig. a. *Mortierella indohii*, CBS 720.71. — Fig. b. *M. chlamydospora*, CBS 529.75. — Figs. c, d. *M. echinosphaera*, CBS 575.75. — c. Young. — d. Mature. — Fig. e. *Trachysphaera fructigena*, CBS 315.31. The scale represents 5 μm.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