STUDIES ON CERTAIN SPECIES OF TAIWAN MUCORALES[1]

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Abstract: This is a continuation of our investigation of the Taiwan mucoraceous fungi. Ten species of Mucorales from soil, air, and animal dung are presented in this paper. They are *Mucor adventitius*, *M. circinelloides*, *M. corticolus*, *M. falcatus*, *M. griseo-lilacinus*, *M. piriformis*, *Pilobolus crystallinus*, *Phycomyces nitens*, *Syncephalis cornu*, and *Syncephalastrum racemosum*, all these are reported as new to the fungal flora of Taiwan. All of them were carefully studied, and specific characters decribed. Tests for zygospore formation were made between the various collections of the same or closely related species, but none were induced. All of the pure cultures which were isolated are therefore presumably heterothallic.

INTRODUCTION

In reviewing the papers and literature on Mucoraceous fungi, it is found that much work has been done in Europe and in America. In Taiwan, the Mucorales have not been widely investigated and studied. K. Sawada made the first contributions to Taiwan fungal flora. In his work (1919-1959) only 4 species of *Rhizopus* and 2 species of *Mucor* were recorded. In addition, 20 species of Mucorales have been reported (Chien, 1972; Yang & Liu, 1972), all of which were new records to Taiwan fungal flora.

The work reported in this paper is an attempt to find more new records of Mucorales and to obtain valuable data to add to what has been previously reported relating to the Taiwan fungal floara.

EXPERIMENTAL WORK

The sources from which the organisms were isolated is recorded in Table I. For isolation the medium used was Martin's modified medium (Synder *et al.*, 1959), for stocking the cultures malt-extract peptone agar (Thompson & Lim, 1965) was used. The synthetic mucor agar (SMA) and potato dextrose agar (PDA) were used respectively for cultivating the culture for microscopic examination and for testing the mating of zygospores throughout this experiment. As to the isolating method the authors preferred the soil plate (Warcup, 1950), since it was easier to prepare.

In isolation an attempt has been made to select a single sporangium for each agar plate. If pure cultures were difficult to obtain, then the single spore isolation technique was adopted.

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Table I. Collection data of species isolated

Species	Substratum & Area	Date of collection	Culture No.
Mucor advantitius	Soil; Grass Mt.	Aug. 10, 1972	12-2, 12-3 12-7
M. circinelloides	Air; NTU campus	Jan. 1973	10-8
M. corticolus	Horse dung; Zoo, Taipei	Dec. 1972	11-6
M. falcatus	Horse dung; Zoo, Taipei	Dec. 1972	11-7
M. griseo-lilacinus	Horse dung; Zoo, Taipei	Dec. 1972	11-8
M. piriformis	Soil; NTU campus	Feb. 29, 1972	1-1
Phycomyces nitens	Horse dung; Zoo, Taipei	Dec. 1972	11-11
Pilobolus crystallinus	Horse dung; Zoo, Taipei	Dec. 1972	11-9
Syncephalis cornu	Parasitic on Mucor; Zoo, Taipei	Dec. 1972	11-10
Syncephalustrum racemosum	Air; Laboratory contaminate	Nov. 1972	10-9

DESCRIPTIONS OF SPECIES FOUND

1. **Mucor advantitius** Oudemans, Oudemans & Koning Extr. Arch. Néerl. Sci. Nat. II. **7**: 278; Gilman 1957; Zycha & Siepmann 1969.
M. advantitius var. aruantiaca Lendner, Mucor. Suisse, p. 64, 1908.
M. vallesiacus Lendner, Bull. Soc. Bot. Géneve, **10**: 376, 1918.
M. humicolus Raillo, Zentrabl. f. Bakt., II, **78**: 523, 1929.

Turf fine and white, 2–3.5 cm high, reverse color white. Sporangiophores long, slender, and twisted, up to 10 μ in diameter, rarely branched, more or less sympodial. Sporangia minute and light in color, globose 35–100 μ (mostly 40–70 μ) in diameter, with diffluent walls. Columellae with an indistinct collarette, subglobose, oval, or campanulate, with flattened base, 15–36×20–47 μ, mostly (2:3), or 15–25 μ in diameter. Spores mostly oblong-elliptical, or elongate, 2–2.5 times as long as broad, 2.5–3×5–7 μ, (the largest 4×8 μ), few are oval or slightly reniform (about 1:1.5). Chlamydospores abundant in substrate mycelium, globose and in chains, also in sporangiophore of 10 days old culture, not globose. Zygospores not found. Culture with sweetish odor. (12–2, 12–3, 12–7). (Plate I, figs. 1–2).

2. **Mucor circinelloides** van Tieghem, Ann. Sc. Nat. Bot. VI. **1**: 94, 1875; Povah 1917; Gilman 1957; Zycha & Siepmann 1969.
M. echinulatus Paine, Mycologia, **19**: 253, 1927.

Turf low, up to 1 cm high, silver white at first, then cream to brown (deep in color), reverse white when very young, becoming bright brownish-cream. Sporangiophores erect, with both tall and short types, the tall ones forming a turf 1 cm high, and short ones attaining only 1–2 mm in height, the tall sporangiophores with long or short branches, short sporangiophores more richly branched, with short and sympodial branches often arising at the point just below the columellae or at place not far below, tapering, or constricted at the apex, 7–19 μ in diameter. Sporangia globose, dark brown, 30–70 μ (90 μ) in diameter, with walls very finely incrusted or smooth (in the persistent sporangia), mostly fragile, undissolved, leaving a large collarette, or the walls breaking down and attached around the columellae. Columellae free, globose or subglobose, often a little wider than high, 13–30 μ in diameter, or 24–44×22.5–43 μ (53×51 μ). Spores globose, broadly oval, or ellipsoidal,

3–5.5 μ in diameter, or 3–5×4–7.5 μ. Chlamydospores and gemmae present in substrate mycelium. Substrate mycelium with many yellow contents. Zygospores not found. (10–8). (Plate I, figs. 3–5).

3. **Mucor corticolus** Hagem, Ann. Mycol., **8**: 277, 1610; Gilman 1957; Zycha & Siepmann 1969.

Colonies light gray becoming gray tinged brown, 1.5 cm high, reverse light gray and yellowish or brownish. Sporangiophores erect, branched once or twice, sympodially, with branches arising immediately below the primary columellae or septa, 10–15 μ thick. Sporangia globose or subglobose, 2.5–5 μ wider than high, with flattened base, 37–70 μ in diameter. Sporangial wall diffluent or slowly dissolved. Columellae subglobose, oval, or obovoid, almost 2–5 μ longer than broad, 15–37.5×17–42 μ, with collarette at base. Spores mostly short oval, globose, or oval, 3–5×3.75–6 μ (7.5 μ). Chlamydospore globose, in substrate mycelium. Zygospore not found. Culture with sweetish odor. (11–6). (Plate I, figs. 7–8).

4. **Mucor falcatus** Schipper, Antonie van Leeuwenhoek, **33**: 195, fig. 3, 1967; Zycha & Siepmann 1969.

M. strictus Hagem sensu Zycha, Krypt. Fl. Mark Brand VI a, p. 80, 1935.

Turf at first white later becoming yellowish to brownish, 2.5–3 cm high, reverse whitish tinged with yellow, quickly collapsing. Sporangiophores sympodially branched, but not richly, 15–20 μ thick (up to 25 μ). Sporangia white or hyaline when young, becoming brown later, globose, 70–120 μ in diameter, wall somewhat echinulate, diffluent, leaving a marked collarette at base. Columellae oval or obpyriform, 30–70×40–90 μ. Spores regular, oval to spherical, 4–6×5–8 μ, mostly 1–2 μ longer than broad. Chlamydospores found in aerial hyphae, but rare in number. (11–7). (Plate I, figs. 6,9).

5. **Mucor griseo-lilacinus** Povah, Bull. Torrey Bot. Cl. **44**: 301, f. 6–10, 1917; Gilman 1957; Zycha & Siepmann 1969.

M. jauchae Lendner, Bull. Soc. Bot. Gébeve, **10**: 37, 1918.

Turf light brownish-gray, not over 2 cm high, reverse brown-cream on margins. Sporangiophores 10–20 μ in diameter, irregularly branched, not richly. The shorter sporangiophores near the substratum usually sympodially branched, tapering and/or constricted just below the sporangia, hyaline, or with many oil droplet contents. Sporangia brown, globose, 40–100 μ in diameter, with walls mostly diffluent and smooth. Columellae globose, subglobose, oval, or obpyriform, with flattened base, 17–47 μ in diameter (of the globose, or the subglobose ones), 35–50×45–70 μ (of the oval, or the obpyriform), with collarette at base, free. Spores mostly broad oval, or oval, 3–7.5×4.4–10 μ, some are globose or subglobose, 4–7 μ in diameter (the ratio of breadth to length 1:1–1.5). Chlamydospores abundant only in substrate mycelium (also in sporangiophore of old culture, but rare in number). Hyphae in substratum with numerous large or small oil droplets. Culture not sweetish in odor. (11–8). (Pltae II, figs. 1–3).

6. **Mucor piriformis** Fischer, Ravenhorst, Krypt. Fl., **1**: 191, 1892; Gilman 1957; Zycha & Siepmann 1969.

M. humilis Naumev, Petersb. Pize. 1915.

Turf about 2–3 cm high, at first white becoming yellowish (light in color), reverse white. Sporangiophores long, up to 3 cm, 15–40 μ thick, easily collapsing, with strong, positive phototropism, unbranched at first, later with short side branches, or once sympodially branched, hyaline, constricted at apex. Sporangia white,

becoming gray tinged brown, then dark gray, large, averaging 100–150 μ in diameter, with echinulate and dissolving wall. Columellae always pyriform, obpyriform, or fiddle-shape, 37.5–59×52–123 μ, with collarette, somewhat adnate. Spore short ovoid. smooth, 3–4×5–6 μ. Chlamydospores not found, Heterotallic. (1–1). (Plate II, figs. 7–8).

7. **Phycomyces nitens** Kunze, Kunze and Schmidt, Mykol. Hefte **2**: 113, fig. 9 (a-e), 1823; Benjamin & Hesseltine, 1959; Gilman, 1959; Zycha & Siepmann, 1969.

Mucor nitens Sprengel, Syst. Veg. **4**: 539, 1827.

Phycomyces splendens Fries, Syst. Myc. **3**: 308, 1832.

Byssus olivacea Which, Trans. Nat. Hist. Soc. Northumberland. Durham and Newcastle-upon Tyne **2**: 121, 1838.

Periconia phycomyces Bonorden, Handb. Allg Myc. p. 113, 1851.

Mucor phycomyces Berkeley, Outl. Brit. Fung., pp. 29, 407, 1860.

M. romanus Carnoy, Bull. Soc. Roy. Bot. Belg. **9**: 162, 1870.

M. violaceus Brefeld, Bot. Unters. Schimmelpilze **4**: 56, 92, 1881.

Phycomyces nitens (Agardh) Schroeter, in Cohn's Krypt.-Fl. Schlesine **3**(1): 209, 1886.

P. pirottianus Morini, Malpighia **10**: 89, figs. 8–12. 1896.

P. splendens Fries emend. Bainier, Bull. Soc. Mycol, France **19**: 166, 1903.

Turf very high, typically at least 4–5 cm in height, loose, brownish-white with silver shade. Sporangiophores erect, single, very long, up to 7 cm, 30–80 μ thick (maximum 92 μ), usually with numerous water droplets adhering to their walls especially when young, constricted somewhat just below the sporangium. Sporangia cream-yellow when the sporangiophores are still very short, becoming olive-green or black later, globose, large, 350–520 μ in diameter. Sporangial wall smooth, diffluent, leaving an indistinct collarette or collarette absent. Columellae oboval, oval, or somewhat obpyriform, 150–230×170–260 μ. Spore oblong-elliptical or elongated, 5–6× 10–15 μ in average (extreme 4.5–10 μ) (almost 1:2–2.5, some are 1:3). Chlamydospores absent in both aerial hyphad and substrate mycelium. Substrate mycelium vigorously branched like a tree, with large and minute swollen thin-walled regions in branch hyphae, these swollen regions are intercalary, oval in form, and contain numerous oil droplet-like bodies. Zygospores not found.

This was a very peculiar culture among our isolates. At first, it was grown in SMA and PDA respectively, but growth was so poor that only one to very few sporangiophores were formed from the substrate mycelium which was very limited in development. The medium of MPA was then tried, the result was good. Therefore all the characters described above are based upon the culture on this medium. (11–11). (Plate III, figs 1–2).

8. **Pilobolus crystallinus** (Wiggers) Tode, Schr. Natfo. Fr. Berlin, **5**: 96, 1783; Grove 1884; Buller 1958; Zycha & Siepmann 1969.

Hydrogera crystallina Wiggers, Primit. Flor. Holsat, p. 110, 1780.

Mucor roridus Bolton, Hist. Fung., **3**; 168, 1789.

P. roridus (Bolton) Persoon, Synops., p. 117, 1801.

P. microsporus (Klein) Brafeld, Bot. Unters., **4**: 70 1881.

P. schmidtii Saccardo, Syll. Fung., **24**: 11, 1926.

Sporangiophore erect, single, 7–12 mm high, 100–150 μ thick. Trophocyst in the substratum, 390 μ in diameter. Subsporangial swelling egg-shape, 450–550 μ wide by 700–800 μ long. Sporangia black, hemispheric, about 300 μ in diameter. Spores

ellipsoidal, regular in form, slightly yellowish, averaging 5×10 μ in diameter. (11–9). (Plate II, figs. 4–6).

9. **Syncephalis cornu** van Tieghem et Le Monnier, Ann. Sci. Nat. V, **17**: 376, pl. 25, figs. 124–125, 1873; Gilman 1957; Indoh 1962; Benjamin 1966; Zycha & Siepmann 1969.
   S. curvata Bainier, Ann. Sci., Nat. VI, **15**: 93–96, 1883.

   Mycelium parasitic on host hyphae. Sporangiophores single, erect, strongly recurved in upper portion, 6–15 μ in diameter at the place of curvature, tapering above and below, with rhizoids at base, swelling at apex to form a globose head, 16–30 μ in diameter. Merosporangia numerous, borne on upper half of the head, cylindrical, 22–30 μ long by 3–5 μ wide, with 3–5 spores in a single merosporangium. Spores oblong, or elliptical, 5–7 μ long. Zygospores not found. (11–10). (Plate III, figs. 3–5).

10. **Syncephalustrum racemosum** Cohn ex Schroter, Kryptogamenfl. Schles. 3, **1**: 217, 1886; Gilman 1957; Benjamin 1966; Zycha & Siepmann 1969.
    S. nigricans Vuillemin, Bull. Soc. Mycol. France, **3**: 111–116, 1887.
    S. elegans Marchal, Rev. Mycol., **14**: 165–167, 1892.
    S. cinereum Bainier, Bull. Soc. Mycol. France **23**: 222, 1907.
    S. fuliginosum Bainier, Bull. Soc. Mycol. France, **23**: 223, 1907.
    S. javanicum Raciborski, Bull. Internat. Acad. Sci. Cracovie, Cl. Sci. Mat. Nat. 1909, p. 347. 1909.
    S. racemosum var. paucisporum Moreau, Bull. Soc. Myco. Frnce, **65**: 146, 1949.

    Turf up to 1 cm high, collapsing with age, at first white, later gray to brownish-gray, reverse light milky yellow. Sporangiophore at first unbranched, later irregularly and abundantly branched, with rhizoids at base, no septa at the place where the branches arise, 10–20 μ thick. Sporangiospores formed within the elongate, cylindrical merosporangia, globose, short oblong, or oblong, 2.5–3×2.5–5 μ. Fruiting head globose or oval, brown, 30–60 μ (the largest 95 μ) in diameter. Merosporangia cylindrical, up to 22 μ long, with 3–7 spores in a single merosporangium. (10–9). (Plate III, figs. 6–7).

DISCUSSION AND CONCLUSION

1. Growth condition. Experience has shown that favorable and uniform growth conditions are very desirable, therefore the SMA medium was used (except for *Phycomyces nitens*) and the optimum temperature of 25°C was maintained throughout this study. Light is an important factor, especially to the height of the turf and somewhat to the spore form. It was found that growth of certain isolates are quicker and the turf is higher under continuous illumination than in the dark.
2. Identification. Povah stated (Povah, 1917) that *Mucor* is composed of a physiologically close group of species, exhibiting only minute cultural variations, thus the identification of these species was based mostly upon morphological differences. In other members of the Mucorales, this is also the case. The characters that has been proven to be more concrete and more reliable are: height and color of turf, nature of the sporangial wall, spore form and size, size and shape of columella, size of sporangium, presence or absence of chlamydospores in sporangiophores, and the form of branching of the sporangiophores can be used as a reference character.

3. *Phycomyces nitens* isolated from horse dung is a very special member in our isolates. It grows well only on MPA (malt-extract peptone agar) medium (Thompson & Lim, 1965) under usual favorable growth temperature (20–25C°). Benjamin & Hesseltine (1959) stated that growth of *P. nitens* on malt-extract agar and on PDA was essentially as described for that on SMA. In our culture, however, sporangiophore and hyphae development was poor and limited, only one to very few sporangiophores formed, on SMA and PDA, and nearly no hyphae developed under either 37°C or 10°C. No zygospores were induced between this isolate and *P. blakeslee* which were bought from the American type culture collection, marked 8743a and 8743b respectively. It is considerded to be *P. nitens*, not only because of its close rcsemblence of charaters: turf height, unbrached sporangiophore, large size of sporangium and of columella, but also the shape (oblong-elliptical) and size (10–17 μ long) of spore. Morini described an isolate under the name of *P. pirottianus* Morini which possessed almost the same size (12–18 μ) of spore as our isolate. He also collected it from horse dung. However Morini's strain was treated as a synonym of *P. nitens* (Benjamin & Hesseltine, 1959) because it showed a strong reaction with the strain of *P. nitens* of the opposite mating type.
4. *Syncephalis cornus* is the only member with parasitic life form in our findings. It is usually parasitic on other members of the mucorales. Therefore an isolation of a pure culture of this species was not obtained during this investigation.
5. No zygospores were found in the isolates reported in this paper, although tests were made in PDA between the various collections of the same and of closely related species. They are therefore supposed to be heterothallic species.
6. In this paper, the authors present 10 species of Mucorales belonging to 5 genera. All of them are new records to Taiwan fungal flora, and are deposited in the mycology laboratory of the Department of Botany of National Taiwan University.

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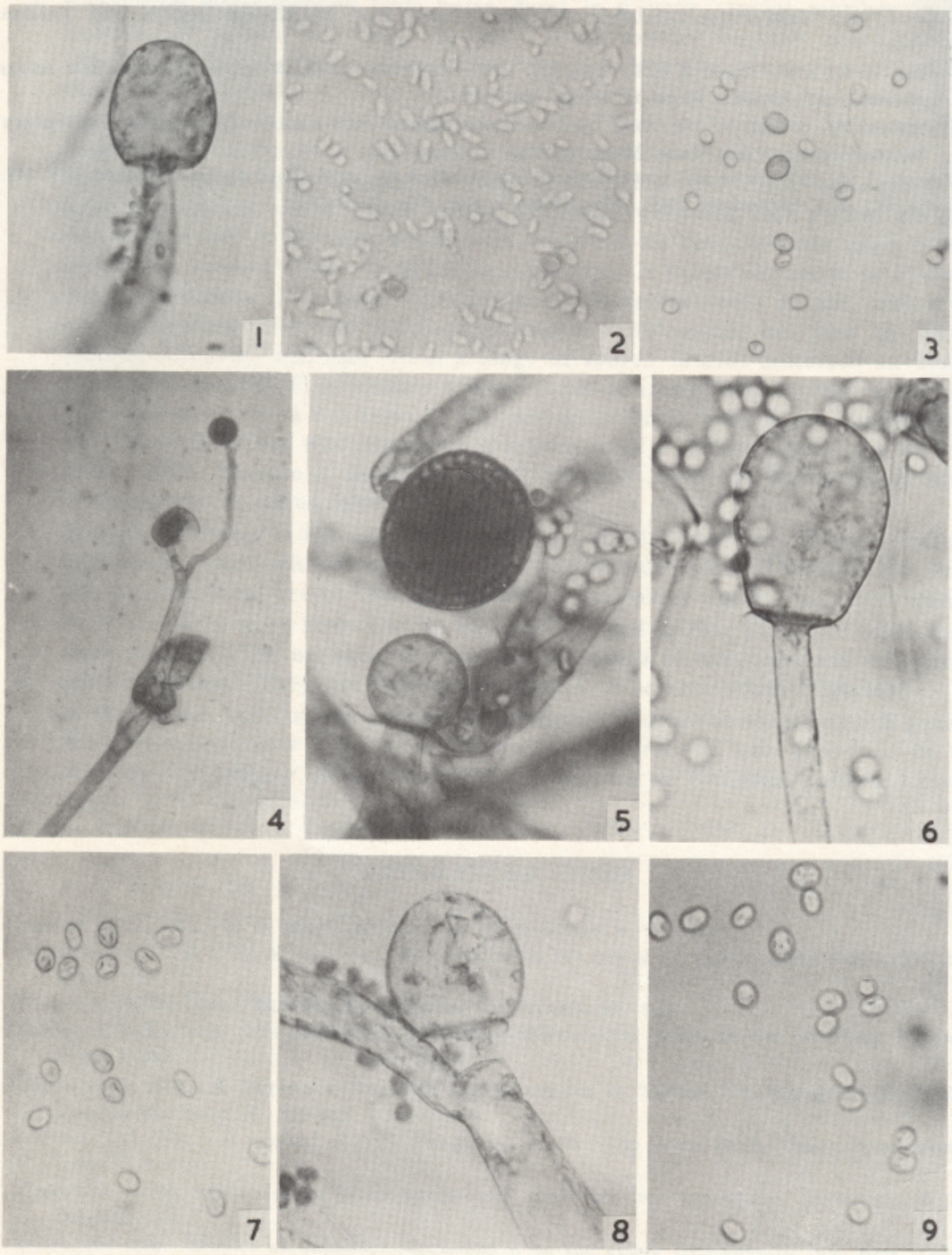

Plate I. 1-2. *Mucor advantitius;* 1. Columella, ×1000; 2. Spores, ×1000; 3-5. *Mucor circinil-loides;* 3. Spores, ×1000; 4. Branched sporangiophores, ×1000; 5. Sporangium (upper) and Columella (lower), showing fragile sporangial wall, ×1000; 6,9. *Mucor falcatus;* 6. Columella, ×1000; 9. Spores, ×1000; 7-8. *Mucor corticolus;* 7. Spores, ×1000; 8. Columella, note the lateral sporangiophore arising very close to it. ×1000.

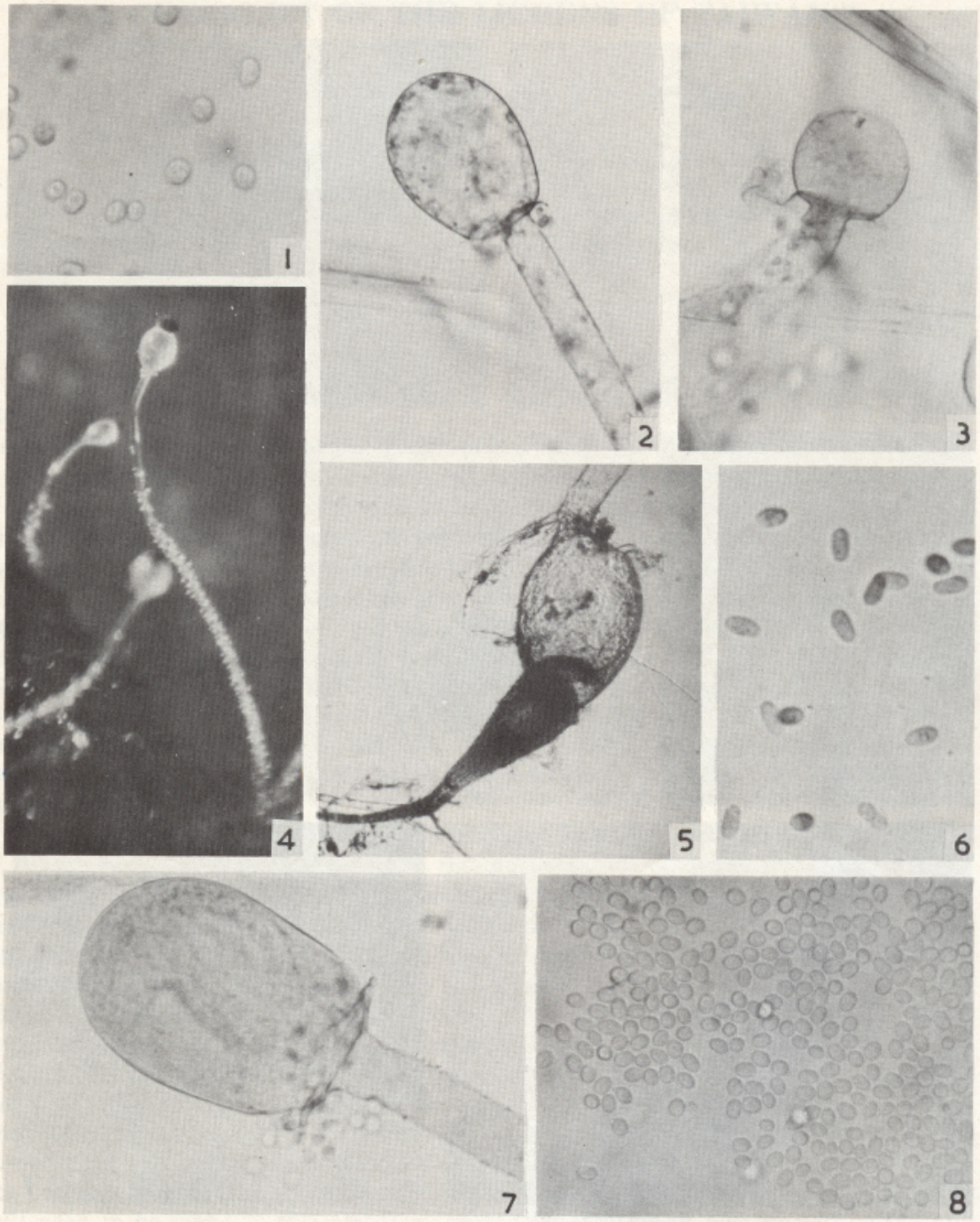

Plate II. 1-3. *Mucor griseo-lilacinus;* 1. Spores, ×1000; 2-3. Columella, ×1000; 4-6. *Pilobolus crystallinus;* 4. Long sporangiophore with a black sporangium at apex, ×38; 5. Trophocyst, ×250; 6. Spores, ×1000; 7-8. *Mucor piriformis;* 7. Large columella with collarette, ×1000; 8. Spores, ×1000.

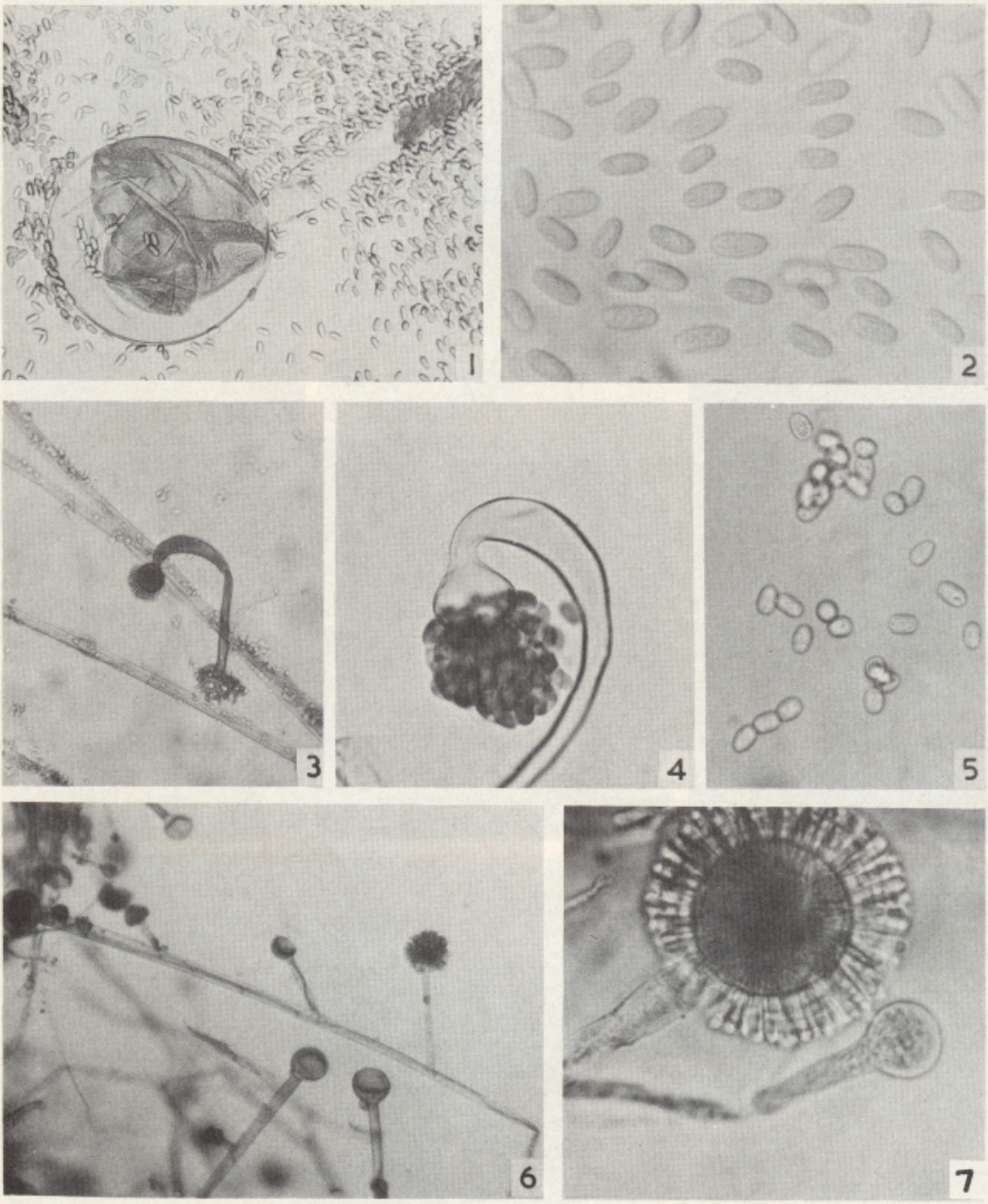

Plate III. 1-2. ***Phycomyces nitens;*** 1. Columella and spores, ×250; 2. Spores, ×1000; 3-5. ***Syncephalis cornu;*** 3. A whole merosporangiophore, ×250; 4. Merosporangial head, ×1000; 5. Merosporangiospores, ×1000; 6-7. ***Syncephalastrum racemosum;*** 6. Sporangiophores, showing branches, ×250; 7. Two heads, one with a spherical mass of merosporangia, the other naked, ×1000.