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A ROBUST CONIDIOBOLUS WITH ZYGO-SPORES CONTAINING GRANULAR PARIETAL PROTOPLASM

CHARLES DRECHSLER [1]

(WITH 6 FIGURES)

In an assortment of entomophthoraceous cultures obtained by canopying maize-meal-agar plates with detritus sifted from slowly decaying plant materials collected in Maryland during March and April, 1963, a robust fungus invited attention because of features not hitherto noted in closely related forms. The fungus, like many others that are readily isolated through similar procedure, is not known to be parasitic; nor, for that matter, is it known to operate in nature as a saprophyte. Mainly because it differs less from *Conidiobolus utriculosus* Brefeld (**1884**) than from *Entomophthora muscae* (Cohn) Fresenius (**1856**) [synonym, *Empusa muscae* Cohn, **1855**] it is presented as a member of the genus typified in the former species.

Conidiobolus eurymitus Drechsler, sp. nov. (*εὐρὺς*, wide; *μίτος*, thread).

Mycelium saepe splendorem aeris materiae ambienti addens; hyphae mycelii filiformes, incoloratae, ramosae, plerumque 3–20 μ latae, in cellulis fere 60–1600 μ longis consistentes, interdum hic illic in glomera enormia saepe 40–200 μ longa et 40–100 μ lata intertextae. Hyphae fertiles incoloratae, simplices, erectae vel acclives, in aerem saepe 25–200 μ ad lucem protendentes, in parte aeria cylindraceae vel medio aliquid inflatae, plerumque 6–14 μ latae, apice unum primiforme conidium ferentes; primiforme conidium violenter absiliens, incoloratum, vulgo globosum vel elongato-ellipsoideum, plerumque 15–54 μ longum, 13–45 μ latum, basi papilla saepius 2–8 μ alta et 3–14 μ lata praeditum, nunc hypham germinationis vulgo 5–13 μ crassam emittens nunc aliud primiforme conidium gignens nunc in apice hyphae ascendentis saepius 3–11 μ longae et 3–6 μ latae conidium formae elongatae ferens; conidia formae elongatae violenter absilientia, incolorata, nonnihil fusiformia, apice glutinosa et obtuse rotundata, saepe 24–41 μ longa et 10–17 μ lata, forsitan interdum multo longiora et latiora. Chlamydosporae vulgo intercalares, incoloratae vel interdum sufflavae, globosae vel elongato-ellipsoideae, plerumque 30–45 μ longae, 25–35 μ latae. Zygosporae vulgo ex copulatione duarum contiguarum cellularum ejusdem hyphae oriundae sed interdum ex copulation duarum cellularum hypharum distinc-

[1] Collaborator, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

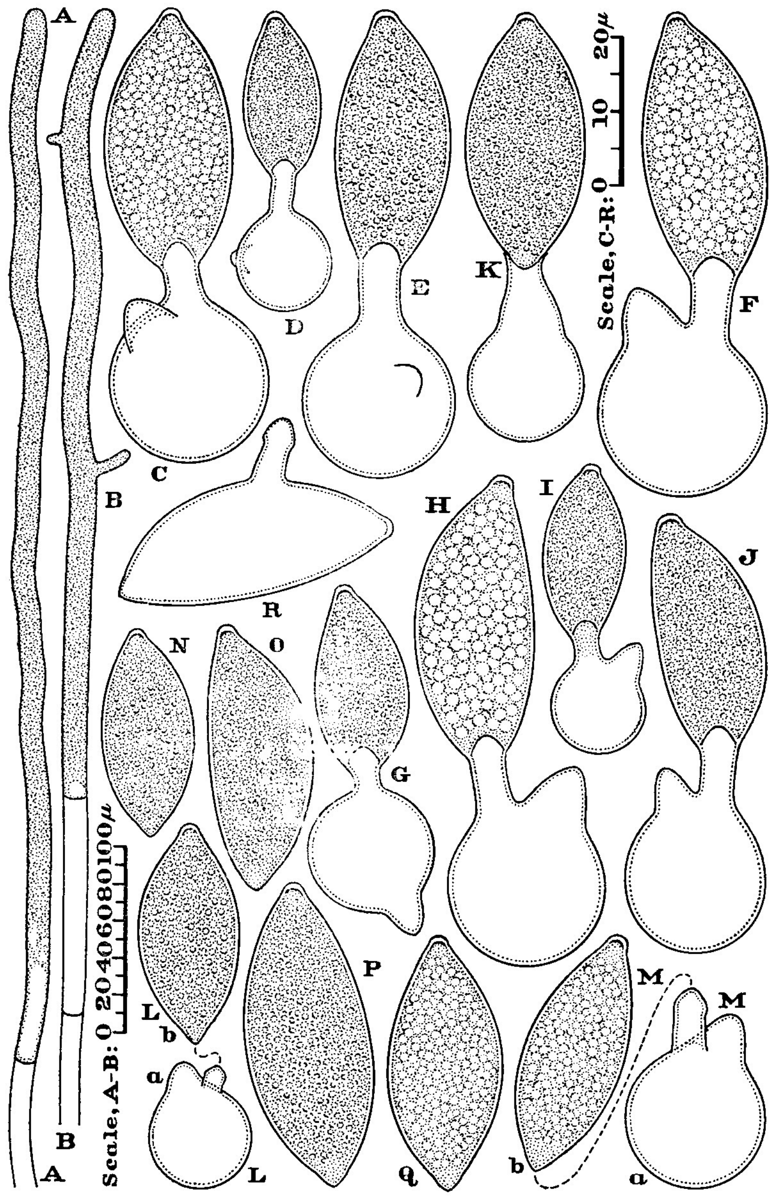

FIG. 1. *Conidiobolus eurymitus:* A, B, × 250; C–R, × 1000. A, B, Distal segments of elongating hyphae at advancing margin of a mycelium in a young maize-meal-agar plate culture. C–J, Empty envelopes of globose conidia that while floating on water have each extended upward a wide conidiophore bearing an

tarum ortae, incoloratae vel raro flavidulae, vulgo globosae vel elongato-ellipsoideae, tum plerumque 20–39 μ longae, 18–35 μ latae, muro 1.6–3.8 μ crasso circumdatae, guttulam oleosam saepius 9.5–20 μ in diametro et protoplasma granulosum continentes, interdum irregulariter cylindratae et pluribus minoribus guttulis oleosis praeditae.

Habitat in materiis plantarum putrescentibus prope Hancock, Maryland, et prope Salisbury, Maryland. Typus: National Fungus Collections No. 71728; American Type Culture Collection No. 16017.

Conidiobolus eurymitus was obtained from decaying plant material collected on March 25, 1963, near Hancock in northwestern Maryland, and also from similar material collected on April 20, 1963, near Salisbury in southeastern Maryland. In the cultures canopied with material from either collection the expanding mycelia of the fungus were made conspicuous by the bronze metallic luster found slowly spreading over the areas of substratum occupied by them. Bright-field microscopical examination provided no evidence of any contaminating microorganism or of any particles of pigment. An impression was gained that the curious sheen results from a liquid secretion which creeps in a very thin layer over the surface of the agar, and thus may cause optical effects much like a thin oil film on a water surface.

On agar substrata of customary firmness growth of *Conidiobolus eurymitus* is often uncertain or very slow. More satisfactory development of the fungus takes place on rather soft culture media as, for example, maize-meal agar prepared with 12 grams of dry agar to the liter. At temperatures near 24 C, even on such soft substratum, a young mycelium usually grows radially only about 4.5 mm in 24 hr. Preference for a readily yielding substratum may here have some explanation in the unusual coarseness of the elongating hyphae at the mycelial forefront, since the terminal segments of these hyphae (FIG. 1, A, B) commonly vary from 10 to 16 μ in width. Meager capability for penetrating firm agar media appears likewise characteristic of the very coarse mycelium of *Basidiobolus magnus* Drechsler (1964).

The large terminal segments at the margin of an expanding mycelium of *Conidiobolus eurymitus,* which often exceed 1 mm in length and usually measure about 12 μ in diam, do not give rise directly to conidia

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elongated conidium, which, being delimited basally by a dome-shaped septum, is ready to spring off. K, Empty conidial envelope with a short conidiophore supporting an elongated conidium that in a wet microscope mount everted its basal membrane. L, M, Empty envelopes of globose conidia, a, each with a short conidiophore from which an elongated conidium, b, has become detached. N–Q, Detached elongated conidia. R, Empty envelope of an elongated conidium with an empty conidiophore from which a new conidium has sprung off.

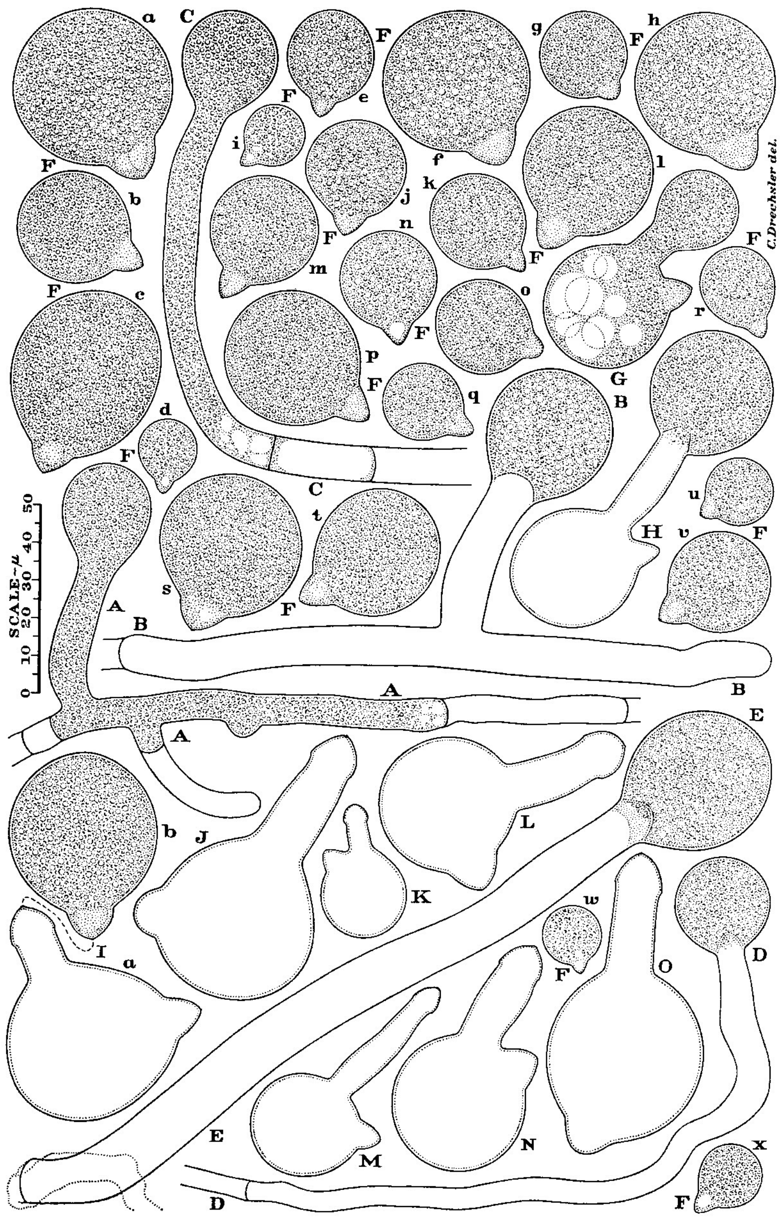

FIG. 2. *Conidiobolus eurymitus,* × 1000. A, Portion of procumbent hypha that has sent up a phototropic conidiophore on which a globose conidium is being formed. B, Portion of procumbent hypha that has become emptied in giving rise

or to zygospores. Reproductive development is initiated by hyphal cells of moderate volume found in older portions of a mycelium, following the emission of narrower lateral branches and deposition of rather numerous cross-walls. As in other species of *Conidiobolus* an aerial phototropic conidiophore may be sent up directly from a procumbent hyphal segment (FIG. 2, A, B) or may constitute the terminal portion of a relatively long branch originating from a submerged segment (FIG. 2, C–E). Soon after the globose conidium has been delimited by a dome-shaped septum (FIG. 2, B, D, E), it springs off through sudden eversion of its basal membrane. The detached conidia (FIG. 2, F, a–x) often put forth germ tubes, which on unoccupied substratum soon grow into young mycelia, but on substratum already permeated by the fungus usually proceed in their courses as migratory hyphae (FIG. 3, A, B). In many instances a detached conidium (FIG. 2, G, H, I, a) extends a germ conidiophore from which a secondary globose conidium (FIG. 2, I, b) is released. Continued recurrence of such repetitional development is accompanied by steady increase in number of empty conidial envelopes (FIG. 2, J–O) and progressive reduction in size of the living conidia.

Conidiobolus eurymitus usually produces secondary conidia of the elongated adhesive type under the wet conditions that can be provided conveniently by transferring portions of an actively sporulating agar culture to a Petri dish containing a shallow layer of distilled water. When the globose conidia formed on the transferred material spring off, many of them alight on the water nearby and remain afloat, evidently being supported on the surface film. Some of the floating spores thereupon give rise on the upper side to a short broad germ conidiophore that soon bears a broadly spindle-shaped conidium (FIG. 1, C–J) delimited basally by a protruding septum. Eversion of the convex wall in a wet mount under a cover glass takes place gradually and without noteworthy effect (FIG. 1, K), but in floating specimens it takes place abruptly and propels the new conidium (FIG. 1, L, b; M, b) from the empty envelope of its parent (FIG. 1, L, a; M, a). The detached broadly fusiform conidia (FIG. 1, N–Q) commonly remain afloat and

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to a phototropic conidiophore and the globose conidium borne on it. C, Young phototropic conidiophore formed distally on a long hypha. D, E, Empty phototropic conidiophores, each bearing a full grown globose conidium. F, Detached globose conidia, a–x. G, H, Detached globose conidia showing an early and an advanced stage, respectively, in the production of a secondary globose conidium. I, Empty globose conidial envelope, a, with a short empty conidiophore from which a secondary globose conidium, b, has become detached. J–O, Empty envelopes of globose conidia, each with an empty conidiophore on which a secondary globose conidium was produced.

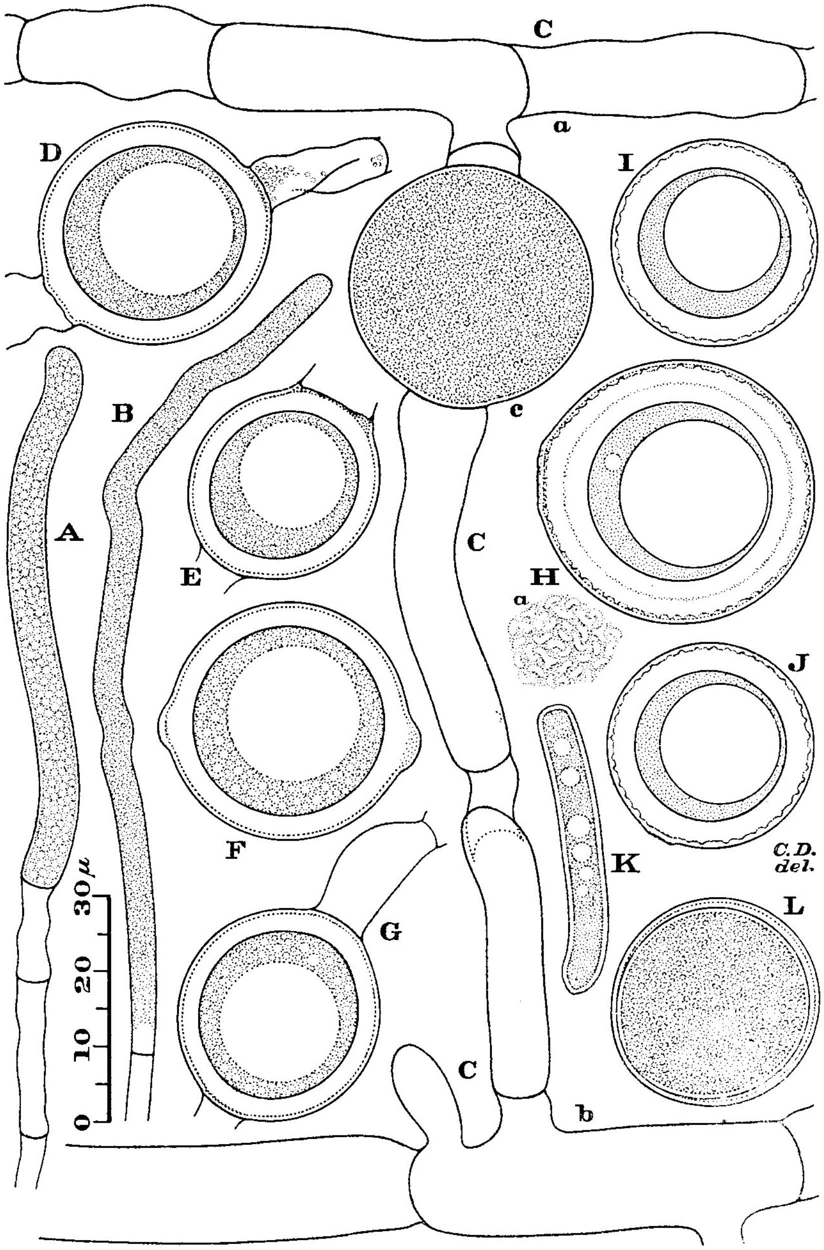

FIG. 3. *Conidiobolus eurymitus*, A–G, and *Conidiobolus stromoideus* (American Type Culture Collection No. 15430), H–J, drawn at same magnification; × 1000. A, B, Migratory hyphae of *C. eurymitus* found in a maize-meal-agar tube

sometimes in turn become emptied (FIG. 1, R) in giving rise to a new conidium of either the elongated or the globose type.

In all wet or watery preparations of *Conidiobolus eurymitus* that were studied only the globose conidia of small or medium size were found participating in the production of elongated secondary spores. Since, nevertheless, the larger globose conidia may, perhaps, under suitable conditions be likewise capable of such participation, it seems probable that the fungus can form elongated conidia of dimensions considerably beyond the ranges given in the diagnosis. At all events the elongated conidia may have contents either of globuliferous (FIG. 1, C; F; H; M, b; Q) or of granular (FIG. 1, D; E; G; I–K; L, b; P) texture. They contribute much to the distinctiveness of the fungus in that they are borne like globose conidia, rather than on tall slender conidiophores like elongated secondary conidia in other members of the genus. In conjunction with their globose parents they make up a sequence of unlike self-propelled conidial types, which, while agreeing with a sequence sometimes occurring in *Entomophthora variabilis* Thaxter (**1888**), represent the reverse of the sequence found in *E. rhizospora* Thaxt., *E. aphidis* Hoffman in Fresenius (**1858**), and *E. dipterigena* Thaxt.

At temperatures near 18 C sexual reproduction usually proceeds on a moderate scale in maize-meal-agar cultures of *Conidiobolus eurymitus.* Conjugation most often takes place between 2 adjacent segments of the same hypha (FIG. 4, A–B, C–E), the contents of one segment migrating into the other through an opening found rather commonly at the periphery of the dividing partition. A distention develops in a position immediately adjoining the perforated cross-wall and increases steadily in size as it receives material from both segments, while at the same time the outlying filamentous portions of the sexual unit become progressively emptied of granular material. Usually a few retaining walls are laid down, which mark successive stages of evacuation (FIG. 4, C–E). A small proportion of the sexual reproductive units of *C. eurymitus*—gen-

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culture 30 days old. C, Diclinous sexual reproductive unit of *C. eurymitus:* 2 separate hyphae, a and b, gave off branches whose fusion resulted in production of the newly delimited full grown zygosporangium, c. D–G, Mature zygospores of *C. eurymitus,* showing granular texture of the parietal layer of protoplasm. H–J, Mature subspherical zygospores of *C. stromoideus,* showing peripheral sculpture in profile and the nearly homogeneous texture of the parietal layer of protoplasm; H, a, appearance of sculpture in the central region of the upper aspect of H, with the unstippled areas representing vermiform and ypsiliform ridges. K, Mature cylindrical zygospore of *C. stromoideus* with thin wall devoid of sculpture. L, After-ripened zygospore of *C. stromoideus* ready to germinate.

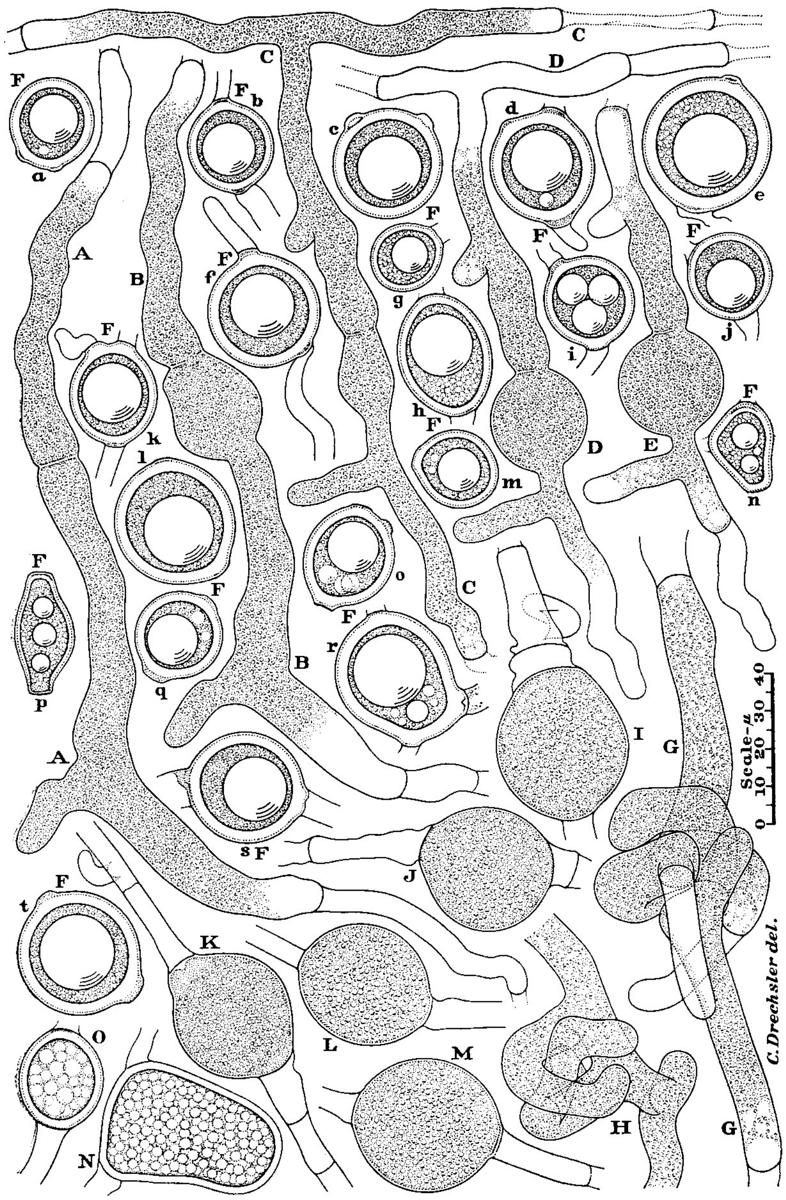

FIG. 4. *Conidiobolus eurymitus* in Petri plates of maize-meal agar, A–F, and of potato-dextrose agar, G–O; × 500. A, B, Two early stages, separated by an interval of 45 minutes, in the migration of protoplasm in a monoclinous sexual reproductive unit. C–E, Three stages, separated by consecutive intervals of 60 and

erally about 10 per cent of them—are of diclinous origin, being formed through conjugation between segments of separate hyphae (FIG. 3, C, a, b). Once the paired segments have become united, development follows the same course as in monoclinous units. When all filamentous parts have been emptied, the distention is delimited as a full-grown globose zygosporangium (FIG. 3, C, c), which is filled throughout with densely granular protoplasm. Through gradual internal changes the cellular contents are converted into a mature zygospore (FIG. 3, D–G; 4, F, a–t; 5, A), which like the zygospores of many related species has a thick wall, a parietal layer of protoplasm and a large reserve globule. The parietal layer here is distinctive in showing a generally granular texture, whereas in most congeneric forms with sexual reproduction the corresponding layer appears to consist of homogeneous material through which a small admixture of granules is sparsely distributed. In the texture of its parietal layer the mature zygospore of *C. eurymitus* resembles somewhat the mature oospores of many *Pythium* species, including, for example, the familiar *P. debaryanum* Hesse and *P. ultimum* Trow.

The zygospores of *Conidiobolus eurymitus* produced in maize-meal-agar tube cultures show only moderate endurance when kept on laboratory shelves at room temperatures ranging during summer mostly between 25 and 38 C. Under such storage conditions they succumb usually within 6 months. At temperatures near 7 C and near 18 C they survive well for more than a year, even if little protected against evaporation. When zygospores taken from maize-meal-agar tube cultures stored for several months at 18 C are placed in distilled water many usually soon reveal the changes whereby germination is initiated. A thick inner layer of the zygote wall proper becomes marked with radial striations (FIG. 5, B) and then undergoes gradual mergence with the parietal layer, which concomitantly acquires globuliferous texture and little by little encroaches on the reserve globule (FIG. 5, C). After the protoplasm has wholly assimilated both the reserve globule and the striate layer of wall (FIG. 5, D), it gradually resumes a granular texture. Thereupon 1 to 3 germ tubes are put forth, but generally only one of them continues to elongate as a germ hypha (FIG. 5, E, t), ultimately receiving the entire mass of protoplasm (FIG. 5, F, t). Consequently

30 minutes, in the migration of protoplasm in another monoclinous sexual reproductive unit. F, Mature and very nearly mature zygospores, a–t, showing usual variations in size, shape, and internal structure. G, H, Hyphal clews in an early stage of growth. I–M, Chlamydospores. N, O, Zygospores in which maturation appears to have been halted before being completed.

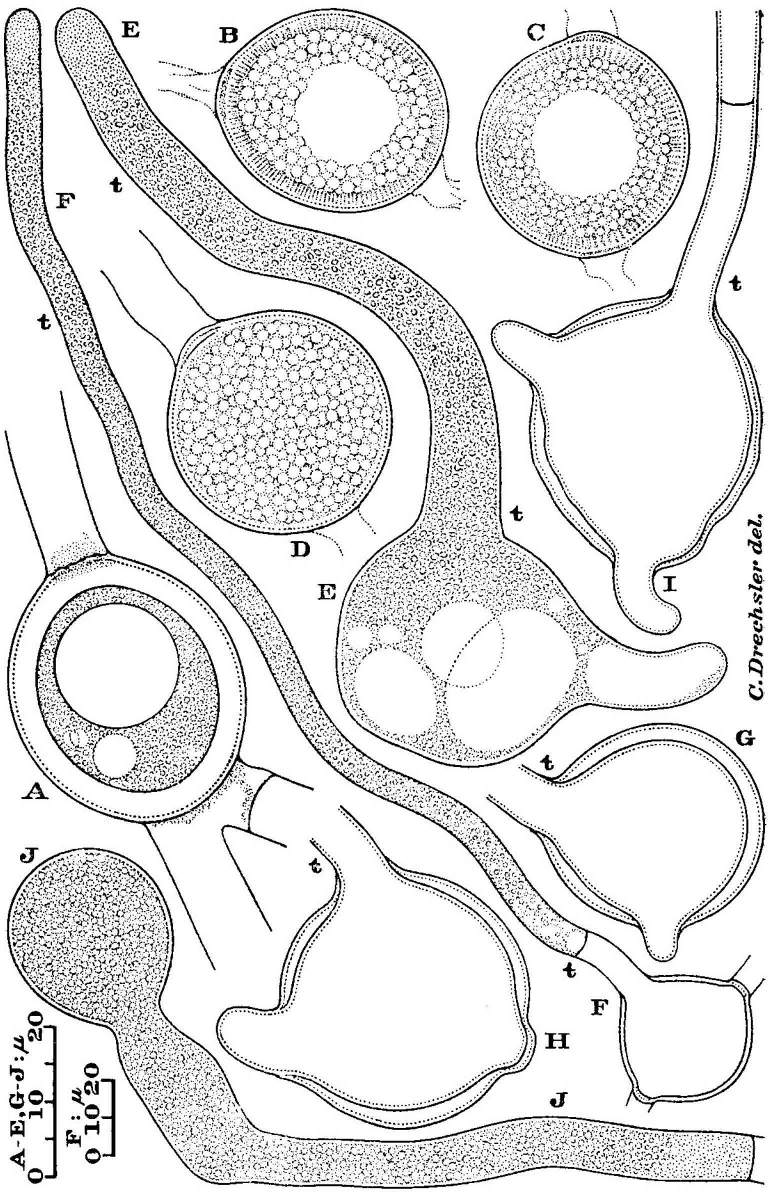

FIG. 5. *Conidiobolus eurymitus:* A–E, G–J, × 1000; F, × 500. A, Zygospore in mature resting state. B-D, Zygospores in irrigated preparations, showing successively more advanced stages of after-ripening. E, Zygospore that has put forth an abortive germ tube as well as a functional germ hypha, t. G–I, Empty mem-

in its empty state the persistent outer layer of the zygospore wall proper, now well separated from the zygosporangial envelope surrounding it, is often found continuous not only with the membrane of the functional germ hypha (FIG. 5, F–I; t) but also with 1 (FIG. 5, G, H) or 2 (FIG. 5, I) empty diverticula left by abortive supernumerary germ tubes.

Since in irrigated preparations fresh unoccupied substratum is not usually available, the functional germ hyphae produced by zygospores of *Conidiobolus eurymitus* often give rise, after rather little elongation, to an ascending phototropic conidiophore that forms a globose conidium (FIG. 5, J). Sometimes, however, the germ hypha migrates extensively on or through old pieces of agar before producing a conidium; or it continues its migration until it becomes apparently too badly exhausted either for continued movement or for reproductive development. Many of the globose conidia produced by germ hyphae originating from zygospores, give rise to globose secondary conidia, but many others, including a large proportion of those that fall on a deposit of water, give rise to elongated secondary conidia.

In potato-dextrose-agar plate cultures a mycelium of *Conidiobolus eurymitus* often has a coarsely branched fibrous appearance (FIG. 6, A) that is caused mainly by the presence of numerous irregular hyphal clews (FIG. 6, B) under and on the surface of the substratum. These clews are formed here and there on the longer mycelial filaments through intertangling of short stout branches arising from positions close together (FIG. 4, G, H). The intertwined primary branches produce secondary branches, which, together with some tertiary ramifications arising from them, form additional components of the intricate mass. When its development comes to an end, the hyphal clew is usually rather compact at the center, while at the periphery a loose disorderly arrangement often prevails. Owing to withdrawal of protoplasmic contents from their distal portions, some component branches even of relatively young clews (FIG. 4, G) are found terminating in an empty segment.

The hyphal clews formed in potato-dextrose-agar cultures of *Conidiobolus eurymitus* have not so far been seen to surpass neighboring discrete mycelial filaments in the production either of zygospores or of prototropic conidiophores. They probably correspond, nevertheless, to the

branous parts left after zygospores have germinated, showing spacial separation of the zygosporangial membrane from the persistent outer layer of the zygospore wall proper—this layer being continuous not only with the functional germ hypha, t, but also with the 1 or 2 empty abortive germ tubes present. J, Distal portion of a germ hypha that is giving rise terminally to a phototropic conidiophore on which a globose conidium is being formed.

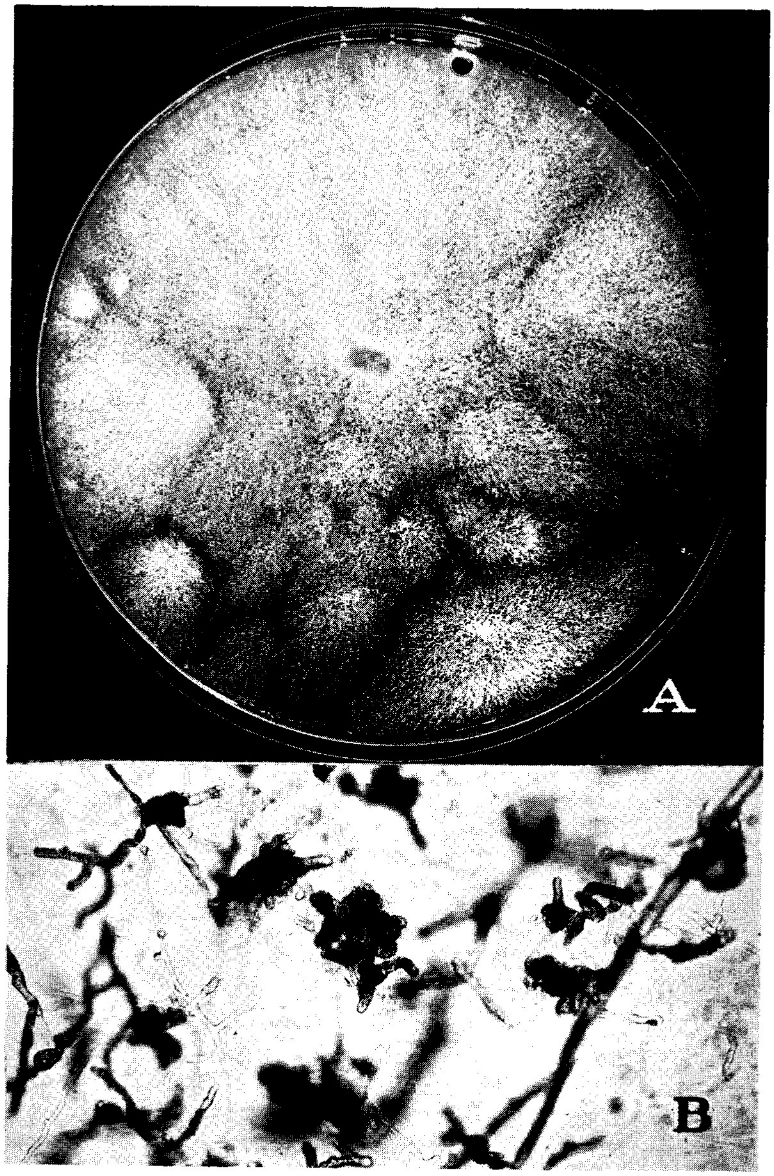

FIG. 6. A, *Conidiobolus eurymitus* in potato-dextrose-agar plate culture 13 days old; numerous hyphal clews give the mycelium a coarse appearance; × 1. B, Hyphal clews, × 100.

intricate "Fadenknäuel" that Brefeld (1884, p. 54) found produced by the luxuriant mycelium which developed when he grew *C. utriculosus* on a rich substraum. They appear, at all events, rather similar to the hyphal knots discussed and figured by Srinivasan and Thirumalachar (1962) in the description of their *C. stromoideus.* The latter species, with mycelium reported as being 7–9 μ wide, is not like *C. eurymitus* conspicuous for coarseness among the members of the genus. It produces migratory hyphae far more abundantly than *C. eurymitus,* with the result that in a maize-meal-agar culture 15 days old the original mycelium is obscured by numerous hyphae in haphazard disorder, each pursuing its own way independently of the others. Its mature zygospores (FIG. 3, H–J) reveal a parietal protoplasmic layer of nearly homogeneous rather than of decidedly granular texture. They are distinguished, besides, by minute peripheral sculpture consisting of many vermiform or ypsiliform ridges (FIG. 3, H, a) that appear to arise on the outer surface of the spore wall proper, though their position in the composite mantle remains somewhat uncertain. No sculpture is visible on the few thin-walled cylindrical zygospores observable here and there (FIG. 3, K). Globose zygospores that have undergone after-ripening (FIG. 3, L) preliminary to germination show a smooth zygosporangial envelope somewhat loosely surrounding a smooth persistent outer layer of the spore wall proper.

In potato-dextrose-agar plate cultures of *Conidiobolus eurymitus* 10–15 days old are often found some scattered intercalary bodies (FIG. 4, I–M) that apparently remain thin-walled and incur no marked change in the densely granular texture of their contents. As these bodies are not known to arise through fusion of adjacent cells and fail to undergo any of the internal reorganization which in young zygotes is initiated very soon after conjugation is completed, they are held interpretable as chlamydospores, even though in their globose or elongate-ellipsoidal shape they bear some resemblance to newly delimited zygosporangia. Interruption or delay in the progress of maturation of zygospores, however, is apparently not uncommon at rather advanced stages, when the contents have attained a globulose condition and are surrounded by a thickened wall (FIG. 4, N, O).

The bronze metallic luster imparted to some substrata occupied by mycelium of *Conidiobolus eurymitus* has not hitherto been found associated with any other member of the genus, nor, indeed, has it been noted in cultures of any less closely related phycomycetes. With respect to coarseness of mycelium *C. eurymitus* exceeds all congeneric fungi except the type species, C. *utriculosus,* of which some filamentous hyphae figured by Brefeld (**1884**, Taf. III, Fig. 1) would seem, from the magnification assigned to them, to reach likewise a maximum width of about 20 μ. However, *C. eurymitus* differs markedly from *C. utriculosus* in its sexual reproduction, since it usually accomplishes such reproduction through conjugation between adjacent hyphal segments, not through conjugation between the swollen tips of 2 lateral branches. The mature zygospores of *C. eurymitus,* which inclose parietal protoplasm of distinctly granular rather than of nearly homogeneous consistency, are smooth and of only moderate size, while those of *C. utriculosus* are abundantly echinulate and exceptionally large, ranging in diameter, according to Brefeld (**1884**, p. 58), from 60 to 100 μ (average 80 μ). In its asexual reproduction *C. eurymitus* is distinguished chiefly by producing elongated secondary conidia on short stout outgrowths from which they soon spring off forcibly, much like globose secondary conidia; whereas in other members of the genus such elongated secondary spores are borne aloft on tall slender filamentous conidiophores, from which they may later become detached passively.

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