

A Small *Conidiobolus* with resting spores that germinate like zygospores¹

Charles Drechsler

Agricultural Research Service, U.S. Department of Agriculture
Plant Industry Station, Beltsville, Maryland

DRECHSLER, CHARLES. (U.S. Dept. Agr., Plant Industry Station, Beltsville, Md.)
A small Conidiobolus with resting spores that germinate like zygospores. Bull. Torrey Bot. Club 89: 233-240. 1962. A new *Conidiobolus* isolated from decaying leaves gathered in deciduous woods near Cumberland, Maryland, is described as *C. parvus*. It belongs among the smallest members of the genus. In maize-meal-agar cultures its mycelia give rise along the slowly advancing margin to some few scattered conidiophores from each of which a globose conidium springs off forcibly. Near the margin are also produced numerous resting spores that most often originate medianly in individual hyphal segments and apparently never result from conjugation. With respect to internal structure these spores during a variable period of dormancy resemble the fully mature zygospores of *C. incongruus*. In their after-ripened stage immediately preceding emission of a germ tube they show marked similarity to after-ripened zygospores of *C. utriculosus* and *Basidiobolus ranarum*. The conidiophores formed in the germination of resting spores commonly produce globose conidia in far greater number than are produced by conidiophores of mycelial origin.

Among the readily culturable members of the Entomophthorales which have been assigned to the genus *Conidiobolus* 4 species that I presented (Drechsler 1955a, 1955b, 1957) under the epithets *pumilus*, *nanodes*, *paulus*, and *undulatus* are all distinguished by relatively slow growth and generally small dimensions. These features are shared also by another unobtrusive species that came to light in Petri plates of maize-meal agar canopied with moist filter paper to which had been affixed some fine detritus sifted from decaying leaves gathered in deciduous woods near Cumberland, Maryland, on November 4, 1961.

Conidiobolus parvus Drechsler sp. nov. Mycelium incoloratum, primo in materiis maeris inconspicuum sed in materiis feracibus saepius lichenoidium postea plus minusve farinulentum; steriles hyphae mediocriter ramosae, aliquid pravae, 1.4-8 μ (vulgo 3.5-5 μ) crassae, in margine mycelii crescentis in cellula plerumque 250-750 μ longa abeuntes, alibi ex cellulis 20-250 μ longis plerumque constantes; fertiles hyphae parce ex cellulis mycelii emissae, simplices, incoloratae, saepius 15-30 μ in aerem ascendentes, erectae vel aelives, vulgo 3-8 μ super basin crassissimae, ibi plerumque 4-6.5 μ latae illinc sursum leviter attenuatae, basi et apice 3-4.5 μ latae, unum conidium ferentes; conidia violenter absilientia, incolorata, globosa, plerumque 4.5-17 μ lata, basi papilla 1.5-4.5 μ alta et 1.5-6 lata praedita, in totum plerumque 6-20 μ longa; sporae perdurantes (azygosporae) copiose

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in hyphis submersis vel procumbentibus prope marginem mycelii crescentis oriundae, vulgo intercalares, flavidae vel paene incoloratae, globosae vel elongato-ellipsoideae, vulgo $10\text{--}25\mu$ longae et $8\text{--}20\mu$ latae sed in materiis feracibus interdum usque 30μ in diametro, primo membrana saepius $0.4\text{--}0.6\mu$ crassa mox in maturitate muro saepius $0.8\text{--}1.2\mu$ crasso stricte circumdatae deinde (membrana cellulae viventis omnino a membrana prima tunc separata) duabus membranis praeditae denique tubulum germinationis qui vulgo in hypham fertilem ascendentem abit emittentes, ita conidia saepe copiose gignentes.

Habitat in foliis arborum putrescentibus prope Cumberland, Maryland. Typus: National Fungus Collections No. 71715; American Type Culture Collection No. 14634.

Mycelium colorless, on substratum low in nutriment often at first rather inconspicuous but on richer substratum then commonly lichenoid or yeast-like and readily seen, later often covered with a whitish coating of detached conidia; sterile hyphae moderately branched, usually somewhat crooked 1.4 to 8μ (commonly 3.5 to 5μ) wide, at the margin of an expanding mycelium often terminating in a cell 250 to 750μ long, elsewhere consisting of cells mostly 20 to 250μ long; conidiophores extended sparingly from hyphal segments, unbranched colorless, often projecting 15 to 30μ into the air, erect or inclined, commonly widest 3 to 8μ above the base, thence gradually tapering upward, 3 to 4.5μ wide at base and tip, bearing terminally a single conidium; conidia springing off forcibly, colorless, globose, mostly 4.5 to 17μ in greatest width, 6 to 20μ in total length inclusive of a basal papilla 1.5 to 4.5μ high and 1.5 to 6μ wide at its attachment; resting spores (azygospores) formed in abundance singly in cells of submerged or procumbent hyphae near the margin of a growing mycelium, commonly intercalary, yellowish or nearly colorless, globose or elongate-ellipsoidal, mostly 10 to 25μ long and 8 to 20μ in greatest width but in rich substratum sometimes as much as 30μ in diameter, at first closely enveloped by a membrane often $0.4\text{--}0.6\mu$ thick, at full maturity surrounded by a two-layered wall mostly 0.8 to 1.2μ thick, later (the membrane of the living cell then everywhere separate from the original membrane) furnished with 2 membranous envelopes, finally germinating in many instances by production of a conidiophore and thereby bringing on abundant formation of conidia.

Observations and Discussion.

A mycelium of *Conidiobolus parvus* growing at 24°C in a Petri plate of moderately soft maize-meal agar advances radially about 1.5 mm in 24 hours. After such a mycelium has developed for several days the central portion of the area occupied by it again looks much like an area of unoccupied substratum, so that the fungus is visible to the naked eye only in a circular peripheral zone 0.8 to 1.5 mm wide. Further growth of the fungus is revealed in radial expansion of the narrow peripheral band and concomitant widening of the featureless central area. In a newly prepared microscope mount the elongating hyphae at the advancing margin are about 4μ wide and rather commonly terminate in a cell more than 500μ long. The terminal cell partly shown in fig. 1, A, for example, measured fully 700μ along its main axis and was continuous with 3 branches measuring 18μ , 22μ , and 103μ in length, respectively. When undergoing microscopic examination in a moist

preparation under a cover glass, the long terminal cell ordinarily soon becomes divided by several cross-walls. Owing to the early onset of reproductive development in maize-meal-agar plate cultures, the shorter hyphal segments backward from the long terminal cell remain in a vegetative state only for a relatively brief period and, as a rule, do not increase markedly in diameter. Some of the small migratory hyphae found in maize-meal-agar tube cultures over 40 days old measure only 1.4μ in width (fig. 1, *B, a, b*).

In tube cultures of potato-dextrose agar *Conidiobolus parvus* produces a firm yeast-like or lichenoid mass of crooked filamentous hyphae (fig. 1 *C-E*) intermixed with irregularly distended segments (fig. 1, *F, a, b; G-H*) as well as with obese ellipsoidal and sphaeroidal cells (fig. 1, *I-N*). Usually no distinct difference with respect to manner of origin can be recognized in the array of cellular components, all of which are filled largely with strongly globuliferous protoplasm. Many of the globose cells (fig. 1, *I-K*), especially those that show a clear space between the contents and the thin outer membrane (fig. 1, *O*), are probably interpretable as resting spores or azygospores. The opulent promiscuous development of the fungus on potato-dextrose agar contrasts markedly with the well-ordered yet vigorous development of *C. polyspermus* Drechsler (1961) on the same substratum.

Conidiobolus parvus shows general parallelism with *Basidiobolus hapto sporus* Drechsler (1956) in the sequence of its growth and reproduction. Sparsely scattered conidiophores are sent up in the narrow peripheral zone of a mycelium growing in a Petri plate of maize-meal agar, most of them (fig. 1, *P, Q*) being extended from a middle position in the parent hyphal cell, though some appear to arise from a proximal or a distal position (fig. 1, *R*). Their upward tapering, though not usually pronounced, contrasts with the upward widening in the conidiophores of *C. paulus* and *C. undulatus*. During the later stages in the enlargement of the conidium a septum is pushed forward from the apical rim of the ascending shaft (fig. 1, *R*; fig. 2, *A-C*). After the septum has been completed at its protruding center and forms a dome-shaped partition (fig. 1, *S-U*; fig. 2, *D*) the newly delimited spore springs off forcibly through eversion of its basal membrane. The detached conidia of *C. parvus* (fig. 1, *V, a-m*; fig. 2, *E, a-v*) show generally more distinct demarcation of the paraboloid basal papilla than is to be seen in the detached conidia of *C. nanodes*, *C. pumilus*, *C. paulus*, or *C. undulatus*. In many instances a primary conidium that has fallen on rather moist substratum sends up a short outgrowth on which is produced a secondary conidium (fig. 2, *F*) that, like its parent, soon springs off forcibly. Through continued recurrence of such development, numerous conidia (fig. 2, *E, c, f, h, i, r, t*) come into being that are smaller than any borne on conidiophores arising from hyphal segments. Some of the smallest conidia concerned in repetitional development (fig. 2, *G, H*) seem furnished with an apical cap of adhesive material.

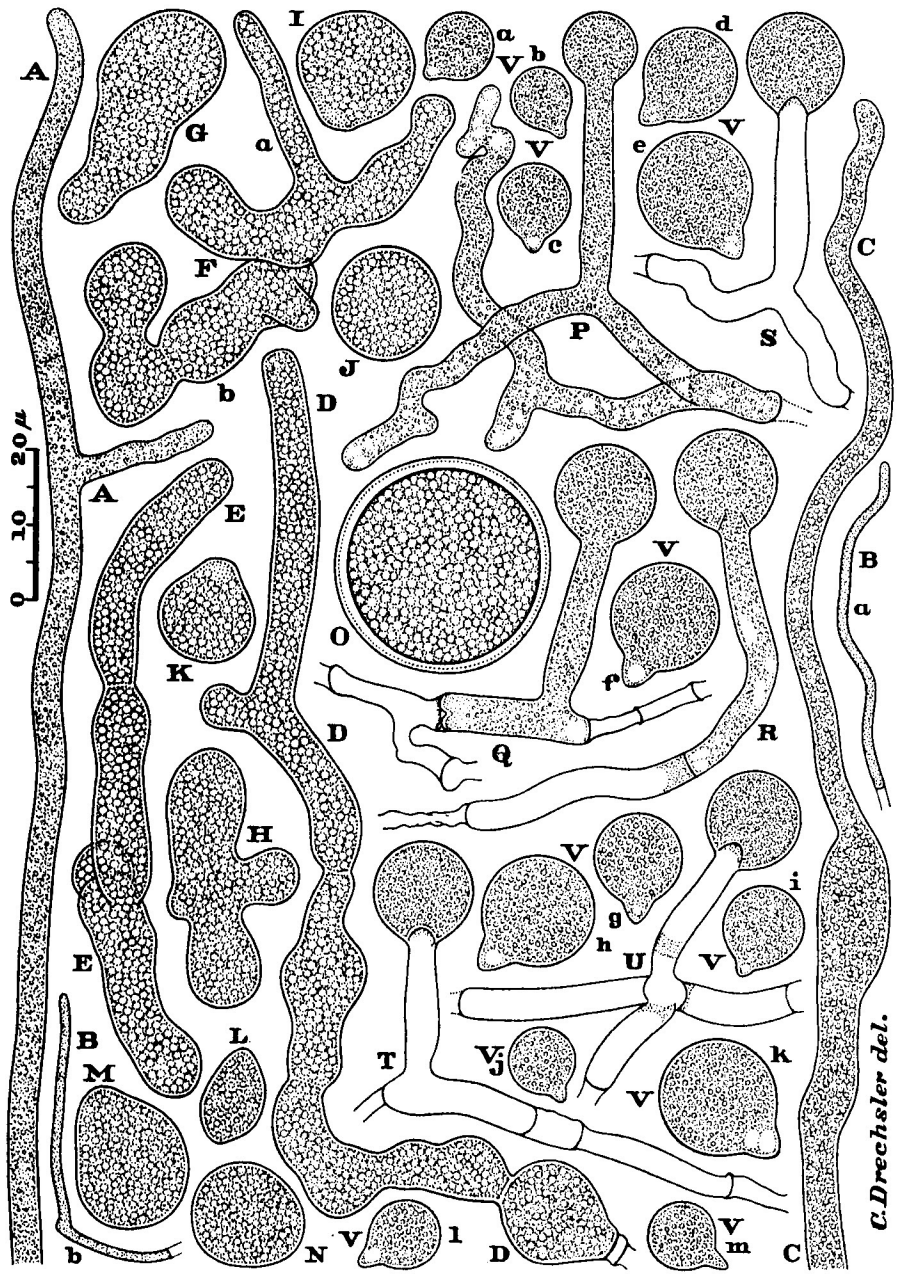


Fig. 1. *Conidiobolus parvus* drawn with the aid of a camera lucida, $\times 1000$.—A, Distal portion of a hypha at margin of a mycelium actively expanding in a maize-meal-agar plate culture.—B, Small migratory hyphal cells, a and b, in a maize-meal-agar tube cul-

Resting spores are produced promptly and abundantly in maize-meal-agar plate cultures of *Conidiobolus parvus*. In such cultures any portion of a mycelium that undergoes conversion into a resting spore becomes invisible to the naked eye. Owing to the promptness and thoroughness with which this conversion takes place, an actively growing mycelium appears usually as a narrow peripheral band that fades from sight along its inner border while it expands along its outer border. The young spore first becomes recognizable as a localized swelling (fig. 2, *I, a*; *J, a*) usually in the middle region of a hyphal segment. The swelling enlarges gradually as it receives protoplasmic materials from the unmodified filamentous portions of the segment (fig. 2, *K*), and when these portions have contributed all their contents it is delimited both proximally and distally by a cross-wall (fig. 2, *L-N*). Thus, in manner of origin, as also in the granular texture of its protoplasm, the newly demarcated reproductive body resembles the chlamydospore of *C. chlamydosporus* Drechsler (1955c). Soon, however, the resemblance diminishes as the contents of the spore take on a somewhat more globuliferous character and at the same time seem to become separated from the enveloping wall by a clear space. In the larger spores (fig. 2, *O, a-m*), where the separation is more pronounced than in the smaller ones (fig. 2, *O, n-p*), the clear space could obviously not persist unless it was occupied by a layer of transparent material. The combined thickness of the outer membrane and the scarcely discernible inner layer varies mostly between 0.8 and 1.2 μ . During the resting period of the spore the curiously reinforced membrane surrounds granular protoplasm in which globules of variable size are indistinctly visible.

At temperatures near 24°C. germination of resting spores usually begins on a noticeable scale in maize-meal-agar plate cultures only 15 to 25 days after inoculation. It proceeds spontaneously, requiring no irrigation of the stale substratum and no addition of fresh water. In the scattered spores that are ready for germination the protoplasm is contained in a thin-walled globose cell lying wholly free within the original thin membranous envelope (fig. 2, *P*). The cell puts forth a protrusion that soon breaks through the envelope and emerges as a germ hypha (fig. 2, *Q-S*). Any germ hypha extended from a submerged spore in an aging culture will usually begin its external development by pushing its way through the overlying agar. On reaching the surface (fig. 2, *T, s*) it elongates as an ascending conidiophore, which, like the conidiophores of mycelial origin produces usually a single

ture 45 days old.—*C-O*, Portions of hyphae (one of them, *F*, consisting of two meagerly connected parts, *a* and *b*) and poorly defined resting spores from lichenoid growth in a potato-dextrose-agar tube culture 15 days old.—*P-U*, Conidiophores arising from hyphal segments near margin of a mycelium actively growing in a maize-meal-agar plate culture.—*V*, Detached conidia, *a-m*, from conidiophores in maize-meal-agar plate culture, showing usual variations in size and shape.

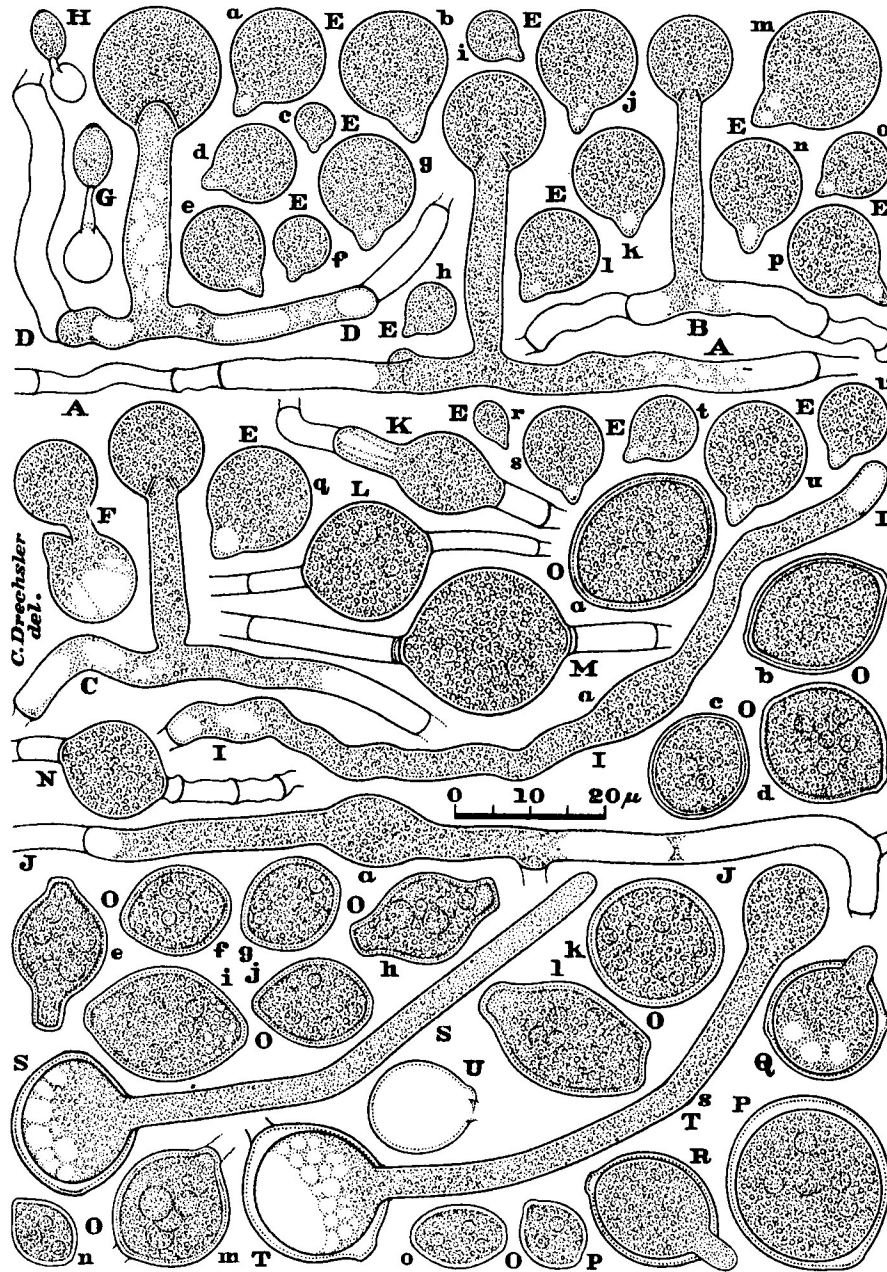


Fig. 2 *Conidiobolus parvus* in maize-meal-agar plate cultures, drawn with the aid of a camera lucida, $\times 1000$.—A-D, Conidiophores arising from hyphal segments near margin of an actively growing mycelium.—E, Detached globose conidia, a-v, from cultures 5 to 49 days old.—F, Detached globose conidium that is producing a secondary conidium on a short ascending outgrowth.—G, H, Small conidia that have each given rise to a small

globose conidium that springs off forcibly. The original or outer membranous envelope of the resting spore (fig. 2, *U*), together sometimes with a short proximal portion of the empty germ hypha, remains visible long after the other membranous parts concerned in germination have faded from sight. In slanted maize-meal-agar tube cultures that have been incubated at temperatures near 24°C. for 50 days most of the resting-spores—usually about four-fifths of them—are represented only by such empty envelopes. The numerous conidia resulting from prolonged germination form a white mealy deposit, especially on the lower portions of the slanted surface. Under like conditions a whitish deposit of conidia usually covers also the lichenoid mycelial growth in potato-dextrose-agar tube cultures of similar age. At temperatures near 10°C. the spores remain much longer in a dormant state, so that cold storage should prove helpful in maintaining cultures of the fungus alive for extended periods.

In the production of a cell that lies free within an envelope representing the outer layer of the thickened wall present during the dormant period, the after-ripening or early germinative development of the resting spores of *Conidiobolus parvus* shows close parallelism with the early germinative development ascribed by Brefeld (1884, p. 59–61, Taf. V, fig. 39–44) to the zygosporangia of his *C. utriculosus*. Similar reorganization of the integument precedes emission of a germ hypha from the zygosporangium not only of the congeneric *C. rhysosporus* Drechsler (1954, p. 572, fig. 54) but also of the less intimately related *Basidiobolus ranarum* Eidam (1886) and *B. haptosporus* Drechsler (1956, p. 666, fig. 3, *G-I*; p. 670, fig. 4, *O*). As the resting spores of *C. parvus* thus display unmistakably the structural rearrangement distinguishing early germinative development in sexual spores of the Entomophthorales they would seem morphologically equivalent to zygosporangia even though they originate without any conjugation of separate hyphal cells. Their conversion from chlamydospores to azygosporangia is probably somehow associated with the reinforcement of the membranous envelope by an inner layer before the period of dormancy. In their resting state they differ rather markedly with respect to internal organization from the mature zygosporangia of most congeneric species known to reproduce sexually. They agree, however, moderately well in their inward make-up with the zygosporangia of *C. incongruus* Drechsler (1960), which clearly originate through conjugation of paired hyphal segments.

daughter conidium apparently provided with an adhesive apical cap.—*I, J*, Hyphal segments, each with a distention, *a*, representing early stage in development of a resting spore.—*K*, Late stage in growth of a resting spore.—*L-N*, Resting spores newly delimited from adjacent portions of empty hyphal membrane.—*O*, Resting spores, *a-p*, in fully mature or dormant stage, from culture 15 days old.—*P*, After-ripened resting spore ready to germinate, from a culture 43 days old.—*Q, R*, Resting spores from each of which a short germ tube has been extended.—*S*, Resting spore with a longer germ hypha.—*T*, Resting spore with a germ hypha that extends above the surface of the substratum, *s*, as a young conidiophore.—*U*, Empty membrane visible rather long after germination was completed, in a culture 43 days old.

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