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TWO NEW SPECIES OF CONIDIOBOLUS

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TWO NEW SPECIES OF CONIDIOBOLUS [1]

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THE TWO readily culturable fungi herein described as new species of *Conidiobolus* were obtained by canopying Petri plates of maize-meal agar with small quantities of finely divided detritus sifted from leaf mold I collected in deciduous woods in northern Wisconsin and northern Virginia. Dried specimens of the fungi have been deposited in the National Fungus Collections, Plant Industry Station, Beltsville, Maryland; and live cultures of them have been transmitted to the American Type Culture Collection in Washington, D. C.

1. CONIDIOBOLUS **megalotocus** Drechsler sp. nov. [3]

Mycelium colorless, branched, usually inconspicuous to the naked eye owing to meager aerial development; intramatrical hyphae filamentous, 1.5–15 μ (mostly 4–12 μ) wide, composed of segments 15–1100 μ (mostly 100–600 μ) long which in many places become disjointed and in other places remain connected only by empty portions of membrane; conidiophores colorless, usually extending 20–125 μ into the air toward the main source of light, the protruding aerial portion being cylindrical or somewhat inflated, mostly 2.5–15 μ in greatest width, on substrata poor in nutrients commonly unbranched and producing a single globose conidium but on richer substrata in many instances bifurcated and then bearing 2 globose conidia simultaneously; globose conidia springing off forcibly, colorless, 10–42 μ wide, 12–44 μ long inclusive of a basal papilla 1.5–8 μ high and 2.5–12 μ wide, some germinating by emission of a germ hypha, others producing individually another globose conidium on a wide phototropic outgrowth, and still others giving rise to microconidia (commonly 2–9) on radially projecting sterigmata; sterigmata usually simple, rarely branched, colorless, commonly 4–18 μ long, 2.5–3.5 μ wide at the base, noticeably tapering upward; microconidia springing off actively, colorless, subspherical or elongate ellipsoidal, usually 9–17 μ in greatest width, 13–23 μ in total length inclusive of a basal papilla about 1.5 μ high.

Isolated from leaf mold gathered in deciduous woods near Mellen, Wisconsin, on November 19, 1954.

In its generally large dimensions *Conidiobolus megalotocus* invites comparison with the congeneric *C. chlamydosporus* Drechsler (1955c) as well as with the closely related and ubiquitous *Delacroixia coronata* (Cost.) Sacc. & Sydow emend. Gallaud (1905). Its elongating terminal segments at the margin of a mycelium that is growing in maize-meal agar usually measure about 10 μ in width (fig. 1). The protoplasm in these terminal segments appears mostly rather homogeneous, showing relatively few granules and little vacuolation. The older hyphal segments 5–10 mm. from the mycelial forefront are, as a rule, noticeable wider, commonly measuring 11–15 μ in diameter (fig. 2); and their protoplasm, which usually seems of moderately granular texture, is in many instances mainly restricted to a parietal layer surrounding very extensive vacuoles. A considerable proportion of the strongly vacuolated segments in maize-meal agar cultures commonly exceed 500 μ in length. Indeed, some strongly vacuolated segments no less than 1 or 1.1 mm. long have come under observation, especially in Petri plate cultures grown in

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[3] Oculo nudo vix visibilis, praecipue in materiis macris inconspicuus; mycelio in aerem parum crescente, incolorato; hyphis sterilibus filiformibus, ramosis, 1.5–15 μ (plerumque 4–12 μ) latis, mox septatis, postea hic illic disjunctis vel inanitis, cellulis 15–1100 μ (plerumque 100–600 μ) longis; hyphis fertilibus incoloratis, in aerem 20–125 μ ad lucem protendentibus, in parte aeria cylindricis vel aliquid inflatis, 2.5–15 μ latis, vulgo simplicibus et unum conidium ferentibus sed interdum praecipue in materiis feracissimis bifurcis denique duo globosa conidia simul gignantibus; globosis conidiis violenter absilientibus, incoloratis, basi papilla 1.5–8 μ alta et 2.5–12 μ lata praeditis, omnino 12–44 μ longis, 10–42 μ latis, nunc hypham germinationis emittentibus nunc aliud conidium gignantibus nunc aliquot (saepe 2–9) microconidia in apicibus sterigmatum ferentibus; sterigmatibus incoloratis, vulgo simplicibus, rarius ramosis, 4–18 μ longis, sursum paulum attenuatis; microconidiis absilientibus, incoloratis, globosis vel elongato-ellipsoideis, basi papilla circa 1.5 μ alta praeditis, ex toto plerumque 13–23 μ longis, 9–17 μ latis.

Habitat in foliis arborum (*Betulae, Aceris, Ulmi*) putrescentibus prope Mellen, Wisconsin.

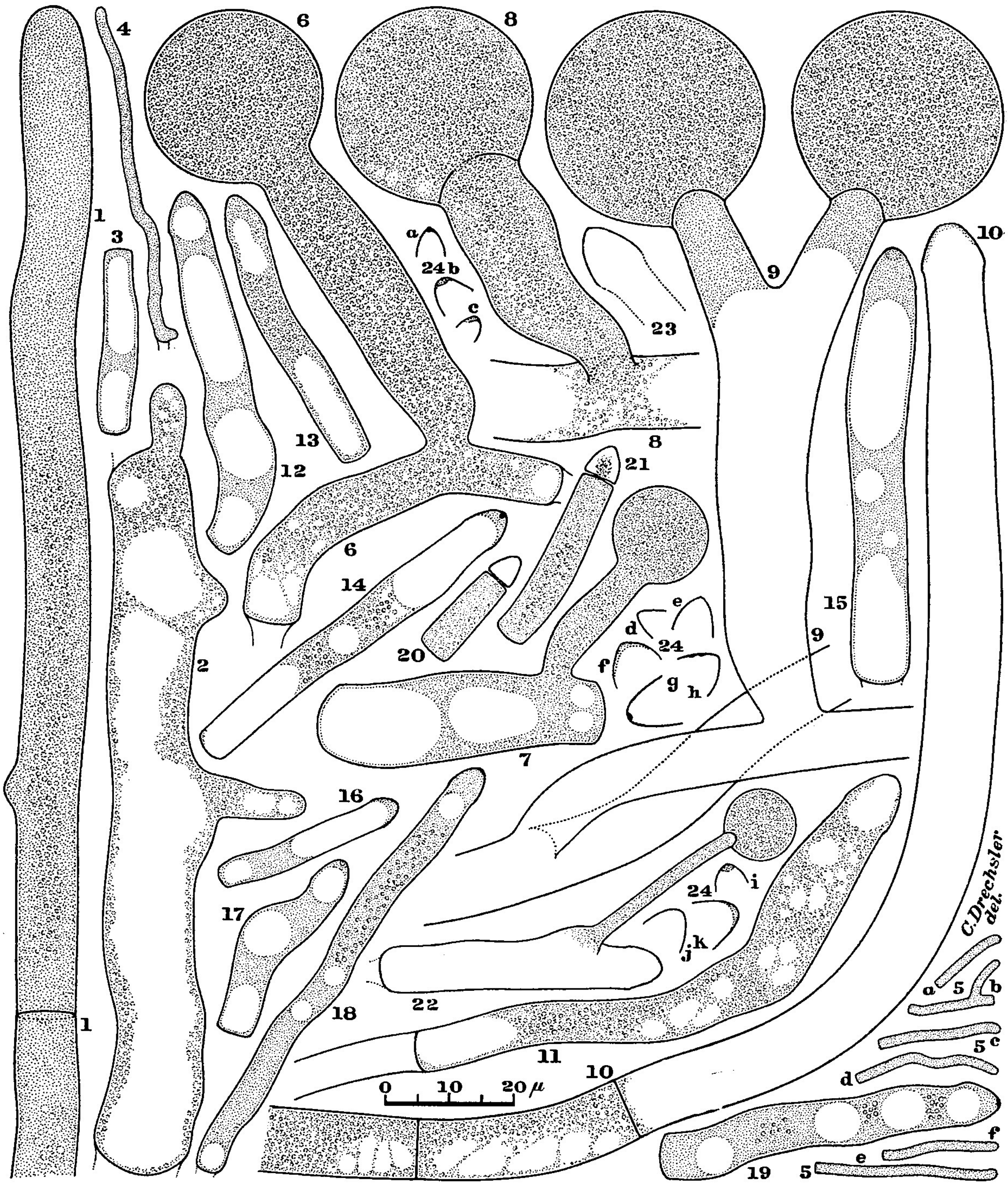


Fig. 1–24. *Conidiobolus megalotocus* as found on maize-meal agar (fig. 1–5, 11–24) and lima-bean agar (fig. 6–10) in Petri plate cultures, ×1000.—Fig. 1. Distal portion of hypha at margin of an actively growing mycelium.—Fig. 2. Wide, strongly vacuolated hyphal segment in a culture 7 days old.—Fig. 3, 4. Smaller segments in a culture 11 days old.—Fig. 5. Very small segments, a–f, in a culture 27 days old.—Fig. 6–8. Broad unbranched conidiophores, each with a young globose conidium.—Fig. 9. Bifurcated broad conidiophore bearing 2 globose conidia.—Fig. 10. Denuded broad conidiophore formed in prolongation of a prostrate hypha.—Fig. 11–19. Living segments formed distally in denuded conidiophores.—Fig. 20, 21. Living segments formed subterminally in denuded conidiophores.—Fig. 22. Terminal segment of a conidiophore with a slender branch bearing a small globose conidium.—Fig. 23. Distal portion of denuded conidiophore with tubular membrane fading from sight.—Fig. 24. Conidiophore caps, a–k.

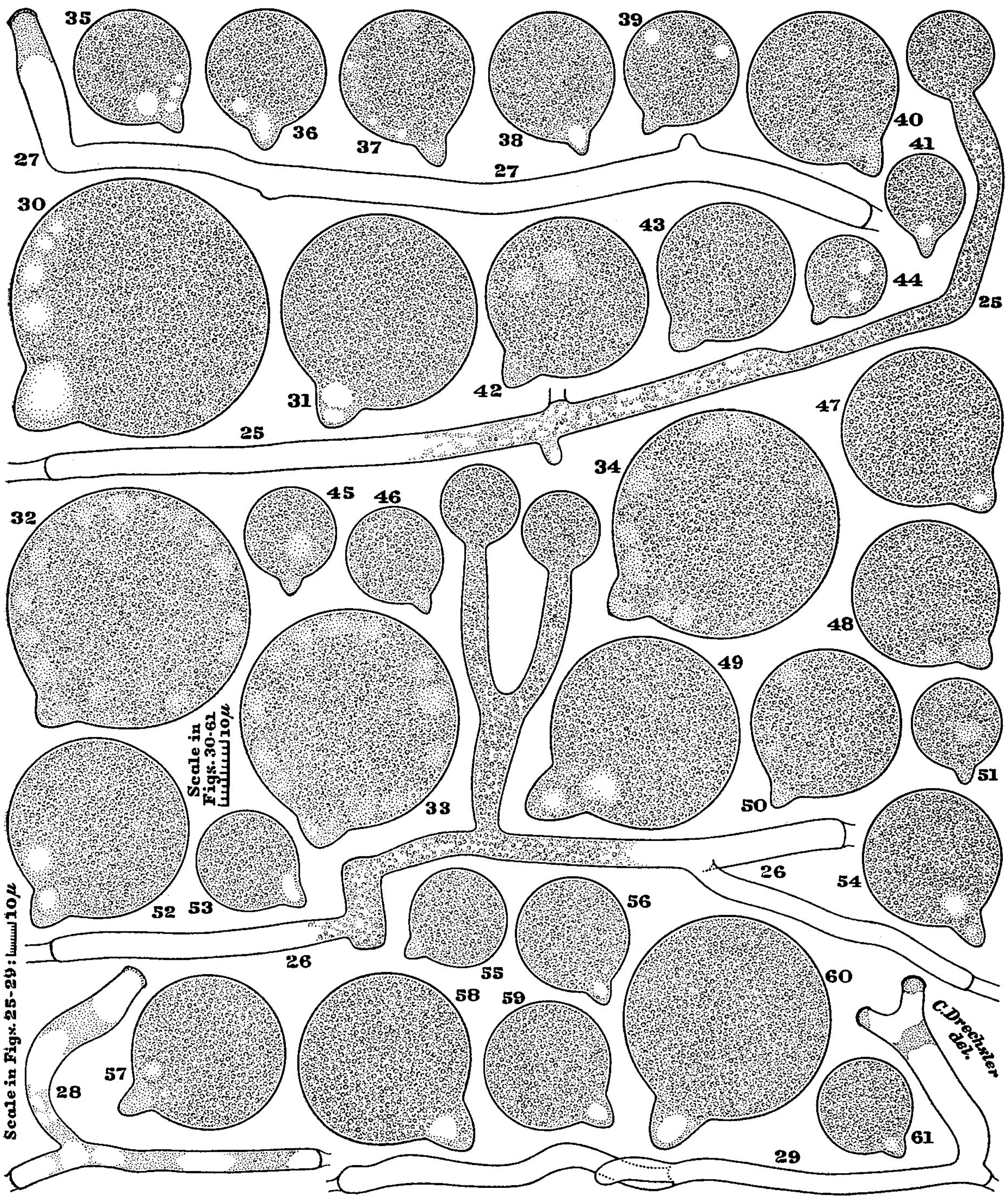


Fig. 25–61. *Conidiobolus megalotocus* as found on lima-bean agar (fig. 25–34) and maize-meal agar (fig. 35–61) in Petri plate cultures; drawn with the aid of a camera lucida.—Fig. 25. Hyphal segment with unbranched conidiophore and young conidium, ×500.—Fig. 26. Branched hyphal segment with a bifurcated conidiophore and 2 young conidia, ×500.—Fig. 27, 28. Hyphal segments, each with a denuded simple conidiophore, ×500.—Fig. 29. Hyphal segment with denuded bifurcated conidiophore, ×500.—Fig. 30–61. Detached conidia showing usual variations in size and shape, ×1000.

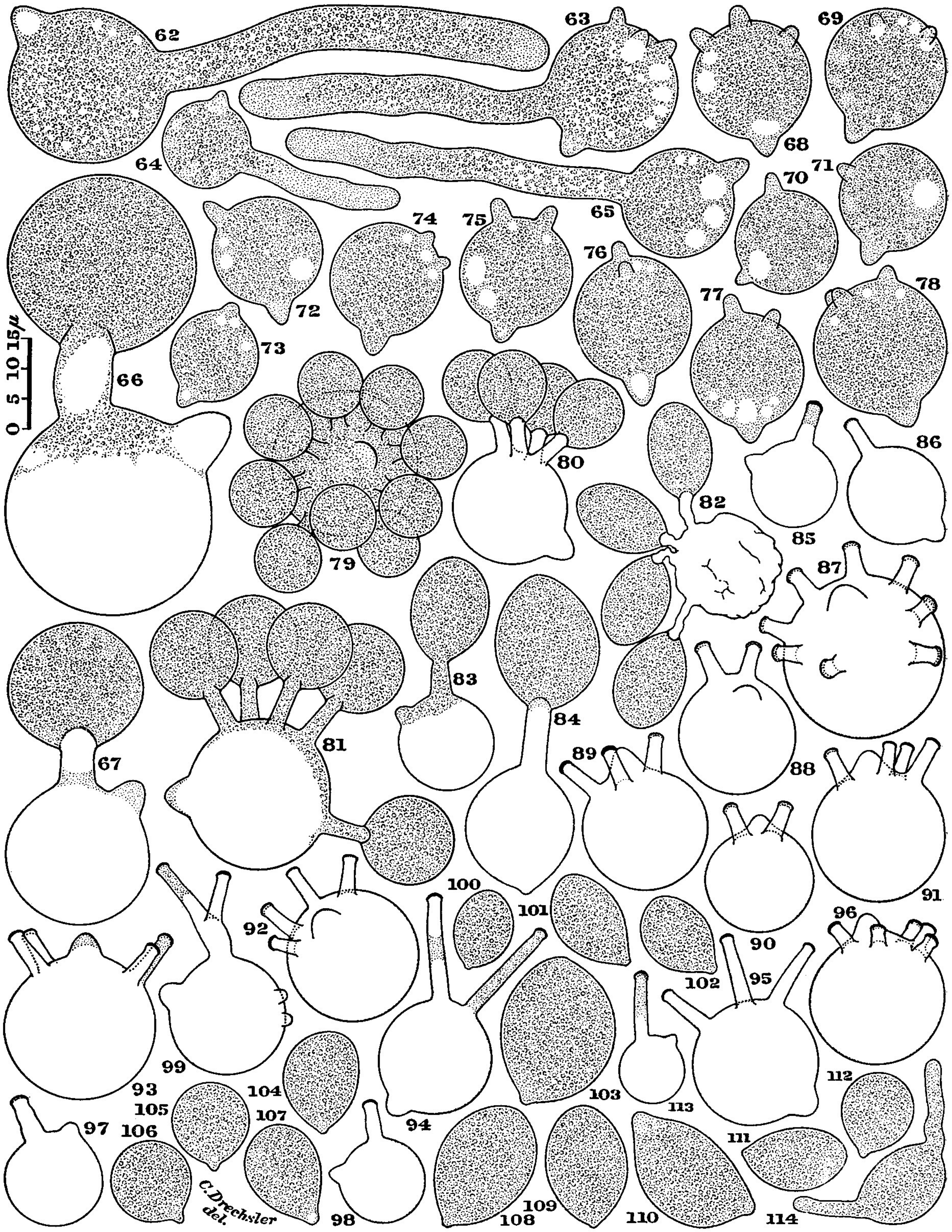
rather dimly lighted surroundings. Small hyphal segments, nevertheless, are also found in cultures of the fungus. When a peripheral portion of an actively growing mycelium is being examined under high magnification, the elongating terminal segments, much as in related species, soon divide into relatively short cells, presumably because of the bright illumination to which they are exposed. Even without exposure to strong light, aging cultures will commonly show some hyphal segments of mycelial origin less than 30 μ long and 6 μ wide (fig. 3). In such cultures filamentous segments less than 2 μ wide (fig. 4) also occur in considerable number, having apparently originated as germ hyphae extended by microconidia into substratum already occupied by the fungus. As these segments migrate promiscuously through the agar (which seemingly supplies them no nourishment) by continuously withdrawing protoplasm from the posterior end while pushing forward at the tip, they expend their substance and thereby diminish in size (fig. 5, a–f) until they may measure only 1.5 μ in width and less than 15 μ in length (fig. 5a).

On lima-bean agar, a substratum richer in nutrients than maize-meal agar, *Conidiobolus megalotocus* produces a luxuriant mycelium in which the older segments usually show only rather moderate vacuolation. The well nourished hyphal segments give rise rather abundantly to broad phototropic conidiophores (fig. 6–10, 25–29), which in general appear shorter than those of the familiar *Delacroixia coronata.* More than a few of the conidiophores produced on lima-bean agar are bifurcated (fig. 9, 26, 29) and bear simultaneously two globose conidia of approximately equal size. The conidiophores formed on maize-meal agar very rarely show similar bifurcation, though some among them that retain a quantity of protoplasm after they have become denuded (fig. 11–21) may later put forth a branch bearing a rather small second conidium (fig. 22). Once a properly emptied conidiophore has fallen over on to the substratum its tubular membrane soon fades from sight (fig. 23), but the dome-shaped distal membrane remains visible, so that in aging cultures numerous caps (fig. 24, a–k) can be seen strewn about haphazardly.

The copious asexual reproduction of *Conidiobolus megalotocus* in lima-bean agar cultures yields globose conidia which in many instances (fig. 30–34) are larger than the conidia formed on maize-meal agar (fig. 35–61). In comparison with the size of the conidium the papilla resulting from the abrupt eversion of the basal membrane whereby the spore is forcibly propelled, is usually smaller in *C. megalotocus* than in *Delacroixia coronata.* Yet as some detached conidia of *C. megalotocus* show a rather strongly protuberant papilla (fig. 30, 49, 58, 60) it is not surprising that in Petri plate cultures such spores are shot toward the main source of light far enough to reach unoccupied substratum beyond the mycelial forefront, and thus after germinating by emission of a stout vegetative hypha (fig. 62–65) can give rise to thickly scattered mycelia. Owing to the growth of these mycelia, and also to the forcible propulsion of secondary globose conidia formed through repetitional development (fig. 66, 67), the fungus spreads more rapidly toward the light than in other directions.

In maize-meal agar cultures a large proportion of globose conidia that fall on substratum already permeated by mycelium of the fungus give rise to microconidia. Seemingly in preparation for such multiplicative development the detached conidium puts forth 1–3 wart-like protuberances (fig. 68–78), and may then remain for some time without undergoing further change. When conditions become suitable it commonly produces 2–9 microconidia on radially oriented sterigmata (fig. 79–82). Some relatively small conidia can produce only one microconidium (fig. 83), and occasionally only a single microconidium (fig. 84) is produced by a parent that would seem large enough to produce two. The sterigmata left denuded on the empty conidial envelope (fig. 85–99) after the microconidia (fig. 100–112) have sprung off through eversion of the basal membrane are longer than those of any congeneric form in which multiplicative reproduction is known. Equally or perhaps even more distinctive of the fungus is the generally large size of its microconidia—a feature intended to be signalized in the epithet compounded of two words (μεγας, τοκος) meaning "large" and "offspring," respectively. Many microconidia give rise on a slender sterigma (fig. 113) to a secondary microconidium of somewhat smaller size, and when this development has been repeated several times the descendents show markedly reduced dimensions. On fresh moist substratum microconidia germinate readily by producing 1 or 2 germ tubes. Elongated microconidia usually extend a germ tube at each end (fig. 114).

Despite the prevalence of multiplicative reproduction in maize-meal agar cultures it appeared limited here to the smaller and medium-sized conidia, and was never observed in conidia more than 28 μ wide. It was wholly absent in lima-bean agar cultures where the largest conidia measured more than 40 μ in diameter. Yet there is no good reason to presume that merely because of their size the larger conidia produced by the fungus are under all circumstances incapable of multiplicative reproduction. If under appropriate conditions the more massive conidia were to give rise to microconidia they could be expected to yield progenies of 20–30 individuals. In view of such potentiality the less numerous progenies that came to light in my cultures should obviously not be held strictly diagnostic of the species.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2. CONIDIOBOLUS **globuliferus** Drechsler sp. nov.[4]

At first usually inconspicuous owing to meager aerial growth, but later usually becoming visible to the naked eye through accumulation of conidia in white deposits; mycelium colorless, branched; assimilative hyphae filamentous, 1.8–12 μ (mostly 3–9 μ) wide, composed of segments 20–200 μ long, which in many places become disjointed and in other places remain connected only by empty portions of tubular membrane. Conidiophores colorless, unbranched, extending 20–35 μ into the air toward the main source of light, the aerial portion usually 5–11 μ in greatest width, bearing commonly a single subglobose conidium; subglobose conidia springing off forcibly, colorless, measuring 11–25 μ in greatest width and 15–30 μ in total length inclusive of a dome-shaped or somewhat conical basal papilla, some germinating by the emission of a germ hypha, others individually producing another subglobose conidium on a broad outgrowth, and still others giving rise on a slender conidiophorous outgrowth to a conidium of elongated type. Slender conidiophorous outgrowths colorless, usually more or less curved, 50–90 μ long, 2–2.5 μ wide at the base, tapering gradually upward, about 1 μ wide at the tip; conidia of the elongated type becoming detached passively, colorless, prolate ellipsoidal, 15–17 μ long, 8–10 μ in greatest width, usually filled with numerous globules about 1 μ in diameter. Chlamydospores formed in submerged and procumbent hyphae, usually intercalary in position but occasionally lateral or terminal, mostly globose or ellipsoidal or barrel-shaped, colorless, measuring usually 15–35 μ in length and 12–25 μ in greatest width, often largely filled with globules about 1 μ wide.

Isolated from leaf mold collected in Arlington, Virginia, on February 28, 1952.

In comparison with other members of its genus *Conidiobolus globuliferus* may be considered a species of moderate dimensions, being noticeably smaller than *C. megalotocus* and *C. chlamydosporus* but more robust than *C. pumilus* Drechsler (1955a) or *C. nanodes* Drechsler (1955b). In a mycelium that is growing actively on an ample expanse of maize-meal agar the elongating segments (fig. 115) at the advancing forefront commonly measure 6 to 7 μ in diameter. On lima-bean agar the hyphae at the forefront appear a little stouter, so that after some widening has taken place the older segments several millimeters from the margin may vary in diameter between 10 and 12 μ (fig. 116). Relatively small segments (fig. 117–125) become fairly numerous in tube cultures a few months old, though owing presumably to the absence of microconidia in the species, migrating cells only about 2 μ in width (fig. 122, 124) appear generally in lesser numbers than in *C. megalotocus*.

Asexual reproduction proceeds rather abundantly in *Conidiobolus globuliferus* on both maize-meal agar and lima-bean agar. The conidiophores, whether arising from a mycelial segment (fig. 126–131) or from the narrow germ hypha of a conidium (fig. 132), are usually widened perceptibly in the relatively short phototropic termination extending above the surface of the substratum (fig. 126–132:s). As a rule the widened part is emptied in producing the subglobose conidium, though occasionally it retains enough protoplasm to permit delimitation of a distal segment (fig. 133) corresponding to the conidiophore segments found more abundantly in *C. megalotocus*. The subglobose conidium, when fully developed, springs off forcibly through eversion of its basal membrane. In detached conidia (fig. 134–158) the papilla resulting from this eversion is not always

[4] Primo inconspicuus, deinde propter congeries conidiorum albas oculo nudo visibilis; mycelio incolorato, ramoso; hyphis sterilibus filiformibus, 1.8–12 μ (plerumque 3–9 μ) latis, cellulis eorum 20–200 μ longis, mox septatis, postea hic illic disjunctis vel inanitis et evanescentibus; hyphis fertilibus incoloratis, in aerem 20–35 μ ad lucem protendentibus, parte aeria 5–11 μ latis, unum primiforme conidium ferentibus; primiformibus conidiis violenter absilientibus, globosis sed basi papilla rotunda vel conica praeditis, omnino 15–30 μ longis, 11–25 μ latis, nunc hypham germinationis emittentibus nunc aliud primiforme conidium ferentibus nunc in apice gracilis fertilis hyphae conidium formae ellipsoideae gigantibus; gracilibus fertilibus hyphis incoloratis, vulgo aliquid curvatis, 50–90 μ longis, basi 2–2.5 μ latis, sursum leniter attenuatis, apice circa 1 μ latis; conidiis formae ellipsoideae incoloratis, interdum 15–17 μ longis, 8–10 μ latis, vulgo multorum globulorum 1 μ crassorum repletis. Chlamydosporis in hyphis submersis vel procumbentibus oriundis, saepissime intercalaribus raro terminalibus vel lateralibus, plerumque globosis vel ellipsoideis vel dolioformibus, incoloratis, 15–35 μ longis, 12–25 μ latis, globulorum circa 1 μ crassorum saepe repletis.
Habitat in humo silvestri in Arlington, Virginia.

Fig. 62–114. *Conidiobolus megalotocus* as found in maize-meal-agar plate cultures; drawn with the aid of a camera lucida at a uniform magnification; ×1000.—Fig. 62–65. Globose conidia, each germinating by emission of a germ hypha.—Fig. 66. Globose conidium giving rise to another globose conidium at the tip of a broad aerial outgrowth.—Fig. 67. Globose conidium with a broad conidiophorous outgrowth bearing a secondary globose conidium.—Fig. 68–78. Detached globose conidia bearing 1–3 wartlike protuberances.—Fig. 79. Globose conidium on which 9 microconidia are being formed.—Fig. 80. Empty globose conidium bearing 4 delimited microconidia, each attached to an empty sterigma.—Fig. 81. Globose conidium with 5 microconidia, of which 1 has been delimited and 4 are nearly ready for delimitation.—Fig. 82. Collapsed envelope of globose conidium with 4 microconidia, each still attached to an empty sterigma.—Fig. 83, 84. Globose conidia, each producing a single microconidium.—Fig. 85–98. Empty envelopes of globose conidia with 1–9 denuded sterigmata.—Fig. 99. Empty envelope of globose conidium with a branched sterigma.—Fig. 100–112. Detached microconidia showing variations in size and shape.—Fig. 113. Empty microconidium with a sterigma from which a secondary microconidium has sprung off.—Fig. 114. Elongate microconidium germinating by emission of 2 green hyphae.

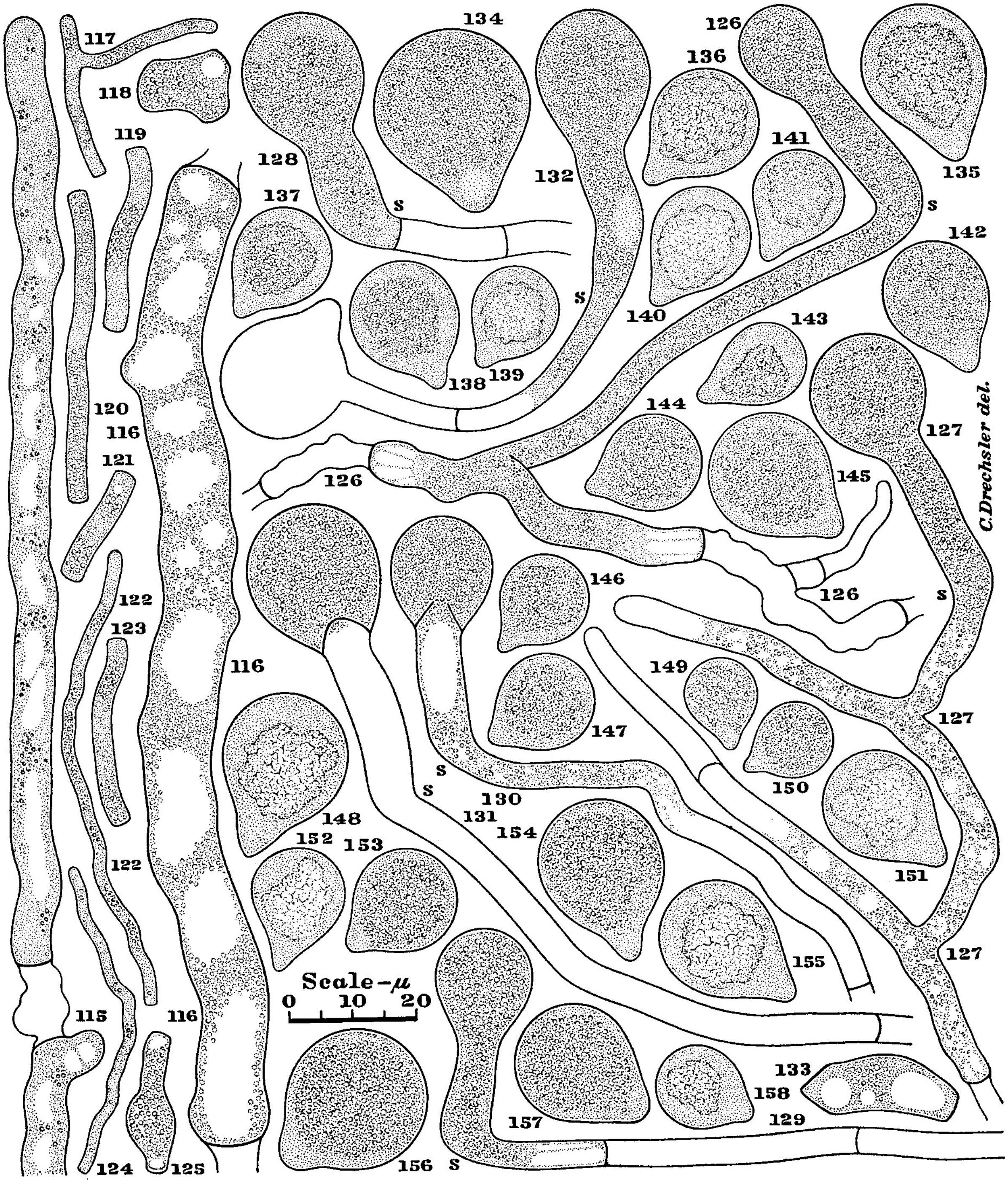

Fig. 115–158. *Conidiobolus globuliferus* as found in maize-meal-agar cultures (fig. 115, 117–158) and in a lima-bean-agar plate culture (fig. 116); drawn at a uniform magnification with the aid of a camera lucida; ×1000.—Fig. 115. Terminal portion of a hypha at margin of an actively growing mycelium.—Fig. 116. Wide intercalary hyphal segment about 5 mm. back of margin of a growing mycelium.—Fig. 117–125. Relatively small segments in a tube culture 78 days old.—Fig. 126–130. Portions of hyphae, each with a broad conidiophore and a young globose conidium; *s*, surface of substratum.—Fig. 131. Empty conidiophore bearing a globose conidium; *s*, surface of substratum.—Fig. 132. Empty conidium with germ hypha terminating in a stout conidiophore; *s*, surface of substratum.—Fig. 133. Living segment formed distally in a stout conidiophore.—Fig. 134–158. Detached conidia showing variations in size, shape, and disposition of granular contents.

sharply demarcated, its contour in many instances tending to merge with the main outline of the spore.

Internally the subglobose conidia of *Conidiobolus globuliferus* show approximately the same variations in the texture of their protoplasmic contents as have been observed in some other species. The newly delimited spore (fig. 131) is usually occupied throughout by coarsely granular material. After the conidium has sprung off, the peripheral layer may become progressively cleared of granules

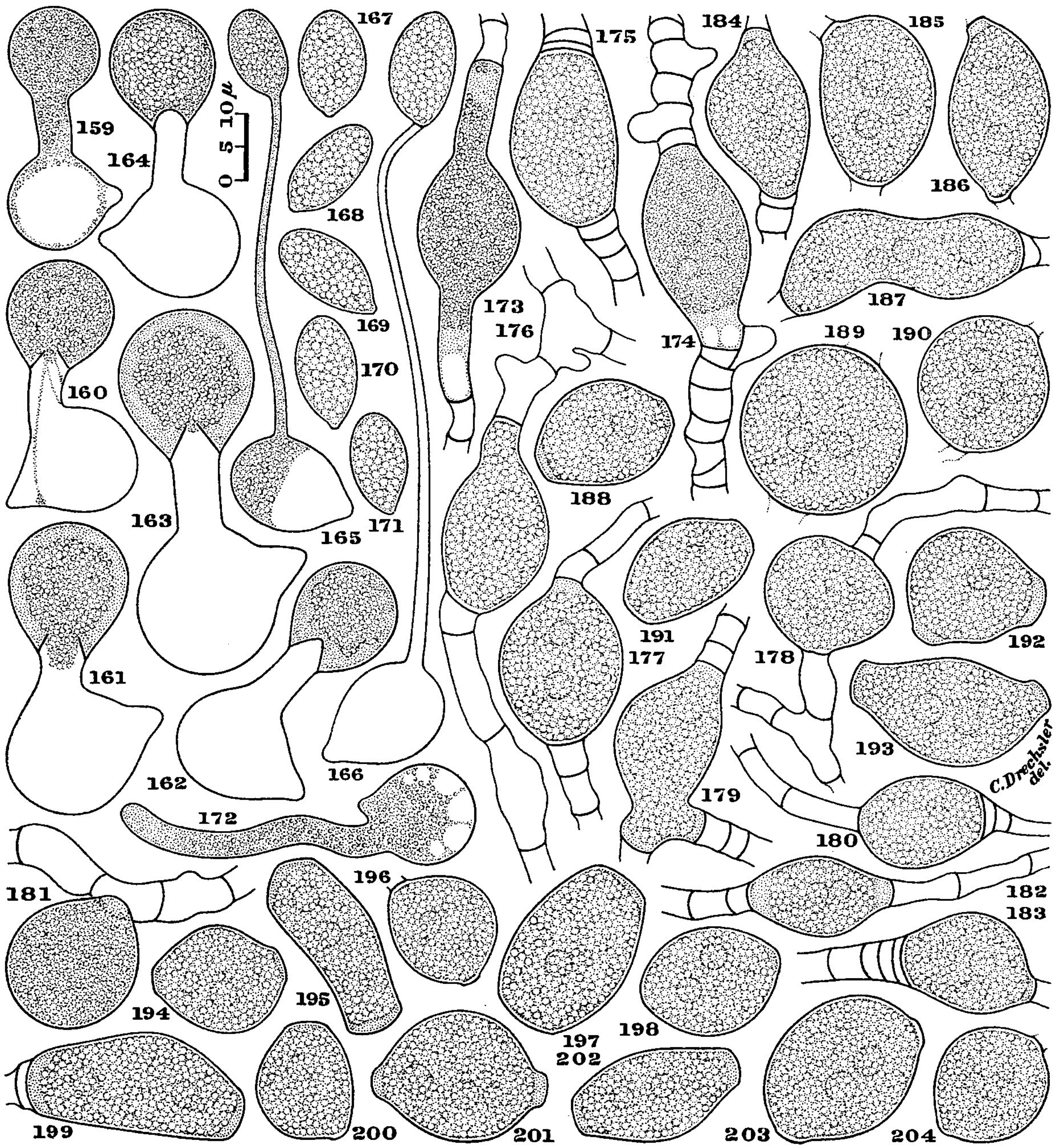

Fig. 159–204. *Conidiobolus globuliferus* as found in maize-meal-agar plate cultures; drawn at a uniform magnification with the aid of a camera lucida; ×1000.—Fig. 159–164. Globose conidia at various stages in the production of secondary globose conidia.—Fig. 165. Globose conidium that is giving rise to an elongate conidium on a slender outgrowth.—Fig. 166. Empty envelope of globose conidium with a slender outgrowth bearing an elongated conidium.—Fig. 167–171. Detached elongated conidia.—Fig. 172. Globose conidium germinating by emission of a germ hypha.—Fig. 173, 174. Chlamydospores in late stage of formation.—Fig. 175–184. Mature chlamydospores with adjacent portions of empty hyphae, showing successively formed retaining walls.—Fig. 185–204. Mature chlamydospores showing variations in size and shape.

while simultaneously in the central region an increasing concentration of granules takes place (fig. 134, 156). Eventually the peripheral layer may show protoplasm of nearly homogeneous texture (fig. 137, 138). In the central region the granules come together in closely crowded arrangement, or unite to form an irregular conglutinated mass (fig. 135, 139–141, 143, 148, 152, 155, 158). The smaller globose conidia formed in some aging cultures may, however, display strongly globuliferous contents that remain unchanged during long periods.

The detached globose conidia of *Conidiobolus globuliferus,* like those of all known congeneric forms, give rise freely to secondary globose conidia on broad aerial outgrowths (fig. 159–164). Since this type of development is usually repeated again and again, with each generation of spores springing in turn toward the main source of light, Petri plate cultures 5–10 days old will usually reveal along one side of the circular dish a white chalk-like coating in which globose conidia are piled several layers deep.

The production of elongated conidia on slender filamentous outgrowths extended upward singly from detached globose conidia (fig. 165) has been found to proceed only sparingly in cultures of *Conidiobolus globuliferus.* In all observed instances the globose conidium serving as parent was relatively small, and the elongated conidium was likewise small (fig. 166–171). If under suitable conditions the larger globose would serve as parents the resulting ellipsoidal conidia could be expected to have considerably greater dimensions than those given in the diagnosis. Moreover, although all the elongated conidia that came under observation were filled with globuliferous protoplasm, it can not be presumed that the present fungus might not under appropriate conditions give rise to elongated conidia containing protoplasm of finely granular texture. As no elongated conidia were found to germinate even in cultures where globose conidia would readily put forth a germ hypha (fig. 172) it seems likely that globuliferous contents denote a state of diminished activity.

Chlamydospores are produced abundantly in cultures of *Conidiobolus globuliferus,* their development, as in *C. chlamydosporus,* proceeding freely at ordinary laboratory temperatures near 20°C. As they are usually formed in a median position the parent hyphal segment is commonly first emptied at the ends and then in portions closer and closer to the middle. Successive stages in the progressive evacuation of protoplasmic contents are marked by deposition of a series of delimiting septa (fig. 173–184). If a chlamydospore is formed intercalarily at the origin of a branch it may receive protoplasm from 3 hyphal elements (fig. 179); if it is formed within a short branch it may appear attached laterally to the axial hypha (fig. 181). During its formative period the chlamydospore contains protoplasm of coarsely granular texture (fig. 173, 174) but soon after its definitive boundary walls have been laid down the contents become pronouncedly globuliferous (fig. 175–204). Except for a few indistinctly visible vacuoles in the center and, in some instances, small regions of homogeneous protoplasm near the hyphal attachments, the mature chlamydospore of *C. globuliferus* appears crowded with globules of relatively uniform size. It shows little tendency to germinate early in the staled substratum in which it was formed. It seems capable of surviving long in a dormant state. When sterile water was added to several maize-meal-agar tube cultures which had been stored more than 4 years at 5°C, and during this period had been in an air-dry condition for about 2 years, many chlamydospores in the softened agar were found in an unmistakably living state. On transferring portions of the softened substratum to tubes of maize-meal agar new cultures of *C. globuliferus* were promptly obtained.

SUMMARY

Two readily culturable entomophthoraceous fungi isolated by canopying Petri plates of maize-meal agar with leaf mold from deciduous woods are described as new species of *Conidiobolus.* Neither species is known to produce zygospores, but in each species the familiar type of asexual reproduction by the formation of subglobose conidia that spring forcibly off broad phototropic conidiophores is accompanied by supplementary asexual sporulation. *Conidiobolus megalotocus,* obtained from northern Wisconsin, is one of the most robust members of the genus, and is distinguished especially by its long sterigmata and large microconidia. *C. globuliferus,* obtained from northern Virginia, gives rise on slender conidiophores to elongated secondary conidia, and at temperatures near 20°C forms numerous chlamydospores with globuliferous contents.

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