MYCOLOGY.—*Two medium-sized species of* Conidiobolus *occurring in Colorado.*

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The two readily culturable entomophthoraceous fungi herein described as new species of *Conidiobolus* were obtained by canopying Petri plates of maize-meal agar with moist filter paper to which had been securely affixed minute quantities (0.03 to 0.05 gram) of decaying plant detritus kindly gathered by W. J. Zaumeyer near Greeley, Colo., in August 1956. Dried portions of Petri plate cultures of the two fungi have been deposited in the National Fungus Collection, Beltsville, Md.; although, unfortunately, the morphological features distinguishing the readily culturable members of the Entomophthorales are for the most part not well revealed in such desiccated material.

1. **Conidiobolus denaeosporus** sp. nov. Mycelium paene omnino in materia alimentaria immersum, parce in aerem assurgens, itaque aliquid inconspicuum; hyphae steriles filiformes, ramosae, rectae vel pravae, vulgo passae sed interdum in pabulo validissimo dense intertextae, plerumque 2.5–10 μ crassae, mox septatae, postea hic illic disjunctae vel inanitae, cellulae eorum plerumque 25–250 μ longae, primo incoloratae, in senio aliquanto sufflavae; primiformes fertiles hyphae singulatim ex cellulis hypharum surgentes, simplices, in aerem saepe 15–50 μ ad lucem protendentes, in parte aeria erectae vel acclives, illic plerumque 4–7.5 μ crassae, apice unum conidium ferentes; primiformia conidia violenter absilientia, saepius in pulverem album visibilem accumulata, primo incolorata, postea in senio aliquid subflava, paulum perdurantia, globosa vel saepius turbinea, apice late rotundata, deorsum in papillam 4–7.5 μ latam et 2–6 μ altam abeuntia, ex toto 13–32 μ longa, 6–21 μ crassa; graciles fertiles hyphae singulatim ex primiformibus vel secundariis conidiis surgentes, incoloratae, simplices, 35–65 μ longae, basi 1.5–3 μ latae, sursum leniter attenuatae, apice circa 1 μ latae, ibi unum secundarium conidium ferentes; secundaria conidia incolorata, elongato-ellipsoidea, 10–18 μ longa, 6–10 μ lata.

Habitat in foliis *Ulmi parvifoliae* putrescentibus prope Greeley, Colorado.

Mycelium almost wholly intramatrical, with little or no aerial development, and thus rather inconspicuous; vegetative hyphae filamentous, branched, almost straight or variously crooked, for the most part spreading out uniformly but in highly nourishing morsels often closely interwoven, mostly 2.5 to 10 μ wide, early divided by cross-walls; the resulting hyphal segments mostly 25 to 250 μ long, often disjointing or becoming separated by portions of emptied membrane, always colorless when young but later sometimes turning yellowish; primary condiophores arising singly from submerged or prostrate hyphal segments, extending 15 to 50 μ into the air toward the main source of light, in the aerial portion erect or inclined, 4 to 7.5 μ wide, bearing a single primary conidium; primary conidia springing off forcibly, often accumulating in whitish deposits visible to the naked eye, relatively long-lived, always colorless when young but when old sometimes yellowish, globose or more often turbinate with spherically rounded distal end and somewhat obconical proximal end, measuring 6 to 21 μ in greatest width and 13 to 32 μ in length inclusive of a basal papilla 4 to 7.5 μ wide and 2 to 6 μ high; slender conidiophores arising singly from globose primary or elongated secondary conidia, colorless, unbranched, 35 to 65 μ long, 1.5 to 3 μ wide at the base, tapering gradually upward, about 1 μ wide at the tip, there bearing a single secondary conidium; secondary conidia colorless, elongate-ellipsoidal, 10 to 18 μ long and 6 to 10 μ wide.

Isolated from decaying leaves of *Ulmus parvifolia* Jacq. collected near Greeley, Colorado.

The main hyphae at the margin of an actively growing mycelium of *Conidiobolus denaeosporus* commonly measure about 7 μ in width and usually terminate in a distal segment (Fig. 1, A) 125 to 250 μ long. As each distal segment pushes forward at the tip it successively cuts off shorter segments, mostly 25 to 100 μ long, at its proximal end. The hyphal segments thus delimited later often undergo changes in shape through emission of lateral branches. In Petri plate cultures of soft agar containing finely divided maize-meal the lateral branches may attain rather extensive

development, and are usually distinguished by conspicuous irregularity of outward form (Fig. 1, B, C). Within and around the larger deposits of maize-meal the branches may become closely interwoven into irregular masses of prosenchyma.

When *Conidiobolus denaeosporus* is well nourished it soon shows abundant asexual reproduction. The numerous phototropic conidiophores (Fig. 1, D–I) then formed, as also the globose or turbinate conidia (Fig. 1, J, a–w) springing off from them, more closely resemble in shape and dimensions the corresponding reproductive structures of *C. rugosus* Drechsler (1955) than those of any other known congeneric form. As might be inferred from the relative size of the basal papilla the conidium commonly springs off with at least moderate force; so that in many instances it falls on unoccupied substratum beyond the limits of the parent mycelium, and thus can readily germinate vegetatively to form a subsidiary mycelium. Accordingly in Petri plate cultures receiving light mainly from one side the fungus spreads more rapidly and disconnectedly toward the source of illumination than in other directions,

Among the numerous globose or turbinate conidia that are found strewn about thickly after asexual reproduction has been proceeding for several days, some scattered individuals are nearly always found giving rise on broad phototropic outgrowths to single conidia (Fig. 1, K–P) which in their turn spring off forcibly. This purely repetitional development begins early and continues for weeks, though usually it proceeds less abundantly than in related species. The emission from globose conidia of slender conidiophores (Fig. 1, Q–S) bearing ellipsoidal secondary conidia (Fig. 1, T, a–o) is not commonly observable in cultures much under 10 days old. Once initiated the latter type of sporulation usually continues for several weeks, even if, for the most part, only rather sparingly. All the globose or turbinate conidia that so far have been observed giving rise to elongated secondary conidia have been of relatively small size. Should some of the larger globose conidia participate in such sporulation the resulting elongated secondary conidia might be expected to exceed the dimen ions given in the diagnosis.

Multiplicative reproduction by the formation of microconidia extended plurally from globose conidia has not hitherto been observed in the several isolations of *Conidiobolus denaeosporus*. None of the isolations has shown any development of chlamydospores. In all series of cultures the several isolations have failed to show sexual reproduction, although cultures of *C. rugosus* on the same medium that were inoculated at the same time and kept at the same temperature consistently formed zygospores in large numbers.

It seems probable that *Conidiobolus denaeosporus* survives unfavorable periods mainly in its globose or turbinate conidia, as these appear considerably more durable than the homologous spores of most congeneric species. In young cultures they are commonly filled throughout with protoplasm that is partly of homogeneous and partly of granular texture (Fig. 1, J, a, f, l–o, q, t). After 60 days they usually show a conspicuous vacuole (Fig. 1, J, b-e, h–k), but their protoplasm, though of diminished volume, remains fully alive, often in Petri plate cultures, despite the presence of alien molds usually ruinous to the asexual spores of some other members of the genus. Similarly vacuolated living conidia (Fig. 1, J, p, s) are usually still abundant in tube cultures 4 months old. When conidia from a nearly dried maize-meal-agar culture 6 months old are moistened a large proportion of them promptly show unmistakably live protoplasm of lumpy texture in a parietal layer surrounding a large central vacuole (Fig. 1, J, u–w). The specific epithet compounded of two words (δηναιος, σπορος) meaning "long-lived" and "seed", respectively, is intended to signalize the rather exceptional endurance of the conidia.

A yellowish coloration often noticeable in vacuolated conidia from maize-meal-agar tube

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FIG. 1.—*Conidiobolus denaeosporus* as found in maize-meal-agar cultures; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1000. A, Terminal portion of hypha at forefront of a growing mycelium. B, C, Irregular hyphal segments near center of a mycelium in a culture 10 days old. D, Distal portion of phototropic conidiophore with conidium nearly ready to be completely delimited; s, surface of substratum. E–I, Phototropic conidiophores, each bearing a fully delimited conidium; s, surface of substratum. J, Detached globose conidia, a-w, from culture 4 days to 6 months old, showing usual variations in size, shape, and internal structure. K-P, Globose conidia, each with a stout phototropic outgrowth bearing another globose conidium. Q-S, Globose conidia that have each sent up a slender conidiophore bearing an elongated conidium. T, Fifteen elongated conidia, a-o, showing usual variations in size and shape. U, Indurated distal portion of a conidiophore found in culture 6 months old.

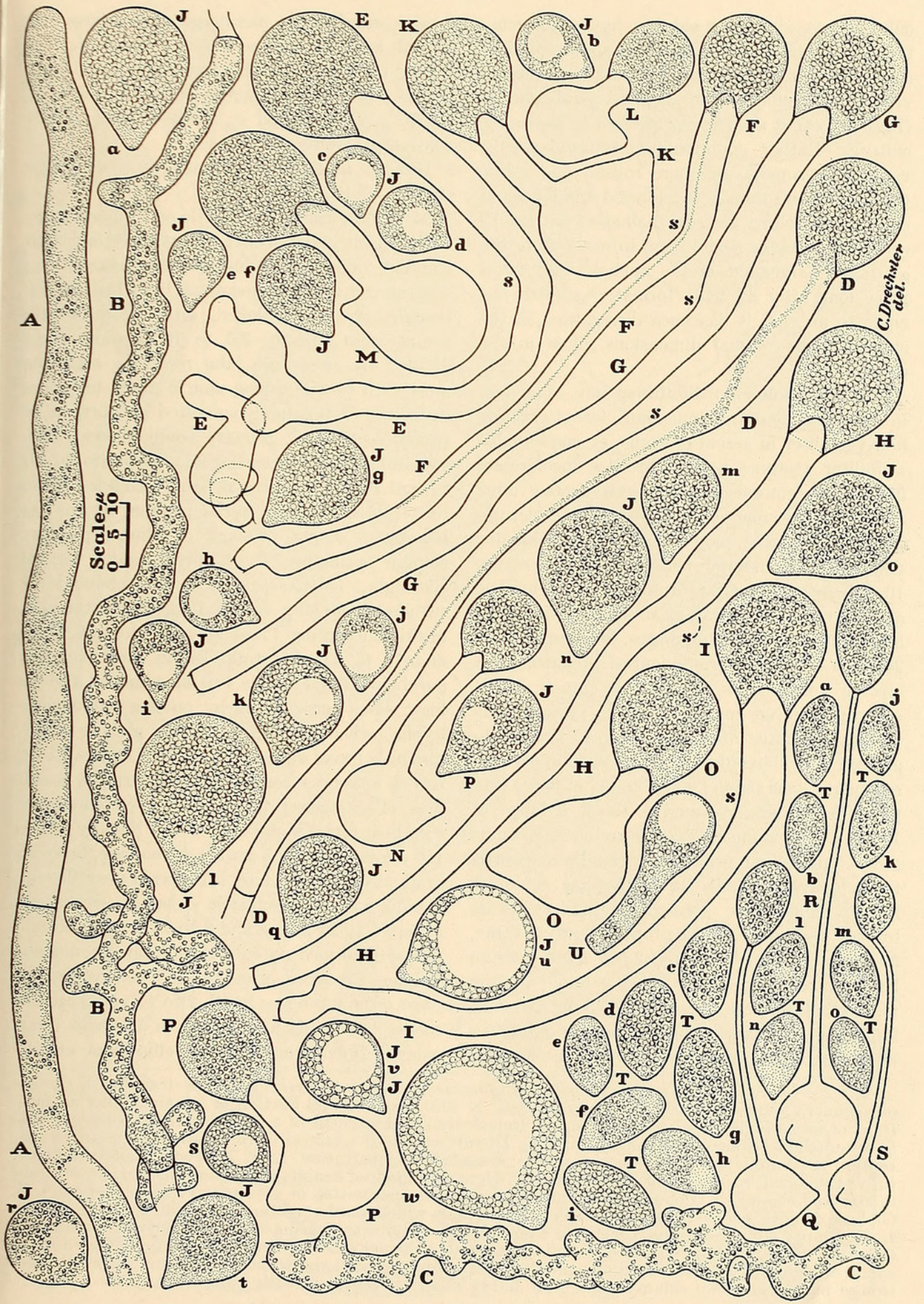

FIG. 1.—(See opposite page for explanation).

cultures several months old may have some relation to the durable character of the spores. Similar coloration permeates also the living segments of vegetative hyphae and of abortive conidiophores (Fig. 1, U) that are likewise present in the aging cultures. Rather commonly the pronouncedly vacuolated conidia are more rotund and wider (Fig. 1, J, u–w) than newly formed conidia, some of them exceeding 26 μ in diameter. As the increase in diameter would seem to result from the gradual enlargement of the vacuole, and takes place long after all truly formative growth has come to an end, it has been disregarded in the statement on conidial dimensions given in the diagnosis.

2. **Conidiobolus inordinatus** sp. nov. Mycelium in materia alimentaria immersum, fere oculo nudo non visibiliter in aerem crescens, et non conidiis visibiliter tectum; hyphae steriles incoloratae, filiformes, ramosae, nunc paene rectae nunc valde pravae, vulgo aliquid inordinatae, plerumque 2.5–10 μ latae, mox septatae, postea hic illic disjunctae vel inanitae, cellulae viventes eorum 10–250 μ longae; primiformibus fertiles hyphae aliquid rarae, incoloratae, simplices, ex cellulis hypharum singulatim surgentes, in aerem saepius 20–60 μ ad lucem protendentes, in parte aeria erectae vel acclives, hic 4–9 μ crassae, apice unum primiforme conidium ferentes; primiformia conidia violenter absilientia, parca, incolorata, globosa vel obovoidea vel turbinea, apice late rotundata, basi papilla 4–9 μ crassa et 3–6 μ alta praedita, ex toto plerumque 15–32 μ longa et 12–25 μ lata, nunc hypham germinationis emittentia nunc aliud primiforme conidium ferentia nunc in apice gracilis fertilis hyphae conidium formae ellipsoideae gignentia; graciles fertiles hyphae incoloratae, saepe prope apicem curvatae, 50–100 μ longae, basi circa 2.5 μ latae, sursum leniter attenuatae, apice 1–1.5 μ latae; conidia formae ellipsoideae incolorata, 15–26 μ longa, 9–13 μ lata; chlamydosporae in hyphis submersis et procumbentibus copiose et festinanter ortae, saepius inordinatae, plerumque intercalares vel aliquid laterales, cylindratae vel globosae vel elongato-ellipsoideae vel bisulcae vel aliquid trifidae, incoloratae, saepius 20–36 μ longae et 12–20 μ latae, vulgo ex parte magna protoplasmatis globuliferi repletae.

Habitat in foliis *Ribis* putrescentibus prope Greeley, Colorado.

Mycelium usually with little or no macroscopically visible aerial growth and not overlaid with conidial deposits visible to the naked eye; vegetative hyphae filamentous, branched, nearly straight or often crooked, often in disorderly arrangement, mostly 2.5 to 10 μ wide, early divided by cross-walls, the resulting segments mostly 10 to 250 μ long and in many instances disjoining or becoming separated by portions of empty membrane; primary condiophores colorless, unbranched, extending 20 to 60 μ into the air toward the main source of light, 4 to 9 μ wide in the erect or inclined aerial portion, at the tip bearing a single primary conidium; primary conidia springing off forcibly, usually formed sparingly, colorless, globose or obovoid or turbinate, broadly rounded at the distal end, mostly measuring 12 to 25 μ in greatest width and 15 to 32 μ in total length inclusive of a paraboloid basal papilla 4 to 9 μ wide and 3 to 6 μ high, some germinating by putting out a germ hypha, others producing another globose conidium on a broad outgrowth, and still others giving rise to a slender condidiophore bearing a conidium of elongated type; slender conidiophores colorless, often somewhat curved distally, 50 to 100 μ long, about 2.5 μ wide at the base, tapering gradually upward, 1 to 1.5 μ wide near the tip; conidia of elongated type colorless, ellipsoidal, often 15 to 26 μ long and 9 to 13 μ wide; chlamdospores formed on submerged and procumbent hyphae, produced promptly and very copiously, most often intercalary with 2 to 4 hyphal attachments but sometimes lateral or laterally intercalary, globose or elongated-ellipsoidal or cyl-

FIG. 2.—*Conidiobolus inordinatus* as found in maize-meal agar cultures; all parts drawn with the aid of a camera lucida at a uniform magnification; × 1000. A, Terminal portion of a main hypha at forefront of an actively growing mycelium. B, Relatively small segment of a lateral branch extended from an intercalary segment of a main hypha. C, Distal portion of a lateral branch with a pronounced distention. D, Young phototropic conidiophore; s, surface of substratum. E, F, Two conidiophores almost fully developed; s, surface of substratum. G, Detached globose conidia, a–z, showing usual variations in size and shape. H, Globose conidium germinating by production of a vegetative germ hypha. I–K, Globose conidia, each with a phototropic outgrowth on which another globose conidium is being formed. L, M, Empty globose conidia, each with a phototropic outgrowth bearing a new globose conidium ready to spring off. N, O, Empty globose conidia, each with a slender conidiophore bearing an elongated ellipsoidal conidium. P–R, Detached elongated conidia showing usual variations in size and shape. S, Portion of hypha with two chlamydospores, a and b, in a completed or nearly completed state.

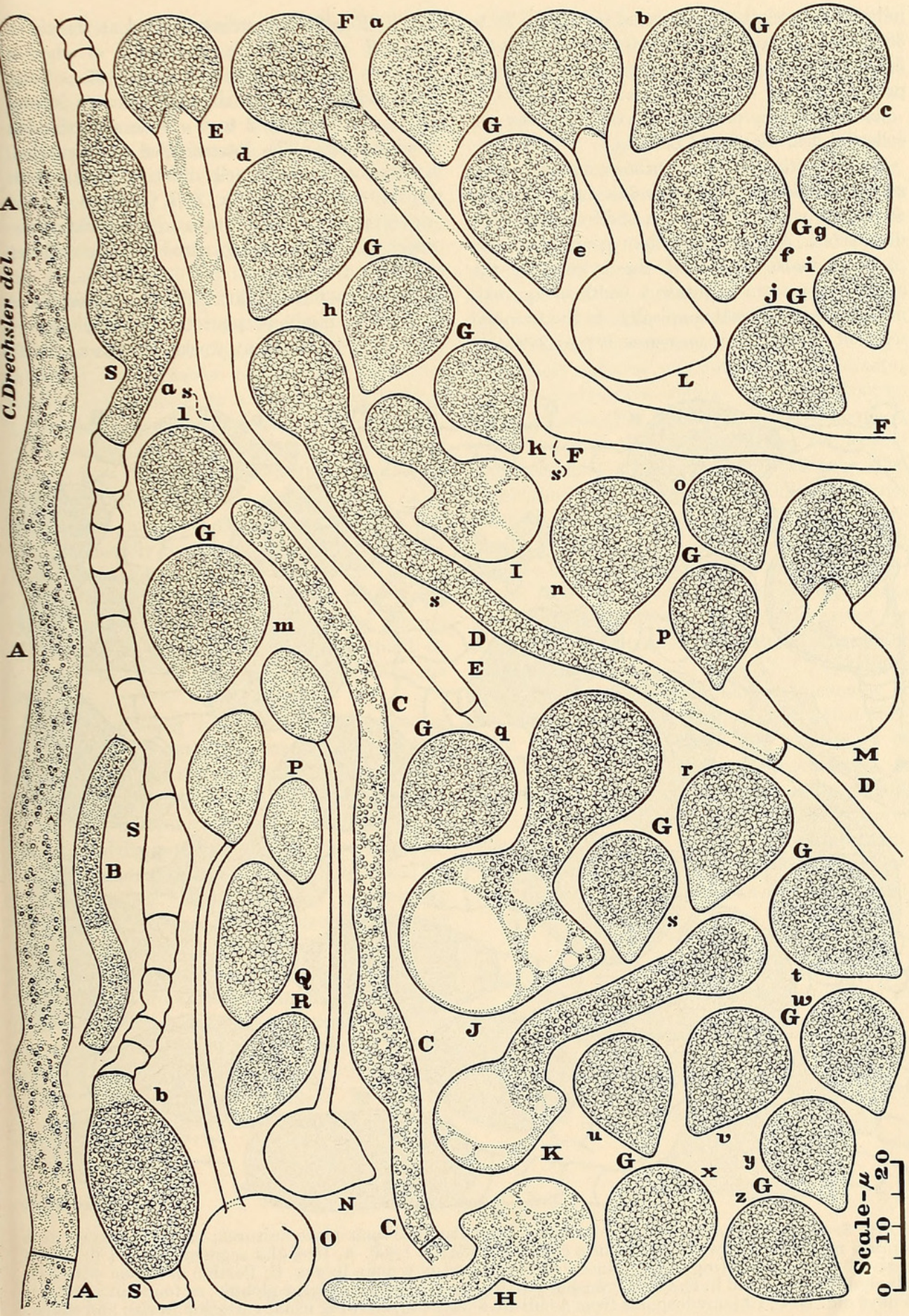

FIG. 2.—(See opposite page for explanation).

indrical or irregularly lobulated, colorless, 20 to 36 μ long, 12 to 20 μ in greatest width, usually fiilled in large part with strongly globuliferous protoplasm.

Isolated from decaying leaves of *Ribes* sp. collected near Greeley, Colorado.

In comparison with the other members of the genus, *Conidiobolus inordinatus* may like *C. denaeosporus* be considered a species of medium dimensions. When its mycelium grows in a Petri plate of maize-meal agar the hyphae at the advancing margin maintain a width of approximately 7 μ in elongating apically. As the terminal segment, which often measures 175 to 250 μ in length (Fig. 2, A), pushes forward at the tip it cuts off proximally a succession of shorter segments mostly between 25 and 125 μ long. From many of these shorter segments lateral branches commonly 3 to 6 μ wide are extended promiscuously. The intermingled ramifications, in which are included cells differing greatly with respect to size, shape (Fig. 2, B, C; Fig. 3, A) and orientation, contribute to a characteristic disorderly appearance signalized in the epithet applied to the species.

In maize-meal agar cultures *Conidiobolus inordinatus* produces phototropic conidiophores (Fig. 2, D–F) for many weeks, though as a rule

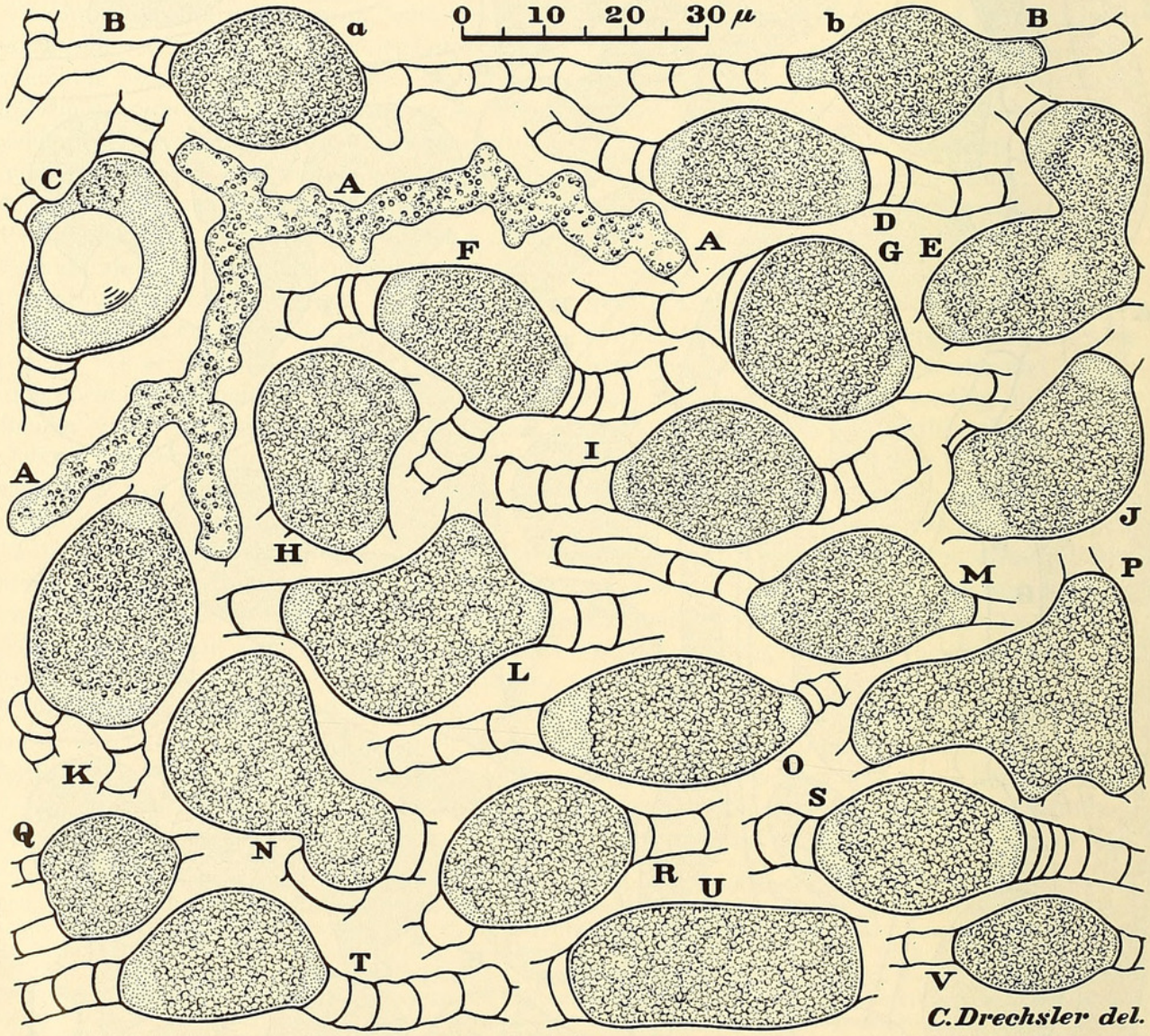

FIG. 3.—*Conidiobolus inordinatus* as found in maize-meal-agar plate cultures; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1,000. A, Irregular segment formed in an extensive branch originating from an intercalary segment of a main hypha. B, Portion of hypha with two chlamydospores, a and b. C. Chlamydospore with large vacuole, or reserve globule, as found in a culture 55 days old. D–V, Chlamydospores from a culture 8 days old, showing usual variation in size and shape.

only rather sparingly. Its conidia (Fig. 2, G, a–z) never were found in such abundance as to be visible collectively to the naked eye. Under a microscope they commonly appear scattered somewhat sparsely over the substratum. On fresh unoccupied substratum the detached conidium may germinate by putting forth a vegetative germ hypha (Fig. 2, H). Often, however, on fresh unoccupied substratum, and also very commonly on substratum already overgrown by the fungus, the detached conidium puts forth a broad phototropic outgrowth (Fig. 2, I–K) on which another globose conidium is formed (Fig. 2, L, M). Cultures well over 10 days old usually show some relatively small globose conidia that have germinated by sending up a slender conidiophore supporting an elongated conidium (Fig. 2, N, O). As in related species the elongated ellipsoidal conidia here (Fig. 2, P–R) becomes detached on slight disturbance. They usually show more pronounced curvature on one side than on the other. Generally they reveal a somewhat oblique basal hilum that is nearly in alignment with the contour of the more strongly curved side. If the larger globose conidia were to serve as parents the elongated conidia then produced could be expected to exceed the dimensions given in the diagnosis.

The outstanding feature of *Conidiobolus inordinatus* is its early and remarkably copious production of chlamydospores (Fig. 2, S; Fig. 3, B-V). Even in cultures only a few days old its disorderly mycelium becomes so abundantly septate and discontinuous that on casual examination the fungus would not ordinarily be taken for a phycomycete. Local swellings representing early stages in chlamydospore development can often be recognized in hyphal segments only 2 to 3 millimeters from the forefront of a growing mycelium. As protoplasmic materials are gathered into the swellings the adjacent portions of hypha are progressively evacuated of contents. Retaining walls are laid down, one after another, at successive stages of this evacuation. These later appear as convex partitions in the emptied portions of tubular membrane, often 50 to 100 μ in length, between neighboring chlamydospores (Fig. 2, S, a, b; Fig. 3, B, a, b). Since in many instances chlamydospores are produced in positions where a hypha gives off 1 or 2 branches it often is supplied from 3 (Fig. 3, C, E, F, H, J–L, Q) and sometimes is supplied from 4 hyphal arms (Fig. 3, P). On attaining their definitive condition the reproductive bodies with the more numerous attachments are especially distinguished by irregularity of shape. Promiscuous variations in outward form are, however, by no means infrequent among mesially intercalary chlamydospores with 2 hyphal attachments.

In most cultures of *Conidiobolus inordinatus* the chlamydospores contain numerous globules about 1 μ wide (Fig. 3, B, a, b; D–V), although in many instances the protoplasm near the hyphal attachments is of homogeneous consistency. The resemblance in texture of cellular contents provides good reason to hold the species more closely related to *C. globuliferus* Drechsler (1956) than to any other congeneric form. Chlamydospores in some old cultures show a single large globule surrounded by a layer which, except for a small quantity of granular material, appears of homogeneous consistency (Fig. 3, C). As these chlamydospores reveal no marked thickening of the enveloping wall it seems unlikely that they have undergone conversion into zygospores or azygospores. Rather similar reorganization of contents has been observed at times in the globose and the elongated conidia of *C. heterosporus* Drechsler (1953). The strongly vacuolated condition of aging conidia of *C. denaeosporus* may denote similar though less pronounced modification.

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The history of a science is the science itself.—GOETHE.