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THREE NEW SPECIES OF CONIDIOBOLUS ISOLATED FROM DECAYING PLANT DETRITUS

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THREE NEW SPECIES OF CONIDIOBOLUS ISOLATED FROM DECAYING PLANT DETRITUS[1]

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IN CONTINUATION of studies reported in recent papers (Drechsler, 1953a, b; 1954), three readily culturable entomophthoraceous fungi are herein described as new species of *Conidiobolus*. I obtained the fungi by canopying maize-meal agar plates with small quantities of plant detritus collected by me in different localities early in 1954. Specimens of the three species have been deposited in the National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

1. CONIDIOBOLUS **rugosus** Drechsler sp. nov.

Mycelium incoloratum, aliquid inconspicuum; hyphis sterilibus mediocriter ramosis, 2.5–11μ crassis, mox septatis, postea hic illic disjunctis vel inanitis, in cellulis 30–200μ longis constantibus; primiformibus fertilibus hyphis simplicibus, in aerem saepe 20–45μ ad lucem protendentibus, in parte aeria 3.5–6μ crassis, apice unum primiforme conidium ferentibus; primiformibus conidiis violenter absilientibus, incoloratis, aliquid globosis, basi papilla 2.5–6μ alta et 3.5–7μ lata praeditis, plerumque ex toto 14–25μ longis, 9–20μ latis; gracilibus fertilibus hyphis singulatim ex primiformibus vel secundariis conidiis surgentibus, incoloratis, saepius 30–75μ longis, basi 2–3μ crassis, sursum leniter attenuatis, apice circa 1μ crassis, ibi unum secundarium conidium ferentibus; secundariis conidiis incoloratis, elongato-ellipsoideis, plerumque 17–27μ longis, 9–18μ latis; zygosporis interdum in singulis hyphis ortis interdum e copulatione cellularum aliae atque aliae hyphae oriundis, flavidis, globosis vel elongato-ellipsoideis, vulgo valde rugosis, plerumque 14–25μ longis, 13–22μ latis, muro simplici vel duplici 1.2–3μ crasso circumdatis.

Habitat in materiis plantarum putrescentibus in Palatka, Florida.

Mycelium colorless, somewhat inconspicuous; assimilative hyphae moderately branched, 2.5–11μ wide, soon becoming septate, in some places later becoming disjointed or evacuated and thus undergoing division into segments 30–200μ long; primary conidiophores unbranched, extending 20–45μ into the air and toward the main source of light, measuring 3.5–6μ in width near the tip, bearing terminally a single primary conidium; primary conidia springing off forcibly, colorless, somewhat globose, 9–20μ wide, measuring 14–25μ in length inclusive of a somewhat conical basal papilla 2.5–6μ high and 3.5–7μ wide; slender secondary conidiophores arising singly from primary or secondary conidia, colorless, mostly 30–75μ long, 2–3μ wide at the base, tapering gradually to a width of 1μ at the tip whereon is borne a single secondary conidium; secondary conidia colorless, elongate-ellipsoidal, commonly 17–27μ long, 9–18μ wide; zygospores sometimes formed through union of segments in the same hypha and sometimes through conjugation of segments belonging to separate hyphae, commonly yellowish, globose or elongate-ellipsoidal, boldly wrinkled, mostly 14–25μ long, 13–22μ wide, surrounded by a wall 1.2–3μ thick that commonly shows a smooth inner layer and an undulated outer layer.

Isolated from decaying plant detritus collected in Palatka, Florida, on January 1, 1954.

Conidiobolus rugosus grows well on maize-meal agar and displays vigorous asexual and sexual reproduction. The robust axial hyphae at the forefront of an expanding mycelium commonly measure 7–10μ in width. In many hyphae the elongating terminal segment (fig. 1) reaches a length of approximately 200μ before it undergoes division in cutting off a new penultimate segment usually between 50 and 125μ long. The segments delimited successively in penultimate position may retain their original dimensions (fig. 2; 3, a, b), or may become shortened through withdrawal of contents from the proximal end when protoplasm is required for emission of a lateral branch. As a rule lateral branches are markedly narrower than the parent hypha. Old cultures in which obviously neither lateral branches nor germ hyphae can find fresh unoccupied substratum usually contain many hyphal segments scarcely 3μ wide (fig. 4).

In young vigorous mycelia procumbent as well as submerged hyphal segments give rise to asexual reproductive apparatus of the primary type. A segment on the agar surface puts forth a phototropic conidiophore directly into the air (fig. 5). The submerged segment extends a branch which pushes its way upward to the surface (fig. 6, s) and then likewise grows into the air to produce a globose conidium terminally. Soon after the conidium has been completely delimited by a conical partition (fig. 6) it springs off forcibly through sudden eversion of its basal membrane. The relatively narrow but strongly protuberant papilla resulting from this eversion gives the discharged conidium (fig. 7–22) a rather distinctive longitudinal profile. After falling on a moist substratum some conidia germinate by emitting a vegetative hypha (fig. 23), while others give rise individually to a short stout conidiophore (fig. 24, 25) bearing another globose conidium (fig. 26) that soon springs off forcibly like its parent. Here and there a globose conidium puts forth a slender conidiophore bearing an ellipsoidal conidium (fig. 27), and this may produce another slender conidiophore with an ellipsoidal conidium at its tip (fig. 28). Ellipsoidal conidia are not shot

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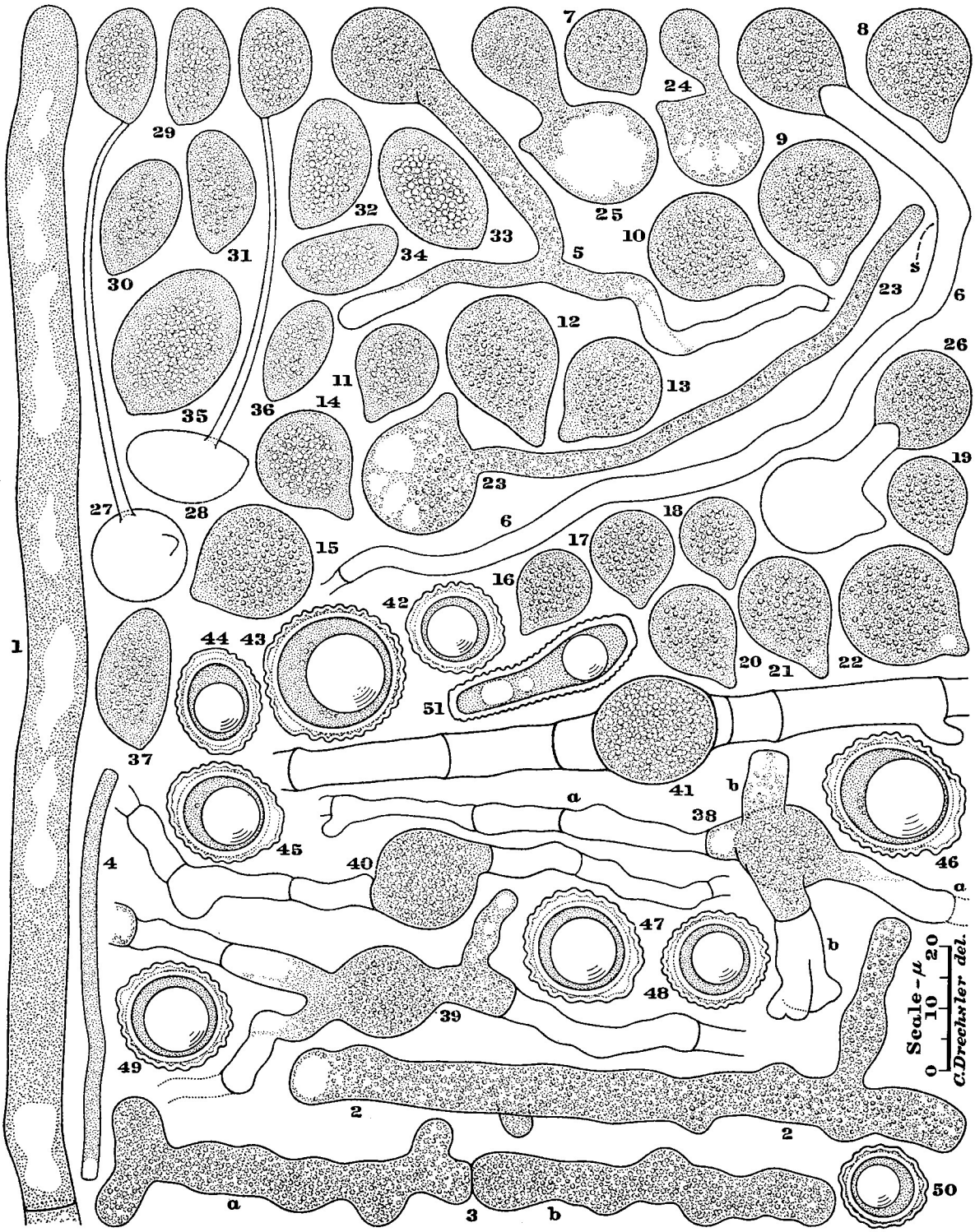

Fig. 1-51. *Conidiobolus rugosus* as found in maize-meal agar cultures; ×1000.—Fig. 1. Terminal segment of an axial hypha at forefront of a growing mycelium.—Fig. 2. Intercalary hyphal segment.—Fig. 3. Two adjacent intercalary segments, a and b, of about medium size.—Fig. 4. Small hyphal segment from a culture six months old.—Fig. 5. Procumbent hyphal segment from which a conidiophore has been extended.—Fig. 6. Distal portion of conidiophore that on reaching surface, *s*, of culture has elongated toward source of light and formed a globose conidium.—Fig. 7–22. Globose conidia showing variations in size and shape.—Fig. 23. Globose conidium germinating by a narrow germ hypha.—Fig. 24, 25.

off forcibly but become detached (fig. 29–37) on slight disturbance.

Sexual reproduction in *Conidiobolus rugosus* may often be observed less than 200–300μ from the forefront of an actively growing mycelium, and must therefore take place freely among hyphal segments only a few hours old. Where conjugation occurs between segments of separate hyphae (fig. 38, a, b) cell fusion is more clearly evident than in the more numerous instances where development is wholly confined to one hypha (fig. 39–41) and where consequently during some stages it gives nearly the same appearance as chlamydospore formation. The mature zygospore (fig. 42–51) with its pure yellow coloration and undulate profile is a strikingly handsome body by which the species can readily be distinguished from all congeneric fungi hitherto described. Despite its smaller size it is more boldly sculptured than the zygospores of my *C. rhysosporus* and my *C. osmodes* (Drechsler, 1954). Its prominent ridges seem connected into a net-like pattern, whereas the minute ridges in *C. rhysosporus* and *C. osmodes* seem generally parallel to one another, all appearing to encircle the zygospore in planes nearly perpendicular to its longitudinal axis.

2. CONIDIOBOLUS **nanodes** Drechsler sp. nov.

Mycelium incoloratum, aliquid inconspicuum; hyphis sterilibus mediocriter ramosis, plerumque 2–7μ crassis, mox septatis, postea hic illic disjunctis vel inanitis, in cellulis 25–150μ longis constantibus; hyphis fertilibus simplicibus, in aerem 10–25μ ad lucem protendentibus, in parte aeria vulgo 3.5–6μ crassis, apice unum condidium ferentibus; conidiis violenter absilientibus, incoloratis, aliquid globosis vel obovoideis, basi papilla 1–2μ alta et 3–5μ lata praeditis, ex toto plerumque 10–17μ longis, 8–14μ latis; zygosporis interdum in singulis hyphis ortis interdum ex conjunctione cellularum aliae atque aliae hyphae oriundis, incoloratis vel flavidulis, vulgo globosis, 7–14μ in diametro, muro levi 0.8–2μ crasso circumdatis.

Habitat in foliis arborum putrescentibus prope Moorestown, New Jersey.

Mycelium colorless, usually somewhat inconspicuous; assimilative hyphae moderately branched, mostly 2–7μ wide, soon becoming partitioned by cross-walls, later in some places becoming disjointed or evacuated, thus undergoing division into segments 25–150μ long; conidiophores unbranched, extending 10–25μ into the air and toward the main source of light, commonly 3.5–6μ wide in the aerial portion, bearing terminally a single conidium; conidia springing off forcibly, colorless, globose or somewhat obovoid, 8–14μ wide, measuring 10–17μ in length inclusive of a basal papilla 1–2μ high and 3–5μ wide; zygospores sometimes formed within one hypha and sometimes arising through conjugation between segments in separate hyphae, colorless or pale yellowish, commonly globose, 7–14μ in diameter, surrounded by a smooth wall 0.8–2μ thick.

Isolated from decaying leaves collected in deciduous woods near Moorestown, New Jersey, on February 25, 1954.

In a mycelium of *Conidiobolus nanodes* that is growing unimpeded on fresh maize-meal agar the axial hyphae at the advancing margin commonly measure about 5μ in width. The terminal segment (fig. 52) in such hyphae may repeatedly regain a length of approximately 150μ after being shortened again and again through delimitation of successive segments at its proximal end. The segments thus cut off one after another become shortened in many instances through withdrawal of all protoplasmic contents from a basal portion of variable size, and subsequently are found connected with their neighbors only by empty hyphal membrane (fig. 52).

Within a few hours after it was formed an individual hyphal segment may expend all its protoplasm in giving rise to a phototropic conidiophore (fig. 53–56) bearing a globose conidium (fig. 57–60). The conidia spring off through eversion of the concave basal wall, though, as might be surmised from the usually rather small size of the papilla present on each of the scattered spores (fig. 61–81), they fly only relatively short distances. After falling on a moist agar surface a conidium may put forth a vegetative germ hypha (fig. 82), or may send up a short stout conidiophore (fig. 83–86) on which another globose conidium (fig. 87) is produced. Secondary conidia of the ellipsoidal type have not been observed in the fungus.

Sexual reproduction proceeds concurrently with asexual reproduction in maize-meal agar cultures of *Conidiobolus nanodes*. Conjugation would seem to take place about as frequently between segments belonging to separate hyphae (fig. 88–90: a, b) as between segments of the same hypha (fig. 91–94). In many instances segments of the same hypha that have become spatially separated from one another (fig. 91, 92) are found conjugating by means of a lateral connection. During the earlier stages in the growth of a zygospore its contents usually change little in texture (fig. 90, c; 91, a) but during later stages (fig. 88–89: c; 92–94: a) the protoplasmic materials begin to show coarsely lumpy or globulose structure. After some further internal reorganization a large reserve globule is found sur-

Globose conidia from each of which another globose conidium is developing.—Fig. 26. Globose conidium that has produced a secondary globose conidium.—Fig. 27. Globose conidium that has sent up a slender conidiophore bearing an elongated conidium.—Fig. 28. Elongated conidium that has sent up a slender conidiophore bearing another elongated conidium.—Fig. 29–37. Detached elongated conidia showing variations in size and shape.—Fig. 38. Late stage in conjugation between segments of two separate hyphae, a and b.—Fig. 39. Late stage in conjugation between adjacent segments of the same hypha.—Fig. 40, 41. Young zygospores.—Fig. 42–51. Mature zygospores showing variations in size and shape.

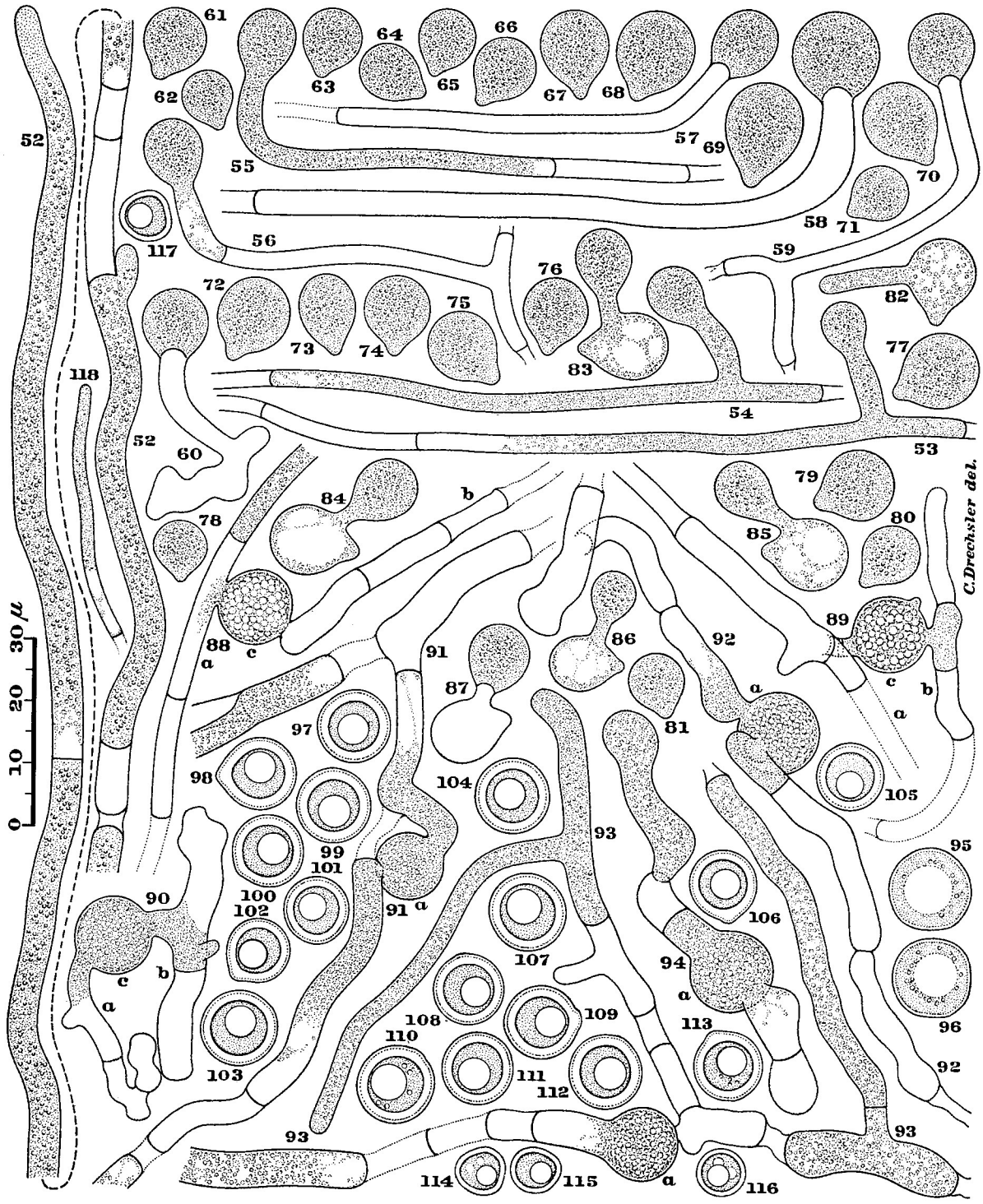

Fig. 52–118. *Conidiobolus nanodes* as found in maize-meal agar cultures; ×1000.—Fig. 52. Terminal portion of hypha at forefront of a growing mycelium; drawn in two parts whose proper connection is indicated by a broken line.—Fig. 53–56. Procumbent hyphal segments that are each giving rise to a conidiophore.—Fig. 57–60. Procumbent segments that have each sent up a conidiophore bearing a conidium ready to spring off.—Fig. 61–81. Conidia showing variations in size and shape.—Fig. 82. Conidium with germ tube.—Fig. 83–86. Conidia that are each giving rise to another conidium.—Fig. 87. Conidium that has produced a secondary conidium now ready to spring off.—Fig. 88–90. Pairs of segments in separate hyphae, a and b, conjugating to form young zygospore, c.—Fig. 91–94. Pairs of segments in individual hyphae conjugating to form young zygospores, a.—Fig. 95–96. Full-grown but immature zygospores.—Fig. 97–113. Mature zygospores from Petri-plate cultures 3–8 days old.—Fig. 114–117. Small mature zygospores from culture 27 days old.—Fig. 118. Small hyphal segment in culture 27 days old.

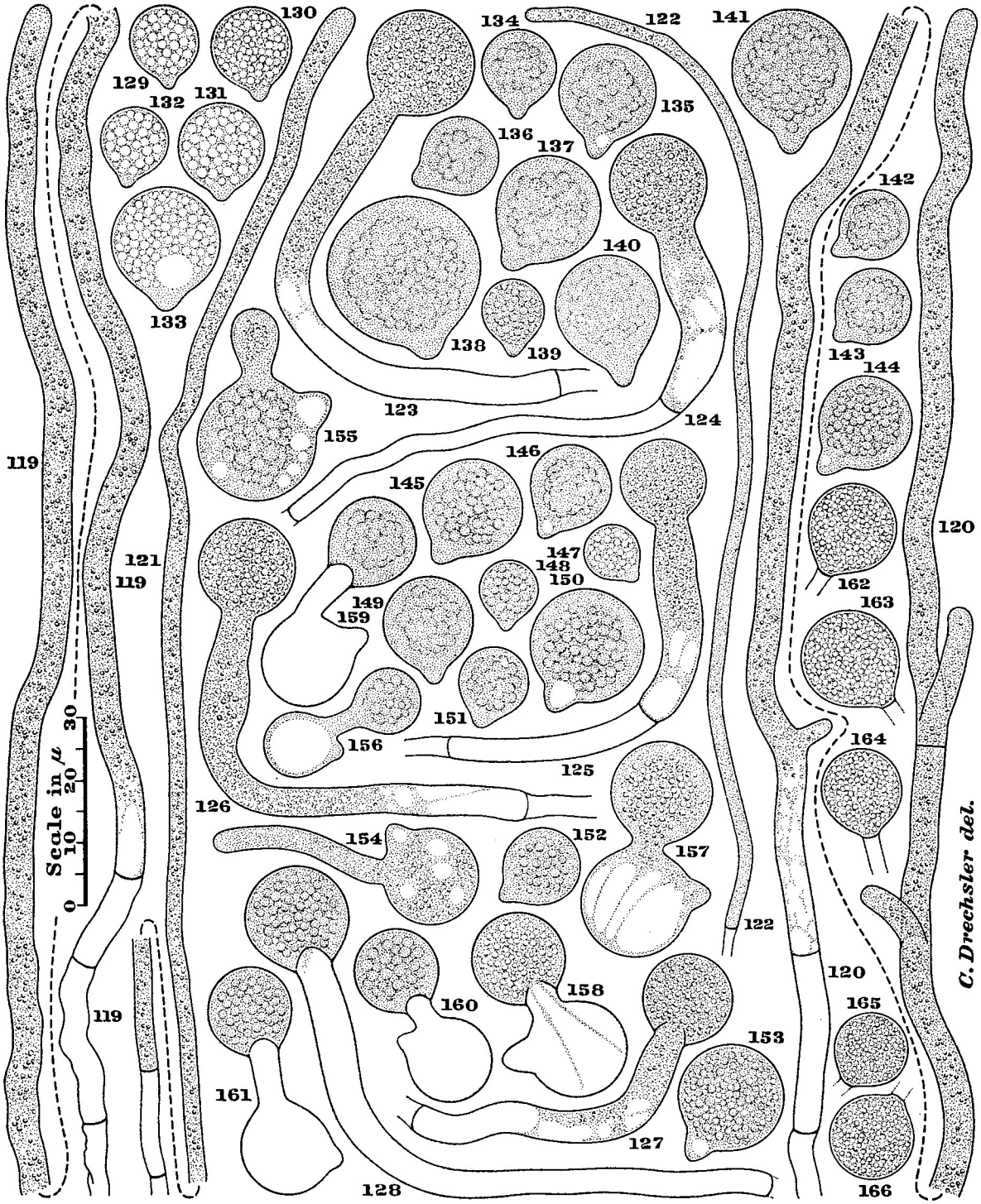

Fig. 119–166. *Conidiobolus lachnodes* as found in maize-meal agar cultures; ×1000.—Fig. 119, 120. Terminal portions of assimilative hyphae at forefront of a growing mycelium, each shown in 2 parts whose proper connection is indicated by broken line.—Fig. 121, 122. Terminal portions of aerial hyphae.—Fig. 123–127. Distal portions of conidiophores with conidia in different stages of development.—Fig. 128. Distal portion of conidiophore with conidium ready to spring off. Fig. 129–153. Conidia showing variations in size and shape.—Fig. 154. Conidium with a germ hypha.—Fig. 155–158. Four conidia, each giving rise to another conidium.—Fig. 159–161. Three conidia, each with a secondary conidium ready to spring off.—Fig. 162–166. Chlamydospores from Petri-plate culture 27 days old.

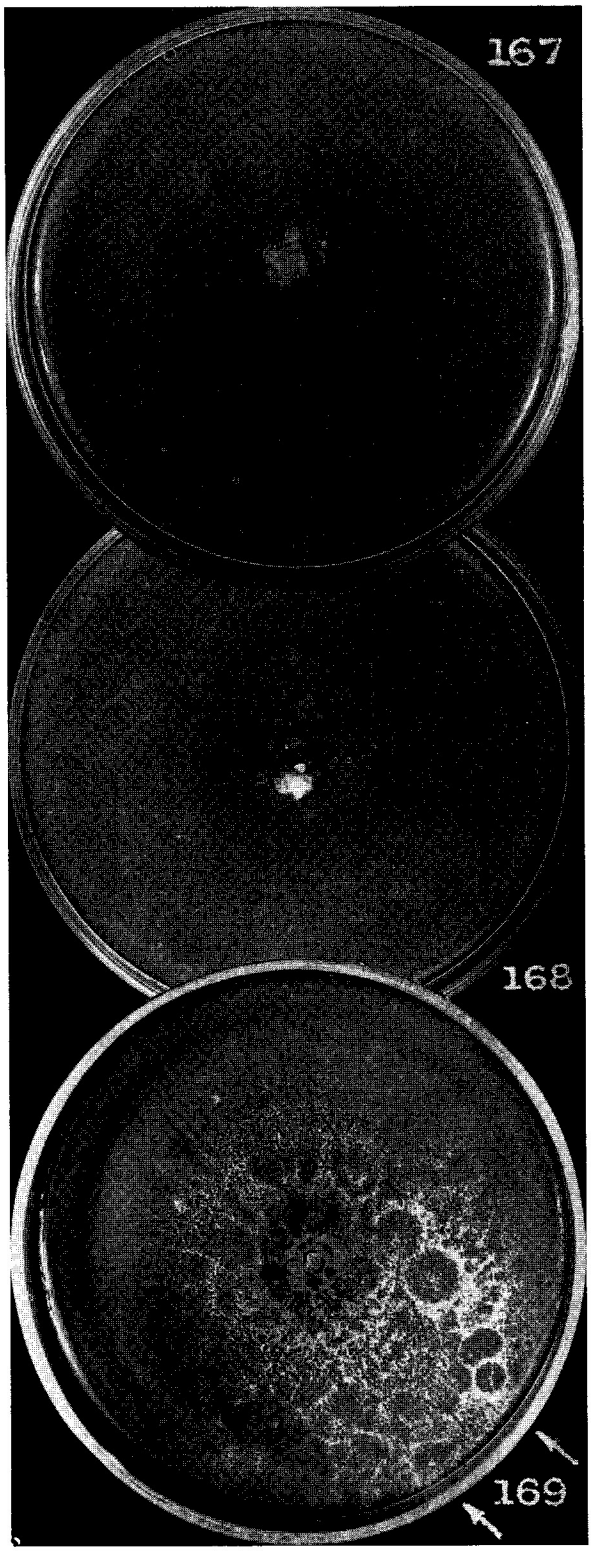

Fig. 167–169. Petri-plate cultures of maize-meal agar showing three species of *Conidiobolus* grown for 9 days at temperatures near 26°C.; ×¾.—Fig. 167. *Conidiobolus rugosus*.—Fig. 168. *Conidiobolus nanodes*.—Fig. 169. *Conidiobolus lachnodes*.—the direction of incident light rays is shown by arrows.

rounded by a parietal layer of nearly homogeneous texture (fig. 95, 96). Mature zygospores produced in a young culture (fig. 97–113) commonly measure about 11–12μ in diameter, and are surrounded by a smooth wall approximately 1.8μ thick. In old cultures smaller zygospores of somewhat angular shape (fig. 114–117) can usually be observed. These apparently result from fusion of rather minute hyphal segments (fig. 118) generally present in aging material.

Even in young cultures, however, the fungus is distinguished from all known congeners by the small dimensions of its reproductive structures. Furthermore, its mycelial hyphae are more delicate than those of any other segmented species hitherto referred to *Conidiobolus*, though slightly coarser than the unsegmented hyphae of the anomalous *C. adiaeretus* Drechsler (1953a). Its specific name—from *νανωδης*, meaning "dwarf-like"—is intended to bring its generally small size into appropriate relief.

3. CONIDIOBOLUS **lachnodes** Drechsler sp. nov.

Mycelium non modo in materiis sed etiam in aerem crescens, saepe lineariter vel ad instar retis albi floccosi surgens, itaque interdum conspicuum; hyphis sterilibus 2–8μ latis, mox septatis, postea hic illic disjunctis vel inanitis, in cellulis 50–350μ longis constantibus; hyphis fertilibus incoloratis, simplicibus, in aerem 15–40μ ad lucem protendentibus, in parte aeria saepius aliquid pravis et irregulariter inflatis, ibi 5–10μ latis, apice unum conidium ferentibus; conidiis violenter absilientibus, incoloratis, globosis sed basi papilla 1–4.5μ alta et 2.5–7μ lata praeditis, ex toto 10–27μ longis, 9–25μ latis; chlamydosporis vulgo in apice hyphis procumbentibus tarde oriundis, incoloratis, globosis, plerumque 11–16μ in diametro.

Habitat in materiis plantarum putrescentibus in Jacksonville, Florida.

Mycelium developing not only in and on the substratum but also producing conspicuous floccose aerial wefts which commonly show an irregularly reticulate design; vegetative hyphae 2–8μ wide, soon becoming partitioned by cross-walls, in some places becoming disjointed or emptied, thus undergoing division into segments 50–350μ long; conidiophores colorless, unbranched, extending 15–40μ toward the main source of light, the aerial part in some instances slightly crooked and somewhat irregularly distended to widths of 5–10μ, bearing terminally a single conidium; conidia springing off forcibly, colorless, globose, measuring 9–25μ in greatest width and 10–27μ in total length inclusive of a basal papilla 1–4.5μ high and 2.5–7μ wide; chlamydospores colorless, globose, 11–16μ in diameter, usually formed terminally on procumbent hyphae.

Isolated from decaying plant detritus collected in Jacksonville, Florida, on January 1, 1954.

Because of the generally inconspicuous character of the mycelium produced on agar substrata by most isolations of *Conidiobolus* it is not usually possible to recognize the separate species of the

genus by their macroscopic appearance in pure culture. Thus, although the maize-meal-agar plate cultures of *C. rugosus* and *C. nanodes* in fig. 167 and 168, respectively, showed under the microscope vigorous mycelium with abundant asexual reproductive apparatus and very numerous zygospores, the two fungi were only rather faintly visible to the naked eye. In producing aerial mycelium in sufficient quantity to arrest attention, *C. lachnodes* (fig. 169) differs markedly from all congeneric forms observed or described in recent times, including *C. brefeldianus* Couch (1939); and, indeed, for like reason it may differ also from *C. utriculosus* Brefeld (1884) since in the detailed account of that much more robust species no mention is made of aerial structures other than conidiophores and conidia. The specific epithet given to the present fungus—from λαχνωδης, meaning "woolly" or "downy"—is intended to signalize its macroscopic aspect.

The submerged and the procumbent hyphae at the margin of a vigorously growing mycelium of *Conidiobolus lachnodes* vary commonly from 4 to 7μ in width. In many instances the terminal segment is more than 300μ long (fig. 119), but in other instances, especially where it has undergone a recent division, its length may not greatly exceed 100μ (fig. 120). Aerial hyphae (fig. 121, 122) mostly range in width from 2.5 to 4μ. They generally arise in somewhat close arrangement without, however, being compacted into felty masses.

Asexual reproduction does not usually take place abundantly in maize-meal-agar cultures of *Conidiobolus lachnodes* at temperatures below 20°C. Temperatures between 25° and 30°C. appear more favorable for development of conidiophores and conidia. Such development would seem promoted also by exposure to somewhat brighter light than ordinarily reaches the secluded nooks preferred in many laboratories for safe storage of Petri plate cultures. The conidiophores (fig. 123–128), as in all congeneric species except *C. adiaeretus*, arise singly from individual segments of procumbent or of somewhat submerged hyphae. The phototropic aerial termination may be little modified in outward form (fig. 123), or may show rather pronounced distention (fig. 124). After the conidium has received all the available protoplasm it becomes completely delimited by a moderately protuberant hemispherical partition (fig. 128). Through sudden eversion of its basal membrane the conidium in many instances obviously springs off forcibly enough to fall on unoccupied substratum beyond the forefront of the mycelium on the side nearest the main source of light, for when actively producing conidia the fungus spreads faster toward the light than in other directions (fig. 169). Newly detached conidia (fig. 129–153) commonly show numerous lumps in somewhat close arrangement within a nearly homogeneous parietal layer. Many of them soon germinate by putting forth a vegetative germ hypha (fig. 154), while others give rise individually to a short conidiophore (fig. 155–158) on which is formed another globose conidium (fig. 159–161). Secondary conidia of the elongate type have not been seen in any material of the fungus.

Some few chlamydospores (fig. 162–166) can usually be found in maize-meal agar cultures of *Conidiobolus lachnodes* 10–15 days after inoculation. They usually increase appreciably in numbers during an ensuing period of 20 to 30 days. In the main they would seem to correspond closely to the chlamydospores of *C. adiaeretus*.

SUMMARY

Three readily culturable entomophthoraceous fungi isolated from decaying plant detritus by the use of canopied agar plates are described as new species of *Conidiobolus*. *C. rugosus* obtained from northeastern Florida is notable mainly because of the boldly undulate sculpturing of its zygospores. *C. lachnodes*, likewise isolated from Florida material, differs from congeneric forms in producing aerial mycelium in readily visible quantity. *C. nanodes*, which was obtained from leaf mold collected in central New Jersey, is distinguished by relatively small dimensions.

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