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AN ODOROUS BASIDIOBOLUS OFTEN PRODUCING CONIDIA PLURALLY AND FORMING SOME DICLINOUS SEXUAL APPARATUS[1]

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A B S T R A C T

A coarse fungus isolated from decaying plant detritus originating in northern Wisconsin and western Maryland is newly described as *Basidiobolus* **magnus**. Like *B. ranarum*, it gives off an odor recalling benzene hexachloride. Its mycelium, especially while young, includes many long segments markedly wider in the middle than at the ends. Its distally phototropic conidiophores sometimes are branched in their procumbent or submerged portions, and each propulsive distention may bear 1–3 globose conidia, which may be shot off forcibly or may fall through collapse of the supporting hypha. The primary globose conidia of *B. magnus* are generally larger than those of *B. ranarum*, from which they differ further in sometimes giving rise to plural secondary conidia of either the globose or the elongated adhesive type. The zygospores, like those of *B. ranarum*, show abundantly undulated sculpture but differ in their generally larger size, their occasional origin through conjugation between cells of separate hyphae, their rather frequent binary development, and their germination by production, often, of 2 globose uninucleated conidia. The elongated conidia, which seem generally no bigger than those of *B. ranarum* and are often only meagerly tipped with adhesive material, sometimes undergo conversion into sporangia through internal segmentation.

IN EARLIER studies *Basidiobolus ranarum* Eidam (1886) was found readily distinguishable by the undulate sculpture of its zygospores not only from *B. meristosporus* Drechsler (1955) and *B. haptosporus* Drechsler (1956) but also from a congeneric fungus (Drechsler, 1958) isolated from a human patient in Indonesia (Emmons et al., 1957). From the 3 congeners mentioned and from *B. microsporus* Benjamin (1962), it is promptly distinguishable also by the production of a *Streptomyces*-like odor resembling the odor associated with benzene hexachloride. Nearly all strongly odorous *Basidiobolus* cultures I have isolated, whether from decaying plant materials or from frog excrement, soon produce zygospores of undulate profile which in their dimension show agreement with Eidam's account of *B. ranarum*—an agreement sustained by close correspondence with respect to morphology of mycelial hyphae, of phototropic conidiophores, and of globose conidia. However, a few odorous cultures with zygospores of undulate profile have been isolated that in several respects disagree so markedly with Eidam's description and diverge so widely from the many cultures held properly referable to Eidam's species as to preclude their assignment to *B. ranarum*.

MATERIALS AND METHODS—Divergence from the familiar developmental habit of *Basidiobolus ranarum* was observed in an odorous culture obtained by canopying a Petri plate of maize-meal agar with a small quantity of decaying plant detritus taken from marshy ground in northern Wisconsin on November 18, 1954. The mycelium invited attention because of its slow growth and unusually coarse hyphae. It gave rise to no conidiophores or conidia, but formed in moderate number large zygospores of undulate sculpture. Asexual reproduction again failed to ensue when the fungus was transferred to several slanted tubes of maize-meal agar containing some finely divided maize-meal in suspension, and again sexual reproduction took place, so that after 20 days the substratum was amply seeded with zygospores of correct internal structure. The several tubes were then stored at room temperature for use eventually in longevity tests. No measures were taken to reduce evaporation from the agar substratum, which accordingly dried to a horny consistency in a few months. In March 1963, when the cultures were more than 8 years old, 5 ml of sterile distilled water were added to each of them, with proper precautions being taken to avoid contamination by alien microorganisms. Within 10 days, renewed development was observable in each culture. Emission of many germ hyphae gave proof that a large proportion of the zygospores had survived. Active production of phototropic conidiophores, of globose conidia, and later of elongated adhesive conidia, indicated that the softened watery substratum furnished conditions more favorable for growth and asexual reproduction of the fungus than had been provided earlier by the unwet, firm culture medium newly prepared with 15 g of agar to the liter.

Three additional isolates of the fungus were obtained from Petri plates of soft maize-meal agar that had been canopied with plant detritus

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sifted from leaf mold collected on September 1, 1963, in woods bordering the old Chesapeake and Ohio Canal near Cumberland, Maryland. Early production of 1–3 conidia on phototropic conidiophores permitted identification of the 3 separate parent mycelial growths soon after they became visible to the naked eye, and at the same time provided good evidence that formation of plural conidia was here a normal character, not a feature resulting from prolonged subsistence on artificial substratum. When the 3 Maryland isolates were grown on several kinds of agar media side by side with the Wisconsin isolate, they always showed very good agreement with the latter in their vegetative development as well as in their asexual and sexual reproduction.

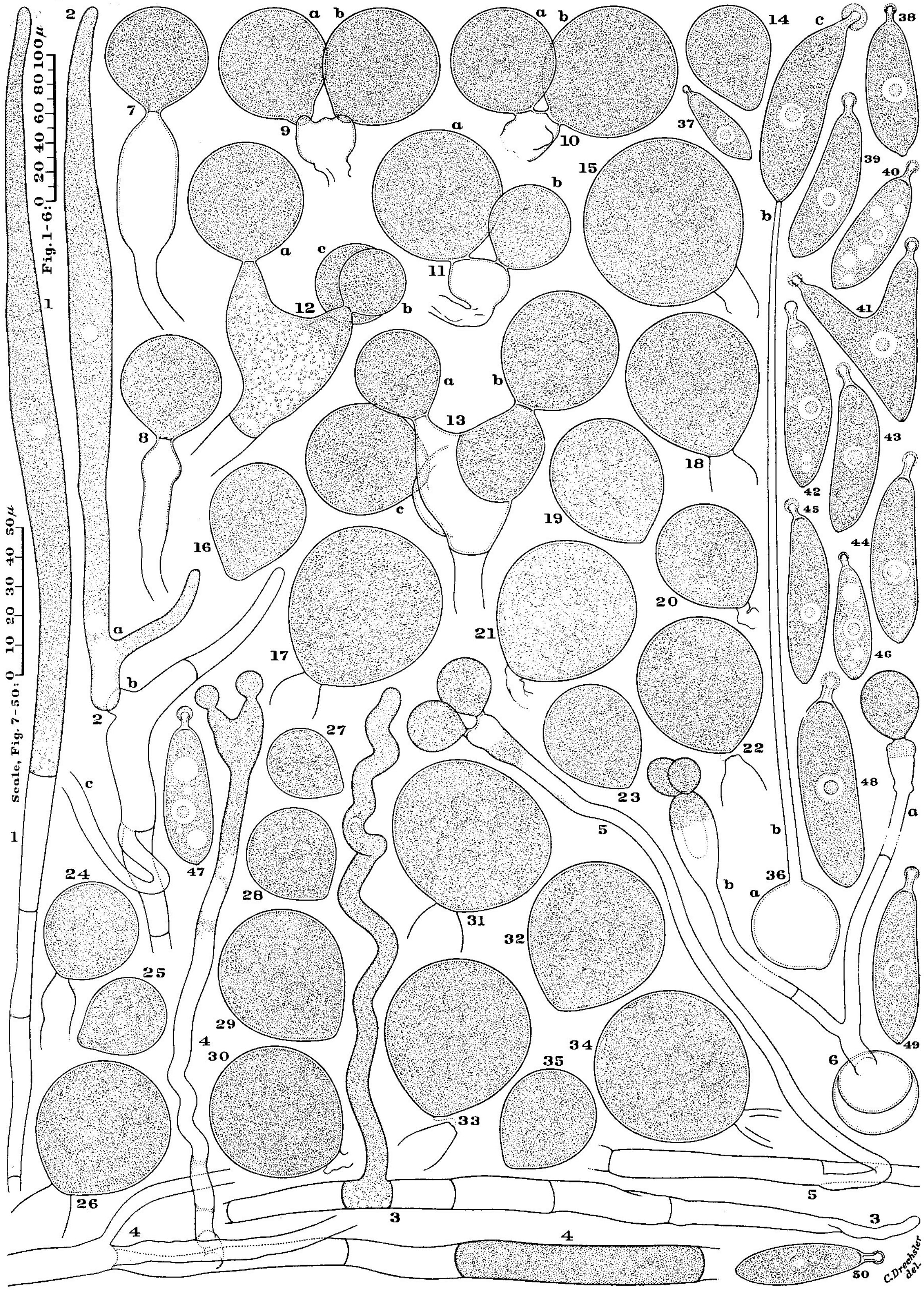
RESULTS—Since all 4 isolates have revealed parallel divergence from *Basidiobolus ranarum*, they are held to be representative of a separate species, for which a term having reference to its large dimensions may serve as an appropriate epithet.

Basidiobolus **magnus** *sp. nov.*[2] (Fig. 1–100)—Mycelium giving off a strong *Streptomyces*-like odor, colorless, usually with little or no aerial covering, composed of branched hyphae, mostly 10–37 μ wide, which contain living cells 30–600 μ long; at the growing margin consisting often largely of medially distended segments 300–600 μ long, 30–35 μ wide at the middle, 15–25 μ wide at the base, and 10–12 μ wide at the tip. Primary conidiophores arising from hyphal segments or conidia or germinating zygospores, colorless, simple or branched in their submerged or procumbent portions; mostly 5–15 μ wide, extending often 35–400 μ into the air, distally directed toward the light, terminally inflated into a propulsive distention that often is elongated ellipsoidal, 12–75 μ long, 8–35 μ wide, and bears 1 globose conidium, but often, too, is variously lobed and then bears 2 or 3 globose conidia. Globose conidia colorless, sometimes subspherical or prolate ellipsoidal or oblate ellipsoidal, more usually broadly obovoid, mostly 18–58 μ long and 16–56 μ wide. Conidiophores of slender type arising singly or in pairs from globose or from elongated conidia, often 150–250 μ long, 3–4.3 μ wide proximally and 1.2–1.8 μ wide distally, each bearing a single elongated conidium. Elongated conidia mostly strobiliform or prolate ellipsoidal, straight or slightly curved, 11–20 μ in greatest width, 33–75 μ in total length inclusive of an apical beak that usually is 4–10 μ long and 2–4 μ wide and is covered distally with yellow adhesive material; sometimes bifurcate, then provided at each tip with an adhesive beak; by internal division sometimes forming cylindrical or somewhat angular sporangiospores often 6–10 μ long and 8–13 μ in greatest width. Zygospores usually formed singly through union of 2 contiguous mycelial segments, much less often through union of contiguous daughter conidia resulting from division of a globose conidium, and sometimes through apical or lateral union of branches extended from separate mycelial hyphae; sometimes formed plurally, more especially in pairs; when formed singly usually subspherical or ellipsoidal, 25–60 μ (mostly 35–55 μ) long, 23–58 μ wide, surrounded by a composite wall 2.5–8 μ (commonly 4–7 μ) thick, that usually has a strongly undulate profile but sometimes is partly or wholly smooth; when formed plurally often somewhat smaller, rounded hemispherical or irregularly ellipsoid, in mature resting state usually showing some clear separation between the thin, colorless zygosporangial envelope and the colorless, undulated outer layer of the zygospore wall proper, as well as between that layer and the smooth, yellowish inner layer.

A mycelium of *Basidiobolus magnus* that is actively expanding on an agar substratum not too rich in nutrients differs markedly from mycelia of *B. ranarum*, as well as of *B. haptosporus* and *B. meristosporus*, in the conspicuous median distention of young terminal and lateral segments (Fig. 1, 2a) at its advancing margin. Since very similar distention of hyphal segments was noted (Drechsler, 1956, p. 658) in a sterile

[2] Mycelium incoloratum, aliquid inconspicuum, saepe benzeni hexachloridum redolens, itaque odore multis speciebus Streptomycetis etiam Basidioboli ranarum simile, vulgo non hyphis aereis vestitum, in hyphis ramosis plerumque 10–37 μ crassis e cellulis 30–600 μ longis constantibus consistens; hyphae prope marginem mycelii crescentis e cellulis elongato-fusiformibus, plerumque 300–600 μ longis, basi 15–25 μ crassis, medio 30–35 μ crassis, apice 10–12 μ crassis constantes. Primiformes fertiles hyphae e conidiis et zygosporis germinantibus et cellulis mycelii surgentes, incoloratae, nunc simplices nunc in partibus immersis vel procumbentibus paulo ramosae, plerumque 5–15 μ crassae, in aerem vulgo 35–400 μ crescentes, apice in tumorem jaculatorium abeuntes. Tumor jaculatorius saepe elongato-ellipsoideus, 12–75 μ longus, 8–35 μ crassus, denique unum conidium ferens; interdum plus minusve lobosus, tunc 2–3 conidia ferens; saepe conidium vel conidia violenter abjiciens, saepe inutiliter cadens. Primformia conidia incolorata, globosa vel ellipsoidea vel saepius late obovoidea, raro ad instar mammiculae basi prominula, 18–58 μ longa, 16–56 μ lata. Hyphae formae gracilis fertiles ex primiformibus vel tenacibus conidiis singulatim vel binatim surgentes, saepe 150–250 μ longae, basi 3–4.3 μ latae, sursum leniter attenuatae, apice 1.2–1.8 μ latae, ibi conidium tenax ferentes. Conidium tenax plerumque strobiliforme vel elongato-ellipsoideum, rectum vel leviter curvatum, apice rostro glutinoso flavido 4–10 μ longo 2–4 μ lato praeditum, ex toto 33–75 μ longum, 11–20 μ latum; interdum bifurcum et 2 rostris glutinosis praeditum; raro in sporangium transiens, tum sporas saepius 6–10 μ longas et 8–13 μ latas intus gignens. Zygosporae plerumque e conjugio duabus contiguarum cellularum in hypha mycelii vel e conidiis bipartitis vel e conjugio cellularum hyphae distinctae singulatim vel binatim oriundae; vulgo globosae vel ellipsoideae, 25–60 μ (plerumque 35–55 μ) longae, 23–58 μ latae, sed interdum rotundo-hemisphaeroideae vel aliquid lobosae; muro saepissime valde unduloso, 2.5–8 μ (plerumque 4–7 μ) crasso, ex interiore leve flavida lamina et incolorata undulata lamina et membrana zygosporangii constante circumdatae.

Habitat in materiis plantarum putrescentibus prope Park Falls, Wisconsin, et prope Cumberland, Maryland. Typus: National Fungus Collections (BPI) 71720; American Type Culture Collection 15379.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odorous culture maintained at the American Type Culture Collection under the name *B. ranarum* (ATCC 11230), there is some possibility that the fungus here presented as a new species may for years have been dealt with under Eidam's binomial. Usually a few of the distended segments soon become emptied of contents (Fig. 2b) in extending a hyphal branch (Fig. 2c), which, often after following a somewhat tortuous course (Fig. 3), may grow into the air as a phototropic conidiophore (Fig. 4). Most distended segments, however, delay their production of conidiophores (Fig. 5) and sexual units (Fig. 51, 52–56) for some days, during which they may undergo cell division, may extend branches laterally or obliquely, may widen noticeably, or may withdraw their contents from outlying parts. Even after many modifications have taken place, the origin of the various components from elongated fusiform segments generally remains recognizable in the older central areas of an extensive mycelium.

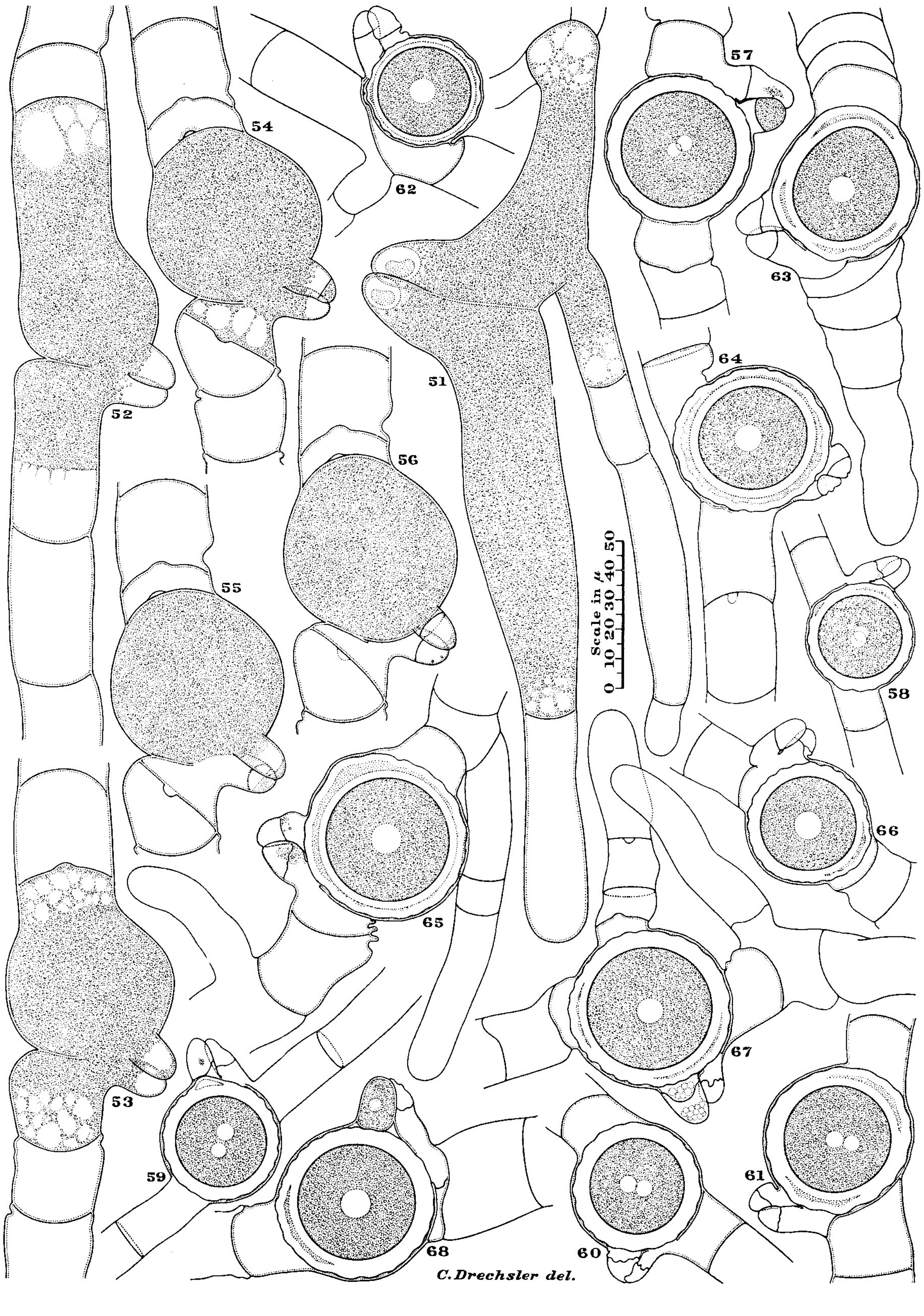
Although phototropic conidiophores (Fig. 6a, 7, 8) bearing solitary conidia are usually formed more abundantly than conidiophores (Fig. 4, 5) or conidiophore branches (Fig. 6b) bearing 2 (Fig. 9–11: a–b) or 3 (Fig. 12–13: a–c) conidia, the production of asexual spores plurally would seem much too frequent in *Basidiobolus magnus* to be considered other than normal for the species. On the other hand the collapse of intact phototropic conidiophores, though more frequent in some material than proper collapse after forcible discharge of their conidia, may well result from unsuitable environal conditions. Forcible discharge, of course, was never absent from any of my cultures or irrigated preparations, and in much material it clearly predominated over premature collapse. Yet the account by Nowak (1930), in which complete absence of forcible discharge was reported, would appear less incredible if it could be assumed that the fungus he investigated was *B. magnus* rather than *B. ranarum*.

The globose conidia (Fig. 14–35) of *Basidiobolus magnus* are in general considerably larger than those of *B. ranarum* or of any other known congeneric species. With respect to shape they vary from oblate-ellipsoidal (Fig. 15, 31) to elongate-ellipsoidal (Fig. 69). Specimens that have fallen on the substratum through collapse of the conidiophore (Fig. 15, 18, 26, 31, 34) show usually a more nearly flat basal end than those that were forcibly shot off (Fig. 19, 22, 23, 29, 32). In some instances a conidium that was forcibly shot off is found lying with its obtusely pointed basal end near the distal piece of empty conidiophore membrane that accompanied it in its flight (Fig. 22, 33, 70). The empty membranous pieces are more often of truncated conical shape (Fig. 71) than of the curious tower-and-cupula design made familiar in Eidam's figures of *B. ranarum*. As usually only few of the detached pieces are distally bilobate (Fig. 72), it may be presumed either that plurally formed conidia are less commonly shot off than single conidia, or that after being forcibly propelled they separate less readily from the shrunken basal appendage.

In not usually displaying a broadly mammiform proximal protrusion as a readily distinguishable feature when they are seen in longitudinal profile, the globose conidia of *Basidiobolus magnus* appear to differ from those of other members of the genus. It seems almost certain that a basal protrusion like that found in globose conidia of *B. ranarum* would merge rather indistinguishably with the obovoid body of the conidium of *B. magnus*, and would thus, much like the basal papilla in conidia of *Conidiobolus osmodes* Drechsler (1954), most often be unrecognizable. The few conidia of *B. magnus* in which a mammiform modification can be made out (Fig. 70) are mainly of subspherical rather than obovoid shape.

After falling on moist unoccupied agar substratum, the globose conidium of *Basidiobolus magnus* usually puts forth a germ hypha (Fig. 73) capable of developing into a mycelium. On agar substratum already occupied by the fungus it sometimes divides lengthwise into 2 cells, which then may conjugate and produce a zygospore (Fig. 74). Much more commonly on such substratum it extends a stout germ hypha and gives rise to 1 or more conidia similarly of the globose type (Fig. 6) and similarly capable of repetitional development. Here and there on occupied substratum a globose conidium (Fig. 36a) sends up 1 (Fig. 36b) or 2 slender conidiophores, each bearing an elongated conidium provided with an apical adhesive beak (Fig. 36c).

Fig. 1–50. Mycelial hyphae and asexual reproductive apparatus of *Basidiobolus magnus*; magnification in Fig. 1–6 is ×250; in Fig. 7–50 magnification is ×500.—Fig. 1. Distal portion of a hypha with a long medially distended terminal cell.—Fig. 2. Distal portion of a hypha; a, long medially distended terminal cell; b, subterminal distended segment emptied through production of a conidiophore of which only a proximal portion, c, is shown.—Fig. 3. Medially distended hyphal part nearly emptied through production of a branch later to develop distally as a conidiophore.—Fig. 4. Hypha with a conidiophore bearing 2 young primary conidia.—Fig. 5. Portion of hypha with conidiophore bearing 2 obovoid conidia.—Fig. 6. Empty conidium from which was extended a germ tube that has given off branches terminating in 2 conidiophores, a-b, bearing, respectively, 1 and 2 conidia.—Fig. 7. Propulsive distention bearing a primary globose conidium.—Fig. 8. Same distention 10 min later, in collapsed state.—Fig. 9–11. Collapsed propulsive distentions, each bearing 2 primary globose conidia, a-b.—Fig. 12, 13. Propulsive distentions, each bearing 3 globose conidia, a-c.—Fig. 14–35. Globose conidia, showing variations in size and shape.—Fig. 36. Empty envelope of globose conidium, a, from which has been extended a slender conidiophore, b, bearing an elongated conidium, c.—Fig. 37–50. Detached elongated conidia, showing variations in size and shape.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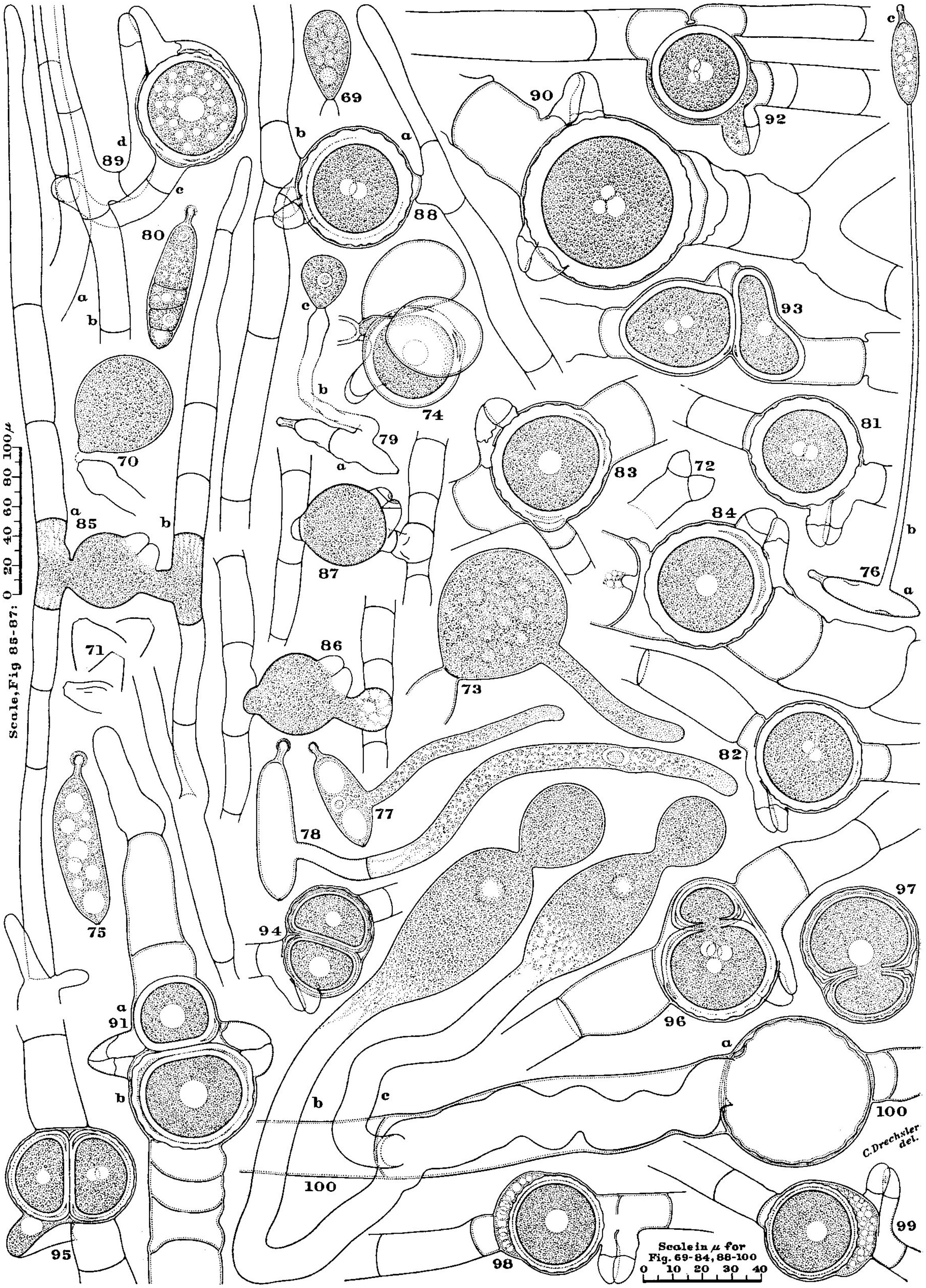
While a nucleus is not usually discernible in the living globose conidium of *Basidiobolus magnus,* the elongated conidia (Fig. 37–50), except when in a strongly vacuolated condition (Fig. 75), ordinarily permit recognition of the single nucleus they each contain. With respect to size and shape they resemble the homologous spores of *B. ranarum,* though among them some few bifurcated individuals (Fig. 41) are reminiscent more especially of the pathogenic Indonesian congener. As a rule they secrete adhesive material less copiously than the similar spores of other known species. On substratum already occupied by the fungus the adhesive conidium (Fig. 76a) often sends up a slender conidiophore (Fig. 76b) eventually surmounted by a secondary adhesive spore (Fig. 76c), which may then repeat the same course of development. Relatively fresh substratum encourages emission of a germ hypha (Fig. 77, 78) capable of growing out and forming a new mycelium. Occasionally an adhesive spore (Fig. 79a) extends a broad germ hypha (Fig. 79b) which after some elongation grows ascendingly into the air in giving rise to a phototropic conidiophore bearing a small globose conidium (Fig. 79c). Now and then, also, an adhesive conidium undergoes conversion into a sporangium through division of its protoplasmic contents (Fig. 80).

Sexual reproduction takes place abundantly in potato-dextrose or maize-meal agar cultures of *Basidiobolus magnus.* Many sexual units come into being through division of a medially distended hyphal segment into 2 approximately equal gametangia that together soon put forth a pair of juxtaposed protuberances, each of which displays a nucleus in its tip (Fig. 51). Movement of protoplasm during the ensuing 2–3 hr (Fig. 52–55) results in formation of a young globose zygospore (Fig. 56) within the envelope of one gametangium usually in a position adjacent to the other, though in exceptional instances (Fig. 66, 98) the zygospore comes to lie at some little distance from the empty contributing gametangium. The protuberances persist usually as paired empty appendages, each of them showing most commonly a transverse partition. A nucleus, as a rule, is not discernible in the variably granular protoplasm of a young, unstained living zygospore. After a few days of relatively slow internal reorganization, the zygospore usually reveals near its center 2 globose bodies that are held to be the nuclei which became paired through the act of conjugation (Fig. 57–61, 81, 82). It is now surrounded by a thick wall displaying usually an abundance of undulations along the periphery. It reaches the fully mature stage 5–15 days later, when its rather finely granular protoplasmic contents show at the center a single globose body presumably interpretable as the fusion nucleus (Fig. 62–68, 83, 84).

According to Eidem (1886, p. 218, lines 32–36) zygospores of *Basidiobolus ranarum* always originate through conjugation of adjacent cells. In all my cultures considered referable to Eidam's species, as also in all my cultures of *B. haptosporus* and *B. meristosporus,* no zygospores ever appeared to have resulted from union of cells not adjacent to each other. However, in cultures of *B. magnus,* conjugation between cells of separate hyphae is evident in approximately 2% of all sexual units. Usually a zygospore of diclinous origin is found lying between 2 hyphae or hyphal branches of sufficient volume to have supplied all the material needed for its development (Fig. 85a, b, 86–87; 88a, b), but in some instances it manifestly must have resulted from union of rather short parallel branches (Fig. 89c, d) extended from 2 separate hyphae (Fig. 89a, b) that supplied most of the protoplasm. The zygospores of diclinous origin, much like those of monoclinous origin, show no nucleus when newly formed (Fig. 87), reveal 2 nuclei at early maturity (Fig. 88), and at late maturity display a largish nucleus in central position (Fig. 89).

After conjugation various departures ıom the usual course of sexual development (Fi 90–97) occur more frequently in *Basidiobolus magnus* than in *B. ranarum.* A largish nucleus, together with 2 smaller nuclei, would sometimes seem present in a zygospore furnished with 2 pairs of septate protuberances (Fig. 90) or in a zygospore with a uniseptate and an unseptate protuberance (Fig. 92). Two juxtaposed zygospores (Fig. 91a, b; 93) may sometimes be produced in a sexual unit, one originating within the membrane of each conjugating cell. Sometimes, on the other hand, 2 zygospores are contained within the envelope of the same gametangium (Fig. 94, 95), where they lie separated from each other along a plane directed parallel or oblique to the axis of the parent filament. It may be of significance that often in such sexual units neither of the juxtaposed protuberances is divided by a cross-wall. Possibly each unseptate protuberance here contributes to the zygote both daughter nuclei originating in its tip. At early maturity a 2-chambered, incompletely divided zygospore, furnished with paired protuberances devoid of cross walls (Fig. 96), may show 4 nuclei, though only a single largish nucleus is ordinarily visible at late maturity (Fig. 97). A more familiar abnormality is encountered here and there in the prolonged retention of a vacuolated deposit of protoplasm between the zygospore wall proper and the thin membrane of the zygosporangium (Fig. 98, 99).

Fig. 51–68. Sexual reproductive apparatus of *Basidiobolus magnus,* ×500.—Fig. 51. Paired adjacent gametangia showing each of the 2 juxtaposed protuberances occupied distally by a nucleus.—Fig. 52–56. Series of 5 successive stages in conjugation of adjacent segments with resultant formation of a young zygospore.—Fig. 57–61. Zygospores showing 2 nuclei at early maturity.—Fig. 62–68. Zygospores showing 1 large nucleus at full maturity.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If slabs excised from potato-dextrose-agar plate cultures of *Basidiobolus magnus* over 20 days old are irrigated with distilled water, some scattered zygospores (Fig. 100a) will usually germinate by putting forth a broad germ tube from which are extended 2 hyphae (Fig. 100b, c), each terminating in a phototropic conidiophore that supplies a single nucleus to the globose conidium formed on its tip. Zygospore germination does not here, as in *B. ranarum*, result in a lone binucleated conidium and thus does not initiate a binucleated condition transmissible through many generations of globose and elongated conidia until a vegetative state supervenes. Because of the frequent production of conidia plurally, the elongated conidia of *B. magnus* are generally no bigger than those of *B. ranarum*, although the zygospores and primary globose conidia serving as their ancestors are conspicuously larger than the corresponding spores of Eidam's species.

Sexual reproductive units of *Basidiobolus magnus* that contain either 2 zygospores (Fig. 94, 95) or a 2-chambered zygospore (Fig. 97, 98) often show clearly some spatial separation of the colorless membranous zygosporangial envelope from the zygospore wall proper, which, moreover, generally can be seen to consist of an outer, colorless, undulated layer and an inner, smooth, yellowish layer. The make-up of the composite mantle thus agrees rather well with the arrangement of parts shown by Fairchild (1896, Taf. XIV, Fig. 15, 16) in figures of *B. ranarum*. At early maturity, and especially if the substratum is rich in nutrients, the 2 layers of the zygospore wall proper are often found indistinguishably merged in all sectors (Fig. 57, 60, 81, 90). Later some separation may become noticeable along arcs of variable lengths (Fig. 58, 61, 63, 67, 68). In some zygospores, separation of the 2 layers becomes evident along the whole circumference (Fig. 64), though the inner boundary of the outer layer often remains indistinct. As the outer contour of the inner layer is usually clearly defined, an appearance much like that of an endogenous spore often results. Occasionally the outer layer is wholly absent (Fig. 91a, 92, 93), so that the composite mantle consists of the zygosporangial membrane and the yellowish smooth inner layer. Somewhat rarely a considerable portion of the outer layer of the zygospore wall proper appears to be smooth (Fig. 96). On the whole, the zygospores of *B. magnus* look much more abundantly undulated than might be inferred from the accompanying figures, in which of necessity they are shown mainly in profile view.

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Fig. 69–100. Asexual and sexual reproductive apparatus of *Basidiobolus magnus*; magnification in Fig. 69–84 and Fig. 88–100 is ×500; in Fig. 85–87 magnification is ×250.—Fig. 69. A small and unusually elongated conidium from a phototropic conidiophore.—Fig. 70. Conidium with prominent basal protuberance near detached membrane of propulsive distention.—Fig. 71. Distal membranous pieces from 4 propulsive distentions.—Fig. 72. Empty membrane of propulsive distention with 2 distal lobes from which separate globose conidia were released.—Fig. 73. Globose conidium with a young germ hypha.—Fig. 74. Pair of adjacent conidia that were emptied in producing a zygospore.—Fig. 75. Strongly vacuolated elongated conidium in which no nucleus is distinguishable.—Fig. 76. Empty elongated conidium, a, from which was extended a slender conidiophore, b, bearing a new elongated conidium, c.—Fig. 77. Elongated conidium with young germ hypha.—Fig. 78. Empty elongated conidium with a procumbent germ hypha containing the single nucleus.—Fig. 79. Empty elongated conidium, a, from which was extended a germ hypha, b, terminating in a phototropic conidiophore bearing an unusually small globose conidium, c.—Fig. 80. Elongated conidium undergoing internal division into sporangiospores.—Fig. 81, 82. Zygospores at early maturity, each showing 2 nuclei.—Fig. 83, 84. Fully mature, uninucleated zygospores.—Fig. 85. Young zygospore developing through conjugation of cells in separate hyphae, a-b.—Fig. 86, 87. Same zygospore after successive intervals of 60 and 45 min, respectively.—Fig. 88. Binucleated zygospore at early maturity, produced through conjugation between cells of 2 separate hyphae, a-b.—Fig. 89. Two hyphae, a-b, that have extended paired branches, c-d, which through conjugation have produced a fully mature uninucleated zygospore.—Fig. 90. Large zygospore at early maturity, showing 2 pairs of protuberances and 3 nuclei.—Fig. 91. Sexual unit furnished with 2 pairs of protuberances and containing 2 fully mature zygospores, a-b, one being found in each gametangium.—Fig. 92. Zygospore containing 3 nuclei at early maturity, but without an undulate layer in its wall.—Fig. 93. Sexual unit with a zygospore, devoid of any undulate wall layer, present in each gametangium.—Fig. 94, 95. Sexual units, each with 2 zygospores in the same gametangial envelope.—Fig. 96. A 2-chambered zygospore with 4 nuclei at early maturity.—Fig. 97. Fully mature uninucleated 2-chambered zygospore.—Fig. 98, 99. Fully mature uninucleated zygospores, each within a zygosporangial envelope that contains, besides, some vacuolated protoplasm.—Fig. 100. Empty zygospore, a, from which was extended a broad germ tube that gave off 2 conidiophores, b-c, each containing 1 nucleus in its propulsive distention and producing 1 conidium.