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SUPPLEMENTARY DEVELOPMENTAL STAGES OF BASIDIOBOLUS RANARUM AND BASIDIOBOLUS HAPTOSPORUS

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(WITH 5 FIGURES)

During the 70 years that have elapsed since *Basidiobolus ranarum* was described by Eidam (**7**) as the type of a new genus of the Entomophthoraceae this fungus has retained widespread interest by virtue of outstanding peculiarities in its development and morphology. The rocket-like action by which the expanded distal portion of its conidiophore propels the globose conidium (**10**) would seem different in principle from the various mechanisms for forcible spore discharge operative in related genera and in other groups of fungi. Its curious sexual reproduction, wherein a preliminary division of the nuclei of adjacent conjugating hyphal segments takes place simultaneously in paired juxtaposed protuberances, has deservedly been set forth in many text-books on mycology. Yet somehow it has not become equally well known that the nuclei of *B. ranarum,* as also those of congeneric species, are for the most part readily visible in unstained living material, being obscured from view only in thick structures, such as young zygospores and large globose conidia, where they lie deeply imbedded in protoplasm of dense, coarsely granular texture. Although the visibility of its nuclei and the ready conjugation of its paired hyphal segments in a period of approximately 2 hours would seem exceptional features that might make *B. ranarum* very desirable for purposes of instruction, the fungus has not been a familiar object in American laboratories. As far as can be determined from the literature it was obtained from American materials only twice during the 65 years directly following its description: Thaxter (**15**) having grown it on frog excrement procured by filtering out sediment from water in which frogs were kept, and Olive (**13**) having brought it into pure culture by allowing conidia formed on contents removed from the intestine of a frog to be shot on to small cubes of sterilized bread. The culture of *B. ranarum* used by Couch (**1**) in experi-

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ments on sexual reactions between entomophthoraceous forms would seem to have originated abroad, having apparently been supplied earlier from a foreign culture collection.

An aversion for killing and dismembering largish animals other than insects may have deterred many American mycologists from acquiring material of *Basidiobolus ranarum* firsthand. Eidam's stern procedure in slaughtering dozens of frogs at a time in order to obtain quickly a generous supply of their intestinal and stomach contents can not be considered at all alluring, and in suburban areas where amphibians seem generally scarce would be against the public interest and difficult to carry out. Fortunately *Basidiobolus* isolations can be obtained readily from much smaller quantities of frog excrement than Eidam believed necessary, so that all destruction of the animals can be obviated. A captured frog confined in a clean glass jar containing 25 to 50 cc of distilled water will commonly void sufficient excrement in 10 to 15 hours, and can then be returned unharmed to its habitat. The excrement, though somewhat gelatinous, may be collected conveniently by passing the liquid contents of the jar through a small paper filter that has been snugly nested in firmly packed absorbent cotton or absorbent paper. If the filter is then flattened out while still moist, and portions of it, soiled with excrement, are affixed, soiled side downward, to the ceiling of sterile Petri dishes containing sterile maize-meal agar, numerous small *Basidiobolus* mycelia, each coming from a separate conidium, will usually appear 24 to 48 hours later in scattered positions on the agar surface. Even the very scanty excrement voided in 15 hours by individual frogs belonging to *Pseudacris nigrita feriarum* (Baird), and weighing only about 5 grams, always yielded many *Basidiobolus* mycelia when brought into a paper canopy over a Petri plate of maize-meal agar. By transferring the small mycelia to sterile agar slants pure cultures were usually obtained at once.

In a collection of 57 *Basidiobolus* cultures obtained from excrement of 15 frogs captured in 4 locations near College Park, Maryland, on June 29 and July 23, 1955, two species were readily distinguished. All isolations, 18 in number, derived from excrement of 2 frogs (*Rana clamitans* Latreille) captured on the earlier date emitted the musty benzene-hexachloride odor familiar in species of *Streptomyces,* and promptly gave rise to numerous propulsive conidiophores as well as to an abundance of zygospores surrounded individually by a wall of undulate outer profile. Among the 39 cultures isolated from excrement of 13 frogs [2] captured on the later date only 2 emitted the musty *Strepto-*

[2] Of these animals 3 belonged to *Rana clamitans* and 10 to *Pseudacris nigrita feriarum.*

myces-like odor and produced zygospores of undulate profile. The remaining 37 cultures emitted no musty odor, and promptly formed numerous smooth zygospores while at the same time giving rise to large numbers of phototropic conidiophores that shot off globose conidia. After 10 days they showed a layer of white aerial mycelium and could thereby be definitely referred to my *B. meristosporus* (**6**). It is of some moment that the 2 cultures of the odorous *Basidiobolus* obtained from the second group of frogs came from separate individual animals, each of which yielded, besides, several cultures of *B. meristosporus*.

As no European writings on *Basidiobolus ranarum* suggested any likelihood that 2 members of the genus might occur in frog excrement, the isolation of 2 species from the excrement voided by individual frogs in the course of one day was wholly unexpected. That the 2 species included *B. meristosporus* was especially contrary to expectations, for though this fungus, through its ready production of elongated adhesive conidia and sporangia, appeared well adapted to colonize the digestive organs of amphibians, it had previously become known to me only from plant detritus collected in Florida, never having developed in cultures prepared with decaying vegetable remains gathered in Maryland and Virginia. However, when Petri plates of maize-meal agar were canopied on July 26, August 1, and August 10, 1955, with leaf mold newly collected near College Park—finely divided forest detritus being affixed to the lids sometimes by means of agar and at other times by means of moist filter paper—many isolations of *B. meristosporus* were obtained. In these canopied plate cultures neither of the 2 species I had previously obtained from plant detritus collected in Maryland and Virginia (**3**, **4**, **6**) came to light. The divergent results have their explanation in the different temperature adaptations of the fungi here concerned. All the earlier work with decaying material from Maryland and Virginia was performed in winter at room temperatures near 20° C, so that the cultures prepared with detritus newly gathered outdoors favored the development of the 2 species, *B. haptosporus* and the odorous form, in which zygospore germination, growth, and reproduction proceed well at such temperatures. The Petri plate cultures canopied with excrement of the 2 frogs captured on June 29, likewise were incubated at temperatures near 20° C, and thus permitted development of the odorous species after its vegetative cells had given rise to phototropic conidiophores and globose conidia. For lack of cooler chambers, the plate cultures canopied with excrement of the 13 frogs captured on July 23, as also the plate cultures canopied with newly collected leaf mold on July 26, August 1, and August 10, were incubated in a basement room at 27° C—a tem-

perature less favorable for the odorous fungus than for the somewhat more thermophilic *B. meristosporus*. Besides, *B. meristosporus* must have been present more abundantly in the materials used for canopying cultures in July and August, as the preceding weeks of hot mid-summer weather could hardly have failed to bring about greater development of the more thermophilic species, and correspondingly more plenteous ingestion of its adhesive conidia by amphibians.

MORPHOLOGICAL FEATURES OF THE ODOROUS FUNGUS AND ITS PRESUMPTIVE IDENTITY WITH BASIDIOBOLUS RANARUM

All the *Basidiobolus* isolations producing zygospores of undulate profile that were obtained from frog excrement seem clearly referable to the same species as the congeneric isolations of like sculpture which I reported earlier (**4**, **5**, **6**) to have been procured from plant detritus collected in New Hampshire, Pennsylvania, Delaware, Maryland, Virginia, North Carolina, and Louisiana. More recently, similar and manifestly conspecific isolations have been obtained also from plant detritus gathered during the third week in November, 1954, in Chicago, Illinois; in Fort Wayne, Indiana; and near Park Falls, Butternut, Mellen, and Grandview in northern Wisconsin. Whether obtained from frog excrement or from plant detritus, the *Basidiobolus* forming undulate zygospores gives off a distinctive benzene-hexachloride odor similar to that emitted by many species of *Streptomyces*. During the period when the mycelium is actively growing, this odor, as a rule, is given off strongly. Only one among more than 150 conspecific isolations in my collection produced the odor so faintly that it might readily have remained undetected. A culture (ATCC*11230*) maintained at the American Type Culture Collection, Washington, D. C., under the binomial *B. ranarum,* which presumably originated in the Old World, also was found to give off a strong *Streptomyces*-like odor. As this culture apparently lacks both sexual and asexual reproduction, and as its hyphal segments often show a rather pronounced median distention, it was held earlier (**5**) to represent a species alien to the odorous cultures isolated in the United States. This opinion may have been incorrect, for if the culture should have lost its reproductive capacities, possibly from long continued propagation on artificial substrata, pronounced modification in outward shape of hyphal segments might have ensued as a direct consequence. When reproduction is temporarily inhibited in my cultures because of unsuitably high temperatures, the hyphae and their segments often show conspicuous modifications. In any case the sterile culture gave little help toward determining the relationship of my odorous isolations to *B.*

ranarum. Eidam's original account of that species makes no mention of any distinctive odor; nor, apparently, is any odor discussed by later European investigators that have dealt with *B. ranarum*.

In its earlier, purely vegetative growth on a transparent substratum the odorous *Basidiobolus* does not differ markedly from *B. meristosporus* and *B. haptosporus*. The main hyphae growing out radially at the margin of a mycelium that is expanding unimpeded in a Petri plate of maize-meal agar commonly measure about 10 μ in width (FIG. 1, *A, B*). As the terminal segment elongates it divides repeatedly in forming one penultimate segment after another. Rather often the segments some distance from the mycelial forefront are noticeably wider than those at the periphery. In a mycelium that has originated from a globose conidium the portions of hyphae near the empty conidial envelope may measure 15 to 20 μ in diameter. Although the mycelium produced in maize-meal agar of low nutrient content is generally robust, it may yet be hardly visible to the naked eye except by reflected light. On maize-meal agar with much fine maize-meal in suspension, as also on Lima-bean agar, a substratum even richer in nutrients, the hyphae are extended in closer arrangement, so that the mycelium is revealed more clearly to the naked eye, usually as a smooth colorless layer of somewhat cartilaginous appearance.

At temperatures near 20° C a mycelium of the odorous *Basidiobolus,* on attaining a diameter of several millimeters, usually begins to produce both conidia and zygospores. As it continues to expand, asexual and sexual reproduction proceed concurrently, some hyphal segments sending up individually a stout conidiophore (FIG. 1, *C*), while others nearby conjugate in pairs. Owing to the strong phototropism of the conidiophores, the single globose conidia they shoot off fall in scattered positions beyond the mycelial forefront on the expanse of unoccupied substratum extending toward the main source of light. Many of the scattered conidia may each give rise directly to a stout phototropic conidiophore that soon shoots off a single globose secondary conidium to a position farther toward the light. Other conidia germinate vegetatively to form small subsidiary mycelia which give rise to plural phototropic conidiophores, each of which likewise shoots off a single globose conidium toward the light. In Petri plate cultures, therefore, the odorous fungus, much like *B. meristosporus,* spreads rapidly and disconnectedly over the areas lying toward the main source of light, while its advance in other directions proceeds more slowly and uniformly through elongation of the hyphae at the mycelial forefront.

In all my isolations of the odorous *Basidiobolus* the globose conidia

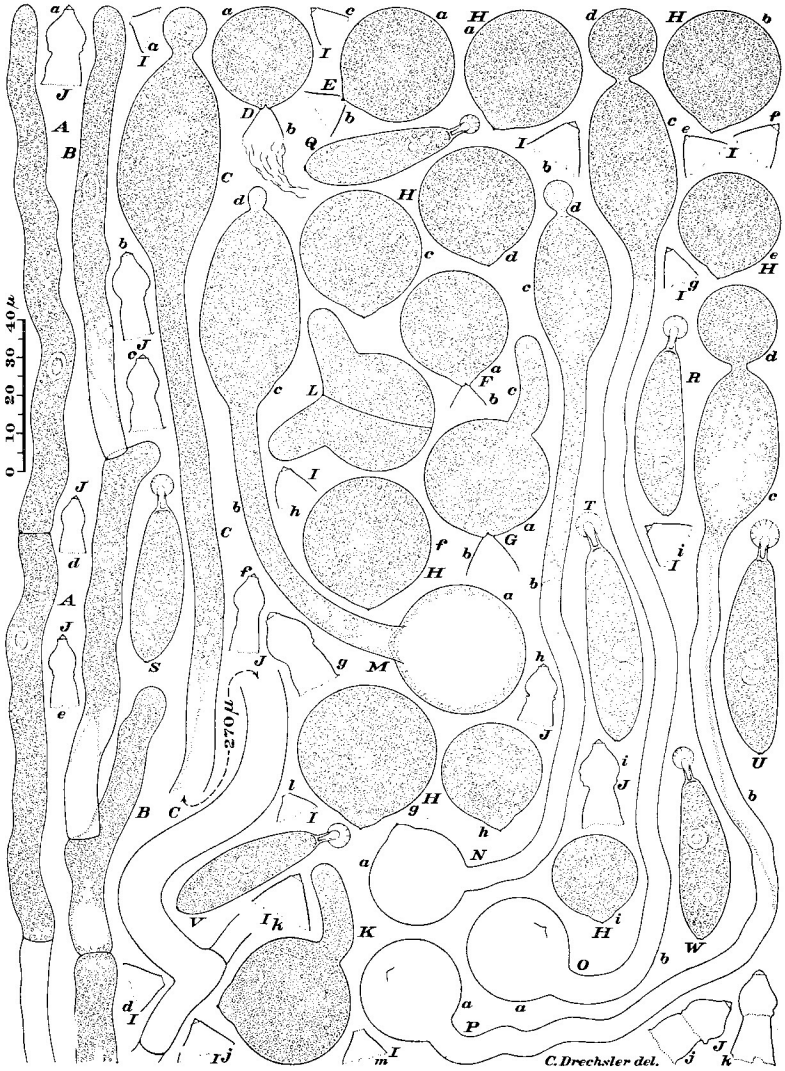

FIG. 1, *A–W*.

FIG. 1. *Basidiobolus ranarum* as found in maize-meal-agar plate cultures, × 500. *A, B*. Distal portions of elongating hyphae at margin of a growing mycelium. *C*. Young conidiophore that grew out from a submerged hyphal segment (a portion 270 μ long being omitted). *D–G*. Globose conidia, *a*, with empty membrane of propulsive enlargement, *b*; the conidium in *G* is extending a germ tube, *c*. *H*. Detached globose conidia, *a–i*. *I*. Detached conical pieces of membrane, *a–m*, from tips of propulsive enlargements. *J*. Empty membranes, *a–k*, of propulsive

were shot off forcibly after the manner set forth in Eidam's original account of *B. ranarum*. As Nowak (12) has contended that in *B. ranarum* the globose conidia are not forcibly discharged, occasion may be taken to emphasize that none of my isolations have under conditions not obviously unfavorable failed to show energetic conidial propulsion. The discharge mechanism, of course, fails to operate in material mounted in water under a cover glass, the fully developed conidium (FIG. 1, *D, a*) in moist preparations remaining attached to the empty conidiophore, which except for a widened distal portion (FIG. 1, *D, b*) soon collapses and evanesces. In some instances a conidium (FIG. 1, *E–G: a*) after being shot off forcibly has attached to its base an empty funnel-shaped membrane (FIG. 1, *E–G: b*). More often, however, the conidium at the end of its flight (FIG. 1, *H, a–i*) is found unencumbered by any membranous attachment. Empty pieces of membrane, some of them conical or funnel-shaped (FIG. 1, *I, a–m*) and others of the curious tower-and-cupola design (FIG. 1, *J, a–k*) made familiar in Eidam's illustrations, become strewn about haphazardly on the substratum. Lying on a moist agar surface many globose conidia (FIG. 1, *G, a; K*) germinate by putting forth a single germ hypha (FIG. 1, *G, c*). A few undergo a division and then extend a germ tube from each of the resulting cells (FIG. 1, *L*). In many instances where a conidium (FIG. 1, *M–P: a*) has put forth a single germ hypha, the hypha (FIG. 1, *M–P: b*) grows upward into the air to give rise on the tip of a propulsive enlargement (FIG. 1, *M–P: c*) to a secondary globose conidium (FIG. 1, *M–P: d*). Such secondary conidia and also all globose conidia of higher orders formed through continued repetitional development, have in my isolations been shot off forcibly, much like primary conidia borne on conidiophores arising from hyphal segments.

Like the hyphal segments from which they are derived, the primary conidia of the odorous *Basidiobolus* vary moderately in size. The globose spores shot off from vigorous young mycelia in maize-meal-agar plate cultures range in length and width between 25 and 40 μ, the averages of the two dimensions in material from separate Petri dishes falling often between 30 and 33 μ. On rich Lima-bean agar larger spores are formed, some primary conidia produced on this medium attaining lengths and widths between 40 and 48 μ, thereby equalling

enlargements. *K*. Detached globose conidium germinating. *L*. Globose conidium extending a germ tube from each of its 2 cells. *M–P*. Four globose conidia, *a*, that have each extended a broad conidiophore, *b*, bearing a propulsive enlargement, *c*, surmounted by a young globose secondary conidium, *d*. *Q–W*. Detached binucleated adhesive conidia.

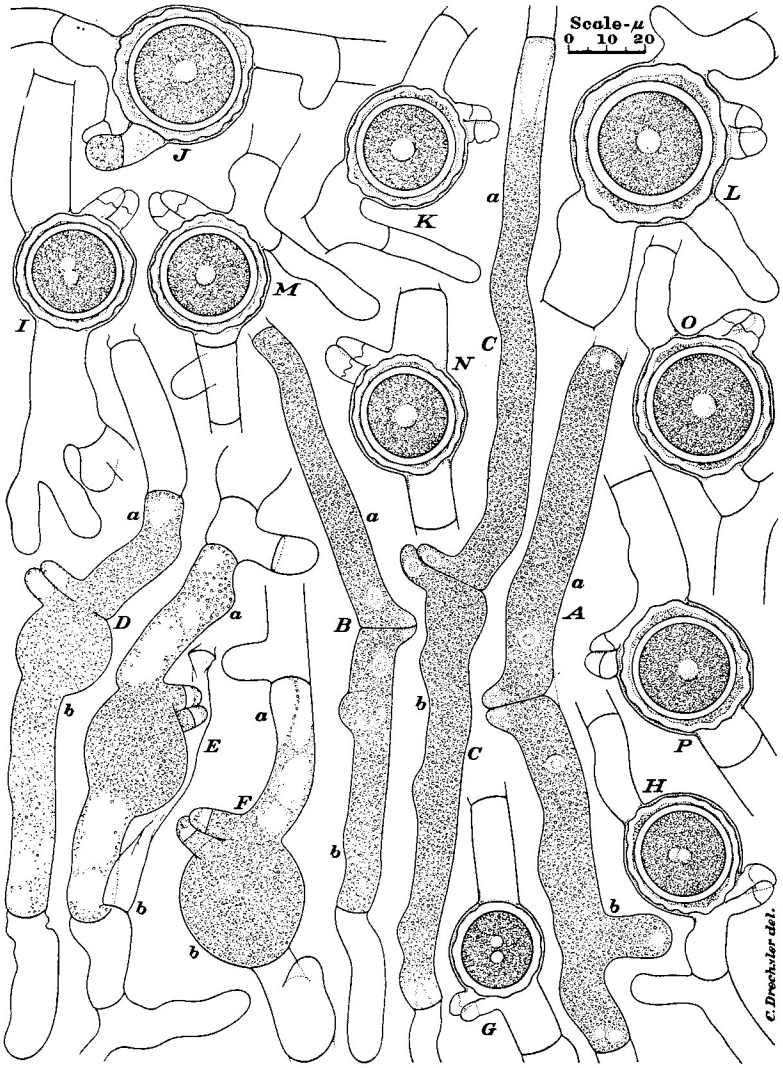

FIG. 2, *A–P*.

FIG. 2. *Basidiobolus ranarum* as found in maize-meal-agar plate cultures, × 500. *A–C*. Three young sexual reproductive units, each consisting of 2 adjacent hyphal segments, *a* and *b*, that have put forth apposed protuberances; in *A* and *B* the cell nuclei are moving toward the protuberances, in *C* they have moved into the protuberances. *D–F*. Sexual reproductive units in each of which one hyphal segment, *a*, is supplying protoplasm for the formation of a zygospore in an adjacent segment, *b*. *G*. Mature zygospore showing no separation of wall into 2 layers.

the maximum measurements indicated in the dimensional ranges (23 to 48 μ for length, 21 to 46 μ for width) that Eidam ascribed to the conidia of *B. ranarum*. It is worthy of note that the several full grown conidia figured by Eidam would seem from the indicated scales of magnification to vary from 28 to 33 μ in length, and thus are generally comparable in size with the dozen globose conidia (FIG. 1, *D–F: a; H, a–i*) figured herein as being tolerably representative of the odorous fungus. These dozen spores and, indeed, all other structures of the odorous species shown in FIGS. 1–3, were drawn from specimens taken from maize-meal-agar plate cultures of an isolation obtained from leaf mold gathered in oak woods near Farmer, North Carolina, late in December, 1951. In view of the rather wide range of variations commonly observable in a single culture, the differences between separate isolations of the odorous species, whether originating from plant detritus or from frog excrement, have not seemed very pronounced.

As has been mentioned, sexual reproduction in the odorous *Basidiobolus* usually proceeds concurrently with asexual reproduction after a young mycelium has attained a diameter of several millimeters. Conjugation always takes place between adjacent hyphal segments (FIG. 2, *A–C: a, b*) and is accomplished in the manner described in Eidam's account of *B. ranarum* through production of juxtaposed protuberances, division of the nuclei in the protuberances, partial solution of the separating wall, and movement of the protoplasm, together with a nucleus, from one of the gametes (FIG. 2, *D–F: a*) into the other (FIG. 2, *D–F: b*). The fusion cell thus formed soon lays down a thick wall with a smooth circular inner contour and an undulated outer contour (FIG. 2, *G;* FIG. 3, *E, F*). Fully mature zygospores of moderate size often show a somewhat thicker wall in which 2 layers, separated here and there, can be distinguished. The largest zygospores commonly show during the resting period a wall composed of a smooth inner layer and an undulated outer layer (FIG. 2, *H–P*). Although the inner contour of the outer layer is usually rather indistinct, the 2 layers seem discrete all around, and in some regions are separated by spaces 1 to 2 μ wide. Some of the wider interstitial pockets seem filled to a greater or lesser extent with granular material.

In respect to size the zygospores of the odorous *Basidiobolus* correspond well with those of *B. ranarum,* the dozen specimens figured herein (FIG. 2, *G–P;* FIG. 3, *E, F*) showing approximately the same range in

H–P. Mature zygospores showing separation of wall into an undulate outer layer and a smoothly spherical inner layer; in *J* the distal segment of one protuberance appears filled with living protoplasm.

diameter—23 to 43 μ—that Eidam ascribed to the zygospores of his frog-inhabiting species. The odorous fungus corresponds well also with the description of *B. ranarum* in the undulate contour of the wall surrounding its zygospore. While Eidam (**7**: 221) noted very briefly that the zygospores he observed developing in nutrient solutions began to show stratification of the endosporium as they matured into resting spores, he gave no details on the nature of the stratification. The peripheral markings in his relevant illustrations (**7**: Pl. 12, Figs. 7–9, 12–14), like the similar markings in a figure of *B. ranarum* given by Thaxter (**15**: Fig. 413), seem more expressive of the undulated appearance of the zygospore wall than of its structural make-up, which probably was not well revealed under the microscopes then in use. Subsequently Fairchild, who presumably employed a European culture in the investigations he carried out at Bonn, Germany, on nuclear division and fertilization in *B. ranarum,* supplied figures (**8**: Pl. 14, Figs. 15, 16) showing two thick layers in the zygospore wall proper, and partial separation of these layers. These figures present much the appearance usual in medium-sized zygospores produced by the odorous American species, though in unstained living material of my fungus it has not been easy to distinguish clearly the additional thin outer layer which Fairchild recognized as the original delimiting membrane secreted by the young zygospore.

During their resting period some zygospores of the odorous *Basidiobolus* have a strongly globuliferous internal structure, while others are largely filled with cytoplasm of uniformly coarse texture. Among zygospores of the latter category the larger number display near the center a single globose, somewhat lustrous body (FIG. 2, *J–P;* FIG. 3, *F*) corresponding in size and shape to the fusion nucleus shown in Fairchild's figures illustrating rather old zygospores of *B. ranarum,* while a smaller number display two such bodies (FIG. 2, *G–I,* FIG. 3, *E*). Two lustrous bodies, or nuclei, are commonly visible in zygospores which from their smoothly spherical shape and thin wall (FIG. 3, *G, H*) are evidently ready to germinate. After a protuberance from the globose zygospore (FIG. 3, *I*) has broken through the enveloping membrane to push forth externally, the 2 nuclei can be observed moving forward in the elongating germ hypha (FIG. 3, *J, K*) separated from each other by an interval of 1 to 6 μ. As a rule the separation is no less evident in short germ hyphae than in long ones. This somewhat aloof companionship merits notice because the 2 nuclei in the germinating zygospore of *B. ranarum,* according to Eidam (**7**: 229, Pl. 12, Figs. 19, 20) regularly emerge from the spore envelope in intimate contact

with one another, and remain in intimate contact as they move forward until the germ hypha has attained sufficient length to become divided by cross-walls. Since the 2 nuclei shown by Eidam (**7**: Pl. 9, Fig. 14) in a conidiophorous hypha arising from a globose conidium produced through germination of a zygospore, appear separated by an interval of nearly 2 μ, it would seem that at least the positional relationship later prevailing in *B. ranarum* is approximately as in the odorous fungus.

Eidam observed that the binucleated condition in germ hypha extended from a zygospore of *Basidiobolus ranarum* is always terminated when mycelial development ensues. In the odorous fungus similarly the uninucleated condition is always restored whenever the vegetative state is resumed. Under natural conditions, therefore, the binucleated condition would usually be of rather short duration. However, in maize-meal-agar plate cultures that are protected from rapid evaporation by being tightly covered with a bell jar, the binucleated condition is by no means ephemeral, but has been found to persist for 5 or 6 months, in many instances eventually even preponderating over the uninucleated state.

Several days after being planted such cultures are permeated so thoroughly by the fungus that virtually all further vegetative growth is precluded. Thenceforth the conidia, unable to produce mycelia, are limited mainly to reproductive development. At first the globose conidia (FIG. 1, *D–F: a; H, a–i*), all of them uninucleated like the hyphal segments from which they originated, nearly always give rise individually to a propulsive conidiophore that eventually shoots off a secondary globose conidium containing the single nucleus of its parent. Later this strictly repetitional development is supplanted in gradually increasing measure by the production of elongated adhesive conidia singly on solitary slender conidiophores. As in *Basidiobolus meristosporus* and *B. haptosporus* these slender conidiophores are not phototropic and apparently are never sent up from a hyphal segment. They are in many instances first sent up from scattered globose conidia (FIG. 3, *A, B*) when the Petri plate culture is 10 to 15 days old and has become overgrown to some extent by alien molds. Each elongated conidium (FIG. 3, *C, a–p*) produced by a uninucleated globose conidium receives the single nucleus of its parent. Although on a fresh substratum a uninucleated adhesive conidium may put forth a broad hypha (FIG. 3, *D*) capable of growing into a mycelium, in aging Petri plate cultures it more usually gives rise to a slender conidiophore on which another uninucleated adhesive conidium is produced. Successive generations of globose and of elongated conidia thus become intermingled everywhere

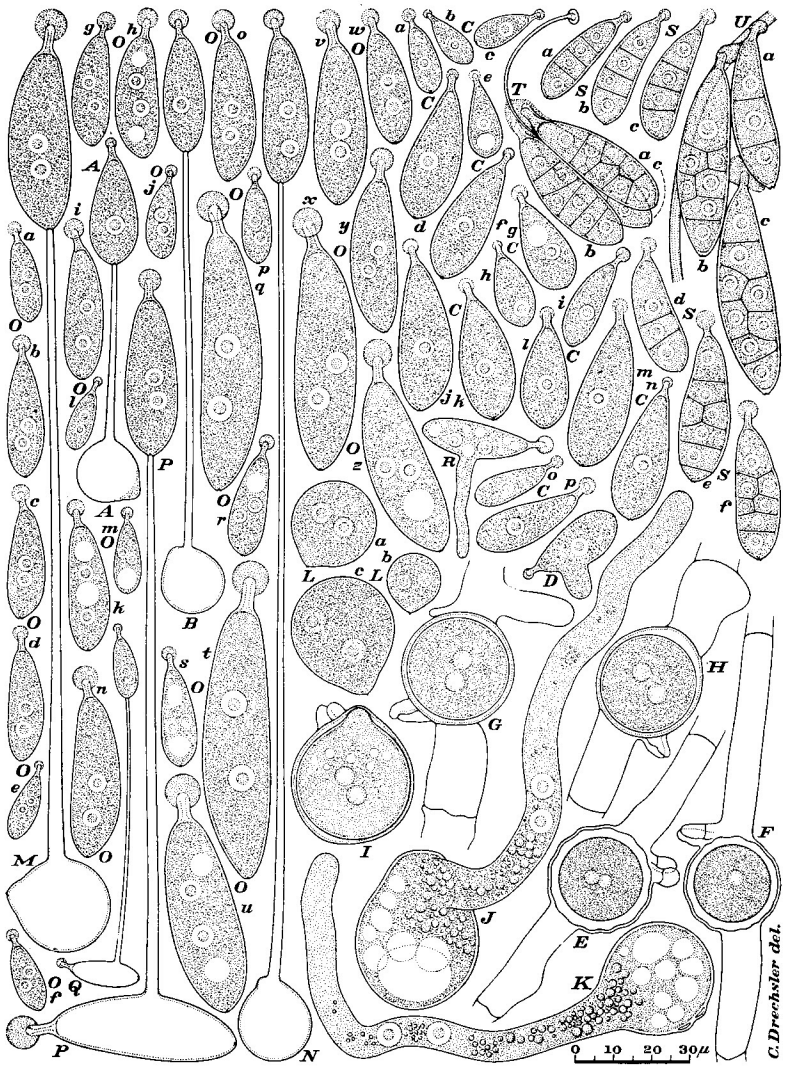

FIG. 3, *A–U.*

FIG. 3. *Basidiobolus ranarum* as found in maize-meal agar cultures, × 500. *A, B.* Globose conidia, each bearing a uninucleated adhesive conidium. *C.* Detached uninucleated adhesive conidia, *a–p.* *D.* Uninucleated adhesive conidium with broad germ tube. *E, F.* Mature zygospores showing no separation of wall into layers. *G, H.* Zygospores ready to germinate. *I.* Zygospore beginning to germinate. *J, K.* Zygospores, each with germ hypha showing 2 nuclei. *L.* Globose conidia, *a–c,* each with 2 nuclei. *M, N.* Globose conidia that have each produced

on the cultures, the uninucleated living specimens being found scattered among the empty membranous envelopes of their ancestors.

After being held for 30 to 40 days at temperatures near 20° C, some maize-meal-agar plate cultures not seriously overgrown with species of *Penicillium* or other strongly antagonistic molds will usually show germinating zygospores here and there. During an ensuing period of 10 to 20 days many other zygospores may likewise germinate, so that in cultures 50 to 60 days old most of the zygospores present originally may be represented only by their empty envelopes. Vegetative development being precluded, the broad germ tube extended in each instance grows out into a phototropic conidiophore which later shoots off a globose binucleated conidium (FIG. 3, *L, a–c*). This conidium, as might be expected, may give rise to a phototropic conidiophore that eventually discharges a secondary globose conidium containing 2 nuclei; or it may send up a slender erect conidiophore bearing aloft an elongated binucleated conidium with an adhesive beak (FIG. 3, *M, N*). Binucleated adhesive conidia thus formed, much like uninucleated adhesive conidia, become detached (FIG. 1, *Q–W;* FIG. 3, *O, a–z*) on relatively slight disturbance. In aging Petri plate cultures they commonly give rise individually to a slender conidiophore on which another binucleated adhesive conidium is produced (FIG. 3, *P, Q*), though they are capable of germinating vegetatively (FIG. 3, *R*) on a fresh substratum. Through continued repetitional development successive generations of binucleated conidia of both the globose and the elongated type are produced among globose and elongated uninucleated conidia derived earlier from hyphal segments. The distinction between uninucleated (FIG. 3, *C, a–c*) and binucleated (FIG. 3, *O, e, f, l, m*) individuals persists recognizably among the dwarfish adhesive conidia found in Petri plate cultures 5 to 6 months old.

While the adhesive conidial state of the odorous *Basidiobolus* appears remarkable in persisting through many generations despite the presence of alien microrganisms, it occurs also in pure cultures. In tube cultures of maize-meal-agar adhesive conidia are formed only rarely and sparingly on the slanted surface of the substratum, but are produced somewhat more often and in greater numbers on the glass

a binucleated adhesive conidium. *O*. Detached binucleated adhesive conidia, *a–z*. *P, Q*. Adhesive conidia that have each produced a binucleated adhesive conidium. *R*. Binucleated adhesive conidium germinating. *S*. Detached adhesive conidia, *a–f*, largely or wholly converted into sporangia. *T, U*. Groups of 3 adhesive conidia, *a–c*, fastened to bristle of a mite, the several conidia being largely or wholly converted into sporangia.

surface opposite the substratum. On the glass surface, besides, they more usually are converted into sporangia. In undergoing such conversion the smaller adhesive conidia become divided by several transverse walls (FIG. 3, *S, a–d*), whereas the larger ones become divided not only by transverse but also by longitudinal walls (FIG. 3, *S, e, f*). The resulting segments, or sporangiospores, always contain a single readily visible nucleus. After their release from the sporangial envelope they usually become more rounded. Many that acquire a nearly globose shape will then measure approximately 10 μ in diameter.

On the whole, sporangial segmentation in the odorous *Basidiobolus* takes place less abundantly and less regularly than in *B. meristosporus*. In Petri plate cultures infested with mites the frequently numerous elongated conidia adhering firmly to the hairs or bristles (FIG. 3, *T, a–c; U, a–c*) of the animals usually are found either converted into sporangia or in process of undergoing segmentation, though the conidia not affixed to mites may show segmentation in only a few instances. It can hardly be doubted that the burdened animals stimulate sporangial development. A chemical stimulus might be received more especially by conidia which are attached directly to a hair or bristle (FIG. 3, *T, a–c; U, a, b*). As segmentation takes place also in conidia (FIG. 3, *U, c*) that adhere to affixed conidia and consequently are not in direct contact with the mite, it seems not impossible that the gentle mechanical disturbance resulting from the locomotion of the burdened animal may have some influence on sporangial development.

If nutrients are wholly lacking, a secondary globose conidium of *Basidiobolus ranarum,* according to Eidam (**7**: 218, lines 4–13), occasionally gives rise on an extraordinarily slender conidiophore to a distal enlargement that after receiving the entire mass of protoplasmic materials puts forth at its apex a small tertiary conidium which may stop developing early in an immature unfinished state. Despite the puzzling implication conveyed in Eidam's text that the tertiary conidium, when not arrested in its development, would normally receive all the protoplasmic contents of the enlargement borne distally on the slender conidiophore, his revelant illustration (**7**: Pl. 9, Fig. 16) unquestionably represents a globose conidium that has given rise on a slender conidiophore to an elongated conidium with an adhesive beak. From the magnification indicated for the illustration the elongated conidium measures about 47 μ in length and 15 μ in greatest width. These measurements, which differ little from the measurements of the 2 conidia shown in FIG. 3, *C, j, m,* are approximately median in the dimensional ranges of the adhesive conidia produced by the odorous *Basidiobolus*—18 to 83 μ for length and 6 to 22 μ for greatest width.

The mites infesting old Petri plate cultures of the odorous *Basidiobolus* usually have numerous adhesive sporangia and conidia attached to their bristles, some of the animals being found laden with more than a hundred of the easily recognizable reproductive bodies. Only occasionally is a globose conidium found attached to a mite, the infrequent instances of such attachment resulting from accidental contact of the globose conidium with the adhesive material at the tip of an affixed elongated conidium. It may be presumed that under natural conditions the mites, spiders, and insects which habitually infest decaying plant materials likewise become somewhat abundantly contaminated with adhesive conidia and sporangia. Infection of the frogs that devour contaminated arthropods would seem therefore to come about very largely from the elongated reproductive bodies, and only in small measure from globose conidia. There is good reason to believe that if Eidam had known the true nature of the distended bodies he found borne aloft on slender conidiophores he would not have been so consistently unsuccessful in recognizing *B. ranarum* in the varied contents taken freshly from the stomach and intestines of frogs, and would have been able to explain much better how the fungus gains entrance into a frog's digestive organs.

The odorous *Basidiobolus,* unlike *B. meristosporus* and *B. haptosporus,* agrees well with *B. ranarum* in the undulated sculpture of its zygospores. It also agrees rather satisfactorily with the original characterization of *B. ranarum* in all other important aspects of morphology. In view of its abundant development in Maryland and Virginia before the onset of hot mid-summer weather, it would seem very well adapted to the cooler summer climate of central Europe where Eidam carried on his investigations. The odorous *Basidiobolus* occurring widely in the United States is accordingly held to be identical with *B. ranarum.*

GROWTH CHARACTERISTICS AND SEXUAL REPRODUCTION OF BASIDIOBOLUS HAPTOSPORUS

The original account (2) wherein *Basidiobolus haptosporus* is presented as a new species was based on an assortment of globose conidia, slender conidiophores, and elongated adhesive conidia found in a maize-meal-agar plate culture that after being planted with leaf mold in February, 1946, was kept tightly covered under a battery jar at temperatures between 18° and 20° C. From the small size of the globose conidia—the empty envelopes of these bodies (2: Figs. 1–4: a) commonly measured less than 25 μ in diameter—and the relatively low temperatures at which they developed, the assortment of asexual reproductive

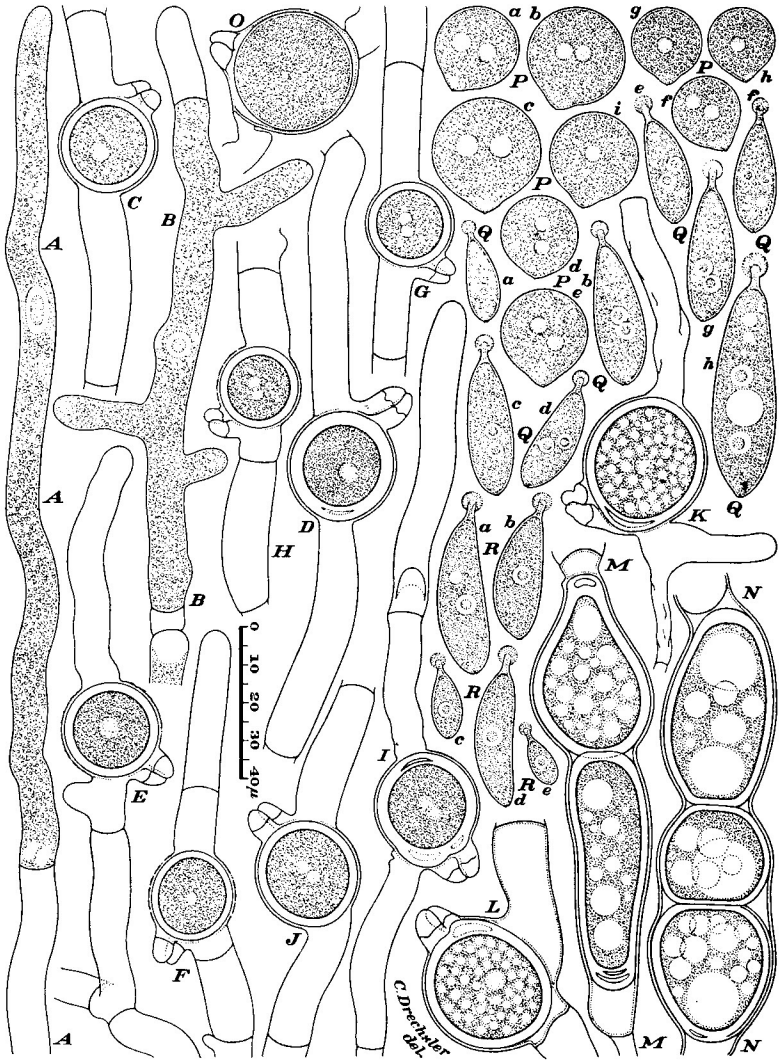

FIG. 4, *A–R*.

FIG. 4. *Basidiobolus haptosporus* (Lubber Run Park isolation) as found in maize-meal agar cultures, × 500. *A*. Terminal portion of hypha at margin of a growing mycelium. *B*. Terminal portion of hypha at margin of a mycelium not actively expanding. *C–J*. Mature zygospores produced in a Petri plate culture. *K, L*. Larger zygospores produced at a depth of 3 mm in a tube culture. *M, N*. Chlamydospores produced in a tube culture at a depth of 5 mm. *O*. Zygospore ready to germinate. *P*. Globose conidia among which six, *a–f*, show 2 nuclei, and

apparatus evidently belonged to the same species as the beaked smooth zygospores I have observed from time to time in maize-meal-agar cultures prepared for the isolation of oomycetous parasites from decaying roots (**6**). The fungus was first obtained in pure culture by canopying Petri plates of maize-meal-agar with leaf mold gathered in moist deciduous woods near Beltsville, Maryland, in December, 1951 (**3, 4**). More than a score of additional isolations have since been obtained from isolation plate cultures canopied with leaf mold or other plant detritus collected in different localities on different dates, as follows: near Fort Myer in Arlington, Virginia, on January 22, 1952; in Lubber Run Park in Arlington, Virginia, on February 28, 1952; near Criglersville, Virginia, on March 23, 1952; near Marriotsville, Maryland, on March 18, 1952; near Middletown, Delaware, on February 27, 1953; and near Gumboro, Delaware, on March 7, 1953.

A mycelium of *Basidiobolus haptosporus* growing actively at temperatures between 18° and 20° C is often hardly visible to the naked eye. On careful scrutiny, however, a somewhat opaque circular band can be distinguished at its periphery. As the mycelium enlarges the circular band expands. The widening central area presents much the same appearance as the unoccupied substratum outside the band. After the band reaches the rim of the Petri dish it vanishes progressively, with the result that soon the culture again looks like a newly poured agar plate. In strong contrast to *B. meristosporus* the fungus does not usually form aerial hyphae in visible quantity. Development of subsidiary mycelia in the expanse of substratum extending toward the main source of light has not been observed often in cultures of *B. haptosporus*.

When the peripheral band of an actively growing mycelium is examined under a microscope, it is found to consist of the vegetative distal portions of radially arranged hyphae. The terminal segments of these hyphae (FIG. 4, *A*) often exceed 200 μ in length, and commonly vary from 8 to 11 μ in thickness. As they elongate in advancing the mycelial forefront they divide repeatedly, thereby cutting off one segment after another. If its elongation is obstructed a terminal segment (FIG. 4, *B*) may like many intercalary segments put forth one or more lateral branches. In a mycelium that has originated from a germinating conidium, the hyphal segments near the empty conidial envelope may measure 15 to 20 μ in width.

Along the inner margin of the visible band at the periphery of a

three, *g–i*, show 1 nucleus. *Q*. Adhesive conidia, *a–h*, each containing 2 nuclei. *R*. Adhesive conidia, *a–e*, each containing a single nucleus.

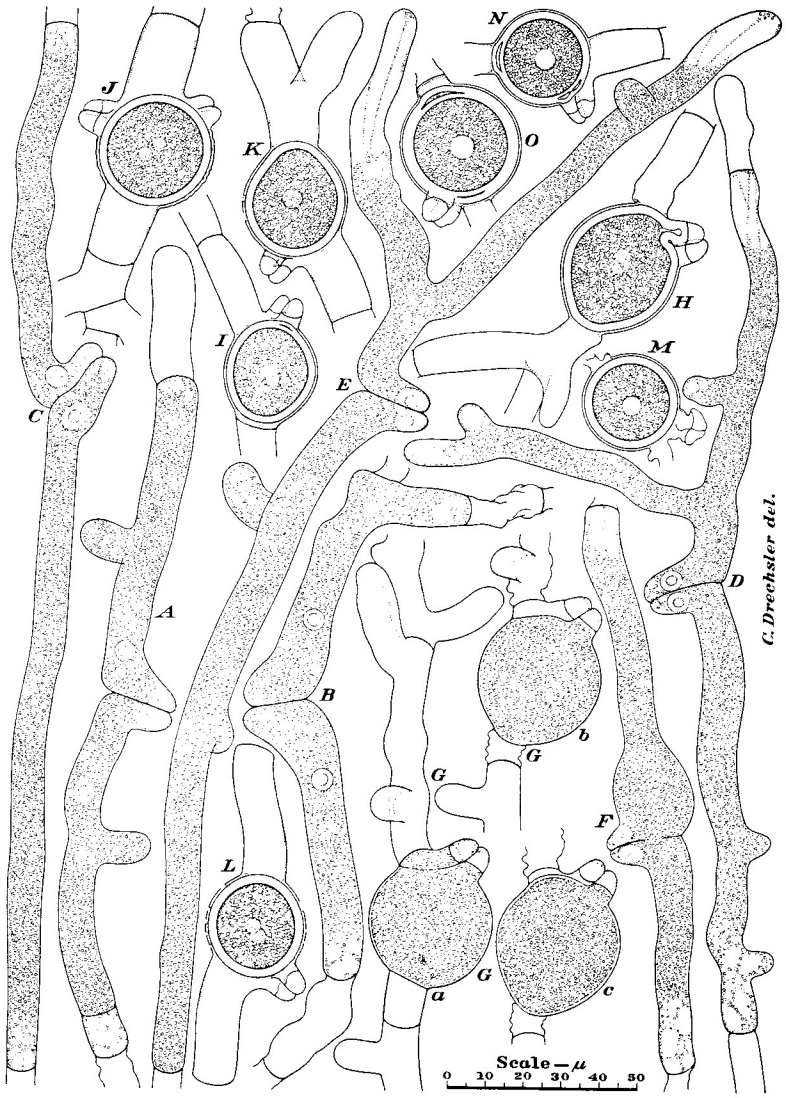

FIG. 5, *A–O*.

FIG. 5. *Basidiobolus haptosporus* (Lubber Run Park isolation) as found in maize-meal agar cultures, × 500. *A–E*. Young units of sexual reproductive apparatus; in *A* and *B* the 2 nuclei are moving toward paired protuberances, in *C* they have reached protuberances, in *D* they have entered protuberances, in *E* they have reached the tips. *F*. Sexual unit showing enlargement in which zygospore will develop. *G*. Sexual unit showing final stages, *a–c*, in conjugation. *H–O*. Zygo-

growing mycelium, paired hyphal segments are found in various stages of conjugation. Much as in other members of the genus, the paired segments extend juxtaposed protuberances (FIG. 5, *A, B*) into which the nuclei migrate (FIG. 5, *C–F*) to undergo division; the tip of each protuberance, partly filled with degenerating nuclear materials, being subsequently walled off. An aperture having meanwhile been formed in the cross-wall between the two segments, the protoplasm and nuclei of both segments collect in an enlargement (FIG. 5, *G, a–c*) on one side of the perforated partition. The enlargement, or fusion cell, now gradually undergoes conversion into a zygospore. At maturity the zygospore (FIG. 4, *C–L;* FIG. 5, *H–O*) is surrounded by a thick smooth wall which in most instances appears closely adnate to the thin hyphal membrane, so that the thin membrane can be distinguished clearly only where it would seem to have become fissured (FIG. 4, *E, F, H;* FIG. 5, *K, L*) or wrinkled (FIG. 5, *J*). The zygospore wall proper is rarely found separated throughout into 2 layers. It commonly either is solid throughout or shows localized separation into 2 layers in relatively small regions at the poles or under the protuberances (FIG. 4, *D, I, K, L;* FIG. 5, *I, N, O*). Some mature zygospores contain protoplasm of coarsely granular texture (FIG. 4, *C–J;* FIG. 5, *H–O*), while others seem filled with strongly globuliferous cytoplasm (FIG. 4, *K, L*). In many zygospores (FIG. 4, *C–F, K, L;* FIG. 5, *K, M, N, O*) a single nucleus can be made out clearly, whereas in others (FIG. 4, *G–J;* FIG. 5, *H–J, L*) two nuclei loom indistinctly through the dense protoplasm.

Sexual reproduction in *Basidiobolus haptosporus* appears to proceed less freely at depths of 2 to 3 mm than near the surface of the substratum. At depths of 4 to 5 mm in slanted tubes of maize-meal agar, zygospore formation is often completely inhibited, the submerged hyphal segments then usually becoming markedly distended and indurated. In many instances the swollen segments appear to contain two or more thick-walled endogenous chlamydospores (FIG. 4, *M, N*). The variously indurated cells seem to resemble rather closely the swollen thick-walled terminal cells that Eidam (**7**: 239, Pl. 12, Fig. 24) observed in preparations of his *B. lacertae*. They invite comparison, besides, with the thick-walled cells Raciborski (**14**: 114) obtained in glycerine cultures of *B. ranarum,* as well as with the resting stage (Dauerzustände) which Levisohn (**11**: 523) observed in cultures of *B. ranarum* amply supplied with air and contaminated with bacteria and molds.

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spores and adjoining empty membranous parts, showing irregular zygospore wall in *H,* minute wrinkling or fissuring of the zygosporangial envelope in *J–L,* and localized separation of zygospore wall into 2 layers in *I, N, O.*

If maize-meal agar plate cultures of *Basidiobolus haptosporus* are protected against excessive evaporation by being covered tightly with a battery jar, many zygospores (FIG. 4, *O*) may be found after 50 to 60 days to have largely resorbed the thick wall present during the resting period. Where contamination by antagonistic molds is not too serious these after-ripened zygospores will germinate freely without irrigation or removal from the staled substratum, each putting forth a germ hypha terminating in a phototropic conidiophore from which eventually a globose conidium is shot off. As far as could be determined from unstained living specimens all globose conidia thus produced contain 2 nuclei (FIG. 4, *P, a–f*). Two nuclei are always discernible in elongated adhesive conidia (FIG. 4, *Q, a-h*) produced on slender conidiophores extended singly from globose conidia of zygosporic origin. Here, as also in *B. meristosporus* and *B. ranarum,* the binucleated condition will persist through many successive generations of repetitional development, being terminated, however, when the conidium becomes transformed into a sporangium through internal segmentation, or undergoes a single division preparatory to the production of a zygospore.

In *Basidiobolus haptosporus* conidia are produced far more abundantly from germinating zygospores than from hyphal segments. Often not a single broad conidiophore can be discovered when an extensive and actively growing mycelium is thoroughly explored under a microscope. Apparently in many Petri plate cultures of *B. haptosporus* asexual reproduction is wholly absent during the period of vegetative growth, all hyphal segments contributing their contents to the formation of zygospores. Yet now and then a small number of detached globose conidia are found scattered about in Petri plate cultures only a few days old. As far as could be determined from examination of unstained living specimens, the globose conidia in such young cultures always contain a single nucleus (FIG. 4, *P, g–i*). Only a single nucleus can be distinguished in the few elongated adhesive conidia (FIG. 4, *R, a–e*) sometimes found in cultures less than 20 days old. Since after-ripened zygospores have never been observed in cultures so young, these adhesive conidia must derive from globose conidia of mycelial origin.

The production of adhesive conidia can obviously no longer be held to distinguish *Basidiobolus haptosporus* as a separate species, as such conidia are now known to be formed also in *B. meristosporus* and *B. ranarum.* Nor can a diagnostic difference be recognized any longer in the difference between the strongly tapering shape of the terminal enlargement figured by Eidam (**7**: Pl. 9, Fig. 16) and the gently tapering

shape of the adhesive conidia present in the material on which my original description of *B. haptosporus* was based. In *B. haptosporus* and *B. meristosporus,* no less than in *B. ranarum,* a strongly tapering shape is usual among adhesive conidia formed on the glass surface opposite the slanted substratum in tube cultures, and would seem to result from dry conditions. Furthermore, in maize-meal-agar cultures of all 3 species a gently tapering shape is usual among adhesive conidia produced by parent conidia lying directly on the moist substratum, and must consequently be held to result from moist conditions. Presumably owing to lack of water the strongly tapering conidia formed on a glass surface by any of the 3 species are very sparingly tipped with sticky material, whereas gently tapering conidia of all 3 species commonly bear a massive globule of yellow adhesive substance at the apex. In *B. haptosporus* the adhesive conidia, as also the globose conidia and zygospores, do not attain maximum dimensions quite as large as in the generally more robust *B. ranarum.* They seem approximately equal in size to the adhesive conidia of *B. meristosporus,* but apparently are less prone to become segmented into sporangia.

Basidiobolus haptosporus is adequately distinguished from *B. ranarum* by its production of smooth rather than undulated zygospores, by its very meager production of conidia from its hyphal segments, and by its lack of any *Streptomyces*-like odor. It is clearly separated from *B. meristosporus* by its adaptation to lower temperatures, by its meager production of conidia from hyphal segments, and by its failure usually to produce aerial mycelium. It differs from the description of *B. myxophilus* R. E. Fries (**9**) in its smooth zygospores, and from the description of *B. lacertae* in its well-developed paired protuberances, which normally show a median septum. A revised diagnosis incorporating information on its vegetative development and its sexual reproduction may prove helpful in construing the species correctly.

BASIDIOBOLUS HAPTOSPORUS Drechsl. emend. Drechsl.

Mycelium inconspicuum, vulgo non in aerem visibiliter crescens, incoloratum; hyphis sterilibus ramosis, 3–20 μ (plerumque 8–11 μ) crassis, mox septatis, hic illic disjunctis, cellulis eorum plerumque 35–250 μ longis, uno nucleo visibili praeditis. Primiformibus fertilibus hyphis singulatim raro ex cellulis mycelii sed saepe ex conidiis vel ex zygosporis surgentibus, incoloratis, simplicibus, basi interdum 3.5–6 μ latis, in aerem vulgo 75–175 μ ad lucem protendentibus, sursum in tumorem jaculatorium aliquando 30–40 μ longum, 15–20 μ latum inflatis, apice unum primiforme conidium ferentibus, denique hoc violenter adjicentibus; primiformibus conidiis globosis sed basi ad instar mammiculae prominulis, plerumque 16–30 μ in diametro. Hyphis formae gracilis fertilibus ex primiformibus vel tenacibus conidiis

nec umquam ex cellulis mycelii surgentibus, incoloratis, rectis, 50–325 μ longis, basi 1.5–4.5 μ crassis, sursum leniter attenuatis, apice 1–2 μ latis, ibi unum conidium tenax ferentibus. Tenacibus conidiis in totum 17–73 μ longis, ex infera viventi cellula et supero glutinoso rostro constantibus; glutinoso rostro flavido, tubulato, 3–8 μ longo, sursum 1–2.5 μ lato, apice vulgo guttula materiae glutinosae flavae 2–10 μ crassa vestito; viventi cellula incolorata, elongato-ellipsoidea, recta vel leviter curvata, 13–61 μ longis, 6–18 μ latis, uno nucleo vel duobus nucleis instructis, quandoque in sporangium transeunte; sporis uno nucleo praeditis, incoloratis, primo disciformibus vel dolioformibus, postea plus minusve rotundatis et saepe circa 10 μ in diametro. Zygosporis ex conjugio duarum cellularum contiguarum oriundis, globosis vel elongato-ellipsoideis, plerumque 23–37 μ longis, 21–34 μ latis, muro levi saepe aliquid flavido 2–3.5 μ crasso circumdatis.

Habitat in materiis plantarum putrescentibus in Virginia, Maryland, Delaware, Wisconsin.

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