

THREE ZOÖPAGACEAE THAT SUBSIST BY CAPTURING SOIL AMOEBAE

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(WITH 4 FIGURES)

As the unusually cool humid weather that prevailed during the summer of 1946 in Maryland, Virginia, and North Carolina furnished conditions of temperature and moisture under which the fungi destructive to eelworms and rhizopods have been found developing most abundantly in the laboratory, it was expected that decaying plant materials collected in later months might yield many zoöpagaceous forms not ordinarily encountered. However, when vegetable detritus taken from woods, fields, and gardens, in various localities within the states mentioned, was planted on Petri plates of maizemeal agar suitably permeated with *Pythium* mycelium, the resulting cultures were in most instances ruined through an overwhelming development of plasmodia belonging to different species of Myxomycetes. Usually these plasmodia suppressed multiplication of rhizopods and eelworms at an early stage, and thereby forestalled virtually all development of fungi habitually subsisting on these animals. In other instances zoöpagaceous fungi became established and began to sporulate in noticeable quantity, but would then be obliterated under shifting streams of slime. In a few cultures, fortunately, plasmodia failed to develop; and here abundant multiplication of rhizopods and eelworms took place, together with development of fungi destructive to them. Among these fungi were observed the three predaceous forms herein described as new members of the Zoöpagaceae.

Trouble from plasmodia of Myxomycetes has been experienced now and then in earlier years, though not on a serious scale. Or-

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dinarily cultures prepared with decaying plant materials collected outdoors in Maryland and Virginia are less likely to be ruined by slime molds than from infestation with mites, annelids, tardigrades, or rotifers. Mites have been found especially troublesome in cultures planted with decaying material taken from greenhouses, or with decaying plant detritus collected outdoors in such warm states as Florida and Louisiana. While these pests do not necessarily prevent vegetative development of nematode-destroying and rhizopod-destroying fungi deep under the surface of an agar culture, they usually grind up the surface layer so badly by their manner of feeding that the reproductive apparatus of all except the most robust Zoöpagaceae is broken up beyond all hope of recognition, and often the conidial apparatus of the sturdiest forms is mangled so severely as to render it unfit for study. The use of herbarium poisons, such as naphthalene and paradichlorobenzene, in mite-infested cultures has not been found advantageous since rhizopods and nematodes succumb quickly in the presence of these volatile compounds. Storage of the cultures at low temperatures similarly entails some disadvantage, for thereby not only the infesting mites but also the microscopic animals and associated fungi similarly preferring warm conditions—the very forms, indeed, that could be expected to be most characteristic of mite-infested material—are largely if not wholly suppressed.

The minute annelids that frequently appear in Petri plate cultures five to ten days after decaying material has been added cause even more serious damage than mites, since they burrow through the agar at all depths, creating havoc in their paths. These unwanted animals seem to hatch most quickly when a rather soft agar medium is employed, or when some free liquid water collects around the material planted, yet may become troublesome even when firm agar is employed and when little moisture condenses. Difficulty with them may be largely obviated, as a rule, by taking care in collecting leaf mold or other decaying plant detritus to include no considerable admixture of sand, clay, loam, or gravel from the underlying soil. Destruction by annelids is exceedingly frequent in cultures planted with ordinary soil from gardens and fields. Despite the manifestly thoroughgoing distribution of the Zoöpagaceae on all arable land, the difficulties attending annelid

infestation have strongly discouraged the use of firm soil in preparing cultures intended for the study of these fungi.

ANOTHER ACAULOPAGE WITH LONG SLENDER ROD-LIKE CONIDIA

Several maize-meal-agar plate cultures that when completely overgrown with mycelium of *Pythium ultimum* Trow had been further planted through addition of decaying detritus taken on August 20, 1946, from an old pile of weeds in a field near Mayo, Maryland, were found after twenty-seven days to contain a sparse branching mycelium composed of narrow colorless aseptate hyphae (FIG. 1, A-G) to which were attached many individual *Amoebae* generally similar to one another. The captives commonly measured from 20 to 40 μ in diameter when drawn into a moderately rounded shape. They were surrounded individually by a thin yet distinctly visible pellicle, which, except in regions where pseudopodia were being extended, was handsomely disposed in delicately rippled folds. Their protoplasm, though colorless and for the most part of only moderately densely granular texture, was reduced in transparency by numerous vacuole-like inclusions of globular or ellipsoidal shape. These inclusions were mostly less than 5 μ wide, but in some animals (FIG. 1, C, D, E) one or two among them measured about 10 μ in their greatest dimension and thus rivaled the contractile vacuole (FIG. 1, A-G: v) in size. Some animals contained, besides, one or more commonplace digestive vacuoles (FIG. 1, A, E). While the single nucleus (FIG. 1, A-G: n) appeared to be generally of prolate ellipsoidal shape, the difference between its length and its width was often relatively small. In comparison with the size of the animal the nucleus seemed unusually large, its length varying commonly from 8 to 12.5 μ , and its width from 7 to 11 μ . It showed, distributed in peripheral positions, some 35 to 50 globose or slightly flattened bodies about 1 μ or slightly less in width. Among the *Amoebae* previously reported as being destroyed by members of the Zoöpagaceae the animal here concerned seemed to resemble most the species found habitually captured by my *Stylopaga rhabdospora* (5: 374-377) and my *S. cephalote* (7: 144-148). However the obscurely globuliferous structure of the protoplasm, together with

the apparently larger number and smaller size of the chromatin bodies, gives reason for presuming that the animal is specifically distinct not only from the one earlier observed serving as prey for the two species of *Stylopage*, but also from the one found parasitized by my *Cochlonema euryblastum* (10: 283-289). In the *Amoeba* parasitized by *C. euryblastum*, besides, the nucleus appeared appreciably smaller relative to the entire animal than in the *Amoeba* of globuliferous protoplasmic texture.

Following narrow perforation of its integument each captured *Amoeba* was soon invaded by a haustorium (FIG. 1, A-E), or at times by two haustoria (FIG. 1, F, a, b; G, a, b). As in *Stylopage rhabdospora* the absorptive organ here was of the familiar pedicellate type. Ramification of the assimilative elements was mainly dichotomous, though irregularity of branching appeared rather often, especially in the more elaborately developed haustoria. The number of terminal branches occasionally exceeded sixteen (FIG. 1, F, b; G, b). The assimilative elements appeared generally to be of about the same width as mycelial hyphae. Owing to the globuliferous character of the animal's protoplasm the haustorium was only somewhat indistinctly visible during the earlier stage of its development, but after most of the protoplasm had been assimilated it emerged more clearly into view (FIG. 1, H, I). Later, when the *Amoeba* was depleted of its digestible substance, the contents of the haustorium were withdrawn backward into the parent hypha; the empty tubular envelope thereupon soon fading from sight together with the empty pellicle loosely surrounding it.

In one of the plate cultures an area occupied by mycelium of the fungus showed a few captured *Amoebae* (FIG. 1, J, K) which, though of about the same size as those taken in large numbers, were distinguished by protoplasm of commonplace granular texture and by a smaller nucleus (FIG. 1, J, n; K, n) that contained a single central endosome instead of many peripheral bodies. Despite careful scrutiny it was not possible to trace any mycelial connection between the hyphae holding fast these few animals of a different species and the hyphae holding fast the much more numerous captives. The fungus represented in these latter hyphae, therefore, cannot yet be reckoned among the small number of zoö-

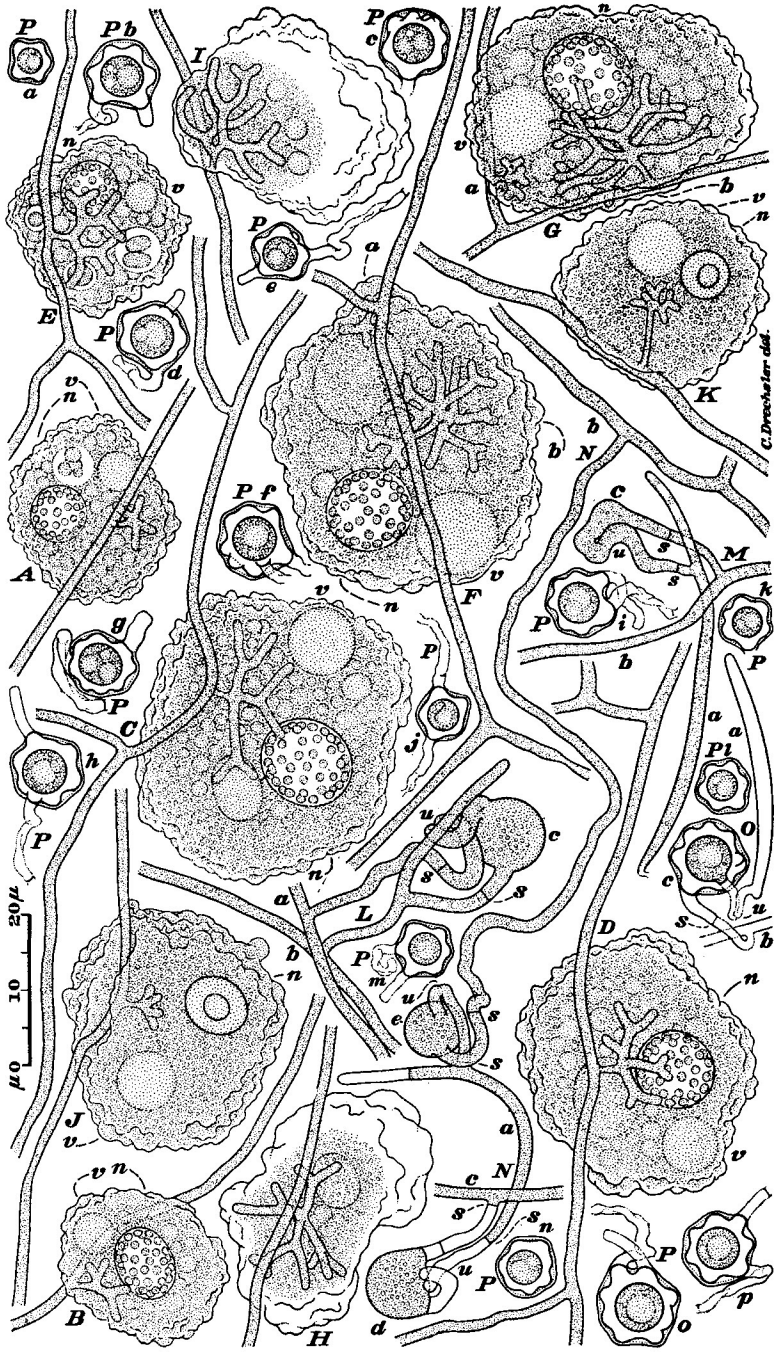


FIG. 1. *Acaulopage baculispora*.

pagaceous forms definitely known to subsist on two or more distinct species of rhizopods.

The fungus gave rise abundantly to both asexual and sexual reproductive apparatus. In initiating asexual reproduction a prostrate mycelial filament would at mostly rather short intervals put forth erect narrow processes that in their upward growth widened out until they had attained a height of about $15\ \mu$ (FIG. 2, *A, a*). Thereupon they continued elongating either at a uniform width, or at a slightly diminishing width, until on attaining a definitive length of 25 to $65\ \mu$ (FIG. 2, *A, b*) a cross-wall was formed a few microns above the base to delimit, in each instance, a rod-shaped or somewhat filamentous erect conidium from the short, often slightly tapering sterigma below (FIG. 2, *A, c-g*; *B, a-e*). Collectively the conidia in an undisturbed sporulating tract offered a handsome bristling display. They readily became disjointed, however, when they were jostled by roving eelworms or rotifers. The procumbent mycelial hyphae, including those beset with numerous denuded sterigmata (FIG. 2, *C, a-e*), came thereby to be partly obscured amid a disorderly confusion of detached spores (FIG. 2, *D, a-z*; *E, a-w*). Many of the spores soon germinated by putting forth a germ tube somewhat obliquely from the basal or the distal end (FIG. 2, *F*).

Although in some measure sexual reproduction took place rather early through union of gametangia borne on branches contributed from two mycelial hyphae (FIG. 1, *L, a, b*), it proceeded far more briskly after the surface of the substratum was liberally strewn with detached conidia. In the species here concerned, as in many related forms, most units of sexual apparatus originated from pairs of gametangia whereof one came from a germinating conidium (FIG. 1, *M, a*; *N, a*; *O, a*. FIG. 2, *Ga, a*; *H, a*; *I, a*) while the other came from a mycelial filament (FIG. 1, *M, b*; *N, b, c*; *O, b*. FIG. 2, *G, b*; *H, b*; *I, b*). The paired gametangia appeared to become delimited by basal cross-walls (FIG. 1, *L, s*; *M, s*; *N, s*; *O, s*. FIG. 2, *G, s*; *H, s*; *I, s*) at about the same time they began to fuse at their tips. Sometimes a gametangium contributed from the mycelium was found borne on a branch so short as to appear virtually sessile on the mycelial hypha (FIG. 1, *N, c*. FIG. 2, *G, b*; *H, b*); frequently it was borne on a branch 5 to $25\ \mu$ in length

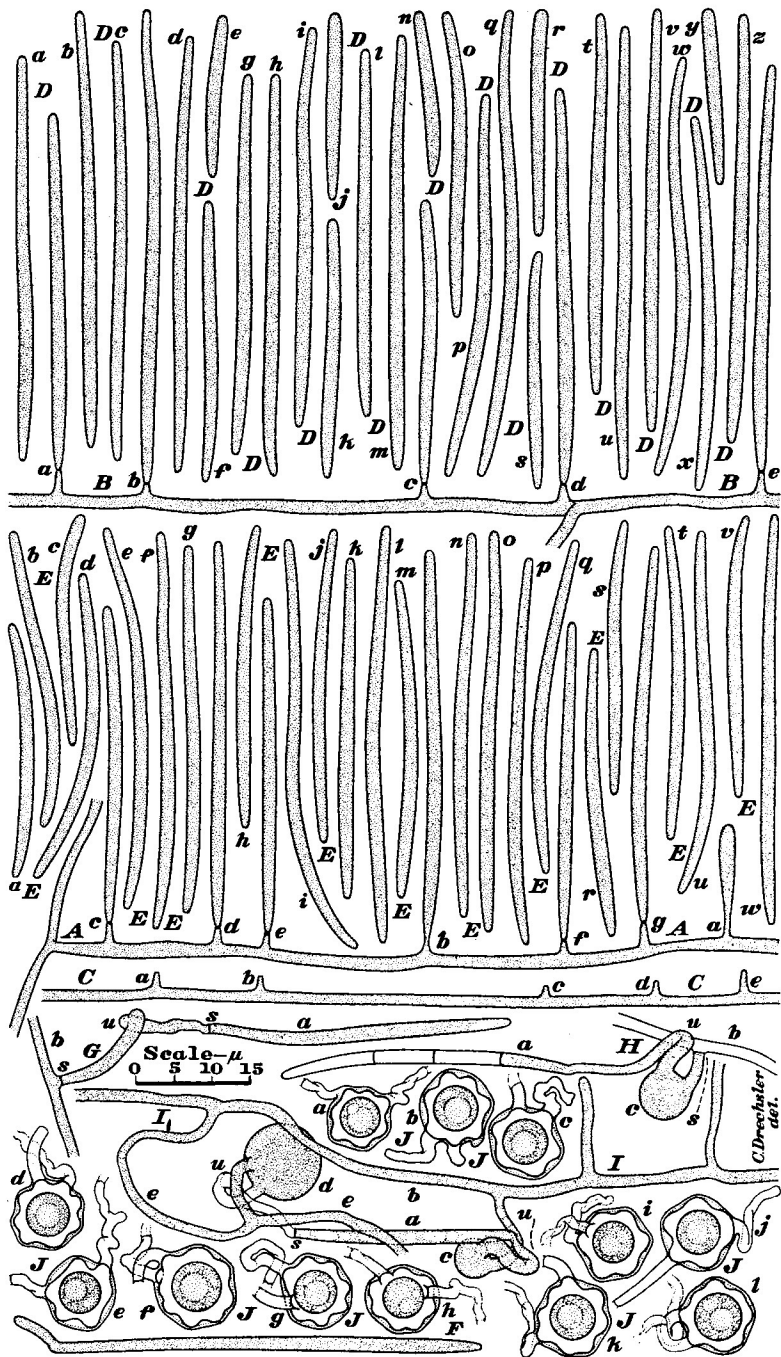


FIG. 2. *Acaulopage baculispora*.

(FIG. 1, *L*, *a*, *b*; *M*, *b*; *O*, *b*); and occasionally it terminated a branch about 100 μ long (FIG. 1, *N*, *b*). Gametangia of conidial origin often were borne on a short proximal portion of the germ hypha (FIG. 1, *M*, *a*), but frequently, again, they included a portion of the spore itself (FIG. 2, *G*, *a*; *I*, *a*), and sometimes, indeed, the entire spore seemed to function as a sexual cell (FIG. 1, *O*, *a*. FIG. 2, *H*, *a*). Often a conidium collaborated in the production of two units of sexual apparatus. In these instances it would sometimes extend a zygothoric germ tube from each end (FIG. 2, *I*, *a*), but at other times it would extend one such germ tube from an end position while putting forth the other from a median position (FIG. 1, *N*, *a*).

After the paired gametangia had fused, one of them—very commonly the one of mycelial origin—would form a globose swelling (FIG. 1, *M*, *c*) usually at a distance of 5 to 10 μ from the place of union (FIG. 1, *M*, *u*). This swelling continued to expand (FIG. 1, *L*, *c*. FIG. 2, *H*, *c*; *I*, *c*, *d*) until the remaining portion of the fusion cell was empty of protoplasmic material. The spherical zygosporangium thus brought into being (FIG. 1, *N*, *d*) then underwent the usual internal changes whereby its cellular contents were converted into a zygospore with a thick, boldly verrucose, distinctly yellowish wall. In mature units of sexual apparatus the zygospore, loosely enveloped by the somewhat collapsed sporangial membrane, contained a spherical protoplast wherein a granular parietal layer surrounded a central reserve globule, or sometimes two reserve globules (FIG. 1, *P*, *a-p*. FIG. 2, *J*, *a-l*). Often, besides, a smaller lustrous body, apparently corresponding to the refringent body present in mature oöspores of many oömycetes, seemed indistinctly visible in the granular layer.

In the genus *Acaulopage*, to which the fungus is manifestly referable, it invites comparison especially with the three species I have described earlier under the binomials *A. macrospora* (2: 189-191), *A. stenospora* (9: 256-258), and *A. ischnospora* (12: 263-269), all of which give rise to narrow erect conidia on short sterigmata. It is clearly distinct from *A. ischnospora* by reason of its shorter conidia and their lack of an empty membranous appendage at the tip. From *A. stenospora* it differs very noticeably in the larger diameter of its conidia, for these bodies measure, on

an average, about 1.9μ in greatest width, whereas the average for the corresponding dimension in *A. stenospora* is about 1.4μ . From the conidia of *A. macrospora* those of the present fungus differ in their lack of any evident tendency either toward distal bifurcation or toward evacuation of contents from one of the ends. Besides, they generally taper upward less markedly, so that despite some measure of intergradation with respect to dimensions, they present a rather different appearance because of their more typically filamentous or rod-like shape. Their outward resemblance to walking sticks suggests for the fungus a specific epithet compounded in part of a word meaning "staff."

Acaulopage baculispora sp. nov.

Mycelium effusum; hyphis continuis, incoloratis, filiformibus, parce ramosis, plerumque $1-2 \mu$ crassis, ad animalia minuta inhaerentibus, pelliculam eorum perforantibus, haustorium (interdum 2 haustoria) intus evolventibus quod protoplasma exhaurit; haustorio pedicellato, pedicello vulgo $2-5 \mu$ longo, $0.6-1 \mu$ crasso, apice abrupte latescente, bis usque quater repetite bifurco, ita $4-16$ (interdum plures) ramulos assumentes divaricatos $2-12 \mu$ longos, $1-1.4 \mu$ crassos ferente. Sterigmata inter se saepe $5-50 \mu$ distantia, erecta, plerumque $1-3.5 \mu$ alta, basi $1-1.5 \mu$ crassa, sursum attenuata, apice circa 0.6μ vel 0.7μ crassa, unicum conidium ferentia; conidiis erectis, incoloratis, aliquid filiformibus, $21-62 \mu$ longis, $1.8-2.1 \mu$ latis, utroque parvulum attenuatis, apice abrupte rotundatis. Hyphae zygosporiferae irregulariter flexuosae, ambae interdum ex duabus hyphis mycelii exeuntes sed saepissime altera ex hypha mycelii altera ex conidio germinanti oriunda, quisque gametangium vulgo $10-20 \mu$ longum, $1.2-2.5 \mu$ crassum ferens. Zygosporangia primo levia, sphaeroidea, plerumque $7-12 \mu$ crassa, membrana eorum in maturitate circa zygosporam laxè collapsa; zygospora aliquantum flavida, globosa, $6-11 \mu$ crassa, valde verrucosa, membrana ejus $1-2.2 \mu$ crassa, cellulam viventem $3.5-6.5 \mu$ crassam circumdante.

Amoebas $20-40 \mu$ latas capiens consumensque habitat in herbis coacervatis putrescentibus prope Mayo, Maryland.

Mycelium spreading; vegetative hyphae aseptate, colorless, filamentous, sparingly branched, mostly $1-2 \mu$ wide, adhering to minute animals, penetrating the pellicle of each animal thus captured, and intruding a haustorium (sometimes 2 haustoria) to appropriate the protoplasmic contents; haustoria pedicellate, the pedicel commonly $2-5 \mu$ long and $0.6-1 \mu$ wide, abruptly enlarging and bifurcating 2 to 4 times and thus bearing 4 to 16 (sometimes more) divergent assimilative branches $2-12 \mu$ long and $1-1.4 \mu$ wide. Sterigmata arising from procumbent hyphae often at intervals of $5-50 \mu$, usually $1-3.5 \mu$ high, $1-1.5 \mu$ wide at the base, tapering upward to a

width of 0.6–0.7 μ at the tip, whereon is borne erectly a single conidium. Conidia colorless, somewhat filamentous, 21–62 μ long, 1.8–2.1 μ wide, tapering only slightly toward both ends, abruptly rounded at the tip, when detached somewhat rounded at the base. Zygothoric hyphae often irregularly flexuous, both of a pair occasionally arising from separate mycelial filaments, but much more often only one of them arising from a mycelial filament, the other being supplied from a germinating conidium, each furnishing a gametangium commonly 10–20 μ long and 1.2–2.5 μ wide; zygosporangium formed usually 5–10 μ from place of union between gametangia, at first smoothly subspherical and mostly measuring 7–12 μ in diameter, but its envelope at maturity collapsing loosely about the zygospore; the latter somewhat yellowish, globose, 6–11 μ in diameter, boldly verrucose, having a wall 1–2.2 μ thick which surrounds a living cell 3.5–6.5 μ in diameter.

Capturing and consuming a species of *Amoeba* commonly 20–40 μ wide it occurs in heaped decaying herbaceous plants near Mayo, Maryland.

In examining young sexual reproductive apparatus of *Acaulopage baculispora* (FIG. 2, I, e) as well as of other small members of the Zoöpagaceae I have been unable to see in some conjugating elements any cross-wall so placed that it might be considered to delimit a gametangium. While failure to observe a delimiting septum may often be held attributable to optical difficulties frequent in the study of minute objects, the possibility is not to be dismissed that in some instances a special delimiting septum may be absent. The function of such septa in the Zoöpagaceae seems a little problematical since in most members they evidently do not isolate the protoplasm of the gametangium from that of the adjoining portion of mycelial branch or adjoining portion of conidium. In most members of the family, as has been intimated previously (11: 26), a pair of conjugating gametangia together contain only a rather small portion of the protoplasmic material eventually needed for the development of the zygosporangium and zygospore; so that of necessity the larger portion must enter the fusion cell after cross-walls have been formed. It may be presumed, therefore, that in the beginning, at least, the delimiting walls here very probably are not complete partitions, but like the centrally perforated cross-walls in the higher fungi (1: 75–167), have an aperture large enough

for slow movement of protoplasm—a feature which assuredly need not be shared by the cross-walls laid down in the progressive evacuation of mycelial hyphae and conidia (FIG. 2, *A*). If the apertures inferred from the sequence of developmental events should be more or less variable with respect to size, it appears possible, in view of the narrowness of hyphae in most Zoöpagaceae, that now and then a wall might for a time consist of a peripheral annular thickening so minute as to escape detection. Indeed, it is conceivable that with very narrow hyphae the measure of seclusion ordinarily provided by delimiting septa might perhaps be available here and there without any special partitions being required.

A SPECIES OF ACAULOPAGE PRODUCING ELLIPSOIDAL CONIDIA WITH
LONG APICAL APPENDAGES

A maize-meal-agar plate culture which after being overgrown by *Pythium helicoides* Drechsl. had been further planted with decaying leaves collected on August 20, 1946, from the floor of deciduous woods near Mayo, Maryland, showed in eighteen days a species of *Acaulopage* reminiscent of the two forms I described earlier under the binomials *A. ceratospora* (2: 193–195) and *A. cercospora* (5: 371–374). Its scanty mycelium consisted of colorless, aseptate, sparingly branched hyphae measuring for the most part slightly less than $1\ \mu$ in width (FIG. 3, *A–D*). To these hyphae were found attached at irregular intervals small specimens of *Amoeba* (FIG. 3, *A*, *a–f*; *B*, *a–c*; *C*, *a*) varying in diameter from 6 to $21\ \mu$ when drawn into a somewhat rounded form. As the captured animals, like many other of the more minute *Amoebae* taken by predaceous hyphomycetes, seemed rather lacking in distinctiveness, they could not be satisfactorily identified. They were each enveloped by a very delicate pellicle, and they bore frequently a variable number of bacteria, which sometimes were visibly surrounded by small digestive vacuoles (FIG. 3, *A*, *d–f*). Within the larger specimens a globose or slightly elongated body measuring commonly 2.5 to $3\ \mu$ in total length and 2 to $2.5\ \mu$ in width (FIG. 3, *A*: *a*, *n*; *c*, *n*; *e*, *n*) seemed interpretable as a nucleus with a relatively large endosome. Many of the better developed captives (FIG. 3, *A*, *d*, *e*) were found invaded by a basally ramified bush-like haustorium composed of three or four assimilative branches

having about the same width as the mycelial hyphae. The smaller captives were often found invaded by only a single assimilative branch (FIG. 3, C, a). The delicate phycomycete was likewise restricted to intrusion of a single assimilative branch when, as occasionally happened, one of the larger specimens (FIG. 3, A, f), soon after being captured, was invaded adventitiously by a robust branch extended from a neighboring mycelial filament (FIG. 3, A, g) of the nematode-capturing hyphomycete *Dactylaria thaumasia* Drechsl. (6: 518-523) also present in the culture. Loss of protoplasm continued in all invaded animals until at a final stage of depletion the contents of the haustorium were withdrawn backward into the parent hypha; whereupon the empty tubular membranes soon faded from sight, together with the empty pellicle surrounding them.

Thus amply nourished on living animals the delicate phycomycete gave rise here and there to comparatively robust conidia. In initiating asexual reproduction the mycelial hyphae would put forth short branches that soon widened out rather abruptly to form an erect elongated-ellipsoidal part often about $15\ \mu$ long and from 5 to $6\ \mu$ wide. Through continued but markedly narrowed growth at its apex this swollen part came to be surmounted by a distal beak commonly 20 to $37\ \mu$ long and 1.3 to $1.9\ \mu$ wide (FIG. 3, B, d; C, b). At first the erect structure was filled uniformly with protoplasm throughout its length (FIG. 3, B, d). Later the beak began to show a vacuolated condition (FIG. 3, C, b); and this condition became more and more pronounced until all the protoplasmic material had been withdrawn from the beak into the swollen part below. A retaining wall was then formed at the base of the empty tubular membrane. At about the same time, if not earlier, another cross-wall was laid down between the swollen part and its supporting stalk (FIG. 3, D, a; E) which meanwhile, like the apical beak, had been emptied of contents. At this cross-wall separation took place on slight disturbance. Eventually numerous conidia consisting individually of an elongated-ellipsoidal living cell together with an empty tubular apical appendage were found strewn about in some abundance (FIG. 3, F, a-z; G, a-e). These detached conidia germinated rather freely by extending, often from a position close to the basal hilum (FIG. 3, H), a delicate vegetative

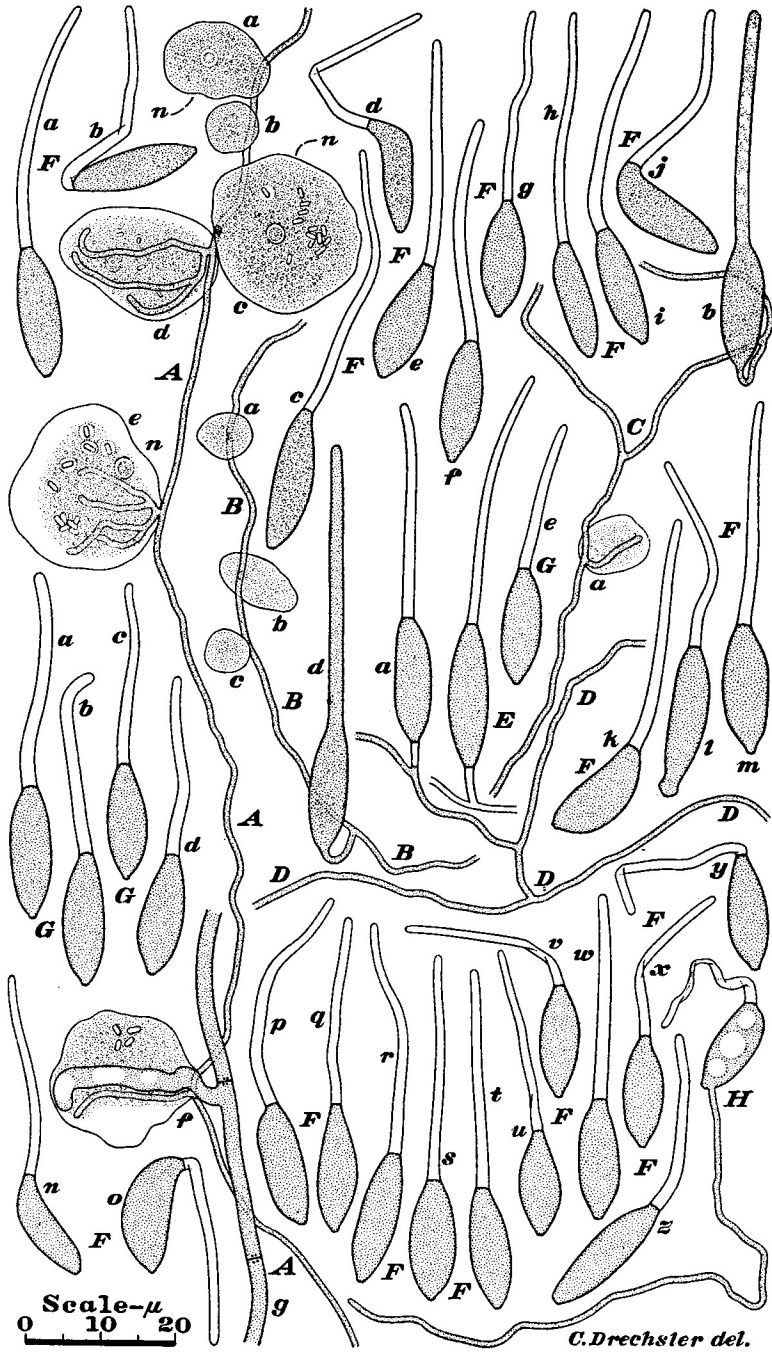


FIG. 3. *Acaulopage gyrinodes*.

hypha evidently capable of capturing any suitable *Amoeba* that might come into contact with it.

Among the species of *Acaulopage* hitherto described only *A. ceratospora* and *A. cercospora* are distinguished by conidia resembling those of the present fungus in having a swollen living cell surmounted by a single narrower tubular appendage. In the present fungus the living cell is scarcely two-thirds as long as in *A. ceratospora*. The apical appendage, too, is shorter than in *A. ceratospora* in approximately the same proportion, besides tapering less pronouncedly; while the basal appendage commonly present in *A. ceratospora* is absent here. By way of contrast the conidia of the present fungus exceed those of *A. cercospora* in all dimensions; the living cell in the former being longer and wider than in the latter by more than a half, while the apical appendage in the former appears about twice as long and three times as wide as in the latter. The fungus obviously represents a new species. A specific term meaning "like a tadpole" may prove helpful in recalling the distinctive shape of its conidia.

***Acaulopage gyrinodes* sp. nov.**

Mycelium sparsum; hyphis continuis, incoloratis, filiformibus, parce ramosis, plerumque 0.8–1 μ crassis, ad animalia minuta inhaerentibus, pelliculam eorum perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio interdum in ramo simplici constante, interdum arbusculiformi tum in 2–4 ramis consistente; ramis assumptibus 5–25 μ longis, 0.8–1 μ latis. Conidia incolorata, erecta, ex ramulis saepe 2–10 μ longis oriunda, in duabus partibus constantia; pars supera vacua, plerumque 20–37 μ longa, basi 1.3–1.9 μ lata, sursum parvulum attenuata, apice 1–1.6 μ lata et ibi abrupte rotundata, saepe plus minusve marcida vel collapsa; pars infera protoplasmatis repleta, plerumque elongato-ellipsoidea, vulgo recta sed interdum curvata, 14–20 μ longa, 4–6.5 μ lata.

Amoebas saepe 6–21 μ latas capiens consumensque habitat in foliis arborum (ex magna parte *Quercus*, *Corni*, *Liriodendri*, *Aceris*) putrescentibus prope Mayo, Maryland.

Mycelium sparse; vegetative hyphae aseptate, colorless, filamentous, sparingly branched, 0.8–1 μ wide, adhering to minute animals, penetrating the pellicle of each animal thus captured, and intruding a haustorium to appropriate the protoplasmic contents; haustorium sometimes consisting of a single assimilative branch, but more often consisting of 2 to 4 assimilative branches, 5–25 μ long and 0.8–1 μ wide, in bush-like arrangement. Conidia color-

less, erect, arising singly from branches 2–10 μ long, each spore consisting of two parts: an upper empty tubular part, 20–37 μ long, 1.3–1.9 μ wide below, 1–1.6 μ wide above, bluntly rounded at its tip, often becoming more or less collapsed; and a lower living part usually of elongated-ellipsoidal shape, usually straight but occasionally somewhat curved, 14–20 μ long, and 4–6.5 μ wide.

Capturing *Amoebae* often 6–21 μ wide it occurs in decaying leaves of deciduous trees (including species of *Quercus*, *Cornus*, *Liriodendron*, and *Acer*) near Mayo, Maryland.

A STYLOPAGE PRODUCING DISTALLY APPENDAGED CONIDIA ON TALL
SLENDER CONIDIOPHORES

Among the members of the genus *Stylopage* that have been set forth as subsisting by capture of *Amoebae*, the two species I described under the binomials *S. araea* (2: 199–201) and *S. rhynchospora* (8: 394–397) are distinctive especially by reason of the unusual height—commonly about 200 μ —of the slender conidiophores on which they produce singly their much wider ellipsoidal conidia. In the stature of their conidiophores as well as in the shape of their conidia these species have supplied a transition to the nematode-capturing forms *S. hadra* Drechsl. (3) and *S. leiohypha* Drechsl. (4) whose robust dimensions might otherwise have made them seem rather alien to the predaceous Zoöpagaceae. A third species manifestly belonging in the same category with *S. araea* and *S. rhynchospora* was recently obtained from leaf mold kindly collected by A. W. Rakosy in oak woods near Frederick, Maryland, on January 15, 1947. The new fungus came to light in several maize-meal-agar plate cultures which after being overgrown by *Pythium irregulare* Buisman had been further planted with small quantities of the forest detritus. When first observed twenty-two days after the leaf mold was added, it was already sporulating somewhat extensively on the agar substratum and must therefore have grown out from the partly decayed oak-leaf fragments nearby, several days—probably between five and ten days—earlier.

The scanty mycelium of the new fungus consisted of meagerly branched aseptate hyphae, mostly about 1 μ wide, along which

specimens of *Amoeba* commonly measuring 10 to 20 μ across were found attached at variable intervals (FIG. 4, A-D). Most of the captured animals were wholly or in large part depleted of their protoplasm; this depletion having evidently been accomplished by rangy bush-like haustoria consisting individually of two to five assimilative branches of about the same width as the parent hyphae. Mycelial filaments on the surface of the agar substratum gave rise in scattered positions to erect solitary conidiophores (FIG. 4, E, a) which from a basal diameter slightly in excess of 1 μ tapered upward very gradually until at a height of approximately 150 μ , where their width often measured only 0.6 or 0.7 μ , they expanded into a rather massive elongated-ellipsoidal or somewhat fusiform part with a narrowing apical beak (FIG. 4, E, b). Later a cross-wall was formed at the base of the swollen part, and the beak was evacuated of contents; so that the individual conidiophore (FIG. 4, F, a), now delimited distally, came to support aloft a fusiform or elongated-ellipsoidal conidium (FIG. 4, F, b) with a membranous apical appendage. Frequently here, as also in *Stylopage araea*, a short distal portion of the conidiophore was seen to be empty of protoplasmic contents while the conidium was still attached (FIG. 4, F, a). In some instances where disarticulation had probably been hastened by the disturbance entailed in mounting material under a coverglass, denuded conidiophores were found filled with protoplasm throughout their length (FIG. 4, G); and prematurely detached conidia likewise were seen filled to the tip of the beak (FIG. 4, H, I). Where the conidia had matured normally, however, the beak seemed regularly represented by an empty tubular appendage often from one-fourth to one-third as long as the living cell. The appendages were clearly visible, though usually in a badly collapsed condition, when undisturbed cultures were examined microscopically by means of a dry objective. In moist preparations they occasionally were revealed with distinctness and in their original shape (FIG. 4, J-N), but more often in such mounts their diaphanous character and badly collapsed condition made them difficult to see even though the truncate distal end of the living cell left no doubt as to their presence (FIG. 4, O, a-z; P, a-z; Q, a-h). Germination of detached conidia was found to take place by emission of a germ hypha close to the basal hilum (FIG. 4,

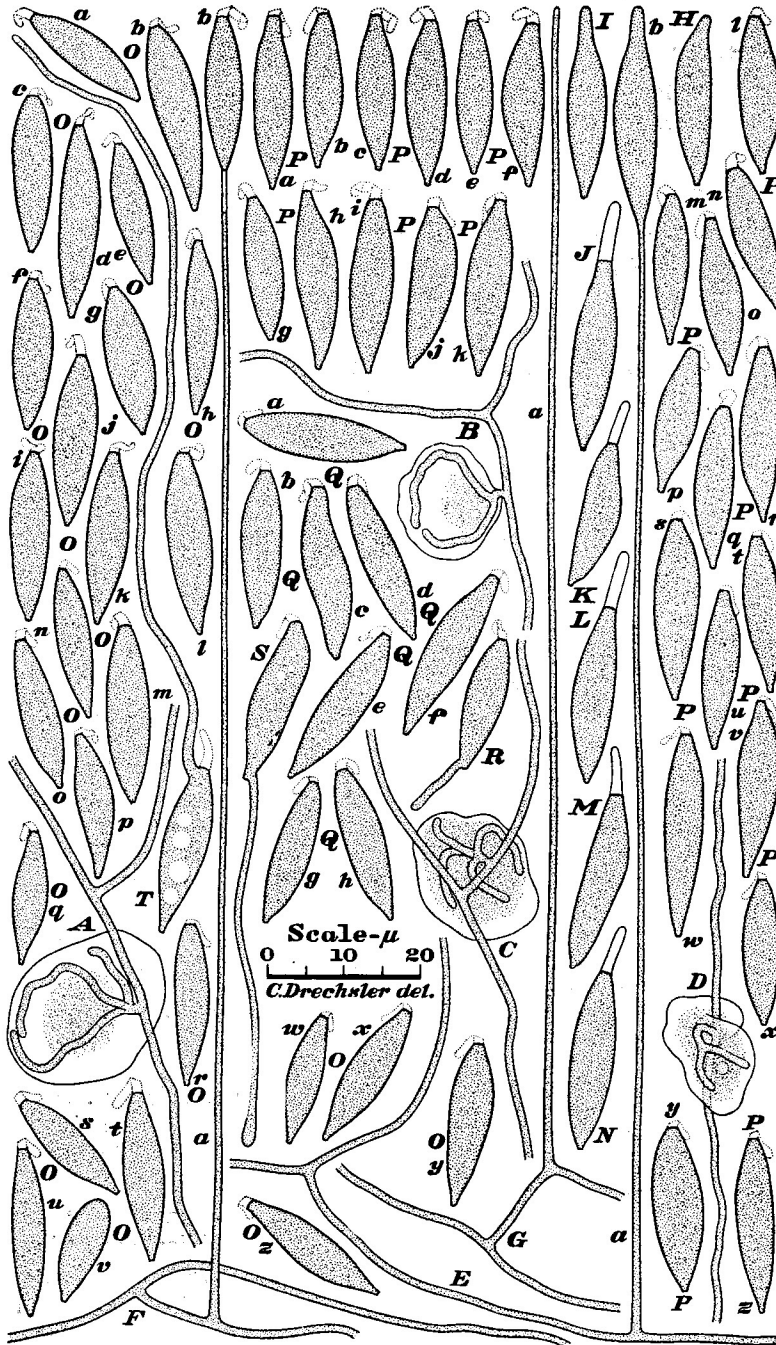


FIG. 4. *Stylopage rhicnaca*.

R, S) or, in other instances, close to the wall setting off the empty appendage (FIG. 4, T).

Development of secondary conidia on germ conidiophores put forth erectly from fallen conidia—a type of repetitional reproduction frequent in *Stylopage rhynchospora*—has never been observed in the new fungus. Nor have conidia of the new fungus ever been observed bearing at the tip any adhesive material such as is present in the yellow globular masses whereby conidia of *S. rhynchospora* often cohere apically in pairs. Owing mainly to their lesser width they are of noticeably more slender shape than the conidia of *S. rhynchospora*. In comparison with the consistently unappendaged conidia of *S. araea* they show little difference with respect to width, but because of their generally greater length offer, again, conspicuously more slender proportions.

A term compounded of two words meaning “withered” and “tip,” respectively, may serve conveniently as an epithet for the fungus in recalling its collapsed conidial appendage.

***Stylopage rhicnacra* sp. nov.**

Mycelium sparsum; hyphis continuis, incoloratis, filiformibus, parce ramosis, saepe 0.9–1.3 μ crassis, ad animalia minuta inhaerentibus, pelliculam eorum perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio arbusculiformi, in aliquot (saepe 2–5) ramis assumptibus vulgo 10–20 μ longis, circa 1 μ crassis constante. Hyphae fertiles incoloratae, simplices, erectae, saepe 140–175 μ longae, basi 1–1.3 μ crassae, sursum leviter attenuatae, apice 0.6–0.7 μ crassae, ibi unicum conidium ferentes; conidiis vulgo in cellula viventi et appendice vacua terminali consistentibus; cellula viventi incolorata, elongato-ellipsoidea vel fusiformi, 17–27 μ longa, 4.5–6.5 μ lata, basi acutula, sursum aliquid applanata; appendice vacua vulgo 5–8 μ longa, basi 1.2–1.8 μ lata, nunc cylindracea nunc sursum attenuata, apice rotundata, saepissime valde marcida et collapsa.

Amoebas saepe 10–20 μ latas capiens consumensque habitat in foliis *Quercus* putrescentibus prope Frederick, Maryland.

Mycelium scanty; vegetative hyphae aseptate, colorless, sparingly branched, often 0.9–1.3 μ wide, adhering to minute animals, penetrating the pellicle of each animal thus captured and intruding a haustorium to appropriate its protoplasmic contents; haustorium bush-like, consisting of several (often 2 to 5) assimilative branches commonly 10–20 μ long and about 1 μ wide. Conidiophores colorless, simple, erect, often 140–175 μ long, 1–1.3 μ wide at the base, tapering very gradually upward, 0.6–0.7 μ wide at the tip whereon

is borne a single conidium. Conidia consisting of a living cell and an empty terminal appendage—the living cell colorless, elongate-ellipsoidal or somewhat spindle-shaped, 17–27 μ long and 4.5–6.5 μ wide, somewhat acute at the narrow basal end, roundly truncate at the wider distal end—the empty terminal appendage commonly 5–8 μ long and 1.2–1.8 μ wide at the base, sometimes virtually cylindrical but at other times tapering upward perceptibly, always rounded at the tip, very often withered and collapsed.

Capturing and consuming *Amoebae* 10 to 20 μ wide it occurs in decaying leaves of *Quercus* near Frederick, Maryland.

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EXPLANATION OF FIGURES

FIG. 1. *Acaulopage baculispora*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. A, B, Portions of mycelial hyphae from each of which a young haustorium has been intruded into a captured specimen of the *Amoeba* sp. most commonly taken as prey. C–E,

Branched portions of mycelium from each of which a fairly well developed haustorium has been intruded into a captured specimen of the *Amoeba* sp. most frequently serving as prey. *F, G*, Branched portions of mycelium from each of which two haustoria have been intruded into a separate specimen of the *Amoeba* sp. most frequently taken as prey; in each instance one haustorium, *a*, is in young condition whereas the other, *b*, is in further developed condition. *H, I*, Portions of mycelial hyphae, each with a richly branched haustorium in a captured *Amoeba* that has been very largely expropriated of its protoplasm. *J, K*, Portions of hyphae possibly belonging to the same predaceous fungus; from each portion a young haustorium has been intruded into a specimen of an *Amoeba* sp. that was not frequently observed taken as prey. *L*, Young unit of sexual reproductive apparatus produced by two mycelial hyphae, *a* and *b*; these hyphae have given off branches bearing gametangia whose conjugation is being followed by development of a zygosporangium, *c*. *M*, Unit of sexual reproductive apparatus contributed jointly by a germinating conidium, *a*, and a mycelial hypha, *b*; the paired gametangia have fused, and a zygosporangium, *c*, is beginning to develop in the gametangium supplied from the mycelial hypha. *N*, Two units of sexual reproductive apparatus formed through interaction of a germinating conidium, *a*, with two mycelial hyphae, *b* and *c*; the conidium has given rise to two gametangia on separate germ hyphae; these gametangia have conjugated with two others borne separately on branches given off by the mycelial hyphae; in the latter gametangia the zygosporangia *d* and *e* are being formed. *O*, Mature unit of sexual reproductive apparatus contributed jointly from a conidium, *a*, and a mycelial hypha, *b*; the zygosporangium *c*, which contains a mature zygospore, was formed in the gametangium supplied from the mycelial hypha. *P*, Mature zygospores, *a-p*, each loosely surrounded by the slightly collapsed zygosporangial envelope; the attachment of the envelope to the empty tubular membranes of the gametangia is shown in most instances. (*n*, nucleus of captured *Amoeba*; *s*, cross-wall delimiting gametangium proximally; *u*, place of union between paired gametangia; *v*, contractile vacuole of captured *Amoeba*.)

FIG. 2. *Acaulopage baculispora*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. *A*, Portion of branched prostrate hypha active in asexual reproduction: *a*, erect conidium in early stage of growth; *b*, erect conidium probably full grown or nearly full grown, though not yet delimited at its base; *c-g*, five sterigmata, each bearing erectly a basally delimited conidium. *B*, Portion of branched prostrate mycelial hypha bearing five sterigmata, *a-e*, each bearing an erect conidium. *C*, Portion of prostrate mycelial hypha bearing five denuded sterigmata, *a-e*. *D (a-s)*, *E (a-w)*, Detached conidia, showing usual variations in size and shape. *F*, Detached conidium germinating obliquely from its distal end. *G*, Young unit of sexual reproductive apparatus contributed from a germinating conidium, *a*, and a mycelial hypha, *b*. *H*, Somewhat older unit of sexual reproductive apparatus likewise contributed from a germinating conidium, *a*, and a mycelial hypha, *b*; *c*, young growing zygosporangium. *I*, Two units of sexual reproductive apparatus resulting from conjugation of two gametangia supplied from the germinating conidium *a* with two other gametangia supplied from the mycelial hypha *b*; in the latter two gametangia the zygo-

sporangia *c* and *d*, respectively, are being formed; one of these gametangia, *e*, is not yet visibly delimited by a cross-wall. *J*, Mature zygospores, *a-l*, each loosely surrounded by the zygosporangial envelope (*s*, cross-wall delimiting gametangium proximally; *u*, place of union between paired gametangia).

FIG. 3. *Acaulopage gyrinodes*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. *A*, Portion of mycelial hypha with six captured *Amoebae*, of which three, *a-c*, have not yet been invaded, whereas the others, *d-f*, have been partly depleted of their protoplasm by haustorial branches intruded into them in numbers of three, four, and one, respectively; one captive, *f*, has been further invaded by a stout branch from a mycelial hypha, *g*, of *Dactylaria thaumasia*; *n*, nucleus of captured animal. *B*, Prostrate mycelial hypha on which three small *Amoebae*, *a-c*, are held captive; the hypha has given rise on a short lateral branch to an erect conidium, *d*, which though full grown has not yet been delimited at its basal end, and is still uniformly filled with protoplasm throughout its apical prolongation. *C*, Portion of prostrate mycelial hypha which has extended an assimilative branch into a small captured *Amoeba*, *a*, and has produced an erect conidium, *b*, from whose vacuolated apical prolongation the protoplasmic contents are being withdrawn. *D*, Portion of prostrate mycelium bearing a mature erect conidium, *a*, whose ellipsoidal living cell is delimited basally from the empty stalk and distally from the empty elongate apical appendage. *E*, Empty portion of prostrate hypha bearing a mature appendaged conidium on a short empty stalk. *F* (*a-z*), *G* (*a-e*), Mature detached conidia showing variations in size and shape of the living cell as well as of the empty apical appendage. *H*, Detached conidium that has put forth a germ hypha obliquely from a position near its base.

FIG. 4. *Stylopaga rhicnacra*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. *A-D*, Portions of mycelial hyphae from each of which a branched haustorium has been extended into a captured *Amoeba*; all the captives have been largely expropriated of their protoplasmic contents. *E*, Portion of prostrate hypha from which has been sent up a slender erect conidiophore, *a*, that is still continuous distally with the conidium, *b*, whose apical prolongation is still filled uniformly with protoplasm. *F*, Portion of prostrate mycelium from which has been sent up an erect conidiophore, *a*, bearing a mature conidium, *b*, that is provided with an empty apical appendage. *G*, Portion of prostrate hypha with an erect conidiophore, *a*, not supporting a conidium. *H*, *I*, Detached conidia in which the apical beak is still filled with protoplasm. *J-N*, Detached conidia wherein the empty apical appendage has not collapsed or shriveled. *O* (*a-z*), *P* (*a-z*), *Q* (*a-h*), Random assortment of detached conidia showing usual variations in size and shape of the living cell; the apical appendage here being in varying degree collapsed or shriveled, and often barely discernible. *R*, *S*, Detached conidia, each with a germ tube arising in close proximity to its base. *T*, Detached conidium with a germ hypha arising in close proximity to its distal end.