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FOUR PHYCOMYCETES DESTRUCTIVE TO NEMATODES AND RHIZOPODS

FOUR PHYCOMYCETES DESTRUCTIVE TO NEMATODES AND RHIZOPODS

CHARLES DRECHSLER

(WITH 5 FIGURES)

Agar plate cultures prepared in the isolation of oömycetes responsible for root rot and allied diseases of the higher plants often display, after aging a week or more, important biotic relationships among some of the various extraneous soil microörganisms that have managed to develop successfully in them. Pure cultures used in connection with studies on the morphology of the oomycetes in question—mainly species of *Pythium, Phytophthora,* and *Aphanomyces*—can often be made to display such relationships likewise if after serving their immediate purpose they receive an accession of decaying vegetable material. Through examination of cultures thus prepared, four additional Phycomycetes habitually subsisting by the destruction of minute terricolous animals have been discovered. While two of the additional forms differ from previously described members of the Zoöpagaceae only with respect to ordinary details of morphology, the other two forms reveal a type of asexual reproduction alien to all seven of the genera hitherto erected in the family. With accession of the four species herein to be described, the recorded membership of the Zoöpagaceae is increased to 42.

A NEMATODE-CAPTURING PHYCOMYCETE WITH INTRAMATRICAL LATERAL CHLAMYDOSPORES

In an earlier summary (1: p. 269, figs. 15C, 15D; p. 270, lines 7 to 19) a *Pythium*-like fungus was briefly set forth that captures nematodes by adhesion to unseptate mycelial filaments about 4 μ wide; the sigillate cushion of adhesive material operative in each instance of capture thereupon being pierced by an infective branch, which then penetrates the animal's integument and gives rise within the fleshy body to a system of assimilative hyphae. As regards

vegetative morphology and predaceous habit the fungus thus shows very obvious parallelism with the two robust nematode-capturing forms that I have elsewhere (**4**, **6**) described as members of the Zoöpagaceae under the binomials *Stylopage hadra* and *S. leiohypha.* However, its asexual reproduction by development of more or less intramatrical, mesially intercalary, globose conidia on creeping mycelial filaments differs rather markedly from the asexual reproduction by development of aerial conidia prevalent among the Zoopagaceae. Because of their resemblance to the asexual spores of some familiar species of *Pythium,* as, for example, *P. ultimum* Trow, the intercalary globose conidia might readily be held to indicate taxonomic relationship in the Pythiaceae, to which family, indeed, the predaceous genus *Zoöphagus* has been assigned by most writers; though a more convincing interpretation of them can be derived by considering the indubitably homologous reproductive bodies produced by another nematode-capturing Phycomycete.

This Phycomycete of less ambiguous morphology developed in some fifty *Pythium* cultures on maizemeal agar, to each of which had been added pinches of leaf mold from collections made during September 1939, near Butternut, Wis., and Haugen, Wis. Its hyphae, while of about the same width as those of *Stylopage hadra,* show little of the haphazard ramification usual in the latter species, but, instead, grow out from their origin in sparse radial arrangement, sometimes pursuing a straightforward course for stretches of 10 mm. without giving off more than a half-dozen branches. It subsists, seemingly to the exclusion of all other sources of nourishment, by preying on eelworms; *Pectus parvus* Bastian being found captured most abundantly in my cultures. Capture is effected by adhesion to outwardly undifferentiated mycelial hyphae, the adhesive material soon becoming visible as a sizeable cushion of sigillate shape and golden yellowish coloration. An infective branch now pierces the adhesive cushion, penetrates the animal's integument, and then ramifies several times (FIG. 1, *A, B*) in giving rise to assimilative hyphae that extend themselves lengthwise through the fleshy body (FIG. 1, *C, a, b; D–F*). Often an eelworm is held in two (FIG. 1, *B, E*) or three (FIG. 1, *F*) places, and is invaded by a corresponding number of haustorial systems. Once the assimilative hyphae, which as in *S. hadra* are perceptibly narrower than the

mycelial threads, have depleted the animal of its digestible substance, their protoplasmic contents are withdrawn into the parent filament by way of the infective branch. Before long the collapsed integument and the evacuated haustorial membranes within it disappear from view completely, so that only a cicatrized stub of the infective branch, more or less imbedded in yellowish material (FIG. 1, *B*), remains as evidence of the animal's destruction. Protuberant stubs of such origin are usually not difficult to distinguish from the small lumps of adhesive substance often secreted by mycelial hyphae in positions near captured animals (FIG. 1, *C, E*).

To initiate development of asexual spores the long mycelial hyphae burgeon forth lateral processes here and there. Each process increases in size as it receives granular protoplasm supplied through progressive evacuation of the adjacent proximal and distal portions of the parent hypha; successive steps in this evacuation being marked by deposition of consecutive retaining septa (FIG. 1, *G, H, I*). Movement of the granular material seems to be rather slow, and apparently becomes still slower after a stage has been reached when all but a short segment of the parent hypha, sometimes not more than 5 μ in length, has been evacuated (FIG. 1, *J, K*). In time, however, through gradual enlargement of a vacuole, this last hyphal segment likewise is emptied of protoplasm (FIG. 1, *L, M*), and a retaining wall is laid down whereby the evacuated filament is delimited from the sessile lateral body that constitutes the asexual spore (FIG. 1, *N–W*). Often, especially in elongated spores relatively narrow at the base, further withdrawal of protoplasm ensues, with the result that a unilocular or bilocular stalk-like basal part is also emptied of contents (FIG. 1, *X, Y, Z, AA*).

It is not evident that the stalk-like part, where present, serves to hold the reproductive body aloft in the air. Apparently the spores, whether stalked or sessile, are never formed as aerial structures, but are produced either in the substratum or directly on its surface. Unlike aerial conidia generally, including those of the Zoöpagaceae, they are not adapted for easy disarticulation. In the presence of active nematodes they sometimes secrete lumps of yellow adhesive material (FIG. 1, *Q*). On being transferred from stale cultures to comparatively fresh nematode-infested cultures, they germinate vegetatively by putting forth a sturdy hypha capable of capturing

prey. Irrigation with fresh water has not brought them to produce zoöspores. Their intramatrical origin, as well as their variability with respect to size and shape, distinguish them from the aerial conidia of the genus *Endocochlus,* which they resemble somewhat in manner of development. In view of their general characteristics they would seem to represent chlamydospores rather than conidia.

The morphology of its vegetative stage, together with its predaceous habit, leaves little doubt that the fungus must belong in the Zoöpagaceae. To make provision in the family for members reproducing asexually by the development of intramatrical chlamydospores, a new genus is now proposed under a name compounded of two words meaning "bladder" and "trap" respectively. The epithet chosen for the species refers, of course, to the position of the reproductive bodies in relation to the hyphae on which they are borne.

Cystopage gen. nov.

Mycelium hyalinum, plerumque parce ramosum; hyphis filiformibus, primo continuis, minuta animalia per adhaesionem capientibus, in ea penetrantibus, carnem eorum assumentibus, chlamydosporas terminalis vel intercalaris vel lateralis in matricem gignentibus.

Mycelium hyaline, usually rather sparingly branched; hyphae filamentous, at first continuous, capturing minute animals by adhesion, then penetrating into them and appropriating their fleshy contents; reproducing asexually by the development of terminal, intercalary, or lateral chlamydospores in or on the substratum.

Cystopage lateralis sp. nov.

Mycelium sparsum; hyphis 2.5–6 μ crassis, saepe recta procurrentibus, sine tuberibus vermiculos nematoideos tenentibus, integumentum eorum perforantibus, ramulos assumentis vulgo 2–3 μ crassos intus evolventibus qui carnem exhauriunt; chlamydosporis incoloratis, globosis, elongato-ellipsoideis, ovoideis, subinde lobulatis, plerumque 25–50 μ longis, 10–28 μ latis, semper a latere hyphae mycelii oriundis, fere sessilibus sed quandoque pediculo evacuato aptis.

Vermiculos nematoideos diversos praesertim *Plectum parvum* capiens consumensque habitat in humo silvestri prope Butternut, Wisconsin, et Haugen, Wisconsin.

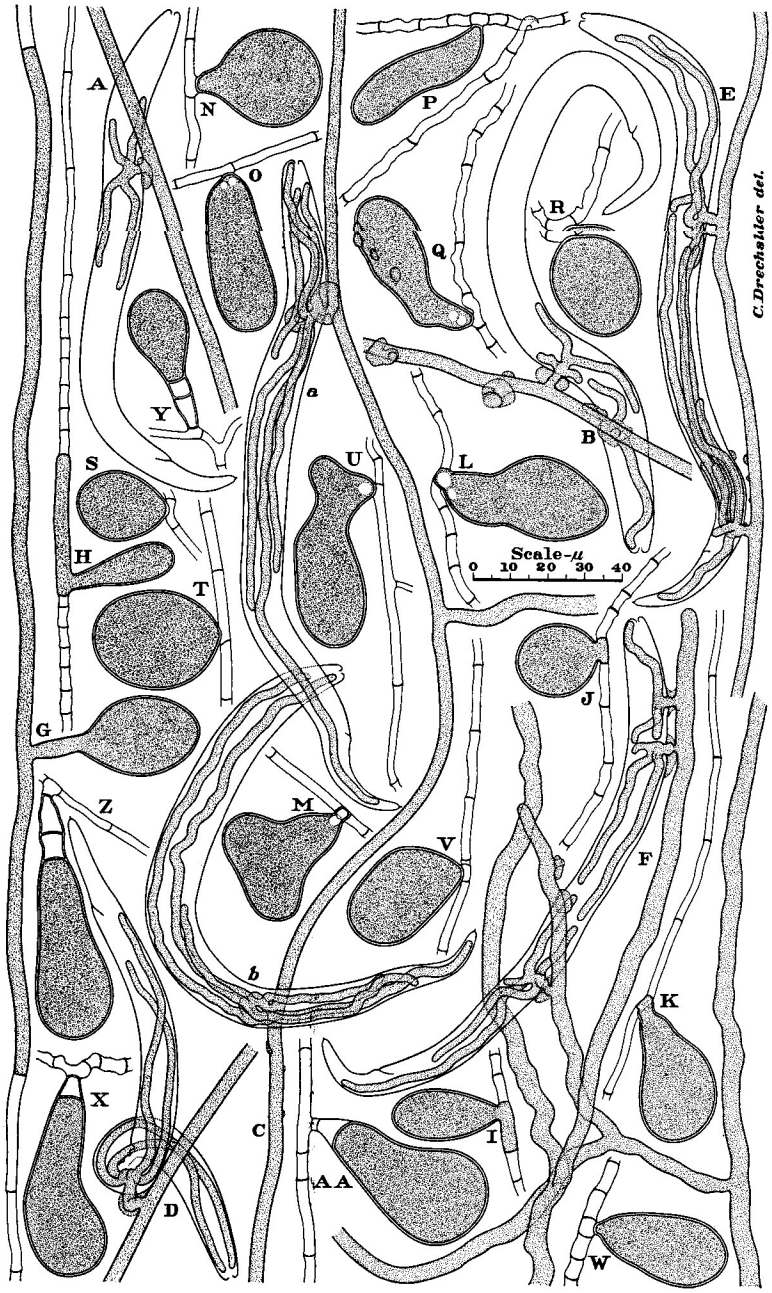

FIG. 1. *Cystopage lateralis.*

Mycelium sparse; hyphae 2.5 μ to 6 μ wide, often straightforward for several millimeters, through adhesion capturing nematodes, then invading them without production of orbicular protuberances, the infective branch giving rise within to haustorial filaments commonly 2 μ to 3 μ wide, which assimilate the fleshy contents. Chlamydospores colorless, globose, elongate-ellipsoidal, ovoid, or somewhat lobate in shape, measuring mostly 25 μ to 50 μ in length by 10 μ to 28 μ in width, always formed laterally on mycelial hyphae, commonly sessile, but sometimes, following evacuation of a narrow proximal part, coming to surmount an empty unilocular or bilocular pedicel.

Capturing various nematodes, including especially *Plectus parvus,* it occurs in leaf mold near Butternut, Wis., and Haugen, Wis.

A RHIZOPOD-CAPTURING PHYCOMYCETE WITH INTRAMATRICAL CHLAMYDOSPORES

One of the cultures in which *Cystopage lateralis* grew out from deposits of leaf mold afforded also the development of a more minute but unquestionably congeneric fungus. This smaller form subsisted, apparently to the exclusion of other nourishment, by capture of a protozoan, elongated elliptical in outline, and measuring mostly 25 μ to 30 μ in length by 11 μ to 15 μ in width. When the animal, present abundantly both on and in the maizemeal-agar medium, first came under observation it was very inactive, and had at its anterior end a median indentation (FIG. 2, *A, B*) that gave it a frontal profile reminiscent of some flagellate protozoans belonging, for example, in the saprozoic genus *Chilomonas.* After portions of the material had been irrigated with fresh water the animal resumed a more active condition by largely obliterating its indentation and by extending from its median anterior region a branching system of delicate pseudopodial strands (FIG. 2, *C, D*). Though a membranous testa was not clearly distinguishable, the relatively stable conformation of the protozoan indicated the presence of a firm envelope. A spherical nucleus, about 7 μ in diameter, and of an indistinctly granular structure throughout, usually occupied a position near the fundus (FIG. 2, *A, C, D*), though occasionally it could be seen nearer the middle of the body (FIG. 2, *B*). The animal apparently is referable to the genus *Lecythium*

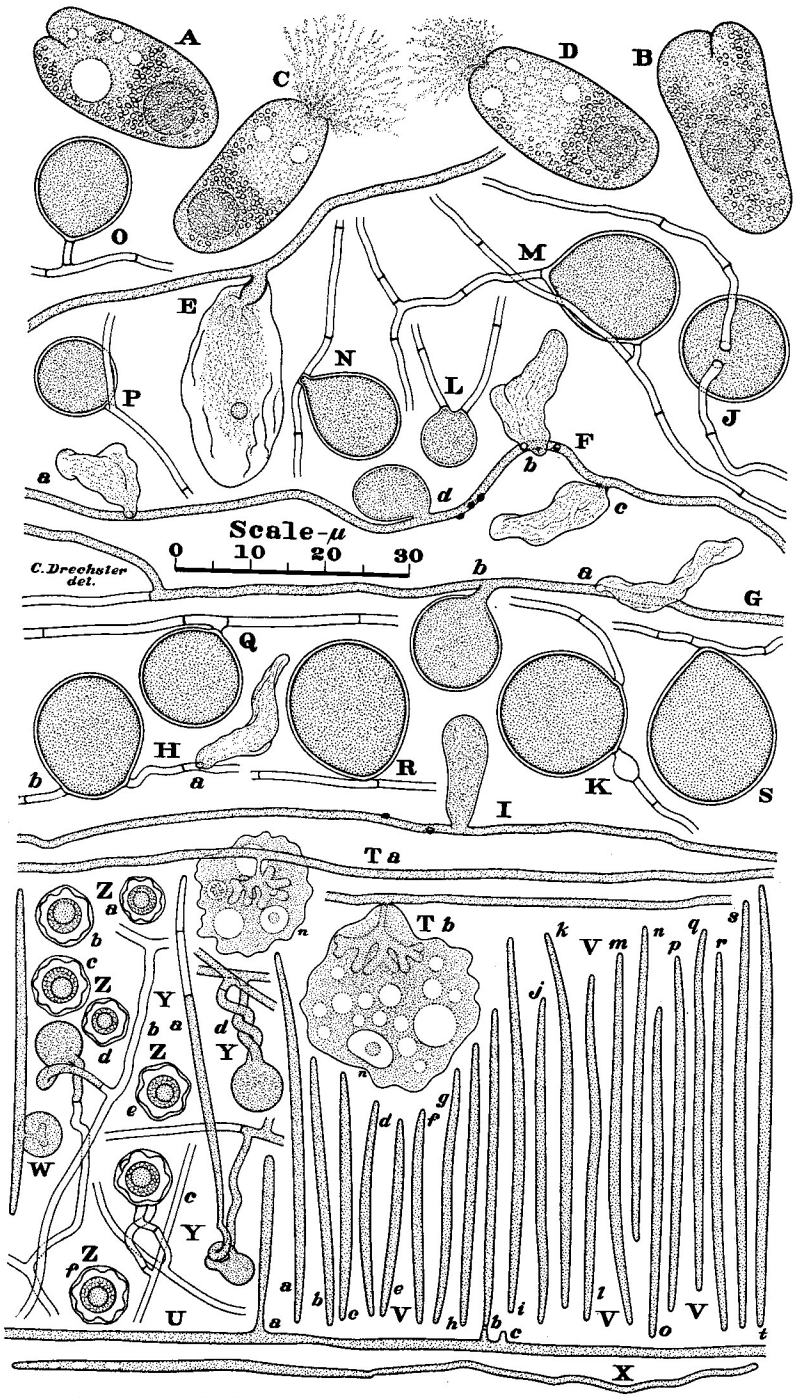

FIG. 2. *A–S, Cystopage subtilis; T–Z, Acaulopage stenospora.*

among the testaceous rhizopods, and in that genus would seem perhaps most similar to *L. mutabile* (Bailey) Hopk.

When specimens of the rhizopod have been captured by adhesion to the delicate mycelial hyphae of the fungus, they are soon expropriated of all protoplasmic contents. This is evidently accomplished by means of commonplace haustorial branches, though, owing to an unfavorable consistency of the sarcode, such elements are in most instances only partially and indistinctly discernible (FIG. 2, *E*). The emptied testae remain attached to the hyphae as collapsed membranous envelopes (FIG. 2, *F, a, b, c; G, a; H, a*) not differing in appearance from collapsed pellicles of soil amoebae.

The fungus often initiates asexual reproduction, much like *Cystopage lateralis,* by putting forth lateral excrescences from its mycelial filaments (FIG. 2, *F, d; G, b; I*). In addition, the protoplasm of vegetative hyphae here often accumulates in intercalary swellings, or in swellings formed terminally on short lateral branches; so that when the adjacent portions of mycelium have yielded up their contents, subspherical chlamydospores are delimited in intercalary (FIG. 2, *H, b; J–M*), laterally intercalary (FIG. 2, *N*), and terminal (FIG. 2, *O*) relationships as well as in lateral (FIG. 2, *P–S*) relationship to the evacuated filaments. A very dense, almost imperceptibly granular internal texture, together with a faint smoky cast, gives the asexual spores an appearance suggestive of the ectoparasitic thalli of *Bdellospora helicoides* Drechsl. (2).

Differing markedly from *Cystopage lateralis* not only by its generally smaller dimensions, but also by the more varied mycelial relationships and more regularly subspherical shape of its chlamydospore, the fungus is described as a new species under a specific name having reference to the slenderness of its hyphae.

Cystopage subtilis sp. nov.

Mycelium sparsum; hyphis 1–1.5 μ crassis, parce ramosis, per adhaesionem animalcula tenentibus, protoplasma eorum assumentibus; chlamydosporis incoloratis vel minime fumidis, lateralibus vel intercalaribus vel rarius terminalibus, globosis vel paululum obovoideis, plerumque 7–20 μ diam.

Speciem *Lecythii* (*Lecythii mutabilis* adfinem) capiens consumensque habitat in humo silvestri prope Haugen, Wisconsin.

Mycelium sparse; hyphae 1 μ to 1.5 μ wide, sparingly branched, capturing minute animals through adhesion, and assimilating their protoplasmic contents; chlamydospores colorless or very faintly smoky, lateral or intercalary or more rarely terminal, subspherical or slightly obovoid, measuring commonly 7 μ to 20 μ in diameter.

Capturing and consuming a species of *Lecythium,* close to or possibly identical with *L. mutabile,* it occurs in leaf mold near Haugen, Wis.

ANOTHER SPECIES OF ACAULOPAGE WITH LONG SLENDER CONIDIA

A maizemeal agar culture to which had been added some pinches of leaf mold collected in Arlington, Va., on Oct. 7, 1936, permitted the development of a species of *Acaulopage* that with respect to morphology would seem approximately intermediate between the two forms I described earlier (3) under the binomials *A. rhaphidospora* and *A. macrospora.* It subsists by capturing amoebae that usually measure 15 μ to 25 μ in diameter, and that usually contain a subspherical or ellipsoidal hyaline nucleus, often 4 μ to 6 μ long and 3.5 μ to 4 μ wide, within which a slightly darker "Binnenkörper," about 1.8 μ in diameter, is distinguishable (FIG. 2, *Ta, n; Tb, n*). Capture is effected by adhesion to mycelial filaments markedly coarser than those of *A. rhaphidospora,* even if the differences in width amounts to less than a micron. The pellicle of the individual captive is soon pierced by a narrow infective branch, which after growing a short distance into the sarcode bifurcates several times to form a pedicellate haustorium with swollen digitate assimilative elements. The animal's contents become increasingly vacuolate (FIG. 2, *T, b*) and eventually disappear completely.

With adequate nourishment the superficial hyphae of the fungus send up erect filamentous processes (FIG. 2, *U, a*) each of which, on attaining definitive length, undergoes division into a long slender aerial conidium and a short basal sterigma (FIG. 2, *U, b, c*). In general, the conidia thus formed (FIG. 2, *V, a–t*) are longer but not wider than the homologous bodies of *Acaulopage rhaphidospora.* On the whole, again, they are appreciably narrower and shorter than the conidia of *A. macrospora,* and would seem to be lacking, besides, in any tendency either toward distal bifurcation or toward

evacuation at the ends. A conidium, after falling on the substratum, often begins predaceous activity directly by intruding a haustorium into an amoeba adhering to it (FIG. 2, *W*). Germination regularly takes place by emission of a germ hypha (FIG. 2, *X*) capable of holding prey.

The germ tube from a conidium often assumes a sexual function by uniting with a zygophoric branch from a mycelial hypha to initiate development of a zygosporangium (FIG. 2, *Y, a*). In these instances, as also when the paired zygophoric branches arise from two separate mycelial hyphae (FIG. 2, *Y, b, c*), some meager reciprocal engagement of the conjugating elements is usual. This engagement only occasionally appears as helicoid intervolution (FIG. 2, *Y, d*). The zygosporangium formed at the junction of the sexual branches is a smooth subspherical body. At maturity its envelope collapses about the prominently warty zygospore formed within it (FIG. 2, *Y, c; Z, a–f*). Both zygosporangium and zygospore are noticeably larger than the corresponding structures of *A. rhaphidospora.*

A term compounded of two words meaning "narrow" and "seed," respectively, is deemed a suitable specific name for the fungus.

Acaulopage stenospora sp. nov.

Mycelium sparsum; hyphis incoloratis, filiformibus, parce ramosis, 1–1.5 μ crassis, ad animalcula haerentibus, pelliculam eorum perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio pediculato, pediculo circa 2–3 μ longo, .7 μ crasso, apice abrupte latescente, vulgo bis vel ter repetite bifurco, ita usque 8 ramulos divaricatos, circa 1.3 μ crassos ferente. Conidia hyalina, filiformia, recta vel leniter curvata, utrimque parvo attenuata; plerumque 25–60 μ longa, 1.2–1.6 μ crassa, ex sterigmatibus circa 2 μ altis et 1 μ latis assurgentia. Hyphae zygosporiferae modo ex hypha mycelii modo ex conidio germinanti oriundae, vulgo 10–30 μ longae, 1–1.5 μ crassae, saepius inter se paulum intricatae; zygosporangio primum levi, sphaeroideo, 6–8 μ diam., maturitate membrana circa zygosporam laxe collapsa; zygospora flavida, globosa, 5.5–7.5 μ diam., membrana .5–1.5 μ crassa, 10–25 verrucis ornata.

Amoebas 5–25 μ latas capiens consumensque habitat in humo silvestri in Arlington, Virginia.

Mycelium sparse; hyphae colorless, filiform, sparingly branched, 1 μ to 1.5 μ wide, adhering to minute animals, perforating the pellicle of each captive, and intruding a haustorium into the sarcode;

haustorium pedicellate, its pedicel, about 2 μ to 3 μ long and 0.7 μ thick, widening abruptly and usually bifurcating successively 2 or 3 times to bear as many as 8 divergent branches about 1.3 μ wide. Conidia colorless, filiform, straight or slightly curved, tapering somewhat at both ends, measuring mostly 25 μ to 60 μ in length by 1.2 μ to 1.6 μ in greatest width, borne erect on sterigmata about 2 μ high and 1 μ wide. Zygophoric hyphae commonly 10 μ to 30 μ long, 1 μ to 1.5 μ wide, usually only rather slightly intricated, arising from mycelial filaments or from germinating conidia; zygosporangium subspherical, 6 μ to 8 μ in diameter, at first smooth, its envelope at maturity collapsing loosely about the zygospore; the latter yellowish, globose, 5.5 μ to 7.5 μ in diameter, surrounded by a wall measuring 0.5 μ to 1.5 μ in thickness inclusive of its 10 to 25 warty protuberances.

Capturing and consuming Amoebae 5 μ to 25 μ wide it occurs in leaf mold in Arlington, Virginia.

A SPECIES OF COCHLONEMA WITH IRREGULARLY CONVOLVED THALLI

In a maizemeal agar culture to which had been added small quantities of leaf mold collected near Haugen, Wis., during September 1939, numerous large amoebae were found parasitized by a species of *Cochlonema* whose luxuriant tufts of conidial chains become visible to the naked eye as minute white flecks distributed here and there over the surface of the medium. The animals attacked commonly measured 60 μ to 75 μ in diameter when drawn into a more or less compact form, and were surrounded by a relatively thick pellicle cast in broad, boldly undulating pseudopodial folds. They consistently revealed imbedded in the granular sarcode a prolate ellipsoidal nucleus (FIG. 3, *A, n; B, n*), often about 15 μ long and 12 μ to 14 μ wide, within which a lighter hyaline outer layer could be distinguished from a slightly darker subspherical central body, or "Binnenkörper," approximately 6 μ in diameter. All the animals attacked clearly belonged to the same species of *Amoeba* that earlier was recorded (**5**, **9**) as being subject to destruction by the two fungi I described under the binomials *Dactylella tylopaga* and *Cochlonema megalosomum*. This species of *Amoeba* was designated in my earlier papers as *A. verrucosa* Ehrenb., and consistency, at least, will be served by applying the same name also in the present account.

The host animal unwittingly initiates its own destruction by ingesting conidia of the parasite strewn about on the surface of the substratum. Each ingested spore soon germinates in extending, usually from near one of its ends, a delicate germ tube that gradually widens as it elongates (FIG. 3, *A, a, b*). However, the young thallus is ordinarily not left to batten undisturbed, for during the earlier stages of infection, the contractile vacuole of the host repeatedly expands in contact with it. In instances of plural infection the expanding vacuole often engages several young thalli, which then usually become more or less entangled with one another (FIG. 3, *B, a, b, c*). Discharge of the contractile vacuole abruptly moves the engaged thalli to a peripheral position in the sarcode, whence the animal seemingly attempts to void them by means of a purse-lipped protrusion (FIG. 3, *A*). On failure of the attempt the enlarging contractile vacuole engages the thalli anew in another effort at their expulsion. Although no instances of successful voidance came under observation, it seems not unlikely that defensive efforts so persistent may at times have a favorable issue. At all events the animal's apparent determination to resist destruction by the parasite under discussion contrasts markedly with its submissive attitude towards *Cochlonema megalosomum,* as well as with the resigned behavior of numerous other rhizopods towards zoopagaceous forms subsisting on them. The show of resolute opposition recalls Penard's (10) early record of successful defensive action by *Amoeba alba* Greeff in eliminating, through abstriction, sizable thalli of the fungus that he designated as *Saprolegnia* B, and that almost certainly must have been a member of the Zoopagaceae.

While it remains uncertain whether any morphological effect can be attributed to the stretching action exerted by the repeated expansion of the contractile vacuole, there can be no doubt that the narrow proximal portion of the thallus is much more prolonged in the fungus under consideration than in related species. It appears more probable that the morphology of the parasite may be influenced somewhat directly by the "rolling" locomotion of the animal. Because of such locomotion the growing thallus is constantly tumbled about, with the result that its tendency toward spiral convolvement, rather clearly expressed through the stages of

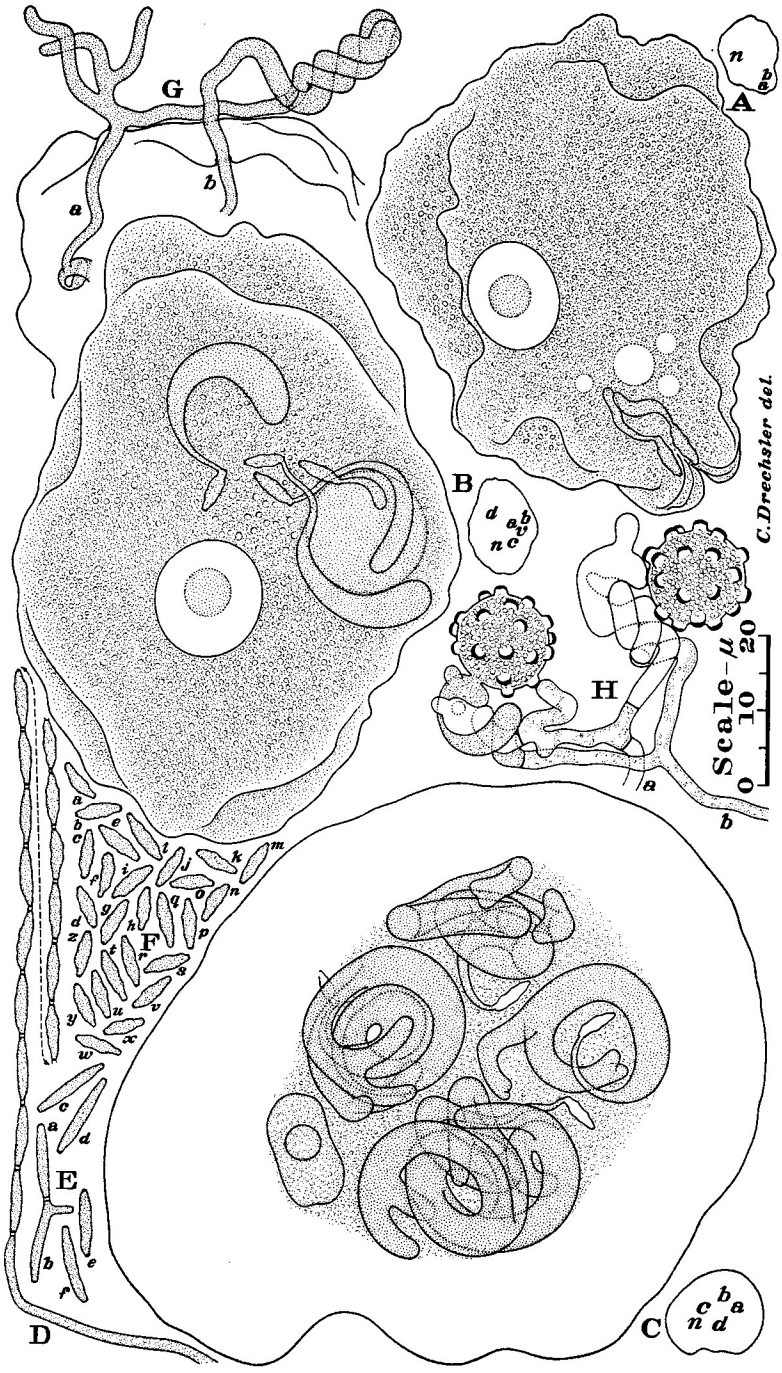

FIG. 3. *Cochlonema symplocum.*

elongation marked by the first and second dichotomies (FIG. 3, *B, d; C, a, b, c*), shows increasing irregularity in the further growth increments present in the relatively large clew-like thallodic coils having three successive bifurcations (FIG. 3, *C, d;* FIG. 4, *A, a, b*). The appearance of disorderly development is heightened in plurally infected animals, since here the several thalli very often become intertangled into a confused snarl. In any case, regardless of the number of thalli at hand, the protoplasm of the amoeba undergoes steady reduction. The degenerating host nucleus remains recognizable until an advanced stage of expropriation has been reached (FIG. 3, *C, n*), but ultimately it, too, disappears from view (FIG. 4, *A*).

As has been intimated, the parasite gives rise to scattered white tufts consisting of long intertangled conidial chains. In the individual chain the component conidia are found united by short empty isthmi (FIG. 3, *D*). The spores in the lower portions of a chain (FIG. 3, *E, a–f*) are generally longer, narrower, and less pronouncedly verrucose than those in the median and distal portions (FIG. 3, *F, a–z*). Examination of asexual reproductive apparatus in earlier stages of development revealed that the chains are formed by segmentation of erect filaments whose smooth basal portions are little given to variations in width, but whose verrucose median and distal portions consist of expanded parts alternating with contrictions (FIG. 4, *B, a*). The number of conidiiferous filaments or of conidial chains in a tuft, often between 8 and 12, is governed mainly by the size of the host animal, rather than by the number of thalli responsible for its destruction. Apparently each thallus puts forth a single reproductive hypha, which, after growing through the host pellicle, branches out laterally to give rise to conidiiferous filaments in such numbers as the quantity of available protoplasm permits: two, three, or perhaps four coming from thalli of moderate size that had been constrained to share the substance of their host with several fellows (FIG. 4, *B, a–c; d–f; g–h*); ten or perhaps more coming from thalli that had undergone no competition.

Sexual reproduction of the parasite was not observed in the culture for several days, until rather suddenly, while the material was being studied, it began simultaneously on an extensive scale. The

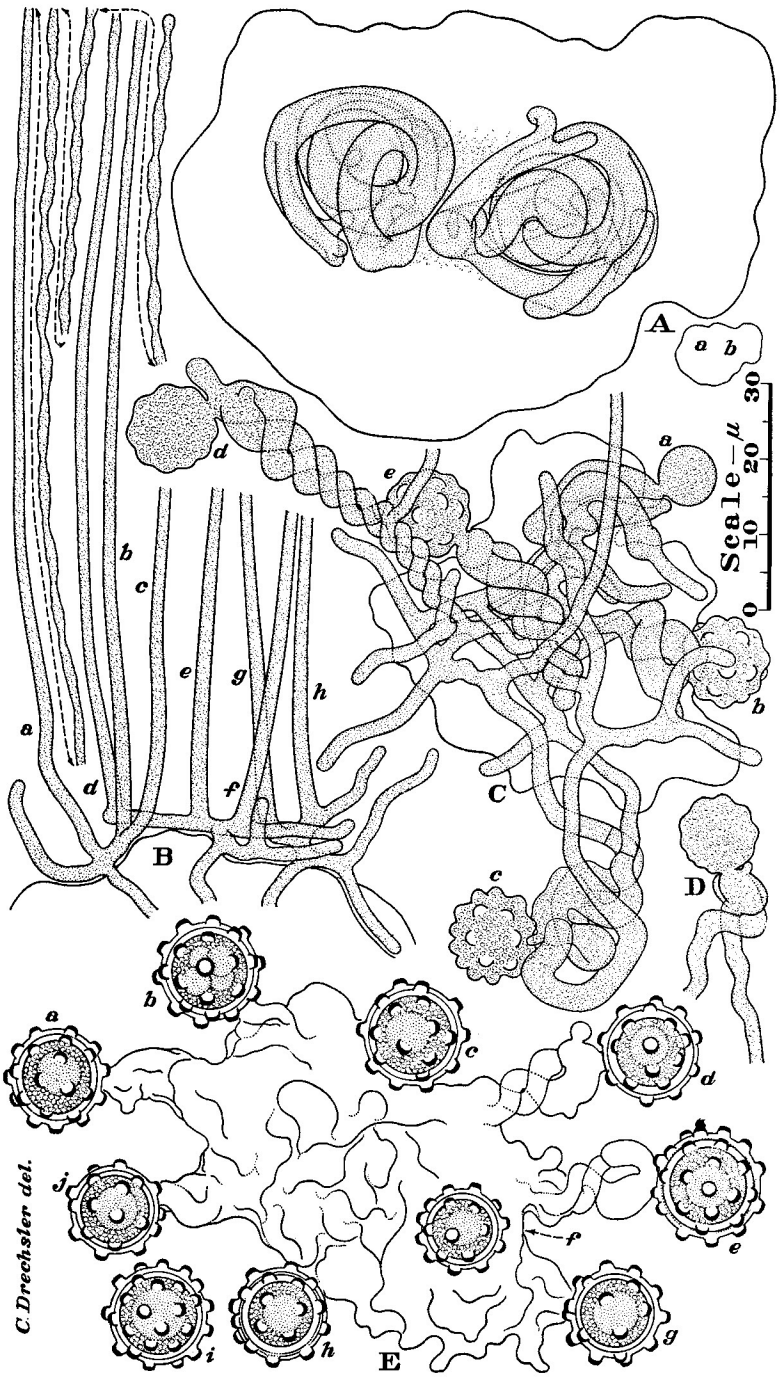

FIG. 4. *Cochlonema symplocum.*

abrupt turn from asexual development probably was due to a marked fall in the temperature of the laboratory, resulting accidentally from failure of the heating system during cold weather. Apparently the fungus, like most other endoparasitic members of the Zoöpagaceae, is heterothallic, since its zygospores have been found produced only around pellicles occupied by plural thalli, and since, moreover, paired zygophoric hyphae have regularly been found arising from separate thalli wherever their connections were not too badly obscured through excessive intrication of vegetative and reproductive parts (FIG. 3, *G, a, b; H, a, b;* FIG. 4, *C*). With respect to origin the zygophoric hypha is closely similar to the conidiiferous filament, as it likewise either represents a branch given off outside the pellicle by the single reproductive filament arising from the proximal portion of a thallus (FIG. 3, *G, a*), or consists of an external prolongation of the reproductive filament (FIG. 3, *G, b*). On making contact with each other paired zygophoric hyphae continue to grow, widening rather markedly and often winding about one another in as many as five helicoid turns before fusing apically (FIG. 3, *G, H;* FIG. 4, *C, d, e*). Where the sexual hyphae are not spirally intervolved, they usually show some reciprocal engagement of a more irregular kind (FIG. 4, *C, a, b, c; D*). A septum is laid down in each hypha, cutting off all or most of its widened intervolved terminal portion as a gametangium (FIG. 3, *G*). From the place of union between the sexual elements, or in close proximity thereto, the zygosporangium buds forth as a subspherical body, smooth during its earlier stages of enlargement (FIG. 4, *C, a*), but later becoming rather boldly verrucose (FIG. 4, *C, b–e; D*); one or both of the gametangia often giving rise, in the meantime, to a distal diverticulum of variable size (FIG. 4, *C, d*). When the zygosporangium has received the entire contents of the two gametangia, it lays down a basal septum (FIG. 3, *H*), and forms internally a zygospore surrounded by a wall rather indistinctly separated from its own (FIG. 4, *E, a–j*). At maturity the zygospore reveals a central reserve globule (FIG. 4, *E, a, c–j*), or occasionally several reserve globules (FIG. 4, *E, b*), surrounded by a coarsely granular parietal layer.

The fungus invites comparison more especially with *Cochlonema verrucosum* Drechsl. (2). As that species was described from a

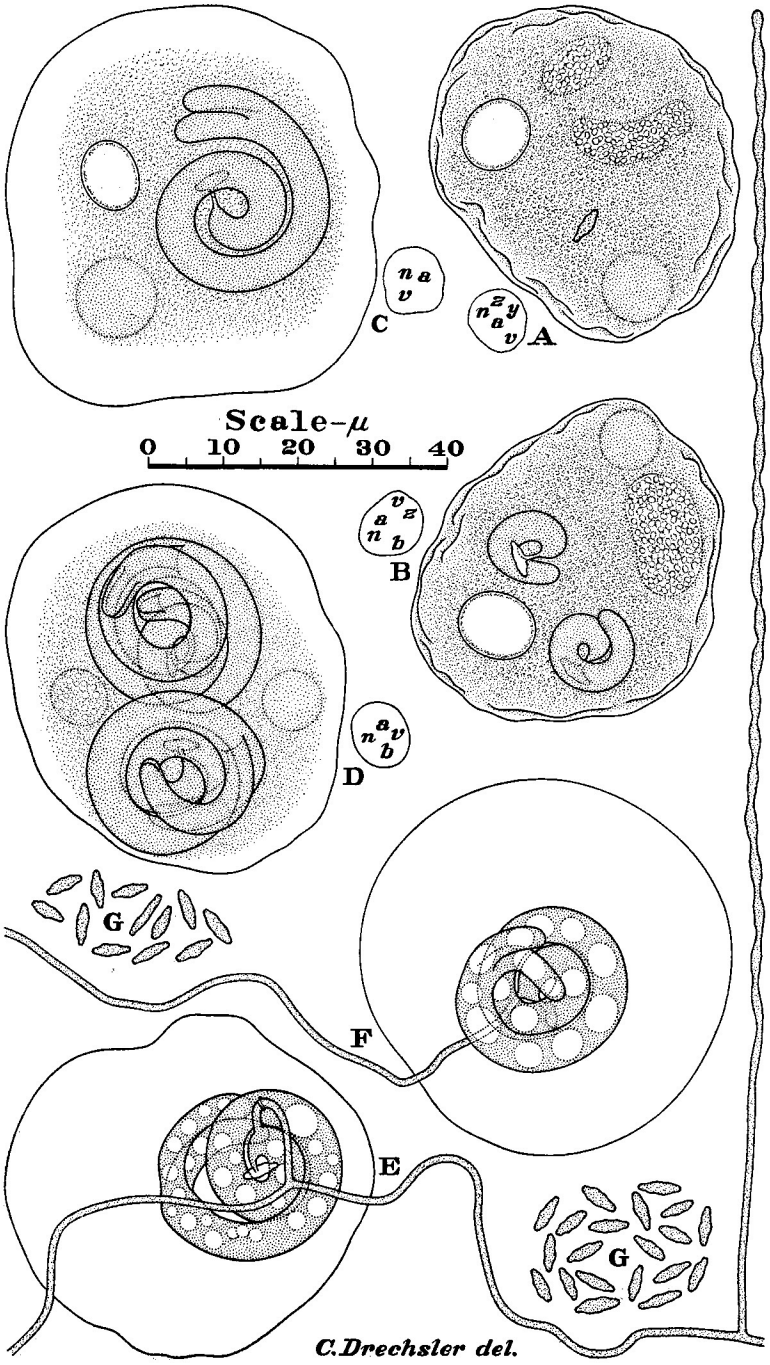

FIG. 5. *Cochlonema verrucosum.*

single culture in which the few specimens of the host animal remaining alive when observations began were already in advanced stages of infection, it may be opportune to give some further details obtained through examination of material in a culture subsequently prepared with leaf mold originating from Arlington, Va., late in October 1937. During early stages of infection, before any pronounced pathological changes had become apparent, the rather slightly prolate nucleus of the host amoeba contained close under its delimiting membrane a narrow, somewhat interrupted layer of perceptibly darker material (FIG. 5, *A, n; B, n; C, n*). In its normal internal organization, therefore, the nucleus here would seem to resemble the larger and conspicuously more prolate nucleus of *Amoeba terricola* Greeff (*sensu strictiore*), the animal set forth in previous papers as subject to destruction by three zoöpagaceous forms I described under the names *Endocochlus gigas* (**7**), *Cochlonema megaspirema* (**8**), and *Acaulopage marantica* (**9**). Most assuredly, at all events, it differs in internal organization from the nucleus of *Amoeba verrucosa*. Apart from nuclear morphology, the host of *C. verrucosum*, provisionally identified as *Amoeba sphaeronucleolus* Greeff, appears clearly distinguishable from both *Amoeba terricola* and *Amoeba verrucosa* by its smaller dimensions and its thinner, more delicately undulous pellicle.

Although in the later material of *Cochlonema verrucosum* some thalli were found that had made nearly three spiral turns (FIG. 5, *D, a, b; E; F*) and had bifurcated once or twice, the distal coils showed no less geometrical symmetry than the proximal coils. At its proximal end the individual thallus always widened out abruptly from a short delicate germ tube. The single reproductive hypha produced by it was appreciably less robust than the corresponding filament in the related parasite from Wisconsin; and the conidia (FIG. 5, *G*) formed through segmentation of aerial branches put forth by this hypha appeared to be of somewhat smaller size than the asexual spores of the congeneric species.

The fungus from Wisconsin is therefore presented as a new member of the genus *Cochlonema*. An epithet meaning "interwoven" or "entwined" is deemed aptly descriptive both of its vegetative and of its sexual stage.

Cochlonema symplocum sp. nov.

Hyphae alitae 2.5–6.5 μ latae, basi paulatim latescentes, vulgo semel vel ter dichotomae, semel vel ter spiraliter convolutae vel saepe irregulariter glomeratae. Conidia hyalina, plerumque verrucosa, fusoidea, utrimque obtusa, 6–12 μ longa, 1.5–2 μ crassa, in catenulas assurgentis saepius circa 500 μ longas digesta, in quaque catenula vulgo quinquagena usque septuagena. Hyphae zygosporiferae 20–50 μ longae, basi circa 2 μ crassae, sursum latescentae, apice 4–5 μ crassae, binae ex duabus hyphis alitis enatae, saepius bis subinde etiam quinquies inter se circumplicantes. Zygosporangia sphaeroidea, saepius 11–14 μ crassa, 20–35 verrucis applanatis 1 μ altis 1.8 μ latis ornata. Zygosporae flavidae, membrana paene cum membrana zygosporangii concreta, loculum 8–10 μ crassum circumdante.

Amoebam verrucosam enecans consumensque habitat in humo silvestri prope Haugen, Wisconsin.

Vegetative hyphae 2.5 μ to 6.5 μ in diameter, usually widening very gradually at the base rather than abruptly, simple or more often repeatedly dichotomous up to 3 times, sometimes wound into a fairly regular spiral coil of 1 to 3 turns, and sometimes convolved into a rather irregular clew. Conidia hyaline, mostly warty, spindle-shaped, blunt at both ends, 6 μ to 12 μ long, 1.5 μ to 2 μ wide, commonly formed in numbers from 50 to 70 in fairly erect chains measuring often about 500 μ in length. Zygophoric hyphae 20 μ to 50 μ long, approximately 2 μ in diameter at the base, widening to a diameter of 4 μ to 5 μ at the apex, those of each conjugating pair arising from separate vegetative hyphae, and often winding about one another in 2 or even as many as 5 helicoid turns. Zygosporangium formed close to junction of the sexual hyphae, usually 11 μ to 14 μ in diameter, ornamented with 20 to 35 warty protuberances which are somewhat flattened on top and measure about 1 μ in height by 1.8 μ in basal width; its envelope often not distinctly separated from the wall of the yellowish zygospore, wherein is contained a spherical protoplast 8 μ to 10 μ in diameter.

Destroying *Amoeba verrucosa* it occurs in leaf mold near Haugen, Wis.

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EXPLANATION OF FIGURES

FIG. 1. *Cystopage lateralis;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portion of mycelial filament with a captured specimen of *Plectus parvus;* an infective branch has penetrated into the nematode, and is extending assimilative hyphae through the fleshy interior. *B,* Portion of mycelial filament with a captured specimen of *P. parvus;* into the animal have been intruded two infective branches both of which are extending assimilative hyphae through the fleshy body; on the mycelial filament are shown imbedded in deposits of adhesive material cicatrized stumps of two infective branches. *C,* Portion of a long mycelial filament on which two specimens, *a* and *b,* of *P. parvus* have been captured; each of the animals is permeated internally by a haustorial system extending from head to tail. *D,* Portion of mycelial filament with a captured specimen of *P. parvus;* assimilative hyphae have been extended almost throughout the contorted body of the captive. *E,* Portion of mycelial filament from which two haustorial systems have been extended into a captured specimen of *P. parvus;* three small lumps of adhesive material are shown attached to the mycelial filament, and one lump is shown adhering to the animal's integument. *F,* Portion of mycelium whereon a specimen of *P. parvus* has been captured by adhesion in three places; three haustorial systems are being extended into the fleshy body. *G,* An extensive portion of mycelial filament that is giving rise to a stalked chlamydospore. *H, I,* Portions of mycelial filaments showing rather advanced stages in migration of hyphal contents into lateral chlamydospores of relatively small size. *J–M,* Late stages in migration of hyphal contents into lateral chlamydospores; only a short seg-

ment of the parent filament remains continuous with each of the developing spores. *N–W*, Chlamydospores wholly lateral in position, no longer including any portion of the parent filament; one specimen, *Q*, has three lumps of adhesive material attached to it. *X*, Chlamydospore connected with the parent filament by an empty basal cell. *Y*, *Z*, *AA*, Chlamydospores connected to the parent filament by two empty basal cells resulting from progressive evacuation of basal portions.

FIG. 2. Drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A–S: Cystopage subtilis*. *A*, *B*, Specimens of the protozoan prey, possibly *Lecythium mutabile* (Bailey) Hopk., as found in a rather dry agar medium. *C*, *D*, Specimens of the protozoan prey 12 hours after moistening the agar medium with fresh water. *E*, Portion of hypha on which a specimen of the protozoan has been captured. *F*, Portion of hypha to which are attached membranous remains of three captured animals, *a–c*, as well as four lumps of adhesive material; a chlamydospore, *d*, is shown in an early stage of development. *G*, Portion of a branching hypha with membranous remains of a captured animal, *a;* a young chlamydospore, *b*, is being formed terminally on a short stalk. *H*, Portion of an empty hypha, to which is attached the collapsed envelope of a captive, *a;* and on which is borne a mature, laterally intercalary chlamydospore, *b*. *I*, Portion of hypha with a chlamydospore in early stage of development; two small lumps of adhesive material are shown attached. *J–M*, Portions of empty hypha, each bearing a mature intercalary chlamydospore. *N*, Portion of empty hypha with a mature chlamydospore in laterally intercalary relationship. *O*, *P*, Portions of empty hyphae, each with a mature chlamydospore borne terminally on a short lateral spur. *Q*, *R*, *S*, Portions of empty hyphae, each bearing laterally a mature sessile chlamydospore.

T–Z: Acaulopage stenospora. *T*, *a*, *b*, Portions of mycelial filament, from each of which a dichotomously branching haustorium has grown into a captured amoeba; *n*, nucleus of each captive. *U*, Portion of superficial hypha bearing a conidium, *a*, in early stage of development, as well as a fully formed conidium, *b*, and a denuded sterigma, *c*. *V*, *a–t*, Conidia showing variations in size and shape. *W*, Conidium from which a small haustorium has been intruded into a minute amoeba adhering to it. *X*, Conidium germinating by the production of a germ hypha. *Y*, Four units of sexual apparatus showing: *a*, early stage in development of a zygosporangium, following union of a sexual hypha contributed by a germinating conidium with a sexual branch arising from a mycelial filament; *b*, immature zygosporangium formed by union of paired sexual branches arising from separate mycelial filaments; *c*, mature zygosporangium originating from conjugation of paired sexual branches contributed by two separate mycelial filaments; *d*, immature zygosporangium formed at junction of two zygophoric branches that wind about one another more extensively than is usual in the species. *Z*, *a–f*, Mature zygospores, enveloped in the zygosporangial membrane, showing differences in size and sculpturing.

FIG. 3. *Cochlonema symplocum*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba verrucosa* infected with two germinating conidia, *a* and *b*, which it apparently is attempting to void; *n*, nucleus of host animal. *B*, Specimen of *A*,

verrucosa infected with four young thalli of the fungus, *a–d; n,* host nucleus; *v,* contractile vacuole of host. *C,* Disabled specimen of *A. verrucosa* whose protoplasmic materials have been largely appropriated by four thalli of the parasite, *a–d; n,* host nucleus in somewhat degenerate condition. *D,* A sporiferous hypha bearing a long chain of conidia, of which only the lowermost 15 individuals are shown. *E, a–f,* Longish conidia from basal portion of conidial chains; the presence of a spur in *b* being due to branching of the sporogenous filament. *F, a–z,* Conidia from median and distal portions of chains, showing variations in size and shape. *G,* Pair of intertwined zygosporic hyphae that have their origin in the separate reproductive filaments *a* and *b*. *H,* Two units of sexual apparatus with full grown zygosporangia, each resulting from union of paired zygophoric hyphae coming from the separate reproductive filaments *a* and *b*.

FIG. 4. *Cochlonema symplocum,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* Pellicle of a specimen of *Amoeba verrucosa,* whose protoplasm, except for a meager remnant, has been consumed in the development of the two large thalli *a* and *b*. *B,* Origins of eight growing conidiiferous hyphae, *a–h; a–c* resulting from ramification, outside the host pellicle, of one reproductive hypha; *d–f* resulting from branching of a second reproductive hypha; *g* and *h* resulting from ramification of a third reproductive hypha. One of the eight hyphae, *a,* is shown completely in four parts connected by broken lines. *C,* Five immature zygosporangia, *a–e,* derived from union of paired zygophoric hyphae having separate origins; at both *a* and *c* a supernumerary zygophoric hypha is present, though not functional. *D,* Young sexual unit with little intervolution of zygophoric hyphae. *E,* Ten mature zygospores, *a–j,* surrounding the collapsed pellicle of a large specimen of *A. verrucosa*.

FIG. 5. *Cochlonema verrucosum,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* Specimen of host amoeba in active condition: *a,* ingested conidium of parasite; *n,* nucleus of host animal; *v,* contractile vacuole; *y, z,* digestive vacuoles containing numerous ingested bacteria. *B,* Specimen of host amoeba in active condition: *a, b,* two small thalli of the parasite, with empty conidial envelopes attached to them; *n,* host nucleus; *v,* contractile vacuole; *z,* large digestive vacuole containing numerous ingested bacteria. *C,* Specimen of host amoeba nearly disabled from loss of contents: *a,* thallus of parasite; *n,* host nucleus; *v,* contractile vacuole. *D,* Specimen of host amoeba nearly disabled as result of infection: *a, b,* two large thalli of parasite; *n,* degenerating host nucleus with lumpy internal structure; *v,* contractile vacuole. *F,* Thallus of parasite from which a reproductive filament has grown out through the enveloping pellicle of the host to produce conidial chains externally. *E,* Well developed thallus from which a reproductive filament has grown out to produce erect conidiiferous hyphae, of which one is shown. *G,* Detached conidia, showing variations in size and shape.