# AN ENTOMOPHTHORACEOUS TARDIGRADE PARASITE PRODUCING SMALL CONIDIA ON PROPULSIVE CELLS IN SPICATE HEADS

CHARLES DRECHSLER[1]

Among the various microscopic animals that multiply in aging agar plate cultures prepared for the isolation of root-rotting fungi, and also in cultures which after being overgrown by a root-rotting oomycete have been further planted with decaying vegetable material, tardigrades, or bear animalcules, often making their appearance. The development of these animals, as has been intimated (Drechsler 1948), is unfortunate where the cultures are intended to be used in the study of fungi destructive to eelworms and rhizopods, for although their slow multiplication and leisurely movement makes them less violently ruinous than annelids and mites, they yet operate persistently like so many minute bulldozers to crush down and obliterate all of the more delicate aerial reproductive apparatus in their paths. They have seemed, in my experience, to be themselves rather little subject to fungus attack. During many years mycelia or other recognizable fungus bodies have been observed within bear animalcules only now and then. Recently, however, several Petri plate cultures revealed progressive destruction of a tardigrade that continued until all motile individuals were killed. The parasite concerned here was a remarkable member of the Entomophthoraceae that like the two related nematode parasites I have described under the binomials *Meristacrum asterospermum* (Drechsler 1940) and *Gonimochaete horridula* (Drechsler 1946) displays a distinctive primal manner of asexual reproduction by giving rise to small spores in comparison with which the familiar large conidia of the insectivorous forms may be regarded as morphologically equivalent to sporangia.

Both the fungus and its host animal were obtained from leaf mold gathered on February 14, 1950, along a roadside bordering a deciduous wood near Oxford, Pennsylvania. On adding small quantities of the forest detritus to maizemeal-agar plate cultures that previously had been inoculated and had become overgrown with mycelium of *Pythium irregulare* Buism., the tardigrade multiplied well for nearly a month to populate the 63 square centimeters of agar expanse in each of the several Petri dishes with three

[1] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; Plant Industry Station, Beltsville, Maryland.

Scale in μ

C. Drechsler del.
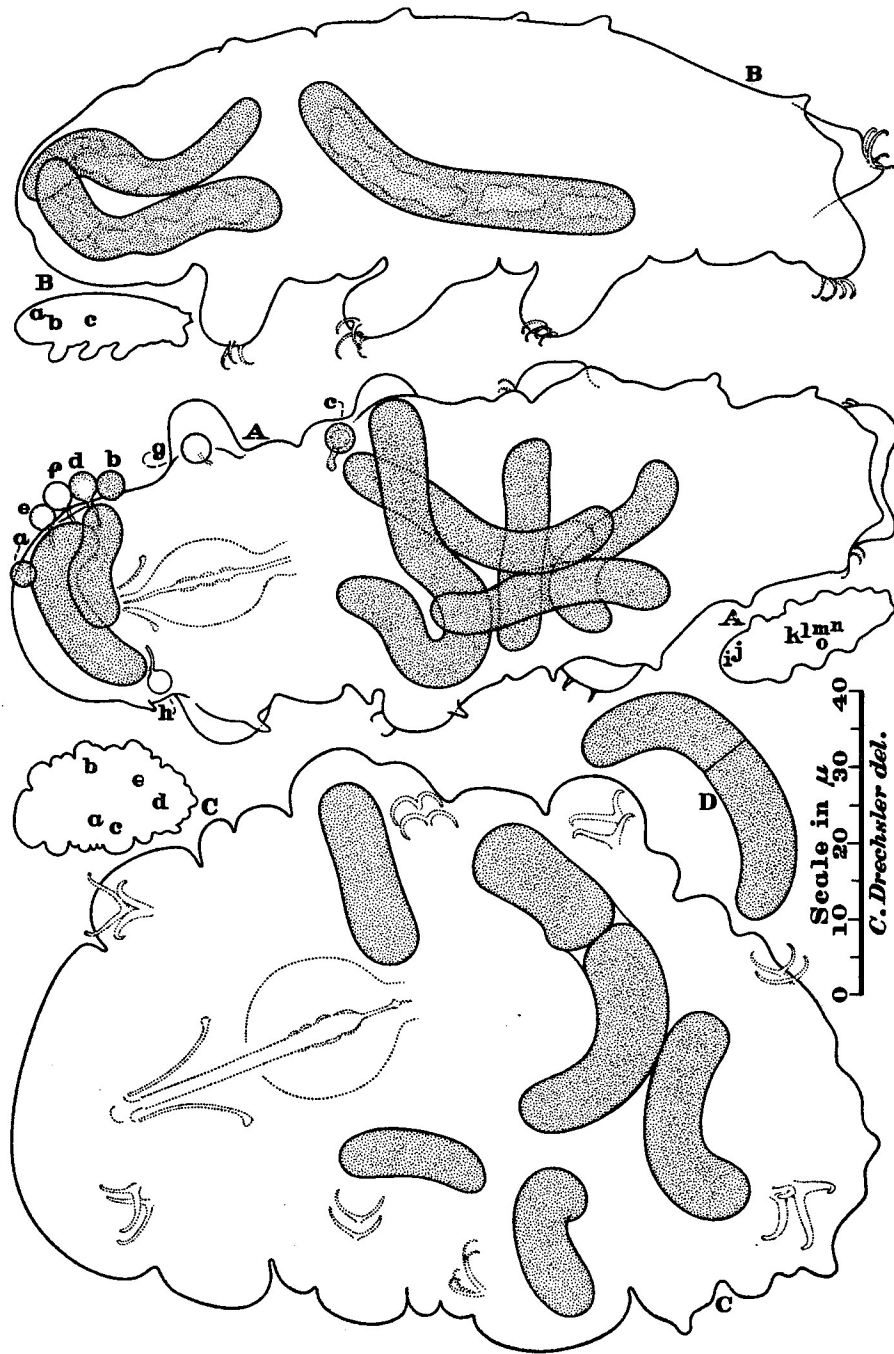
hundred to five hundred individuals of varying ages. Full grown animals measured about 200 μ in length and 65 μ in width. Cuticles of approximately these dimensions were found here and there, loosely enveloping clusters of five, six, or seven smooth eggs. Except for their legs and a considerable number of somewhat pointed warty projections on the dorsal side, the animals seemed lacking in noteworthy protuberances. According to the classification given in the fairly recent monograph by Marcus (1936) the species belongs clearly in the family Macrobiotidae of the order Eutardigrada. Since the two claws on each foot appeared generally of about equal size, it seems better referable to the genus *Macrobiotus* than to *Hysibius*, though I was not able to identify it with any one of the many species compiled under *Macrobiotus* by Marcus. Since the accompanying illustrations (figs. 1–5) are intended primarily to set forth the development of the parasite they can show little more than the outline of the animal's body as it appeared in each instance under the microscope. When the animal was already disabled by the fungus at the time it was mounted with the subjacent agar under a cover glass, its body outline, whether presented in dorsal (fig. 1, A) or in lateral view (fig. 1, B), kept approximately normal dimensions. Infected animals in whom development of the parasite was less advanced, often writhed beneath the cover glass, on exposure to upward illumination, for more than an hour in order to turn upside down, thereby bringing into view all four pairs of claws; and in the struggle they usually became flattened much beyond their normal width (fig. 1, C). As the animal's bare outline, especially in dorsal aspect, often showed too little difference between the front and rear profiles, the head end was marked in the figures, wherever space allowed, by indicating the position of the stylet and pharynx.

Infection of tardigrades always proceeds from externally adhering globose spores varying in diameter from 3 to 4.5 μ. In the region of contact between the individual spore and the animal's cuticle no deposit of adhesive secretion, nor any outward structural modification, is to be seen. While the spores seem capable of sticking to the cuticle about equally well in all parts, they usually beset the frontal region most abundantly, as, indeed, might be expected from the greater local frequency of encounter resulting from the forward locomotion of the animal (fig. 1, A; fig. 2, A; fig. 3, A). For some


FIG. 1. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Infected specimen of *Macrobiotus* sp. (dorsal view) showing two adhering conidia, a and b, that have not begun to germinate; two germinating conidia, c and d; three empty conidial envelopes, e-g; an empty conidial envelope with denuded germ tube, h; and seven assimilative hyphal cells, i-o. B, Infected specimen of *Marcrobiotus* sp. (lateral view) showing three assimilative hyphal cells, a-c. C, Infected specimen of *Macrobiotus* sp. (ventral view, somewhat flattened) containing four unicellular assimilative hyphae, a-d, and a uniseptate hypha, e, which is nearly ready to separate into its component cells. D, Same hypha as shown in C, e, but drawn four hours earlier, soon after cross-wall was laid down.

Scale in μ

0   10   20

C.Drechsler del.
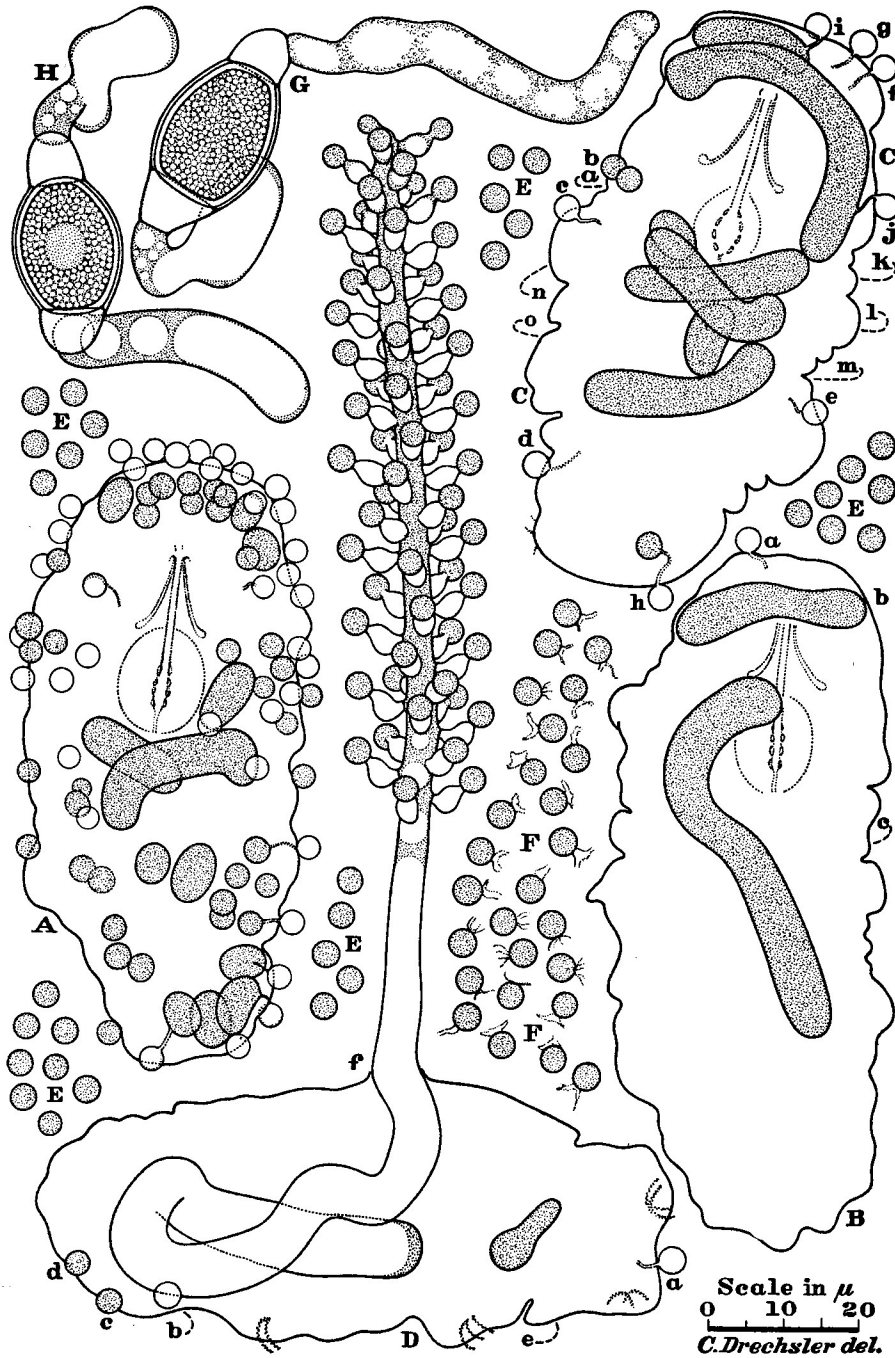
time after becoming attached the spore shows no germ tube, and remains filled throughout with protoplasm (fig. 1, A, a, b. fig. 2, A; C, a, b; D, c, d. fig. 3, A, a–j; B, a. fig. 4, A, a. fig. 5, B, a). Sooner or later a narrow germ tube burgeons forth from the adhering surface of the spore, perforates the animal's cuticle, and penetrates into the underlying fleshy tissue, while concomitantly a vacuole enlarges within the spore (fig. 1, A, c, d). Along the sides of the host animal as also at its rear and down its legs, the body wall is only a few microns thick, so that virtually all germ tubes intruded from spores affixed to the legs or adhering in lateral or posterior regions reach the body cavity before they cease elongating. Once its tip is in the body cavity each germ tube swells out terminally to form a globose infection bladder (fig. 3, B, b, c), into which are received, as a rule, the entire contents of the spore (fig. 2, A; C, h. fig. 3, B, d. fig. 5, B, b). The infection bladder now begins autonomous development as a thallus or assimilative hypha. In many instances it soon becomes abjointed through disturbances due mainly to the animal's locomotion, and gradually is floated away in the host haemolymph, leaving behind the empty spore envelope and slender germ tube (fig. 1, A, h. fig. 2, B, a; C, c–g; D, a, b. fig. 3, B, g–j. fig. 5, A) from which it originated. In the frontal region of the animal the infection bladder is rather often formed in the tissue under the cuticle (fig. 2, A; fig. 3, B, c, d), as the tissue here makes up a considerably thicker layer than elsewhere. Yet even in the fleshy head some empty germ tubes are to be found from which young assimilative hyphae have been abjointed (fig. 2, B, a; C, f, g. fig. 3, A, k, l). Often the germ tubes become indiscernible early, so that the connection between the empty spore envelope and the young assimilative hypha imbedded in the fleshy head, is no longer visible, though presumably still existent (fig.

FIG. 2. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Small specimen of *Marcrobiotus* sp. (dorsal view) to which are attached many living conidia as well as many conidial envelopes from which infection bladders have been intruded, though germ tubes are visible only in a few instances; within the animal are shown many small infection bladders and about a dozen somewhat larger hyphal cells. B, Small specimen of *Macrobiotus* sp. (dorsal view) showing an empty conidial envelope with denuded germ tube, a, and two hyphal cells, b and c. C, Small specimen of *Marcrobiotus* sp. (dorsal view) showing two adhering conidia, a and b; five empty conidial envelopes, c-g, each with a denuded germ tube; an affixed empty envelope, h, with a germ tube bearing an infective bladder at its tip; two empty affixed conidial envelopes, i and j, each having a germ tube connected with a sizable hyphal cell; and five separate assimilative hyphal cells of different sizes, k-o. D, Small specimen of *Macrobiotus* sp. showing an affixed empty conidial envelope, a, with denuded germ tube; an empty conidial envelope, b, whose germ tube is not visible; two adhering conidia, c and d; a small assimilative hyphal cell, e; and a large assimilative hypha, f, from which a conidiophore bearing about sixty conidiiferous branches has been extended. E, Random assortment of conidia as appearing on a moist substratum. F, Random assortment of conidia as appearing on rather dry agar soon after their discharge, each with tattered membrane of the lateral cell on whose tip it was produced. G, Unit of sexual apparatus with a nearly mature zygospore. H, Unit of sexual apparatus with a fully mature zygospore.

Scale in μ
0 10 20 30

C. Drechsler del.
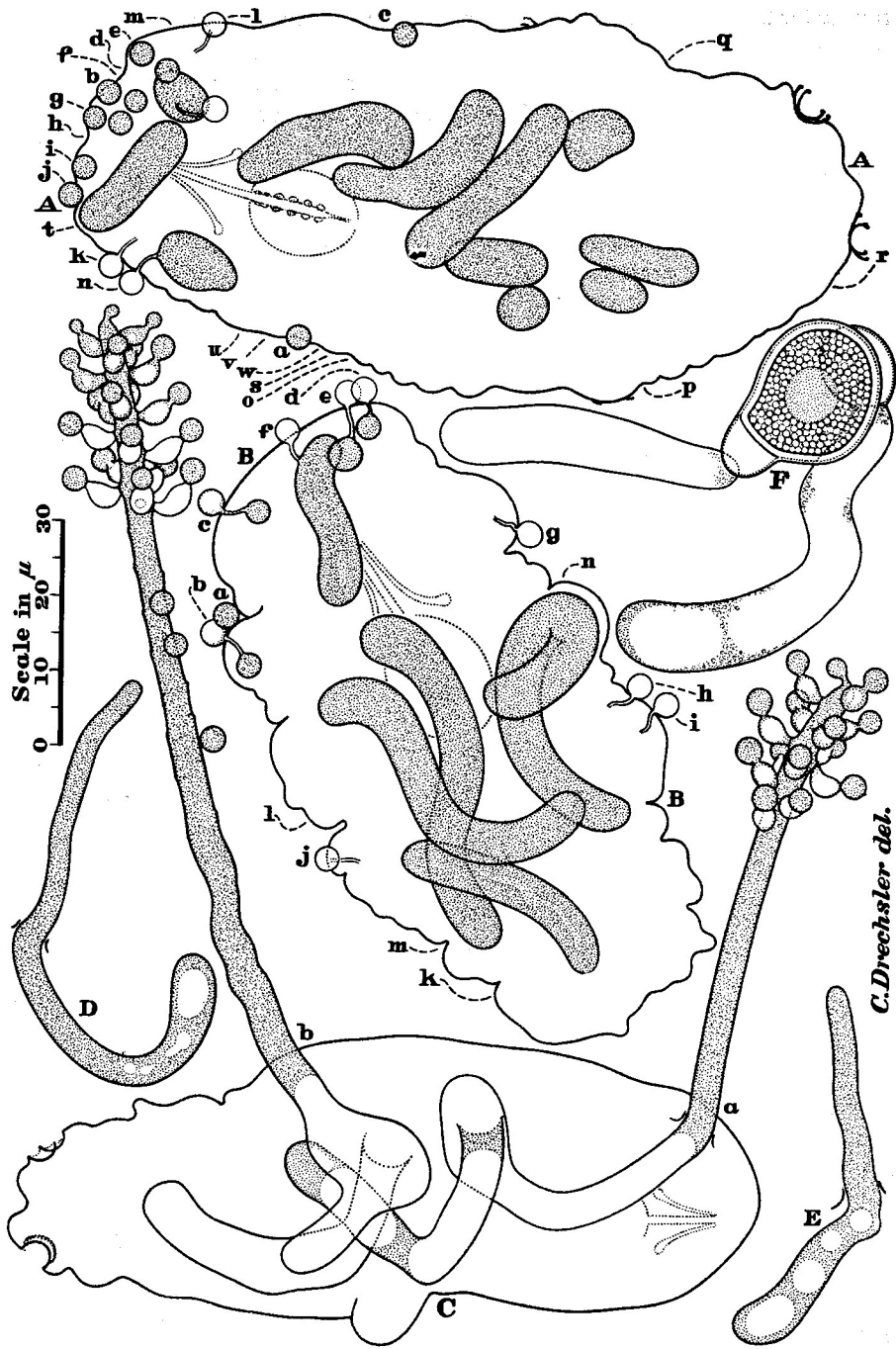
2, A). On the other hand, germ tubes not infrequently persist as visible connections at more advanced stages of growth, not only in the frontal region (fig. 2, C, i. fig. 3, A, m; B, e, f. fig. 5, A, a, b, g) but also in positions farther back (fig. 2, A; C, j. fig. 3, A, n. fig. 5, A, c–f). Where, in instances of such persistence, the thallus has grown into a short thick hypha, the germ tube is commonly found attached not centrally to one end of the hypha, but to the side of the hypha at some little distance from one end. In this respect the fungus shows noteworthy parallelism with *Meristacrum asterospermum*.

As has been intimated the thalli or assimilative hyphae achieve their early growth mainly in the haemolymph of the animal's body cavity. In this liquid ambient they are bustled around promiscuously until because of increasing size they become lodged between the organs of the host and its body wall. They thereby become settled in a pronouncedly haphazard arrangement (fig. 1, i–o; B, a–c; C, a–e. fig. 2, B, b, c; C, i–o. fig. 3, A, m–w; B, f; k–n. fig. 4, A, b–f. fig. 5, A, a–v; B, c–e), which, in conjunction with their frequently crooked elongation, makes for a disorderly appearance that contrast strongly with the neat orderliness usual in the vegetative hyphae of *Meristacrum asterospermum*. Unicellular assimilative hyphae of the tardigrade parasite are not often found exceeding 80 μ in length, for segmentation here is not delayed as in *M. asterospermum*, until vegetative growth has been virtually concluded, but may take place, especially where a large animal host has been infected from a single spore, during all except the earliest stages of growth. In some instances a hypha only about 40 μ long may show a median cross-wall (fig. 1, C, e; D). More often, however, median partitions are laid down in hyphae ranging from 50 to 75 μ in length (fig. 4, A, f). After a cross-wall has been formed the daughter segments become convexly rounded at the place of division (fig. 1, C, e), and then are easily broken apart by rather slight disturbance. When the dead or moribund host is no longer capable of furnishing any disturbance, disjunction would seem to ensue less freely, so that several segments may remain united and thus

FIG. 3. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Small specimen of *Macrobiotus* sp. (dorsal view) showing ten conidia, a–j, adhering to its cuticle; two affixed empty conidial envelopes, k and l, each with a denuded germ tube; two empty conidial envelopes, m and n, each with a germ tube to which a growing assimilative hypha is attached; and nine detached assimilative hyphal cells of various sizes, o–w. B, Small specimen of *Macrobiotus* sp. (dorsal view) showing an adhering conidium, a; two nearly empty conidial envelopes, b and c, each with a germ tube terminating in an infection bladder; an affixed empty conidial envelope, d, with a germ tube terminating in infection bladder; two empty conidial envelopes, e and f, each connected by a germ tube to a growing assimilative hyphal cell; four affixed empty conidial envelopes, g–j, each with a denuded germ tube; and four separate assimilative hyphal cells, k–n. C, Small specimen of *Macrobiotus* sp. with two assimilative hyphal cells, a and b, each of which has given rise to a growing conidiophore; the conidiophore of b shows serrations where propulsive cells were attached earlier. D, E, Small assimilative hyphal cells, each of which has begun to put forth a conidiophore. F, Unit of sexual apparatus, with a fully mature zygospore.

Scale-μ
0   10   20

C. Drechsler del.

may appear as a sparsely septate, simple or branched filament extending crookedly through the animal (fig. 5, A, v). Naturally no hyphal segmentation occurs in host animals, which, as the result of being infected by many adhering spores at nearly the same time, harbor from the beginning a larger number of thalli than can develop to full size on the limited supply of nutrients available. The frequency of septation is manifestly governed by the size of the animal host as well as by the number of competing thalli present originally, since a small young tardigrade can provide nourishment for only 1 or 2 full-sized unicellular hyphae (fig. 2, D, e, f; fig. 3, C, a, b), whereas a large tardigrade is often found to contain 25 to 35 such bodies.

Asexual reproduction is initiated by the fungus some time after the death of the host, usually when a considerable portion of its substance still remains to be assimilated. The unicellular hypha or hyphal segment grows out broadly at one end (fig. 3, C, a; D; E. fig. 4, B, C. fig. 5, B, d, e; C), or less often puts forth a stout lateral branch (fig. 3, C, b; fig. 4, D). On reaching the host cuticle, the prolongation or branch forces its way through this barrier and continues growth upward into the air. At a height frequently of 25 to 50 μ, its apical growth is modified by the production at rather short intervals of conidiiferous branches outwardly much resembling the phialides familiar, for example, among the species of *Acrostalagmus, Cephalosporium,* and *Spicaria* parasitic on nematodes and insects. These branches are burgeoned forth one by one at the elongating bluntly rounded tip. In a moist microscopical mount under a cover glass they first become recognizable individually as a small globose or saccate protuberance connected narrowly with the hyphal tip (fig. 3, C, a, b; fig. 4, B, C). Very commonly the protuberance is found directed at nearly a right angle to the axis of the hypha. Although such posture might result from accidental bending incurred in the preparation of the mount it appears rather more likely that the protuberance is normally budded forth at an abrupt angle. In any event the growing protuberance very soon occupies an unquestionably lateral position as the hypha continues to elongate straightforwardly. On reaching definitive size it forms an ellipsoidal utriform branch usually curved in such wise that when viewed from the side the proximal portion dips noticeably downward, whereas the distal portion is directed markedly upward. The branch now becomes delimited basally through deposition of a cross-wall at its narrow

FIG. 4. *Ballocephala sphaerospora,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Specimen of *Macrobiotus* sp. (dorsal view) showing an adhering conidium, a; four unicellular assimilative hyphae, b-e; and a uniseptate assimilative hypha, f. C, Assimilative hyphal cell that has given rise to a growing conidiophore from which forty-six conidiiferous branches have already been put forth. D, Small assimilative hyphal cell that has grown out laterally to produce a young conidiophore. E, Denuded conidiophore and nearly empty assimilative hyphal cell from which it arose. F, Empty assimilative hyphal cell and denuded conidiophore arising from it; for lack of space, shown in parts whose proper connection is indicated by dotted lines. G, Unit of sexual apparatus with nearly mature zygospore.
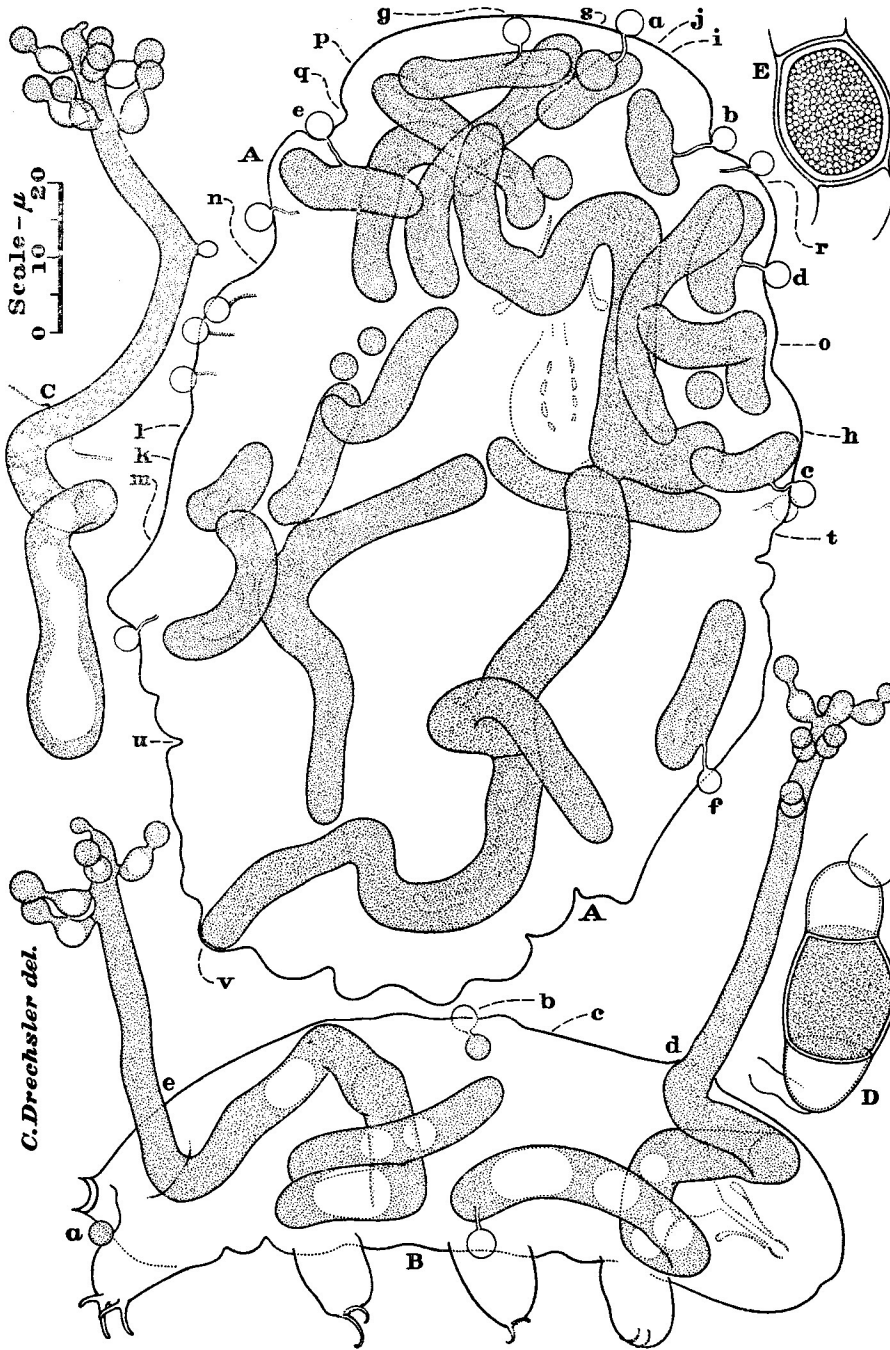
attachment, while at about the same time it puts forth a globose bud distally. This globose bud gradually expands as it receives protoplasmic material from below. Eventually, when after increasing vacuolization the utriform part has yielded all its granular contents, the bud is walled off proximally as a full grown conidium. In the undisturbed humid atmosphere of a closed Petri plate culture the conidium may remain in place for many hours or, perhaps, for a few days, as the utriform cell, filled with clear liquid, retains its shape unchanged.

While the sequence of events in the production of the first lateral branch and of the conidium borne on it are taking place, the parent hypha continues its upward growth and at intervals of about 1.5 or 2 μ keeps on putting forth at its apex one utriform protuberance after another. Each successive protuberance then likewise undergoes development into a lateral branch and terminal conidium. Consequently when the first conidiiferous branch is completely formed, from 5 to 10 (as a rule, perhaps, 8 or 9) such branches have been put forth and have reached varying stages of development,—the lowermost one being nearly mature while the uppermost one is present in an incipient state. A progression of development corresponding approximately to that of an indeterminate inflorescence in phanerogamic plants is thus inaugurated. This progression is maintained as elongation of the conidiophorous hypha continues, until all the protoplasmic material elaborated below in the assimilative hypha or hyphal segment has been expended. Where the underlying assimilative unit is fairly large, from 40 to 80 conidia may eventually be produced (fig. 2, D, f; fig. 4, C). Transfer of protoplasmic materials upward is accompanied by increasing vacuolization below. It seems very probable, nevertheless, that the protoplasm available in the hyphal cell at the time the conidiophore begins to grow out is augmented considerably through continued expropriation of the host, for when reproductive development from all hyphae and hyphal segments is completed, little is left of the tardigrade except its cuticle and claws.

FIG. 5. *Ballocephala sphaerospora,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Specimen of *Macrobiotus* sp. (dorsal view, somewhat flattened) showing seven affixed empty conidial envelopes, each with a denuded germ tube, and seven other such envelopes, a-g, each with a germ tube to which is attached a growing assimilative hypha; fourteen unicellular assimilative hyphal cells, h-u, of different sizes; and one branched biseptate assimilative hypha, v, whose lowermost segment seems ready to push a conidiophore through the host cuticle. B, Small specimen of *Macrobiotus* sp. (lateral view) showing an adhering conidium, a; empty conidial envelope, b, with a germ tube terminating in an infection bladder; a separate assimilative hyphal segment, c; an empty conidial envelope with a germ tube to which is attached a rather large assimilative hyphal cell that has put forth a young conidiophore, d; and a separate assimilative hyphal cell which likewise has put forth a young conidiophore, e. C, Another assimilative hyphal cell from which has arisen a conidiophore in rather early stage of development. D, Zygosporangium showing between the empty locules at its ends a young zygospore filled with finely granular protoplasm. E, Zygospore in nearly mature condition.

Although the axial hypha grows out about 1.5 to 2 μ before extending each successive protuberance from the apex, the resulting branch does not usually come off directly above its immediate predecessor, but is attached to a different sector, and thus in a plane parallel to the surface of the substratum makes a rather wide angle with its immediate predecessor. Generally the individual conidiiferous branches are separated by intervals of approximately 10 μ from their nearest fellows that project out in the same direction and that arise, above or below, from the same radial sector of the parent hypha. As the branches collectively occupy only an estimated one-third or one-fourth of the space available on the hypha, their arrangement is a loosely rather than a compactly spicate one. Viewed from above, that is from a position in alignment with the axial hypha, they show often some tendency toward pentastichy. Their rather even distribution and fairly regular spacing adds to the handsome appearance offered by a well-laden conidiophore in lateral view.

Since in a quiet humid atmosphere a full-developed conidiiferous branch may remain unchanged for a somewhat extended period, Petri plate cultures that have been kept closed for several days will contain here and there the cuticle of a dead host tardigrade from which perhaps more than twenty well-developed conidiophores and some smaller ones are found arising. Often nearly all of the more robust conidiophores erupt near the margin of the cuticle, and lean slightly away from it; so that together they make up, as it were, a palisade having a roughly elliptical form below and flaring out perceptibly above. The striking display thereby provided does not long endure when the cover of the Petri dish is removed. After a few minutes the handsome conidial apparatus will begin to disintegrate, and in less than half an hour may become desolate beyond recognition. Every conidiophore will then usually be found denuded not only of all mature conidia but also of the utriform lateral branches on which they were borne. Conidiophores that had completed their development will have been reduced to bare empty hyphae, with slight serrations marking the places where branches had been attached (fig. 4, E, F). Conidiophores that had been well advanced in their development but were still actively elongating, will have retained only the immature branches, usually numbering from 5 to 10, at different stages of production at the tip. Only rather young conidiophores without any mature conidiiferous branches will have remained unchanged. The agar substratum surrounding denuded conidiophores will have become thickly strewn with detached conidia. Mostly such scattered conidia appear as globose bodies with a clean circular profile (fig. 2, E). In some areas, however, where the agar would seem a little too dry at the surface for ready wetting of any small objects that may fall on it, the conidia show individually a film-like appendage, highly variable with respect to size and outline (fig. 2, F). This appendage evidently represents the tattered membrane of the lateral cell on

whose tip the conidium was produced. Since nothing with the shape and size and content of the lateral cells is ever observable among the scattered conidia, it seems certain that these cells undergo destruction when the spores are being shed.

The results achieved through their destruction may be inferred from the distribution of the conidia over the substratum. In instances where a single conidiophore had developed from a small tardigrade that succumbed in a rather isolated position, the detached spores will not be found limited to a small area around the base of the denuded hyphal shaft where they would have fallen under gravitation alone, but will be scattered over a considerably wider expanse. About four-fifths of the conidia will usually lie somewhat evenly distributed in a circular tract having a radius of 200 μ. By far most of the remaining spores will be sprinkled more sparsely in the adjacent concentric zone having its outer circumference 300 μ from the denuded hypha, while a few will be seen at distances between 300 and 400 μ. Evidently the conidia of the fungus do not merely fall from their attachments, but are forcibly thrown off. The propulsive energy is obviously supplied on rupture of the turgid envelope of the unicellular supporting branch. Unfortunately the bursting of the cell, as also the flight of the spore, is wholly indiscernible under the microscope. Judging from the membranous remnants attached to the discharged spores, the cell envelope probably ruptures at its basal end. It seems at least possible that momentum is imparted to the spore through recoil from violent backward squirting of sap. The mechanism operative here may therefore be of the "rocket" type whereby, according to Ingold (1934), the inflated basidium of *Basidiobolus ranarum* Eidam is projected into the air. As the distal portion of the supporting cell is directed noticeably upward, there is reason to presume that the conidium is shot off ascendingly, and thus has a longer trajectory than if it were shot off horizontally.

Shortly after all their mature lateral cells have been discharged, conidiophores—especially conidiophores closely surrounded by many others—are often found with individual spores adhering to them, or with chains of 2 or 3 or 4 spores drooping from their tips. Many such adhering spores unquestionably come from neighboring conidiophores on normal discharge of the lateral cells that produced them. Lodgment of spores on their own conidiophores can perhaps take place only through mishap or faulty operation of the discharge mechanism. The generous spacing of the lateral cells on the hyphal shaft strongly recalls the uniform separation of basidia noted by Buller (1909) in the hymenium of species of *Coprinus*. If, as Buller suggests with reason, the wide spacing of basidia serves helpfully in preventing the sticky basidiospores from touching one another during development and discharge, the separation of the lateral cells in the present fungus can well be interpreted as an adaptational feature of like utility. Of course, strong adhesiveness of

the conidia is here a character of very obvious necessity, for through it alone can the fungus get and keep a foothold on the cuticle of the host animal preliminary to infection.

When the one or two conidiophores usually found produced from small young tardigrades have discharged their conidia, the substratum over an area of about .2 square millimeter is bestrewn with spores so thickly that any visiting host animal would seem to have little prospect of not becoming infected. Where a fully grown tardigrade gives rise to a score of conidiophores arranged in a flaring palisade, the ensuing discharge provides a similarly effective cover over an area of approximately .6 square millimeter. These areas are, indeed, small in comparison with the macroscopically visible fields of heavy conidial contamination that often surround insects killed by entomophthoraceous parasites of the robust type long familiar. Pronounced disparity in range of projection is not surprising in view of the wide difference in size of both the spores and the associated propellent parts. From assigned linear dimensions it may be computed that the conidia of the tardigrade parasite are exceeded in volume fully two hundred times by those of the house-fly fungus, *Entomophthora (Empusa) muscae* (Cohn) Nowak. and from one thousand to two thousand times by those of *Entomophthora (Empusa)papillata* Thaxter (1888). The disparity in range of projection seems expressive of adaptation to wide differences with respect to unobstructed space available under natural conditions. Infected insects usually succumb in exposed positions, where ordinarily there is ample scope for long trajectories, while terricolous tardigrades of the kind here concerned live and die mainly in the narrow interstices within accumulations of decaying vegetable materials.

In agar plate cultures of customary firmness the tardigrades move about and succumb to infection mainly on the surface. Therefore, when the prolongations from the hyphal segments break through the host cuticle at the front or rear, along the sides, or from the back, they usually emerge directly into the air and so are favorably situated to begin forming conidia after only little additional growth. Yet in limited areas where the agar has become softened under small deposits of water, infected tardigrades occasionally bog down and thus eventually succumb in submerged positions. The hyphal prolongations emerging from such animal hosts first push their way through the overlying ambient before they begin to produce conidia. As in *Meristocrum asterospermum*, conidia here are formed only on aerial terminal portions of the outgrowing hyphae. Although under natural conditions the host animal with its weak legs and broad frontal conformation seems unlikely to bore into the individual firm pieces that make up plant detritus, infected specimens undoubtedly become submerged now and then through the ordinary accidents of water movement. A noteworthy postural feature of the

fungous comes to light when infected animals succumb on the vertical faces of an agar block that has been placed on the flat expanse of a Petri plate culture. The conidiophores, in these instances, grow out more or less horizontally, that is, in a general direction normal to the vertical surface concerned.

The fungus shows distinctive departures from the developmental trends displayed by *Gonimochaeta horridula* and *Meristacrum asterospermum*. In *G. horridula* all segmentation takes place rather soon after the host has been infected, the products of repeated fission there being only a little larger than the infective bodies intruded from the adhering conidia, and consequently much smaller than the thallodic cells that eventually will put forth reproductive outgrowths. In *M. asterospermum*, on the other hand, hyphal growth proceeds without segmentation until virtually all the digestible substance of the host has been assimilated; the hyphae thereupon dividing by fission until the resulting segments have the reduced length at which asexual or sexual reproduction is habitually initiated. With respect to vegetative division the tardigrade parasite reveals a condition intermediate between the extremes represented in the two related forms; for, as has been noted, fission here occurs concomitantly with assimilation and growth, so that the hyphal cells never come to exceed the dimensions suitable for reproductive development.

However, in the organization of its conidial apparatus the tardigrade parasite is not intermediate between the two related small-spored fungi, but clearly shows an advance beyond *Meristacrum asterospermum*, just as that fungus shows an advance beyond *Gonimochaete horridula*. In *G. horridula* the aerial hyphal outgrowths into which the protoplasmic contents of the thallodic segments migrate, reveal a sequence of development like that of a determinate inflorescence. Spore formation there is always delayed until the outgrowth has reached its definitive length. The endogenous spores are first individualized at the tip of the outgrowth, and later in successively more proximal portions. They are also discharged in a more or less basipetal sequence, all owing their rather feeble propulsion to the pressure exerted by the clear liquid filling the thallodic segment below. In *M. asterospermum* development of the sporophorous outgrowth again proceeds as in a determinate inflorescence, but here the outgrowth, instead of forming spores endogenously, becomes partitioned at short intervals, and each of the resulting segments burgeons forth a conidium laterally. The condium evidently is shot away somewhat feebly through bursting of the parent segment, whose wall thereby collapses utterly. Apparently as a device to keep conidia from sticking to one another the fertile distal portion of the sporophorous hypha is convolved in a corkscrew-like spiral, and the conidia arise along the outer curvature of the helix. Development of the conidiophore in the tardigrade

parasite is distinctive in that it proceeds after the manner of an indeterminate inflorescence. The aerial shaft here does not become partitioned transversely but instead puts forth many phialide-like lateral cells, each of which produces and later shoots off a distal conidium. With respect to arrangement of axial hypha and conidiiferous cells the tardigrade parasite invites comparison, among nematode-killing hyphomycetes, with various species of *Acrostalagmus*, whereas *M. asterospermum* more nearly resembles my *Meria coniospora* (Drechsler 1941). Despite their different cellular relationships, the conidia of the tardigrade parasite seem strictly equivalent to those of *M. asterospermum*, which from similarities in size, shape, and plural development, appear homologous with the small secondary spores that are produced plurally from the large primary conidia of *Delacroixia coronata* (Costantin 1897) Saccardo & Sydow 1899. These secondary spores, or "microconidia" as Kevorkian (1937) termed them, seem interpretable on grounds set forth earlier (Drechsler 1946) as being equivalent to the zoospores of the Oomycetes, and the sporongiospores of the Mucorales. In accordance with such interpretation the large conidia of *D. coronata*, and by extension more especially also the large multinucleate conidia borne on unbranched conidiophores of the insectivorous forms commonly referred to *Empusa*, would correspond in a morphological sense to the sporangia of the Oomycetes and Mucorales. The conidia of the tardigrade parasite thus appear to be of a more primitive type than the large derivative conidia familiar among the Entomophthoraceae.

The fungus was found producing zygospores rather sparingly. In most infected animals no zygospores were formed at all. Usually only one or two developed within an individual tardigrade host, though in a few animals as many as seven were observed. Among the many intertangled thallodic segments often present, units of sexual apparatus are in large part commonly obscured from view. Sometimes the parent segments become largely indiscernible even while the zygospore is still in a young condition (fig. 5, D); or frequently again, a zygosporangium containing a nearly mature zygospore will be indistinguishable at either end (fig. 5, E). Where a reproductive unit in a nearly mature (fig. 2, G; fig. 4, G) or fully mature (fig. 2, H; fig. 3, F) state is clearly visible throughout, it reveals a hyphal segment at each of the two ends of an elongated ellipsoidal zygosporangium. The two hyphal segments, which sometimes are virtually empty of living material, and at other times retain a substantial quantity of protoplasm, evidently represent the parent cells from which were delimited the gametangia whose fusion resulted in the zygosporangium. At each end the ripening zygosporangium is usually empty for a distance equal to about one-fifth of its length, the empty portions appearing as dome-shaped locules. Occasionally a locule may keep a considerable remnant of protoplasm (fig. 4, G). Between the two

locules is contained the zygospore proper. Of prolate ellipsoidal, or dolioform shape, it occupies the full width of the zygosporangium, usually, indeed, causing that structure to bulge out noticeably. Except at the ends its thickish brown wall is adnate to the thin colorless zygosporangial envelope. At maturity the zygospore has a distinctly brownish coloration. In its interior a parietal layer of coarsely granular texture surrounds a homogeneous reserve globule.

To signalize a characteristic feature of its conidial apparatus the fungus is described under a generic name compounded of two words meaning "to throw" and "head", respectively.

Ballocephala Drechsler, gen. nov. Hyphae assumentes intra animalia viva crescentes, simplices vel aliquanto ramosae, interdum aliquid septatae, denique cellulis eorum saepe disjungentibus; cellula assumenti aut hypham fertilem aut sporam perdurantem (zygosporam vel azygosporam) gignente; hypha fertili in aerem se porrigente, in apice ramum lateralem pullulascente, deinde identidem recrescente multos alios ramos laterales deinceps proferente; ramo laterali in una cellula constante, in apice unum conidium ferente et postea id violenter propellente.

Assimilative hyphae growing parasitically within living animals, simple or somewhat branched, sometimes rather sparingly septate, the cells later often separating from one another; the assimilative cells each eventually putting forth a conidiophore or participating in the development of a zygospore or of an azygospore; the conidiophore after growing some distance into the air giving rise at its tip to a lateral branch, and then on repeated elongation burgeoning forth many other such branches, one by one, in successively more distal positions; each lateral branch made up of a single cell, and forming distally a single conidium which subsequently it propels forcibly into the air.

Ballocephala sphaerospora Drechsler, sp. nov. Hyphis assumentibus incoloratis, continuis vel paululum septatis, in singulis vel aliquot cellulis 5–80 μ longis et 5–10 μ crassis consistentibus, cellulis vulgo facile secedentibus; hyphis fertilibus incoloratis, simplicibus, plerumque 50-175 μ longis, basi saepius 4–5 μ crassis, sursum leniter attenuatis, apice circa 3 μ crassis, 20–80 ramulos conidiferos in laxum spicum ferentibus; ramulis conidiferis utriformibus, aliquid curvatis, 4–7 μ longis, 3–4.5 μ latis; conidiis laevibus, incoloratis, globosis, 3–4.5 μ in diametro, tubulum germinationis 2–5 μ longum et circa .5 μ crassum in tardigradum intrudentibus; zygosporangio inter duas cellulas assumentes oriundo, elongato-ellipsoideo, plerumque 28–33 μ longo, 14–16.5 μ lato, membrana ejus incolorata, .3–.4 μ crassa; zygospora elongato-ellipsoidea vel dolioformi, 17–20 μ longa, 13.5–16 μ lata, membrana ejus brunnea, .6–.8 μ crassa, cum membrana zygosporangii late concreta.

Speciem Macrobioti enecans habitat in humo silvestri prope Oxford, Pennsylvania.

Assimilative hyphae colorless, most often consisting of a single cell 5 to 80 μ long and 5 to 10 μ wide, but sometimes found composed of 2 or 3 or 4 such cells; the growing cells, whether separate or united, commonly dividing by means of a cross-wall on reaching a length between 40 and 80 μ, with the resulting segments usually soon becoming disjointed. Conidiophores

colorless, unbranched, mostly 50 to 175 μ long, 4 to 5 μ wide at the base, gradually tapering upward, near the tip about 3 μ wide, usually bearing 20 to 80 propulsive conidiiferous branches in open spicate arrangement; the propulsive branches unicellular, of utriform shape, mostly 4 to 7 μ long and 3 to 4.5 μ wide, usually curved with the distal portion directed upward; conidia colorless, globose, smooth, 3 to 4.5 μ in diameter, on adhering to a tardigrade host intruding an infective body by means of a germ tube usually 2 to 5 μ long and about .5 μ wide. Zygosporangium produced between two assimilative hyphal cells, of elongated ellipsoidal shape, measuring commonly 28 to 33 μ in length and 14 to 16.5 μ in greatest width, having a colorless wall, .3 to .4 μ thick, which is extensively adnate to the zygospore wall; zygospore elongated ellipsoidal or dolioform, often 17 to 20 μ long and 13.5 to 16 μ in greatest width, its proper wall distinctly brownish, and .6 to .8 μ thick, its protoplast at maturity consisting of a coarsely granular parietal layer and a central reserve globule 4 to 5 μ in diameter.

Parasitic on *Macrobiotus* sp. it occurs in deciduous leaf mold near Oxford, Pennsylvania.

Literature Cited

Buller, A. H. R. 1909. Researches on fungi. 1. London.

Costantin, J. 1897. Sur une Entomophthorée nouvelle. Bull. Soc. Mycol. Fr. **13**: 38–43.

Drechsler, C. 1940. Three fungi destructive to free-living terricolous nematodes. Jour. Washington Acad. Sci. **30**: 240–254.

——————. 1941. Some hyphomycetes parasitic on free-living terricolous nematodes. Phytopathology **31**: 773–802.

——————. 1946. A nematode-destroying phycomycete forming immotile spores in aerial evacuation tubes. Bull. Torrey Club **73**: 1–17.

——————. 1948. Three Zoöpagaceae that subsist by capturing soil *Amoebae*. Mycologia **40**: 85–105.

Ingold, C. T. 1934. The spore discharge mechanism in *Basidiobolus ranarum*. New Phytologist **33**: 274–277.

Kevorkian, A. G. 1937. Studies in the Entomophthoraceae I. Observations on the genus *Conidiobolus*. Jour. Agr. Univ. Puerto Rico **21**: 191–200.

Marcus E. 1936. Tardigrada. *In* Schulze, F. E., W. Kükenthal, K. Heider, R. Hesse, and C. Apstein: Das Tierreich, Lieferung 66. Berlin and Leipzig.

Saccardo & Sydow. 1899. *Delacroixia. In* Saccardo, P. A.: Sylloge fungorum **14**: 457. Patavii.

Thaxter, R. 1888. The Entomophthoreae of the United States. Mem. Boston Soc. Nat. Hist. **4**: 133–201.