MONOCHYTRIUM, A NEW GENUS OF THE CHYTRIDIALES, ITS LIFE HISTORY AND CYTOLOGY.*

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In working over sections of leaves and stems of the common Ragweed, Ambrosia artemisifolia, infested with Rhodochytrium spilanthis the cytology of which had interested the writer in connection with his work on Synchytrium, he found that there was present along with the Rhodochytrium another parasite. It was at first supposed that the new plant was an early stage of Rhodochytrium but it was soon found that it had nothing in common with Rhodochytrium except its host plant, being distinct in all of the details of its cytology as well as in its method of parasitism and its life history. Whereas Rhodochytrium is an intercellular parasite infesting the fibrovascular bundles of its host into which it sends numerous haustoria to gather its nutrition, the new plant which I shall term *Monochytrium* leads an intracellular existence within the epidermal, hypodermal or more rarely the chlorenchyma cells of its host thus resembling in its mode of life such species of Synchytrium as S. taraxici, a resemblance which is further increased by the absence of haustoria. From these plants, however, Monochytrium differs markedly in the binucleate sexual resting spores and in the solitary zoosporangia in allusion to which the generic name has been chosen.

After Monochytrium was discovered a considerable amount of the Ragweed infested with Rhodochytrium was examined in the hope of detecting the new parasite in the living state and of observing its grosser characters and its zoospores. This search was, however, fruitless, which is not surprising in view of the habits of the fungus. For while the parasite is extraordinarily abundant in certain small areas of the sections (Fig. 1), such areas are seldom found. Out of 200 slides Monochytrium was observed in only 10. Furthermore, the parasite deforms its host only very slightly so that infested areas would not be easy to find unless they were abundant. The Rhodochytrium material from which the slides were made was supplied me by the kindness of my good friend, Professor F. L. Stevens, and his colleague, Mr. J. G. Hall of the North Carolina Agricultural Experiment Station. It was collected at Raleigh on July 3, 11, and 18, 1908, and was a portion of the material sent by Dr. Stevens to Professor Atkinson from which he published his two notes on Rhodochytrium. It was killed in Chromacetic acid, imbedded in paraffine

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in the usual way, sectioned 5–10μ thick, and stained on the slide with Heidenhein's Iron Alum Haematoxylon and with Analin Safranin and Gentian Violet. Either stain is satisfactory but most of the drawings have been made from material stained with the Safranin-Violet combination.

The youngest stages of the parasite found were imbedded in the cytoplasm of the host cell (Fig. 2). They were minute amoeboid cells whose size (3μ) corresponds rather closely with that of the segments of the zoosporangium. Not infrequently the perforations by which the young parasite had entered the host cell may be seen in section as thickenings on the inside of the wall of the host (Figs. 8, 10). In rare instances slight thickenings may also be observed on the outside surface of the wall (Fig. 13). In other cells cut tangentially so as to allow one to look through the perforations (Fig. 3) one sees that the holes are surrounded by irregular cellulose thickenings. In appearance these collars resemble somewhat the irregular growths of bark around a healing wound on a tree trunk and suggest that they were secreted by the cytoplasm of the host cell in an attempt to repair the damage; in many instances there are indications that such repair is completed for in most of the infected cells the points of entrance appear to be simply thickened places on the walls and no perforation can be observed by focusing up and down.

In favorable locations the young amoebulae imbedded in the host cytoplasm are extremely abundant, hardly a cell being free from parasites (Fig. 1). Moreover, there are frequently nearly a dozen in a single cell (Fig. 2). Their minute size precludes very exact observations as to their structure but as compared with the segments of the zoosporangia from which they are supposed to have come their cytoplasm is less dense, containing, apparently almost from the beginning, several relatively large vacuoles (Fig. 5), between the meshes of the reticulate cytoplasm. Of the nucleus little can be seen beyond the deeply staining nucleolus and the nuclear membrane, though by analogy with the larger nuclei of later stages it may be supposed to have more or less of a chromatin reticulum in addition. Lying in the cytoplasm close beside the nucleus there is frequently a deeply staining body (Figs. 5, 6) whose general appearance at once suggests a centrosome. No such structure was observed at any other stage of the life history but it is not impossible that one may be associated with the flagella of the zoospores. But as long as the zoospores themselves remain unknown it is idle to speculate on the matter. The deeply staining bodies in question occur, however, with sufficient frequency to make it very desirable to be able to offer some interpretation of their presence.

In almost every cell in which there are several of these amoebulae they may be seen to become associated in pairs, (Fig. 4), coming into closer and closer contact until the plasma membrane between them breaks down and the cytoplasm of the two fuses (Figs. 5, 6). All stages in this conjugation except the disappearance of the plasma membrane are very easy to observe, many dozen of them being found in my slides. The nuclei, however, do not fuse though they may in the early stages lie close together. Immediately after conjugation which seems to take place soon after the amoebulae have entered their host, growth begins and seems to proceed rather rapidly judging from the fact that conjugating forms are much more abundant than such stages as are shown in Figs. 8 and 9, which immediately follow. Without any further change in structure the zygote continues to grow until it has completed its active life when it encysts and becomes a binucleate resting spore.

Though there are frequently two or even more than two zygotes in a single cell all of the amoebulae do not succeed in conjugating. Such as fail become large coenocytes which ultimately segment into zoosporangia. The very early stages in the division of the nuclei of these zoosporangia are so minute and difficult to follow that one can hardly be certain of the correctness of his conclusions. But apparently the nucleus fragments by constriction into about four daughter nuclei while the parasite is yet very small (Fig. 13, a). These do not further subdivide until a considerable enlargement both in nuclei and cytoplasm of the parasite has taken place. (Fig. 13, b). Such quadrinucleate parasites are fairly abundant and from this stage on the course of development is easy to follow. The parasite increases from 10–15μ, usually to about 70μ and the nuclei multiply until they become exceedingly numerous and very minute (Figs. 14–17). No spindles were observed at any time in this process of multiplication, while some clear cases of amitosis were seen (Fig. 14). The nuclei are so minute however, that it cannot be stated positively that amitosis is the sole method of nuclear division. At the end of this vegetative period the cyst segments into a zoosporangium (Fig. 18), with an immense number of spores so minute (2.5μ) that their finer structure cannot be made out.

In the intermediate stages of the active cycle both of the resting spores and the zoosporangia there is a strong tendency for the vacuoles of the cytoplasm to coalesce to form one large central vacuole (Figs. 14–17), traversed only by very fine strands of cytoplasm. This central vacuole may appear very early (Fig. 14) or it may not appear at all (Figs. 9–13). During these stages also refringement deeply staining granules frequently appear on the strands of cytoplasm (Figs. 11, 16). These resemble closely the

similar granules found in the cytoplasm of many species of Synchytrium.

Resting spores and zoosporangia are likewise entirely similar in their relations to the host cell. As already indicated the parasites in their first stages lie imbedded in the cytoplasm of the host cell. As they grow older they continue to be surrounded by a more or less definite layer of host cytoplasm but soon establish definite relations with the host nucleus also which becomes so appressed against the parasite as to be markedly deformed (Figs. 8, 11, 15). There is no indication, however, that the immediate injury to the nucleus is very great. Though death is the ultimate result to the host cell the relations of parasite and host appear to be to a certain extent mutualistic. The host nucleus maintains its finer structure and staining reaction unchanged to the end and gives no indication of such abnormal behavior as Von Gutenberg, Kusano and others have reported in the nuclei of the host cells surrounding the galls of Synchytrium. The presence of the parasite causes some hypertrophy of the host cell which gradually enlarges to dimensions considerably in excess of its original size (Cf. Figs. 8, 13, with Figs. 12, 18). The enlargement is however very seldom sufficiently great to cause galls such as occur in Synchytrium. For the most part the hypertrophied cells find room not by swelling out from the surface of the host but by pushing aside the adjacent cells (Figs. 1, 16). These compressed cells are however, only slightly injured considering the degree to which they are distorted (cf. Fig. 2 which shows a cell lying adjacent to a large zoosporangium and distorted by it.) There is surprisingly little of the disorganization of the tissues which is usually met with in such cases but the nuclei and chloroplasts of the affected cells retain their characteristic form and staining reaction even when the cell walls are so crowded that the outlines of the individual cells are no longer discernible as in cases like Fig. 16.

The size which is attained before the active life is completed and encystment takes place varies from 30 to 50μ depending probably on the amount of nutriment available for the parasite. When it first appears (Fig. 11) the wall of the resting spore is a thin transparent membrane secreted around the periphery of the parasite. When older it becomes a thick yellow wall (Fig. 12) which is homogeneous, one layered and smooth on the outer surface except for irregular roughenings due apparently to the adherent debris from the contents of the host cell. The spore wall is certainly not composed of cellulose; at no stage in its formation does it take the stain as do the walls of the host or the three layered cellulose walls of the resting spores of Rhodochytrium which are found together with it in the same slides. Its

general appearance is identical with that of the resting spores of Synchytrium which Von Gutenberg has recently determined to be chitinous. On account of the scarcity of material, however, michrochemical tests to determine its composition were not undertaken.

General Considerations.

The relationships of Monochytrium are in the present state of our knowledge regarding the Chytrids somewhat obscure. Its method of parasitism and general structure are similar to those of Synchytrium and, had the present plant been described without reference to its cytology, the only difference between the two genera that would have been noticed is the difference in segmentation which in Synchyrium results in the formation of zoosporangia each of which in turn gives rise to numerous zoospores while in Monochytrium the zoospores are formed directly, each cyst becoming a single zoosporangium. This difference is however of itself sufficient to remove the plant from the Synchytriaceae and place it among the Olpidiaceae. From all the genera of this family Monochytrium may be separated at once by its habitat. All the other genera are parasites of aquatic plants or animals except Asterocystis which infests the roots of the seed plants.

So far as the writer is aware in no other plant has a conjugation of gametes been reported to occur after the young parasites have infected their host. But when the cytology of the lower organisms especially of their early stages is better known it may be found that such a conjugation is not so rare as now appears. It is quite possible that many forms now supposed to be non-sexual may conjugate after infecting their host. The life history of most species of Synchytrium for example would seem to demand some difference in constitution between the summer sori and the resting spores similar to this belated conjugation of Monochytrium; but if such a sexual act exists it is obvious that in these cases the nuclei also must fuse. The continued independence of the nuclei of the zygote may be more unusual but when it is recalled in how few of the zygospores of the lower plants are the actual conditions of the nuclei known, it is evident that such a plasma conjugation may be more common than now suspected. This long continuance of the apylogamic phase in Monochytrium cannot fail to recall the similar phenomena in the nuclei of the higher fungi. Nothing could be of greater interest than to determine the fate of these two nuclei in the germination of the resting spore. Attempts at germination must however wait upon more abundant material than is now available.

It is hoped that an opportunity may also be presented to observe the zoospores in the living condition in order to deter-

mine their behavior and their structure, particularly the characters of their organs of locomotion. For it will be recalled that while in many groups the number and position of the flagella are so constant as to be made the basis of distinctions of ordinal or of even higher rank, in the Chytridiales they are very variable for one finds in genera undoubtedly closely related great diversity in this regard. The zoospores of Synchytrium for example have one flagellum while those of Woroninella have two. The behavior of the zoospores of some of the Chytrids goes to show that the flagella of this group may be of very indefinite organization. Atkinson has shown that when liberated inside the sporangium the zoospores swim actively forward until they strike the wall of the sporangium when the flagella are retracted and the zoospore puts out pseudopodia by which it gropes for the opening of the sporangium. In case it is located too far from the ostiole to reach it with its pseudopodia it resumes its flagellate form and swims about again until it finally escapes. Such behavior indicates very plainly that the flagella of these zoospores resemble the long actively lashing pseudopodia present in such of the Protozoa as Mastigamoeba more than the definite highly specialized motile organs of the Protococcoid forms. In the latter group the zoospores have no power of retracting and again putting forth their flagella but retain the same ones throughout their active stage. Comparisons of flagella based on analogies to the highly specialized organs of other groups must obviously be of somewhat doubtful value.

Indications are not lacking that the spores of Monochytrium are even more widely different from the typical flagellate zoospore than those of other Chytrids. For it seems probable from the habits of the fungus that the motile organs of Monochytrium spores are very inefficient as compared with those of the Synchytria. In each area where it has been found the abundance of the individual parasites was very great. At the same time the infested areas are narrowly circumscribed. This is in strong contrast to the habit of Synchytrium which is always widely distributed over the plant and seldom so excessively abundant as Monochytrium. This is especially evident when one considers the young stages of the parasite. Such a complete series of young stages as here figured for Monochytrium would be exceedingly difficult to assemble for any species of Synchytrium with which I am familiar; in very much more extensive work with Synchytrium decipiens in all stages the writer has never seen so much as one percent of the young stages that he has in Monochytrium. The reason is that the parasites are so much more widely scattered that their detection when very small is difficult. Nevertheless,

Monochytrium presumably has as great an opportunity for the dispersal of its spores in dewdrops and spattering rain as has Synchytrium. The writer is therefore led to expect that when the zoospores of Monochytrium are observed they will be found to be amoeboid rather than flagellate.

For a summary of the most important points in the life history of Monochytrium a condensed technical description may be offered. The type and only known species I propose to name in honor of Professor F. L. Stevens who has made notable contributions to the cytology of the lower fungi.

Monochytrium gen. nov.

Mycelium nullum, plasmodium rotundatum; sporae perdurantes 30-50μ, globosae, ortae a copulationis zoosporarum intra cellulas matricis, binucleatae, exosporio crasso, paene levi non echinulata; zoosporangia circa 70μ, formata a zoosporis sine copulatione, unum a quoque plasmodio, sine membrana, sine collo; zoosporae numerosissimae, 2.5μ, moto ignoto.
Intra cellulas epidermicas aut hypodermicas aut raro chlorenchymatas plantarum viventium.

Monochytrium stevensianum sp. nov.

Characteribus generis. Intra cellulas foliarum petiolorumque Ambrosiae artemisiifoliae in Raleigh, Carolina boreali; Stevens & Hall Julio 1908.

The slides containing the type specimens are deposited in the herbarium of the Ohio State University. With them are index cards giving the location of the cysts drawn, in vernier readings of the Spencer Lens Company's mechanical stage No. 490 with the verniers set to read 30 and 90 respectively when the aperture in the centering slide accompanying the instrument occupies the optical axis of the microscope. The originals of all the figures may therefore be quickly found with any microscope equipped with a No. 490 mechanical stage, or with any mechanical stage with a vernier reading to tenths of millimeters for after one is found and the differences in reading between stage No. 490 and the one employed are determined, all may be located by simple additions to the readings given.

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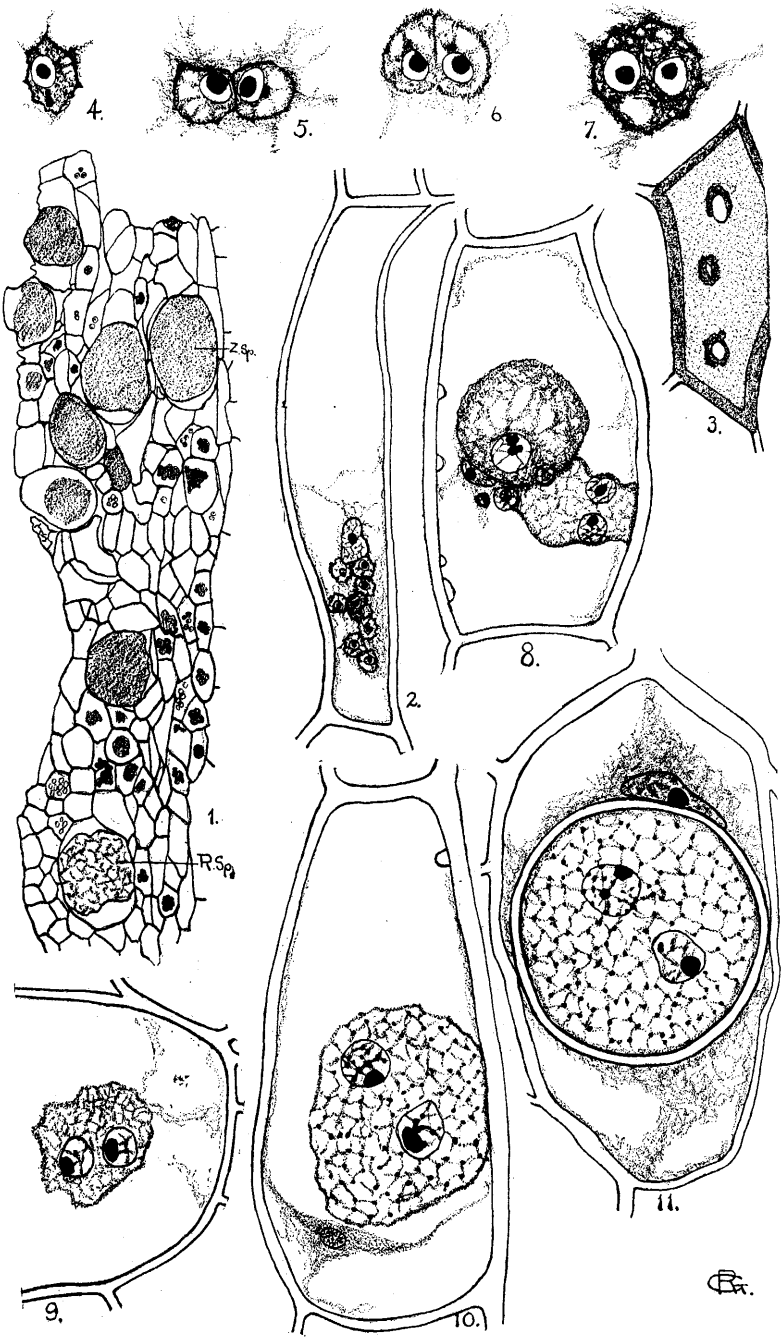
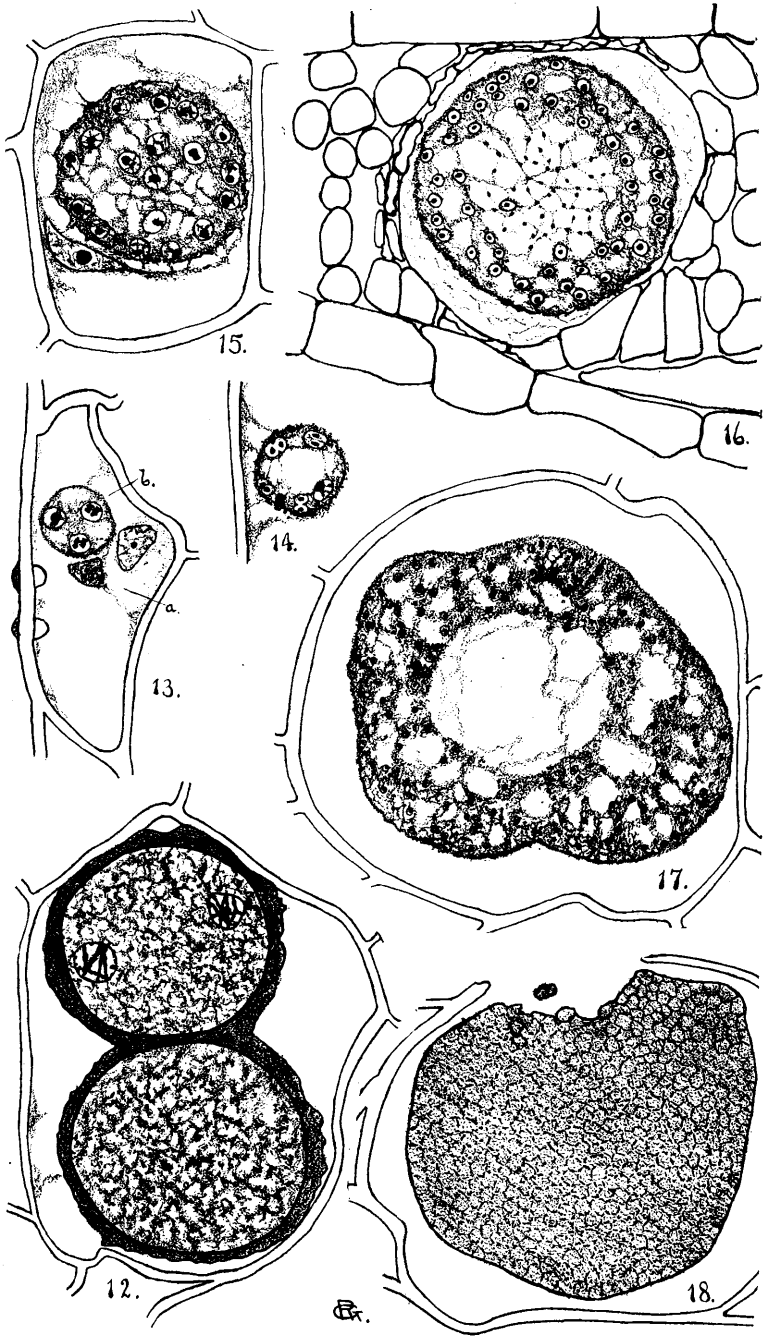


GRIGGS on "Monochytrium."



GRIGGS on "Monochytrium."

EXPLANATION OF PLATES III AND IV.

All figures are camera drawings from sections. All except Figs. 1, 3–7, were made with a Spencer 1.5 mm. immersion objective and compensating ocular 4 giving a magnification of approximately 670 diameters. Fig. 1 was drawn with an 8 mm. objective and 4 ocular giving an approximate magnification of 125 diameters; Fig. 3 with the 1.5 mm. objective and 3 ocular, magnification 533; Figs. 4–7 with objective 1.5 and ocular 12, magnification 2130. The plates were reduced to 2-3 of their original size exactly eliminating the enlargement due to the camera and rendering them the same size as seen in the microscope.

PLATE III.

Fig. 1. A tangential section through the hypodermis of the wing of the petiole of the Ragweed, showing the general relations of the parasites to the tissue of the host. R. Sp. Resting Spores, Z. Sp. Zoosporangia.

Fig. 2. A cell with numerous amoebid zoospores imbedded in the host cytoplasm; one pair of zoospores conjugating; cell distorted by an adjacent zoosporangium measuring 45 x 60 μ, note slight degree of injury.

Fig. 3. A tangential section of a host cell wall showing perforations where the parasites entered.

Fig. 4. One of the zoospores from Fig. 2.

Fig. 5. Zoospores just beginning to conjugate.

Fig. 6. Conjugating zoospores.

Fig. 7. Conjugation complete.

Fig. 8. A cell with two young zygotes, each binucleate, and several unconjugated zoospores; note cellulose plugs marking the points where the parasites entered.

Fig. 9. A young zygote.

Fig. 10. Zygote nearly full grown.

Fig. 11. A young resting spore; note slight injury to the nucleus and cytoplasm of the host cell.

Fig. 12. Two ripe resting spores within same host cell; each binucleate though the nuclei of the lower spore do not lie within the plane of section.

Fig. 13. A cell with two young parasites; *a* probably the first division (amitotic) of the zoospore; *b*, a plasmodium with four nuclei; on the wall are shown the plugs marking the points of entrance.

Fig. 14. A young plasmodium with eight nuclei most of which are in process of amitosis; central vacuole developed unusually early.

Fig. 15. A plasmodium with about 60 nuclei; central vacuole beginning to appear; note relations of parasite and host nucleus.

Fig. 16. A larger plasmodium with well developed central vacuole lying in the chlorenchyma of its host; note slight injury beyond mechanical distortion.

Fig. 17. A full sized plasmodium with very many nuclei.

Fig. 18. A ripe zoosporangium; opening at top may be natural or due to knife.