The Plasmodiophoraceae and their Relationship to the Mycetozoa and the Chytrideae.[1]

BY

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With Plate XII.

SINCE the publication in the Annals of Botany, in 1911, of an investiga-
tion of the life-history and cytology of *Sorosphaera Graminis* (25), a
great deal of work has been done on the Mycetozoa and Plasmodiophoraceae,
and several papers have been published describing the cytology of various
species and discussing the relationship between these two families. Pavillard,
in the 'Progressus Rei Botanicae', 1910, gives an excellent account of the
position at that date, and concludes that the Plasmodiophoraceae are
a branch of the Mycetozoa that has become modified owing to having
taken to a parasitic mode of life. Maire and Tison (13 and 14), in 1911,
combat this view, and suggest that the Plasmodiophoraceae should form
a separate family, which they consider probably owes its origin to the
Chytrideae. Osborn (20), on the other hand, in his paper on *Spongospora*,
lays stress on the similarities between the Plasmodiophoraceae and Myce-
tozoa, and describes a karyogamy similar to that observed by Jahn and
Kränzlin in the Mycetozoa.

The present paper is the outcome of the study of many species of both
the Plasmodiophoraceae and the Mycetozoa, and, while admitting that
these families are related, I am in agreement with the opinion expressed
by Maire and Tison that their differences are so great that the Plasmodio-
phoraceae should form a separate order intermediate between the Mycetozoa
and the Chytrideae. I do not think that the differences between these
families can be accounted for by the parasitic mode of life adopted by the
Plasmodiophoraceae.

THE PLASMODIOPHORACEAE.

This family comprises a number of multinucleate amoeboid organisms,
which live as parasites on various Phanerogamic plants; the best known,
and perhaps the commonest, member of the family is *P. Brassicae*. The

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[1] This thesis, together with the work on the Plasmodiophoraceae previously published, viz.
Root Diseases of the Juncaceae; Tumours on *Veronica Chamaedrys*; Life-history and Cytology of
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members of this family are characterized by a vegetative stage, in which the nuclei of the amoebae divide by a process that has been described by Maire and Tison as 'an intranuclear karyokinesis combined with an amitosis', and by a reproductive stage which closes with two karyokinetic nuclear divisions leading to spore formation.

The family includes the following seven genera:

1. *Plasmodiophora*, with free spores.
2. *Sorosphaera*, with spores collected into hollow spheres.
3. *Tetramyxa*, with spores in tetrads.
4. *Sorodiscus*, with spores collected into hollow, flattened spheres and ellipsoids.
5. *Molliardia*, with spores at present unknown.
6. *Spongospora*, with spores collected into spongy masses.
7. *Ligniera*, with spores collected into masses of varying shapes (e. g. spheres and ellipsoids).

Of these genera, the first five possess certain common characteristics in that they each contain only a single species, the presence of which in the host-plant always gives rise to hypertrophy of tissue with the formation of tumours or swellings either in the stem, leaf, inflorescence, or root. The genus *Spongospora*, represented by *S. subterranea*, causes no apparent hypertrophy to the tissues of the potato, on which it is parasitic.

The genus Plasmodiophora, Wor.

The cytology of *P. Brassicae* has been fully described by Nawaschin (16), Prowazek (23), and Maire and Tison (11). The statement made by Marchand, and quoted by me in my paper on *S. Graminis* (25), to the effect that the roots of Celery, Sorrel, and Melon had been attacked by this species, has been found by Griffon and Maublanc (2) to be an error. Nawaschin states that the groups of diseased cells are formed by the division of single or small groups of infected cells at the growing apices of the roots, and that the amoebae have no power to pass from cell to cell by penetration of the cell-wall; this appears to me to be the case in all the genera. The spores are said to germinate readily, but I do not find this to be the case. Although I have tried to germinate spores of various ages, including some which had been kept through the winter, in no case have I seen an actual germination. In cultures of the spores I have occasionally seen a number of small, pear-shaped flagellate bodies, but these may very probably have been due to other organisms, as the culture was not pure. Prowazek has described a karyogamy taking place on spore formation; this, however, has not been confirmed.

The genus Sorosphaera, Schröt.

This genus is marked by the spores being collected into hollow spherical masses, enclosed by a common membrane. It is represented by the single species *S. Veronicae*, which causes swellings on the stems, leaves, or inflorescences of various species of *Veronica*. I have found this parasite only in *V. Chamaedrys*, although it is stated by Lagerheim to be found also in *V. hederifolia* and in other species. I have seen *V. hederifolia* growing in the immediate vicinity of diseased *Chamaedrys* plants, the former remaining quite healthy. Diseased *Veronica* plants are not commonly met with, and can only be identified with the aid of the microscope. Tumours caused by eel-worms are indistinguishable to the naked eye from those caused by the *Sorosphaera*. These eel-worm tumours, which are much more common, I have seen on *V. Chamaedrys* and other species of *Veronica*.

The cytology and life-history of *S. Veronicae* has been described by Maire and Tison (11) and by Blomfield and myself (1). The first-named authors, in a recent paper (14), confirm the opinion expressed by Blomfield and myself that the infection takes place at the growing apex of the stem.

Winge (26), in his paper on 'Cytological Studies in the Plasmodiophoraceae', in remarking on the akaryote stage of the organism, states that the karyosomes of the nuclei of the amoebae disappear at the close of the vegetative period of its life-history, and that subsequently no fresh karyosomes are formed. He is of opinion that the akaryote stage is formed by the bulk of the chromatin passing out from the nuclei into the plasma of the amoebae. He does not, however, consider that the organism is at any period devoid of nuclei, since some of the chromatin always remains on the site of each nucleus. Subsequently fresh nuclei are formed on the sites of the former ones; these, however, contain no karyosome, and form the reproductive nuclei of the organism. This confirms the statement made in my paper on the cytology of *S. Graminis* (25), that the reproductive nuclei occupied the same sites as the early vegetative ones. Osborn (20), however, in his paper on *Spongospora*, states that there is a total disappearance of the vegetative nuclei, and that the reproductive nuclei are formed *de novo* on adjoining sites.

The genus Tetramyxa, Göbel.

This genus, which is represented by the single species *T. parasitica*, is characterized by the formation of spores in tetrads. Its life-history and cytology have been studied by Maire and Tison (14), who state that the nuclear phenomena are for the most part similar to those met with in the other genera, with the exception that no akaryote stage appears to follow the close of the schizont stage. They have, however, observed and figured

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some forms resembling the akaryote stage in the cells of young tumours, in which the disease was in an early stage and the infection recent. Should these forms prove to be the akaryote stage, this stage would seem to be displaced and would appear earlier than is the case in the other genera.

T. parasitica causes tumours on *Ruppia rostellata* ; it has also been stated to have been found on *Zannichellia palustris* by Hisinger in 1887. Maire and Tison, however, have recently found it plentifully in *R. rostellata*, growing in close proximity to *Z. palustris,* which was uninfected. This throws doubt on the statement that the latter is a host-plant. I have found multinucleate amoebae in the root-hairs of plants of *Z. palustris* which were otherwise free from infection ; these amoebae might be a species of *Ligniera*, or, on the other hand, they might be *T. parasitica.*

The genus Molliardia, M. and T.

In *M. Triglochinis*, M. and T., which is the sole representative of this genus, neither the akaryote stage nor spore clusters have been observed as yet. This species was formerly known as *Tetramyxa Triglochinis*, but has been removed from that genus, since the latter is characterized by the presence of tetrads of spores. It is the cause of swellings on the stems and inflorescences of *Triglochin maritimum* and *T. palustre* ; these swellings are similar in structure and development to those of the *Veronica.*. It has been described by Maire and Tison (14), who state that the mitoses in the schizont stage are similar to those of the other genera, with the exception that abnormal mitoses are not uncommon, and the presence of uninucleate amoebae in the plant-cells is frequent. It is not unlikely that a spore stage will be found, in which case *M. Triglochinis* will possibly be transferred to one of the other genera.

The genus Sorodiscus, Lager and Winge.

S. Callitrichis, Lager and Winge, is the cause of tumours on the stems of *Callitriche autumnalis,* and is the only species of this genus. It has been described by Winge (26), who states that it is very closely related to *Sorosphaera Veronicae,* from which it differs in having the spores arranged in flattened hollow spheres or ellipsoids. Winge has observed in *S. Callitrichis* the typical vegetative mitoses which, he states, occur simultaneously in all the nuclei of an amoeba ; this statement applies also to the other genera. He states further that the axes of the dividing nuclei (i.e. their elongated karyosomes) are generally parallel ; this is certainly not the case in the other genera I have examined. Winge also observed the two mitoses which occur just before spore formation, the number of chromosomes showing a reduction from sixteen to eight.

The genus Sporigospora, Brunch.

S. subterranea has been described by Osborn (20), and agrees in most respects with other genera. As before stated, Osborn maintains that there is a complete disappearance of nuclei in the akaryote stage, and that fresh nuclei (reproductive) are formed on different sites. These reproductive nuclei fuse in pairs, and the resulting nuclei undergo two mitoses prior to the formation of spores. This karyogamy, observed by Osborn, has not been noted in any of the other genera. The vegetative nuclei divide in the manner peculiar to the Plasmodiophoraceae. Osborn, however, states that the chromatin is in the form of a ring around the elongated karyosome, whereas careful focusing shows that it is in reality an equatorial plate. Osborn was unable to get the spores to germinate, but Massee (15) states that he has seen a germination, the spore giving rise to a single myxamoeba.

The genus Ligniera, M. and T.

This genus, which is closely allied to *Sorosphaera*, was formed by Maire and Tison (13) to include those members of the Plasmodiophoraceae in which the spore-clusters are most usually of ellipsoidal and irregular shapes and are rarely spherical. A common characteristic of the members of this genus is that they do not give rise either to hypertrophy of tissue or to nuclear degeneration in the host-plant. To this genus the species described by myself and named *Sorosphaera Junci* and *S. Graminis* have been transferred.

Other species at present included in the genus are: *L. radicalis*, M. and T., found on roots of *Callitriche stagnalis*; *L. verrucosa*, M. and T., found on roots of *Veronica arvensis*.

To the above species three new ones, recently found by myself, have now to be added—viz. *L. Bellidis*, *L. Menthae*, and *L. Alismatis*. All the *Ligniera* agree closely in their life-histories and cytology, but, owing to the fact that they do not cause hypertrophy in their host-plants, and for other reasons referred to later, they are not so suitable for the observation of cytological details as are the members of the other genera. All the species of this genus are root parasites, and the infection in all cases takes place near the root apices. The infected cells, which are confined to the outer cortex of the root, are frequently found in longitudinal rows. In many cases the amoeboid stage of the organism is only to be found in the youngest roots. Root-hairs may also serve as channels for infection, but in this case the amoeba does not spread the infection beyond the cell which gives rise to the hair. The parasite may complete its life-history in the root-hair by the formation therein of a row of spores. Schizogony is rare, and the akaryote stage is seldom seem, it being probably of but short duration, as Osborn suggests.

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In the roots of *Poa annua* the root-hairs are frequently swollen at their apices to form club-shaped chambers, in which the amoebae or spores of *L. Graminis* may be seen. The nuclei, however, of these amoebae are smaller than those in the cortical cells of the root. Figs. 9 and 10 show these hairs. It is to be noted that many of the *Ligniera* are parasitic on the roots of marsh- and water-plants, and further search will probably result in the addition of more new species. I have also observed that these parasites are frequently associated in the roots with typical mycorhiza. This I find to be the case in the roots of *Poa annua, Bellis perennis, Chrysanthemum Leucanthemum, Mentha Pulegium*, and, at times, *Juncus articulatus*. The mycorhizal cells have their nuclei enlarged and degenerate, and, as viewed under the microscope, do not give a healthy appearance.

The three new species of *Ligniera* are described below.

Ligniera Bellidis Schwartz, sp. nov. Amoebis raris in cellulis hospitis; sporis 5–6 μ × 4–5 μ in acervulos diversiformes conjunctis. Hab. in radicibus Compositarum. Sevenoaks.

This species, which is found in the roots of daisies, is closely related to *L. Graminis* and *L. Junci*, but appears to be distinct, since grass and rush-roots in the immediate vicinity of diseased daisies were found free from infection. The habitat from which the diseased plants were obtained was a moist one on Fawke Common, Sevenoaks; the soil, however, was very poor and stony. The daisies were plentiful but the plants were very small; about 40 per cent. of them were infected. Microscopic examination of the diseased roots showed that the cortical cells were for the most part filled with spore-clusters of various shapes, of which the ellipsoid was the most common. Very rarely were any amoebae to be seen even in the youngest roots. The same parasite has been found by me on the roots of *Chrysanthemum Leucanthemum*, but the roots of other Composites growing in the diseased area were found to be unaffected. The disease could be imparted from the *Chrysanthemum* to *Bellis*, and vice versa. The structure of the diseased roots in both plants was similar, and the reserve plant-food in the root-cells was inulin.

Infection probably takes place at the growing root apices, but even in these regions of diseased roots only masses of spores are usually to be found. This is in striking contrast to the case of *M. Triglochinis*, in which amoebae only are to be seen. The vegetative amoeboid stage of *L. Bellidis* is shown in Fig. 7, and the akaryote stage in Fig. 8. This species, on account of the lack of amoeboid forms, is not suitable for the observation of nuclear divisions; the modes of division are probably similar to those met with in other genera.

Ligniera Menthae Schwartz, sp. nov. Amoebis in cellulis radicis hospitis; sporarum soris globosis aut acervulos diversiformes efformantibus; sporis 3–5 μ × 4–5 μ. Hab. in radicibus plantae 'Mentha Pulegium' dictae.

This species I found at Chislehurst, in the roots of some plants of *Mentha Pulegium* which were growing in profusion on the border of a pond on the Common. Other marsh-plants growing with the *Mentha*, such as *Alisma ranunculoides, Bellis perennis*, and various species of *Juncus*, were examined, but were found to be free from the *Ligniera*.

Microscopical examination of the older roots showed the infection to be confined to the outer cortex, the cells of which were largely filled with spore-clusters. These clusters were mostly ellipsoidal in shape; spherical ones were also common, and sometimes a tetrad or a single or double row of spores were to be seen. The individual spore is similar to that of other species. In the young branch-roots amoebae were sometimes to be seen, although the *L. Menthae* displayed the same tendency to early spore formation as I observed in *L. Bellidis*, but not in so marked a degree. The vegetative nuclei are typical ones; they are, however, somewhat smaller than those of *L. Graminis*. I have not observed the typical ' cruciform '· method of division of these nuclei, although the usual method of division is probably of that form. I have, however, observed in one of the amoebae some abnormal vegetative nuclear divisions in which the division was effected by an ordinary karyokinesis, as is shown in Fig. 6. This confirms the observation of Maire and Tison, who found a case of typical karyokinetic division in the schizont stage of *Molliardia*. The akaryote stage shown in Fig. 2 is comparatively common. I have not observed any karyogamy prior to spore formation, but the form is not well suited for cytological study owing to the difficulty of finding diseased material.

The amoebae are small and irregular in shape, and several of them may be seen in a plant-cell, so that schizogony is not uncommon. The infected cells occupied by the organism in its amoeboid form occur in small groups and are rarely isolated. The amoebae are shown in Fig. 1. I have only found this disease in the one locality mentioned above, but, on the other hand, I have not had the opportunity of examining plants from more than two other localities, so I am unable to speak as to the rarity or otherwise of its occurrence.

Ligniera Alismatis, Schwartz, sp. nov. Amoebis raris in cellulis hospitis; sporarum soris, iis *Spongosporae* similibus, aut sporis in ordinibus aut globose dispositis; sporis $3\,\mu \times 4\,\mu$. Hab. in radicibus plantae ' Alisma Plantago ' dictae.

This species I found parasitic in the roots of *Alisma Plantago* growing in some trenches near Dunton Green, Kent. It resembles *L. Bellidis* in that the amoeboid form is very rarely seen. The spore-clusters are not exactly similar to those of other species of *Ligniera*, since in the latter the clusters usually exhibit an axial symmetry, while in *L. Alismatis* the boundaries of the spore-clusters are not, as a rule, so definite as in the other

species, and to some extent they resemble those of *Spongospora*. The spores are very rarely collected into spheres or ellipsoids, which are the forms commonly met with in other species. The individual spores are approximately of the same size as those of *Spongospora*.

The life-history and cytology of the various members of the Plasmodiophoraceae is remarkably uniform, except for the case of *M. Triglochinis*, in which, apparently, there is no spore formation by the parasite in the host-plant. With regard to the karyogamy described by Osborn as taking place in *Spongospora*, this has not been observed in any of the other genera. I have re-examined my slides of *S. Veronicae* with a view of confirming Osborn's observation, but have found no such karyogamy as he describes, although this species is a favourable one for the observation of nuclear changes, the akaryote and early spore forms being well marked and plentiful. It would, I think, be easy to mistake overlapping nuclei or the close of a mitosis for a karyogamy. Winge also fails to see any signs of a karyogamy in *Sorodiscus Callitrichis*, which is also a favourable subject for observation. Of the karyogamy described by Osborn, Winge remarks : ' If a karyogamy at this stage is really found in *Spongospora*, we should rather conclude that this organism is not a Plasmodiophoracea.' Maire and Tison have also failed to find a karyogamy in either *S. Veronicae* or the other species studied and described by them ; they suggest, as also does Winge, that a conjugation of amoebae follows the germination of the spores.' This suggestion I also made in my paper on *S. Junci*. The refusal of the spores to germinate under artificial conditions is unfortunate ; probably the germinated spore gives rise to a pear-shaped swarm-cell, and it is at this stage that a conjugation should be looked for.

MYCETOZOA.

In 1884 Strasburger observed a simultaneous mitosis of all the nuclei in the developing sporangium of *Trichia fallax* ; this mitosis gives rise to the nuclei of the spores. This simultaneous nuclear division has since been observed in other species, and is stated by Pavillard (21) to be probably constant throughout the endosporous Mycetozoa.

In 1907 Jahn and Helene Kränzlin (8) identified this division as a heterotypic mitosis ; according to this view, the reduction would be completed on the germination of the spore. In *Arcyria* and *Trichia* Kränzlin states that just prior to spore formation the nuclei in the sporangium associate in pairs and a karyogamy takes place, and that any nuclei which have not paired quickly degenerate. Kränzlin states further that a temporary enlargement of the nucleus follows, and that then synapsis takes place. In the exosporous genus *Ceratiomyxa* a karyogamy has been described by Olive (19) and Jahn (6), but their accounts do not agree.

Quite recently Jahn (7) has observed a conjugation of the myxamoebae formed after the germination of the spores, and has stated that his original contention of a karogamy prior to spore formation was due to an error. The nuclear fusions he had previously seen he now considers as a concomitant of nuclear degeneration, and not as sexual fusions. The reasons he assigns for this view are ample—viz. that the fusions are occasional, and do not give the appearance of a universal or simultaneous karyogamy; the fusions are also to be found in the stalk and in the neighbourhood of the membrane, and are accompanied by degeneration. Similar nuclear fusions have been observed by Winkler in the disorganizing tapetal cells of developing anthers. In Jahn's paper (7) on the sexuality of the Mycetozoa, published in 1911, he states that the swarm-cells are haploid, whereas the nuclei of the plasmodia are diploid, so that the sexual fusion is to be looked for at the commencement of plasmodium formation, and not at the stage preceding spore development. He figures mitoses in the haploid amoebae of *Physarum didermoides*, and diploid mitoses in young plasmodia, and he also figures a nuclear fusion of two haploid myxamoebae; he finds also typical karyokinetic divisions of the plasmodium nuclei.

The spores of the Mycetozoa, when germinated, as a rule give rise to ciliate swarm-cells, one of which emerges from each spore. In the exosporous *Ceratiomyxa*, however, each spore gives rise to eight swarm-cells, and in a few species of the endosporous Mycetozoa we get two or four swarm-cells from each spore. This I have observed to be the case in *Comatricha obtusata*, the spores of which each give rise to two swarm-cells. I have germinated the spores of *Fuligo septica* and *Lycogala miniatum*; the swarm-cells of the former are shown in Fig. 11. The *Fuligo* spores germinate in half an hour in rain-water if they are previously broken up by crushing them on a microscope slide, otherwise the germination is extremely uncertain, only one culture out of ten showing germination after three days; the spores, if kept for a week or more, lose their power of rapid germination. The swarm-cell is pear-shaped, and has a long flagellum at the pointed end, at which end also the nucleus is situated. The swarming lasts for about thirty hours and is followed by temporary encystment; after two or three days a few amoebae or small plasmodia may occasionally be seen. I have not succeeded in cultivating the organism beyond this stage.

Strasburger's statement that a simultaneous division of the nuclei of the developing sporangium takes place just prior to spore formation I have verified in the case of *Lycogala miniatum*, and this karyokinetic division is shown in Figs. 12 and 13.

The development of the sporangium of *Arcyria ferruginea* shows considerable difference to that of a typical endosporous Mycetozoon. In place of the universal karyokinetic division of the nuclei and consequent simultaneous formation and ripening of the spores we get a gradual or successive

ripening from the outside inwards. Thus the innermost part of the young sporangium is plasmodial with nuclei having well-marked karyosomes; contiguous to this region is a narrow belt containing nuclei in which the karyosomes are replaced by granules of chromatin, and in the outer portion of this belt the plasma becomes discrete, and beyond this point is replaced by spores, as may be seen in Figs. 14 and 15. A slightly earlier stage of development to that just described is to be seen in Fig. 16, in which the karyokinetic nuclear divisions, which precede the spore formation, are to be observed in place of the spores. Among these dividing nuclei some are to be seen in the ' synapsis' stage ; these are shown in Fig. 20.

In *Spumaria alba* and *Arcyria albida* I have observed the enlarged and degenerate nuclei described by Helene Kränzlin, but have not seen them approaching in pairs, nor have I seen any nuclear fusions.

In *Comatricha obtusata* I find that a karyokinetic nuclear division takes place within the spore, so that the ripe spore is usually binucleate. This division, which is not, however, quite universal, is shown in Fig. 18.

COMPARISON OF THE PLASMODIOPHORACEAE AND MYCETOZOA.

In the vegetative stage of the Plasmodiophoraceae we get the well-known intranuclear 'cruciform' type of nuclear division. This is altogether lacking in the Mycetozoa, where nothing resembling it has been observed. In the vegetative or plasmodial stage of the Mycetozoa Jahn has observed typical karyokinetic figures; Lister also has observed karyokinesis in the plasmodium of *Badhamia utricularis*, although he inclines to the opinion that direct nuclear division is the usual form. The akaryote stage which follows the vegetative stage in the Plasmodiophoraceae is also wanting in the Mycetozoa. These considerations seem to me of sufficient importance to justify the separation of the Plasmodiophoraceae from the Mycetozoa.

Osborn laid stress on the karyogamy observed by him in *Spongospora*, and, on the strength of its similarity to that described by Jahn and Kränzlin in the Mycetozoa, considered these processes as homologous and as evidence of close relationship. Since, however, Jahn has retracted his view, the existence of a karyogamy in the Mycetozoa becomes, to say the least, doubtful, and the argument used by Osborn has little weight. Jahn, however, as I stated above, also observed a karyogamy or conjugation of the nuclei of the young uninucleate amoebae developed from the swarm-cells. If, as is possible, a conjugation of the myxamoebae be found in the Plasmodiophoraceae, this would furnish evidence of relationship between this family and the Mycetozoa.

THE CHYTRIDEAE.

Němec (17) has described, in *Sorolpidium Betae*, a member of this family which is quite evidently closely allied to the Plasmodiophoraceae.

Among the points of resemblance between them may be mentioned the fact that *Sorolpidium* possesses two distinct forms of nuclei—viz. vegetative nuclei in which the chromatin is collected into a central karyosome, and reproductive nuclei in which the karyosomes are lacking. The resemblance is rendered the more evident by the mode of division of the vegetative nuclei which Němec states is by an amitotic division of the karyosome combined with an equatorial plate of idiochromatin. In the Plasmodiophoraceae the spores are, however, formed after two karyokinetic divisions, whereas in *Sorolpidium* the spore mother-cells form the resting stage, and later each cell nucleus undergoes two karyokineses, thus giving rise to four zoospores. The amoeboid vegetative stage in *Sorolpidium* is succeeded by the reproductive phase in which the organism divides into a number of uninucleate myxamoebae which form the spore mother-cells which are contained in a sporangium with an enclosing wall. Thus in the Plasmodiophoraceae we get clusters of spores, each spore of which is, in most genera, formed from a uninucleate amoeba; these clusters are usually enclosed by a common membrane, and constitute the resting stage of the organism. For the resting stage in *Sorolpidium* we get collections of spore mother-cells, each cell of which is formed from a uninucleate amoeba; these collections are enclosed in a membrane, and each spore mother-cell produces four zoospores.

Kusano (9) has also described a species of *Olpidium* parasitic on *Vicia unijuga*. He states that in the amoeboid vegetative stage the nuclei, which contain a prominent karyosome, divide amitotically, there being, however, no plate of idiochromatin as in the previous species. Later on the nuclei lose their karyosomes and become reproductive nuclei. These, like the corresponding ones in the Plasmodiophoraceae, undergo karyokinetic divisions, but give rise to the mother-cells of the zoospores. The zoospores conjugate in pairs, and the zygote gives rise to resting sporangia, which at first are binucleate, fusion of the nuclei taking place later. During the development of the resting sporangia the nuclei pass through a phase similar to the akaryote stage in the Plasmodiophoraceae.

I have observed in some few of the roots of *Mentha Pulegium* the presence of an *Olpidium* similar to those described above, but on account of inability to obtain sufficient material I have been unable to cover completely its life-history. This *Olpidium* is found in the cells in the form of amoebae of varying size, which apparently fuse to form a plasmodium. These amoebae differ from those of the *Ligniera* in being much larger, in having power of penetrating the cell-walls, and in their nuclei being much smaller; also they are not found in groups as are those of the *Ligniera*, and they are usually seen in the neighbourhood of the sporangia to which they give rise. The sporangium, which is surrounded by a membrane, contains the reproductive nuclei which probably give rise to the zoospores. This organism is shown in Figs. 21 and 22.

From a cytological point of view it is clear that these organisms are closely related to the Plasmodiophoraceae. The great similarity of the vegetative nuclear division, the somewhat similar akaryote stages, the karyokinetic divisions of the reproductive nuclei are all evidence of relationship. As differences we may note the formation of a membrane around the developing sporangium, and the fact that this body contains the spore mother-cells, and not the spores themselves. On germination the nuclei of these mother-cells undergo the karyokinetic divisions, whereas in the Plasmodiophoraceae these two divisions preceded the resting stage. Like the Mycetozoa, these species of *Olpidium* have both the vegetative and the reproductive forms of nuclei. The so-called spores of some of the Mycetozoa may, in reality, be 'sporangia', in that they give rise to more than one swarm-cell on germination, as for example *Ceratiomyxa* or *Comatricha*. The balance of the evidence of relationship seems to me to show that the Plasmodiophoraceae should form a separate class intermediate between the Chytrideae and Mycetozoa.

SUMMARY AND CONCLUSIONS.

1. The root parasites of *Bellis perennis, Mentha Pulegium,* and *Alisma Plantago* are members of the Plasmodiophoraceae belonging to the genus *Ligniera.*

2. The ' cruciform ' type of vegetative nuclear division, and the presence of the akaryote stage is constant throughout the Plasmodiophoraceae, but both are lacking in the Mycetozoa, but are found in *Olpidium.*

3. The nuclei of the Plasmodiophoraceae, Mycetozoa, and Chytrideae are of two types: vegetative and reproductive.

4. There is an absence of karyogamy prior to spore formation in all three families.

5. In the Mycetozoa the vegetative and reproductive nuclear divisions are karyokinetic.

6. The Plasmodiophoraceae, though closely related to both the Mycetozoa and Chytrideae, are best considered as a separate class.

7. The spore mother-cells in the zoosporangium of *Olpidium* each give rise to four zoospores.

8. There is a conjugation in pairs of the zoospores of the Chytrideae.

9. There is a conjugation of the myxamoebae and a nuclear fusion in the Mycetozoa.

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EXPLANATION OF PLATE XII.

Illustrating the paper by Mr. Schwartz on the Plasmodiophoraceae and their relationship to the Mycetozoa and the Chytrideae.

Fig. 1. Longitudinal section of portion of outer cortex of diseased root of *Mentha Pulegium*. × 800.

Fig. 2. The akaryote stage of *Ligniera Menthae*. × 800.

Fig. 3. Karyokinetic nuclear divisions of reproductive nuclei of *L. Menthae*. × 800.

Fig. 4. Karyokinesis leading to spore formation in *L. Menthae*. × 800.

Fig. 5. Spore clusters of *L. Menthae*. × 800.

Fig. 6. Karyokinesis in vegetative phase of *L. Menthae*. × 800.

Fig. 7. Amoeboid stage of *L. Bellidis*. × 800.

Fig. 8. Akaryote stage and spores of *L Bellidis.* × 800.

Fig. 9. Apex of root-hair of *Poa annua* infected by *L. Graminis.* × 800.

Fig. 10. Empty spores of *L. Graminis* in apex of root-hair of *Poa annua.* × 800.

Fig. 11. Swarm-cells, myxamoebae, and spore of *Fuligo septica.* × 800.

Fig. 12. Karyokinesis of nuclei in sporangium of *Lycogala miniatum.* × 800.

Fig. 13. Anaphase of mitoses in sporangium of *Lycogala miniatum.* × 800.

Fig. 14. Central plasmodial portion of sporangium of *Arcyria ferruginea.* × 800.·

Fig. 15. Middle belt of sporangium of *A. ferruginea,* showing transition to reproductive nuclei and spores. × 800.

Fig. 16. Portion of sporangium of *A. ferruginea,* showing karyokinesis of reproductive nuclei prior to spore formation. × 800.

Fig. 17. Section of sporangium of *A. ferruginea,* showing central plasmodial portion and outer sporogenous portion. × 500.

Fig. 18. Spores of *Comatricha obtusata,* showing nuclear division. × 800.

Fig. 19. Portion of sporangium of *Arcyria albida,* showing enlarged nuclei. × 800.·

Fig. 20. Karyokinesis and synapsis in sporangium of *Arcyria ferruginea.* × 1,200.

Fig. 21. Developing sporangium of *Olpidium* in root of *M. Pulegium.* × 800.

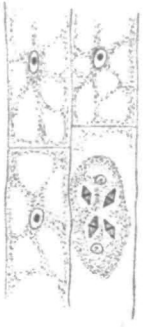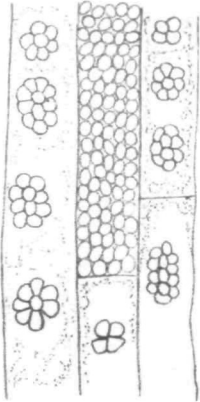Fig. 22. Ripe sporangium of *Olpidium.* × 800.
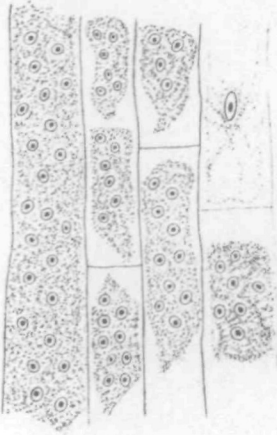
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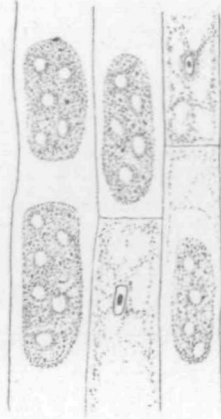
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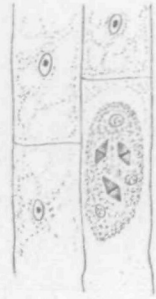
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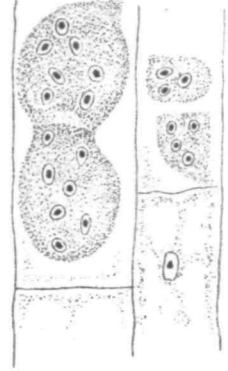
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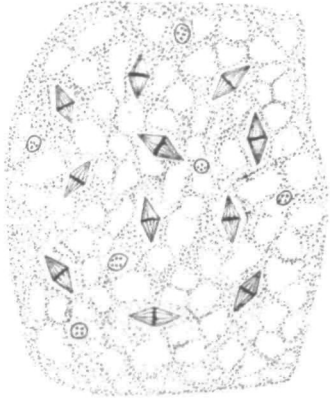
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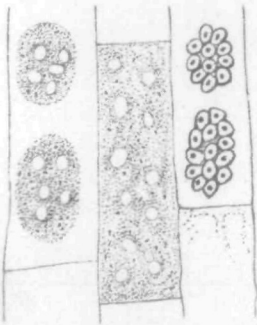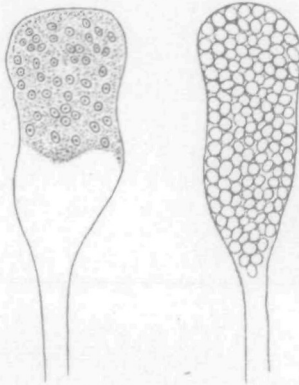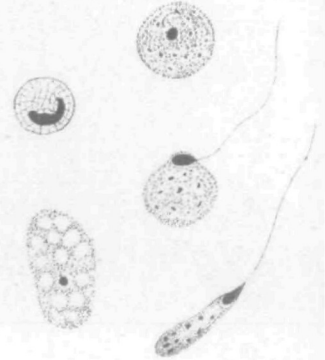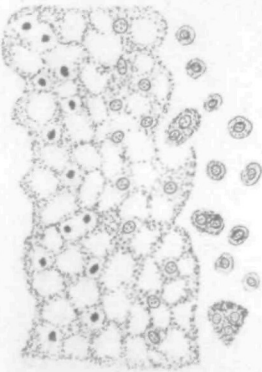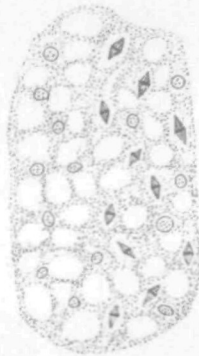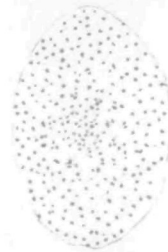
E.J.S.del.

SCHWARTZ — PLASMODIOPHORA.

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