On a new Sporozoon from the vesiculæ seminales of Perichaeta.

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With Plate XXII.

The vesiculæ seminales of the common earthworm (*Lumbricus*) are almost, if not quite invariably, found to be crowded with Gregarines. The species which inhabit *Lumbricus* seem to be certainly more than one. Lankester (1) considers that the Gregarines of *Lumbricus* may be safely referred to two distinct species (*Monocystis magna* and *M. lumbrici*); Ruschhaupt however (3) allows no less than five distinct species. The vesiculæ seminales of all the different genera and species of exotic earthworms which I have had the opportunity of examining, were invariably found to contain Gregarines; I have not hitherto studied these different forms very carefully; but for the most part I have not observed any striking differences between those species which occur in *Acanthodrilus*, *Perichaeta* and other genera and those which infest *Lumbricus*. But this is not the case with a Gregarine which I have recently noted in the vesiculæ seminales of a species of *Perichaeta*.

The *Perichaeta* appears to belong to an undescribed species; I have lately received it with a number of other earthworms from New Zealand, which Mr. W. W. Smith was so good as to collect for me.

The vesiculæ seminales of this worm were crowded with cysts of varying dimensions — some very large — which undoubted-

belong to some form of Gregarine. They were in many cases plainly distinguishable from the cysts of the common *Monocystis lumbrici* by the fact that one end of the cyst was prolonged (see pl. XXII, fig. 1 *c*) into a stalk of attachment; in some cases two cysts were attached by a single stalk (pl. XXII, fig. 1 *c*). Moreover the cyst is very much thicker in the form under consideration than in any of the species that have hitherto been described as occurring in the earthworm.

My notes upon this Gregarine by no means form a complete account of its life-history. I have however been able to observe three stages, which are not without interest.

In figs. 1, 2 are depicted a number of the encysted individuals; the figure illustrates the very remarkable variations in the form of these cysts. But of a very large number which I examined only two or three had an unbroken rounded or oval contour. In every case when the cyst had this regular form it contained (fig. 2) apparently two Gregarines. The individuals were not completely separated from each other by a septum; there were only indications of such a septum at the periphery; centrally it was even difficult to distinguish the boundary between the two individuals. Such cysts, which were by no means common, are not unlike the double cysts of *Porospora gigantea* figured by VAN BENEDEN. In by far the greater number of cases the cysts were fusiform or stalked; sometimes (fig. 1 *b*) the stalk was exceedingly short; in other cases (fig. 1 *c*) it was very much longer than the cyst. Frequently each end of the cyst was prolonged into a short stalk (fig. 1 *d*), or one stalk might be very much longer than the other (fig. 1 *e*). The most characteristic form of the cysts is illustrated in fig. 1 *c*, here two cysts are seen to be connected by the extremity of their stalk; when two or even more cysts were thus connected, there were considerable variations in the length of the stalk.

1) Membranes of Cyst.

The most remarkable feature about these cysts is the structure of the cyst itself, that is of the outer membrane which encloses the parasite.

The cyst as in most of the Septata (LANKESTER, 1) is made up of two layers. The inner layer is very fine and shows no structure (fig. 3). The outer layer forms a very complicated membrane which is best seen in transverse sections (fig. 4). I have already referred to the stalked Gregarine cysts; in these the outer membrane is very

much thicker both relatively and absolutely on the stalk; the membrane is in fact usually prolonged for some distance beyond the contents of the cyst; sometimes (as in fig. 1 *c*) the stalk is entirely formed by the membrane.

This membrane is very distinctly laminated; it has even an irregularly fibrous structure; the fibres are for the most part disposed concentrically — but not always. The thickness of the cyst recalls that of *Gamocystis* (Schneider, 6) and *Clepsidrina* (Schneider, 6) in which forms however it is quite transparent; in these genera there is a laminated membrane of some thickness lying within the outer layer; I imagine that the two together are the equivalent of the outer membrane of my Gregarine, and that a fine innermost membrane in *Clepsidrina* and other genera which ultimately bears the sporoducts represents the inner membrane of the species described in the present paper. It seems to me however that the innermost of the two membranes is only the cuticle of the free form (see below p. 785).

This outer cyst membrane contains imbedded in its substance numerous round bodies which I cannot but regard as nuclei (fig. 4 *n*). In transverse sections through the stalk of one of the cysts these nuclei had such a regular arrangement that the membrane presented a certain resemblance to a layer of columnar epithelium. I may remark that in such preparations there was a row of very darkly stained dots just outside the inner membrane; these are shown in the figure referred to. I regard them as the expression of a layer of specially thick fibrillæ (see below). With regard to the nuclei of the outer cyst membrane I may quote the following remarks by Waldenburg (8) about the Gregarine cysts of *Lumbricus*.

„Man unterscheidet gewöhnlich eine doppelte Membran: die innere ist ganz der bei den Fischcysten beobachteten ähnlich, sie besteht aus vielen durchsichtigen, structurlosen, sehr zarten Lamellen, welche man häufig bei reifen Psorospermiencysten von einander einzeln abgelöst, geblättert findet. In seltenen Fällen sieht man hier und da vereinzelte Kerne in derselben. Die äussere Haut, die bei manchen Cysten fehlt, sieht dem jungen Bindegewebe der Fische sehr ähnlich: man erkennt bei näherer Betrachtung in einer hyalinen Grundsubstanz spindelförmige, grosskernige, durch Fortsätze mit einander communicirende Zellen“ etc.

It is true that Bütschli (2, p. 536 etc.), who is a well known authority upon this group, is disinclined to accept Waldenburg's

statements, remarking of them that they are „sehr wenig vertrauenerweckend". It seems to me however to be just possible that WALDENBURG has met with Gregarine cysts in *Lumbricus* like those of *Perichaeta*.

The nature of the outer cyst membrane in the *Perichaeta*-Gregarine is such that it cannot be regarded as certain that the membrane is excreted by the parasite; it is possible that it is a pathological formation induced by the presence of the parasite. Among the true Gregarines however such formations do not appear to have been met with.

Among the Myxosporidia on the other hand — for example in *Myxobolus mülleri,* which is parasitic upon the gills of certain fishes — BÜTSCHLI (2, p. 592) describes the spores as being enclosed in a delicate cyst which is formed of a nucleated protoplasmic layer; and WALDENBURG, as will be seen from the extract quoted above, indicates the resemblance in this particular which his supposed Gregarine cysts bear to the cysts of „fish-psorosperms".

I am not inclined to regard the parasite described in the present paper as a Myxosporidian for the reason that another stage in its life-history, which will presently be described, agrees with that of certain Gregarines; were it not for this reason, the apparent resemblance of the cyst in the two cases would lead me to refer this parasite to the Myxosporidia.

2) Contents of Cyst.

All the cysts that I examined were completely filled with round or oval bodies (fig. 5) with a hard outline, but perfectly transparent and structureless. The figure in question illustrates some of the contents of a crushed cyst mounted in Glycerine. Variously sized granules of this kind are found in other Gregarines.

When a portion of the vesicula seminalis containing cysts was stained in logwood and cut by the ordinary paraffine method, the contents of the cyst showed a very different appearance illustrated in fig. 6. The greater part of the cyst contents were unstained and had a very finely granular appearance; imbedded in this were a vast number of small bodies usually comma-shaped.

In transverse sections of the cyst which had been previously stained with logwood the nucleus of the parasite was frequently to be observed; in several cysts there was only a single nucleus present which is represented in fig. 6, *n*. The nucleus in these cases was of

considerable size and provided with a large nucleolus. The logwood stain had not affected the nucleus itself, but had tinged the nucleolus of a yellowish brown.

In glycerine preparations (fig. 7 *a*) a single nucleus was frequently seen when it happened to be situated in the taillike process of the Gregarine; in such preparations no nucleus could ever be seen in the central region owing to the great thickness of this part. In other individuals transverse sections showed numerous nuclei scattered through the parasite; these were smaller and had an obvious nucleolus; they are no doubt produced by the division of the at first single nucleus. This very early stage in sporulation is unfortunately the only stage which my preparations afford.

In one instance (fig. 14) I was so fortunate as to notice the formation of karyokinetic figures in nuclear division. I believe that this process has as yet been observed in but few Gregarinidæ. I am disposed however to value this discovery not at all with the idea that it is of great importance, but because it appears to show that my specimens are well preserved and that I may therefore have confidence in describing the details of the structure of this form.

The division of the nuclei in the encysted parasites is accompanied by a division of the cyst contents which are separated into a number of masses, much fewer however in number than their nuclei.

3) Young stages.

In fig. 8 are presented a number of small Gregarines from the vesiculæ seminales of the same earthworm which I regard (at least for the present) as young stages of the same parasite; the figure comprises sketches of a series of individuals indicating the principal forms which I observed.

It will be noticed that the general shape is oval or round with one or two long processes; where there are two processes one is considerably longer than the other.

It may be that these different shapes are merely due to the fact that the parasites were killed when in movement, and were therefore fixed in different attitudes. But it seems more likely that this is not the case.

The resemblances between my figures and those which VAN BENEDEN (5) gives of a corresponding stage in *Porospora gigantea* is not a little striking. This resemblance cannot however be more than superficial; there can of course be no „pseudofilaria“ stage since the

cysts have approximately the same form as the free parasite. Moreover it seems to be clear that the cyst (that is to say the outermost cellular cyst) may be formed when the parasite is comparatively small and therefore goes on growing *pari passu* with the increase in size of the contained parasite; and this seems to go to prove that the cyst is a pathological formation caused by the presence of the parasite. In fig. 9 is represented a young cyst in which the nucleus happened to be very distinct; it is intermediate in size between the fully developed cysts (fig. 1) and the young forms (fig. 8), but comes nearest to the latter; the cyst membrane has numerous nuclei, but is comparatively thin and hyaline in appearance. There is a considerable space between the cyst membrane and the contained parasite, which is further evidence of the truth of my assumption that the cyst is a pathological formation.

There are some reasons which point to the conclusion that these young Gregarines really retain the form which they had during life. In the first place the similarity of their shape to the encysted adults is remarkable; if it be assumed that the reagent used in the preservation of the earthworm has caused the young free Gregarines to contract unequally so as to acquire the shape illustrated in fig. 8, this explanation will hardly do for the (presumably) more rigid cysts. The vesiculæ seminales of the worm also contained numerous cysts of the common *Monocystis lumbrici* or at least of some form very closely allied to this. These cysts were in various stages of development; some were filled with spores: in others the cyst had been only recently formed, and the nucleus of the parasites was undivided. In all these cases the cyst had the typical rounded form, and had been so far unaffected by the reagent. There seems to be no particular reason why the cysts of one species should be more affected by the reagent and altered in shape than those of another.

I furthermore took the opportunity of subjecting the living *Monocystis lumbrici* and *M. magna* to the influence of various reagents, such as Alcohol, Corrosive Sublimate, Methyl Green, Iodine and found that their shape was hardly perceptibly altered by a prolonged immersion in these fluids.

These reasons taken together seem to me to prove that the shape of this Gregarine is during life that which is represented in the figures illustrating this paper. It is important to endeavour to prove that this is so, because the shape of Gregarines usually differs characteristically in well marked species. At the same time the nature of the cyst is so peculiar in this form, that any further description

of the unencysted parasite would be unnecessary, were it merely desired to show its specific distinctness from any known form.

Besides the young stages described above which are of about the same size as *M. lumbrici*, I met with a number of individuals intermediate between these and the encysted form.

I have already pointed out one intermediate stage between the encysted parasite and the young individuals. It appears however to be rarely the case that the parasite becomes encysted before attaining to greater dimensions than the specimen illustrated in fig. 9.

In the body cavity, particularly in the posterior region, were numerous Gregarines of which examples are illustrated in figs. 10, 12, 13. I also found individuals belonging to this stage in the vesicula seminalis; they agree in their general form both with the very young specimens and with the encysted individuals; in my opinion they are undoubtedly the mature unencysted stage.

The granules filling the body were sometimes confined to the central region, and sometimes extended into one or both of the processes. The granules (figs. 5, 6) were identical in character with those of the encysted form being much larger than those of the young specimens.

In those cases in which the granules of the entoplasm do not extend into the processes of the Gregarine their contents consists of finely granular protoplasm; this is continuous with a layer of finely granular protoplasm of excessive thinness which surrounds the coarsely granular entoplasm of the central region of the body. This finely granular layer is probably to be looked upon as the ectoplasm. Even when the large granules of the entoplasm do extend into the processes of the body, they form but a narrow layer, the ectoplasm being relatively of great thickness. It seems to be very possible that the movements of the body, if there are any in the living Gregarine, are brought about by the contractions of the granular ectoplasm; if so its extreme thickness in the two processes of the body would seem to indicate that these are more especially organs of locomotion. In *Conorhynchus* (Greeff, 8) the body is furnished with numerous processes which are chiefly composed of ectoplasm. These facts still further favour the supposition that the form of the spirit preserved examples of this Gregarine is that of the living form.

The cuticle was proportionately thicker than in the early stages. On the two processes of the body (figs. 11, 12) were a series of rather coarse striations running in a direction transverse to that of the long axis of the Gregarine at an angle of about 45°. These were not visible in

the central region of the body owing to the opacity caused by the granules. On crushing an individual by pressing on the coverslip these striations were seen to extend on to the central region of the body.

Careful focussing showed that these striations were due to the presence of fine ridges lying quite superficially; at the edges of the body they could be seen to form a distinct layer outside the cuticle though formed in all probability by local thickenings of it.

It is quite common for Gregarines to exhibit a striation of the cuticle though the direction of the striæ seems to distinguish the present species. In describing the encysted form I have called attention to the fact that the cyst has the appearance of being composed of innumerable closely felted fibres. In sections of the ‚tail' of the encysted parasite it was always possible to recognize (fig. 4) a layer of specially thick fibres immediately overlying the structureless cuticle of the Gregarine; it may be that these are the same as the striæ of the unencysted parasite; but in this case it will obviously be necessary to assume that the rest of the cyst is formed by the tissues of the host.

In any case it must be noted that the constitution of the cyst in this parasite differs as regards its relation to the cuticle of the unencysted form, from that of other Gregarines.

There is some difference of opinion as to the formation of the cyst in other Gregarines. By some it is stated that the cyst is a new formation altogether, by others that it is simply the persistent cuticle of the free form. Bütschli (2) states that in the encysting *Clepsidrina blattarum* the cuticle of the free form disappeared. In *Adelea* according to Schneider (6) the cuticular cyst is formed underneath the original cuticle and cannot therefore possibly be confounded with it.

Ruschhaupt however gives a rather different description of the formation of the cyst in *Monocystis;* it consists, according to him, of two layers; the outer layer is the persistent cuticle of the free stage, the inner layer is formed anew.

In the present species it seems to me that, whatever may be the origin of the portion of the cyst membrane that contains nuclei, the delicate inner cyst membrane at least, if not also the layer of fibres outside it, is the persistent cuticle of the free form.

Fig. 11 illustrates the taillike process of a Gregarine of this stage highly magnified to show the cuticle and the striæ; it will be noticed that numerous cells of the perivisceral fluid are adherent to the outside. This I always found to be the case with individuals of this stage. It occurred to me at first that the tendency of these cells to attach themselves to the Gregarine might have some relation to the formation of the cellular cyst. I do not however think that any particular weight can be attached to the fact that these cells become adherent to the parasite, as the same thing occurs with other foreign bodies in the coelom — for example with detached setæ.

4) Multiplication by fission.

Bütschli (2, p. 504) remarks that the propagation of Gregarines invariably take place by spore formation, and that a simple fission of the free parasite never takes place.

Since Bütschli's account of the Sporozoa was written Ruschhaupt (2) has discovered that fission does occur in the Gregarines of the earthworm. This process cannot however be at all common in the group as it has been so seldom observed.

In the Gregarine which forms the subject of the present paper I have observed at least two stages of what appears to me to be a process of division by fission.

So far as this process can be safely interpreted by these two stages it seems to be rather different from cell division in *Monocystis*. In the latter, according to Ruschhaupt, a constriction appears in the middle of the Gregarine; the two halves are for a short time connected by a thin bridge which ultimately breaks through.

In fig. 2 is illustrated what I believe to be an early stage in the process of division. At the extremity of the body a rounded swelling is formed which is filled with large granules. I observed a similar condition (see fig. 8) in an individual belonging to the youngest stage. In the next stage (fig. 3) the swelling at the extremity of the process has increased so as to be equal in size to the parent form and a process has grown out from the free extremity of this.

These appearances may of course be delusive, but they seem to indicate that a division of the free parasite occurs which is in its nature something between budding and fission.

I would interpret the facts as indicating that the formation of new individuals by division takes place as follows.

During life the entoplasm is probably in motion and flows along the prolongation of the body; the large granules of the entoplasm are carried along with it. One of the extremities of the body becomes enlarged by a kind of budding and in this bud collects a quantity of entoplasm granules [I can say nothing as to the behaviour of the nucleus]. The bud gradually increases in size until it becomes as large as the parent form. It is then separated off by a constriction in the process connecting it with the parent having previously (in some cases) developed a corresponding process at the opposite pole of the body.

5) Affinities.

These then are the principal facts in the structure of the Sporozoon. It remains now to be considered whether it is a true Gregarine or a Myxosporidian.

As has been already stated the cellular cyst is so far evidence in favour of referring this Sporozoon to the Myxosporidia. Indeed there appears to be no Gregarine in which there is a cyst of this kind. In the Myxosporidia the parasite surrounds itself with no clear structureles cyst like that of Gregarines, and usually it breakes up into spores in an uncncysted condition, that is to say without being surrounded by the cellular cyst which is found in certain forms. Accordingly in the Myxosporidia the mature individual is often of an irregular form. This appears to be at first sight a further point of resemblance between the Sporozoon described in the present paper and the Myxosporidia; I have figured numerous cysts (fig. 1) of this parasite which are very different in form.

The form however does not vary within wide limits and corresponds in every case to that of the free individuals; it is, as I am disposed to think, the characteristic form of the species and is not due to the fact that the individuals can perform active amœboid movements while within the cellular cyst: the presence of a structureless delicate cyst membrane surrounding the parasite as it lies within the cellular cyst, is however decidedly against the probability of its being a Myxosporidian.

The young stages represented in fig. 8 furnish a very strong argument in favour of regarding this organism as a Gregarine. They have a fixed and definite form like that of most Gregarines. Bütschli has summed up (p. 2) the resemblances and differences between the

Gregarines and the Myxosporidia and has shown that there is at least no wide gulf between the two groups.

I would suggest that the Sporozoon described in the present paper still further bridges over the line of separation.

The general structure of the parasite shows that it should be placed among the Gregarines, while the cellular cyst, though not perhaps exactly like that of any Myxosporidian, is yet more comparable to what is found in that group.

As my account of this species is necessarily very incomplete, I refrain from any further discussion of its systematic position among the Sporozoa.

Resumé.

The following is a brief resumé of the principal facts contained in this paper.

1) In the youngest stage the Gregarine has a spherical body with one or two long processes; if there are two processes they are placed at opposite poles. There is a delicate cuticle, and the ectoplasm and entoplasm can be distinguished.

2) In the next stage the parasite is much larger but of the same form. The granules of the entoplasm are for the most part large and oval in form, but there are also smaller granules interspersed among them. The ectoplasm is especially thick in the processes of the body. The cuticle is raised into fine ridges which run obliquely to the long axis.

a) In this stage and in the last multiplication by fission occurs. A swelling appears at the extremity of one of the processes. This gradually grows, develops at process at its free extremity, and becomes separated as a new individual.

3) In the third stage the Gregarine still retains the same form. The cuticle of the free stage persists, but is covered by a cyst membrane consisting of a fibrous substance in which are imbedded numerous nuclei.

a) Sporulation commences by a rapid division of the at first single nucleus; karyokinetic figures are formed during the division of the nuclei. The protoplasm also divides, but not so rapidly as the nucleus.

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Explanation of Plate XXII.

Fig. 1. Encysted form of Gregarine from seminal vesicles of a *Perichaeta*.
Fig. 2. Double cyst.
Fig. 3. Extremity of a stalk of a cyst.
Fig. 4. Tranverse section through d°; *n* nuclei of cyst.
Fig. 5. Contents of cyst in glycerine preparation.
Fig. 6. do. from a transverse section after staining with logwood: *n* nucleus.
Fig. 7. Extremity of the stalk of a cyst to show *a*, nucleus.
Fig. 8. Young stages of the same Gregarine.
Fig. 9. Newly formed cyst of do.
Fig. 10. Two individuals of stage 2.
Fig. 11. Extremity of one of the processes of same highly magnified.
Fig. 12. First stage in transverse fission of mature unencysted individual.
Fig. 13. Second stage of the same.
Fig. 14. Dividing nucleus of encysted individual showing karyokinetic figures.
Fig. 15. Nuclei (*n*) and granules of entoplasm from section of encysted individual.
Fig. 16. Portion of an encysted individual undergoing division.