On *Kalpidorhynchus arenicolae* a new Gregarine, parasitic in *Arenicola ecaudata.*

By

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(With Plates VI and VII.)

The parasite here described was discovered in the autumn of 1905. At that time, during the absence of Prof. E. A. Minchin in Uganda, I was entrusted with a course of lectures in advanced zoology in University College, London, and one of my students, Mr. W. de Morgan, when dissecting specimens of *Arenicola ecaudata* called my attention to numerous small white cysts which occurred in the coelomic cavity. We found that large elongated Gregarines were also present, attached by one extremity to the walls of the coelom, and that the cysts belonged to this parasite. It proved to be an entirely new genus of Gregarines and I have made as thorough an investigation of it as my time and opportunities allowed. Mr. W. de Morgan was associated with me in the earlier stages of the investigation and I have much pleasure in acknowledging here the assistance which he gave me, and the credit that is due to him as the actual discoverer of the organism. I have also received much kind help from Mr. H. B. Fantham who works beside me at University College, and whose special knowledge of the technique of researches on Protozoa, and of the literature of the group, has been always freely placed at my disposal.

Methods.

My observations have beeu made partly on fresh specimeus partly on preserved material. All the specimens of *Arenicola* were abtained from the Laboratory of the Marine Biological Association at Plymouth, and my thanks are due to Dr. Allen, the Director, and his Staff for the trouble they have takeu to seud the auimals to me alive and iu good condition. For fixing I have nsed chiefly a saturated solutiou of corrosive sublimate with 10% acetic acid; for staining nsnally the irou haematoxylin method or saffranin. Other staius have been tried including Giemsa's mixture but the two first mentioned have giveu the best results. Iu additiou to prepariug sections in the usual way I have made many preparatious by fixiug and staining on the slide or by breaking cysts already fixed and staining their conteuts on the slide.

The Trophozoites.

The trophozoites are of an elegant, elougated form and occur in considerable numbers, of various sizes, attached to the coelomic surface of the nephridial tnbes, and to the body wall in the neighbourhood of these tubes. They are not usually found on the processes of the edges of the uephrostome. The trophozoites are large and conspicuous, reaching a length of 1,5 mm, when matnre, bnt younger forms of various sizes also occur. Their appearance in the fresh condition under a low power is shown iu Fig. 1, in which they are seen attached to the coelomic epithelinm covering a uephridium. The attached extremity forms a very distinct epimerite, separated from the rest of the cell by a constriczed ueck, and having the form of a shallow cup. The geueric name which I have given to the organism refers to the character of the epimerite (*κάλπις* au urn or cup, *ῥύγχος*, snont). There are no appendages on the external surface of the epimerite, but within the edge there is a band of fine striations parallel to the long axis of the trophozoite: these belong to the iuternal protoplasm. When the trophozoite is fixed and mounted in the entire state a small mass of the coelomic epithelinm of the host usually remains attached to the end of the epimerite, as seeu in Figs. 2, 3. The exact nature of the attachment is somewhat uncertain. Iu examiuing fresh specimens free from any coelomic cells a slight projection of protoplasm is some-

times seen in the centre of the terminal hollow of the epimerite, bnt this does not form a very definite or conspicuous structure.

The nephridial tubes are attached to the wall of the coelom on either side of the ventral nerve cord by a longitudinal membrane which extends throngh a gap in the layer of longitndinal muscles to the layer of circular mnscles. The inner surface of this membrane, that is the snrface turned towards the nerve cord, is a favourite site for the attachment of the trophozoites. I have cnt ont a portion of the ventral region of the worm with a nephridium attached, and cnt it into a series of sections in order to examine the attachment of the epimerite to the epithelium in section. The appearance of snch a section nnder a high power is shown in Fig. 4. The section was stained with HEIDENHAIN's iron-haematoxylin method. The preparation shows that the cuticle of the trophozoite is continned over the terminal snrface of the epimerite, and I have not been able to discover any perforation in this cnticle. The concavity at the terminal surface of the epimerite has become everted in the process of preparation, so that the section of that surface is convex instead of being concave. One of the most important points brought ont by the sections is that the fibrillar character of the protoplasm is not merely confined to the periphery of the epimerite, bnt extends throughont its thickness. At first I thonght that this longitudinal striation belonged to the cuticle, and was similar to the ridges of the epimerite in *Schneideria mucronata* [LÉGER]. It is now clear however that in *Kalpidorhynchus* the cnticle of the epimerite is qnite smooth and simple, and that the striation is due to the fibrillation of the cytoplasm. The fibrillate structure therefore is a specialisation of the ectoplasm, and is probably related to special contractility, the function of which may be to draw in the terminal surface of the epimerite and thus form a sucker. As seen in Fig. 4 the fibrillated cytoplasm in the section has contracted away from the cnticle, except at the central point where it still adheres to the latter.

The membrane referred to above, which connects the nephridium of the worm with the body-wall, consists of a thin sheet of fibrous connective tissue, containing a nucleus here and there, and bearing on either face an irregular coelomic epithelium. This epithelinm is thicker on the side towards the nerve-cord than on the other side of the membrane, and only the nnclei of its cells are conspicuous, the cell-outlines being very indistinct. The epimerite of the Gregarine is attached to a protuberance of the surface of the membrane,

as seen in Fig. 4, a protuberance which seems at first sight to be derived from the connective tissne of the membrane rather than from the epithelial cells. The protuberance consists of an nnstained mass of fibrous appearance, separated from the membrane by a clear space traversed by fibres. This space seems to be due to contraction in the process of preparation. On either side of the fibrons mass are epithelinm cells, and within it, in contact with the cuticle of the epimerite, are two large "giant" nuclei. These are nuclei which have undergone hypertrophy in conseqnence of the presence of the epimerite. Fnrther within the mass is a stained fragment of chromatin, which seems to be the remnant of a nncleus which has degenerated. Whether the epimerite is, at an earlier stage, as in other cases which have been described, contained within an epithelial cell, I do not know, as I have not yet traced the attachment of the trophozoite in its earlier stages.

A mode of attachment somewhat similar to that of *Kalpidorhynchus* is described and figured by Brasil (Arch. Zool. exper. 1904, p. 223) in a Gregarine which he identifies as *Doliocystis pellucida* [Köll.], bnt which, as I have pointed ont below in discussing the affinities of *Kalpidorhynchus* does not seem to me to agree with other descriptions of *Doliocystis*. In Brasil's figure the trophozoite, though not possessing a definite epimerite, is attached by its narrower extremity, which is concave, to a single cell of the intestinal epithelium; and the nncleus of this cell is close to the surface and is much enlarged, having nndergone hypertrophic degeneration. The character of this nnclens is quite similar to that of those which occur in the tissne to which the epimerite of *Kalpidorhynchus* is attached. It is therefore more probable that these nnclei belong to modified epithelial cells than to connective tissue, but the epithelial cells of the coelomic wall in *Arenicola* seem to be so feebly attached that it is difficult to nnderstand how the parasite could be kept in place by them.

Gametocytes and Gametocyst.

The trophozoites when mature become detached from the coelomic wall and adhere together in pairs, or gronps of more than two, by their thicker extremities from which the epimerites have disappeared. I am nnable to say what becomes of the epimerite, whether it separates and is lost as is stated to be the case in other Gregarines, or whether it is absorbed into the body of the gametocyte. An early stage of association is shown in Fig. 7 in which

it will he seen that the attached ends of the gametocytes have become enlarged and terminally flattened; hnt the candal extremities are still present and no cyst is yet formed. The figure is drawn from a fixed and stained preparation. On the surface of the gametocytes is seen a somewhat irregular layer of coelomic cells, which are entirely ahsent from the surface of the trophozoite when it is attached to the wall of the coelom. The gametocyte is surronnded hy a thick layer of snch cells during the rest of its development. A similar investment of the cysts hy coelomic cells has heen described in the case of other coelomic Gregarines, for example in *Urospora lagidis* [St. Joseph], hy Brasil (1904), who terms the cells indifferently phagocytes or amoehocytes. I can see no difference between these cells and those of the coelomic epithelium, and it seems to me that the free cells of the coelomic liquid are derived from those of the coelomic epithelinm which mnltiply and, becoming detached, are suspended in the liqnid. In *Kalpidorhynchus* as in *Urospora lagidis* according to Brasil, the trophozoites when attached to the wall of the coelom are not surrounded hy coelomic cells, althongh I cannot agree with Brasil's expression that "la forme vegetative n'est jamais la proie des phagocytes", becanse the surrounding of the cyst hy coelomic cells is not the same thing as an attack hy phagocytes, since the cyst is alive and developing normally and is not injured or destroyed by the cells which surround it.

The coelomic cells accnmnlate on the surface of the trophozoite even hefore it has hecome associated with another individnal, as soon as it has separated from the coelomic wall. Fig. 5, from fresh material shows a trophozoite which still possesses an epimerite and has not yet entered npon the association stage. Incidentally this specimen seems to prove that the epimerite is not separated from the rest of the trophozoite hut is detached from the surface of the coelomic wall. The coelomic cells form a thick mass surronnding the Gregarine like a belt about its middle region, just hehind the anterior portion which is becoming glohular. These coelomic cells in their behaviour follow closely the changes of the gametocytes in the process of encystment, although they cannot he considered to be in any way essential to the formation of the cyst, since association and encystment occur in other Gregarines, snch as intra-intestinal forms, which have no snch cellnlar investment. When the gametocytes nnite together their cellnlar investments advance towards the attached extremities and become continuons, so as to surround the enlarged portions of the gametocytes, leaving the

slender caudal portions protrnding. This happens before the cyst well is deposited.

I have not recognised cases in which one trophozoite encysted alone withont association, bnt cases of the association of more than two individnals are very common. I have seen cases of nnion of two, three, fonr and five. Examples of the union of three and four gametocytes are represented in Figs. 6, 8 Pl. V. Brasil (Arch. Zool. exper. 1905, p. 24) states that in *Gonospora* and *Urospora* solitary encystment is exceptional and does not seem to be followed by normal mnltiplication of nnclei. He asserts also that the cyst formed by a single individnal is soon attacked by phagocytes and indnbitably becomes their prey. In view of the fact that all cysts are snrronnded by the so-called phagocytes this assertion seems difficult to accept without further evidence. In a note Brasil states that he has seen a cyst of *Urospora* in which one of the gametocytes was dead and in course of degeneration, while the other seemed to be developing normally: the surface of the cyst corresponding to the dead individual was covered with a thick mantle of phagocytes, the rest was entirely withont them. But Brasil does not explain how his arguments are to be reconciled with the presence of the 'phagocytes' aronnd the whole surface of the normal cysts, except that in *Urospora* the cells are stated to snrround the cyst only at the end of the development going on within it, i. e. when the gametes begin to be formed. This is not the case in the new form which I am describing.

The next question to be considered is whether cysts formed from more than two individnals are capable of development. My own observations do not decide this qnestion in the affirmative, for in my preparations all the cysts in later stages of development show only two gametocytes. The qnestion is briefly discnssed with regard to triple associations by Woodcock (Q. J. M. S. 1906, p. 69). He concIndes that the balance of evidence seems to point to the subseqnent degeneration of such cysts. Berndt found that in *Gregarina cuneata* associations of more than two individnals invariably came to nothing. Cuénot instances rare cases of triple association in *Diplocystis*, one of which apparently prodnced sporoblasts (gametes); bnt Woodcock judges from the figure that these were not normal and healthy. In one specimen of *Arenicola ecaudata* which I opened, associating gametocytes were very numerous and a large proportion of them were multiple, that is consisted of three, four, and even five individnals. It is certain therefore that snch cases frequently

occur, bnt I am nnable to decide whether snch associations undergo normal, healthy development.

With regard to the coelomic cells snrrounding the cysts I have only to add that they certainly have no phagocytic action on the normal living gametocytes or cysts. They attach themselves to the gametocytes before the cyst is formed in the fashion shown in my figures. Shortly afterwards the cyst wall is formed, and it may be that the presence of this wall protects the living contents from the phagocytic action. Bnt on the other hand the trophozoites are not surrounded by coelomic cells at all and the gametocytes are surronnded before the cyst is formed. It is possible that if the protozoan dies within its cyst, it is removed by the phagocytic action of its cellular envelope, but on this point we have at present no evidence. When the cyst wall is formed, as in Figs. 8 and 9, a clear space is seen, in the fresh condition, between the wall and the gametocytes which it encloses; bnt in sections of preserved material in later stages no such space is present.

Formation of the Gametes.

The two gametocytes remain distinct within the gametocyst, separated by a membrane consisting of the cnticles of the gametocytes, during the nuclear divisions which lead to the formation of the gametes. The dividing membrane is donble, and is in fact formed merely by the parts of the cuticles of the gametocytes which are in contact with each other. The cytoplasm during this stage is vacuolated and finely granular. The nnclei are minute and scattered, and may be seen in various stages of mitosis and also in the resting condition. It is noticeable that the two gametocytes within the same cyst are never in exactly the same phase of development. As is shown in Fig. 10, one of them has denser cytoplasm and fewer nnclei than the other, the one with more numerous nuclei and larger vacuoles being more advanced in development. It seems possible that this difference between the gametocytes in the process of nnclear division might lead to sone degree of ineqnality between the gametes prodnced, some degree of anisogamy. One gametocyte might prodnce a larger nnmber of gametes of smaller size, the other a smaller number of larger size. I have not however been able np to the present time to detect any difference between the nnclei of the conjugating gametes. The dividing nuclei in the gametocytes are extremely small, and do not take stains deeply.

At the end of the process, after conjugation, the cyst is filled with zygotes with large deeply stained nuclei. It is evident therefore that a great increase in the amount of chromatin present takes place: the chromatin grows at the expense of the protoplasm. It will be seen that the various stages which I have described are closely similar to those described by Siedlecki in *Lankesteria ascidiae* in 1899. I have not stndied the changes in the original nucleus of the gametocyte which lead to the formation of the first mitotic figure. These stages including the degeneration of the karyosomes and the formation of the chromosomes are not represented in my preparations. They have been fully described by Cuénot in *Monocystis* by Siedlecki in *Lankesteria*, and by other observers in other Gregarines.

The next stage I have to mention is the end of nnclear division when the partition between the gametocytes has disappeared, and each of them consists of a labyrinth of protoplasmic cords, each having a sinnons course with nnclei chiefly on the edges. (Fig. 11 Pl. VI.) Brasil has described in this stage in *Urospora* and *Gonospora*, distinct evidence of anisogamy, and has lately extended the observation to *Monocystis agilis* of the earthworm. I have hitherto not been able to discover any constant difference between the nuclei of the two gametocytes at this stage in my preparations, although I have examined them repeatedly with regard to this special point, with the most powerful apochromatic lenses of Zeiss. The nuclei appear to be pyriform, with granules of chromatin at the periphery. Brasil resolves the pyriform shape into a circular nncleus connected by an attractive cone with an exceedingly small centriole. I have not been able to confirm entirely this interpretation. It is true that in some nuclei there is in the thicker basal portion a circular clear space, but this seems to me merely the vacnolated part of the nncleus, surronnded by the chromatin. The conical projection appears to consist of chromatin, and to be part of the nnclens, not a cone of attraction ontside it. In some cases I have seen what appears to be a stained particle at the apex of the cone, bnt this particle differs in no respect from the series of similar particles which form everywhere the boundaries of the areas of cytoplasm in which the nnclei are sitnated. These boundary-lines of dots or stained particles are very distinct in sections stained with iron-haematoxylin, and the elements into which the gametocyte is thus divided are the gametes themselves. In fact the cords of protoplasm at the stage we are considering, represented in Figs. 11 and 12, consist

of numbers of gametes still attached to each other. If the figures are compared it will be seen that the nuclei in this stage of *Kalpidorhynchus* as they appear in my Fig. 12, agree very closely in structure with those of *Monocystis agilis* in Fig. 27 Pl. X of Brasil's paper in the Arch. Zool. exper. 1905, that is to say with the nuclei of the npper gametocyte in that figure. In neither of the gametocytes in my preparations do the nnclei agree with those in the lower gametocyte of Brasil's figure.

With regard to the question of anisogamy there is in my preparations a slight difference between the two gametocytes in the stage of Fig. 11. One gametocyte, that on the right in the figure has narrower bands or cords of protoplasm, and is slightly more deeply stained, that is to say the protoplasm is probably denser. On the other hand after the most careful examination with the highest powers I can find no other constant difference between the nnclei in the two gametocytes, nor between the gametes as indicated by the boundary lines, either in size or structnre. After all, the slight difference in appearance between the two gametocytes may be only a difference of phase or stage of development: one may be a little more mature than the other. The cords of protoplasm break np into gametes each with its own nucleus. The ontlines of these gametes in my preparations are usually indistinct, although the nnclens is deeply stained and conspicnous. In sections of a gronp of cysts examples containing the varions stages here described can be distinguished, and usnally all the contents of one cyst are in the same stage or nearly so. Steps in the process of conjngation I have not been able to follow ont quite satisfactorily, but the large cells with conspicuons nuclei and definite outlines shown in Fig. 15 are identified without difficulty as zygotes. Some cysts contain definite cells with very inconspicnous nnclei; by careful focnssing two groups of chromatin but slightly stained can be made ont in each, as shown in Fig. 14. I have been in some donbt whether these represented a stage in conjngation, or in the division of the nucleus of the zygote after conjngation, in the process of the formation of the sporozoites. At present I am convinced that the former conclusion is correct, as I have cysts which contain nnmerous gametes with bright deeply stained nuclei and cells in this other stage in each of which there are two indistinct masses of chromatin. In the same cyst occur sometimes these binncleate cells, a certain number of the typical gametes, and also pairs of gametes in contact, and here and there binncleate cells in which the two nuclei are fairly distinct

and conspicnous. It seems therfore that in the process of conjugation the nnclei become expanded, the chromatin being more diffused and staining less deeply, although after conjugation concentration occurs again and the nucleus becomes conspicuous. Such a change is similar to that which occurs in the process of fertilisation in the Metazoa, in which the pronnclei are large and pale. So far as I have been able to discover no previous observer has mentioned this condition of the nnclei in conjngation, which makes it difficult to compare, as Brasil does, the sizes of the conjngating nnclei. I can only say that I have seen no evidence of difference between the separate gametes, or between the members of the conjugating pairs, or their nnclei.

The zygotes after conjngation assume a pyriform shape, and often adhere together in conples by their narrower ends as seen in Fig. 16. I have stndied the zygotes in this stage and their development into meture spores in preparations made by breaking cysts on the slide and then fixing and staining the contents. Figs. 17, 18 show the successive stages in the division of the nncleus of the zygote as seen in a successful preparation of several cysts made in this way, the material being fixed with corrosive sublimate and acetic acid, and stained with saffranin. It is remarkable that the zygote does not retain the pyriform shape during its development, bnt becomes ellipsoidal, resnming the pyriform condition in the mature spore. From this it must be inferred that the cytoplasm nndergoes movements of contraction during the division of the nncleus of the zygote. Precisely the same kind of thing has been observed by Brasil. In reference to the development of the zygote of *Urospora lagidis* he writes "Le sporozoite, d'abord piriforme, se developpe rapidement. Il perd son prolongement et devient ovoide en meme temps qne son noyan se dedouble par une mitose longitndinale". The division of the nuclens however is not according to my observations a typical mitosis, but is amitotic and the nnclei change their positions considerably, as is evident from the figures. In the two-nncleus and fonr-nncleus stages there are nnclei at the extremities of the elongated zygote, not only in the eqnatorial region. In the 8-nnclens stage also the nuclei are distributed thronghout the length of the yonng spore (Fig. 18 b). In the mature spore on the other hand (Fig. 19), the nuclei are narrow and elongated, and are grouped in the well known manner, like the staves of a barrel, in the eqnatorial region of the spore. At the broad end of the spore is a little mass of grannlar snbstance, the residual protoplasm, and the rest of the protoplasm is divided, so that the boundaries of the sporozoites can

be distinguished. In one of the nnclei figured in the binncleate stage it will be seen that each nucleus shows a division into four fragments, and that the two nnclei are connected by strands, presumably of chromatin. In this case therefore the division of the chromatin into 8 parts has already taken place, although these parts are only subsequently separated.

The rounded zygote after conjngation is about 9 μ in diameter, the matnre spore is 15 μ in length. I have fonnd it almost impossible to see the sporocyst or envelope snrrounding the spore, in preparations mounted in Canada balsam. It is so transparent as to be difficnlt to see even in the fresh state. It is drawn ont into a protjecting spont at the narrow end of the spore, and is considerably thickened at the broad end. In the former character, the presence of a spout or funnel, the sporocyst agrees with that of *Cystobia* as described by Minchin and Woodcock and also with *Lithocystis* Giard, both of which occur in Echinoderms. The thickening of the sporocyst at the opposite end (probably a thickening of the onter coat or epispore), also resembles the caudal projection at the same end in the spore of *Cystobia holothuriae* and in *Lithocystis schneideri*.

The history of the spores I have not followed further, bnt it seems probable that the gametocysts escepe to the exterior through the nephridia with the generative products. I have examined specimens of *Arenicola ecaudata* from Plymouth on many occasions in the last two winter seasons, from October to May, and never found a specimen entirely free from the parasite; but the cysts were very abnndant in specimens containing large quantities of the generative prodncts, so that there is some reason for conclnding that the cysts escape chiefly if not exclusively when the worms discharge their ova and spermatozoa. In the summer season May to October, I have not made many observations but I have found the parasite present in Jnne.

Messrs. Gamble and Ashworth in their elaborate and beautifully illustrated memoir on the species of *Arenicola*, make no mention of any parasite resembling the Gregarine I have described, although in all the specimens of *A. ecaudata* which I have examined it is so conspicnous that it cannot escape notice. I have shown my preparations to Mr. Ashworth and he is convinced that if the parasite had been present in the specimens of *A. ecaudata* which he dissected he conld not have failed to notice it. Messrs. Gamble and Ashworth obtained their specimens from the coast of Lancashire, while all

mine came from the shores of Plymouth Sound. It appears therefore that the parasite is of local occurrence, and that while every specimen of the host-species at Plymouth is infected, it is absent from those on the Lancashire coast.

Systematic Position and Affinities.

It is difficult to find a position for this new Gregarine in the Classifications hitherto proposed, such as that of Labbé as modified by Minchin in his account of the Sporozoa in Lankester's Treatise of Zoology. The possession of an epimerite would seem to exclude it from the Acephalina or Monocystidea, but on the other hand it does not agree very well with the characters of any family of the Cephalina. The absence of a septum suggests at first that it might belong to the Doliocystidae but there are several reasons against this conclusion. *Doliocystis* is intraintestinal, not coelomic, and its epimerite is described as long narrow and intracellular, contained within a single epithelial cell. The genus *Doliocystis* was named by Léger in 1893 and he describes the epimerite as contained in a single epithelial cell. In one species *D. nereidis* living in the intestine of *N. cultrifera* the epimerite is described as a simple little button. In another, *D. polydorae*, the epimerite is stated to have the form of a cone continued directly into the anterior extremity of the body of the Gregarine which is elongated into a neck. Lankester figured as long ago as 1863 a form which is now regarded as a species of *Doliocystis* from the gut of *Aphrodite*, and in this figure the epimerite is elongated and cylindrical and united with the body by a narrow neck.

On the other hand L. Brasil, in his paper on the intestinal epithelium of *Lagis koreni* [Mgr.], in reference to the question of the effect of Gregarines on cells of the host, describes and figures the attachment of *Doliocystis pellucida* [Kölliker], which, as it lives in the digestive tube of *Nereis* (*Lipephile*) *cultrifera* [Gr.] is apparently a synonym of *D. nereidis* [Léger]. Neither description nor figures of Brasil give any indication of an epimerite, and it is impossible to reconcile them with Léger's description. Brasil states that *Doliocystis* is "fixée sur une cellule epitheliale de son hote", and that the attachment determines the attraction and hypertrophy (hypertrophic degeneration) of the nucleus of the supporting cell. The latter statement is considered in another part of this paper, here I have only to point out that if Brasil's description is correct,

either the Gregarine to which it refers is not a *Doliocystis* at all, or else LÉGER's description is not to be trusted. It appears therefore that a thorough investigation of *Doliocystis* is still required.

I have shown that the epimerite of *Kalpidorhynchus* is not intracellular and as it differs in shape from anything described in *Doliocystis* and as the parasite is coelomic instead of being intestinal, it is obvious that there is no close resemblance between the two genera in the trophozoite stage. We will next consider the characters of the spores. LÉGER describes the spores of *Doliocystis* as oval, measuring 7 μ by 5 μ, and states that they present a notable thickening at one of the poles. In this latter character they resemble the spores of *Kalpidorhynchus*, bnt on the other hand the latter possess, in the funnel-shaped projection of the sporocyst at the pole opposite the thickening, a character which is not described in *Doliocystis* and which occnrs in several other genera of Gregarines, all of which belong to the Monocystidea. DOGIEL (1906) points out that, as Prof. MINCHIN had previonsly suggested, the Gregarines parasitic in Echinoderms resemble one another in the fact that their spores possess a more or less well-developed funnel at one pole. With regard to the opposite pole the spores of these genera form a series, the sporocyst at this pole showing different degrees of modification in the same or similar directions. Thus in *Cystobia irregularis* [MINCHIN] the pole in qnestion is not thickened at all, in *Cystobia holothuriae* [SCHNEIDER] this pole is produced into a long flat process, in *Cystobia chiridotae* [DOGIEL] the corresponding process is slender and pointed, in *Urospora*, species of which live in Holothnrians (*Synapta*), Oligochaeta (*Tubifex rivulorum*), Nemerteans, Gephyreans (*Sipunculus*) and Polychaeta, the process is similar but much longer, and at the same time the edge of the funnel is prodnced into four small teeth. The spore of *Urospora sipunculi* was figured by LANKESTER as long ago as 1872. In *Lithocystis* the process opposite the funnel, instead of being a filament is long and tubnlar, with a blnnt extremity. *Lithocystis schneideri*, the only species, occurs in the coelom of Echinids and has not been found in other hosts. According to LÉGER, teste MINCHIN, the tubular process in the spore of *Lithocystis* occurs only in the nnripe spore, becoming filamentous when the spore is mature. The spore of *Urospora lagidis* as figured by BRASIL shows, not a hollow funnel opposite the process as above described, bnt merely two short teeth: the figures suggest that these may be really the sides of the funnel in optical section, bnt I have no reason to doubt the accuracy of BRASIL's ohservation and must conclnde that this

species presents a modification of the character. Another genus of Monocystidea which may be added to this series is *Gonospora*, which at any rate in some of its species has at one pole a funnel-like projection whose edges are produced into teeth. Brasil (1905) figures a spore of *Gonospora varia* [Léger), parasite of the coelom of the Polychaete *Audouinia tenteculata* [Mont.]. It is not qnite certain that the projection of the sporocyst in this case is a hollow funnel. Brasil describes it as merely "une couronne de fines pointes hyalines". *Ceratospora* which is also a coelomic Gregarine occurring in a Polychaete (*Glycera*) and a Monocystid, althongh aberrant is probably a more remote member of the same gronp. The spores have a collar-like expansion at one extremity, with the opposite pole of the sporocyst prodnced into two long slender filaments.

It is evident that in the character of its spores *Kalpidorhynchus* belongs to this group, all the other members of which are Monocystids. I see no alternative therefore but to create for it a new family *Kalpidorhynchidae*, having its closest affinities with the Monocystids above mentioned but differing from them in the possession of a well-developed epimerite. The fact that the gametocysts of the new genus are surrounded by a thick envelope of coelomic cells is another point in which it agrees with *Urospora*, *Gonospora* etc., but this is of less importance as an indication of affinity, being merely a physiological condition characteristic of the coelomic Gregarines of Chaetopoda.

Summary.

The paper describes a new Gregarine which lives in the coelom of *Arenicola ecaudata* in Plymouth Sonnd, and which I have named *Kalpidorhynchus arenicolae*.

The trophozoite is of considerable size, long and slender in shape and attached by a distinct cnp-shaped epimerite to the wall of the coelom, especially to the outside of the nephridia and in their neighbourhood.

The protoplasm of the epimerite shows a distinct fibrillar structure the fibres running in a direction parallel to the long axis of the trophozoite.

The gametocytes associate together by their thicker extremities from which the epimerites have disappeared. The attached ends expand and become hemispherical, while the caudal extremities diminish and disappear.

Three, four, or five gametocystes may unite together in association, and an accumulation of coelomic cells becomes attached to them even before the commencement of association.

The gametocysts are surrounded by a thick layer of coelomic cells, and the two gametocytes are separated by a partition formed of their cuticles.

During the multiplication of nuclei the two gametocytes are in different phases and show differences of structure, but anisogamy or a constant difference between the conjugating gametes has not been observed.

The spores are octozoic and their development is of the normal type.

The sporocyst is pyriform, with a projecting funnel at one pole and a simple thickening at the other. In this respect *Kalpidorhynchus* shows affinities with *Urospora*, *Gonospora*, *Cystobia*, and other Monocystid Gregarines, from which it differs in the presence of an epimerite in the trophozoite stage. It is therefore made the type of a new family *Kalpidorhynchidae*.

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Explanation of Plates.

Plate VI.

Fig. 1. Two trophozoites of *Kalpidorhynchus* attached to coelomic surface of a nephridium. Fresh state. ZEISS obj. A, oc. 2.

Fig. 2. A trophozoite of *Kalpidorhynchus*, with a clump of coelomic cells attached to the epimerite. From preparation fixed on slide with corrosive subl. and acetic acid, stained with saffranin. ZEISS obj. A, oc. 3.

Fig. 3. Anterior end of a trophozoite more highly magnified, showing the striation at the end of the epimerite and the structure of the nucleus. ZEISS 3,0 mm, apochrom. obj., compens. oc. 8.

Fig. 4. Epimerite of trophozoite attached to surface of suspensory membrane of nephridium. From a section. ZEISS 3,0 mm, apochrom. obj., compens. oc. 8.

Fig. 5. Detached trophozoite with mass of coelomic cells adhering about the middle region. Fresh state. Low power.

Fig. 6. Three gametocytes at commencement of association, with swollen extremities surrounde by coelomic cells. Fresh state. Low power.

Fig. 7. Two gametocytes associating, coelomic cells on surface, cyst not yet formed. From preparation fixed and stained on slide. Low power.

Fig. 8. Four gametocytes associating, surrounded by cyst and coelomic cells. Fresh state. Low power.

Fig. 9. Two gametocytes associated, surrounded by cyst. Fresh state. Low power.

Plate VII.

Fig. 10. Gametocyst in course of development surrounded by coelomic cells, the two gametocytes entirely distinct, and showing differences in the cytoplasmic reticulum and number of nuclei. ZEISS 16 mm, apochrom. obj., compens. oc. 8.

Fig. 11. Gametocyst at later stage showing the two gametocytes distinct with cytoplasm in cords and nuclei at surface of the cords. ZEISS 8 mm, apochrom. obj., compens. oc. 8.

Fig. 12. Portion of gametocyst in same condition as Fig. 11, showing small portions of the two gametocytes more highly magnified. ZEISS 2 mm, apochrom. obj., compens. oc. 18.

Fig. 13. Gametes from another cyst. Same combination.

Fig. 14. Conjugation stage, from another cyst, zygotes showing two indistinct slightly stained nuclei. Same combination.

Fig. 15. Zygotes from another cyst, with definite outline and deeply stained definite nuclei. Same combination.

Fig. 16. Zygotes or sporoblasts, showing pyriform condition with expanded nuclei: two united by their narrow ends. From cyst burst and prepared on slide. Same combination.

Fig. 17. Sporoblasts with two nuclei, from same preparation as Fig. 16. Same combination.

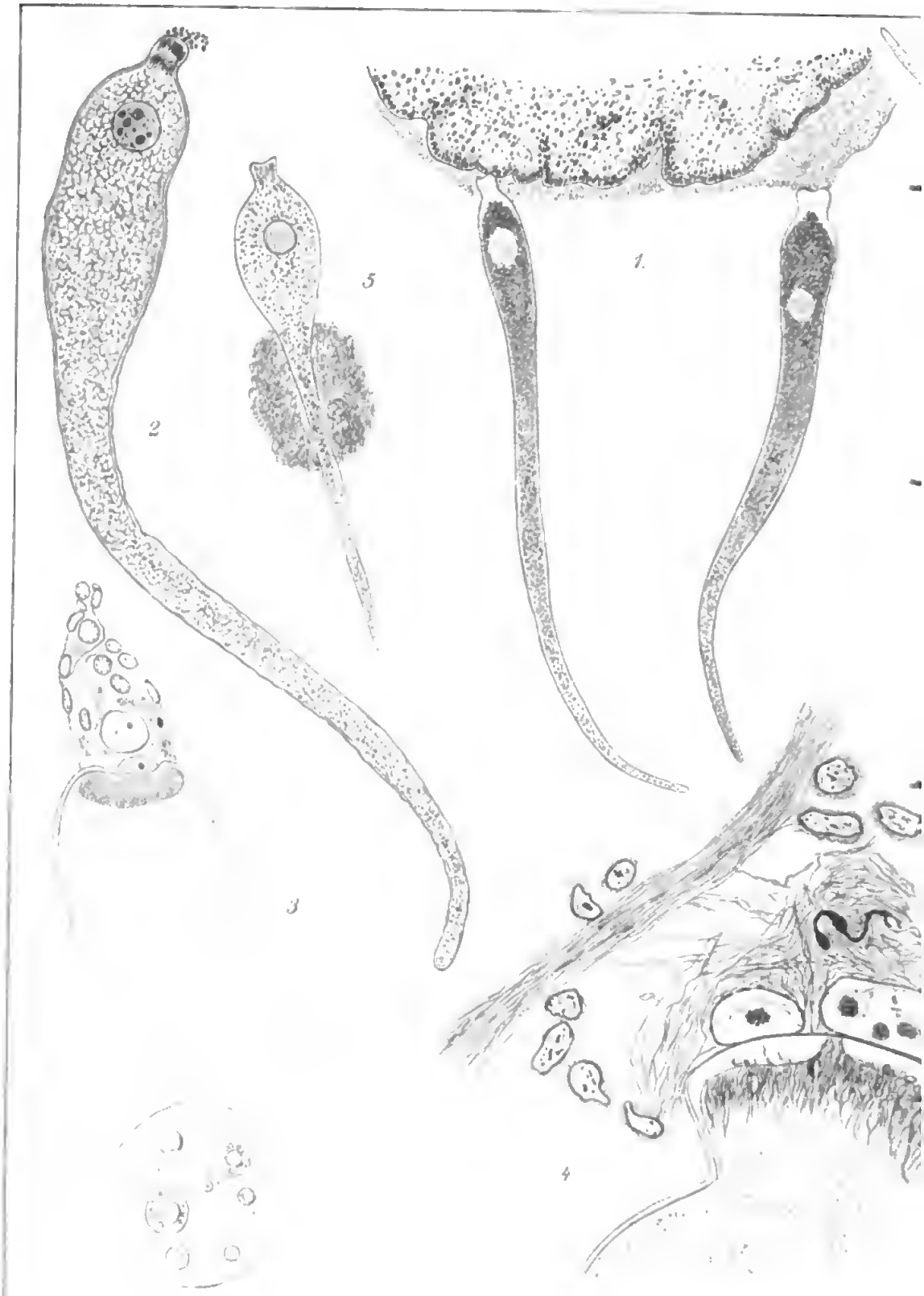
Fig. 18. Sporoblasts, a, with four nuclei, b, with eight nuclei. From same preparation. Same combination.

Fig. 19. Mature spores showing eight sporozoites and sporal residuum. The sporocyst is not visible in the Canada balsam. From same preparation. Same combination.

Fig. 20. Mature spores from a section, showing the eight nuclei in two, the four upper ones only visible in the third. Same combination.

Fig. 21. Spores from a burst cyst in the fresh state to show the sporocyst: the nuclei are not visible. The granular matter at the blunt end of the cyst in the lower figures is the sporal residuum. Same combination.

Fig. 22. Mature spores from a cyst burst and prepared on the slide, stained with GIEMSA's stain and examined in alcohol. Same combination.



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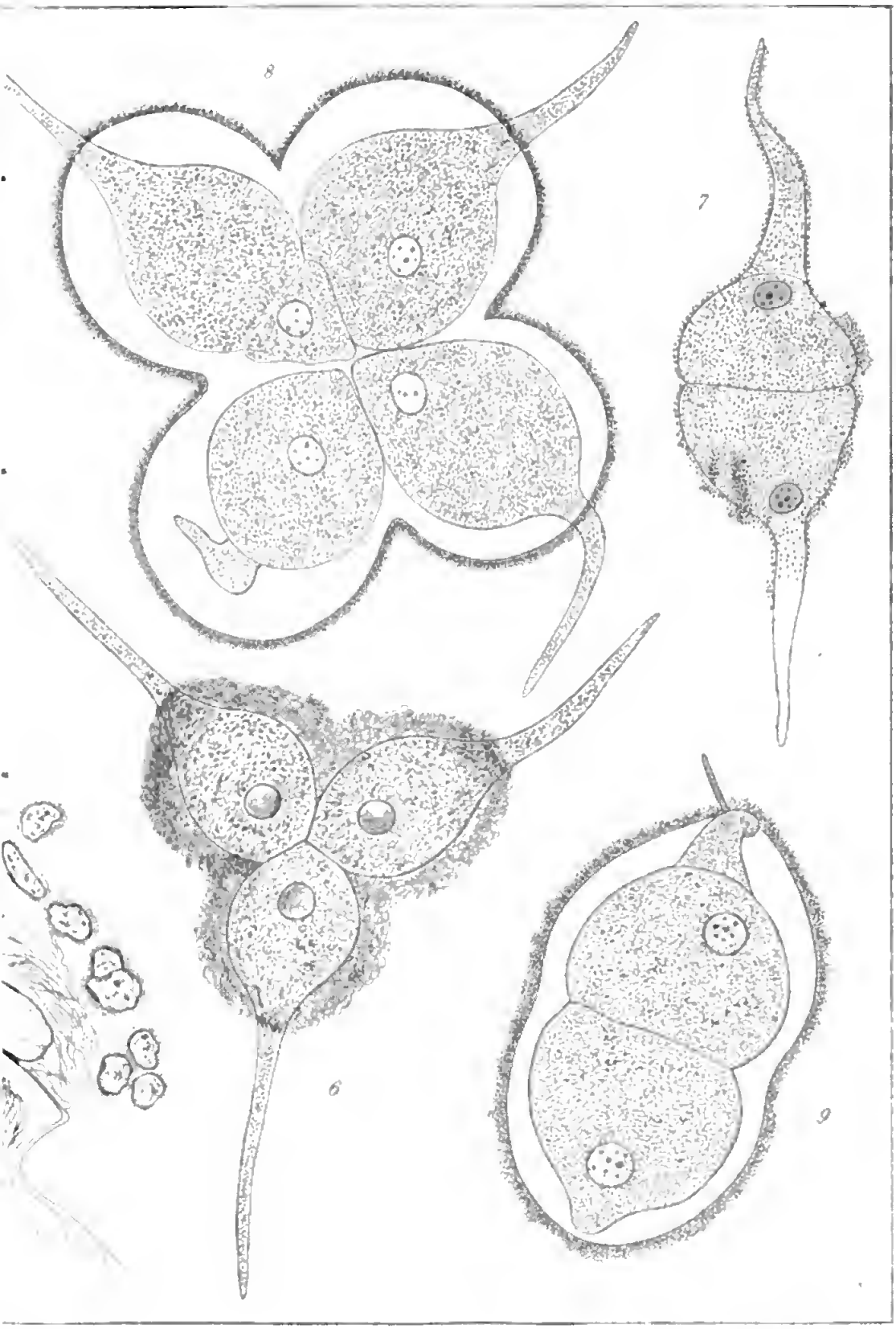

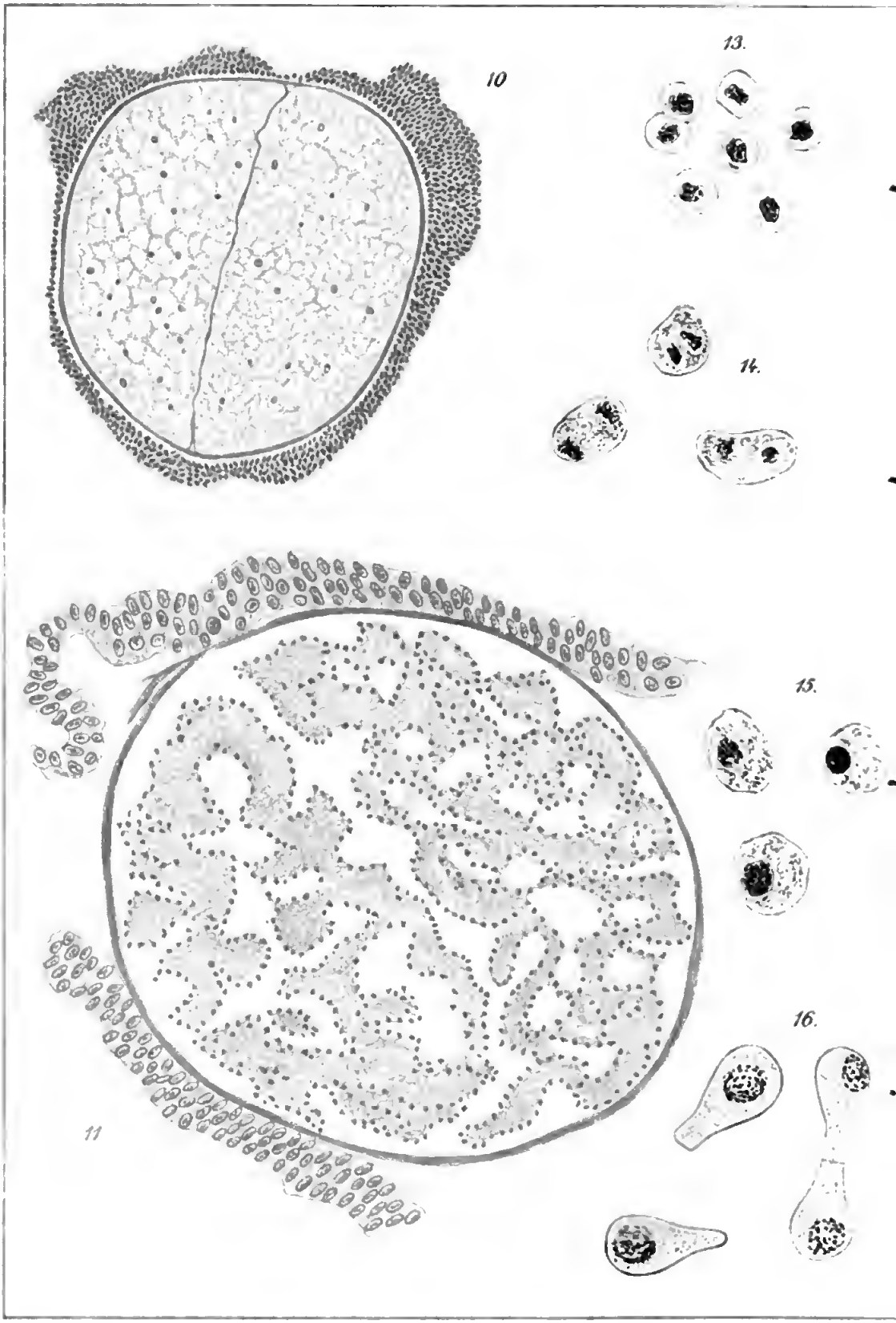

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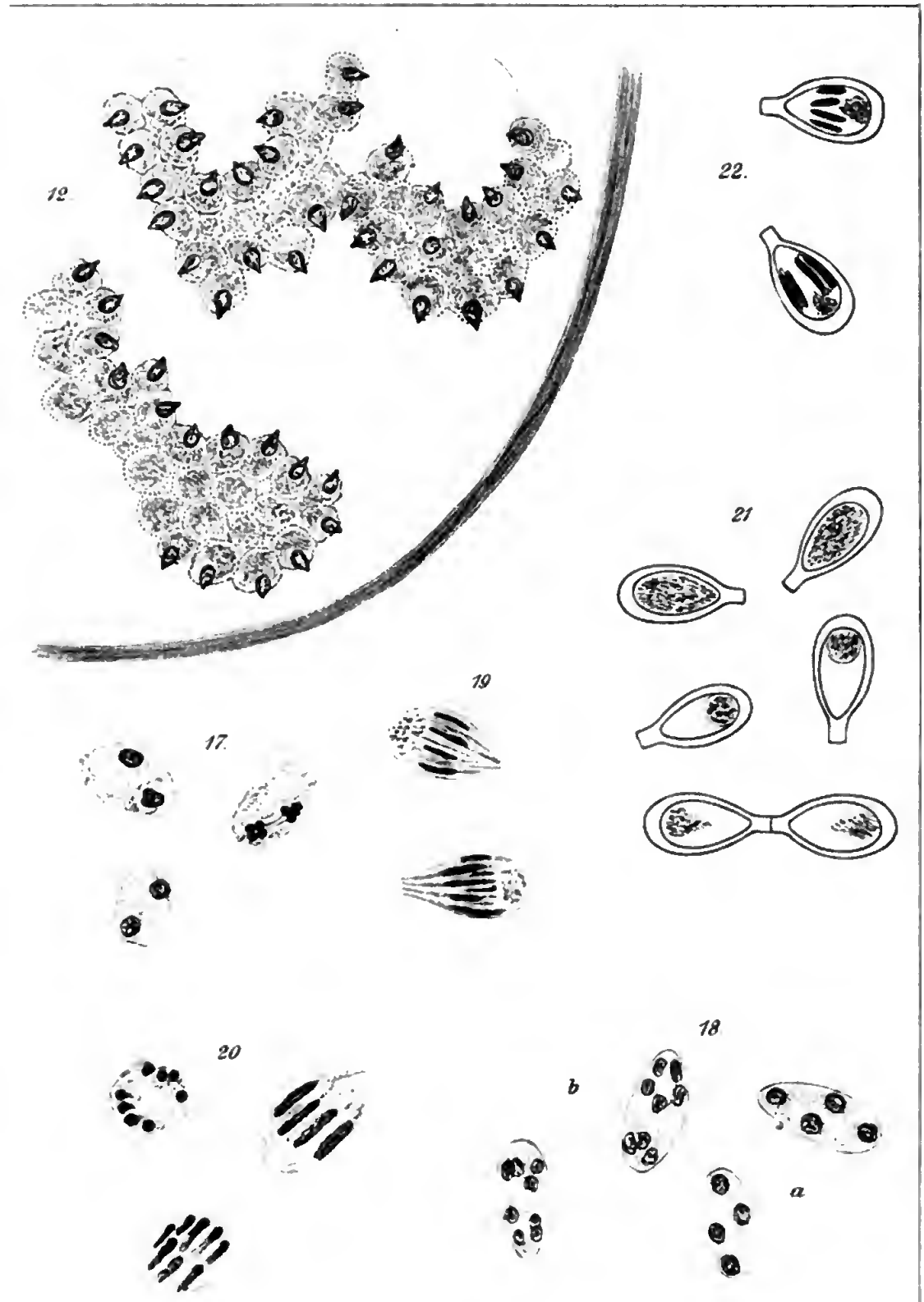

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