Studies on three new Gregarines from Orthoptera in Japan.

by

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(With 2 Plates and 2 Text-figures)

I. Introduction

During the year 1949 to 1950, the writer observed the Gregarines parasitic in Orthoptera taken from the suburbs of Hikari and several other places in Yamaguti Prefecture. Twentynine species of Orthoptera were examined and seventeen of them were parasitized with gregarines which have not been reported in Japan.

Among them I want to report in this paper three new species, dealing with their life-histories and the morphological characters of the trophozoites, sporonts, cysts and spores.

To Prof. Y. Ozaki, I express my most hearty appreciation for his valuable suggestions and critisisms throughout this investigation.

II. Material and Methods

All the hosts used in this investigation were collected chiefly in and aroumd Hikari. Some were taken from Naruto, Yanai and Tabuse in Yamaguti Pref..

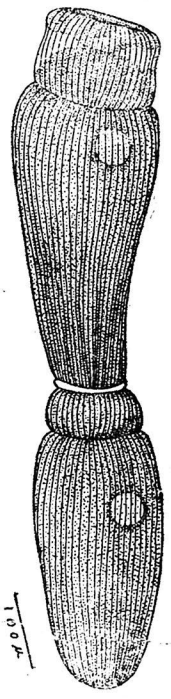
For studying the live parasites, the following method was used. The host brought to the laboratory is slit lengthwise on the abdominal side with fine scissors after its head have been clipped off. The alimentary tract is drawn out and put quickly into the dish containing Ringer's solution. It is torn up gently with a pair of needles and then the free parasites will drop to the bottom of the dish. The parasites may now be picked out with a capillary pipet. When they

are placed on the slide glass, they are to be measured and sketched with a camera lucida as soon as possible, because the animals, being now in an unnatural medium, will indicate deformation before long. Ringer's solution was found to be the best medium in which the live gregarines were observed.

Smears and sections were also made, and the following fixative agents were used for both methods : Schaudinn's, Bouin's and Bouin-Allen's fluids. Bouin's fluid was found most useful for fixing smears. The stains employed were Heidenhain's iron haematoxylin and Delafield's haematoxylin. Various counterstain were employed for the haematoxylin preparations.

To study the gamete-formation and sporulation, the cysts were separated from host faecal matters and kept in a hollow slide covered with a glass piece. Then the slide was placed in a moist chamber in which the parasites continued healthy to produced spores in about four to six days, although some of them degenerated away under such changed conditions.

II. Gregarina concava sp. nov.



Text-figure. An association of Gregarina concava sp. nov. in living.

Host : Gampsocleis burgeri de Haan.

The hosts were collected from the suburbs of Hikari and Obatake in the summer and autumn of 1950. The gastric caeca was the usual seat of infection although the intestine was frequently found to contain parasites. Nearly all grasshoppers examined in these seasons were parasitized, and sometimes the infection was heavy, as many as two hundreds parasites being in each several hosts especially during the fall.

The trophozoite

Whether such polycystid gregarines have the completely intracellular stage or pass only attaching to the epitherial cells of the host intestine through its trophozoic stages, has been already studied and discussed by many previous workers as Schneider, Laveran and Mesnil, Leger and Duboscq, Minchin, Ellis and Watoson. But this subject is so difficult to solve that the definite solution has not been given by this time. Attempting to acquire any data for solving this problem, the writer has investigated many sections of the alimentary tracts of the hosts. The youngest stage found by him was, however, trophozoites inserting its anterior extrmity into the host cell. The rest of the body was laid among the cillia in the lumen of the intestine. This new species has no complete intracellular stages as Gregarina acridiorum observed by Leger and Duboscq ('02).

The youngest trophozoite measuring 15 μ in length is subspherical in outline. The pellicle is thin. The cytoplasm uniformly granulated is almost transparent and no septum is found in it. The nucleus with a definite nuclear membrane contains a karyosome and also a certain amount of chromatin granules distributing within the nuclear sap (Pl. I, Fig.2).

As soon as the trophozoite absorbs nourishment, it grows to an ovoidal cephalont. The cephalont attaining a size of about 25 μ by 10 μ, differentiates the body into two definite parts, the deutomerite and the protomerite (Pl. I, Fig. 2). The anterior end of protomerite remains in the epitherial cell as before. This portion becomes the epimerite, a small spherical papilla without a stalk, by which the parasite may be able to hang on the host cell. The anterior region of the protomerite just behind the epimerite presents a hyaline consistency of the cytoplasm and various shapes in sections. By expanding and contracting this part, the parasite may be able to regulate its situation on the host cell (Pl. I, Fig. 3).

The cephalonts, undergoing a certain amount of growth chiefly in length and attaining about 150 μ

x 40 μ in size, detach themselves from the surface of the host cell. The parasite liberated, drops off its unuseful epimerite and becomes a sporont(Pl. I, Fig. 4, 5).

The sporont

The sporonts are biassociative, ellipsoidal to cylindrical in shape. The maximum length of an association found was 950 μ. The largest primite was 520 μ long, and 200 μ wide. Ratio length protomerite: total length primite = 1 : 3···4.5 :: width protomerite : width deutomerite = 1 : 1.1···1.2. Length protomerite : total length satellite = 1 : 4···6 :: width protomerite width deutomerite = 1 : 1.1···1.4. The protomerite of the primite is short cylindrical and slightly wider than high. It is widest in the central portion, concaved on the top and square cornered. The concaved anterior terminal is generally fringed by a wavy margin and its bottom upheaves like a cone. The constriction is very conspicuous and fairy deep in the adult. The deutomerite is elongate ovoidal, widest in the slightly posterior to the septum. From here the deutomerite is tapering and ending in a rather broadly rounded extremity. This posterior end of the primite fits into a very deep concavity of the anterior end of the satellite.

The satellite is often less longer than the primite, ovoidal in shape. The anterior extremity of the protomerite as above mentioned comes in contact with the primite. Then the protomerite of the satellite is compressed, flattened at the base and consequently reduced from 1½ to 2 times in width in comparison with its height. There is a slight constriction at the septum. The deutomerite is ovoidal, a little wider than the protomerite, widest at the middle portion and is well rounded at the posteror end (Pl. 1, Fig. 1, Text-Fig. 1).

A table of measurements in which all dimentions are given in microns is as follows.

Table. 1.

	TL	LP	LD	LP/TL	WP	WD	WP/WD
Prim. 1	233	70	163	1:3.3	80	98	1:1.2
2	235	73	162	1:3.2	72	90	1:1.3
3	250	80	170	1:3.1	80	92	1:1.2
4	320	110	210	1:2.9	120	128	1:1.1
5	500	110	390	1:4.5	152	180	1:1.2
6	500	130	370	1:3.8	170	180	1:1.1
7	450	100	350	1:4.5	150	170	1:1.1
Sat. 1	190	32	158	1:5.9	60	75	1:1.3
2	184	40	144	1:4.6	55	62	1:1.1
3	262	50	212	1:5.2	70	78	1:1.1
4	295	48	247	1:6.2	88	90	1:1.1
5	410	75	335	1:5.5	120	160	1:1.3
6	420	110	310	1:3.8	125	175	1:1.4
7	480	73	407	1:6.6	125	175	1:1.4

TL=total length, LP=length epimerite, LD=length deutomerite, WP=width protomerite, WD=width deutomerite, Prim.=primite, Sat.=satellite.

The endocyte is very dense in both protomerite and deutomerite, and is yellowish brown in colour. The granules in the protomerite are somewhat larger and fewer than those in the deutomerite, nevertheless the protomerite is seen black in transmitted light. The primite is generally less dense than the satellite.

The epicyte is fairly thick throughout, but the anterior end of the primite is much thicker than elsewhere. As above written, this portion concaves in a characteritsic funnel shape, so the protomerite shows as if being separated into another two chambers when it is stained··· the anterior funnel-shaped part and the posterior dense flattened one (Text-Fig. 1).

Fine parallel longitudinal striations are recognized in the epicyte of both protomerite and deutomerite. They are easily discernible with the aid of an intravitam stain, or after releasing the dense endocyte by crushing the body. If a host is starved for a few days, we may be able to collect the parasites becoming more or less transparent and indicating the striations clearly.

The nucleus is spherical and contains one to three karyosomes. It is average 30 μ in diameter but is not visible in the dense adults in vivo.

The young sporonts are slender and their endocytes are less dense than those of the matured ones. They actively move forward in gliding and bend often their bodies. With age they increase their size and density and finally become practically the black swelled bodies. Movement of such syzygy is no longer the active motion of transration. They have become sluggish and tend to revolve for cyst formation (Pl.

II, Fig. 11).

The cyst and its development.

The mature sporonts are found in the intestine, especially in the posterior parts of it. When they were taken from the intestine by means of a fine pippet and placed into a petri dish containing Ringer's solution, they still continued their slow rotation. In rotating around a common axis, the pair came closer and closer together, finally constituted a shperical cyst. After an hour the cystwall already had made its appearance, yet I could discern that both sporonts remaining the septum between the deutomerite and protomerite were still rotating very slowly. About two to three hours later, movement had ceased and other than the line of separation between two individuals could not be distinguished.

Cysts are spherical, average 350 μ in dianeter, opaque yellowish brown in colour. The cystwall consists of two membranes. The outer one being 40 μ to 50 μ in thickness is a transparent, geratinous layer in which very fine concentric threads are arranged. The inner one, average 10 μ in thickness, enclosing directly the two individuals is thin and more transparent than the former membrane(Pl. II, Fig. 12).

These completely formed cysts collected from faecal masses or from the intstine were crushed or sectioned at various developmental stages and stained to be observed. Further development of the gametocysts takes place outside of the host as follows. Two or three hours after cysts have been formed the nuclei show a little changes and they elongate slightly and the karyosomes are disolved. Afterwards the nuclear membranes disappear and each nucleus begins to repeat the nuclear division. There is a distinction between the two gametocytes of which the one is female, and the other is male.

The former is more deeply stained than the later. By repeated divisions a number of nuclei are formed and they lie scattered in the cytoplasm. Consequently this half is separated into several lobes consisting of many nuclei in them. Various stages of mitosis are seen at those lobes. In the metaphse two fine centrioles connected by the spindlefibers occupy the opposite poles of the nucleus. Four or five sickle shaped chromosomes are visible in the center of the spindle. Unfortunately I have not been able to discern the exact number of chromosomes, but finally the female gamete having a nucleus and surrounded by a bit of cytoplasm is fully formed. In the latter half, the male, the nucleus undergoes the repeated divisions more speedy than in the female part, and this time those divisions are equally taken place all over the whole cytoplasm (Pl. II, Fig. 14).

While these nuclear divisions are going on within the two individuals, the separation membrane between them dissolves. Owing to this disappearance the female-gamate groups scatter in the whole cyst and are surrounded by the male gamates (Pl. II, Fig. 15).

There is little distinction in shape between male and female gamates, and both are spherical and possess spherical nucleus. The female gamate having a traverse diameter of 2 μ is a little larger than the male.

After the conjugation of two gametes has occured, the two gamete-nuclei separate for a while but soon fuse in a zygote (Pl. II, Fig. 19, a-b). This is the sporoblast. The zygotes gradually assembe to the center of the cyst (Pl. II, Fig. 17).

The sporoblast containing a nucleus is ellipsoidal in shape and is covered with a thin wall. Divisions of the nucleus in the sporoblast are done mitotically accompanying with the growth of the sporoblast. Cytoplasmic strands connecting the two divided products are cleary visible. The nuclei are divided further two times to produce eight nuclei in it. In this way the sporocyst with eight sporozoites is completely formed (Pl. II, Fig. 19, c-g).

One or two days after the cyst has been formed, three to five orange coloured discs appeared on the cyst surface. The disc from which a sporoduct is constructed toward the center of the cyst, where numerous sporocysts are collected, comes out by dense accumulation of protoplasm on the periphery of it. When such cysts packing numerous spores had been irritated by shocks or drying, sometimes they began to project the ducts outward and to discharge the spores. The extended duct is very long, 800 μ to 1 m. m., or 2½ or 3 times the radius of the cyst. The anterior 1/5 portion of the duct is orange and along the side of the side of the portion many orange oil globulars adhered are seen (Pl. II, Fig. 16).

The sporocyst

The perfectly formed sporocysts, spores are extruded

from the ducts in chains. The spore in which eight sporozoites are formed is barrel-shaped, 4 μ by 3 μ. The spore wall which is constructed by two membranes, epispore and endospore, is discerned well in the fresh condition. There is a corona of very delicate spines at each end (Pl. 1, Fig. 8, h).

Remarks

Among the member of the genus Gregaina, this species resembles G. rigida Ellis in having the nearly same size and ratio of body, but differs from the latter in having a different size of the cyst, spore and a different number of the sporeducts. Although Watson('16)described in a G.rigida that the two satellites formed an association upon rare occasions and the protomerite was sunk at its anterior end. But this species shows always the protomerite of primite concaved as above mentioned.

Such other species as G. acridiorum Leger, G. longiducta Ellis, G. consobrina Ellis, G. illinens Watoson and G. nigra Watoson are easily distinguished from this new species in the shape of the protomerite and in the length and number of the sporeducts.

IV. Gregarina acantholobae sp. nov.

Host : Acantholobus japonicus de Haan

This species was taken at Naruto and Tabuse, Yamaguti pref. in March to May 1950. The parasite lives in the intestine of the host. The infection was considerably heavy, as many as hundred being found in each of several hosts. About 70% of the hosts examined contained twenty to thirty parasites.

The sporonts are biassociative and associations are oftn formed early in sporont life while they are very small. The maximum length of an association seen was 500 μ, the maximum length of the primite 270 μ, its width 115 μ. The average ratio of length protomerite : total length primite 1:5 and the ratio width protomerite: width deutomerite 1:1.8.

A table of measurments in which all dimensions are given in microns is as follows.

Table. 2.

	TL	LP	LD	LP/TL	WP	WD	WP/WD
Pri. 1	250	52	198	1:4.8	61	110	1:1.8
2	185	35	150	1:5.3	50	90	1:1.8
3	160	38	122	1:4.2	50	85	1:1.7
4	150	50	100	1:5.0	47	84	1:1.8
5	185	40	145	1:4.6	50	93	1:1.8
6	174	37	137	1:4.7	50	100	1:2.0
Sat. 1	211	5	206	1:4.2	70	100	1:1.4
2	150	4	146	1:3.7	61	93	1:1.5
3	150	4	146	1:3.7	58	90	1:1.6
4	141	5	136	1:2.8	60	77	1:1.3
5	155	7	148	1:2.2	52	100	1:1.9
6	153	7	146	1:2.1	54	100	1:1.9

The protomerite of the primite is dome shaped or somewhat pentagonal, slightly wider than height, widest at the base and terminates in a small cone. The anterior end of the protomerite in all sporonts shows a thickened area of the epicyte, in the center of which a canal leads through to the interior of the protomerite. There is a constriction at the septum. The deutomerite is elongate ovoidal, widest at the posterior portion and terminates in a broadly rounded extrimity. The protomerite of the satellite is so greatly flattened that it shows a thin plate shape. In association the end of the primite fits into a shallow dish of the protomerite of satellite. At the jointing plane, the center of the anterior surface of the protomerite, there is a small lense shaped body corresponding to the thickened epicyte of the primite. This body seemes to make the union firmer by fitting into the end of the primite. The deutomerite is also elongate ovoidal but widest at shoulder and very slightly constricted in the middle portion. It is well rounded at the end (Text-Fig. 10).

The endocyte is very dense and brownish yellow in the deutomerite. In the protomerite it is much paler than that of the deutomerite. The portion which is directly under the thick epicyte of the protomerite is stained conspicuously by haematoxylin. Longitudinal striations are easily discernible with the aid of an intravitam stain. The fine circular striations of myoneme are also seen when the parasite is moving. The nucleus is spherical, 20 μ to 25 μ in diameter, contains one karyosome. It is seen in vivo as a clear granule (Pl. 1, Fig. 7).

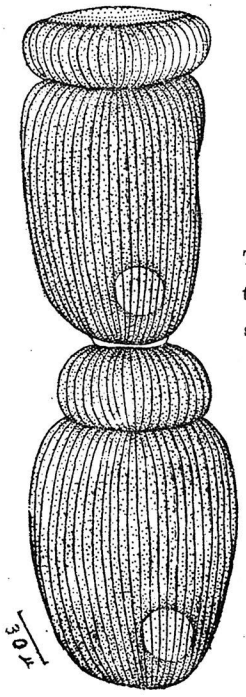
Trophozoites attached to the cells of the intestine with their epimerites are taken and they are transparent or nearly so in vivo. The epimerite is a simple spherical hyaline knob set upon a short slender stalk.

Movement is active. Cysts were not found.

Remarks

Among the members of the genus Gregarina, this species bears some resemblance to G. stigia Watson and G. udeopsyllae Watson in having almost same shape and size of the primite, but this speecies differs from the others in having a conspicious canal through the thickened epicyte of the anterior end of the protomerite and in having the thin plate-shaped protomerite of the satellite.

V. Gregarina korogi sp. nov.



Text-figure 2. An association of Gregarina korogi sp. nov. in living.

Host : Gryllus mitratus de Saussure

The parasites were collected at Hikari and Naruto, Yamaguti Pref., during the summer and fall of 1949. The intestine is the usual seat of infection.

Sometimes in the gastric caeca are present in large numbers, and nearly every cricket examined at this season was parasitized.

The sporonts are biassociative. The maximum length of an association found was 400 μ, the maximum width 110 μ. The average ratio of length protomerite: total length primite was 1:4 and the ratio width protomerite : width deutomerite was 1 : 0.9. The sporont is short cylindrical or elongate ovoidal in shape. The protomerite of the primite is generally wider than the deutomerite. The protomerite is broad and low, slightly concaved or flat at the anterior end. It is from two to three times as wide as high, the average being two, and is widest at the middle. There is a deep constriction at the septum. The deutomerite is widest at the shoulder and from here tapering to the broadly rounded posterior end. The protomerite of the satellite considerably narrower than that of the primite. It is flattened, 1½ to 2 times as wide as high and depressed at the anterior end into which concavity is formed (Pl. 1, Fig. 8, Text-Fig. 2).

A table of measurements in which all dimensions are given in microns is as follows.

Table. 3.

	TL	LP	LD	LP/TL	WP	WD	WP/WD
Prim. 1	165	50	115	1:3.3	100	100	1:1.0
2	165	30	135	1:5.5	92	55	1:0.6
3	160	50	110	1:3.2	70	70	1:1.0
4	160	40	120	1:4.0	90	75	1:0.8
5	100	25	75	1:4.0	45	38	1:0.8
Sate. 1	190	42	148	1:4.5	88	130	1:1.5
2	140	35	105	1:4.0	60	65	1:1.1
3	145	32	113	1:4.5	44	77	1:1.8
4	180	40	140	1:4.5	85	115	1:1.4
5	100	30	70	1:3.3	38	40	1:1.1

The endocyte is very dense in both deutomerite and protomerite and is black in transmitted light. The nucleus is spherical, 20 μ in diameter, and is not visible in the dease adults, but is seen in vivo in the younger sporonts and in the trophozoites. It contains one karyosome.

The immatured sporonts are much slender than the adults and they are elongate cylindrical in shape. The cepharont has a hyaline knobshaped epimerite as other gregarines. Fine longitudinal striations are clearly seen on their surfaces.

Cyst of 200 μ to 300 μ in diameter has the transparent envelope of 30 μ in thickness when the cyst is new. Dehiscence is done by sporeducts which are from two to four in number. Sporeducts being swelled at the basal portions, are 800 μ long. Spores are extended in chain, barrel shaped and measured 5 μ by 3 μ (Pl. 1, Fig. 9, 10).

Remarks

This species bears some resemblance to G. galliveri,

Watson from Gryllus abreviatus Serv. in having the nearly same shape of body. But this species differs from the other in having differnt size of body, cyst and spore, and in having a differnt number of sporeducts.

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Explanation of Plates

Plate I.

Figures 1—6. Gregarina concava sp. nov.

Fig. 1. Mature association.

Fig. 2. Trophozoites attached to the epitherial wall of the host intestine.

Fig. 3. Two trophozoites attached to the host cells,
(a) Showing the protomerite is somewhat contracted
(b) the protomerite is extended.

Fig. 4. A cephalont.

Fig. 5. A trophozoite, free in lumen of the intestine without her epimerite.

Fig. 6. Protomerite of primite, showing that it is concaved in apex.

Fig. 7. Mature association of Gregarina acantholobae sp. nov.

Fig. 8. An association of Gregarina korogi sp. nov.

Fig. 9. A cyst of Gregarina korogi sp. nov. with two spore ducts in the process of extruding spores.

Fig. 10. Four spores of Gregarina korogi sp. nov.

Plate II.

Figures 11—19. Gregarina concava sp. nov.

Fig. 11. Association in process of rotation.

Fig. 12. Cross section of cyst, showing two individuals are surrounded with common membranes.

Fig. 13. Sveral nuclear divisions are seen at a separated lobe of female gametocyte.

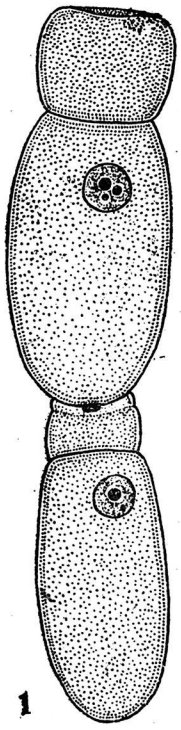
Fig. 14. Cross section of cyst, showing the nuclear divisions are taken place in both gametocytes, (M), male, (F), female.

Fig. 15. Section of Cyst in which the conjugation are taken place.

Fig. 16. A cyst with two sporeducts in the process of extruding spores. Two spore ducts are seen.

Fig. 18. Enlarged view of the basal portion of a spore duct.

Fig. 19. The sporuration.
a. Two gametes having just fused.
b. A zygote formed by the fusion of the two gamates.
c—f. Nuclear divisions in the sporocyst.
g—h. Ripe spore with eight sporozoites in in.



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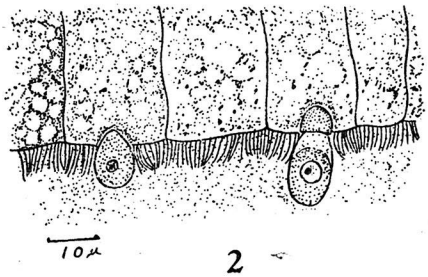

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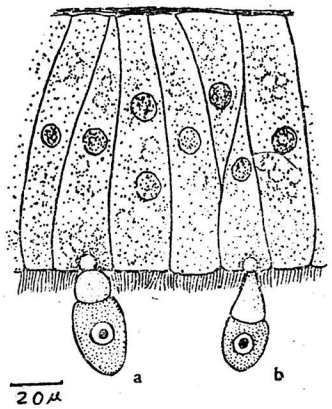

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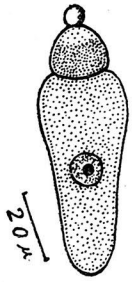

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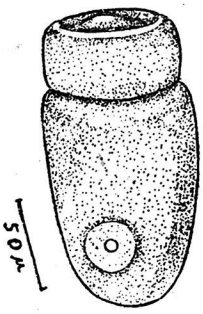

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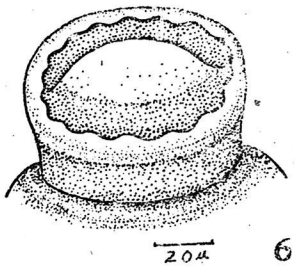

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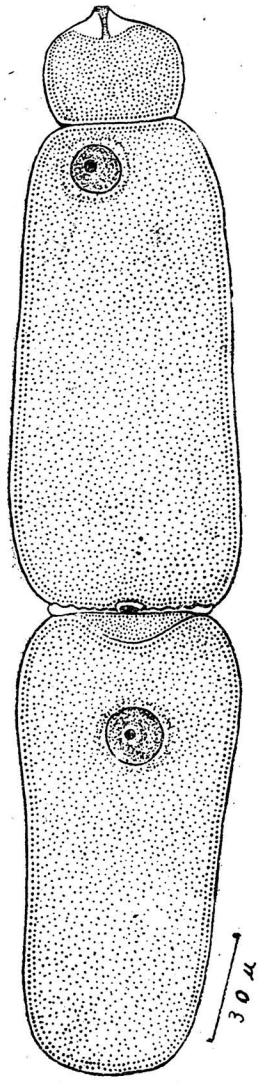

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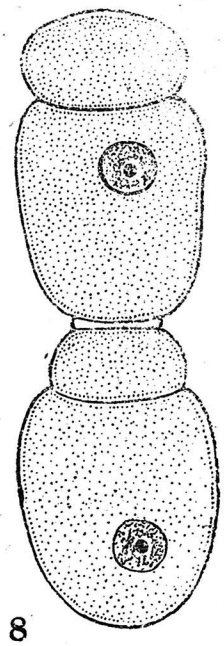

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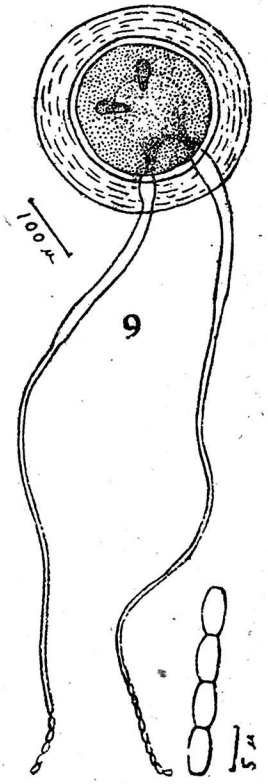

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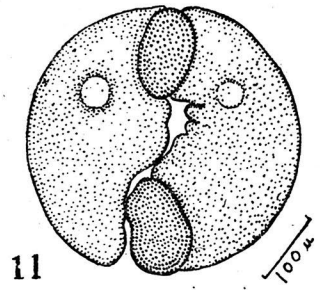

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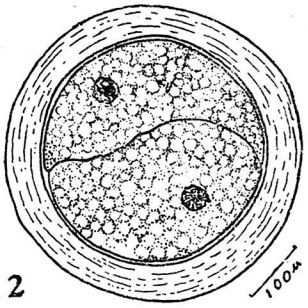

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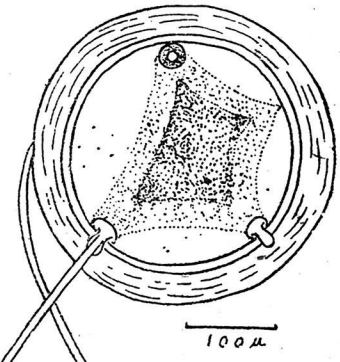

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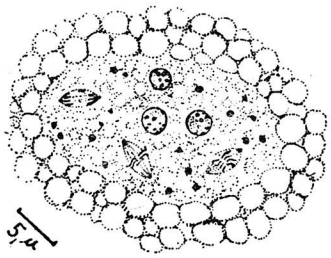

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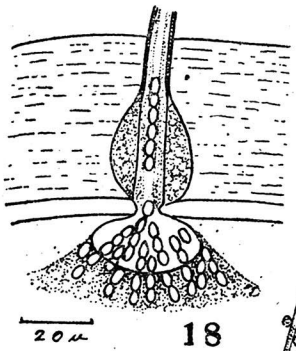

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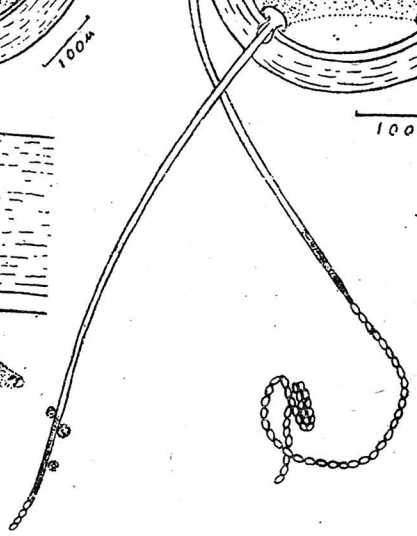

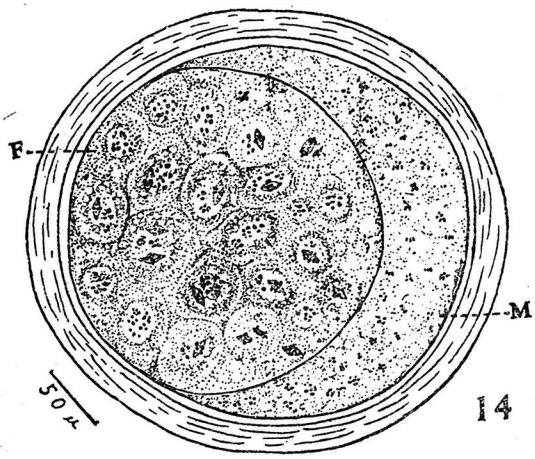

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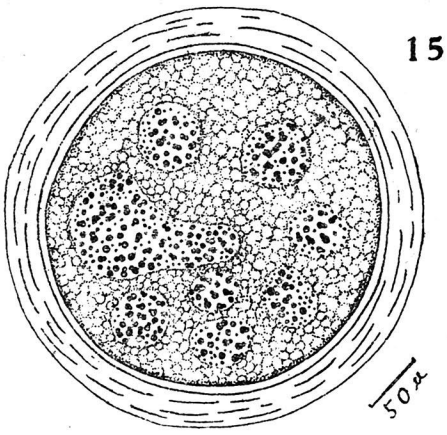

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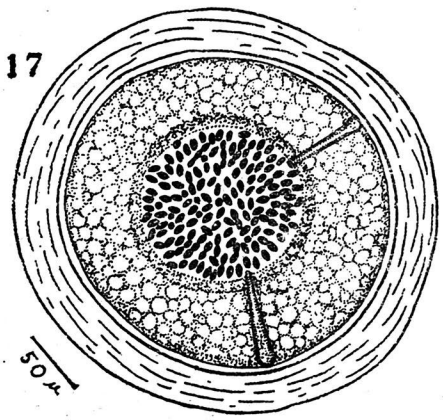

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