Gonospora minchinii, n. sp., a Gregarine inhabiting the egg of Arenicola.

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With Plates 5 and 6.

When examining the contents of the coelom of an Arenicola ecaudata Johnston, at the Marine Biological Laboratory in Plymouth last winter, we discovered a new Gregarine of considerable interest, since it appears to be the first instance on record of such a parasite inhabiting the ovum of its host.[1]

This gregarine belongs to the genus Gonospora. It does not seem to occur at all in the male worm, and of the females examined only about 30 per cent. were infected. However, since the parasite was not found in any but female worms whose ovaries were fairly ripe and had begun to shed their products into the coelom, it is probable that it often inhabits less mature hosts, but in some situation not yet determined. We have looked for it without success in the immature ovary. Frequently it occurs simultaneously with the larger and well-known coelomic gregarine Gonospora (Kalpidorhynchus) arenicolae Cunningham.

The immature ovary of Arenicola ecaudata is a lobulated organ with finger-shaped processes (see Gamble and Ashworth, 1). Inside it the germ-cells multiply, accumulating in its lumen, and later bursting through its wall. The ova thus escape into the coelom at various stages in development; some quite small and oval, others larger, more rounded, loaded with yolk, and surrounded by a thick covering. This shell is formed of two

[1] Since this was written we have learnt from Sir Ray Lankester that many years ago he discovered a somewhat similar parasite in the eggs of Thalassema. From an inspection of some unpublished drawings of the trophozoite, which he kindly sent to us, we conclude that it is not the Gregarine described in this paper.

distinct layers : an outer thin refringent membrane, the original vitelline membrane ; and an inner much thicker and probably less dense perivitelline layer (fig. 10). A full-grown ovum with its covering is about 120 to 130 microns in longest diameter.

Young Trophozoites.—The youngest stages of the parasite observed were small rounded trophozoites embedded in the egg close to its nucleus. Fig. 1 shows such a stage where the gregarine is 12 μ in diameter ; far smaller than the nucleus of the immature egg it has invaded, and indeed only about twice the diameter of its nucleolus. It will be noticed that even at this early stage the nucleus of the parasite is distinguished from that of the ovum by the possession of two karyosomes, while the latter is almost invariably provided with only one nucleolus. The trophozoite continues to grow at the expense of the egg, enlarging and becoming stored with granules of paraglycogen (figs. 2, 3). As it acquires the shape and size of the adult (fig. 7) the egg and its nucleus become more and more compressed against the surrounding membranes.

Penetration into the egg.—It has been stated above that the ovum of Arenicola is protected not only by a vitelline membrane, but also when full-grown by a thick perivitelline layer. How does the parasite penetrate into the egg ? is a question which at once suggests itself. Now it is probable that fully-developed eggs are safe from invasion, since infected eggs are rarely, if ever, found with the perivitelline layer fully formed. By far the greater number of eggs infected are provided with a vitelline membrane only (figs. 4, 7), or with but a thin perivitelline layer as well (figs. 6, 11). The parasite enters the egg by boring a round hole through these membranes, and usually the margin of the hole is found turned inwards (figs. 2, 4). The aperture so formed may remain open ; but sometimes it seems to close up almost entirely (fig. 6), presumably when the egg is invaded at a very young stage.

Position and growth of trophozoite in eggs. —It is often very difficult to decide whether the parasite, having pierced the egg-membranes, really enters the egg-cell or merely bulges into it. Except perhaps in the very earliest stages it

certainly lies as a rule outside the egg-cell, between it and the membranes (figs. 2, 4). It compresses the egg more and more as it grows and is separated from it by a space, except at that one region opposite the point of entrance where the epimerite of the parasite adheres closely to the egg-cytoplasm near the germinal vesicle (fig. 9). Here are developed, in that part of the gregarine which is fixed to its host, small club-shaped bodies staining deeply in haematoxylin or fuchsin. They appear to be hollow, with long narrow necks reaching to the surface (fig. 9). These strange structures somewhat resemble the 'lamelles mucoïdes' described by Léger and Duboscq in Nina (2); but their function would appear to be connected with the absorption of nutriment from the egg, or possibly merely with fixation. Meanwhile, as the parasite grows it enlarges the deep depression it causes in the egg; the margin of this hollow is at first smooth (fig. 2), it soon becomes notched, and finally drawn out into delicate protoplasmic processes converging towards the point of entrance (figs. 3, 4, 5).

Effect of parasite on host egg.—The very young ovum has little or no yolk; but with advancing age the yolk granules increase in number until the fully-developed egg becomes so heavily loaded that it looks quite opaque. In parasitized eggs, however, the yolk is absorbed by the gregarine almost as fast as it is laid down, so that in late stages the compressed ovum is relatively clear, while the parasite on the contrary is densely granular (fig. 4). The nucleus of the egg is also influenced, for its nucleolus, instead of undergoing the orderly series of changes seen to occur in normal eggs, lags behind in differentiation, remaining in fact apparently at that stage of development it had reached when the egg was invaded. Thus the nucleolus in most parasitized ova resembles that of the quite young ovum when it is still small and has but little yolk (figs. 2, 7).

Another peculiar and somewhat similar effect is seen on the egg-envelopes. There is no reason to think that the perivitelline layer when once formed can be reabsorbed, and since it is, as a rule, almost or quite absent from parasitized eggs,

even when these have reached full size, there can be little doubt that the presence of the gregarine checks its deposition. Never have we observed full-sized eggs without parasites in which this layer was not present.

Emergence of parasite from egg.—When the trophozoite has completed its growth it emerges from the egg-shell by a round hole, which is probably the enlarged original opening through which it entered, or at least formed afresh in the same place (figs. 5, 8). The gregarine first pushes out its pointed 'tail' end, the rest of the body following after.

Fate of parasitized egg.—As soon as the parasite has thus abandoned the egg, leaving a large space partially surrounded by the emaciated host-cell and communicating with the exterior by an aperture of considerable size, leucocytes from the coelomic fluid make their way in (figs. 8, 11, 12). They gather in large numbers in the cavity, and proceed to attack the already depleted ovum, the cytoplasm of which becomes vacuolated. Strange thread-like structures, which stain in acid-fuchsin, are now visible round the edge of the egg (*th.*, fig. 12) before its final breaking up.

The free trophozoite.—The full-grown trophozoite free in the coelomic fluid is usually pear-shaped, the epimerite being at the blunt end. As a rule the nucleus is provided with two conspicuous karyosomes, but additional small granules may be present. Often the gregarines hang together in groups, sometimes in masses of ten or twelve individuals.

Association and spore-formation.—The association of two trophozoites is terminal (fig. 13), the 'head' end of one penetrating deeply into that of the other in the manner so characteristic of the genus Gonospora (**3, 4**). At the extremity of the embedded epimerite may be seen in sections a cap of dense substance tipped with a deeply-staining granule, possibly of nuclear origin (fig. 14). At this stage, before the formation of a cyst, the two associates can still be separated by pressure. As soon as the cyst wall is secreted round the pair their opposed faces flatten out. Gamete formation and syngamy then take place as usual in these gregarines.

A spore with its eight sporozoites is shown in fig. 15 ; it is from 8 to 10 μ in length. The sporocyst is thin, one pole being rounded and the other provided with a slight thickening, but there is no well-developed funnel such as occurs in Gonospora glycerae (**3**).

For this new gregarine we propose the name Gonospora minchinii.

Summary.—The new species of gregarine described above, and to which we have given the name Gonospora minchinii, occurs in the coelomic fluid of the female Arenicola ecaudata. The adult trophozoite is pear-shaped, and the ripe spore has a thin cyst without distinct funnel. The young trophozoite lives in the egg floating in the coelomic fluid of the Arenicola, where it grows at the expense of the food-material stored in the ovum. To reach the ovum it pierces the vitelline membrane and perivitelline layer. The growing trophozoite occupies a deep depression it causes in the egg, to which it adheres by its epimerite. The margin of this depression becomes drawn out into delicate protoplasmic processes. The cytoplasm and nucleus of the host-cell, and also the development of the perivitelline layer, are affected by the presence of the parasite. When full-grown the trophozoite escapes from the egg by a hole pierced in its envelopes, and leucocytes then enter the space so left to complete the destruction of the ovum.

References.

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3. Pixell Goodrich, H. L. M.—"The Gregarines of Glycera siphonostoma", 'Quart. Journ. Micros. Sci.', vol. lxi, 1916.
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EXPLANATION OF PLATES 5 AND 6.

Fig. 1.—Young egg of Arenicola with small trophozoite inside it. Whole preparation; Formol-iodine, Paracarmine. × 500.

Fig. 2.—Later stage showing opening in vitelline membrane, and depression in egg in which lies the parasite. Whole preparation; Formol-corrosive, Paracarmine. × 500.

Fig. 3.—Nearly full-grown parasite in egg; from the living. × 500.

Fig. 4.—Semi-diagrammatic optical section of egg with contained parasite. × 500.

Fig. 5.—Trophozoite emerging from egg; from the living × 500.

Fig. 6.—Portion of a section of an infected egg showing the young trophozoite. Bouin, Iron-Haematoxylin. × 1,100.

Fig. 7.—Optical section of whole preparation of egg with full-grown trophozoite. Formol-corrosive-acetic, Paracarmine. × 500.

Fig. 8.—Infected egg from which the parasite has escaped. Leucocytes are making their way into the cavity. From the living. × 500.

Fig. 9.—Portion of a section of full-grown trophozoite which is fixed to host-cell near flattened nucleus, and showing deeply-staining bodies, *a*. Chrom-osmic; Iron-haemat., Light-green. × 1,100.

Fig. 10.—Part of section of uninfected egg, showing normal development of vitelline and subvitelline membranes. Bouin, Iron-haemat. × 1,100.

Fig. 11.—Section of an infected egg from which parasite has escaped. Chrom-osmic; Iron-haemat. × 500.

Fig. 12.—Similar egg at later stage showing its destruction by invading leucocytes. Chrom-osmic, Iron-haemat., Light-green.

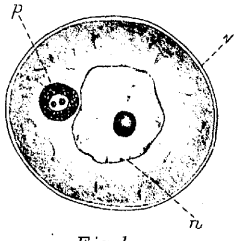
Fig. 13.—Two Gonospora minchinii in association. Whole preparation. × 120.

Fig. 14.—Section through dovetailing epimerites of associates. Chrom-osmic; Iron-haemat., Light-green. × 1,100.

Fig. 15.—Spore with eight sporozoites. × 3,000.

Reference Letters.

a. = deeply-staining bodies at edge of trophozoite fixed to ovum. *c.* = cytoplasm of ovum. *cp.* = cytoplasm of parasite. *l.* = leucocyte in cavity vacated by parasite. *lt.* = limit between associated trophozoites. *mp.* = minute pore, probably contracted pore of entrance. *n.* = nucleus of egg. *nc.* = nucleolus. *np.* = nucleus of parasite. *o.* = ovum. *op.* = opening. *p.* = parasite. p^1 and p^2. = associates. *pr.* = protoplasmic process. *sp.* = space left by parasite. *sv.* = perivitelline layer. *t.* = 'tail' end of trophozoite. *th.* = threadlike structures in outer zone of egg. *v.* = vitelline membrane



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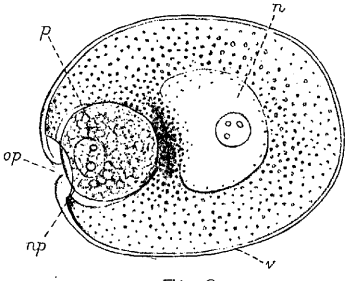

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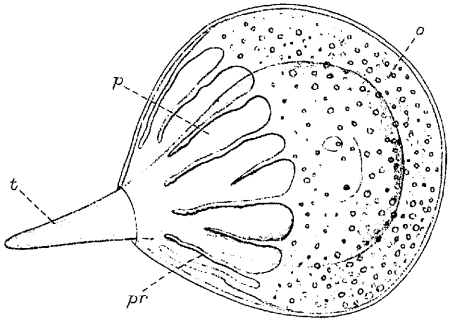

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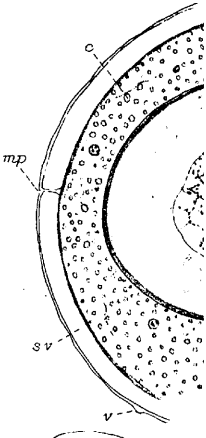

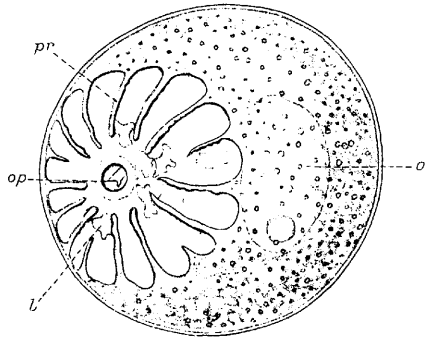

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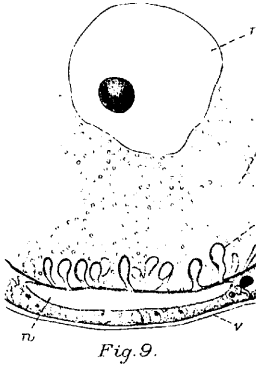

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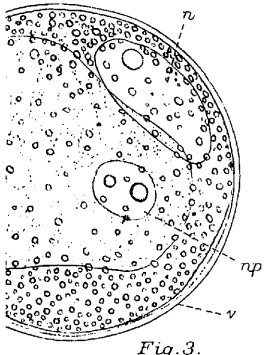

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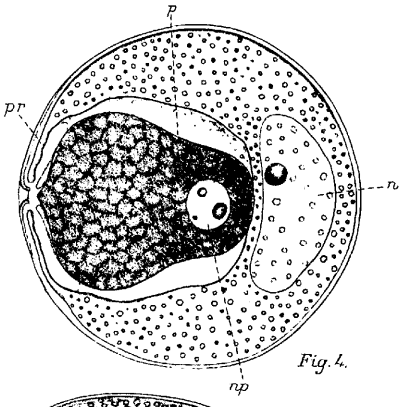

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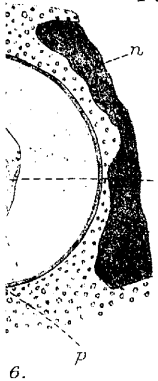

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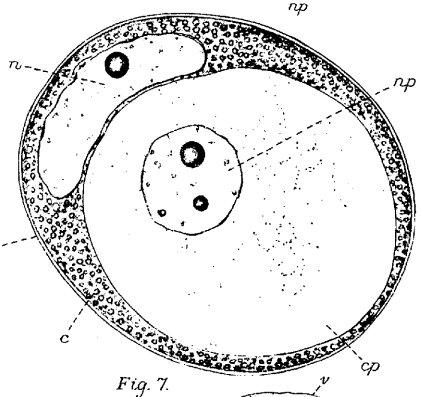

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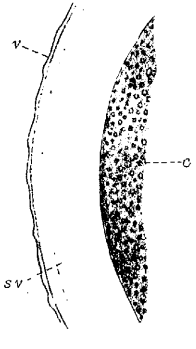

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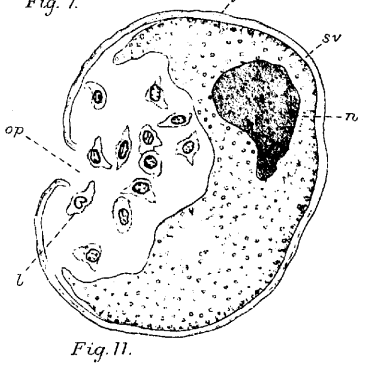

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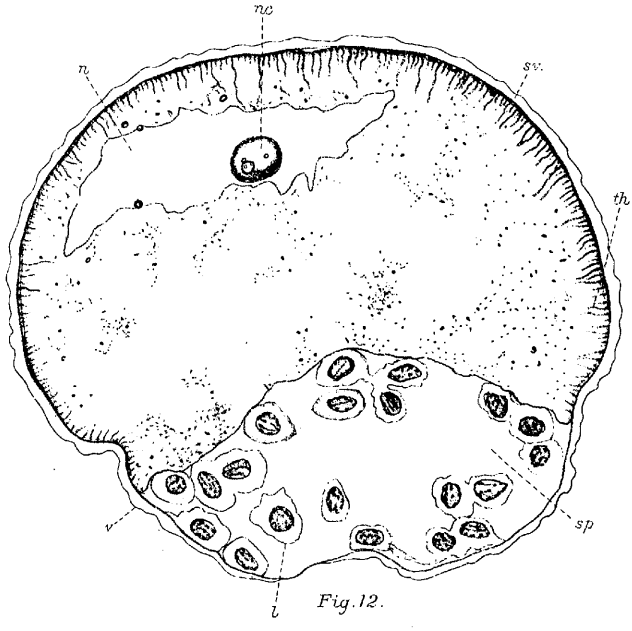

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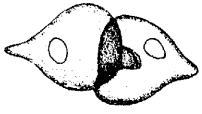

Fig. 13.

Fig. 15.

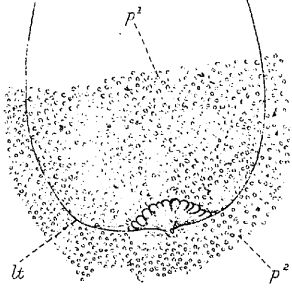

Fig. 14.

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