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On a Remarkable New Nematode, Tylenchinema oscinellae gen. et sp. n., Parasitic in the Frit-Fly, Oscinella Frit L., Attacking Oats

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VII. *On a Remarkable New Nematode,* Tylenchinema oscinellæ *gen. et sp. n., Parasitic in the Frit-fly,* Oscinella frit *L., attacking Oats.*

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[Plates 22–26.]

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Introduction.

On June 3, 1929, some young oat plants, lifted from an experimental plot at Winches Farm, St. Albans, were brought into the laboratory and examined for the presence of "tulip-root," caused by the nematode worm *Tylenchus dipsaci*. The "tulip-root" condition was not found, nor were any examples of its causative agent seen, but the stunted and swollen stems of the young plants were attacked by frit-fly. On breaking open some of the stems, larvæ of the fly were found surrounded by the débris of the destroyed tissues, and in this material a few nematodes were discovered which attracted the writer's attention because they appeared Tylenchus-like and yet could not be identified with any of the plant-parasitic or free-living Tylenchus species known to the writer.

Most of them were in an ensheathed condition, but one or two fully developed males were found having caudal alæ but lacking any recognisable buccal stylet. The appearance of the ensheathed forms called to mind certain figures by Wülker (10), p. 410, illustrating the development of *Allantonema mirabile*, a nematode parasite of the pine weevil, *Hylobius abietis*, and the view was tentatively formed that the newly-found worms were in some way connected with the frit-fly, possibly as parasites of it. This view received support from the fact that none of them were observed in dissections of healthy stems, although specimens of certain free-living nematodes were found in these.

Several more affected stems were opened up and from many, but not all, of them the new worms were obtained. By June 5 it was clear that the male worms always lacked a mouth stylet, whilst the females were provided with a comparatively massive one. The females also were found to have the uterus crowded with very small spermatozoa, a feature in which they resembled the young spermatised females of *Allantonema* described by Wülker.

This fact further confirmed the view, which by now had become fully formed, that the worms would turn out to be parasitic in the frit-fly. Proof that this provisional hypothesis was well founded was obtained on June 11, when a frit-fly larva, previously washed to rid it of any free nematodes which might be clinging to it, was opened with needles, and from its body-cavity there floated out seven female worms identical in structure with those already found in the destroyed plant tissues surrounding the fly larvæ.

From this time onwards more frit larvæ were collected and dissected, and worms were found in them. Some of the larvæ were found to contain the larvæ of Hymenopterous parasites in addition to the new worm. What the ultimate fate of the latter is, under this state of parasitism of the host, it is impossible to say. In due course pupæ were collected; some were opened so as to yield various stages in the development of the worm and many more were kept for the study of the parasite in the adult flies which would emerge from them later on.

During the month of July large numbers of male and female frit-flies were dissected, and it was found that the worm in most cases brings about the complete sterilisation of both sexes, since the testes and ovaries of parasitised flies remain rudimentary and undeveloped. Later in the summer, flies of the next generation, which develop in the panicles of the oat plant, were dissected and the worms were found in these also.

A study of the relevant literature revealed the fact that there was no previous record of a parasitic nematode worm in the frit-fly, and it was therefore concluded that the worms were new to science.

Life-History.

(a) *Life-history of the frit-fly in its Seasonal Generations.*

In order to render the account of the new parasite quite clear, the writer has considered it advisable to give a brief outline of the life-history of the frit-fly, *Oscinella frit*, as it occurs throughout the year on oats and wild grasses.

The adult flies appear in the spring from overwintering larvæ and the females lay eggs on spring-sown oats whilst the seedlings are still small, generally during May. The eggs hatch and give rise to small legless larvæ, which make their way into the shoots the central tissues of which are destroyed by them. As a result of this the plant is incapable of producing an ear, and though fresh tillers or shoots are frequently produced these may become attacked and the whole plant remains small and stunted. The central leaf of a shoot turns yellow and the outer sheaths become swollen and thickened.

The larvæ when fully grown measure some 3 to 4 mm. long by about 0·5 mm. wide. They pupate on the plant and the pupæ are usually found just under the outer leaf sheaths, generally fairly close to the ground. They are light to dark brown in colour and occur in large numbers on affected plants during the latter half of June.

Flies emerge during July, their period of maximum numbers being round about July 17, and after pairing, the females lay eggs on the panicles of oats which are showing at this time. The adult flies are small, shining, black insects, the females measuring about 2 mm. and the males about 1·5 mm. long, and have a characteristic short hopping flight.

The generation just described may be termed the spring or stem generation. The eggs laid on the panicles again give rise to larvæ, which attack the tissues of the growing soft corn. Great damage is done and a light yield of oats results. The larvæ pupate and in due course flies emerge, as a rule during August and early September. This may be called the summer or panicle generation.

The females again lay eggs, but this time it is on various species of wild grasses, and the same course of development is passed through on the wild host plant as on the oat. The rate of development is much slower, however, during the colder season and the winter is passed in the larval condition. The larvæ continue to feed on the soft tissues of grass stems which are damaged by them. In the spring, pupation takes place and the adult flies emerge in time for eggs to be laid on the seedlings of spring-sown oats, thus bringing us round to the point at which we started.

(b) *Life-history of the nematode linked to that of the frit-fly.*

Commencing with the larvæ of the frit-fly within the tissues of an oat shoot, we find almost completely developed larvæ of *Tylenchinema oscinellæ* undergoing their last two moults, and young adult males and females living in the free state amongst the débris of the plant tissues. The female worms are impregnated with spermatozoa by the males and the latter then die out. The females now enter frit-fly larvæ and one or more take up their residence in the body-cavity of the host.

Here they remain during pupation and are within the fly on its final emergence from the pupal case. Males and females are both attacked. By the time the flies emerge the parasite has increased in size and has become a sausage-shaped organism lying freely coiled within the abdominal cavity of the host. Here it becomes viviparous and produces large numbers of small larval nematodes which are shed into the cavity. These larvæ are motile, and since the cavities of the abdomen, thorax and head of the fly communicate with one another the larvæ move freely into all three cavities. Here they pass through at least one, and probably two moults, whilst at the same time the reproductive organs undergo considerable development. It is now necessary for them to reach the exterior in order that the life-cycle may be completed. This is effected by their passage from the body-cavity into the gut of the fly and so out *via* the anus.

Parasitised flies of both sexes, having failed to develop their sex cells, fly about and instead of taking part in the normal process of reproduction are able only to deposit larvæ of the nematode parasite. Normal females go to oat panicles and there lay eggs; similarly, the parasitised flies responding to the same urge of the life-cycle rhythm also fly to the oat panicles, but, instead of eggs, deposit larvæ of the nematode parasite. These find their way, possibly in response to some chemotactic stimulus, into the plant tissues surrounding the fly larvæ. Here they undergo two more moults and become young adult males and females. The latter, after receiving spermatozoa from the males, enter frit-fly larvæ and in this way the cycle of growth within the host is carried on.

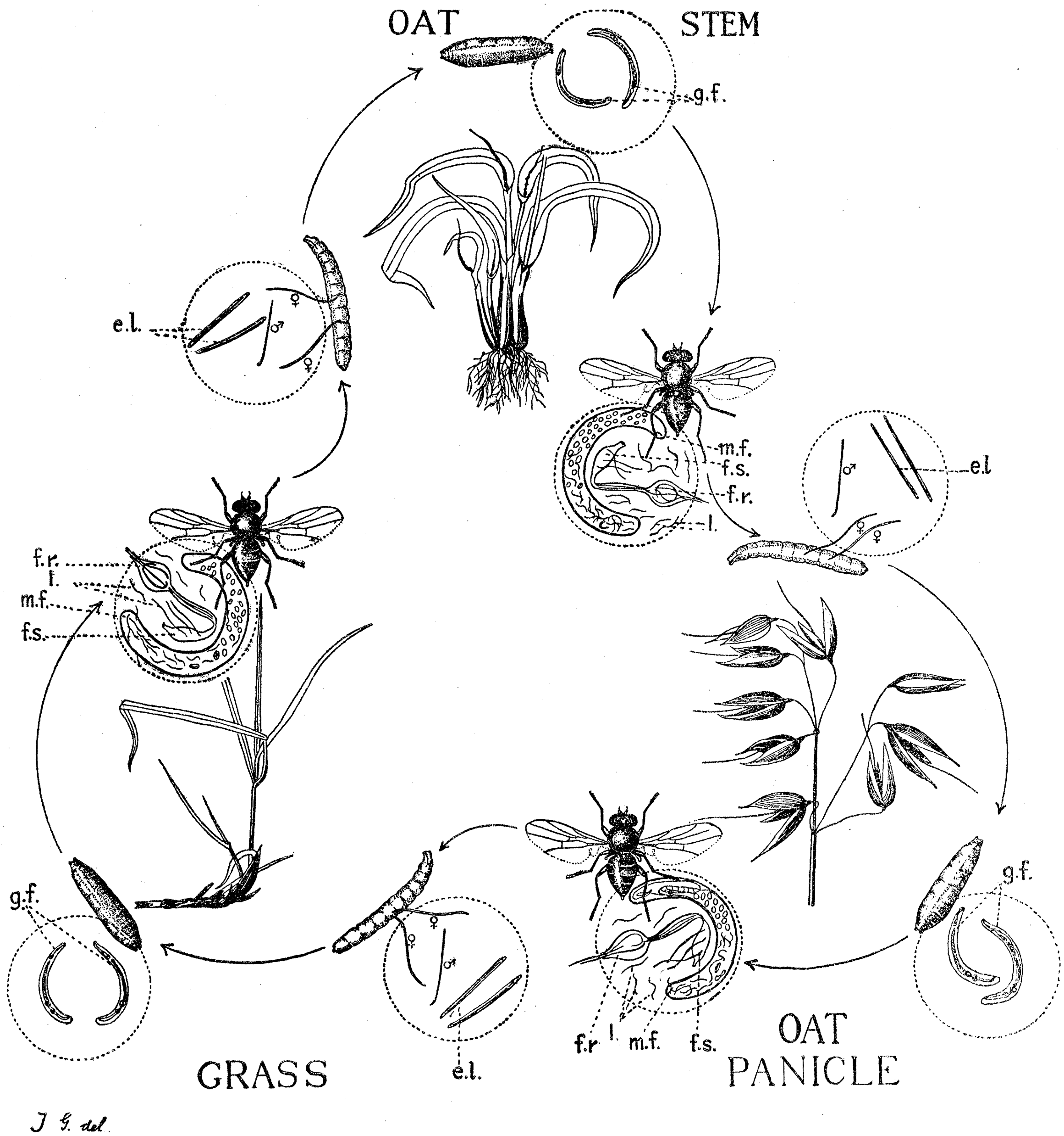
So far the events described above have been determined for the stem and panicle generations of flies, but it is evident that an exactly similar cycle must be passed through in the case of the overwintering generation on grass. That this is so is proved by the fact that the first worms found by the writer in oat stems were ensheathed larvæ undergoing their last two moults, and these must have come from parasitised flies of the overwintering generation.

The accompanying diagram (text-fig. 1) has been drawn to illustrate the life-history of the fly and the parasite.

Structure and Development.

(a) *Technique.*

Oat stems attacked by frit-fly were opened with stout needles in tap-water and the free-living stages of *Tylenchinema* were picked up by means of a capillary pipette under



TEXT-FIG. 1.—Schematic drawing illustrating the life-history of the frit-fly in its three seasonal generations coupled with that of its parasite, *Tylenchinema oscinellæ*. The various stages of the fly and worm are shown greatly enlarged, whilst drawings of the oats and grass are smaller than natural size. Although the female fly only is shown it should be understood that the male also harbours the worm. The dotted circles contain stages of the parasite related to the corresponding stage of the host. The circles cut into the pupa and adult fly, but not into the fly larva, in each case, thus indicating that the parasite is within the pupa and fly but outside the larva. *e.l.*, ensheathed larvæ; *f.r.*, fly's rectum; *f.s.*, fly's stomach; *g.f.*, growing females; *m.f.*, mature female; *l.*, larvæ in body-cavity.

a binocular microscope. Transferred to microscope slides, the worms were studied in detail after being carefully killed by heat over a very small by-pass flame of a bunsenburner. The edges of the coverslip applied to the drop containing the worms were sealed down with hot wax from the wick of a candle recently extinguished. The detailed structure of the anterior region of young adult females and of the tail end of the males was studied under the oil-immersion in preparations of the above description.

For the study of preserved specimens, free-living and parasitic stages of the worm were fixed in hot 70 per cent. alcohol containing 2 to 3 per cent. of glycerine. The worms were isolated in glass capsules in the smallest possible quantity of water or Ringer's solution and the hot fixative was poured quickly over them. A trace of aqueous solution of Nile blue was added immediately (just enough to give a faint blue tint to the liquid), and the capsule was set aside, at room temperature, to allow the alcohol to evaporate slowly. By this means the nematodes were left in a weak solution of glycerine, in which they were afterwards mounted on slides. Permanent mounts of this kind had the cover-slips sealed first with glycerine jelly and then, when this was set, with gold size.

The addition of the Nile blue proved very valuable, as it brought out the details of the developing gonad in the larvæ and the position of the intestinal gland in the young females. If more than a trace of it is used the fat globules in the intestinal wall are stained by it. The reproductive organs of normal and parasitised flies were fixed and stained by the same method.

A few examples of rudimentary testes and ovaries were fixed in Bouin's fluid and subsequently embedded in paraffin wax. This was carried out by the method shown to the writer several years ago by the late Prof. E. A. Minchin. A thin slice of goat's liver, which had been kept for some time in 95 per cent. alcohol, was placed in a shallow dish of alcohol of the same strength. The testes and ovaries were brought into this dish and arranged in the desired position on the surface of the liver by means of needles, the whole operation being carried out under the binocular microscope.

In order to attach the objects to the liver a small drop of Mayer's egg-albumen was allowed to fall from a needle through the alcohol above the objects. In so doing it spread out over them, became coagulated and fixed them to the liver. The latter was then placed for 3 or 4 hours in butyl alcohol and from this taken straight to melted paraffin wax in an embedding bath (Twort's method). Sections were cut 6 μ thick and after mounting were finally stained with Ehrlich's hæmatoxylin and counter-stained with Biebrich scarlet.

Flies of both generations were anæsthetised with chloroform in a suitable glass vessel and dissected under the binocular microscope with fine cutting needles in Ringer's solution. In this, the sausage-shaped females and the larvæ continued to live for quite half-an-hour.

Flies of the stem generation were obtained from collected pupæ and by sweeping with a fine muslin net through standing oats during July. Those of the panicle generation were obtained from panicles of oats borne on stems cut from the plot and placed in a large breeding box from which light was completely excluded, except for a 1-inch hole in the top. Over this was placed an inverted glass cylinder, about 5 inches high, and since the flies come towards the light they were obtained in large numbers in the cylinder.

(*b*) *Larvæ within Fly and Free-living.*

1. *General.*—The larvæ passed by the gravid female into the body-cavity of the fly are very small and measure 0·21 to 0·23 mm. in length by 0·008 to 0·01 mm. in greatest width.

The body tapers slightly anteriorly and posteriorly. The head is knob-like with rounded sides and is separated from the body by a slight constriction. The tail is bluntly rounded. The cuticle carries very fine transverse striations, and faint indications of lateral fields are discernible under high magnification. Separate lips or papillæ have not been found on the head.

The mouth is terminal and leads into a very narrow cuticularised buccal tube, a little longer than the width of the head. It is not a stylet or spear, such as occurs in *Aphelenchus*, *Tylenchus* and *Heterodera* species, and basal thickenings are not found on it.

The buccal region is followed by a small fusiform œsophagus, about 0·03 mm. long, which narrows where the nerve-ring crosses it and then expands again slightly. The intestine is distinctly visible at this early stage of growth and is made up of about 16 cells, each having a prominent nucleus. Posteriorly the intestine is connected by a short rectum to the anus, which is situated about 0·02 mm. from the tip of the tail. The above account of the general structure applies to male and female larvæ. As growth proceeds and the larvæ attain a length of from 0·46 to 0·5 mm. the cuticle of the head shows signs of six equidistant longitudinal ridges and the œsophagus becomes correspondingly longer and more sharply defined. In neither male nor female does it ever show a rounded or oval muscular bulb, such as is found in *Aphelenchus* and *Tylenchus* species.

The larvæ probably feed on the fluid contents of the body-cavity of the host, and the walls of the intestine become well stocked with globules of reserve fats.

As in most other nematodes growth is accompanied by a series of moults, probably four in number. Three of these have been observed, one taking place within the body-cavity when the larvæ are about 0·46 mm. long (Plate 22, figs. 20 and 21) and the last two in the free-living condition. The one which has been noted on larvæ from within the host is probably the second moult; the writer having failed as yet to see the first. It is possible that this takes place whilst the larvæ are still within the uterus.

The third and fourth moults undergone in the free-living condition are illustrated in Plate 22, figs. 11, 12, 24 and 25, from which it is apparent that the final one takes place whilst the worm is still within the cast cuticle of the third ecdysis. It would seem that during these free larval stages, the worms do not take in food, but make use of the fatty reserves stored in the intestinal walls.

At the earliest stage within the body-cavity of the host the male and female larvæ are distinguishable from each other owing to slight differences in the appearance of the group of sex cells, known as the genital primordium, and because of the presence in the females of a specialised granular cell, just in advance of the genital primordium, destined to become the intestinal gland.

II. *Male.*—The genital primordium in the youngest male larvæ (Plate 22, figs. 1 and 2) consists of an elongated group of cells lying about midway down the body, closely applied to the intestine but sharply distinguishable from it, measuring about 0·04 mm. long by 0·005 mm. wide. The 12 to 14 cells composing it are polygonal in shape and each contains a large nucleus, having a central karyosome surrounded by a clear zone enclosed in a nuclear membrane, on which there are small chromatinic thickenings. The protoplasm of the cells is very finely granular. At the anterior and posterior ends there is a cell with a somewhat smaller nucleus. These are the terminal cells which belong to the tunica propria covering the cell mass. The anterior one by its division supplies the material to cover the gradually enlarging gonad. The posterior one gives rise by successive divisions to a group of cells from which the vas deferens is produced.

As the larva grows there is a gradual increase in the length of the developing gonad and in the number of cells composing it. It grows anteriorly and posteriorly in the body. Figs. 3 and 4 (Plate 22) show its appearance in a larva 0·253 mm. long, in which the posterior terminal cell has multiplied and given rise to a group of cells distinguishable from the rest of the gonad. A stage of growth a little more advanced is shown in figs. 5 and 6, of the larva 0·3 mm. long, in which the mass of sex cells has attained a length of 0·125 mm., the anterior end of it lying a little in advance of the middle of the body and the posterior end well down towards the anal region. The cells composing the rudiment of the vas deferens now form a well-defined group, in which the outlines of the separate cells are rather indistinct and the protoplasm has a slightly clearer appearance than that of the cells making up the testis. In the latter the cells of the anterior germinal zone are distinctly larger than those half-way down, and these again are larger than those at the posterior end where division proceeds rapidly.

A further stage in growth is represented in figs. 7 and 8 from a larva 0·37 mm. long, in which the front end of the testis reaches into the anterior third of the body, whilst the posterior end is within a short distance of the two groups of cells, now discernible, from which the spicules will develop.

A slightly more advanced stage of development is shown in figs. 9 and 10, from a larva 0·463 mm. long. Here the rudiment of the vas deferens lies quite close to the spicule mother-cells, whilst the anterior end of the germinal zone of the testis extends almost to the œsophagus. The posterior region of the testis is occupied with quantities of very small rounded spermatozoa, whilst immediately in front of these is a zone in which the spermatocytes, which are smaller than the anterior spermatogonia, are undergoing division.

The spicule mother-cells now have the form of two distinct, rather elongated groups of large nucleated cells. Larvæ at the stage of the development just described are ready to make their way out from the host and they have been found in large numbers within the rectum and hind intestine of male and female flies (figs. 45 to 48, Plate 25).

During the two final ecdyses (figs. 11 and 12) there is very little further extension of the testis anteriorly, but the posterior half or two-thirds becomes occupied with spermatozoa. The vas deferens reaches further back, becomes narrowed down and connected up with the anal opening. The spicules and gubernaculum become fully formed and take up their adult position. At the same time the œsophagus loses its fusiform outline and all trace of a definite lumen within it disappears. These internal changes bring us to the structural condition found in the adult worm.

III. *Female.*—In the case of the youngest female larvæ within the body-cavity of the host, the genital primordium is smaller and consists of fewer cells than that of the male. Figs. 16 and 17 show its condition, in a larva measuring just over 0·2 mm. long, as a lens-shaped area composed of two large central cells with a smaller cell at each end. The large cells are the ovarian primordium cells; each has a correspondingly large nucleus with a central karyosome surrounded by a nuclear membrane carrying small thickenings of chromatin. The smaller cells at each are "terminal cells"; the anterior one is destined to provide the tunica propria of the future ovary and the posterior one the future uterus and oviduct.

As the larva grows in length the whole genital primordium gradually shifts posteriorly in the body, from its original mid-way position, and takes up a position on the ventral side of the intestine a little in advance of the rectum. Multiplication of the posterior terminal cell takes place, whereby a narrow elongated column of cells is produced (figs. 18 and 19), the separating walls of the several cells often being rather indistinct.

As the incipient uterus approaches the anal region a plate of cells is formed on the ventral body-wall a short distance in advance of the anus. These cells are ectodermal in origin, and after becoming ridged and folded in the central region they give rise to the vagina and vulva. By the time this stage of development is reached the larva has undergone an ecdysis and is ready to enter the gut of the host preparatory to making its way out.

The condition met with in a larva from the gut is shown in fig. 22 (Plate 22). The ovarian group consists of four cells and the posterior end of the uterine group is connected to the centre of the vaginal group. At this time also the rectal cells are clearly visible on either side of what will ultimately become the adult rectum.

During the last two moults, passed in the free-living condition, the final stages in the development of the vulva, vagina and uterus are passed through and a condition is reached such as is shown in fig. 23. Here the lumen of the long uterus is indicated as a line. A few cells at the anterior end are destined to form the future oviduct. The vagina and vulva are practically complete. With the casting of the last cuticle the worm becomes the young adult female and is ready to receive her stock of spermatozoa from the male.

In the case of the female larva there are two special structures, in addition to the reproductive organs, which must be dealt with, viz., the intestinal gland and the buccal stylet.

Intestinal Gland.—In the smallest larvæ found within the body-cavity of the host, the primordium of the gland is found as a single elongated cell on the dorsal side of the intestine, just in advance of the genital primordium, as shown in figs. 16 and 17. It is readily distinguished from the adjacent intestinal cells on account of its coarsely granular contents.

It also has a distinct large nucleus containing a central karyosome separated by a clear zone from the nuclear membrane. The cell remains in the same relative position during the whole of the larval development within the host; increasing a little in size as the larva grows. During the two final ecdyses it grows forwards along the dorsal side of the intestine and œsophagus, and the duct is laid down connecting it with the lumen of the anterior region of the œsophagus.

The buccal stylet is not found during the larval development and is only formed as the worm is undergoing its last moult, as shown in fig. 25.

IV. *Transmission from Fly.*—As already mentioned in the account of the life-history of the worm, when the larvæ have reached a certain stage of development in the body-cavity of the host, they make their way into the intestine and so reach the exterior *via* the anus. A similar course is taken by the larvæ of *Allantonema mirabile* and *Bradynema strasseni* in escaping from their beetle hosts, as Wülker (10), p. 468, makes clear. On the other hand Cobb (2), p. 669, says that the larvæ of *Howardula benigna* pass out with the eggs of cucumber beetles (*Diabrotica* spp.).

It is at present impossible to say exactly how entry into the lumen of the gut is effected, since no larvæ have actually been discovered in the process. It may be conjectured, however, that it is accomplished by the larvæ actively boring through the gut wall, in such a way that no injury is done to the wall, the hole through which a larva passes immediately closing up.

Schneider (8), p. 247, says that he found larvæ of *Sphærularia bombi* within the intestine of queen bumble bees and in the wall of the intestine. Wülker (*loc. cit.*) says that in *Allantonema* the larvæ only enter the intestine in sexually mature examples of *Hylobius abietis*. He records that Leuckart once found dead larvæ, which had apparently not succeeded in penetrating the wall, held fast in it.

It is also probable that the larvæ enter between the cells of the wall of the stomach or mid-intestine, since both fore- and hind-intestine are lined throughout with a thin layer of chitin. Larvæ have been found in the stomach, in the region of the gut just anterior to the point where the Malpighian tubules arise, and also in the long colon and pyriform rectum, which make up the hind-intestine.

In no case have larvæ been found within the Malpighian tubules. The photomicrographs, figs. 45 and 46 (Plate 25), show them in the stomach, colon and rectum, whilst figs. 47 and 48 show larvæ passing out from male and female flies.

(*c*) *Adults, free-living.*

I. *Male* (figs. 13, 14 and 15 (Plate 22)).—Principal measurements :—Length, 0·55 to 0·65 mm. ; width, 0·015 to 0·02 mm. ; anus to tip of tail, 0·034 mm. ; anterior end to excretory pore, 0·12 mm. ; spicules, 0·011 mm. long ; gubernaculum, 0·003 mm. long. Proportions, length to breadth, $\alpha = 36$–37 ; length to length of œsophagus $\beta = 4$–5 ; length to length of tail, $\gamma = 16$–18. The body tapers gradually from the region of the excretory pore to the head end, whilst posteriorly the tail tapers from a little in front of the anus to the tip.

The cuticle is finely striated transversely and the striations are interrupted by the lateral fields, which are well defined and have a width in the middle of the body of 0·006 mm. By careful focusing, under the oil-immersion, it can be seen that the lateral field presents a double contour at each edge, due to its slightly winged character, whilst towards the middle two longitudinal lateral lines can be distinguished.

The head is rounded and is set off from the body by a slight constriction. No papillæ or separate lips could be discovered on its surface. A mouth aperture is lacking and no buccal rods or other specialised mouth structures are present—features doubtless connected with the fact that the adult male does not feed. The outline of the fusiform œsophagus present in the larval condition is now lost, but an œsophageal region is present, extending from the head to a short distance behind the excretory pore, as a faintly vacuolate and rather ill-defined area. The nerve-ring crosses the œsophagus just in front of the excretory pore. There is no intestinal gland in the male.

At the tail end on each side of the body the cuticle is produced into a narrow wing-like expansion or ala, which arises a little anterior to the anus from the ventral edge of the lateral field and extends to the tip of the tail, as in many species of *Tylenchus*. Its free edge is slightly crenate in appearance.

The spicules are paired and shaped as shown in fig. 15. Each is broad anteriorly and tapers towards the tip. There is a short, simple gubernaculum. The gonad has been dealt with in the account of the larval development.

II. *Female* (figs. 26, 27 and 28 (Plates 22 and 23)).—Principal measurements :—Length, 0·5 to 0·6 mm. ; width, 0·015 to 0·02 mm. ; anterior end to end of œsophageal region, 0·125 to 0·128 mm. ; anterior end to excretory pore, 0·117 to 0·12 mm. ; anus to tip of tail, 0·03 to 0·032 mm. ; vulva to tip of tail, 0·058 to 0·06 mm. ; length of buccal stylet, 0·02 to 0·021 mm. Proportions, $\alpha = 33$ to 36, $\beta = 4·4$ to $4·6$, $\gamma = 18$ to 19.

The body is of slender proportions and tapers before and behind. The cuticle bears faint longitudinal and fine transverse striations, and the lateral fields are of the same width and appearance as in the male. The head loses the knob-like shape found in the larval condition and becomes bluntly conical. It is still possible in some cases to find a line of constriction separating it from the body. The tail tapers slightly posterior to the anus and carries one or two small irregular processes on the ventral side at the tip. When killed by heat the young female does not straighten out completely, but takes up a curved position, concave on the ventral side, with the head end tilted slightly to the dorsal side (fig. 27).

The mouth aperture is terminal in position and is very small. The stylet is strongly developed and is, for the most part, cylindrical in shape, as shown in fig. 28. From this it can be seen that it is made up of a short anterior conical portion attached to the long cylindrical posterior part. It has stout walls and the lumen is quite distinct. At its posterior end it narrows down a little with a gradual thinning of the walls, and here it is always turned slightly towards the dorsal side of the body. The opening is not at the extreme tip of the stylet, but on the ventral side, about half-way down the conical portion. There are no swellings or thickenings of any kind at the base of the stylet such as are found in most species of *Tylenchus*, *Aphelenchus* and *Heterodera*.

As far as can be judged from the low-power drawings of *Allantonema* and *Bradynema* given by Wülker (10), figs. 12 and 19, the stylet in these species is not shaped like that just described, but has weak basal swellings. A clear zone surrounds the stylet and is limited behind by a distinct line. Possibly this is a muscular region as in *Tylenchus* species. The next section of the œsophagus is traversed by a narrow lumen, with thin but very clear walls, into which, at about 0·03 mm. from the base of the stylet, the intestinal gland opens on the dorsal side. This is in the form of a short lateral duct at right angles to the lumen, as shown in fig. 28, and several fine branches of the wall radiate into the substance of the gland for a short distance.

The narrow lumen is continued a little way beyond the opening of the duct and then expands into the lumen of the œsophagus. This is generally rather irregular in shape, and, as a rule, contains small groups of refractive granules. As in the larval condition there is no muscular œsophageal bulb.

The nerve-ring crosses the fore part of the intestinal gland and the œsophagus at about the level of the excretory pore. The intestine, the beginning of which is not clearly marked off from the œsophagus except for the presence of fatty food globules, extends backwards throughout the rest of the body and is connected with the anus by a short rectum.

The intestinal gland extends a little further forwards than its duct and has a rounded end. Posteriorly it is closely applied to the intestine, so that it is practically impossible, in freshly killed specimens, to distinguish a line of demarcation between them, but in specimens stained with Nile blue the gland shows up better and has a more finely granular appearance than the intestine. In freshly killed specimens the substance of the gland seems to be made up of small lightly refractive spheres. Its single nucleus remains a prominent feature in stained worms.

Wülker (10), pp. 419–431, shows a similar uni-nucleate gland in young females of *Allantonema mirabile* and *Bradynema strasseni*, but does not figure a definite duct connecting the gland with the alimentary canal, as has been described above for *Tylenchinema oscinellæ*.

True œsophageal glands of the usual type, *i.e.*, a single dorsal and a pair of sub-ventral glands, are not present in the œsophagus, and it is not clear with what the gland can be homologised in more typical nematodes. As already shown in the account of the larval development, it arises from a single specialised cell on the dorsal side of the intestine, and during the final ecdyses grows forwards into the œsophageal region The writer has, therefore, given it a locational name in calling it "intestinal gland." Wülker calls it "Schlunddrüse" in *Allantonema* and *Bradynema*, and homologises it with a uni-nucleate gland in another nematode, *Ichthyonema* (*Philometra*), a parasite of certain fishes.

The development of the genitalia has already been dealt with and little remains to be added about them in the young adult female. The vulva is often very difficult to see, since it is merely a very small orifice and prominent surrounding lips are absent. The vagina is short and has rather thick clear walls surrounding its very narrow lumen, which leads into the uterus practically at its posterior end. In the fertilised worm the uterus is densely crowded with very small rounded spermatozoa, which cause it to expand and become pushed forward in the body. Its anterior end narrows where it is connected to the ovarian group of cells by the group forming the incipient oviduct.

III. *Infection of Fly Larvæ.*—It was impossible to make direct observations on the manner in which the fertilised females get into the fly larvæ. It may, however, be safely assumed that they are not eaten by the larvæ but penetrate through the body-wall and so come to lie in the body-cavity. Skin penetration amongst nematodes, as a means of infecting a mammalian host, is a well established and widespread phenomenon upon which a considerable amount of research has been carried out in recent years. In all such cases it is an infective larval stage which enters the host, whereas in the case of *Tylenchinema* it is an adult worm which has to enter the body. Nevertheless it is quite reasonable to suppose that the fertilised worm does bore through the cuticle and body-wall of the fly larva. Whether the stylet is actually used in the process as a puncturing organ must remain for the present a matter of conjecture, as must also the question whether the secretion of the intestinal gland acts as a solvent of the larval cuticle. Both may, of course, function in the manner suggested, but their mere presence in the worm need not imply that they are so used.

In this connection it may be pointed out that the infective larvæ of *Ancylostoma duodenale* and *Strongyloides stercoralis*, the former an ensheathed and the latter a sheathless larva, bore their way through intact mammalian skin by sheer muscular activity, whilst both lack a buccal stylet or specialised glands directed to subserve this purpose.

The suggestion that the females enter through the skin, and not *via* the mouth, also receives support from the fact that in making dissections of fly larvæ, the worms were never found in any part of the alimentary canal of the host. They floated out freely from the body-cavity of the larvæ when these were opened carefully by means of fine needles in Ringer's solution or tap-water.

Further, an additional argument against their entering by way of the mouth is that the mouth-parts of the fly larva are strong, toothed, chitinised structures, working very closely together, which would, in all probability, rupture the worms if they came into contact with them.

(*d*) *Parasitic Female.*

I. *General.*—Having entered the body-cavity of a frit-fly larva as a slender worm, measuring about 0·55 mm. long by 0·015 mm. wide, the female gradually develops into a much larger sausage-shaped worm, which by the time the adult fly emerges from the pupal case may measure 1·6 mm. long by 0·13 mm. wide. As it has already attained the adult condition before entering the larva its growth is accomplished without further ecdyses. The cuticle stretches and the underlying structures of the body-wall become very vacuolate in appearance, at least during the early stages of growth in the host. The ultimate size reached by the worm is partly determined by the space available for growth in the abdomen of the fly, which is quite limited, and, as will be explained in a later section, on the state of development of the host gonad. Moreover, where only one worm is present in a fly it is always larger than when two occur, and each individual where two are present is larger than when three or four are found.

How growth takes place in *Tylenchinema* is not very clear, but from the examination of worms not yet showing much increase in size taken from frit-fly larvæ it seems that the cells composing the body-wall increase gradually in size. In some specimens it has been possible to distinguish a pavement of polygonal cells underlying the cuticle. The nuclei of these cells also swell up, the cells gradually expand and their contents become vacuolate in appearance, a feature which was quite marked in specimens freshly teased-out from frit-fly larvæ. A musculature of some sort must be present, since the full-grown worms are capable of movement when teased-out in Ringer's solution.

Wülker (10), p. 450, says that in *Bradynema strasseni* a musculature is present, though weakly developed, but is entirely absent from the adult *Allantonema mirabile*. In the atter also there is a syncytium, occupying much of the body of the worm, in which an outer layer with large nuclei and an inner one with small nuclei can be distinguished.

The stylet is retained throughout life and can be seen in quite large worms in the fresh condition ; it is difficult to observe, however, in fixed specimens. The remains of the intestinal gland, or its nucleus, can be seen in smaller specimens and a granular region representing the intestine can also be made out, so that it is quite probable that *Tylenchinema* continues to take in food *via* the alimentary canal, at any rate for the greater part of its period of growth if not for the whole of its parasitic existence. In this it differs from *Allantonema* and *Bradynema*, in which the stylet and all trace of the alimentary canal are lost, along with the excretory canal and the nerve ring, during their parasitic stage of life.

The cuticle retains transverse striations which can be distinguished on gravid worms, and, in addition, the anterior region of the worm frequently presents an annulate appearance of the cuticle, due to contraction of the body along the longitudinal axis. The gradual increase in size and the general softening of the tissues accompanying the parasitic mode of life are necessitated by the great enlargement which takes place in the reproductive organs of the worm. Several stages in the growth of the gonad have been worked out and are dealt with in the next section.

II. *Reproductive Organs.*—The earliest discoverable change within the young parasitic female taken from a frit-fly is an increase in the length of the ovarian primordium and in the number of cells composing it. Fig. 29 (Plate 23) shows the appearance of the ovarian group and the oviduct in such a worm, practically identical in size and appearance with a free-living female.

The ovarian cells have increased to six in number and each cell is large with a large nucleus. The cells of the oviduct have multiplied, the group has grown forward and become folded on itself for a short distance. The anterior terminal cell gives rise by a series of divisions to the covering membrane of the ovary. At this stage the spermatozoa are still distributed throughout the length of the uterus.

In the stage of development shown in fig. 30, also from a worm of practically the same size and shape as a free-living female, the ovary has grown very considerably and is composed of a large number of cells, whilst the oviduct is also longer and is still more folded on itself anteriorly. Its posterior end is now showing a distinct flask-shaped enlargement, which is destined to become the definitive receptaculum seminis.

The body soon begins to increase in length and width, and at the same time both head and tail become more or less rounded. Fig. 31 represents the principal features of a specimen 0·683 mm. long, taken from a fly pupa, and shows the various regions of the reproductive organs in a further stage of growth. The stylet and the principal structures of the œsophageal region are still discernible as well as the excretory pore. In the gonad the receptaculum seminis has become fully formed and practically all the spermatozoa are congregated in it, though some are still to be seen within the uterus. The walls of the latter are rather thick and the cells composing them are distinctly visible. The oviduct is still more looped on itself and the ovary occupies the bulk of the central region of the body. Its anterior end has pushed forward into the base of the intestinal gland and displaced it towards the ventral side of the body.

The next stage of growth comes with the enlargement of the cells towards the posterior end of the ovary, whereby separate ova are produced. These pass down the oviduct, through the receptaculum seminis, into the uterus where they undergo segmentation leading to the production of embryos (fig. 32). This process goes on, and more and more ova pass into the uterus, with the result that it gradually enlarges and pushes the receptaculum seminis forwards in the body, till finally the bulk of the body is occupied by the uterus. This stage is shown in fig. 33 (Plate 24) drawn from a worm 1·5 mm. long by 0·12 mm. wide. The receptaculum seminis is now found in an anterior position and the oviduct, still folded on itself, is filled with large unfertilised ova. The ovary extends close up to the head and is reflexed on itself anteriorly. Coiled embryos, closely crowded together, occupy the posterior half of the uterus. The vulva and very short vagina are in most cases still discernible in a subterminal ventral position, and in some cases the anus can be made out by careful focusing.

The specimen drawn does not represent the extreme extent of the forward growth of the uterus. This is so great in the majority of cases that the receptaculum seminis, the folds of the oviduct, and the loops of the ovary, become closely packed together towards the anterior end of the body, and it becomes extremely difficult to differentiate the separate regions.

The structure and development of the larvæ passed into the body-cavity of the host have already been dealt with.

Ethology.

(a) *Parasitism.*

I. *Effect on Host.*—Mention has already been made of certain nematode parasites of insects and a brief account of the effect of these on their respective hosts may be given here, by way of preface to the discussion of the subject in the case of *Tylenchinema* in the frit-fly. Fuchs (3), p. 175, considers that *Tylenchus contortus* occasionally kills its host, *Ips typographus*, whilst in most cases it reduces the egg output of the female to about half the normal. In the case of *Allantonema mirabile* parasitic in *Hylobius abietis*, Wülker (10), p. 464, considers that the action of the worm is negligible, as he frequently found parasitised beetles *in coitu* which had reproductive organs well stocked with ripe sex-cells. He never found any decrease in the size of parasitised beetles as compared with normal ones. zur Strassen (9), p. 7, says that the presence of from 10 to 20 *Bradynema rigidum* has practically no effect on its host, *Aphodius fimetarius*, the females of which remain capable of developing their eggs to maturity.

Howardula benigna studied by Cobb (2) from the cucumber beetles, *Diabrotica vittata* and *D. trivittata*, is said to effect a reduction in size and weight of the host and at the same time to diminish its fecundity. There is no evidence in this case, however, of the parasite causing the suppression of the ovaries, for Cobb speaks of the larvæ, from a few to about fifty, being deposited with each egg. Wülker (10), p. 465, says that *Atractonema gibbosum*, parasitic in the larvæ of *Cecidomyia pini*, may lead to the death of the latter or delay the metamorphosis.

Sphærularia bombi, the females of which occur in queen bumble bees, may have a marked effect on the host and cause sterility, as a result of which parasitised queens are unable to found new colonies. Pantel (7), pp. 163–180, has studied the changes set up in the ovaries in great detail. The worm enters the queen only when the latter is full-grown and her ovaries are already developed with the result that, instead of being able to lay eggs, a gradual degeneration of the oocytes and nutritive cells sets in. The onset of this acute stage occurs when a large number of larvæ of the parasite are present in the body-cavity of the host. The oldest oocytes and nutritive cells at the bases of the ovarioles are the first to become degenerate and from these the process travels upwards to the younger ones.

The writer has been unable to detect any effect on the external characters of the host due to the presence of *Tylenchinema oscinellæ*. Small and large specimens of both male and female flies have been found harbouring the parasite, and the careful comparison of these with normal flies has failed to reveal any alteration on the head characters, in the shape of the pincer-like organs on the terminal abdominal segment of the male, or in the number and distribution of the bristles at the tail end of the female. Internally, however, the parasite exercises a profound effect, in that it prevents the growth of the gonads in both male and female flies which are consequently sterilised.

The reproductive organs of normal frit-flies appear not to have been described previously; it will consequently be necessary to give an account of them in each sex, and also as they are found in parasitised flies. The male is considered first (figs. 34 and 53). There is a pair of pyriform testes, light to dark brown in colour, about 0·4 mm. long by 0·2 mm. in greatest width. From the base of each there is a comparatively short vas deferens, which leads to the anterior end of the somewhat club-shaped common ejaculatory duct. Two accessory glands arise, on the other side of this duct, a little behind the points of attachment of the vasa deferentia. Each is a tubular, thick walled cellular structure, and is usually folded on itself and about the testes. The ejaculatory duct gradually narrows as it proceeds posteriorly to its external opening, and is, as a rule, closely applied to the wall of the rectum.

Each testis is covered with a thin clear membrane which carries the brown coloration. Within this, at the apex, is found a small rounded group of very small cells, the *germarium*, from which the sex cells arise. This is succeeded by a zone of growth made up of a series of layers of cells, each a little broader than the one nearer the apex. Then follows a transitional zone in which the large cells are undergoing division and elongation, and finally there is the zone, making up the bulk of the organ, in which masses of long spermatozoa can be seen lying coiled in the horizontal plane. The germarium and the growing sex cells do not rest on the covering membrane but on layers of flattened epithelial cells, which give the appearance of partitions between the various groups of cells. At the base these epithelial cells become continuous with those forming the walls of the vasa deferentia. The latter, the accessory glands, and the ejaculatory duct, are all covered by a thin clear membrane and the cells composing their rather thick walls rest on this.

Figs. 35 and 36 show the reproductive organs from *parasitised male* flies, drawn to the same scale as fig. 34. There is a great reduction in size, accompanied by complete suppression of the two accessory glands. The testes are very small and in a rudimentary condition. They may be pyriform or rounded in shape, whilst the vasa deferentia may be short, as in fig. 35, or long as in fig. 36. Within each there may be found either a compact group of very small cells, the individual members of which have remained undifferentiated (fig. 35), or the testis may appear almost hollow and empty as in the left one of fig. 34. The enclosing wall is frequently comparatively thick and rather irregular in outline. Transverse sections across such an apparently hollow testis show that the cavity contains a scattered net-work of protoplasm, probably from degenerate cells, with numerous deeply staining nuclei of irregular shape. This is surrounded by a dense layer on which rests the enclosing wall of cells; these are large and irregular in shape. Such a section is shown in fig. 42. Apart from being smaller than normal, the vasa deferentia and ejaculatory duct do not seem to have their individual cells affected by the parasite.

The *normal female* organs in a newly emerged fly (figs. 37, 49 and 51) consist of two pyriform ovaries, from the base of each of which leads an oviduct. The oviducts unite and form a common oviduct, which joins posteriorly with the vagina in a Y- or T-shaped arrangement. These ducts are generally found closely applied to the rectum, and where the latter begins to narrow down into the terminal abdominal segments the vagina is swollen for a short distance. Here arise the two ducts leading to the spermathecæ. They are very long tubes with clear walls, which gradually diminish in diameter and finally become wrapped in many coils round two cellular structures, which are the spermathecæ. It may be put on record that when flies, anæsthetised by chloroform, are dissected in Ringer's solution, these ducts show a most remarkable power of violent spasmodic movement, which is retained for several minutes. They are suddenly pulled backwards and then quickly released, and then after a few seconds the process is repeated.

To return to the structure of the ovaries: they are covered by a clear membrane continuous with that covering the oviducts and vagina. Each is made up of 10 to 12 ovarioles, which are of the " polytrophic " type (*vide* IMMS (6), p. 150). Each ovariole (figs. 39 and 51) is surrounded by a thin transparent membrane, within which is an epithelial layer. At the apex is a group of small cells forming the *germarium*, from which sex cells and nutritive cells become differentiated. A little posterior to this the cells are slightly enlarged, but it is impossible to differentiate them with certainty into epithelial, nutritive and oocytic cells until about half-way down the ovariole. Here we find a group of nutritive cells, which are larger than those of the preceding one and completely obscure the contained oocyte. The last swelling contains a much larger oocyte, visible as a coarse granular mass at its lower end, which is almost enclosed by large nutritive cells with very large nuclei. The whole again is surrounded by a columnar epithelium. The oviducts, the common oviduct, and the vagina have fairly stout walls in which there are circular and longitudinal muscles.

In *parasitised females* the oviducts, common oviduct, vagina and spermathecæ, with their coiled ducts, are unaffected, but the ovaries remain small and undeveloped, as shown in fig. 38, which is drawn to the same scale as the normal organs in fig. 37. They show a good deal of variation in shape and a certain amount in size, and may be just small club-like swellings at the ends of the oviducts, or well-defined rounded or almost pyriform bodies.

Although each ovary is in a rudimentary condition and has a rather thick wall, the outlines of what were destined to become ovarioles can be distinguished within (figs. 40 and 52). Each undeveloped ovariole is surrounded by a thin membrane within which lies a mass of small undifferentiated cells. The individual cells vary in size from those making up the germarium to those a short way down a normal ovariole. Transverse sections across such rudimentary ovaries show that each ovariole is made up of a mass of small cells, the walls of which are closely pressed against one another, whilst the bulk of each cell is occupied with a rounded reticulate nucleus (fig. 41).

It is clear from these observations that *Tylenchinema* exerts an action definitely inhibitory to the growth of the sex cells in both male and female frit-flies; in the male also it prevents the growth of the accessory glands. These are the effects, but the way in which the parasite brings them about can only be conjectured. It may be suggested that it is due to the action of some substance secreted or excreted by the worm into the body-cavity of the fly. The subject is discussed further in the following section.

II. *Effect on Parasite.*—A few cases were observed, in both male and female flies, in which from one to three or four worms were present in the abdominal cavity and yet the reproductive organs of the host were healthy and of normal size. In every such case the worms were small and only partly developed; the ovary had grown forward in the body to a variable extent sometimes reaching almost to the level of the excretory-pore, but in no case was it fully grown. In all cases the definitive receptaculum seminis had been formed and the spermatozoa had congregated in it. There were signs of degeneration within the body of the worm, revealed by the presence of large scattered fat globules and an appearance suggestive of disintegration setting in, in the intestine and in the cells making up the body-wall. When fixed in hot alcohol and stained with Nile blue, the worms did not take the stain well, but appeared a very faint greenish blue in colour. Such a worm is shown in the photomicrograph fig. 44.

In all the cases examined, if more than one worm were present in a fly, whose reproductive organs were normal, then all were in a degenerate undeveloped condition. No example was found in which one worm was healthy and two or three degenerate. Again, no case was found in which one ovary or testis of a fly was normal and the other rudimentary. It is always a case of either the parasite gaining the ascendancy, along with a check to the reproductive organs of the host, or *vice versa*.

In the great majority of cases the worm manages to get the upper hand and grows to sexual maturity within the host, but occasionally the fly, during its final metamorphosis, is able, by some means, to build up its gonads in the normal manner. When this happens the worm fails to grow, remains non-functional and becomes degenerate. There appears, in fact, to be a race between the development of the worm within the pupal fly and the gonads of the latter. Generally the former, but sometimes the latter, wins. These relationships may possibly be explained on the supposition that the worm secretes or excretes something, perhaps from the intestinal gland, which prevents the normal growth of the host's sex-cells. At the same time it is quite likely that the same may be true of the host; if once its reproductive organs become sufficiently developed, then it is able to pour out some substance which definitely inhibits the growth of the worm. Such an " all or none " reaction is not suggestive of a competition for a common source of food between the parasite and the gonads of the host.

(b) *Incidence of Infection.*

I. *Stem Generation of Fly.*—Large numbers of flies of this generation were dissected to determine the degree of infestation with *Tylenchinema*. Some of these were flies bred-out from collected pupæ, but the bulk of them were taken by sweeping through the plot of oats with a muslin net on July 17 and 20. A few were dissected immediately after being anæsthetised by chloroform, but most of them were preserved in 70 per cent. alcohol and dissected later.

Number dissected.	Females.	Males.	Infected.	Females.	Males.	Percentage infected.
966	607	359	142	68	74	14·7

The numbers reveal the fact that although amongst the flies dissected there was a large preponderance of females, yet amongst the infected ones there was a slightly larger number of males than females. The writer cannot advance any adequate explanation of this. It may be that normal males are more active on the wing than parasitised ones and so escaped capture. The data available from flies bred out at Harpenden, and from those of the panicle generation discussed later, show that there are normally about equal numbers of males and females. Consequently the 966 flies, made up of 607 females and 359 males, cannot be regarded as a truly representative or normal collection.

Of the 142 infected flies, 4 females contained degenerate worms and had normal ovaries, which leaves 138 flies, or 14·27 per cent. of the total, in which effective sterilisation had been brought about by *Tylenchinema*.

Flies of the stem generation, collected at Harpenden, were obtained from Dr. A. D. Imms. The results of the dissection of these gave the following numbers :—

Number dissected.	Females.	Males.	Infected.	Females.	Males.	Percentage infected.
476	249	227	27	13	14	5·67

Here the degree of infestation is considerably lower than at Winches Farm, but the number of flies examined is far fewer and it is possible that had more been available for examination a different degree of infestation might have been obtained. The figures show that here the numbers of males and females are practically equal and that the incidence of infection is equally distributed amongst them.

II. *Panicle Generation of Fly.*—As explained on p. 320, flies of this generation were obtained from oat stems bearing panicles placed in a breeding-box. The material was put in the box on August 2. Flies began to emerge on August 8 and continued to appear until September 6. They were collected daily, exclusive of Sundays, and after being anæsthetised, some were dissected, but the bulk were preserved in 70 per cent. alcohol and put aside for later examination. Flies collected on a Monday included those which had come out on Sunday and were preserved together.

Again, owing to pressure of work, all the flies emerging from August 17 to 24 inclusive were preserved together, and for this reason it is only possible to obtain an average daily count for this period. As will be seen from the accompanying table, not all the flies collected during the period of high numbers were dissected, but a sufficiently large number was examined to give a reliable figure for the percentage infection.

Date.	Number of flies.	Number dissected.	Number infected.	Percentage infection.	Average percentages.
August 8, 9 and 10	9	9	0	0	1·85
,, 11 and 12	80	80	2	2·5	
,, 13	85	85	1	1·17	
,, 14	290	290	4	1·36	
,, 15	181	181	6	3·3	
,, 16	165	165	2	1·2	
August 17–24	1648	814	34	4·2	4·2
August 25 and 26	738	309	21	6·8	7·07
,, 27	554	400	29	7·25	
,, 28	351	300	26	8·6	
,, 29	409	300	18	6·0	
,, 30	232	232	15	6·46	
August 31, September 1 and 2	100	100	11	11·0	7·0
September 3	117	117	5	4·27	
,, 4	61	61	5	8·2	
,, 5	22	22	1	4·5	
,, 6	5	5	0	0	

The number of flies emerging is low at first, but rises, with some fluctuations, to a maximum on August 27, and then falls off rather steeply, again with a certain amount of fluctuation, to the cessation of appearance on September 6.

The daily percentage infection also varies, but on the whole is low during the first nine days. It is higher, however, during the following eight days, and rises still higher during the next six days, and remains at about the same height throughout the remainder of the period of emergence. By basing the figures for percentage infection in blocks over periods of days, as indicated in the final column, it is possible to overcome the error involved in determining the percentage infection on comparatively small numbers of flies. The results obtained in this way show that the degree of infestation reached during the period of maximum emergence of flies is maintained to the end. The figures also show that in this generation the degree of infestation is not nearly so high as for the stem generation, being just over 5 per cent. as compared with a little over 14 per cent.

One other point worthy of notice is the distribution of the infestation between male and female flies, as shown in the accompanying figures :—

Number dissected.	Female.	Male.	Number infected.	Female.	Male.	Percentage infection.
3472	1926	1546	180	85	95	5·18

It is revealed that the proportion of the sexes is more nearly equal than in the stem generation, but again there is preponderance of female flies. The infestation with *Tylenchinema* is about equally divided between them, with a slight preponderance amongst the males. There appears to be a significant difference in the incidence of infection as between the sexes, both in this and the stem generation, of flies collected at Winches Farm, the males in each case showing a higher degree of infection than the females.

Geographical Distribution.

Through the kindness of Dr. A. D. Imms, frit-flies of the panicle generation were obtained, which had been bred out from oats sent to Rothamsted Laboratory, Harpenden, from various places in England and Wales.

As a result of the dissection of these it was found that *Tylenchinema oscinellæ* occurs at the following places : Daresbury (Cheshire), Cambridge, Bridgewater (Somerset), near Cardiff (Glamorganshire), Aberystwyth (Cardiganshire), Rugby (Warwickshire), and Sandford (Oxfordshire). To these must be added St. Albans and Harpenden (Hertfordshire). The parasite, therefore, has a fairly wide distribution in England and Wales.

Systematics.

A recent paper by van Zwaluwenburg (11) ably summarises most of the earlier accounts of insects harbouring nematodes, whether as vectors or as final hosts, and a perusal of this shows that there is no previous record of a nematode from the frit-fly. It becomes necessary, therefore, to determine the systematic position of the new parasite.

Sphærularia bombi Dufour, 1837, parasitic in queen bumble bees of various species, and *Atractonema gibbosum* Leuckart, 1887, parasitic in the larvæ of the gall midge, *Cecidomyia pini,* are ruled out of consideration, as the final stage of the parasitic female in each case is highly specialised and changed in appearance ; the vagina and uterus assuming large proportions, whilst the body of the worm remains small.

The characters exhibited by the mature parasitic stages of nematode parasites of insects are not very serviceable for taxonomic purposes, and the writer has therefore made use of the anatomical features shown by the young free-living adults of both sexes. When *Tylenchinema* is compared with young adults of *Allantonema* and *Bradynema* it is found to differ from them in the following points :—

1. *Tylenchinema* male has no buccal stylet, whereas one is present in the male of both *Allantonema* and *Bradynema*.
2. The young female of *Tylenchinema* is much slenderer than that of either *Allantonema* or *Bradynema*, as depicted by WÜLKER (10). When examined side by side, specimens of all three would easily be distinguished from one another.
3. *Tylenchinema* female has a large stylet, without basal swellings, followed by a narrow duct into which opens the duct of the intestinal gland. Similar features have not so far been revealed in *Allantonema* and *Bradynema*.
4. The tail of the young adult female of *Tylenchinema* tapers gradually and there are one or two small terminal processes, whereas in *Bradynema* it is short and rounded and in *Allantonema* it tapers evenly to a sharp point.

If in addition to these points the mature parasitic females of *Allantonema*, *Bradynema* and *Tylenchinema* are compared, the new parasite would be excluded from the two earlier genera, on the following grounds : (1) *Allantonema* is a large rounded motionless organism, *Bradynema* is large and sausage-shaped with bluntly rounded head and tail ; (2) in *Allantonema* and *Bradynema* the stylet and anus are completely lost, whereas in *Tylenchinema* they are retained.

Howardula benigna COBB, 1921, parasitic in species of the cucumber-beetle, *Diabrotica*, has not been described in sufficient detail to enable one to make a close comparison with *Tylenchinema*. COBB says that the male is unknown and that the female is syngonic, *i.e.*, produces both sperms and ova. The life-history is, however, similar in essentials to that of *Allantonema* and *Bradynema*. In view also of WÜLKER's researches on these two genera and the results presented in this paper, it seems highly probable that males will be found to occur in *Howardula*, especially as COBB says the young forms which enter the larvæ of the host are " already spermatised individuals."

The genus *Tylenchus* remains for consideration. FUCHS (3 and 4) made the two species *T. contortus* and *T. dispar*, with two varieties of the former and five of the latter, for worms found in the body-cavity of the bark-boring beetles, *Ips typographus* and *Cryphalus picœ*. WÜLKER (10) made the species *T. hylastis* for a worm from the beetle *Hylastes ateri*. In general appearance, and in the great growth of the female gonad, these all resemble *Tylenchinema*. In both male and female of all three species, however, a stylet is present, which is more or less knobbed posteriorly and tapers to a fine point anteriorly. This feature alone suffices to differentiate them from *Tylenchinema*.

A few words are necessary on the genus *Tylenchus* and its relation to these beetle parasites and to *Tylenchinema*. It is a well-known and widely distributed genus, containing many free-living and plant-parasitic species. It has well defined characters which reveal its kinship to and difference from two other well-known genera, *Aphelenchus* and *Heterodera*, both of which contain plant-parasitic species [*vide* GOODEY (5)]. In *Tylenchus* the stylet is made up of two parts, usually of about equal length ; an anterior conical portion attached to a posterior cylindrical portion, the base of which always carries three thickenings.

Another important generic character is that the œsophagus always shows a median, rounded, or oval muscular bulb, containing three central cuticular thickenings, whilst its posterior region is made up of a group of three œsophageal glands. The openings of these glands are always constant and afford characters of generic value. The males are distinguished from those of *Aphelenchus* and *Heterodera* by the presence, on either side of the tail, of a winged expansion of the cuticle, and also by the shape of the spicules. In possessing such caudal alæ the male of *Tylenchinema* closely resembles the males of many species of *Tylenchus*, and if the male alone had been found it would doubtless have been placed in the genus *Tylenchus* as an aberrant species. The character of the stylet in the female, and the shape and structure of the œsophagus, however, in the larvæ and female of the new parasite, exclude it from *Tylenchus*. At no stage in its development does it possess a muscular bulb, and true œsophageal glands appear to be absent.

As a result of these differences the writer has felt justified in establishing a new genus for the worm from the frit-fly. Kinship to *Tylenchus* is shown by the male having caudal alæ, and for this reason the name given has been compounded of *Tylenchi* and *nema*. The latter brings it into line with *Allantonema* and *Bradynema*, to which it is closely akin structurally and in mode of development.

DIAGNOSIS.

Tylenchinema n. gen.

Free-living forms, small slender worms, about 0·5 mm. long. Male without mouth or stylet and with degenerate œsophagus ; tail tapering behind anus and bearing caudal alæ ; spicules paired, gubernaculum simple and small. Testis single and anterior. Female with well-developed stylet, without basal thickenings, mainly cylindrical and having a short conical anterior region ; œsophagus without muscular bulb but with a narrow lumen in anterior region, into which opens the duct of the large intestinal gland ; latter extending from median part of intestine forwards into the œsophageal region. Vulva close to anus ; vagina very short, leading into tubular uterus serving temporarily as receptaculum seminis ; oviduct and ovarian cells few in number. Fertilised female enters larva of host fly.

Parasitic stage a sausage-shaped form usually more or less coiled, having greatly developed ovary ; spermatozoa becoming localised in a special swelling at end of oviduct, the definitive receptaculum seminis; uterus becoming of great size and occupying bulk of body. Viviparous.

Genotype—*Tylenchinema oscinellæ.*

Parasitic in body-cavity of the Frit-fly, *Oscinella frit* L., on oats. Free-living forms in débris of stem or panicle of oats in vicinity of frit-fly larvæ.

Summary.

1. Ensheathed larvæ and young adult males and females of a small Tylenchoid nematode were found in oat stems attacked by frit-fly. They were present only in the débris surrounding the larvæ of the fly.

2. The male worm has no mouth or buccal stylet and its œsophagus is degenerate. Its tail is Tylenchoid and has lateral caudal alæ. A pair of spicules and a gubernaculum are present.

The female has a large buccal stylet connected posteriorly with the narrow lumen of the œsophagus, into which opens the duct of a large intestinal gland. The reproductive organs consist of a small ovarian primordium, connected behind with a tubular uterus, which serves temporarily as a receptaculum seminis.

3. The males die after impregnating the females with spermatozoa, and these females then enter frit-fly larvæ, probably through the skin. They remain within the host through its metamorphoses, becoming parasitic in the abdominal cavity of the male and female flies which finally emerge.

4. The female grows within the host to a comparatively large sausage-shaped organism. Its reproductive organs occupy most of its body and it becomes viviparous.

5. The larvæ passed into the body-cavity of the fly are recognisable quite early as potential males and females, owing to the different appearance of the respective genital primordia, and to the fact that the female possesses a special cell on the dorsal side of the intestine destined to become the intestinal gland. Growth takes place in these larvæ, and they make their way after a time into the intestine of the fly, whence they pass to the exterior *via* the anus.

6. The development of the gonad has been studied in detail in the growing male and female larvæ, as well as the subsequent growth of the female's reproductive organs after she has become parasitic in the fly.

7. The presence of the parasite within the host generally results in the sterilisation of the latter, by preventing the growth of the gonads in either sex. A few cases have been observed in which the reproductive organs of the host are normal, and the parasite in such cases is degenerate.

8. Flies of the stem generation of frit-fly have been found parasitised to the extent of 14 per cent. and of the panicle generation to about 5 per cent.

9. Flies have been examined from various localities in England and Wales, with the result that the parasite has been found to have a wide distribution.

10. The systematic position of the worm and its affinities to other nematode parasites of insects are discussed. A new genus is erected for its reception and the name *Tylenchinema oscinellæ* has been given to it.

It is a pleasure to the writer to acknowledge his indebtedness to Dr. A. D. IMMS, F.R.S., for his kindness in identifying flies from Winches Farm, and for supplying frit-flies from Harpenden and various other places. Thanks must be also given to Prof. R. T. LEIPER, F.R.S., for the keen interest he has shown throughout the work.

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Explanation of Plates.

Abbreviations.

a., anus.
a.g., accessory gland.
a.t.c., anterior terminal cell.
b.w., body-wall.
c.a., caudal ala.
d.o., degenerate œsophagus.
e., embryos.
e.d., ejaculatory duct.
e.p., excretory pore.
g., gubernaculum.
g.p., genital primordium.
int., intestine.
i.g., intestinal gland.
n.b.w., nuclei of body-wall.
n.i.g., nucleus of intestinal gland.
n.or., normal ovariole.
n.r., nerve-ring.
o., ovary.
o.c., ovarian cells.
oo., oocyte.
œs., œsophagus.
ov., oviduct.
p.i.g., primordium of intestinal gland.
p.t.c., posterior terminal cell or cells.
r.c., rectal cells.
r.o., rudimentary ovary.
r.or., rudimentary ovariole.
r.s., receptaculum seminis.
r.ts., rudimentary testes.
s., spermatozoa.
sp., spicules.
sp. m.c., spicule mother-cells.
st., stylet.
sth., spermathecæ.
sth.d., spermathecal duct.
ts., testis.
ut., uterus.
v., vulva.
v.d., vas deferens.
vg., vagina.

Plate 22.

Figs. 1–10.—Illustrate various stages in the growth of the gonad in male larvæ from the body-cavity of the host.

Fig. 1.—Outline of very young larva, showing position of genital primordium. Scale D.

Fig. 2.—Genital primordium of above highly magnified. Scale E.

Fig. 3.—Outline of slightly older larva. Scale D.

Fig. 4.—Genital primordium of same highly magnified. Scale E.

Fig. 5.—Outline of still older larva. Scale C.

Fig. 6.—Genital primordium of same ; the posterior terminal cell has now multiplied and given rise to the group of cells destined to become the vas deferens. Scale E.

Fig. 7.—Outline of an older larva. Scale A.

Fig. 8.—Gonad of same ; the spicule mother-cells are now discernible. Scale E.

Fig. 9.—Outline of a larva in the stage ready to leave body-cavity of host. Scale A.

Fig. 10.—Gonad of same ; spermatozoa now found in testis and vas deferens almost reaching to spicule mother-cells. Scale E.

Fig. 11.—Anterior end of free-living male larva showing two cast cuticles. Scale E.

Fig. 12.—Posterior end of same, showing spicules already formed ; the larva extends to the end of the second cuticle. Ventral view. Scale E.

Fig. 13.—Entire male in lateral view to show general proportions and principal structures Scale D.

Fig. 14.—Œsophageal region of male, highly magnified, showing absence of stylet and degenerate character of œsophagus. Lateral view. Scale E.

Fig. 15.—Tail of male, highly magnified, showing one spicule, gubernaculum, caudal ala, vas deferens and end of intestine. Lateral view. Scale E.

Fig. 16.—Outline of very young female larva, showing location of genital primordium and intestinal gland-cell. Scale D.

Fig. 17.—Genital primordium and earliest stage of intestinal gland-cell, highly magnified. Lateral view. Scale E.

Fig. 18.—Slightly older female larva, under low magnification showing genital primordium more posteriorly situated. Scale A.

Fig. 19.—Genital primordium of above highly magnified. Note multiplication of the posterior terminal cells. Scale E.

Fig. 20.—Female larva from body-cavity of fly undergoing an ecdysis. Lateral view. Scale A.

Fig. 21.—Anterior end of same under high magnification. Scale E.

Fig. 22.—Posterior end of an older larva, ready to leave host, showing position and degree of growth of reproductive system. The uterus is joining up with the centre of the plate of ectodermal cells to form vagina and vulva. Rectal cells well marked. Scale E.

Fig. 23.—Reproductive system from a free-living ensheathed larva, practically in its final condition. Scale E.

Fig. 24.—Free-living female larva, showing two cast cuticles. Scale B.

Fig. 25.—Anterior region of above, highly magnified, showing stylet in adult worm within second cuticle. Scale E.

Fig. 26.—Posterior region of young spermatised adult female; the relations of the ovarian and oviduct cells to the uterus filled with spermatozoa are shown. Lateral view. Scale E.

Plate 23.

Fig. 27.—Young adult female in lateral view, showing the general appearance of the worm and the principal anatomical features.

Fig. 28.—Anterior region of young adult female, more highly magnified, in lateral view.

Fig. 29.—Early stage in growth of ovary and oviduct in a female from a Frit-fly larva.

Fig. 30.—A more advanced stage of growth of ovary and oviduct from another female from a Frit-fly larva. Note the beginnings of the receptaculum seminis as a swelling at the posterior end of the oviduct.

Fig. 31.—Parasitic female from a Frit-fly pupa, showing more advanced growth of reproductive organs. The ovary by its forward growth has pushed the intestinal gland into a ventral position.

Fig. 32.—Another parasitic female with the reproductive system still further developed. The uterus now contains developing eggs and a few larvæ. The receptaculum seminis is being pushed anteriorly by the filling up of the uterus.

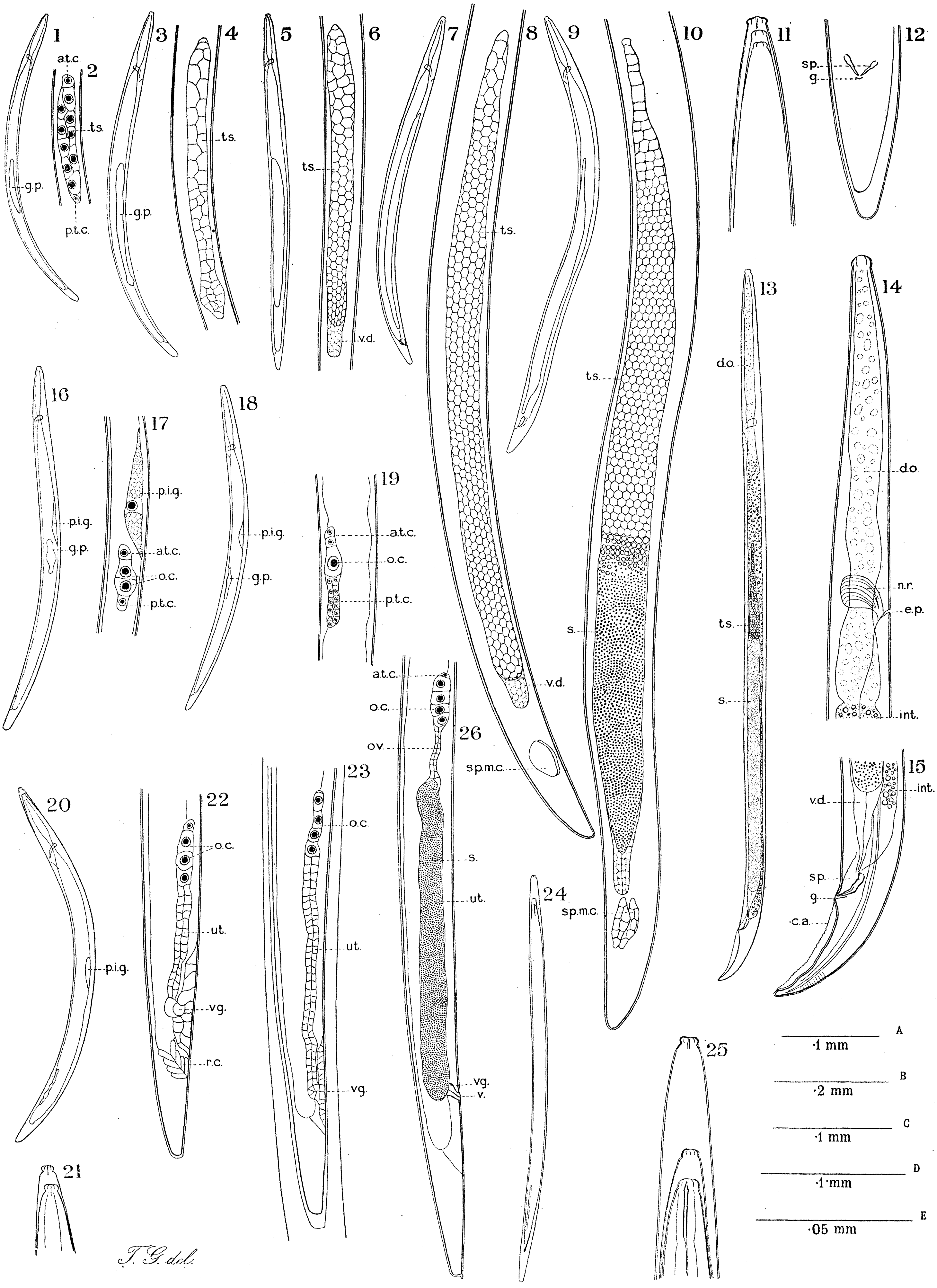
Plate 24.

Fig. 33.—A gravid parasitic female from abdominal cavity of a Frit-fly. The uterus has become filled with developing eggs and larvæ and has carried the receptaculum seminis into the fore part of the worm.

Fig. 34.—Normal male reproductive organs of Frit-fly, showing the testes, vasa deferentia, accessory glands and ejaculatory duct.

Figs. 35 and 36.—Rudimentary male reproductive organs from Frit-flies parasitised by *Tylenchinema*, drawn to same scale as fig. 34. Note the absence of accessory glands and the dwarfed testes.

Fig. 37.—Normal female reproductive organs of Frit-fly, showing ovaries, oviducts, common oviduct and vagina. Arising from latter the long coiled ducts of the spermathecæ.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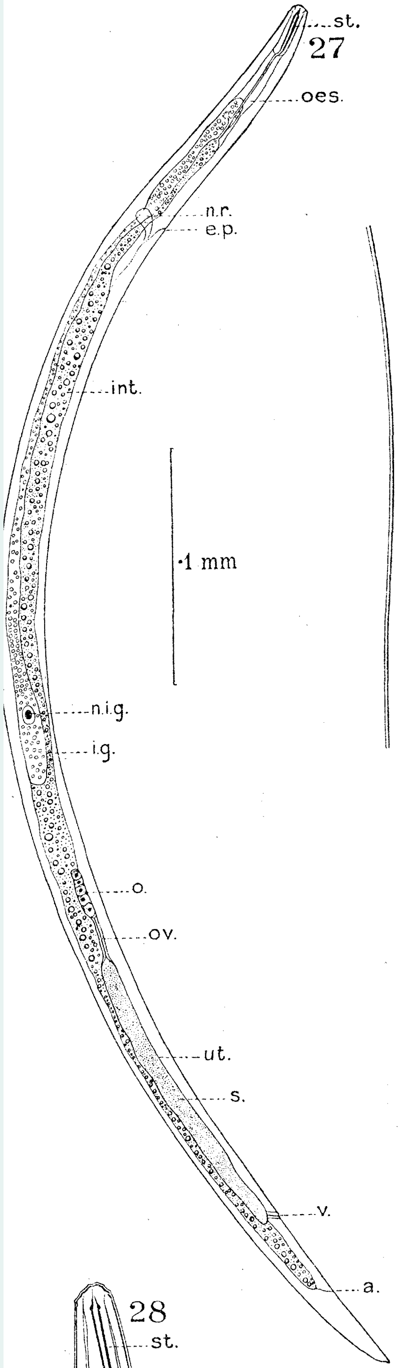
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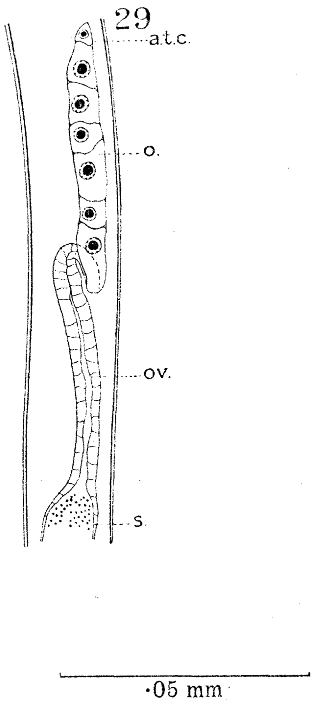
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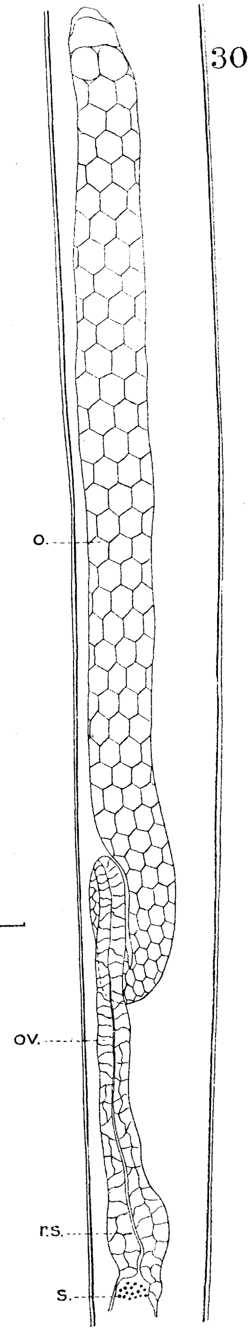
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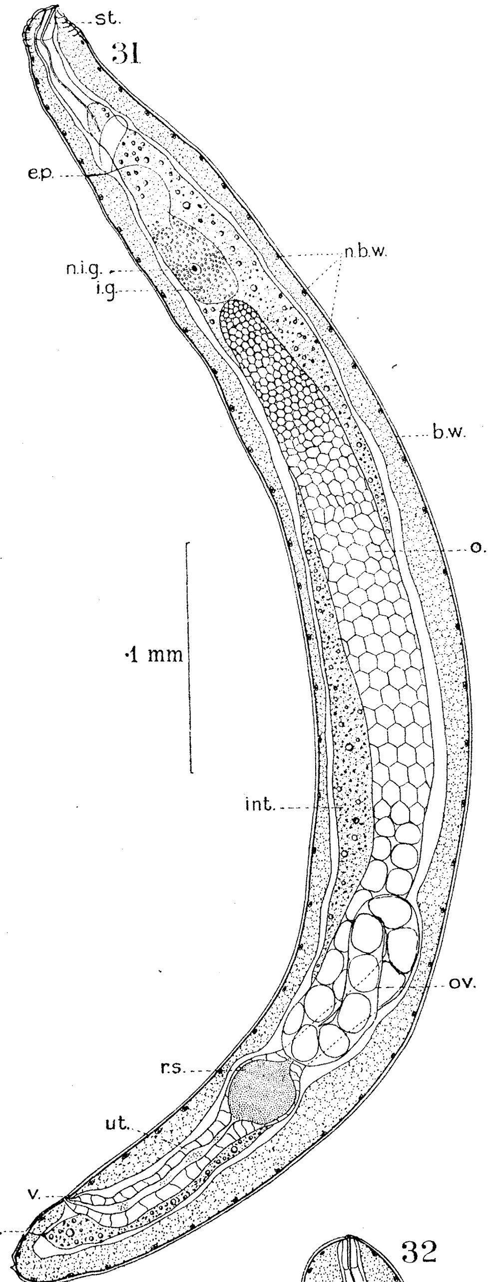
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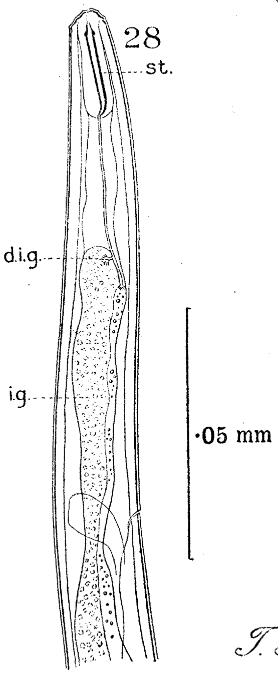
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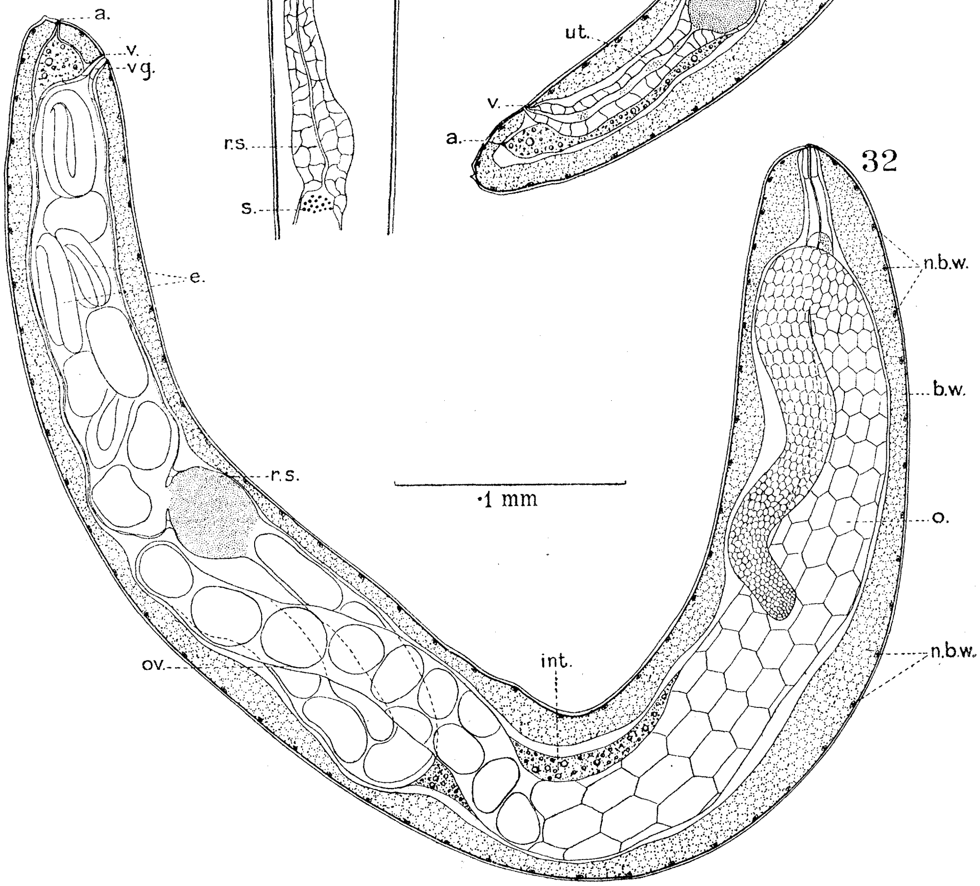
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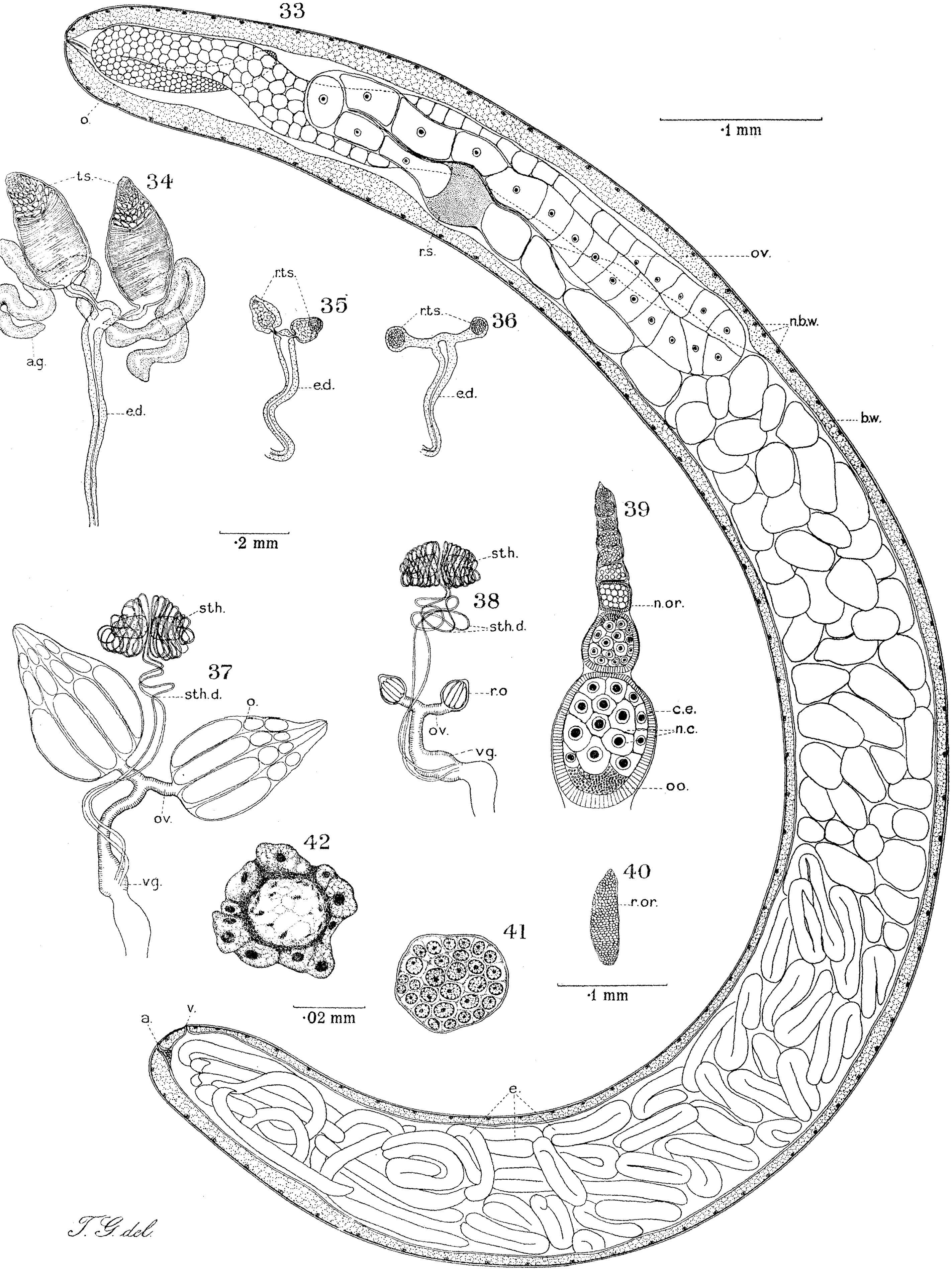

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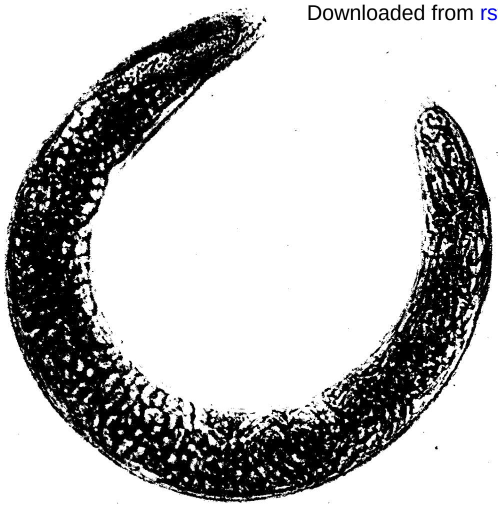

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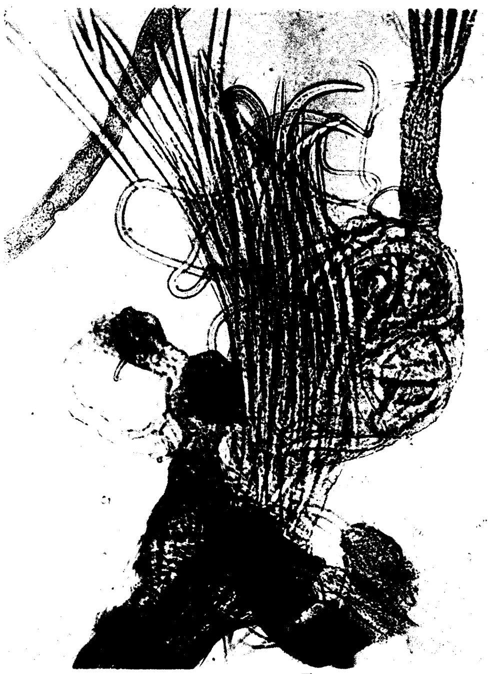

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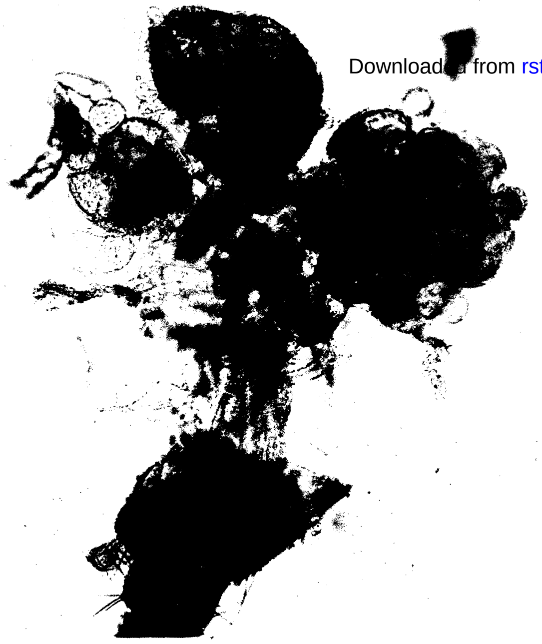

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FIG. 38.—Rudimentary female reproductive organs from a Frit-fly parasitised by *Tylenchinema*, drawn to same scale as fig. 37. Note that the outlines of the ovarioles can be seen and that the spermathecæ and their ducts are unaffected by the parasite.

FIG. 39.—A single polytrophic ovariole from a normal ovary of Frit-fly, to show its structure.

FIG. 40.—An ovariole from a parasitised Frit-fly, showing mass of undifferentiated cells within. Same scale as fig. 39.

FIG. 41.—Transverse section across a single ovariole from a parasitised Frit-fly. The cells are all small and each is practically filled by its reticulate nucleus. Drawn under oil-immersion.

FIG. 42.—Transverse section of rudimentary testis from parasitised male Frit-fly. The wall is made up of large irregular cells the inner walls of which are pigmented. A faint reticulum of granular material containing scattered nuclear masses occupies the centre of the organ. Same magnification as fig. 41.

PLATE 25.

FIG. 43.—A mature parasitic female of *Tylenchinema oscinellæ* from abdominal-cavity of Frit-fly. × 100.

FIG. 44.—A degenerating, incompletely developed example of *Tylenchinema oscinellæ* from abdominal-cavity of a Frit-fly with normal gonads. × 100.

FIG. 45.—Rectum of Frit-fly containing larvæ of *Tylenchinema oscinellæ*. The larvæ which are sticking out at the side were all packed tightly within the rectum, the wall of which ruptured when the coverslip was applied in mounting the preparation. × 92.

FIG. 46.—Colon and stomach, showing larvæ within ; from same preparation as fig. 45. × 92.

FIG. 47.—Tail end of male Frit-fly, showing a larva just being passed out. × 162.

FIG. 48.—Tail end of female Frit-fly, showing two larvæ being passed out and several within terminal abdominal segments. × 162.

PLATE 26.

FIG. 49.—Normal ovaries of a female Frit-fly ; the ovarioles have been somewhat damaged in the process of dissection. × 75.

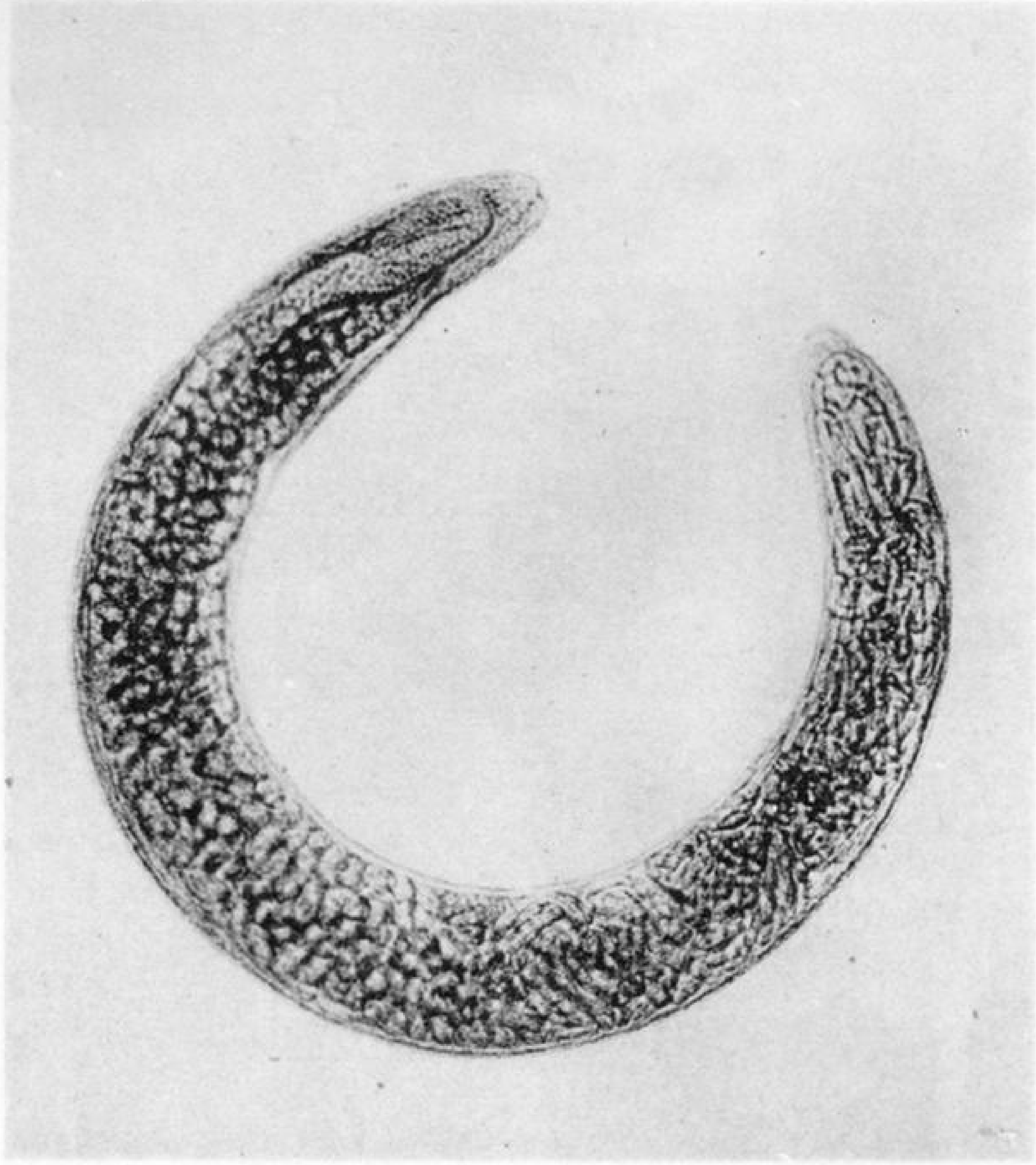
FIG. 50.—Rudimentary ovaries from a parasitised Frit-fly. × 75.

FIG. 51.—A single normal ovary, showing the arrangement of the ovarioles. × 190.

FIG. 52.—Rudimentary ovaries from a parasitised Frit-fly, showing the outlines of the undeveloped ovarioles. × 320.

FIG. 53.—Normal male reproductive organs from a healthy Frit-fly, showing testes, accessory glands and ejaculatory duct. × 92.

FIG. 54.—Rudimentary male reproductive organs from a parasitised Frit-fly, showing dwarfed testes and absence of accessory glands. × 92.



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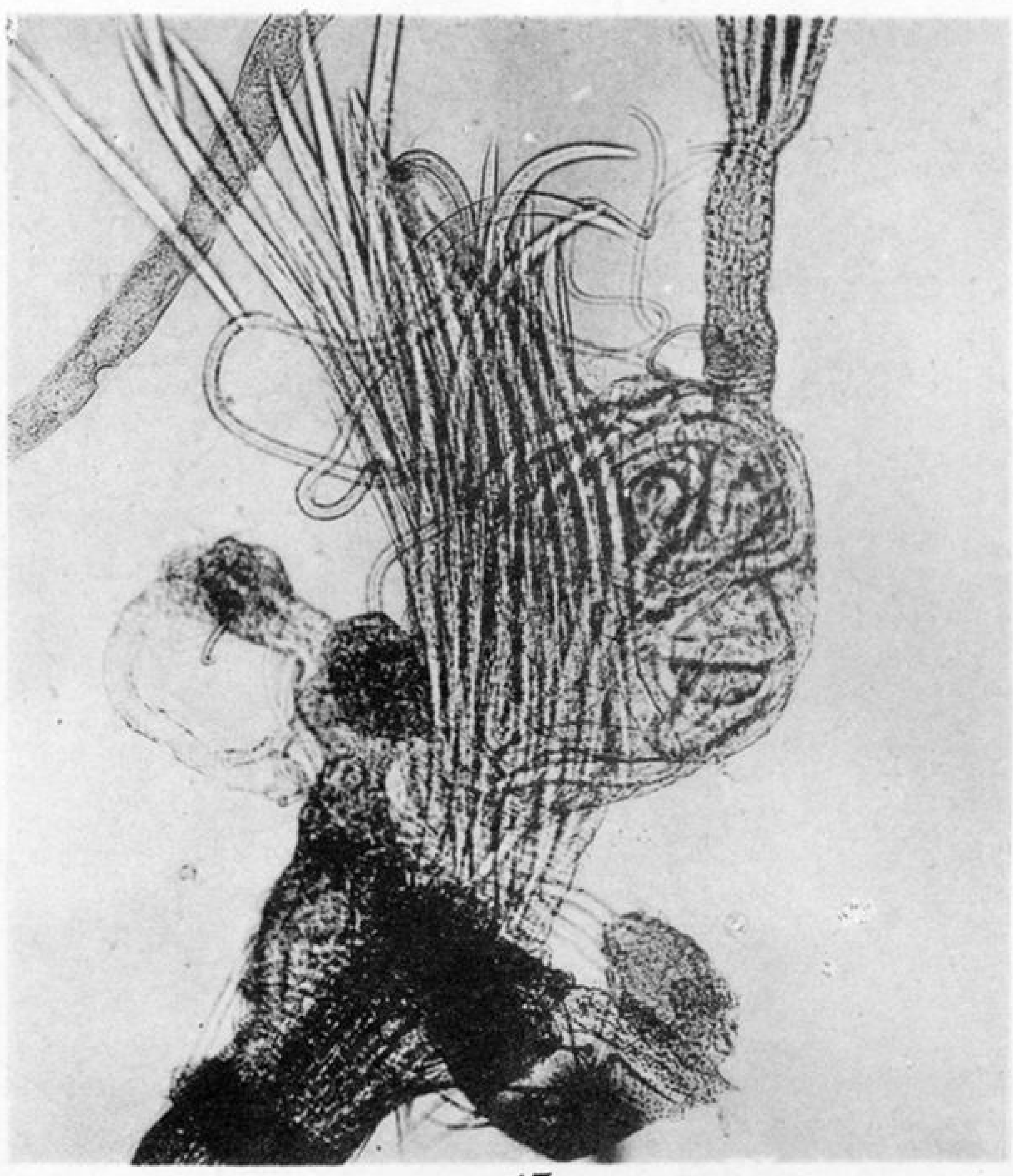

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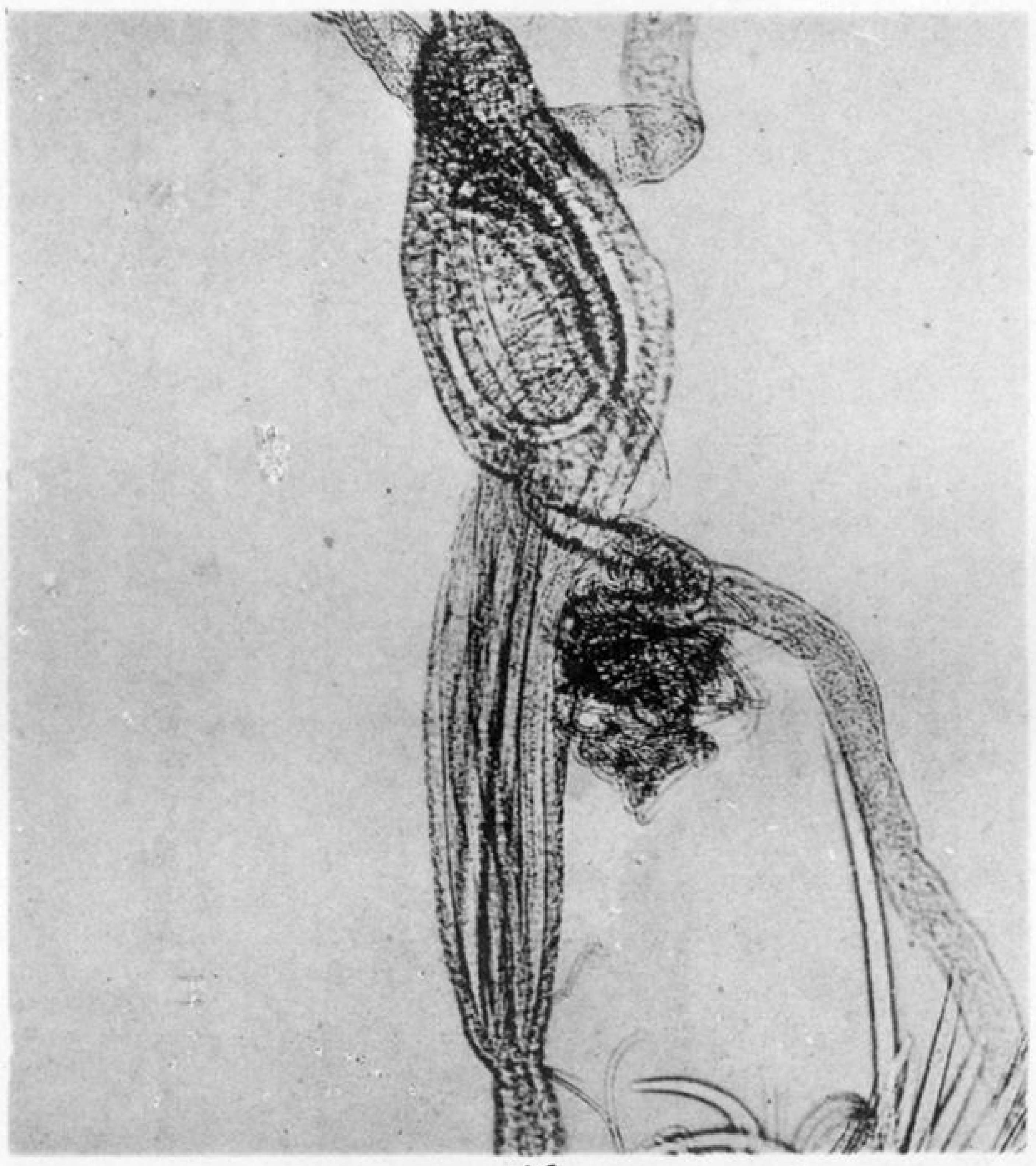

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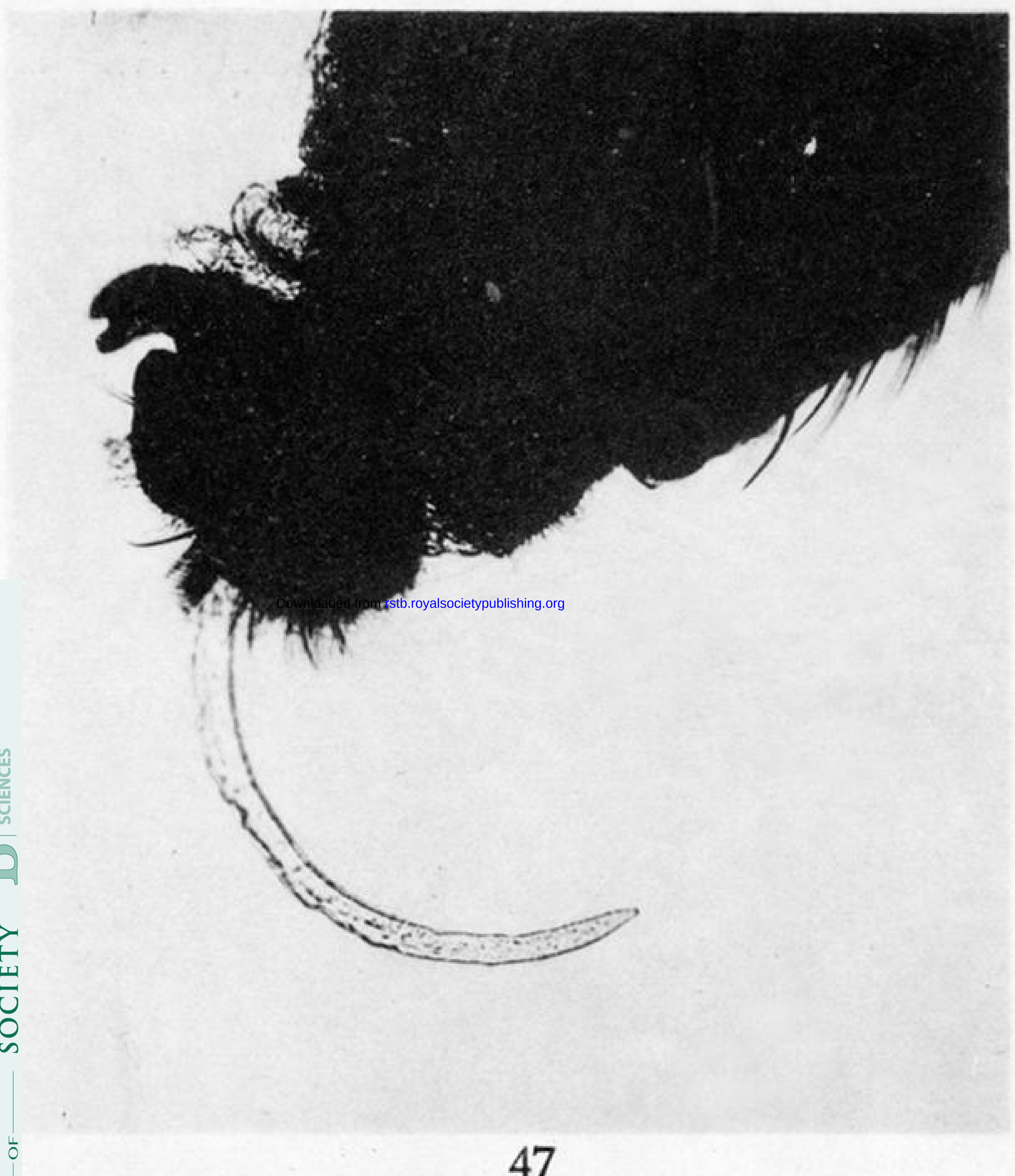

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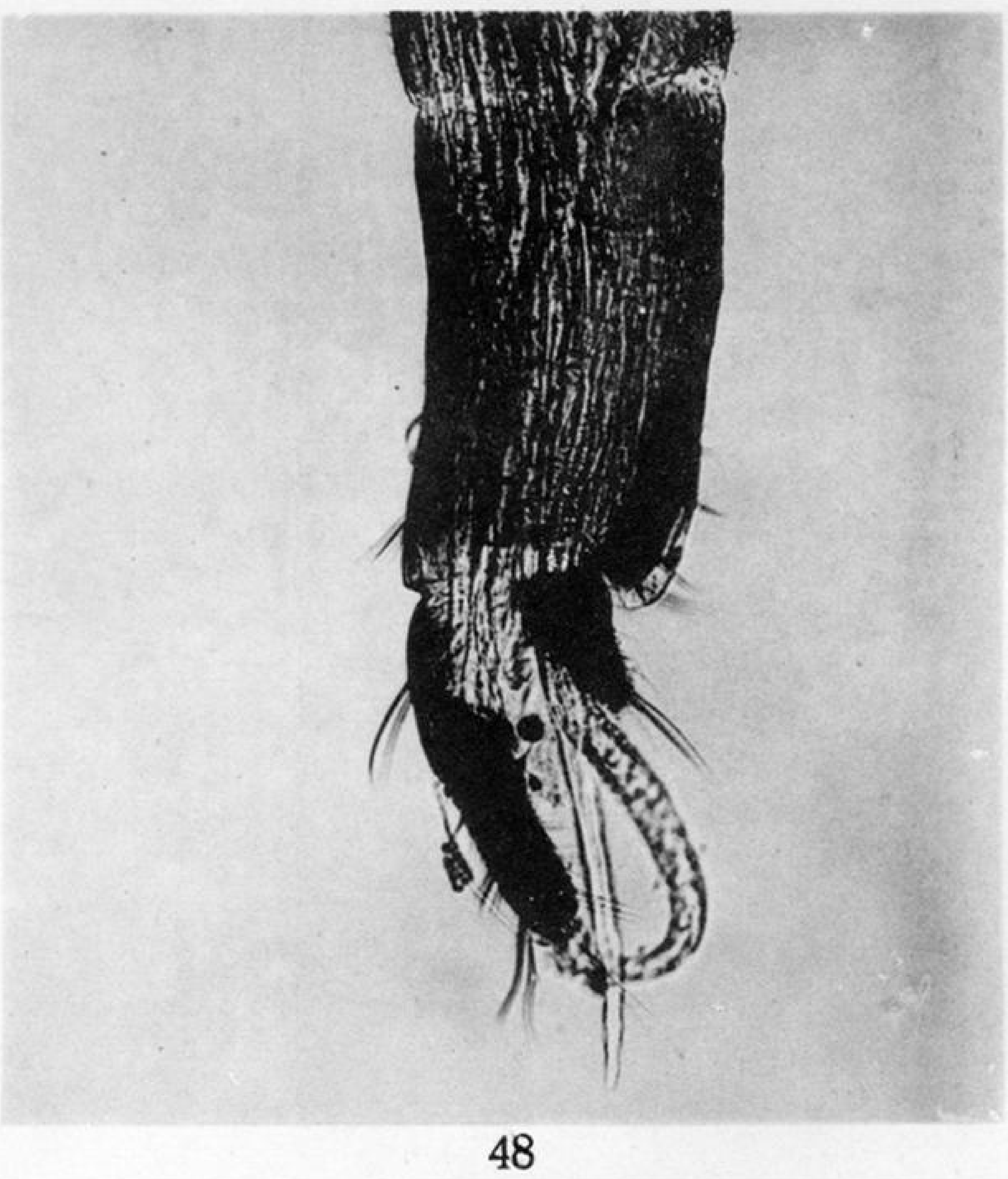

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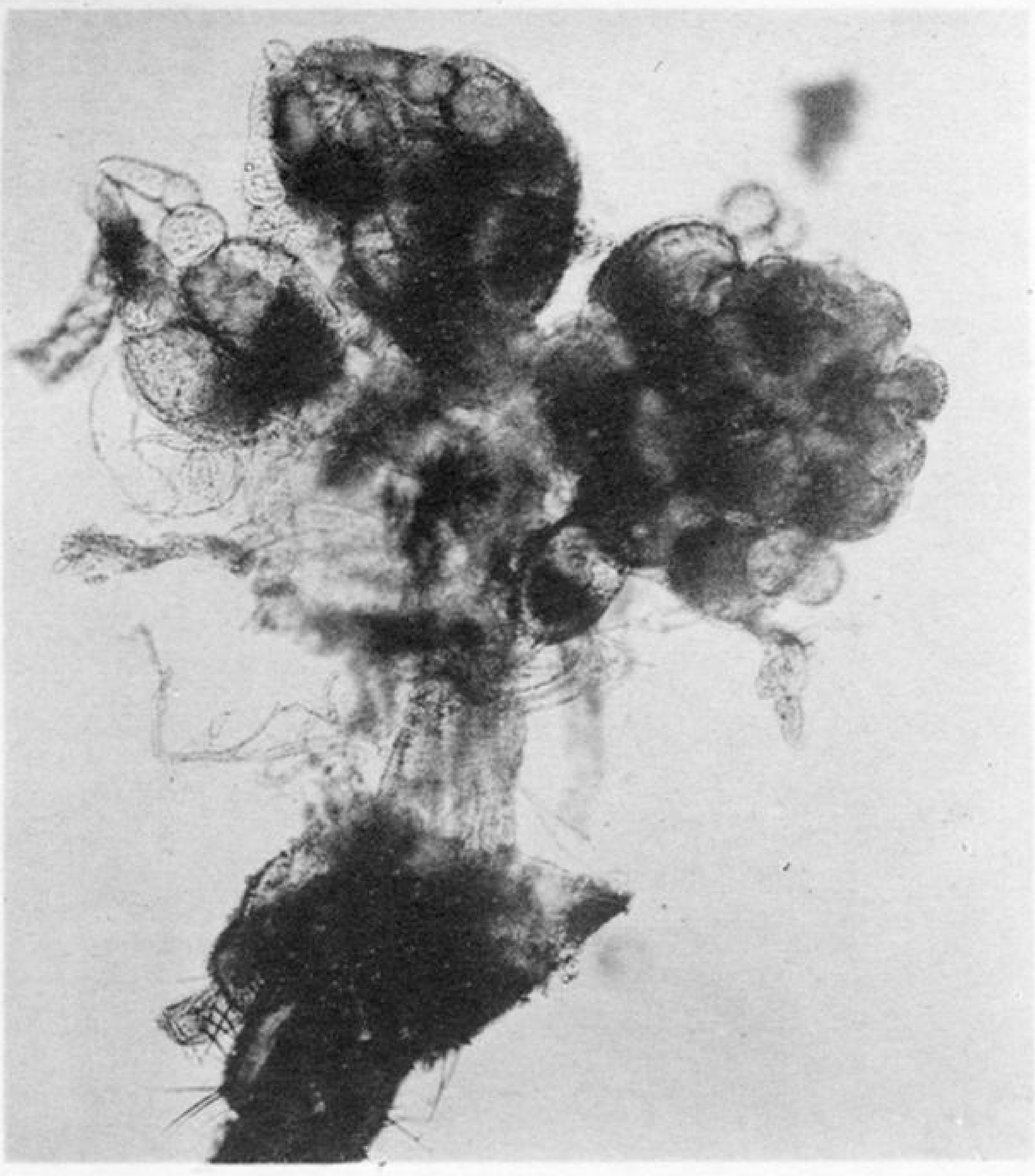

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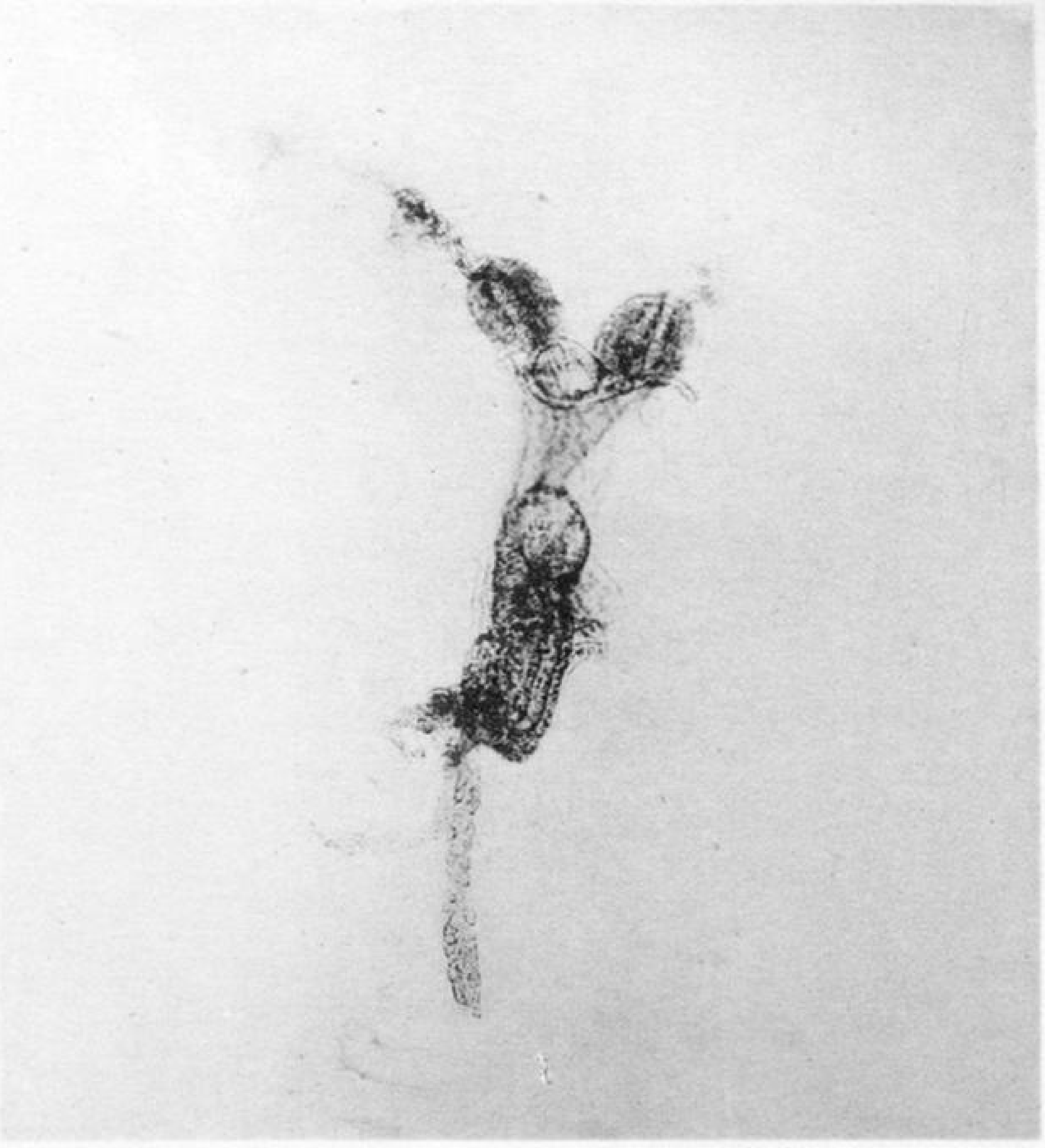

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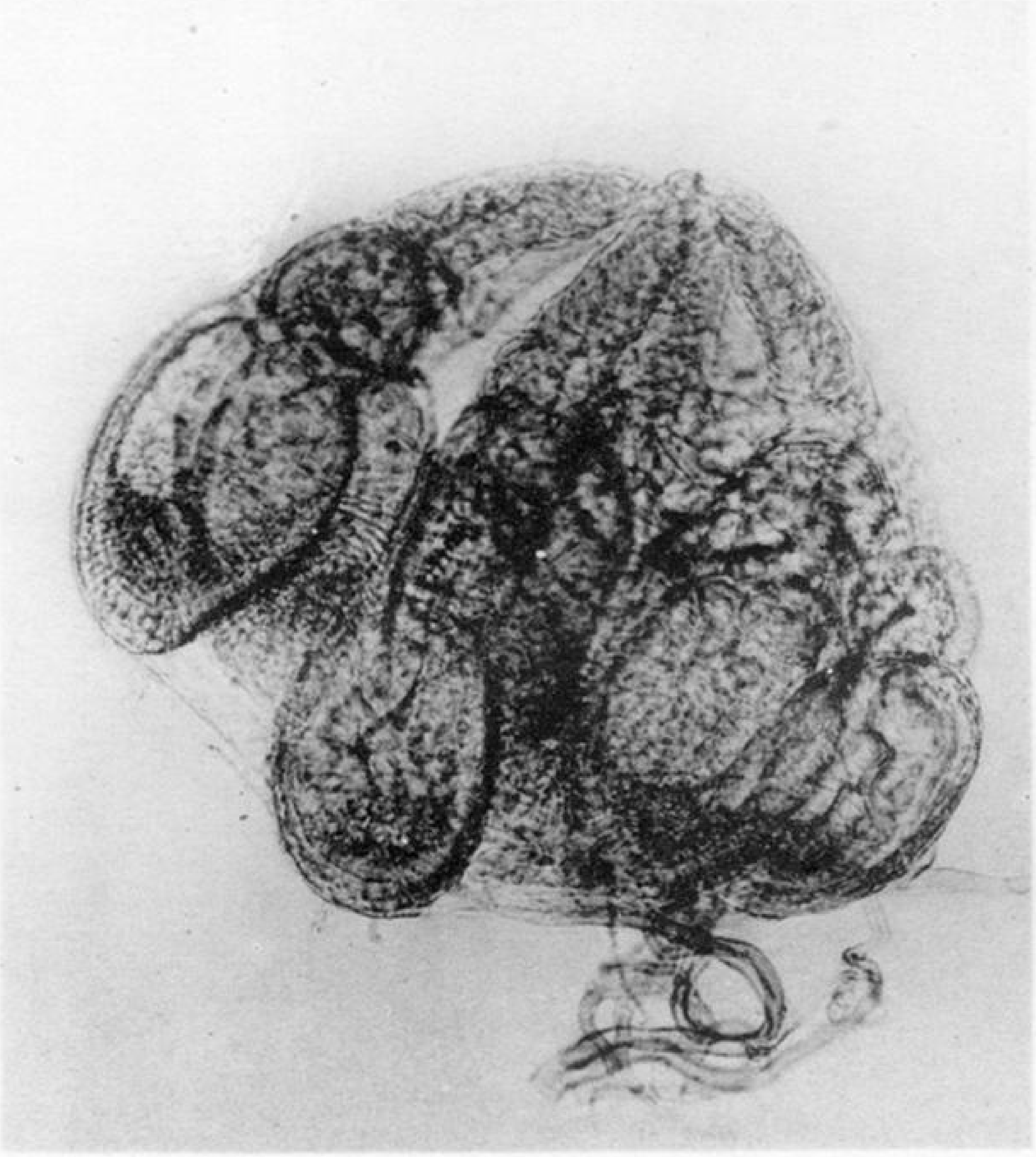

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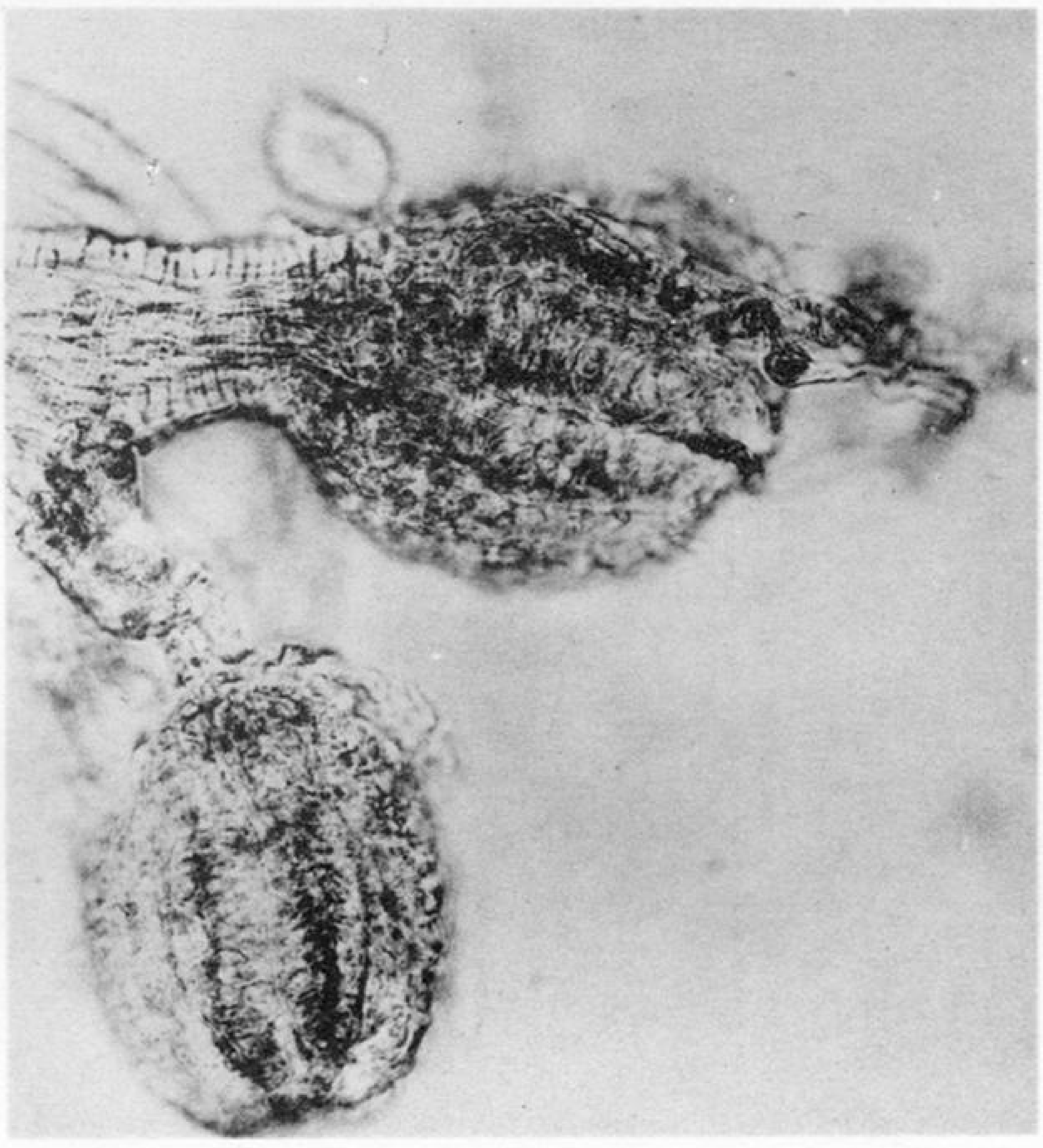

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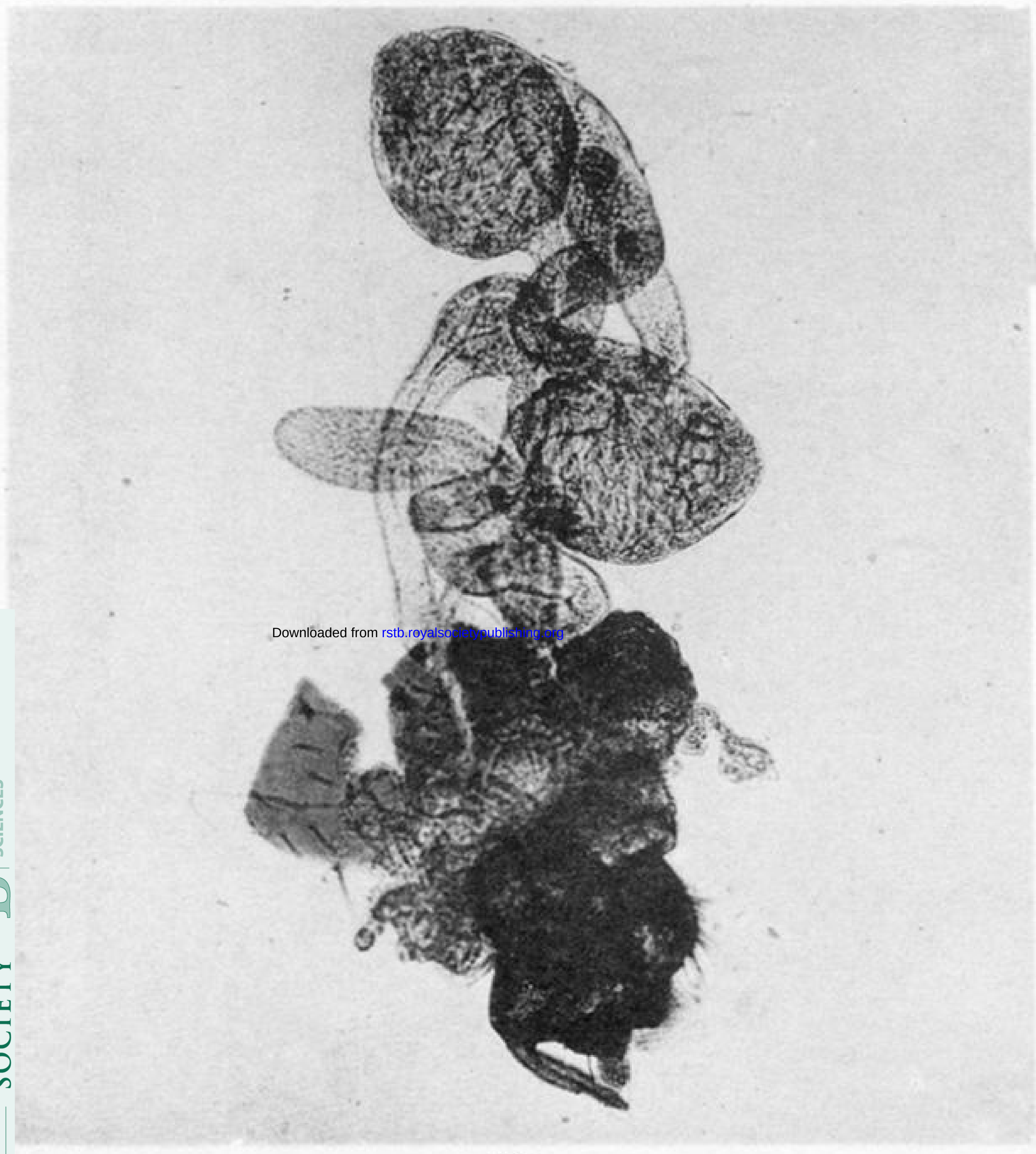

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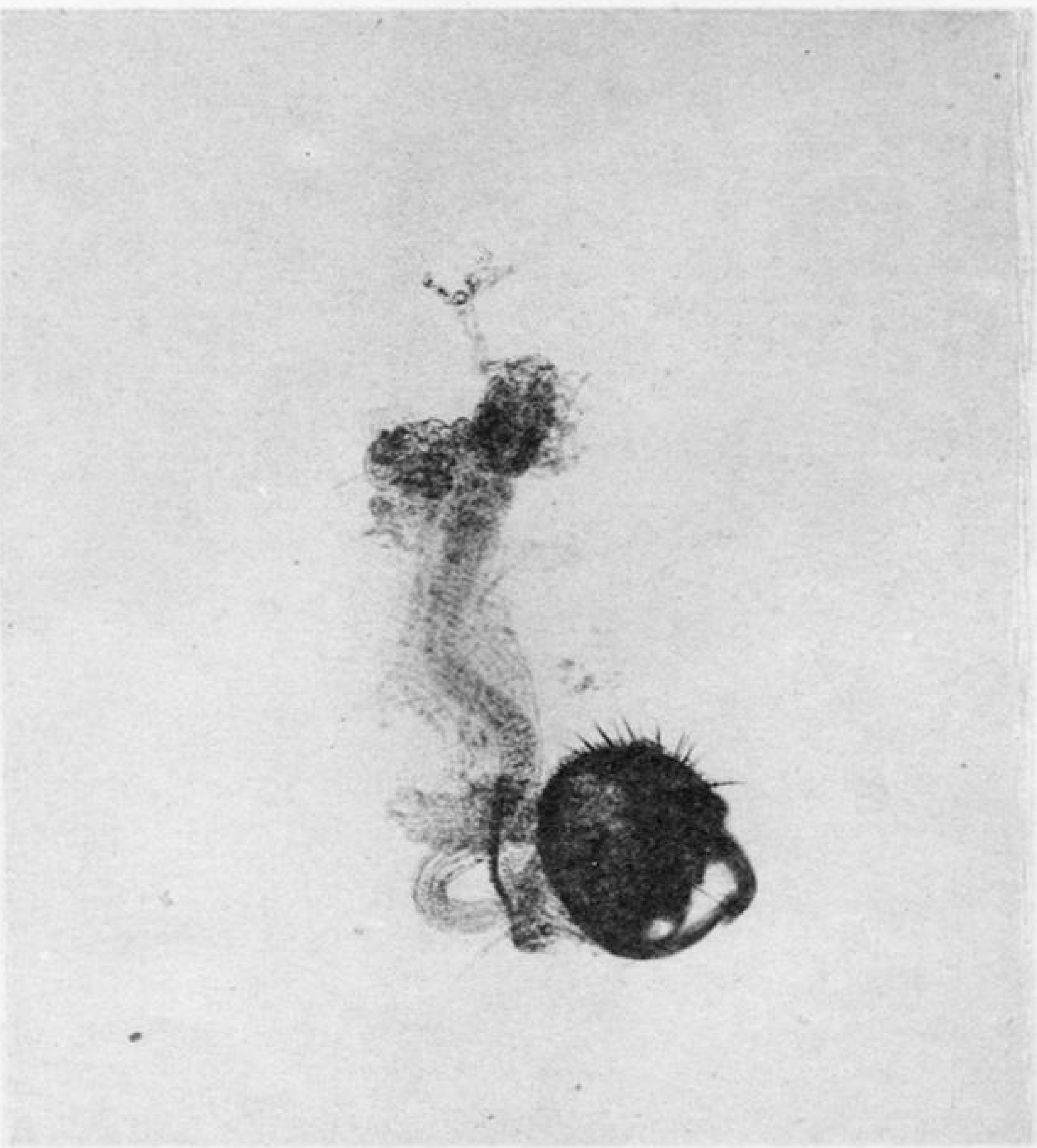

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