VOLUME VIII SEPTEMBER, 1915 No. 2

THE LIFE HISTORY OF *NEMATODIRUS FILICOLLIS* RUD., A NEMATODE PARASITE OF THE SHEEP'S INTESTINE.

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(With Plates VIII and IX and 5 Text-figures.)

CONTENTS

Introduction.

SHEEP are probably more frequently affected by diseases due to parasitic worms than any other of our domestic animals, and of Nematodes alone at least twenty-eight species have been recorded from the alimentary tract, chiefly from the fourth stomach (abomasum) and the intestines.

These round-worms have in recent years formed the subject of careful study by a number of investigators, the latter, however, have confined themselves largely to the systematic and anatomical features of the adult individuals, the larval forms and life histories generally receiving only brief and occasional notice. Thus of the Strongylidae inhabiting the sheep's alimentary canal and to which family the majority of the most injurious Nematodes belong, there is only a single species, *Haemonchus contortus*, whose life history has received adequate attention during the last few years. Our knowledge of the development of the other forms is very incomplete and in most cases confined to scanty descriptions of isolated larval stages.

The presence of *Haemonchus contortus*, the well-known "Twisted Wireworm," in the fourth stomach of sheep has been proved to be responsible for very serious losses among flocks, both in this country and abroad; opinions vary, however, as to the amount of damage caused by the other common Strongylid round-worms of the alimentary tract, although most agree that when occurring in large numbers the majority are capable of inflicting very serious, if not fatal injuries, whilst lighter infections may lead to weakness and loss of condition, especially among lambs and yearlings.

Vermifuges and other medicinal remedies have not proved entirely satisfactory, and of late years the efforts of agriculturists have been directed towards preventing or reducing the infection by these parasites. Effective prophylactic measures against Nematodes cannot however be devised until a thorough knowledge of their life histories is obtained and this should include the biology as well as the anatomy of the various larval forms.

It was with the intention of filling up one of the numerous gaps in our knowledge of sheep Nematodes that the observations recorded here were undertaken. *Nematodirus filicollis* was selected for study for a number of reasons: (*a*) in spite of the few records of its occurrence it proved to be a very common species in different parts of England, (*b*) our knowledge of its life history is confined to short and incomplete descriptions of the newly-hatched larva, and (*c*) the ease with which material could be obtained and the large size of the eggs in this species make it a very convenient form for study.

My observations on the parasite were commenced in August, 1913, at the South-Eastern Agricultural College at Wye in Kent[1], the greater

[1] I wish to take this opportunity of expressing my indebtedness to Mr H. E. Hornby of this institution for placing a quantity of *Nematodirus* material at my disposal;

part of the work was however carried out in the new Agricultural Zoology Laboratory in the University of Birmingham. *Nematodirus filicollis* has generally been considered to be a somewhat uncommon parasite in Europe, but both in Kent and in the Birmingham district this form proved extremely abundant at all seasons and was found in a large percentage of lambs and yearlings suffering from gastro-intestinal troubles, as well as in a number of apparently healthy animals.

The worms usually occur in the duodenum but in the case of heavy infection are also found in other parts of the small intestine; they have been recorded from the fourth stomach as well[1], I have however never come across them in this position.

Whilst in the majority of cases the parasite occurs in relatively small numbers, I have occasionally observed thousands in the duodenum alone, the contents of the latter seeming nothing else than a writhing mass of worms. Lambs thus heavily infected always exhibited symptoms of helminthiasis, to what extent these were due to the presence of *Nematodirus filicollis* is difficult to say as this worm was never found by itself but was always associated with other parasitic worms, both in the small intestine and in other parts of the alimentary tract. Among the worms found associated with *Nematodirus filicollis* in the small intestine were the tape-worm, *Moniezia expansa*, and the following Nematodes: *Bunostomum trigonocephalum* Rud., *Ostertagia circumcincta* Stad., *Cooperia oncophora* Raill., *Trichostrongylus vitrinus* Looss, and *Strongyloides papillosus* Wedl.

Historical.

The worm now under consideration was first described by Rudolphi in 1802 under the name of *Ascaris filicollis*, the type specimens having been obtained from sheep in Germany. The same investigator removed the species to the genus *Strongylus* in 1803, and under the name of *Strongylus filicollis* we find descriptions of the parasite in many helminthological publications, including those of Schneider (1866), Curtice (1890), Railliet (1893), and Stödter (1901).

Ransom in 1907 took the species as type of a new genus *Nematodirus*, and in his monograph on the Nematodes parasitic in the alimentary

I desire, also, to express my thanks to the Birmingham Natural History and Philosophical Society for assistance, by means of a grant from the Endowment of Research Fund, in defraying the cost of the illustrations of this paper.

[1] Cf. Neumann (1905), p. 361.

tract of Ruminants, published in 1911, we find the following generic diagnosis:

Metastrongylinae: Head not over 50μ in diameter; circumoral papillae inconspicuous. Cuticle of head may be slightly inflated and in cervical region striated transversely. Cervical papillae apparently absent. Bursa without unpaired dorsal median ray. Dorsal lobe of bursa reduced to two small short lobules, each supported by a dorsal ray. Ventro-ventral and latero-ventral rays of each lateral lobe of bursa close together, parallel. Six supporting rays in each lateral lobe. Medio-lateral and postero-lateral rays close together, parallel. Externo-lateral ray distally diverges widely from the other lateral rays. Spicules more than 0·5 mm. in length, slender, tubular, filiform, united by a membrane throughout their length, or only in their distal portion. Gubernaculum absent. Vulva of female behind the middle of the body. Ovijectors well developed. Eggs large, generally over 150μ long.

In this monograph Ransom mentions three species of *Nematodirus* as occurring in Ruminants: *N. filicollis* Rud. in cattle, sheep, goats, prong-horned antelope, roe-deer and fallow-deer, *N. spathiger* Railliet in the dromedary and *N. digitatus* Linstow in the zebu. To these must be added *N. roscidus* Brumpt (1911) from deer, *N. mauritanicus* Maupas and Seurat (1912) from the dromedary, and *N. fordi* Daniels (1908) from cattle as well as from man and the pig. Railliet and Henry in a recent paper (1912) have shown that *N. digitatus* and *N. fordi* differ in many respects from the other species and have divided the genus into two sub-genera *Nematodirus* and *Mecistocirrus*[1], the latter to include these two species. The sub-genus *Nematodirus* is defined as follows:

Corps capillaire, longuement effilé dans sa partie antérieure; extrémité céphalique munie d'un léger renflement vésiculeux souvent strié en travers; tégument rayé par 18 arêtes longitudinales assez nettes; pas de papilles cervicales apparentes. Bourse caudale bilobée, à côtes d'égale importance; les postérieures séparées, sans tronc commun; les antérieures dédoublées; la pointe des antérieures externes à égale distance des antérieures et des moyennes. Spicules grêles, longs d'au moins 500μ (et au plus du $\frac{1}{12}$ du corps). Queue de la femelle tronquée et mucronée; vulve vers le $\frac{1}{3}$ ou le $\frac{1}{4}$ postérieur du corps; vagin très court. Oeufs ellipsoïdes, grands, à coque plutôt épaisse, segmentés au moment

[1] The differences between *Nematodirus* and *Mecistocirrus* have recently been emphasised by Neveu-Lemaire (1914) who suggests raising these to generic rank.

de la ponte; l'embryon se développe à l'intérieur de la coque et y subit deux mues, après quoi il est apte à rentrer directement dans l'organisme sans phase de liberté dans le milieu extérieur. Habitat: ordinairement le duodénum des Ruminants.

Railliet and Henry also show that there has been in the past considerable confusion between the two species *N. filicollis* and *N. spathiger*, and point out that whilst Schneider (1860) correctly figured the true *N. filicollis* Rud., the specimens described under that name by Curtice (1890), Stödter (1901), and Ransom (1911) are to be referred to *N. spathiger* Railliet.

Maupas and Seurat recently (1912) described *N. filicollis* from the dromedary and sheep in Algeria; from the descriptions given by the authors there can be little doubt that these worms are also to be included in Railliet's *N. spathiger*[1].

On account of this confusion between the two species I have considered it advisable to give a short description of the adult individuals observed by me in England before proceeding to the account of their development; it will be seen that these worms belong to the true *Nematodirus filicollis* Rud. as defined by Railliet and Henry.

Specific Diagnosis.

In the adults the body is slender and considerably attenuated anteriorly; the living worms are semi-transparent and either colourless or, more frequently, tinged with a blood-red colouring matter, the latter often most intense at the anterior and posterior extremities and apparently lying in the body-cavity and not in the alimentary canal. The cuticle is nearly always inflated at the anterior extremity, and in the region of the neck is marked with transverse striations, absent from the rest of the body, which is provided with eighteen longitudinal cuticular lines. The head has a breadth of 30 to 60μ and bears six small papillae surrounding the circular mouth. Cervical papillae are absent. The oesophagus measures 450–600μ in length and is broadest posteriorly (30–40μ). The nerve-ring is situated about 300μ from the anterior extremity, the excretory pore 50–70μ in front of the junction of oesophagus and intestine.

Male. The average length of the male was found to be 11·5 mm., the smallest mature specimen measured 7·5 mm., the largest 13·5 mm.

[1] Seurat has recently (1913) described the Rodent *Ctenodactylus gundi* as a host of *Nematodirus filicollis* in Tunisia. The figures of the worms given by the author show that these also do not belong to this species.

The maximum diameter of the body varies between 90 and 130μ. The lateral lobes of the bursa are without distinct dorsal lobules such as occur in *N. spathiger*. The postero-lateral ray is the thickest; the externo-dorsal is extremely slender. It was noticed that the posterior branch of the dorsal ray is frequently bifurcated at the tip, a point not previously recorded. The long slender spicules have an average length of 810μ, varying between 750 and 925μ. The terminal membrane of the spicules is lanceolate, ending in a fine point.

Female. The average length of the adult female is 18 mm., the specimens observed varying from 13 mm. to 21 mm. The maximum breadth of the body just anterior to the vulva measures 150–225μ. The tail is truncated posteriorly and from the tip projects a very slender rod-shaped process 12–18μ in length. The truncate tip of the tail has a breadth of 20–25μ and the cuticle surrounding it is slightly inflated and frequently presents faint transverse striations similar to those in the neck region. The anus is situated 65–80μ from the posterior extremity, the body at this level having a breadth of 45–60μ. The vulva is a little less than a third of the length of the body from the posterior end. The vagina is very short, the muscular portions of the two ovijectors measure 400–500μ. The average size of the egg is 160μ × 80.

The Egg.

The eggs of *Nematodirus filicollis* are considerably larger than those of most Nematodes which inhabit the alimentary tract of Ruminants in this country. I have measured a large number both from the sexual organs of adult females and from washings of faeces; as mentioned in the specific diagnosis it was found that they have an average length of 160μ by an average breadth of 80μ. The smallest egg observed had a length of 130μ, the largest 210μ, the breadth varying similarly between 65 and 95μ. It is worth noting that the eggs from the faeces were always found to be a little larger than those taken from the maternal uteri.

The shape of the eggs is fairly constant, appearing elliptical in side view, circular in cross section. The egg-shell is thick but quite transparent and colourless; the thickness, approximately 3μ, is uniform and not increased at the two poles as in *N. spathiger* (according to Railliet and Henry). The surface of the shell is quite smooth. The eggs commence their development within the uterus and when laid usually contain seven or eight cells; more advanced eggs were never found in fresh faeces, although earlier stages were occasionally met with. The egg contents are dark in colour, opaque and filled with coarse yolk granules; the vitelline membrane is usually conspicuous.

Development of the Egg outside the Host.

The development of *Nematodirus* has received little attention from previous investigators. Ransom (1911) seems the first to point out that the embryo within the egg-shell develops into a larva with the filariform type of oesophagus before hatching, thus differing from other Strongyles, such as *Haemonchus contortus*, in which the newly-hatched embryo possesses a rhabditiform oesophagus with a posterior bulb, not developing into the larval stage with filariform oesophagus until later. Railliet and Henry (1912) confirm this statement and add the information that the embryo undergoes two moults within the egg-shell before hatching. Maupas and Seurat (1913) describe the early development in more detail and figure the newly-hatched larva; they show that the latter is provided with a sheath formed by the second ecdysis and that the skin cast during the first moult can also be seen surrounding the mature larva within the egg-shell.

In order to study the development outside the host it was necessary to obtain eggs in large numbers and, as far as possible, in pure cultures, i.e. free from the eggs of other parasitic or free-living Nematodes. Such cultures could be obtained from two sources: (*a*) from the sexual organs of the adult worms, (*b*) from the fresh faeces of infected sheep. A certain number of cultures were made by teasing up the bodies of mature females; by this method, however, the eggs obtained were not numerous, moreover they included a very large percentage which failed to continue their development; it was, therefore, found more convenient to obtain the material from the second of the two sources just mentioned.

From the fresh faeces of heavily infected sheep eggs could be obtained in very large numbers by the usual methods of sieving and sedimenting[1]: by the use of very fine sieves it was found possible to separate the large *Nematodirus* eggs from the smaller ones of other genera.

The eggs of *Nematodirus filicollis* were found to develop equally well in tap-water and in moist faeces, in the case of the faecal cultures it was found necessary to provide sufficient aeration in order to prevent the "poisoning" of the eggs by decomposition products. Eggs in water

[1] For a comparative account of the methods of examining faeces cf. Hall (1911).

seemed not to require much air and developed well in solid watch-glasses with sealed covers or in small corked tubes.

The presence or absence of daylight has little or no effect on the development, eggs developing equally well whether exposed to light or kept in a dark chamber.

A certain amount of moisture was found to be essential, in the earlier stages of development the eggs were invariably killed by desiccation; excess of moisture is not harmful to the cultures, for, as already mentioned, development proceeds perfectly well in pure water.

The rate of development of the eggs, always slow as compared with other Strongylids, is very strongly influenced by temperature conditions which are certainly of the greatest importance. Extensive series of experiments concerning this factor could unfortunately not be made, cultures were however kept at a number of different temperatures and the general effects of these conditions ascertained.

The best results were obtained at temperatures between 19° C. and 27° C., i.e. warm summer temperatures, under these conditions eggs were found to contain mature larvae ready to hatch 24–28 days after leaving the body of the host. At ordinary laboratory temperatures (13°–15° C.) development proceeded more slowly and with less regularity, eggs often taking 5–6 weeks to produce mature larvae. At low temperatures development is suspended, freezing kills the eggs in their early stages.

Several cultures were kept in an incubator at a temperature of 38° C., under these conditions the eggs developed rapidly as far as the morula stage, after which the majority died; no mature embryos were formed, thus indicating that complete development cannot take place at the blood temperature of the host and that eggs swallowed by sheep during the earlier stages would not survive.

As already mentioned the eggs usually contain 7–8 cells when laid, they do not develop further until they leave the body of the host. In water or moist faeces the development of the embryo continues and is of quite the normal type, resembling that of other Nematode worms. Under favourable temperature conditions the morula stage is reached in 3–4 days and is soon followed by the characteristic "tadpole" stage, in which the embryo is flexed and considerably thickened at the anterior extremity. The first movements of the embryo now become apparent, the latter continues elongating and by the end of the first fortnight has reached a length of approximately five times that of the egg in which it lies coiled; the thickening at the anterior extremity has disappeared and the embryo is approximately cylindrical in shape, except posteriorly where the body tapers to a long slender tail.

The embryo at this stage performs continuous, although somewhat sluggish, movements within the egg-shell; its internal organisation is not visible, the whole of the body with the exception of a small spot at the cephalic extremity being filled with dark, yolk-like granules.

During the second fortnight important changes take place, the opaque granules of reserve substances are slowly absorbed and the internal organisation of the embryo gradually revealed, the latter moreover undergoes two ecdyses before appearing in its final condition ready for liberation from the egg-shell. The mature embryo is quite transparent and appears highly refractive, it is enclosed in a tightly fitting sheath, the uncast skin derived from the second ecdysis, and is still surrounded by the shed skin from the first moult. The mature embryo is extremely lively at ordinary laboratory temperatures and is in continual motion within the egg-shell.

The Hatching of the Embryos.

For some time great difficulty was experienced in getting the embryos to hatch from the eggs, many mature eggs were kept for weeks in the laboratory without a single larva being freed, whilst in some cultures even after seven months more than 90 per cent. of the embryos were still unhatched although apparently healthy and moving actively within the egg-shells.

Mr H. E. Hornby, who had made some observations on this species at the South-Eastern Agricultural College at Wye, informed me that he had had the same difficulty in obtaining larvae from *Nematodirus* eggs kept in moist faecal cultures, but had been more successful with some cultures which had been dried for a short period. I therefore tried the effect of submitting some of my cultures to alternate desiccation and remoistening; this yielded somewhat better results, a few larvae hatching at each remoistening but still forming a very small percentage.

It is, of course, well known that the larvae of many Nematodes hatch only when the eggs are taken into the body of their host, this led me to try the effect of temperatures approximating to that of the blood on the mature eggs of *Nematodirus filicollis*. In an oven at a temperature of 38° C. the embryos with only few exceptions hatched within a very short period[1], thus suggesting the possibility that in this species also the liberation of the larvae normally takes place within the alimentary canal of the host. Although a quite conceivable solution of the difficulty it struck me as a highly improbable one, since the study of some liberated larvae had shown that these are admirably adapted for leading a free existence and, moreover, that their structure and habits are precisely similar to those of other Nematode worms known to have free-living stages in their life histories.

I only accidentally became aware of the true facts of the case; during a particularly warm, sunny day in the spring one of my cultures was left exposed to the sun's rays for several hours and examination of a small sample showed that a considerable number of larvae had been liberated. The temperature in the sun was approximately 25° C.

Other cultures were then subjected to various temperatures between 24° C. and 32° C., both in ovens and in the open, and these experiments showed that the larvae are quite capable of hatching so long as the temperature is sufficiently high. During a warm spell in the early summer (24°–28° C. in the shade during the middle of the day) nearly all the larvae in my cultures were released.

Description of the "Ensheathed" Larva.

The newly-hatched larvae of *Nematodirus* are easily distinguished from those of other Nematode parasites of the sheep by their comparatively large size and by the great length and tenuity of the tail-region.

It must be noted that these larvae on hatching have already undergone two ecdyses, one complete and one partial, within the egg-shell; as pointed out by previous observers, they are therefore in a considerably more advanced condition than the newly-hatched larvae of the majority of Strongylid Nematodes, e.g. *Haemonchus contortus* or *Anchylostoma duodenale,* in fact they have reached a state of development comparable with that of the latter worms at the end of their period of free life. According to Looss' nomenclature (1911, p. 345) the *Nematodirus* larvae when liberated from the egg are in a state of transition between the second and third larval stages; in structure they have reached the third stage of development, but are still enclosed in the loose skin

[1] In these experiments the liberation of the larvae took place quite independently of the chemical composition of the fluid in which the eggs were kept. The media used were those suggested by Martin (1913, p. 64), namely, distilled water, ·2 per cent. HCl, ·2 per cent. $NaHCO_3$ and ·8 per cent. NaCl.

of the second stage. The general shape of the larvae is therefore determined by this outer skin, and in describing and measuring them it is necessary to distinguish carefully between this sheath and the enclosed larva which almost completely fills it.

The total length of the free larvae, including the tail, varies from ·85 to 1·15 mm., the average length being about 1 mm. The body has a maximum breadth of 25–30μ and is approximately cylindrical, tapering however anteriorly and posteriorly but only moderately towards the cephalic extremity which terminates in a rounded head about 15μ in width. Posteriorly the body passes gradually into the very slender tail which has a length of about one-third of the total length of the

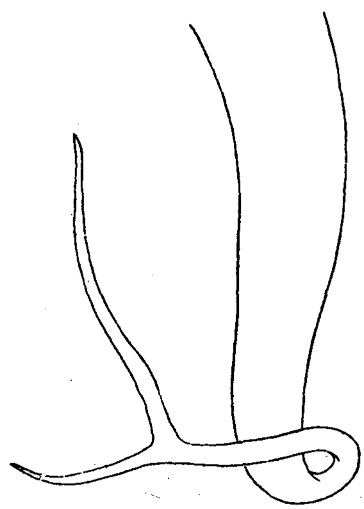


Fig. 1. Tail of larva with fork-like malformation of posterior end. × 750.

larva. In many of the larvae a slight constriction is to be seen a short distance behind the commencement of the tail, the posterior portion of which is often difficult to see except under high magnifications, having a thickness of little more than 1μ.

Abnormalities of the caudal region were frequently observed, the commonest being a fork-like malformation of the extremity, as shown in Text-fig. 1.

The skin or "sheath" is comparatively thick and provided with closely set transverse striae, only visible under high powers of the microscope; it is completely closed, the position of the mouth is however indicated by a slight thickening at the anterior extremity.

Under normal conditions and when uncontracted the enclosed larva completely fills the ensheathing skin with the exception of the slender tail, for this reason the sheath is sometimes inconspicuous, it can however be readily observed when the body is flexed, being thrown into distinct and regular folds on the concave surface; the larva has moreover considerable powers of contraction and when contracted the outer skin can be seen projecting at each extremity.

As mentioned above the enclosed larvae do not project into the tail-regions of the sheaths, they are therefore considerably shorter than the latter, measuring 5·3–7 mm. in length.

The shape of the head differs somewhat from that of the cephalic extremity of the sheath, being slightly truncated anteriorly, not rounded. There are no distinct lips, cephalic papillae are however present, in the form of six small but highly refractive spots.

The tiny mouth leads into a short buccal cavity. The oesophagus is slender, 180–220μ in length, it increases in breadth posteriorly but does not terminate in an oesophageal bulb and is not provided with a "dental" apparatus. The intestine is also narrow and consists of eight cells only, four dorsal and four ventral alternating with one another; these intestinal cells are rather dark in colour and usually filled with reserve granules and vacuoles which conceal the nuclei, the latter are however occasionally visible. From the termination of the intestine a very slender rectum leads to the anus, a tiny opening situated on the ventral surface about 50μ from the posterior extremity of the larva.

The tail of the larva is peculiar and extremely characteristic, differing markedly in shape from that of the sheath; it is divided at the posterior extremity into dorsal and ventral lobes in such a way as to appear deeply forked when seen in a lateral view. The two lobes vary somewhat in shape and size in different individuals, the dorsal is however usually somewhat larger than the ventral which it conceals in a dorsal view (Text-fig. 2). Between the dorsal and ventral lobes is an elongated rod-shaped process recalling the tail-spike of the adult *Nematodirus* female, it has a length of about 13μ and projects well beyond the lobes of the tail.

The excretory apparatus is difficult to make out in the ensheathed larva, the terminal canal can however be seen in the majority of individuals leading to the minute excretory pore, which opens on the ventral surface a little anterior to the junction of intestine and oesophagus and about 150μ from the anterior extremity of the body.

The central portion of the nervous system can be seen in many individuals and takes the form of a ring encircling the oesophagus at a point just anterior to the level of the excretory opening.

The genital rudiment is quite conspicuous, forming a lens-shaped body situated ventrally to the intestine and opposite the fifth intestinal cell. Under a high magnification it can be seen to consist of a number of cells (usually about 8–12) with conspicuous nuclei.

The Biology of the "Ensheathed" Larvae.

The free larvae are extremely active and in a liquid medium at laboratory temperatures (13°–15° C.) perform vigorous, snake-like movements similar to those described in the mature larvae of other

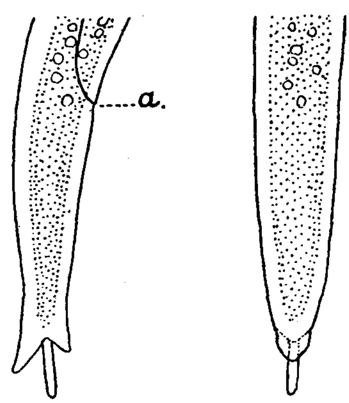


Fig. 2. Posterior end of body of "exsheathed" larva, viewed from right side and from dorsal side. *a.* anus. × 750.

Strongylid worms. When placed in a watch-glass or tube containing water they immediately sink to the bottom and are evidently incapable of keeping afloat by swimming movements.

The larvae of *Nematodirus filicollis* possess the same migratory instincts as the larvae of *Haemonchus contortus* and *Anchylostoma duodenale* and are able to climb vertical surfaces if these are kept sufficiently moist. The majority of my cultures were kept in a moist chamber in small glass dishes about 40 mm. in diameter and with walls 25 mm. high, the larvae as they hatched during warm weather immediately commenced to ascend the sides of the dishes and after a few weeks were present in such numbers in this position as to appear to the naked eye as a whitish deposit covering the whole of the inner surface of the vertical walls. When the dishes were removed from the moist chamber the larvae did not return to the damp faecal mass at the bottom, but dried and adhered to the walls as the moisture evaporated. The larvae also ascended blades of grass and seedlings placed in the dishes.

Ransom has already pointed out in the case of *Haemonchus contortus* (1906) how this upward migration of the ensheathed larvae is connected with a method of infection most peculiarly adapted to the food habits of sheep and other herbivorous animals; the similar migratory instincts of the larvae of *Nematodirus filicollis* suggest that these reach the alimentary canals of their hosts in the same manner.

The ensheathed *Nematodirus* larvae are evidently able to maintain their existence in water for a considerable period, I have kept specimens in small watch-glasses for over eleven months.

Perhaps the most striking feature in the biology of the larvae is their power to withstand desiccation for long periods. This phenomenon can be studied by allowing a drop of water containing larvae to evaporate on a glass slide; as the moisture of their surroundings becomes reduced the larvae contract somewhat within the sheaths and usually coil themselves into a spiral, the fluid within the sheaths is still present for a short time after the water on the slide has completely evaporated, this however soon disappears and the larvae become much shrivelled, assume a glassy appearance and become extremely brittle. When remoistened the larvae quickly absorb water through their sheaths, regain their shape and gradually uncoil, the whole process often taking less than twenty minutes.

The larvae can in most cases endure repeated drying and remoistening and in one experiment I successfully dried and revived a number of individuals twelve times in six days.

The *Nematodirus* larvae are able to withstand complete desiccation equally well whilst still within the egg-shell, this however only applies to the mature embryos which are already provided with sheaths.

The maximum period for which the larvae can retain their vitality when subjected to complete desiccation was not ascertained, my experiments however showed that they can do so for a very long time. I will quote one interesting experiment in support of this statement. A small mass of faeces, known to contain numerous eggs enclosing mature larvae, was placed in a small open box on August 30th, 1913, and then left untouched until September 27th, 1914. By this date, i.e. more than twelve months afterwards, the lump of faeces was as hard as stone and had to be broken with a hammer; a small piece was chipped off and soaked in water in a watch-glass, as it softened it was broken up with a needle revealing numerous eggs containing shrivelled larvae. After about fifteen minutes the latter had appreciably swollen and soon regained their normal shape, they were moving actively within the egg-shells less than forty minutes after the commencement of the experiment. Another sample from the same dried mass was treated in a similar manner on April 30th, 1915, and yielded a number of live larvae which seemed none the worse for having been dried for twenty months.

A number of experiments were also made to determine the effect of different temperatures on the ensheathed larvae, it was found that these, when perfectly dry, are able to withstand very high temperatures without losing their vitality. On one occasion seven dried larvae were kept for ten hours in an oven at a temperature of 60° C., when afterwards cooled and placed in water they all revived in less than half an hour and were still alive and active twenty-four hours after being remoistened. In the course of another experiment three dry larvae were subjected to a temperature of 70° C. for two hours, of these two revived and were swimming about actively twenty-five minutes after remoistening.

Other experiments showed that this power of resisting high temperatures was confined to larvae in the dry condition, larvae in water are quickly killed if subjected to temperatures much over 50° C.

The dried larvae of *Nematodirus filicollis* are equally resistant to cold, and several experiments showed that these could be left outdoors during sharp frosts in the winter without fatal consequences.

The Completion of the Second Ecdysis.

As already mentioned the ensheathed larvae of *Nematodirus filicollis* can live in water for many months without undergoing any change, and in this medium at laboratory temperatures make no efforts to rid themselves of the closely-fitting sheaths formed during the second ecdysis within the egg-shell. This fact together with the similarity in structure and habits of these larvae with the ensheathed larvae of other better known worms of the same family (e.g. *Haemonchus contortus* and *Anchylostoma duodenale*) leads to the conclusion that at this stage they have reached maturity and are ready to pass into the body of their definite host.

Looss (1911) has shown that the mature larvae of *Anchylostoma* cast their protective skins as soon as they enter the body of their host, whether through the skin or through the mouth; he also showed that the completion of the second ecdysis can also take place in the open under certain artificial conditions. According to this investigator the process is always a purely mechanical one and is performed either actively by the larvae (e.g. under certain chemotactical stimuli when they unmistakably exert themselves to leave their envelopes) or passively (e.g. in certain media, such as gelatine or water containing numerous foreign particles, when the rending of the outer skin results from its mechanical retention by the medium whilst the larva itself attempts to move forwards).

The larvae of *Nematodirus* are also able to complete their moults in the open at laboratory temperatures when subjected to certain conditions. Thus it was found that ecdysis occurred simultaneously in a number of ensheathed larvae living in a culture of faeces which had been repeatedly dried and remoistened during the course of some experiments on the resistance of the larvae to drought.

A similar phenomenon was observed on two occasions in moist cultures of faeces which had been attacked by fungi, and I was able to watch several individuals in the act of moulting. In these cases the hyphae of the fungus had enveloped the larvae and arrested their progress, resulting in violent serpentine movements which finally led to the rupturing of the sheaths near the anterior extremities.

On all occasions when the completion of the moult was observed in the *Nematodirus* larvae the process took place in a purely passive manner; I never succeeded in inducing active ecdysis by means of chemical stimuli, although the effects of various stains as well as of weak solutions of neutral salts, acids and alkalis were tried.

The influence of temperature on the ensheathed larvae is, however, very marked, and repeated experiments showed that these when subjected to temperatures approximating to blood-temperature, with few exceptions, complete their ecdyses within a few hours. The experiments were performed by means of a small electric oven at a temperature of 38° C., the larvae being placed in small stoppered tubes in water and various solutions. The casting of the sheaths took place quite independently of the chemical composition of the fluid in which the larvae were kept, pure water as well as dilute neutral acid and alkaline solutions were used, always however with similar results.

The above experiments show that whilst at low temperatures the larvae of *Nematodirus filicollis* are occasionally able to complete their moults under certain somewhat abnormal conditions—they normally do so when subjected to a temperature of 38° C.—we may assume therefore that they would behave in a similar manner when taken into the body of a sheep or other host.

Structure of the "Exsheathed" Larva.

Whilst retained within the sheaths the larvae of the third stage undergo no further development, and just after the completion of the second ecdysis show practically the same structure as those described on page 143; in most individuals however the food granules and vacuoles have almost entirely disappeared from the eight intestinal cells, revealing the nuclei as spherical bodies of a rather lighter colour than the protoplasm surrounding them.

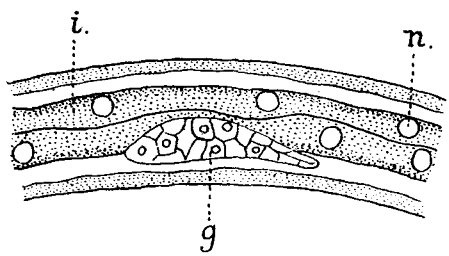


Fig. 3. Genital rudiment and adjacent parts of body of larva fifteen days after completion of the second ecdysis. *g*. genital rudiment. *i*. intestine. *n*. nucleus of intestinal cell. × 750.

In their behaviour these larvae differ markedly from those in the ensheathed stage, they have lost their agility of movement and at laboratory temperatures are exceedingly sluggish, often remaining motionless for hours. They show no tendency to climb vertical surfaces.

In water or cultures of faeces the "exsheathed" larvae usually die within a few days, I however succeeded in keeping a few individuals for fifteen days in an oven at 38° C. These larvae showed little change except in the structure of the intestine and genital rudiment.

By the fifteenth day the number of intestinal cells had increased considerably and fourteen nuclei could be counted; the cell limits were at this stage scarcely discernible and moreover the intestine now showed a distinct lumen.

The genital rudiment also showed a considerable increase not only in actual size but also in the number of its constituent cells (Text fig. 3).

Mode of Infection of Sheep.

It was unfortunately found impossible for various reasons to perform infection experiments on sheep, I am therefore not in a position to produce direct proofs that these animals become infected by swallowing the sheathed larvae of *Nematodirus filicollis*. The circumstantial evidence in favour of such direct infection is, however, very strong: in the first place, as already pointed out, in their structure, general behaviour and especially in their migratory instincts, these larvae are precisely similar to those of forms known to infect warm-blooded animals directly; the conditions under which the second ecdysis is completed also greatly favour this view; whilst lastly, as will be shown in the next paragraph, examination of the intestinal contents of sheep harbouring adult *Nematodirus* revealed practically all stages between mature larvae and adult individuals, the youngest of these intermediate stages showing but little advance in structure over the "exsheathed" larvae just described.

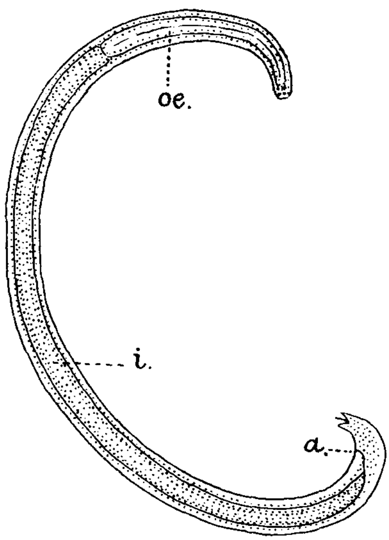
Whilst the larvae in the free condition are probably the more usual source of infection it must not be forgotten that these are no doubt infective whilst still within the egg-shell, being already ensheathed before hatching. That the mature eggs are a possible source of infection was indicated by Railliet and Henry (1912) when in their definition of the genus they stated "l'embryon...est apte à rentrer directement dans l'organisme sans phase de liberté dans le milieu extérieur."

Young Stages in the Sheep.

The alimentary tracts of infected sheep and lambs were carefully examined for young parasites, and a number of interesting early stages of *Nematodirus filicollis* were obtained; the youngest of these were only little more advanced than the "exsheathed" larvae described in a preceding paragraph.

The youngest stage was found in a lamb killed in March, 1914, it measured only 1·2 mm. in length and except for its greater relative breadth and for an increase in the number of intestinal and genital cells is very similar to a mature larva just after ecdysis (Text-fig. 4). The body has a maximum thickness of 40μ and is approximately cylindrical in shape, tapering however at both extremities. The truncated head measures about 15μ in breadth and is provided with six small papillae. The oesophagus has a length of 270μ, the intestine is multicellular and the genital rudiment no longer exists as a lens-shaped body. The most interesting organ at this stage is the tail, which retains all the characteristic larval features, namely the forked extremity and the rod-like terminal process.

The first traces of external sex characters were seen in specimens 2·5 mm. in length, these very closely resemble the earlier stage just described and do not show any appreciable increase in breadth, in a few



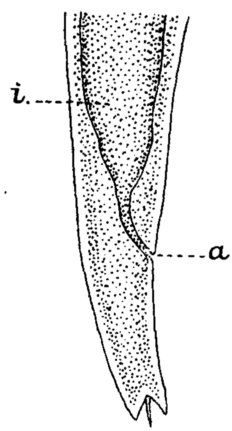


Fig. 4. Young stage from intestine of lamb. *a.* anus. *i.* intestine. *oe.* oesophagus. × 100.
Fig. 5. Posterior end of similar but slightly older stage from intestine of lamb. *a.* anus. *i.* intestine. × 300.

individuals however a distinct swelling is visible, just anterior to the tail-fork and at about the level of the anus, which probably represents the first sign of a male bursa.

The bursal rudiment is much more conspicuous in a specimen 3·3 mm. long, here it forms a hollow swelling about 60μ broad in the same position below the larval skin, the latter is very loose in the anterior part of the body and is evidently about to be cast; this is probably the final moult before the adult stage is reached. In this specimen the body has an almost uniform thickness of 50μ, tapering however at the two extremities but only slightly posteriorly, the region just in front of the rudimentary bursa measuring 45μ. The head is 20μ broad and is provided with a circular mouth and six cephalic papillae, the latter now more conspicuous than in the earlier stages. The caudal extremity of this specimen bears a striking resemblance to that of an early stage of a Strongylid figured by Leuckart (1876) under the name of *Strongylus polygyrus*.

With the final moult the characteristic tail-fork and caudal process are lost in the male specimens, the bodies of the latter now terminating in the bursae, at first small and with very faint and inconspicuous rays. The smallest specimen in which all the rays as well as the spicules were visible measured 5 mm. in length.

In the young females the caudal fork remains for a longer period and seems to pass gradually into the truncated extremity of the adult, the rod-like process of the larva is evidently retained as the terminal spike so characteristic of the mature female.

Summary of Life History.

1. The eggs of *Nematodirus filicollis* when laid contain an embryo with seven or eight cells, they pass out of the infested host with the faeces.

2. Even under favourable conditions development takes place slowly and the embryos are not ready to hatch until 24–28 days have elapsed. In their early stages the embryos are not able to withstand desiccation and are killed if frozen or subjected to high temperatures.

3. Whilst still within the egg-shell the embryo undergoes two ecdyses and when ready to hatch is enclosed in a tightly fitting sheath formed by the incompletely cast skin of the second moult; the larvae on liberation from the egg-shell are therefore in a more advanced condition than those of most other Strongylids (e.g. *Haemonchus* or *Anchylostoma*) and are in a stage comparable with that reached by the latter at the end of their period of free existence.

4. The sheathed larvae are often retained for a long time within the egg-shells and both in this position and after hatching can resist complete desiccation for considerable periods (twenty months or even longer); when dried they are able to withstand freezing as well as temperatures much above those likely to be met with in the open.

5. The free larvae will live for a considerable time in water, they possess well-developed migratory instincts and climb vertical surfaces, such as grass stems and blades, and the glass walls of the vessels in which they are kept.

6. The sheaths are cast off by the larvae when these are subjected to temperatures approximating to the blood-temperature of the host; completion of the second moult occasionally also takes place at laboratory temperatures under certain abnormal conditions.

7. No infection experiments were made on sheep, but other evidence shows that these animals must become infected by swallowing the sheathed larvae, either when free or whilst still enclosed in the egg-shells.

8. A number of young stages of the parasite were met with in the intestines of sheep, the smallest of these being only little more advanced in structure than the larvae just after ecdysis.

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EXPLANATION OF PLATES.

PLATE VIII.

Nematodirus filicollis Rudolphi.

Fig. 1. Adult female, ×20.

Fig. 2. Posterior end of body of female, viewed from right side, ×450.

Fig. 3. Anterior end of body of female, viewed from left side, ×200.

Fig. 4. Posterior end of body of male, viewed from right side, to show bursa and spicules, ×170.

Fig. 5. Posterior end of united spicules of male, ventral view, ×950.

Fig. 6. Egg, taken from fresh faeces of lamb, ×400.

PLATE IX.

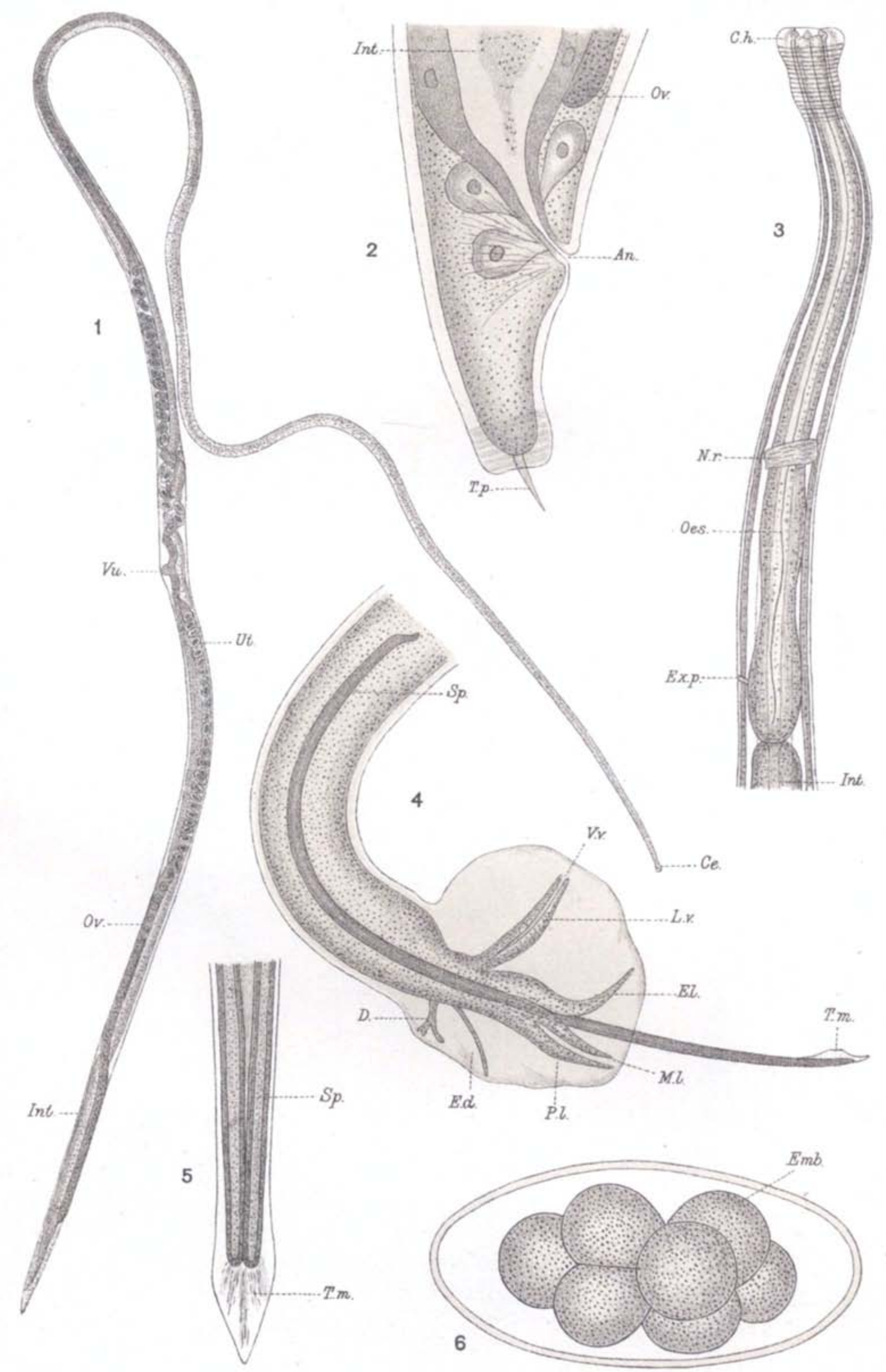
Nematodirus filicollis Rudolphi.

Fig. 1. Newly-hatched larva, ×350.

Fig. 2. Larva after completion of second ecdysis, ×350.

Fig. 3. Young stage (3·3 mm. long) from intestine of lamb, ×60.

Fig. 4. Posterior end of same specimen, showing the rudimentary bursa enclosed by the larval skin, ×400.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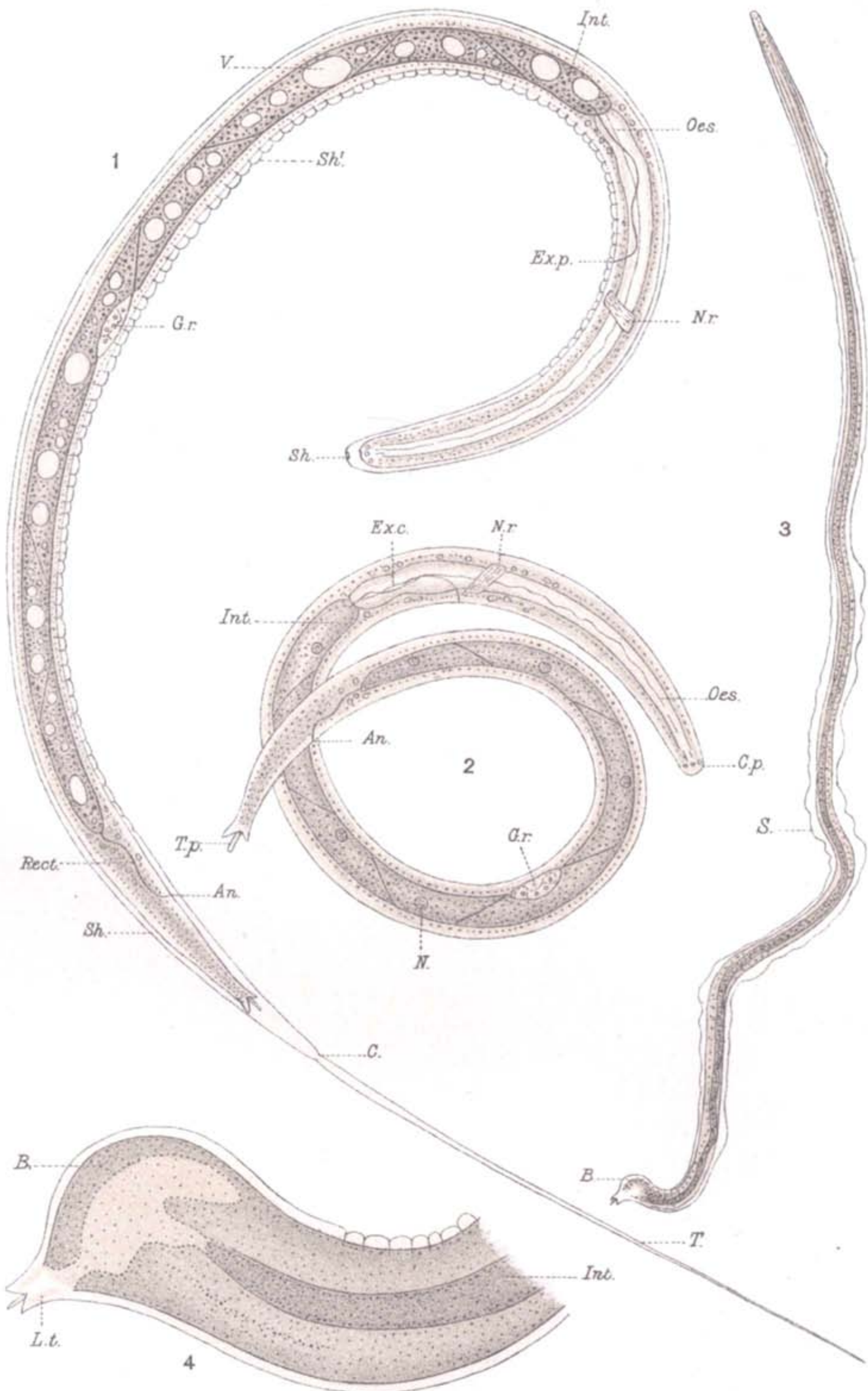
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INDEX TO LETTERING.

An.	Anus.
B.	Rudimentary bursa enclosed by larval skin.
C.	Constriction in tail of sheathed larva.
Ce.	Head.
C.h.	Inflated cuticle of head of adult.
C.p.	Head papillae.
D.	Dorsal ray of bursa.
E.d.	Externo-dorsal ray of bursa.
E.l.	Externo-lateral ray of bursa.
Emb.	Embryo within egg-shell.
Ex.c.	Excretory canal.
Ex.p.	Excretory pore.
G.r.	Genital rudiment of larva.
Int.	Intestine.
L.t.	Larval tail.
L.v.	Latero-ventral ray of bursa.
M.l.	Medio-lateral ray of bursa.
N.	Nucleus of intestinal cell.
N.r.	Nerve ring.
Oes.	Oesophagus.
Ov.	Ovary.
P.l.	Postero-lateral ray of bursa.
Rect.	Rectum.
S.	Larval skin about to be cast.
Sh.	Sheath of free larva.
Sh'.	Sheath of larva thrown into folds.
Sp.	Spicules of male.
T.	Tail of sheathed larva.
T.m.	Terminal membrane of united spicules.
T.p.	Rod-like terminal process of tail.
Ut.	Uterus.
V.	Vacuole in intestinal cell.
Vu.	Vulva of female.
V.v.	Ventro-ventral ray of bursa.