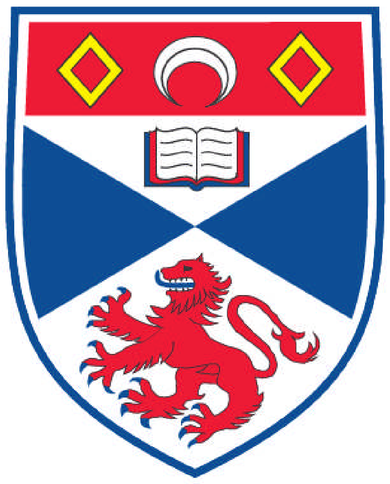
PARASITOLOGICAL STUDIES : ON THE STRUCTURE, BIOLOGY AND TAXONOMY OF NUCELLICOLA KILRYMONTIS GEN. ET. SP., NOV., (CRUSTACEA : COPEPODA) PARASITIC IN NUCELLA LAPILLUS (L.), (GASTROPODA : PROSOBRANCHIA)

Charles Edward Fitches

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews



1966

PARASITOLOGICAL STUDIES :

ON THE STRUCTURE, BIOLOGY AND TAXONOMY OF NUCELLICOLA KILRYMONTIS gen. et sp. nov., (CRUSTACEA : COPEPODA), PARASITIC IN NUCELLA LAPILLUS (L.), (GASTROPODA : PROSOBRANCHIA).

being a Thesis presented

by

CHARLES EDWARD FITCHES

to the University of St. Andrews

in application for the degree of

Doctor of Philosophy.

St. Andrews, 1966.

DECLARATION

I hereby declare that this thesis is based on the results of observations and experiments carried out by me, that the thesis is my own composition, and that it has not previously been presented for a Higher Degree.

(C. E. Fitches)

RESEARCH TRAINING

My first training in parasitology was during the academic year October 1960 to June 1961 when I studied the ecto- and endo-parasites of the starling, Sturnus vulgaris L., in the district of St. Andrews. The results of these studies were presented in the form of a thesis as part of the requirements for the Degree of Bachelor of Science with Honours in Zoology at the University of St. Andrews. My supervisor for the year was Mr. D.R.R. Burt of the Department of Natural History.

Mr. Burt was again my supervisor when I enrolled both as a Research Student, under Ordinance 350 of the University Courts of the Universities of St. Andrews, Glasgow, Aberdeen and Edinburgh (General No. 12), and as a candidate for the Degree of Doctor of Philosophy, under University Court Ordinance 79 (St. Andrews No. 16), on the 10th October, 1961. The results of my researches are here submitted as a Ph.D. Thesis.

CERTIFICATE

I hereby certify that Charles Edward Fitches was admitted as a Research Student in the Department of Natural History, University of St. Andrews on 10th October, 1961. He has spent nine terms on research on Parasitology, has fulfilled the conditions of Ordinance No. 16 (St. Andrews) and is qualified to submit the following thesis in application for the degree of Doctor of Philosophy.

David R.R. Burt, Supervisor.

On the Structure, Biology, and Taxonomy of *Nucellicola kilrymontis* gen. et sp. nov., (Crustacea : Copepoda), parasitic in *Nucella lapillus* (L.), (Gastropoda : Prosobranchia).

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INTRODUCTION

Nucellicola kilrymontis gen. et sp. nov., was first discovered in 1951 by Professor H.G. Callan of the department of Natural History when working at the Gatty Marine Laboratory St. Andrews. The animal came to his notice when he was conducting fertilization experiments on *Nucella* (= *Purpura*) *lapillus* (L.); in particular, he noticed that the embryos within the egg-string of *N. kilrymontis* displayed movements of a 'twitching' nature. Mr. D.R.R. Burt, of the same department, carried out a few brief preliminary observations and came to the conclusion that this animal was in fact a parasitic copepod of a very specialised nature which had not hitherto been recorded.

In 1954 Miss M. Murdoch was given the task of studying *N. kilrymontis* as a research topic for her Honours thesis. Miss Murdoch obtained the larval stages from the nauplius within the egg-string, to what she described as the second copepodid stage, and was able to draw the adult, having successfully separated it from the host tissues. She ascribed this parasite to the genus *Cerastocheres* Monod and Dollfus, and placed it in the family Lernaeidae but her description was incomplete and was not published.

Although my description of *N. kilrymontis* differs in many respects from that given by Miss Murdoch, I am most grateful for the help given by her earlier work. In fairness it must be stated that Miss Murdoch had little time at her disposal and was consequently unable to detect as much

detail as is recorded here. I will not give a summary of her conclusions but will discuss these where they are relevant to my findings.

Sections I and II of this thesis contain the techniques used in the dissection and histological preparation of N. kilrymontis, and section II the various experiments carried out in connection with the rearing of the larval stages.

The anatomy and morphology of the adult male and female have been investigated and are described and discussed in section III.

The development of the egg, the nauplius, the metanauplius and the copepodid stages, together with the development of the larval muscular system, are described and discussed in section IV.

In section V I have discussed, within the limits of present day copepodid systematics, the identity and classification of N. kilrymontis. Although the diagnosis of this new genus and species is not given until section V, I have used the new name Nucellicola kilrymontis throughout this thesis.

The relationship between host and parasite, and the phenomenon of host specificity with respect to this parasite and its host, are discussed in section VI.

I have conducted a survey of the geographical distribution of N. kilrymontis in the Bay of St. Andrews, along the coastline north and south of St. Andrews and of various sites around the British Isles. I also investigated the possible existence of a seasonal breeding cycle in this

parasite by examining host specimens taken from the same site at frequent intervals over a period of two years. The results of these investigations appear in section VII.

Section VIII contains a summary of my findings and I express my thanks to the various people who have rendered assistance in section IX.

I TECHNIQUES

1. Dissection of Host

The shell of each specimen of Nucella lapillus was fractured using a "Mole" self-grip wrench. The pieces of shell were carefully removed from the specimen and the latter was superficially examined for the presence of N. kilrymontis. The parasite is usually detected quite readily due to the coiling egg-string which lies just beneath the host's epithelium, usually in the region of the digestive gland (see Plate 1 figs 1 & 2).

If, after this examination, the host did not appear to be infested, it was placed in a dissecting dish and examined with the aid of a 'Binomax' Binocular dissection microscope. This provided for a more thorough examination and often revealed the presence of N. kilrymontis where it had previously escaped the naked eye. Often the parasite was almost completely embedded within the tissues of the host, only a small section of the egg-string being visible - generally the most mature part which is most often found in the tissue of the mantle.

2. Removal of N. kilrymontis from the host

The separation of the parasite from the host is a most difficult operation. I have been unable to dissect one specimen with the complete egg-string intact. As will be seen later, the parasite has a very thin epithelium and this is in turn surrounded by a thin epithelium of parasitic origin. This latter epithelium is closely adposed to the host tissue and thus renders dissection a very exacting task.

The parasitised whelk is pinned out in a small dissecting dish and covered with Berkefeld-filtered sea-water. The parasite is then carefully dissected out with the aid of two pairs of very fine watchmaker's forceps. When N. kilrymontis was found in the digestive gland or gonad, or in both of these tissues, then rupture of the host tissue during dissection caused local clouding of the water by egg or sperm material. In such cases the filtered sea-water was continually flushed over the preparation using a glass pipette.

After many unsuccessful attempts I was able finally to dissect out several parasites from their hosts, although rarely was I able to remove the parasite with the outer epithelium intact. This outer epithelium is continuous with that covering the egg-string and is most delicate. Where it does surround the egg-string it is even more delicate, so that even disturbing the host tissue close to it, almost without exception, results in its rupture. Add to this the fact that the egg-string coils many times after leaving the trunk of N. kilrymontis and the great difficulty of a complete dissection becomes apparent. As a consequence I am unable to show either a drawing or a photograph of this parasite with its complete egg-string intact.

Several of the dissected parasites were further dissected in order that the internal organs might be studied more closely. In these cases the dissection was carried out in Berkefeld-filtered sea-water under a high-magnification Beck-Greenhough dissection microscope using reflected light. As will be seen later, one or more dwarf males are present at the posterior

end of the female, and several of these were dissected out. In a few cases the ovaries and oviducts were dissected out and used in aceto-orcein squash preparations for examination of the chromosomes.

3. Preservation of specimens

Several techniques were employed in the preparation of the parasite for microscopical examination. Whole parasitised host specimens, pieces of host tissue with the parasite embedded, and dissected parasites were all fixed in various fluids. The fixative most often employed was Zenker's Fluid (Carleton and Drury, 1957) dissolved in 3 per cent. sodium sulphate to make it isotonic with sea-water. The material was fixed in Zenker's Fluid for 12 to 24 hours, depending on the size of the tissue, and then thoroughly washed in running water for a time equal to that of the length of fixation. As with all fixatives involving mercuric chloride, the material, after fixation and washing, was immersed in a solution of iodine in 70 per cent. alcohol in order to dissolve any excess mercuric chloride present and thus eliminate the possibility of crystals of mercuric chloride forming in the Canada balsam of mounted specimens (Vade Mecum, 1950).

Both 5 and 10 per cent. formaldehyde saline (Carleton and Drury, 1957) were used when the material was subsequently to be stained by a haematoxylin method, since formaldehyde intensifies the different varieties of haematoxylin (Carleton and Drury, 1957).

Carnoy's acetic alcohol fixative (Vade Mecum, 1950) was used for pieces of egg-string, ovaries and oviducts, and for the larval stages. It was found that this fluid was better than all others tried for the preservation of yolk material.

Alcoholic sublimate (Vade Mecum, 1950) was generally unsatisfactory, whereas Bouin's fluid (Vade Mecum, 1950) proved a good fixative, especially when the material was subsequently stained with a method involving acid fuchsin.

4. Preparation of whole mounts

After dissection, the adults of N. kilrymontis were treated in various ways. Some were fixed and later sectioned, and some were squashed, stained, and examined as whole mounts. The method which proved most satisfactory in squashing the parasite was to place it, well surrounded by Berkefeld-filtered sea-water, on a microscope slide with a smear of vaseline at each end of the slide. Another slide was then lowered gently onto this surface and the two slides gently compressed together until the parasite became flattened. The whole was then placed in the fixative, which was generally either formaldehyde saline or Bouin's fluid. This method gave a much more controlled "squash" than did earlier attempts using cover-slips tied together with thread, or held together by paper-clips. These latter methods were haphazard, there being no way of controlling the pressure exerted, and often they resulted in the rupture of the delicate epithelium of the adult.

The same squash technique was used for several males which had been dissected out of their females, but it was difficult to orientate these because of their small size and the results were not so good as those for the complete female with included male. Similarly this technique proved unsatisfactory with the egg-string - although the eggs were satisfactorily squashed, the outer epithelium was ruptured in every case, and when the two slides were separated the eggs floated away in all directions.

After washing away the fixative the squashed preparations were stained in one of the following ways. Early attempts were made with alcoholic borax camine, a stain used by Murdoch for unsquashed whole mounts. This method was not very satisfactory since it is a monochromatic stain and there is very little differentiation between tissues.

The method I employed most often was Mayer's haemalum technique (Pantin, 1959). This involves taking the preparation down through the various grades of alcohol to distilled water and then staining in the haemalum until the nuclei are bright red; this usually took between two and five minutes. The specimen was then washed and blued in running tap-water for about 20 minutes before being passed through an ascending series of alcohols to 90 per cent. alcohol. It was then counter-stained in a saturated solution of eosin in 90 per cent. alcohol for two minutes. Differentiation of the eosin was carried out in 90 per cent. alcohol and the specimen then dehydrated thoroughly in absolute alcohol, cleared in either xylol or cedar-wood oil, and mounted in Canada balsam. This technique gave an excellent representation of the general anatomy, the nuclei being stained deep blue, and the cytoplasm pink.

A similar, though not so striking, dichromatic effect was obtained using Erlich's haematoxylin in the manner often used for cestode and trematode material. The specimen was left in a dilute solution of Erlich's haematoxylin (eight drops to 10 ml. of distilled water) for 24 hours, and then dehydrated to 70 per cent. alcohol when differentiation was carried out in dilute acid alcohol. After washing in three changes of 70 per cent. alcohol the specimen was 'blued' in a weak solution of ammonia in 70 per cent. alcohol for two hours before being dehydrated, cleared and mounted in the manner described above.

Adapting a technique described by Perkins (1956), several adults were each placed in polyvinyl alcohol, in which some chlorazol black E had been dissolved, on a microscope slide and a cover-slip placed immediately on top. Some of these preparations were slightly squashed and some were not. This stain was employed to investigate the distribution, if any, of chitin in the tissues of N. kilrymontis. It is a very straightforward technique to use since the fixation, staining, and mounting are all carried out in the same medium. The results obtained by this technique were conclusive and will be discussed later.

The same reagent, chlorazol black E, was dissolved in Berlese's gum chloral (Vade Mecum, 1950) and the same technique applied. This did not give such good results, although Berlese's fluid seems to be a better agent for the clearing of non-stained tissues, the chlorazol black E did not appear to stain the chitinous structures as well as when dissolved in polyvinyl alcohol.

5. Histological Techniques

After fixation and washing (where necessary), whole parasitised whelks, pieces of host with the parasite embedded, and dissected parasites, were dehydrated, cleared, and embedded in paraffin wax (54°C. M.P.) in the usual way. It was found necessary to keep the whole whelks in the molten wax in the oven for up to three hours to ensure full penetration of the tissues, whereas 40 minutes usually sufficed for the dissected parasite.

The embedded material was made into blocks and serial sections were cut at thicknesses ranging from 5 μ to 25 μ using a Leitz Rotary microtome. The pieces of ribbon thus obtained were mounted on microscope slides using a dilute gelatine-bichromate solution in a bath maintained at 45° to 48° C.

Several different staining techniques were employed with the sectioned material. Both haematoxylin methods described above for the squashed preparations were used, although the times of immersion of the sections in the haematoxylin solutions were shorter:- two minutes for Mayer's haemalum, and 12 hours for Erlich's haematoxylin. The method most frequently used was Mallory's triple technique (Vade Mecum, 1950) and it proved a most reliable general-purpose stain. It was, however, incompatible with formaldehyde-fixed material, the acid fuchsin not being taken up by the tissues in these cases.

Some sections were stained by the Heidenhain Iron Haematoxylin method (Vade Mecum, 1950) and counter-stained with eosin; this method gave very good differentiation between nuclear and cytoplasmic materials. However it proved no better than Mayer's haemalum counter-stained with eosin

(described above), and since this latter method is less complicated it was more frequently used.

Some of the dissected parasites were embedded in celloidin and sections cut at 50 μ and 100 μ using a Jung sledge microtome following the technique described by Pantin, 1959. It was hoped that this would provide a means of building a composite picture of the parasite from serial sections since there were less sections involved than with the material embedded in paraffin wax. However the staining techniques attempted did not prove at all successful. Both the Mallory triple stain and chlorazol black E stain advised by Pantin did not give satisfactory results and the technique was abandoned.

The ovaries, oviducts, testes, and portions of the egg-string of several specimens were dissected out and fixed in Carnoy's fluid (Vade Mecum, 1950) for 30 minutes. These pieces of tissue were then placed on microscope slides and flooded with a freshly-filtered solution of 1 per cent. orcein in 45 per cent. acetic acid and left for 15 minutes. A coverslip was then placed on each piece of tissue and compressed strongly between two filter papers (to remove excess fluid), using the thumbs. By this means some satisfactory temporary squash preparations displaying chromosomes were obtained. When reasonable preparations were obtained, the coverslips were ringed with vaseline in order to preserve them for several weeks.

6. Treatment of Larvae

Larvae reared experimentally in the cold-room were subjected to different techniques in their preparation for microscopic examination.

Alcoholic sublimate (Vade Mecum, 1950) was the first fixative tried. It was not altogether satisfactory in that considerable precipitation occurred, due perhaps to its reaction with salts present in the sea-water. It was abandoned in favour of the less complicated Carnoy method (Vade Mecum, 1950) which does not involve mercuric chloride and hence requires no subsequent washings in iodine solution as are necessary with alcoholic sublimate. The more simple method was obviously preferable in view of the small size of the larvae, but apart from this consideration, Carnoy's fluid proved to be the better of the two fixatives. Bouin's fluid (Vade Mecum, 1950) recommended by Heegaard (1947) for copepod larvae, also proved a very good fixative.

After fixation some larvae were stained in one or other of the two haematoxylin stains described above for the adult parasite, the times of immersion in the various solutions being the same as for the sectioned adult material. Mallory's triple stain was also used, but although this method, and the two haematoxylin techniques, successfully stained the larvae, the results were not satisfactory due to the thickness of the larvae, and very little detail could be distinguished in the specimens so stained.

In view of the small size of the larvae of N. kilrymontis, for sectioning purposes I adopted a technique used by Dr. J. S. Scott in this department for sectioning small cysts of Paricterotaenia paradoxa (Rudolphi 1802) (Cestoda). The larvae, after they had been dehydrated and placed in cedar wood oil, were placed in the empty puparia of Drosophila melanogaster, previously dehydrated and kept in cedar wood oil. The pupa containing the

larvae is then embedded in wax and sectioned as for normal wax blocks. The results obtained were not satisfactory - the larvae collapsed on sectioning due to poor wax penetration, this because of the impermeability of the chitinous exoskeleton.

The technique described by Daniel (1927) for staining the muscular systems of crustacea was employed with a view to tracing the development of the muscles through the various larval stages. After complete dehydration the larvae are placed in a 0.05 per cent. solution of benzoquinone in absolute alcohol and left over-night. They are then taken through a mixture of equal parts of absolute alcohol and methyl salicylate to pure methyl salicylate and finally mounted in Canada balsam. Daniel used this method on the shrimp, Crangon vulgaris, and obtained excellent results, the muscles being stained red while the remainder of the animal was transparent. My results were disappointing, although the muscles did take up the stain they became brown in colour, not red as in Crangon, furthermore, the other tissues of the body also took up the same brown colouration, to varying extents, so that in most cases it was difficult to distinguish the muscle bands. Daniel used the same technique on various crustacea and found that they took up the stain to different degrees, none of them being as distinct as in Crangon. He states that there are individual differences in reaction to the stain and suggests that the concentration of the benzoquinone solution should be varied for different crustacea. Although I tried two further concentrations, 0.01 per cent. and 0.1 per cent., the results were no better than for the 0.05 per cent. solution.

In many cases larvae were placed directly into a dilute solution of

acid fuchsin in polyvinyl alcohol on a microscope slide and a cover-slip lowered onto the preparation. As stated earlier, this fluid acts as fixative, clearing agent and mountant, and is accordingly a most convenient method to employ. After the first few attempts with this method it was found that the larvae were difficult to locate on the slide and, furthermore, their structures were indistinct, being poorly stained. The method described above for adult preparations, involving chlorazol black E dissolved in the polyvinyl alcohol, was finally adopted. The chitin present in the body wall of the larvae readily took up the blue-black stain as did the appendages, although to a somewhat lesser degree. Again this stain was used in solution in Berlese fluid and the results were again not as good as when dissolved in polyvinyl alcohol.

I tried the technique used by Cannon (1941) and described by Gurr (1960) in his Encyclopaeidia of Microscopic Stains. This involves both lignin pink and chlorazol black E in solution in distilled water. After fixing the larvae for 18 to 48 hours in sea-water Bouin and thoroughly washing out the picric acid in 50 and 70 per cent. alcohols, followed by washing in running water to remove all traces of alcohol, the specimens were immersed in the stain for 15 minutes. This method also gives a distinct demonstration of chitin. I also dissolved these two dyes in both polyvinyl alcohol and Berlese fluid as in the method described above. I hoped, by using these methods, that the appendages of the larvae would be stained to a better degree than that obtained using chlorazol black E alone. This expectation was not however realised, the quality of staining being no better than that previously obtained. Accordingly the examinations and drawings of the

larvae and their appendages were all made from live specimens mounted in Berkefeld-filtered sea-water under a cover-slip. I found that using welled slides the larvae were free to move about and were consequently virtually impossible to draw using a camera lucida. The method I adopted was to use an ordinary microscope slide and "trap" the larvae between it and the cover-slip without causing any compression of the specimens and hence avoiding distorting them.

7. Drawings and Photographs

Drawings were made with the aid of a Cooke, Troughton and Simms camera lucida. Photographs were taken using either an Edixa Standard 35 mm camera or a Cooke, Troughton and Simms 35 mm single frame camera attached to the microscope.

II EXPERIMENTAL PROCEDURES

In an attempt to obtain all the stages of the life cycle of N. kilrymontis, several series of experiments were carried out in a constant temperature room. The temperature of this room was maintained at a steady 10°C., varying occasionally within the limits of 7°C. and 12°C.

I thought it advisable to try and simulate natural conditions as far as possible, and Dr. C. Muir of this department kindly constructed an automatic time switch which, operating a lamp above the experimental tanks, produced 12 hours of light alternating with 12 hours of darkness every 24 hours.

In early experiments I used two large aquaria (24 inches long by 12 inches broad by 15 inches high) filled with sea-water which had been filtered through Whatman No. 1 filter paper. In each tank was suspended a bank of eight pyrex tubes (6 inches long by 2 inches diameter), open at both ends. The tubes were supported by non-corrosive polythene-coated spring clips screwed to a horizontal wooden bar. Each tube contained four whelks and a fine mesh gauze (gauge 185) was held in place over the bottom of each tube by an elastic band, the top of each tube being covered by a coarse mesh gauze in the same manner. Thus the larvae of N. kilrymontis could not escape through the bottom of the tube and the host specimens could not escape through the top. Each tube was so placed that the bottom half was submerged while the top half was above the water surface.

I did think of the effects of the tide on the host and considered constructing some device for rhythmically raising and lowering either the

water level or the tubes. However the whelks could move about inside the tubes and be either submerged or above the water level. In fact I had observed that they passed most of the time above the water level in this and all subsequent experiments and consequently I abandoned the idea of a "tide machine".

Initially I was concerned over the problem of a food supply for the whelks. According to Moore (1938), Nucella lapillus feeds on either the barnacles Balanus balanoides or Chthamalus stellatus, or the mussel Mytilus edulis, depending on which is most abundant in any particular area. The whelks I used for experimental purposes were taken from rocks in the Bay of St. Andrews and were observed to be feeding on B. balanoides. Moore states that N. lapillus will change from one food supply to the other but that this change is a slow process. In the first instance I used small specimens of Mytilus edulis gathered from the same locality as the whelks. There were two reasons for this choice: first, specimens of M. edulis are easy to obtain singly whereas B. balanoides are found as clusters on rock surfaces and are impossible to remove intact without also taking some of the rock substratum. Secondly, M. edulis was a much "cleaner" food source, since the rock fragments taken with B. balanoides contained many impurities.

I found that neither animal would survive for more than 48 hours in the tubes - they are both filter-feeders - and during this time they were not attacked by the whelks. I finally decided to abandon entirely the idea of a food supply and in fact this appeared to have no adverse effect on the whelks. Some whelks were even maintained without a food supply for

as long as eight months. On removing the shells of these whelks after this period I found that the outward appearance of the organs was quite normal, except in some instances where the digestive gland of a few specimens appeared to be reduced in size.

Perhaps the most important factor in all of the experiments was the condition of the sea-water used. Since there was no available supply of running sea-water in the department I brought it in polythene containers from the Gatty Marine Laboratory to the cold room.

The difficulties involved in keeping marine animals in stagnant sea-water are obvious; in the tanks where I kept the specimens of N. lapillus I used "Reliant" aeration-filtration corner units in order to keep the water both aerated and as clean as possible. In this apparatus air is blown down a small-bore tube, the end of which is well below the water surface and is upturned into the bottom of a wider tube. The air passes up this latter tube and carries water with it. This water is expelled onto a wad of glass wool through which it passes to the perforated base of the container and hence returns to the water in the tank. The entire apparatus is supported at a corner of the tank by two suction cups.

Using this apparatus it was found that the whelks could be kept alive for several months in non-filtered sea-water. Generally, however, it was seen that much detritus accumulated on the bottoms of the tanks within a few weeks and accordingly the water in each tank was changed every four weeks, as was the glass wool pad. The latter by this time was usually covered with a brown alga. In each tank the water level was marked at the

beginning of each experiment and the level was maintained at this mark by the addition of distilled water to compensate for evaporation losses.

Sea-water filtered through Whatman No. 1 filter paper was also employed in these large tanks but this did not appear to confer any advantage over un-filtered sea-water and was finally discarded in favour of the latter. In fact N. lapillus is a most robust animal and will survive quite wide fluctuations of salinity and oxygen tension. In support of this I can add the following observation.

About 30 specimens of N. lapillus were placed in a tank (14" long by 9" broad and 9" high) in un-filtered sea-water and without a corner unit, only a normal aeration candle being immersed in the water. These whelks survived in the same water for seven months (distilled water being added from time to time to counter losses by evaporation) during which time about 40 egg cases were deposited on the sides of the tank. Furthermore these egg cases were viable and young N. lapillus specimens were seen to emerge from them. By the end of the seven-month period the water was still quite clear although the bottom and sides of the tank were covered with green and brown algae. Only three of the original 30 whelks died during these seven months; the remainder were removed from their shells in the normal manner and they all appeared to be quite healthy, although none was subjected to histological techniques and examined microscopically.

The early experiments using Pyrex tubes half-immersed in sea-water were designed to show which whelks were parasitised (by observing the emergence of metanauplii from the parasitised hosts) and the behaviour of

the larvae once hatched. It was soon realised that the apparatus was not only clumsy but unnecessary. Further, where larvae were present in a tube they were virtually impossible to detect once they had settled on the white gauze on the bottom of the tube.

The detection of parasitised whelks was readily accomplished by placing four in a 4½" diameter Pyrex bowl in sea-water, with the top of the bowl almost completely covered by a glass plate, a small gap being left to afford an air supply. After 24 hours the water in the bowl was examined, under a binocular dissection microscope, for the presence of larvae. If these were found then the whelks were divided into two lots of two whelks and each lot placed in a separate bowl. These were again examined after 24 hours and when larvae were present the two whelks were each placed in a single bowl. After a further 24 hours those whelks not producing larvae were eliminated and there remained a definite known source of N. kilrymontis. This technique was carried out several times on a large scale; usually 40 whelks were taken at the beginning and, due to the fairly high rate of infestation, these often produced between 15 and 20 parasitised specimens.

Having thus obtained a source of larvae I was now faced with the problem of keeping them alive so that they might undergo their normal metamorphoses. Since the larvae are delicate in structure they are readily attacked by bacteria, especially during the periods when they are at rest on the bottom of the container prior to, and during, a moult. It was found that using non-filtered sea-water very few cases of development to the first copepodid stage were obtained.

The larvae were kept in small containers, 14 cm. diameter by 4 cm. high petri dishes and small Pyrex bowls ($4\frac{1}{2}$" diameter), all being covered with either glass plates or inverted Pyrex bowls to keep out dust but leaving a ready access for air. Due to the fact that these vessels afforded a large surface area of water it was thought unnecessary to aerate the water. It was not realised at first that some water movement is necessary, since accumulation of the larvae on the bottom causes overcrowding and subsequent mortality. Consequently, in later experiments, the $4\frac{1}{2}$" diameter Pyrex bowls were well filled with water and air was slowly bubbled in though a fine glass tube. This had the double effect of providing some water circulation together with aeration, the latter now being deemed necessary due to the increase in water volume without an accompanying increase in surface area. I found, as did Heegard (1947), that too much aeration resulted in high mortality among the larvae and consequently the rate of air flow through the glass tube required fine adjustment.

The water first used in these experiments was filtered through Whatman No. 1 filter paper. This did not seem to have any advantages over un-filtered sea-water, the bacteria passing quite freely through the filter. I therefore used a Berkefeld Filtration Apparatus, the sea-water being first passed through Whatman No. 1 filter paper and then through the Berkefeld filter to remove bacteria. Using this water, development to the copepodid stages was readily accomplished.

A further attempt to keep down bacteria was the use of antibiotics following the technique used by Shelbourne (1963) for the hatching of plaice (Pleuronectes platessa, L) eggs. This involves the use of a sodium

penicillin G and streptomycin sulphate mixture (50 international units and 0.05 mg/ml respectively). These salts were dissolved in Berkefeld-filtered sea-water and, using the same Pyrex bowls, this time thoroughly sterilized in an oven at 130°C., the larvae were introduced. All glass-ware involved was sterilized and the larvae were washed several times in different changes of Berkefeld-filtered sea-water before being placed in the anti-biotic medium. Although these precautions were taken, larval development did not proceed any further by this method than by the one using untreated Berkefeld-filtered sea-water.

I also used Berkefeld-filtered sea-water which had been pasteurised by heating to 60°C. and then slowly cooled to the cold-room temperature; both plain and 'antibiotic' Berkefeld-filtered sea-water were pasteurised, but again in these media larval development did not proceed further than when untreated Berkefeld-filtered sea-water was used.

Moyse (1960) found, when rearing barnacle larvae in the laboratory, that the bubbling of air from a glass jet to keep the larvae moving and their food supply (diatoms) suspended, did not produce significantly better results than in stagnant cultures where satisfactory conditions have been established. Similarly he found that penicillin and streptomycin did not produce significantly better results when added to healthy cultures, although they help in poor culture conditions where many larvae have died.

My results generally support these observations, the main exception being that of water agitation. I found that when the water was not agitated then the larvae settled on the bottom of the container and when many larvae were present this produced overcrowding and a high mortality with consequent

bacterial growth. In using diatom and flagellate cultures as possible food supplies (see section IX) each day I stirred the water in the containers with a glass rod - a technique used by Moyse, but although this was sufficient to keep the diatoms and flagellates suspended it did not in any way assist the development of the copepodid larvae.

The standard method finally adopted for rearing the larvae was that of untreated Berkefeld-filtered sea-water with gentle bubbling and daily water change, with the addition that, before introduction to the observation bowl, the larvae were thoroughly washed in several changes of Berkefeld-filtered sea-water.

Miss Murdoch detected slight movements in the interior of the cephalic region of the second copepodid stage and thought that these might be the movements of the gut contents as has been sometimes observed in other crustacea. She commenced experiments on the feeding of these larvae using cultures of Nitzschia closterium and Chlamydomonas sp., obtained from the Gatty Marine Laboratory, but was unfortunately unable to make any observations.

I decided to follow this line of investigation and six cultures (three diatoms and three flagellates) were kindly supplied by Dr. M. Parke of the Plymouth Marine Laboratory. Stock cultures of these organisms were set up and maintained in the same cold-room as used for the experiments on Nucella lapillus and N. kilrymontis. These stocks were sub-cultured every month using the "Erd-Schreiber" culture solution as prepared at the Plymouth Laboratory, all the usual sterilizing precautions being observed. The results of these investigations, together with other experiments carried out in the cold-room, will be discussed in section IV.

III THE MATURE ADULT

1. Habitat

The adult of N. kilrymontis is found in the tissues of Nucella (Purpura) lapillus (L.), the common dog-whelk of rocky shores, and is fully endoparasitic in nature. The most frequent sites for the adult are found to be the digestive gland and the junction of this gland with the gonad (Plate 1, figs 1 and 2). However I have found specimens in almost all of the tissues of the host.

2. External appearance

The body of the parasite is semi-transparent and the cement glands and oviducts within it can usually be seen through the integument as white structures (Plate 2, fig. 3). The covering of the egg-string is fully transparent and the eggs and embryos are clearly visible. N. kilrymontis is cylindrical in shape although its axis is more often curved to varying degrees and occasionally even 'S'-shaped (Plate 2, figs 3 and 4). The egg-string arises at the posterior end of the female (Plate 2, fig. 4), and no matter where the body of N. kilrymontis is situated in the host, the egg-string of a mature parasite passes towards the mantle of the host (Plate 1, figs 1 and 2), where it terminates. It coils unceasingly from beginning to end (Plate 1, fig. 2) and is full of eggs and developing embryos arranged in a multiseriate manner. In the young part of the egg-string one can see the spherical eggs and in the oldest part the young larvae can be seen performing a twitching motion.

Posterior to the point of origin of the egg-string, and situated beneath the integument of the female, one or more dwarf males can be seen. In live specimens these are only detected under the microscope, the parasite being slightly squashed and mounted in filtered sea-water under a cover-slip. When viewed under the binocular dissection microscope the male appears as a small knob at the posterior end of the female (Plate 2, fig. 3).

3. Dimensions

There is great diversity in the sizes of different specimens of N. kilrymontis. Using the Vernier scale on the microscope stage, measurements were taken of several unsquashed adults and the following results were obtained: 36 mature females (including their males) varied between the limits 2.5 mm to 7.1 mm long by 0.5 mm to 1.8 mm broad. These adults were mature in that they originally had egg-strings attached which contained active embryos. Three females (including their males) which had no egg-strings and were thus not fully mature, varied between the limits 1.7 mm to 5.2 mm long by 0.7 mm to 1.0 mm broad. Two immature females which had neither egg-strings nor males, measured 2.4 mm long by 0.5 mm broad, and 2.7 mm long by 0.7 mm broad. In all cases the breadth was measured across the anterior portion of the female, this more often than not proving, where there was any variation, to be the broadest part.

From the above measurements it can be seen that there is no sharp division, with respect to size, between the three stages of the adult, namely the mature female with one or more males, the immature female with

male, and the immature female without male. There is, in fact, some considerable overlap in the lengths and breadths of these three stages. It is regrettable that due to the paucity of immature specimens of N. kilrymontis I was unable to obtain sufficient measurements to carry out a statistical analysis of all three stages. The immature forms are considered in section IV and are not discussed in this section.

Fourteen males which had been dissected out of their females were measured. They varied between the limits 0.53 mm to 0.69 mm long by 0.34 mm to 0.42 mm broad.

I am unable to give any exact measurements for the egg-strings due to the impossibility in dissecting one intact. They are much longer than the body of N. kilrymontis (Plate 1, fig. 2.) and I estimate that they can be as much as ten times the body-length, i.e. in the region of 50 mm to 70 mm. They appear to be longer and broader in specimens where there is more than one male.

There is no correlation between the size of N. kilrymontis and the size of the host, nor is there any difference in the sizes of parasites taken from male and female hosts.

4. Body Wall

The appearance of N. kilrymontis conveys little or no suggestion of a typical copepod; if anything one would suspect, from its external appearance, that it had affinities with the Trematodes, were it not for the presence of the egg-string.

The integument is of a soft nature over the whole of the body; there is no indication of the presence of hardened chitinous structures of any description. By using the technique involving polyvinyl alcohol and chlorazol black E, I was able to show definitely that there is in fact, no trace of chitin in the body wall. Only two structures take up the deep blue stain thereby displaying the presence of chitin; a pair of appendages in the adult male, and the exuviae of the male, situated between the integument of the female and the adult male (Plate 6, figs 10 and 11).

As stated earlier, there are two integuments surrounding N. kilrymontis: the outer one surrounds the complete body and egg-string and is of a delicate nature. Observations of stained sections of mature adults using the X100 oil immersion lens show that this integument has no identifiable form of cellular organisation. Similarly the inner integument, which surrounds only the body of the adult, has no apparent structure under the light microscope. The two integuments are merely sheets of tissue surrounding the parasite. In sections of younger adults the integuments are noticeably thicker and apparently do have some level of organisation; this is discussed later.

When I first viewed the adult under the microscope, I was unable to determine which was the anterior end. I was not aware that the male did exist associated with the female. Murdoch also had difficulty with this and did not in fact reach a conclusion as to which was the anterior, and which the posterior end of the parasite. When I examined sections stained with haematoxylin I was able to detect the presence of spermatozoa in what proved to be the testes and the vas deferens, and this finally resolved the problem. In fact the male is a separate organism and can be completely

removed from the female, if care is taken not to rupture the delicate integument which surrounds it.

The internal organisation of the male and female are discussed separately.

5. The Female

Within the integument of the mature female there are only four types of organ. These are the ovaries, the oviducts, the cement glands and the accessory glands. These are all suspended in the haemocoel which in live specimens is a colourless fluid, but when fixed and stained it is seen to contain nucleated cells. The female, in fact, is virtually reduced to the level of a genital segment.

There are two ovaries (Plate 3, fig. 5.e) which, in the living state appear as completely transparent, colourless organs which stain deeply with haematoxylin (Plate 4, fig. 6 and Plate 5, figs 8 and 9). The ovaries are generally situated in the posterior half of the female and are completely separate. Each ovary is continuous with an oviduct and there is no sharp distinction between the gland and its duct. I define the posterior limit of the ovary as that point where the formation of yolk commences in the oocytes but this is merely a matter of convenience in nomenclature. It is possible that the entire tube which I have named ovary and oviduct could be the ovary alone, although the arrangement of the oviduct is so closely similar to the maturation tubules (=oviducts) of other parasitic copepods, notably Xenocoeloma brumpti Caullery and Mesnil, that I feel

justified in making this distinction.

In the ovary the cells are tightly packed together and each cell has a large nucleus, containing a large central nucleolus, with little cytoplasm, thus accounting for the intense staining reaction with haematoxylin. I have been unable here to distinguish between such cells as oogonia and oocytes, although in some of the sections I noticed that there appeared to be some trace of a central lumen in the ovary. This would suggest that the cells on the outside of the ovary, i.e. those nearest its outer wall, could be oogonia, and the cells towards the centre could be the developing oocytes.

In mature females the oviducts are packed with oocytes from the level of the ovary to their termination in the cement gland. I have assumed that, since there is a steady process of egg-delivery into the egg-string, there must be a continuous movement of oocytes along the oviducts towards the cement glands. This is borne out by the observation that at different levels further and further away from the ovary, the oocytes are larger in size. This is due to the fact that on leaving the ovary each oocyte begins to lay down yolk in the cytoplasm, and this process is apparently continuous all the way to the cement gland. Towards the distal end of the oviduct nuclei are rarely visible in the oocytes due to the massive accumulation of yolk.

The oviducts, as was the case for the ovaries, are completely separated from each other. They do, however, follow the same route, that of undulating to the anterior region of the body and then returning in the opposite direction, passing the ovaries and finally terminating in the cement glands

at the posterior end of the body (Plate 3, fig. 5b). Each oviduct twists and turns on its route so that when dissected out of the body and completely unravelled, they are each more than twice the body length.

Both the ovaries and oviducts are thin-walled in mature adults whereas the cement glands have thick walls. The two cement glands are pear-shaped and, in comparison with the normal copepod arrangement, they unite to form a single duct, the vagina or metraterm, through which the eggs pass to the exterior.

Each cement gland has a thick wall consisting of elongated cells in which the large nuclei are situated at the outer border of the gland (Plate 14, fig. 31). At the inner border of the gland the cells stain deeply with haematoxylin (Plate 14, fig. 31), and this suggests the presence of mitochondria involved in some secretory mechanism (in some way similar to the arrangement of mitochondria in mammalian kidney tubule cells). Using Mallory's triple stain a blue-red colour is seen in the lumen of these glands (Plate 7, fig. 12), which could perhaps be the actual secretion itself since it displays no cellular structure. However the possibility that this is a precipitate formed during staining must not be discounted, although all washings between and after the different stains were thoroughly carried out.

Although this body is called the cement gland it must be remembered that it is here that fertilization of the oocyte occurs. In the lumen, and mostly at the anterior end of the gland in mature specimens stained with haematoxylin, sperms are abundant (Plate 14, figs 30 and 31). The

oocyte must pass through this region; thus the cement glands might be considered as a pair of receptacula seminales. In Caligidae the receptacula are medially joined and open into a single egg canal. Furthermore, the cement glands of parasitic copepods are normally found as diverticula of the oviduct. However the walls of this gland bear a great resemblance to the cement glands of other parasitic copepods, notably Lernanthropus, and, moreover, if it is a receptaculum seminis then I cannot think of a function for its thick wall.

The fertilized oocytes pass singly through this gland down through the vagina to the egg-string, and the eggs do not adhere to each other until they arrive in the egg-string itself. It is perhaps the case that each egg is covered with some secretion as it passes through the cement gland and that this secretion aids the adhesion of the eggs as they enter the egg-string.

There is another type of gland in this region which I name the accessory gland. It is a racemose gland consisting of a large number of secretory cells which open into the end of a short duct (Plate 8, figs 14 and 15; Plate 9, figs 16 to 18). The secretory cells are slightly elongated and surround the lumen of the gland. There is a long filament attached to each cell on the end furthest away from the lumen (Plate 8, figs 14 and 15). A narrow duct leads from each accessory gland to connect either with the posterior end of the cement gland or directly with the vagina. In most of the mature specimens examined there were between four and six of these glands.

The accessory glands are obviously secretory in function - sometimes they appear to be distended (Plate 11, fig 22) and other times they are empty. They are not visible in unstained preparations nor are they seen in the live animal; sometimes they do not appear even in stained preparations. The true nature of their action is unknown; it is possible that they might even be the cement glands, if the cement glands described above are really the sperm receptacles, although their structure is dissimilar to the normal cement glands of parasitic copepods. One possibility is that they secrete a substance which activates or catalyses the secretion of the cement glands which surrounds the egg so that it becomes 'sticky' as it passes into the egg-string.

6. <u>The Male</u> (Plate 12, figs 24 and 25)

The somewhat ovoid male is situated beneath the integument at the posterior end of the female. There may be as many as five males attached to one female in this same region, and in fact the occurrence of two or more males is as frequent as that of only one male. In cases where more than one male occurs, the egg-string appears to be much larger and to contain more eggs and embryos. There is no obvious reason for this, especially since the presence of two or more males does not appear to influence the rate of oocyte production.

Although the male, like the female, is reduced almost to a system entirely concerned with reproduction, it does display more organisation than the female. The number of appendages occurring in the copepodid stages is reduced in the adult male to one pair; in the adult female there

is none. Anterior and dorsal to these appendages are two pairs of lobes which are the modified first and second antennae, the larger pair being the first antennae. There is a small projection anterior and dorsal to the first antennae which I call the rostrum. I can ascribe only one function to the modified first antennae, that of acting as a form of anchorage for the male in the female by virtue of their shape and bulk. The modified second antennae might also be involved in anchorage although they are very small by comparison with the first antennae.

I identify the sub-chelate chitinous appendages (Plate 13, figs 26 and 27) as the maxillipeds for two reasons. First they are so similar to the maxillipeds of the second copepodid stage that they can only have been derived from these. Secondly, they are closely similar in structure to the maxillipeds of many Lernaeopodidae, notably the genera Achtheres, Clavella and Brachiella.

There is a considerable supporting network of chitin associated with the maxillipeds and this is apparent at the anterior end of the male. This network is the endophragma to which the muscles of the maxillipeds are attached, but in the mature adult male these muscles are no longer apparent. It is difficult to see the maxillipeds in living specimens unless they are squashed beneath a cover-slip, and it is virtually impossible to learn the nature of their function. It appears that, as in Lernaeopodidae, they act as organs of attachment, and are concerned with maintaining the male in its position close to the vagina. In Lernaeopodidae the male uses the maxillipeds, in conjunction with the second maxillae, as organs of attachment for clinging to the female.

The organs within the body of the male are concerned solely with reproduction. The two short pyriform testes (Plate 12, figs 24 and 25h) are situated dorsally at the posterior end of the body and are the largest structures in the male. They are entirely independent of each other and each gives rise to a vas deferens which passes dorsally to the anterior end of the body. The walls of the vas deferens consist of large cells with large nuclei and surround a lumen which invariably contains spermatozoa. The spermatozoa can also be seen accumulating in the testis around the beginning of the vas deferens.

At the anterior end of the male the vas deferens turns ventrally and in this region the cells of which its wall is composed become much hypertrophied forming an almost spherical region of the duct (Plate 12, fig. 25e). The nuclei of these cells are also larger than elsewhere in the vas deferens and the cells themselves have the appearance of secretory cells. It is in this modified area of the vas deferens that the spermatozoa are accumulated into spermatophores (Plate 13, fig. 28). This, then, is the region of spermatophore formation, a modification of the vas deferens which occurs frequently in parasitic copepods, in which group the possession of definite spermatophores seems to be a universal character (Calman, 1909).

The spermatophores are crescent-shaped and each contains many elongate pyriform spermatozoa. The spermatozoa are 5.4 μ to 7.5 μ long (from the measurements of ten spermatozoa), and each has a flagellum 3 μ to 3.5 μ long (five measurements). The flagellum is not always detectible with the light microscope, but is obvious when viewed under the phase contrast microscope. The spermatophores vary between 120 μ to 200 μ long by 16.5 μ to 26.5 μ at their broadest diameters.

The spermatophores pass from their region of formation through a thin-walled tube to one of two ventral pores on the antero-lateral border of the body, just posterior to, and to each side of, the maxillipeds (Plate 12, fig. 25).

Sperm transference and Fertilization

Although I have never observed the actual mode of transference of the spermatophores from the male to the female, on several occasions I have noticed that the rostrum of the male projects into the vagina (Plate 11, fig. 23b) and I assume that the spermatophores are released into this canal, there being no other duct in this region. It is not apparent when the spermatozoa are actually released from the spermatophores as I have never detected a spermatophore in any part of the female. Calman (1909) states that the spermatophore contains a substance which by its expansion serves to expel the spermatozoa, and that usually there is a mass of cementing substance surrounding the "neck" of the spermatophore for attaching the latter to the copulatory aperture of the female. Neither of these substances is apparent in N. kilrymontis. Since only free spermatozoa are found in the female it must be that they are released as soon as the spermatophore is introduced into the vagina.

In cases where there are more than one male, spermatophores are sometimes seen dispersed outside the male from which they arose (Plate 4, fig. 7 and Plate 14, fig. 30a).

There are three possible reasons for this phenomenon. First, the female exerts some control over the number of spermatophores she can accept

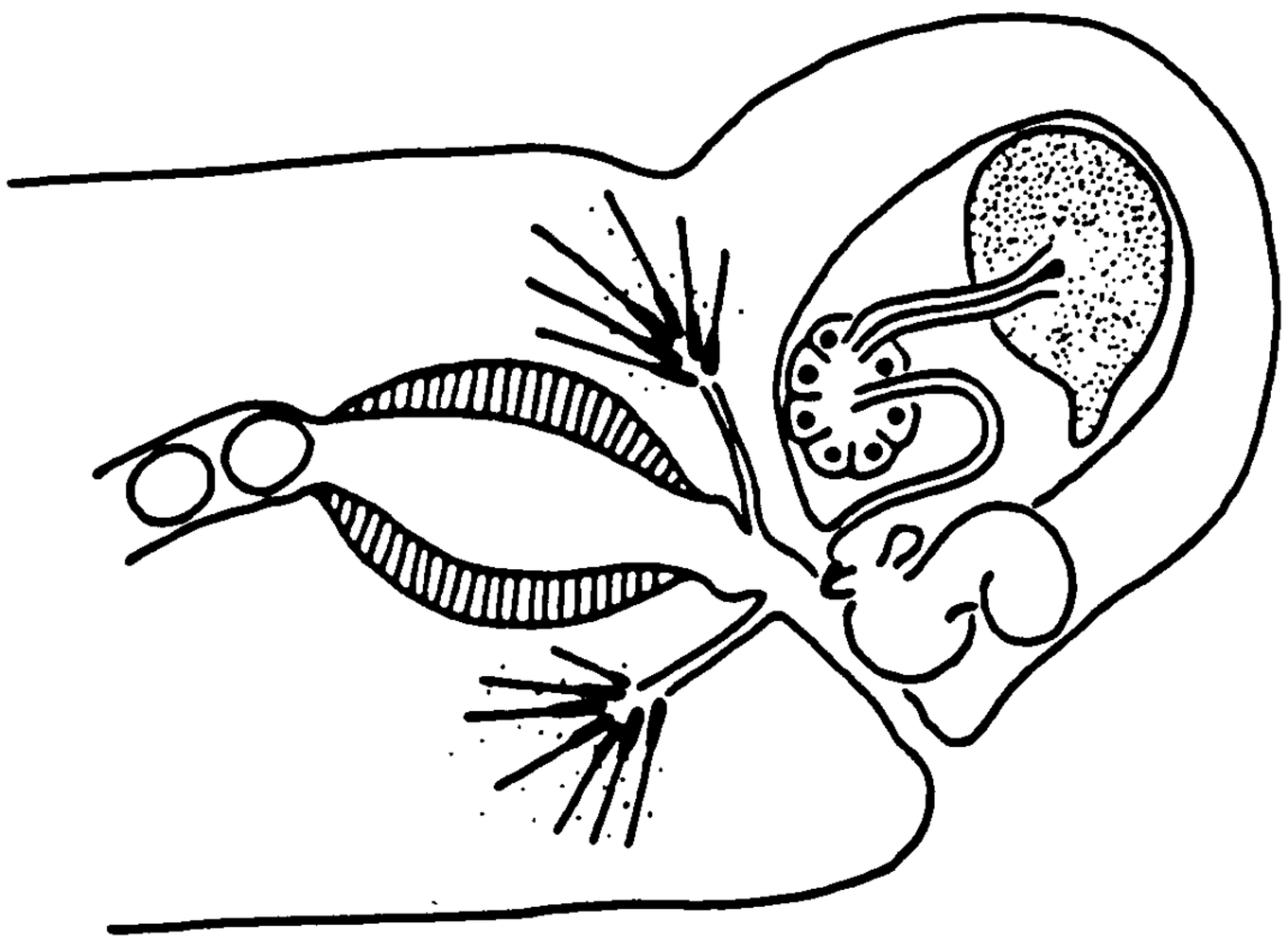
at any time; secondly, the first male to arrive and develop fertilizes the female and no further fertilization takes place, any other males producing spermatophores after this are thereby redundant and there is no demand for their spermatophores; thirdly, there is a small limited area where the males can effectively place themselves in relation to the vagina and thus only one or two will successfully release their spermatophores, the remaining males being out of position thereby having no means of transferring their spermatophores.

I believe that a union of the latter two suggestions is the most plausible for these reasons. First, one fertilization, or one donation of spermatophores, is a common phenomenon among parasitic copepods. Secondly, once egg production has commenced it would be difficult for the spermatophores to pass up the vagina when the eggs are passing down, although the vagina does not always contain an egg. Furthermore it must be accepted that individual spermatozoa could pass up the vagina if they had previously escaped from their spermatophore and this view is supported by the observation that spermatophores have never been seen in the female. Thirdly, the very presence of spermatophores around some of the males suggests that they have no means of access. Fourthly, in cases where there are four or five males, never more than two rostra have been observed in the region of the vagina, and these corresponded to the males which did not have spermatophores around them.

It is difficult to see how one fertilization can be effective enough to confer life-long fertility on the female. I have maintained parasitised

whelks for as long as four months in the cold room and during this time they continually produced larvae at a rate varying between 10 and 100 per day. This represents an egg production of between 1,200 and 12,000 during the period and I have never seen more than 200 to 400 spermatozoa in one cement gland at any time. It is perhaps more probable that a re-injection of spermatophores occurs from time to time but this has not been seen in my material.

The orientation of the male to the female is such that the ventral surface of the male is closely applied to the posterior surface of the female in such a manner that the openings of the vasa deferentia and the rostrum, or part of the rostrum, are all applied to the dorsal wall of the vagina. This is shown diagrammatically in Text fig. 1.



TEXT FIG. 1.

There are no other structures within the male, apart from a central mass of oil droplets, lying between the vasa deferentia, which are seen in some living specimens when viewed under the microscope (Plate 12, fig. 24). This mass does not stain by any of the methods I have used and its nature is, accordingly, not determined.

The only remaining structure concerning the mature adult male is the empty exoskeleton of the final male copepodid stage - the male exuviae. I concluded that these were the exuviae of the final copepodid stage of the male since, in specimens containing more than one male, there are exuviae for each male present (Plate 6, figs 10 and 11). A description of the exuviae is given in section IV.

7. The Egg-string

Little mention of the egg-string will be made at this stage. The integument surrounding it is continuous with the outer integument of the female, and the eggs inside are bound together by some secretion, although this is not a very strong adhesive since the eggs freely float apart in Berkefeld-filtered sea-water in the dissection dish after rupture of the integument.

One point worthy of note is the occurrence in N. kilrymontis of only one egg-string. The normal arrangement in copepods is that the oviducts are separate and lead to two openings, usually laterally situated on the genital segment, to each of which is attached a single egg-string or egg-sac. This is a striking difference between other known free-living, commensal, phoretic and parasitic copepods.

It often happens that two or more females are found in one host and these may be close together in the same tissue or in completely separate parts of the body. The highest number of females that I have recorded from one host is six, and the occurrence of two or three females in one host is frequent. In such cases the volume of bodies and egg-strings of N. kilrymontis, especially the latter, can be quite high, and the parasites can occupy more than 50 per cent. of the volume of the visceral mass. When this occurs the egg-strings from the different closely situated females generally coil around each other and pass together to the mantle. When the females are quite widely situated in different parts of the host then, generally, each egg-string passes independently to the mantle.

8. Nutrition

In neither the adult male nor the female is there any evidence of a digestive tract or feeding mechanism. There appears to be only one reasonable explanation here - that the adult satisfies its food requirements by absorption from the host's tissues. It is not possible that, as happens in the Monstrilloida, the fully developed adult must subsist upon nourishment accumulated during its juvenile stages. The vast difference in size between the adult and the larval stages opposes this view; furthermore, much energy must be expended by N. kilrymontis as it enters the host and develops into the adult form, apart from the energy required for the high rate of egg-production maintained by the adult. In any case in the Monstrilloida the juvenile stages are parasitic and feed on their hosts whilst the adults are free-living. Not only is this situation reversed in

N. kilrymontis, but the early larval stages also do not possess an alimentary tract, and they appear to depend entirely on the yolk reserve laid down during the development of the oocyte.

It must be that N. kilrymontis absorbs food through its two integuments, which have been shown to be simple structures in the mature adult. The fact that the parasite is most often found in contact with the digestive gland, the host's own food reserve, is indicative of this. This is discussed further in section IV.

9. Excretion

The excretory gland of Copepoda is the maxillary gland, which is wanting in N. kilrymontis. This is not unexpected as it is reduced and even absent in many marine forms. It is perhaps the case that excretion is carried out in the same manner as is probably for nutrition, that is, through the integuments.

10. Muscular System

There is no trace, either in the adult male or the adult female, of a muscular system.

11. Nervous System

An attempt was made to discover if N. kilrymontis possesses a nervous system in the adult form, since no trace of any such system could be seen in sections stained by the usual general techniques. The technique employed was the Intra-vitam methylene blue method, using MacConnell's modification of Unna's method as recommended by Pantin.

The live animal is placed in about 25 ml of water and 1 to 2 ml of stain added. The water changes to dark-blue and this is the colour taken up by the animal; nerve cells, when present, take on a purple colour. The time of immersion in the stain varies with different animals, usually from a few seconds to two hours, depending on the size of the animal and the age of the solution.

I used this technique on ten dissected parasites and immersed these for periods of time varying between a few seconds to four hours (after which time the condition of the animal appeared to deteriorate). I was unable, in any instance, to detect the presence of nerve cells in these specimens. This does not preclude the possibility of their being present since the technique is not a predictable one. Furthermore, I used the stain in Berkefeld-filtered sea-water, there being neither any mention of the type of water used by previous authors, nor whether sea-water has any adverse effect on the stain itself.

12. Locomotion

Murdoch states that, "when it was prodded with forceps the parasite was capable of slight movement, and in one case the anterior end bent through 180° to become applied to a region near the posterior end".

I have tried many times, using forceps, needles and pins, to stimulate movement in parasites removed from their hosts, and on every occasion I failed to elicit the slightest response. It is my belief that in its natural environment, the animal, once established in the host, cannot move

independently of the host since it is so completely enclosed. In the absence of a muscular system it would appear likely that the only type of motion N. kilrymontis could perform would come about as a result of its internal turgor.

Murdoch's observation remains as the only record of locomotion in the adult stage of N. kilrymontis.

13. Chromosomes

As stated in section II, several regions of the reproductive system were used in aceto-orcein squash preparations to examine the chromosomes with a view to finding the number of chromosomes present in this parasite. The ovaries, different regions of the oviducts, the testes, and the very first portion of the egg-string, were all treated in this manner.

Several attempts were made with each tissue and the results obtained were generally of a poor standard, although one or two reasonable preparations were obtained which provided a good opportunity to count the chromosomes (Plate 15, figs 32 and 33). More refined methods, for example the Feulgen staining technique, would doubtless provide better results.

I was unable to detect the presence of chromosomes in the ovary due to the closely-packed arrangement of so many small cells. The nuclear elements were so condensed that separate chromosomes were not distinguishable. The same was true for squashes of the testes, again I could not distinguish separate chromosomes in the cells of this organ; although they were unmistakably present, it proved impossible to count them.

In the early part of the oviduct, that is, the first half of the ascending loop, the cells are larger, having commenced yolk production, and here I was successful in obtaining many oocytes with well-separated chromosomes, the large nucleolus also being clearly distinguishable. I counted the chromosomes in 40 such oocytes and the number obtained varied between 20 and 24 with a mean and standard deviation of 22 ± 0.35. In the second half of the ascending loop of the oviduct I again counted the chromosomes in 40 oocytes and obtained the mean 22 ± 0.05. The oocytes in this region were larger and the nuclei less frequently visible due to the presence of more yolk in the cytoplasm.

Thus it would appear that the chromosome number in the ascending limb of the oviduct is 22, any small deviation above or below this figure being due to fragmentation or loss of some chromosomes during the compression of the cover-slip when the preparation was made.

It is difficult to identify the stage at which these chromosomes are seen. I believe that they are in early prophase of first meiosis, pairing of the chromosomes not yet having occurred. In fact some preparations do show the chromosomes with a granular appearance, characteristic of chromosomes in leptotene. The chromosomes could be in prophase of oogonial mitosis, prior to the formation of oocytes, but it is difficult to think of this occurring at this level since the proliferation of cells appears to take place in the ovary. However, if it were a mitotic prophase then it would support the view that the number of 22 chromosomes is in fact the female diploid number.

This idea gains further support from examination of the preparations of the region of the oviduct just before it enters the cement gland. Here can be seen several formations of equatorial plates in which there appear to be 11 pairs of chromatids. Forty of these plates were counted, the average number of pairs of chromatids being 11 $\pm$ 0.2.

In these preparations the oocytes are almost certainly in metaphase of the second meiotic division; this will result in the oocyte, just prior to fertilization, having a chromosome complement of 11.

I have found no instances of chiasmata in these equatorial plates and this is normal for the metaphase of second meiosis. Chiasmata normally arise during diplotene of the first meiotic division, and although I thoroughly searched the preparations of the early oviducts, I could not find any trace of chiasmata nor of division of the chromosomes into chromatids, which occurs in early diplotene. Examination of squash preparations of the descending limb of the oviduct revealed no additional stages. In this portion of the oviduct were seen occasional groups of chromosomes at a stage identifiable with that occurring in the early oviduct, but their frequency was much lower due to the great accumulation of yolk which tended to obliterate the nuclei. Furthermore the number of oocytes in this part of the oviduct is considerably lower.

I made several squash preparations of the egg-string adjacent to the posterior portion of the female parasite, each preparation involving roughly the first 20 to 40 eggs in the egg-string. These preparations were made in an attempt to see, if possible, a mitotic division so that I might verify the diploid chromosome number as 22. The eggs at this stage are developing

rapidly and the cells are still quite large and I accordingly thought there was a strong possibility of obtaining at least one good preparation. However this proved not to be the ease, I did not see one cell with clearly distinguishable chromosomes. Therefore I have only the evidence described above on which to base my conclusion that the diploid chromosome number for the female of N. kilrymontis is 22.

This number does seem to correspond quite well with the diploid numbers of the few parasitic copepods so far known. Hersilia apodiformis (Cyclopoida) has a diploid number of 24; Lichomolgus forficula and Sapphira sp., both Cyclopoida, have diploid numbers of 20 and 16 respectively. The caligoid Mytilicola intestinalis has the diploid number 22, and Pandarus sinuatus also a caligoid, has a diploid number of 16. None of these copepods produces heterogametes, both male and female having the same diploid chromosome number. It has been possible only to examine chromosomes of the female of N. kilrymontis and I am accordingly unable to comment on the diploid chromosome number of the male.

IV DEVELOPMENT AND LIFE HISTORY

1. Development in the ovaries and oviducts (Plate 16, figs 34-37)

The cells in the ovaries of Nucellicola kilrymontis are very tightly packed together (Plate 16, fig. 34) and there is no indication of division into regions of oogonia and oocytes. Neither do aceto-orcein squash preparations of this region show any identifiable stages of meiosis, in fact the chromosomes are indistinguishable. The cells are 2.5 μ to 5.5 μ in diameter and the nucleus occupies almost all of this space, staining deep blue with haematoxylin. There is one large central nucleolus.

The mechanism of oocyte formation was not apparent but it is worth mentioning that in one or two instances I did detect traces of a lumen in the ovary and it might be that the oocytes are shed or extruded into a lumen from whence they are passed to the oviduct. In the absence of definite evidence of this phenomenon I will comment no further. In a similar way I have noticed occasionally evidence of "strings" of five to nine cells in sections of the ovary cut at 5 μ. This somewhat recalls the situation in the Lernaeopodidae where strings of developing oocytes are connected to filaments inside the ovary, the oocyte at the distal end being the most mature and the first to leave the filament (Wilson C.B. 1911). I did, in fact, notice that the cells of such "strings" in N. kilrymontis were not uniform in size, there being an increase in diameter of the cells from one end to the other.

As stated previously, there is no sharp distinction between ovary and oviduct. The beginning of yolk formation in the oocytes is my criterion

for the beginning of the oviduct, although it must be pointed out that the cells do not all commence yolk formation at the same place in the tube. I made a few preliminary examinations of different regions of the ovary and oviduct using the electron microscope and in this region I noticed that the first yolk globules to appear were situated round the periphery of the nuclear membrane giving rise to a typical centrolecithal egg. The yolk content of the oocyte continues to increase up to the point of fertilization in the cement glands.

In the early part of the oviduct (Plate 16, fig. 35) the oocytes differ considerably in diameter according to the degree of yolk content. At this stage also the vitelline membrane becomes quite distinct since the oocytes are now more widely dispersed. In this region the nuclei are 3.5 μ to 6.5 μ in diameter and the large central nucleolus is now quite distinct with chromosomes surrounding it within the nuclear membrane.

The oocytes increase in size and yolk content as they pass along the ascending and descending limbs of the oviduct. In the terminal portion of the oviduct the oocytes are large and either spherical or slightly ovoid (Plates 4-5, figs 6-8) depending upon whether or not they are closely packed. The largest diameter is between 80 μ and 110 μ, that is, approximately 30 times the volume of the early cells in the ovary.

As the oocytes pass through the cement glands to the egg-string they are fertilized and covered with a layer of cement substance forming an outer egg membrane, or shell, with is separated from the vitelline membrane.

2. Development in the egg-string (Plates 17-18, figs 38-45)

The eggs pass from each cement gland to a single, median vagina, and from here into the egg-string where they tend to adhere to each other in the early stages. The presence of only one egg-string is an unusual occurrence in copepods and this will be discussed later.

In the early part of the egg-string development is very rapid, the egg passing through 2-, 4-, and 8- cell stages almost immediately and the "blastula" stage (Plate 17, fig. 38) is reached very quickly. The "blastula" is spherical, 85 μ to 120 μ in diameter, and consists of a single layer of cells enclosing a ball of yolk material.

There is no orientation of the developing eggs in the egg-string as is the case in many other parasitic copepods where the egg-strings are external. It is thought that in such cases where the larvae are orientated in a definite fashion, they benefit from a closer contact with the surrounding water and hence an improved oxygen supply. This is not the case in N. kilrymontis (where the egg-string is internal in the host) where the arrangement of eggs appears to be quite haphazard, and as the anterior face of each developing larva becomes apparent it seldom faces in the same direction as that of its neighbour.

As the "blastula" stage develops further the cells at one end divide more rapidly and a "pad" of cells is formed which is later seen to be the anterior end of the embryo (Plate 17, fig. 39). These embryos are between 120 μ and 130 μ long. Later still the cells at the opposite end of the embryo divide more rapidly than the lateral cells and another, smaller, pad

is formed, which will eventually become the thorax and abdomen of the later larva (Plate 17, fig. 40).

The next structures to appear are the buds of the first antennae (Plate 17, fig. 41a). Normally in copepod development the three pairs of nauplius limb buds appear simultaneously, but in N. kilrymontis the buds of the first antennae appear shortly before those of the second antennae and mandibles. When all three pairs of limb buds are present (Plate 17, fig. 41b), the embryo measures 160 μ to 170 μ long by 80 μ to 90 μ broad.

When dissected alive out of the egg-string and viewed under the high power dissection microscope the appendages have the appearance of strings of pearls, each consisting of a row of cells which easily floats away from the embryo. At this stage also faint traces of muscle strands, running antero-posteriorly, can be seen. The state of development so far attained occurs in the proximal centimetre of the egg-string (which may be as long as 5 to 7 cm.). The rate of development of the larvae now appears to slow down and continues at this slower rate to the end of the egg-string.

After this level of development has been reached it becomes almost impossible to pick out definite stages in subsequent development in the egg-string. It is known that in the Lernaeopodidae the nauplius stages appear in the embryo and are often suppressed or very transitory (Gurney 1934, Wilson C.B. 1911). This phenomenon appears also in N. kilrymontis; as will be seen later, the earliest free-swimming larva is the second metanauplius.

The limb buds acquire more cells and become stronger in appearance while two dorsal longitudinal muscle bands are laid down. Figure 42, Plate 18 shows the final nauplius stage in horizontal section. The head,

and appendages of the head, are well differentiated, although the appendages are not yet fully-formed, there being no trace of setae, although a trace of segmentation can sometimes be discerned. The beginning of thoracic segmentation occurs at this stage and a first trace of the abdomen can be seen. There is still a considerable amount of yolk in the internal cavity.

The first metanauplius stage, which is still embryonic, follows, and here there is less yolk than in the third nauplius stage. When seen in latero-sagittal section, three thoracic limb buds can be identified (Plate 26, figs 55-56). There are two distinct bands of muscle fibres running longitudinally, with their origin on the internal dorsal surface of the anterior end of the head, and insertion on the internal dorsal surface of the anterior end of the thorax. There are also, on each side, three distinct, but smaller, bundles of muscle fibres passing from the dorsal surface of the head, in the same region where the longitudinal dorsal muscles are attached, to the ventral surface of the head where they pass to the bases of the head appendages. Three pairs of slender muscle fibres pass to the thoracic limb buds from the dorsal surface of the thorax.

Figure 43, Plate 18, is a sagittal section through the second metanauplius in the egg-string where further differentiation can be seen to have taken place. Parts of the outer shell membrane can be seen in this section and in others in figure 45, Plate 19. This membrane persists to the distal end of the egg-string, although in this region it is not always present around the second metanauplii. In the second metanauplius there are four pairs of thoracic limbs, pairs three and four being somewhat rudimentary while pairs one and two bear setae. The appendages of the head also bear setae and the muscle strands supplying the second antennae and the mandibles have

each become divided into two strands (these two pairs of appendages are biramous as will be seen later) (Plate 27, fig. 57). The maxillipeds are present in rudimentary form and the early abdomen is now clearly distinguishable.

Figures 44 and 45, Plate 19, are of horizontal sections through the distal portion of the egg-string in situ. The larvae in these photographs are seen to be at all stages of naupliar and early metanaupliar development. It is noticeable that at this stage the larvae are more widely dispersed than in the proximal portion of the egg-string (containing "blastulae" etc.). This may perhaps be due in part to the "twitching" of the second metanauplii which occurs in this region, and which first drew the attention of Professor Callan to this parasite.

I observed this "twitching" behaviour closely and came to the conclusion that it was due entirely to the movement of the two pairs of antennae and the mandibles, there being, as yet, no body movements such as occur in the first copepodid stage. The movements of these appendages within the egg-string are identical with the movements they display in the free-swimming metanauplius stages.

3. <u>The free-swimming second Metanauplius stage</u> (Plate 20, fig. 46: Plate 23, figs 49-50)

The first free-living stage in the sea is the second metanauplius. The means whereby it emerges from the host remain unknown despite many careful observations of living specimens and close examinations of preserved and sectioned material. I have been unable to detect a terminal pore in the egg-string whereby the larvae could escape to the exterior, neither in live,

intact specimens in situ, nor in sections taken through this particular region.

A close examination of the mantle cavity of a parasitised host reveals second metanauplii entangled in the mucus secreted by the host - they are often seen to be still so entangled on the bottoms of dishes used in my experiments. The second metanauplii of the egg-string and the free-living metanauplii found in the mucus, may both be either free from the outer (shell) membrane, or still enclosed within it. The former situation is more common for the free-living metanauplii. It would accordingly follow that the second metanauplii do not force or break their way out by the "twitching" movements they perform in the egg-string. If this were the case then one would not expect to see free larvae still surrounded by a shell membrane. Yet in the absence of a pore this would seem to be the only possible solution.

The second metanauplius is ovoid and measures 195 μ to 210 μ long by 80 μ to 90 μ broad (across the widest part of the head). It has three pairs of appendages on the head, the first and second antennae and the mandibles. Beneath the cuticle, on the ventral surface of the head, can be seen the rudimentary maxillipeds. The thorax bears four pairs of appendages. The abdomen is present and consists, at this stage, of one segment.

The first antenna (Plate 30, fig. 63) is uniramous and bears two long, strong, terminal setae. It consists of one segment but beneath the cuticle can be seen the two segments of the antenna of the next instar. The second antenna (Plate 30, fig. 67) is biramous, the protopodite is of two segments, the exopodite of four segments, and the endopodite of two segments.

On the distal segment of the endopodite there are two terminal setae, while on the exopodite the distal segment also bears two terminal setae and the penultimate segment has one seta on its distal, ventral border. The exopodite is completely lacking in internal structure. The four segments of the uniramous second antenna of later stages can be seen beneath the cuticle of the protopodite and endopodite of this second metanauplius stage. The mandible (Plate 30, fig. 71) is biramous, the protopodite having two segments, the exopodite four segments, and the endopodite two segments. The distal segment of the endopodite has two terminal setae, while on the exopodite the distal segment bears two terminal setae and the penultimate segment has one seta on its distal, ventral border. Internally the exopodite has no structure, but a one-segmented process can be seen passing through the protopodite and extending into the first segment of the endopodite.

The maxilliped (Plate 20, fig. 46e) is a single, inwardly curved process and beneath its cuticle can be seen the two segments of later stages. The segment which bears it is the first thoracic segment which is coalesced with the head forming the cephalothorax.

The remainder of the thorax consists of four free segments, each segment bearing a pair of appendages, the first three biramous and the fourth rudimentary. The first thoracic appendage (Plate 31, fig. 74) consists of a one-segmented protopodite and an exopodite and endopodite each of one segment. The exopodite bears four long setae distally and the endopodite has two distal setae. In the second thoracic appendage (Plate 31, fig. 75) the protopodite, endopodite and exopodite each consist of one segment.

The exopodite bears three setae distally, and the endopodite has one distal seta. The third thoracic appendage (Plate 20, fig. 46f) has a protopodite, endopodite and exopodite each consisting of one segment and setae are lacking. The fourth thoracic appendage (Plate 20, fig. 46f) is rudimentary, consisting of one segment with no setae. The thoracic appendages of the second metanauplius are all situated beneath the cuticle and are not functional in swimming although they do display occasional movements. The thorax is not movably articulated with the head as it is in later larval stages.

Internally the second metanauplius contains much yolk, and since this diminishes in quantity through the subsequent larval stages I conclude that it must be the larval food supply. The absence of mouthparts and a digestive system of any description support this. The muscular system is quite clearly defined and is discussed later. The only other distinguishable structure within the second metanauplius is a sac, containing a granular substance, at the anterior end of the head (Plate 20, fig. 46a). The sac is pyriform in shape with the tip closely applied to the frontal cuticular margin. The function of this body is not apparent but as it persists through the three succeeding stages it appears to be homologous with the frontal gland of the Lernaeopodidae.

When the second metanauplius hatches its stadium is very short, varying from 30 minutes to six hours, depending mainly on the time it spends in the mucus of the host. After this interval it moults giving rise to the third metanauplius. During this short stadium the second metanauplius displays intermittent swimming activity, this being brought about by the "twitching" movements of the two pairs of antennae and the mandibles.

The duration of any such swimming activity is extremely short, the larva generally only rises in the water and then sinks back to the substratum. Due to this fact and to the short duration of the second metanauplius stage it is reasonable to assume that this is not a dispersal stage in the life cycle of N. kilrymontis. Probably the benefit derived from this swimming activity is that of a fresh oxygen supply. The second metanauplius settles on the substratum and is quiescent for a while before moulting to the third metanauplius stage.

4. The third Metanauplius stage (Plate 21, fig. 47: Plate 24, fig. 51)

This stage hatches from the second metanauplius and, as in that stage, the head appendages are distinct, there being two pairs of antennae, one pair of mandibles and one pair of maxillipeds. The other thoracic appendages are again visible beneath the cuticle. The abdomen consists of two segments which are visible beneath the cuticle.

The third metanauplius measures 195 μ to 240 μ long by 90 μ to 95 μ broad (across the head). Beneath the cuticle the thorax is seen to be narrower than the head, this being the beginning of the movable articulation between head and thorax which occurs in later stages.

The first antenna (Plate 30, fig. 64) is uniramous and two-segmented with two long, powerful setae on the distal border of the terminal segment. Beneath the cuticle of this appendage can be seen the three segments which occur in this appendage in the next larval stage, the terminal internal segment bearing three short setae distally. The second antenna (Plate 30, fig. 68) is biramous, the protopodite having two segments, the endopodite three segments and the exopodite six segments. The endopodite bears two

setae distally on the terminal segment, and the exopodite also has two setae similarly situated and one seta on the distal, ventral border of the penultimate segment. Internally the structure of the replacing appendage of the next instar can be seen in the form of four segments passing through the protopodite and endopodite, the exopodite being empty. The mandible (Plate 30, fig. 72) is biramous, with protopodite of two segments, endopodite of three segments, and exopodite of four segments. There are two setae distally on the terminal segment of the endopodite while the exopodite bears two setae distally on the terminal segment and one seta on the distal, ventral border of the penultimate segment. Internally there is merely a structureless process extending into the protopodite, the exopodite and endopodite being completely devoid of replacing structures.

The maxilliped (Plate 21, fig. 47) is two-segmented and these two segments are duplicated internally. Anterior to the maxillipeds the ventral surface of the cuticle, in the mid-line, is somewhat thickened in the shape of an inverted 'T' (Plate 21, fig. 47).

The remainder of the thorax consists of four free segments each of which bears a pair of biramous appendages. The protopodite, exopodite and endopodite of all thoracic appendages of the third metanauplius each consists of one segment. The exopodites of the first and second thoracic appendages (Plate 31, figs 76 and 77) each bears four long setae while on the endopodites of each there are two long setae. The exopodite of the third thoracic appendage (Plate 31, fig. 78) has three long setae and the endopodite has two long setae. The fourth thoracic appendage (Plate 21, fig. 47) has two short setae on the exopodite and two on the endopodite.

The thoracic appendages of the third metanauplius are not used in swimming and they are completely enclosed by the cuticle, including the tips of the setae of the first two appendages which cause an extension of the cuticle in this region. These appendages, as in the second metanauplius, display occasional movements.

The third metanauplius again displays little internal structure. The muscles are well-differentiated (see later) and there is much yolk. The only other structure present is the frontal gland which has the same appearance as in the second metanauplius.

The third metanauplius displays little swimming activity. Such swimming movements as it does perform are brough about by the two pairs of antennae and the mandibles. These movements result in the larva rising in the water and it sinks back motionless to the substratum. Again, as in the second metanauplius, I do not consider this stage to be in any way distributive; especially so since the duration of the third metanauplius is, in suitable conditions, of the order of one to ten hours. In unsuitable media or conditions (as were discussed in section II) this stage is the most susceptible; where conditions proved unsuitable then the highest mortality occurred in the third metanauplii, not the second metanauplii.

5. <u>The first Copepodid stage</u> (Plate 22, fig. 48: Plate 24, fig. 52: Plate 25, figs 53 and 54)

The first copepodid measures 195 μ to 230 μ long from the anterior border of the head to the posterior extremity of the fourth thoracic segment. The abdomen, which is directed downwards, is 20 μ to 30 μ long. The head is 80 μ to 95 μ broad.

The first antenna (Plate 30, fig. 65) is uniramous and three-segmented, four segments being visible beneath the cuticle. There are four short terminal setae on the last segment and each of the two preceeding segments bears two setae distally on the ventral border. The second antenna (Plate 30, fig. 69) is uniramous and three-segmented, although often a small cuticular projection of two segments can be seen attached distally to the second segment, this presumably being the remains of the exopodite of this appendage in the third metanauplius. There are no setae and internally four segments can be seen beneath the cuticle. The last of these segments bears a claw distally. In the first copepodid the mandible (Plate 22, fig. 48c) appears as merely a small projection beneath the cuticle.

The maxilliped (Plate 30, fig. 73) is two-segmented with the distal segment flexed towards the posterior end of the body. Internally there are again two segments. The maxillipeds "hang down" from the ventral surface.

The free thorax again consists of four segments, each segment bearing a pair of biramous appendages in which the protopodites, exopodites and endopodites all consist of one segment. The first and second thoracic appendages (Plate 31, figs 79 and 80) each has four long setae on the exopodite and two on the endopodite. The exopodite of the third thoracic appendage (Plate 31, fig. 81) bears three setae and the endopodite two setae. The exopodite and endopodite of the fourth thoracic appendage (Plate 31, fig. 82) each bear two short, stout setae.

The abdomen (Plate 31, fig. 87), although still of only one segment, is longer than in the third metanauplius. Three segments can be seen beneath the cuticle, the distal segment bearing four short setae which project through the cuticle.

Internally the first copepodid possesses a well-developed musculature (see later) and yolk material. The frontal gland is also present but again these are the only discernible internal structures.

The first copepodid emerges from the third metanauplius through the dorsal surface behind the first antennae of the latter (Plate 24, figs 51 and 52). It is almost immediately a vigorous swimmer, the thoracic appendages now playing an active role in this capacity. Swimming is accomplished by the first and second antennae moving quickly forwards and backwards in such a way that they pull the body through the water. The first antennae are now directed forwards and curved slightly downwards, whereas in the metanauplius they were attached ventro-laterally to the head and directed sideways. In the metanauplius the motion of the first and second antennae could best be described as a "rowing" action. The thoracic appendages of the first copepodid exhibit a rapid and jerky paddling motion and the thorax is articulated with the head and "jack-knifes" in the dorso-ventral plane as the larva swims. The articulation occurs at the junction of the first and second thoracic segments so that the first thoracic segment (i.e. bearing the maxillipeds) is fused with the head, and the first free thoracic segment is in fact the second true thoracic segment.

6. The second Copepodid stage

As very few copepodids survived to the second copepodid stage I am unable to give as complete a description of this stage as of the preceeding stages. I have many times successfully maintained the first copepodid alive in the constant temperature room for as long as eight days, yet during this time I only obtained three specimens of the second copepodid stage. These appeared within three to four days of the emergence of the first copepodid stage.

The second copepodid is little changed in appearance from the first copepodid. The first antenna is uniramous and four-segmented with the same arrangement of setae as in the first copepodid. The second antenna is glabrous and four-segmented with a claw on the terminal segment. The mandible is completely absent and the maxilliped again has two segments although the flexion of the distal segment is more pronounced at this stage.

I was unable to make a thorough examination of the free thoracic appendages, they are all biramous and the protopodite, exopodite and endopodite of each appendage consist of one segment. The abdomen appears not to have changed from that of the first copepodid state.

7. Experiments on the feeding of the larval stages

Although I could not find any trace of a digestive system, mouth, or anus in any of the larval stages, I made several attempts to induce the larvae to feed on various flagellates and diatoms. Murdoch states

that she noticed movements in the anterior end of the head in the region of the second antennae and thought that these might be gut movements. I closely examined this region in the first copepodid and although I did notice movements here, they were the contractions and extensions of the muscles supplying the first and second antennae.

The cultures used were three flagellates, Dunaliella primolecta (ex Chlamydomonas I) (Plymouth culture no. 81), Isochrysis galbana (culture no. I), and Coccolithus huxleyi (culture no. 92), and three diatoms, Nitzschia closterium (culture no. 170), Skeletonema costatum (culture no. 106), and Phaeodactylum tricarnectum (culture no. 100). These cultures were supplied singly, and mixed in various combinations, to considerable numbers of first copepodids (I deemed it unnecessary to try the metanauplii in view of their short duration). In no instance was I able to trace any of the diatoms or flagellates inside the larvae. The longest time during which first copepodid larvae were subjected to concentrations of the diatoms and flagellates was eight days - after this time there was still no trace of them within the larvae. Since the first copepodids generally begin to degenerate on about the seventh day, I did not proceed further with these experiments.

During the longer experiments the water was changed every 24 hours and each time fresh culture was added to the larvae. In shorter experiments, when left for more than one day, the dishes were stirred with a glass rod every 24 hours in order to resuspend the culture. Precautions were taken at all times to ensure that all the glassware used in these

experiments, as in the sub-culturing carried out every month, was thoroughly sterilized by heating to 120°C in an oven.

8. <u>The muscular system</u> (Plates 26, 27, 28, figs 55-60)

Muscles first appear in the nauplius stages as two dorso-lateral bands extending almost the whole length of the body. At the first meta-nauplius stage these two bands are further differentiated and appear as two broad ribbon-like bands of striated muscle fibres (Plate 26, figs 55 and 56). Both ends of these bands have their origins and insertions on the inner dorsal surface of the larva at points near to the posterior and anterior faces. Each band contains several striated fibres. The fibres which pass to the three pairs of head appendages have their origin at a point just posterior to, and in between, the insertions of the two powerful dorsal muscle bands. These three pairs of muscle bundles, each of two or three striated fibres, have their insertions in the bases of the head appendages. Three pairs of single fibres can be seen at the posterior end of the larva. These fibres have their origin at a point just posterior to the origins of the two large dorsal bands, and their insertion is in the base of each of the first three thoracic limb buds. I was able to trace these fibres only in the first metanauplius. In plate 26, figs 55 and 56 are drawings made from serial sections of such stages in the egg-string in situ. All of the remaining drawings of muscle systems are from live specimens and from specimens stained by Daniel's method - described earlier as unsatisfactory with this material.

By neither of these two methods was I able to trace any thoracic musculature in subsequent larval stages.

The free-living second metanauplius (Plate 27, fig. 57) is altered but little from the preceeding stage. The two dorsal bands are slightly broader and their origins are on the internal dorsal border of the first thoracic segment. The muscle bands supplying the second antennae and the mandibles have each divided into two bands, each consisting of two striated fibres. The attachment of the muscles bands to the three pairs of head appendages is not clear, but presumably the division of the bundles passing to the second antennae and the mandibles is connected with the biramous condition of these appendages.

In the third metanauplius (Plate 27, fig. 58 and Plate 28, fig. 59) an extra pair of muscle bands is present. They have theirorigins at the same posterior point as the origins of the dorsal muscle bands and they pass forwards and downwards on each side to their insertion at a point between the second antenna and the mandible. Each band consists of two broad striated fibres and the striations are further apart than in the other fibres. The dorsal muscle bands have not changed in appearance from those of the second metanauplius. I am of the opinion that these two pairs of longitudinal muscle bands, i.e. the dorsal bands and the lateral bands, are concerned with the extensions and flexions of the body seen in the swimming of the first copepodid stage.

The three pairs of muscles supplying the first and second antennae and the mandibles appear to be weaker than in the second metanauplius. The

muscles supplying the first antennae are each of a single striated fibre, and the two pairs of muscles supplying the second antennae and the mandibles each still consist of two separate bands, although each band now consists of only one fibre. It is also apparent that in each pair of these fibres the posterior fibre is broader than the anterior one.

In the first copepodid (Plate 28, fig. 60) the two pairs of large muscles remain much the same. In the head region however, the fibres supplying the appendages have changed considerably. The first antenna still receives only one fibre, whereas the second antenna also is now supplied by a single fibre. Furthermore the muscle supply to the mandibles (which are now lacking) has disappeared altogether. I have found no trace of a muscle supply to the maxillipeds. I am of the opinion that the gradual diminution of the fibres supplying the head appendages is connected with the gradual lessening of the function of these appendages in swimming, in turn concurrent with the increase in the work done by the thoracic appendages in this capacity. I have not however been able to trace a parallel increase in strength of the muscle fibres supplying the thoracic appendages.

9. The Larval Nervous System

I attempted to show the presence of a nervous system in all of the larval stages obtained experimentally by using the intra-vitam methylene blue technique described earlier for the adult. The results again were negative and I am consequently unable to give an account of this system in the larvae. In view of the presence of a strong muscular system one would expect to find an associated nervous system.

10. Effect of Light on Larvae

Although there is no trace of an eye spot in any of the larval stages I conducted several experiments to determine whether or not the larvae reacted to light. Crustacean larvae generally are positively phototropic and swim towards the surface of the sea, in so doing they benefit in the presence of an abundant food supply in the plankton and this behaviour also assists in their distribution.

First of all I used a circular glass dish, 5½ inches in diameter with vertical side 1½ inches high. Half of the bottom of this dish was covered with black paper and the other half with white paper. A mixture of about 100 metanauplii and 100 copepodids were introduced in Berkefeld-filtered seawater and evenly distributed over the whole area of the bottom of the dish. The source of illumination was the strip lighting on the ceiling and this was kept on throughout the experiment. After 24 hours the larvae over each half of the dish were counted and it was found that there was no significant difference in the distribution over these two backgrounds.

In the next experiment I blackened out completely one half of the dish by sticking black paper round the sides for as far as the black paper beneath extended. I also covered the top of this half of the dish with glass which was also blackened with paper. The source of illumination was the same as in the previous experiment and again about 200 larvae were used. After 24 and 48 hours there was again no significant difference in the number of larvae in each half of the dish.

This experiment was repeated using a stronger light source with a 60 watt bulb held in position two feet above the dish. Again there was no significant difference in the number of larvae counted on the two backgrounds.

Moyse (1960), when rearing barnacle larvae experimentally, removed ripe embryos from adults and placed these in sea-water in a dish which was completely blackened off except for a vertical strip on one side through which a light was projected. The embryos were placed on the side away from the light and as the nauplii hatched they swam towards the light thereby leaving behind most of the protozoa present in the egg masses. In this way he obtained nauplii relatively free from contamination. I decided to use a similar dish in order to test the response of larvae of N. kilrymontis to light. Accordingly I blackened out the top, bottom, and sides of the dish, except for a ½ inch vertical strip down one side through which was shone a 60 watt bulb. I again used about 100 each of metanauplii and copepodids. After 24 and 48 hours there was neither an accumulation of larvae around the lightened strip, nor was this area devoid of larvae, the larvae remaining evenly distributed over the bottom of the dish.

I therefore conclude that the larval stages, at least up to the first copepodid, show neither positive nor negative response to light stimulation.

11. Further attempts to obtain later larval stages

I tried many times to rear the larvae beyond the first copepodid stage in the constant temperature room but every effort resulted in the death of the larvae, usually at the first copepodid stage, between six to eight days after the emergence of the first copepodid. In order to complete

the life cycle of N. kilrymontis I tried several methods of locating further larval stages but without success.

Two series of experiments were conducted in an attempt to infest non-parasitised specimens of Nucella lapillus with N. kilrymontis. In the first series I used a tank, 14 inches long by 9 inches broad and 9 inches high, containing Berkefeld-filtered sea-water, aerated and filtered with a "Reliant" corner unit. Into this tank were placed five parasitised Nucella, the shells of which were marked with black ink, and 30 apparently non-parasitised (as they had not liberated any larvae of N. kilrymontis) Nucella. Both the parasitised and non-parasitised Nucella were obtained by the method described previously.

The experiment was begun on the 4th July, 1963, and on the 11th, 18th, and 25th July, and on the 1st August, six unmarked Nucella were removed and examined for the presence of N. kilrymontis both in their tissues and mantle cavity. At the same time some of the sediment was pipetted from the bottom of the tank and examined for the presence of larvae.

On the 11th July, of the six Nucella examined, two were found to be parasitised, each containing a fully mature parasite with egg-string intact. On the 18th July two more Nucella were found to be parasitised and although these parasites were small they did possess viable egg-strings. On the 25th July and on the 1st August the samples each revealed one parasitised Nucella and in each case the parasite was large and possessed a large egg-string. On each of the examination dates the samples taken from the bottom of the tank contained many metanauplii and first copepodids but no later stages.

From the results obtained by this experiment I concluded that there was here no evidence of experimental infestation. I considered it extremely doubtful that a fully mature adult parasite could develop within one week and I came to the conclusion that the so-called "non-parasitised" Nucella used in the experiment had in fact been parasitised before the commencement of the experiment. Although the method I used to separate parasitised Nucella was successful in finding parasitised specimens, it is of no use for determining non-parasitised ones. Apparently N. kilrymontis does not produce larvae continually - although there is certainly no evidence of a cyclical egg-laying.

On the 27th March, 1964 I commenced a more controlled series of experiments based on the same lines as the one described above. I used specimens of Nucella kindly supplied by the Director of the marine laboratory at Millport, having earlier established that in this locality the Nucella were free from infestation by N. kilrymontis (see section VII). About 1,000 mature Nucella were received from Millport and 400 of these were examined to check the findings made earlier. None of the Nucella examined was infested with N. kilrymontis.

Four glass-fibre tanks, 18 inches long by 12 inches broad by 9 inches high were fitted with "Reliant" corner units. Into Tank 1 were placed 20 litres of Berkefeld-filtered sea-water containing 1.0 gram of Streptomycin sulphate G and 10^6 international units of sodium penicillin G (a concentration of 0.05 mg./ml. and 50 int. units/ml. respectively - the same concentration used by Shelbourne in his work on the hatching of plaice larvae). Tank 2 contained 20 litres of untreated Berkefeld-filtered sea-water. Tank 3

contained 20 litres of unfiltered sea-water with the same concentrations of antibiotics as in Tank 1. Tank 4 contained 20 litres of untreated and unfiltered sea-water.

Into each of these tanks were placed 150 Nucella from Newport, together with seven parasitised Nucella, marked with black ink to avoid their being examined in error. On subsequent dates a number of Nucella was removed from each tank and examined for the presence of N. kilrymontis. At the same time pipettings from the bottom of each tank were examined. The results of these examinations are to be found in Table 1. As seen in the table, not one case of an experimental infestation was obtained, and the bottom samples produced no larval stages beyond the first copepodid. The only conclusion I can draw from this experiment is that the Nucella in the tanks containing antibiotics tended to survive better than those in the other tanks - thus the presence of antibiotics prolongs the life of the animals under these conditions.

Another approach tried in the search for further larval stages was to examine several samples of both top and bottom plankton dragged in the Bay of St. Andrews, in the region of the rocks fronting the castle ruins, where the percentage infestation is highest. Although these samples contained many different crustacean larvae there was not a single specimen which bore a resemblance to the first copepodid, or the male exuviae of N. kilrymontis, to warrant further investigation.

I took many hand-net samples of the rock pools in the same area, but again these yielded no larvae similar to the larvae of N. kilrymontis that I had reared.

TABLE 1

Attempt to experimentally infest *Nucella lapillus* with *Nucellicola kilrymontis*

Date	Tank Number	Number of *Nucella* examined	Number parasitised	Larvae present in bottom sample	Number of dead *Nucella* removed	Number of dead parasitised *Nucella* removed	Comments
5.4.64	1	20	0	1st copepodids	1	0	
	2	20	0	1st copepodids	1	0	
	3	20	0	1st copepodids	3	0	
	4	20	0	1st copepodids	0	0	
12.4.64	1	20	0	1st copepodids	0	0	
	2	20	0	metanauplii	18	2	
	3	20	0	1st copepodids	3	0	
	4	20	0	1st copepodids	8	0	
23.4.64	1	20	0	1st copepodids	0	0	
	2	8	0	metanauplii	83	5	Discontinued
	3	20	0	1st copepodids	0	1	
	4	20	0	1st copepodids	12	4	
11.5.64	1	20	0	1st copepodids	0	0	
	3	20	0	1st copepodids	2	2	
	4	20	0	metanauplii	16	0	
20.5.64	1	20	0	1st copepodids	2	1	
	3	20	0	1st copepodids	8	0	
	4	0	0	nil	34	3	Discontinued
28.5.64	1	20	0	1st copepodids	5	2	
	3	20	0	metanauplii	8	0	

Although it is generally the case that parasites of invertebrate hosts do not have intermediate hosts, I examined about 500 specimens each of Balanus balanoides and Mytilus edulis, the food sources of Nucella lapillus, on the chance that I might find further larval stages. Again my examinations did not show parasitic copepods.

As a result of these negative findings I cannot give a description of the complete life history of N. kilrymontis. The only 'link' in the gap between the second copepodid and the adult is represented by the male exuviae which lie trapped between the integuments of the adult male and female. I am of the opinion that these exuviae are the exoskeleton of the final male copepodid stage.

12. The male exuviae (Plate 6,figs 10-11; Plate 29, figs 61 and 62)

These are completely devoid of internal organisation consisting only of the cuticle of the final copepodid (male) stage. They are, without exception, found in the presence of every male attached to a female. Where more than one male is present, then the same number of exuviae occurs. Their removal intact is achieved with some considerable difficulty since when freed from the surrounding tissues they readily float away in the filtered sea-water. Several specimens were mounted in polyvinyl alcohol containing chlorazol black E.

From the frontal margin of the head to the posterior margin of the thorax the exuviae measure 155 μ to 182 μ long. The abdomen, from its base on the thorax to the base of the four setae, measures 43 μ to 49 μ long.

The head is 112 μ to 130 μ long by 100 μ to 112 μ broad. From the dorsal to the ventral surface of the head the depth is 78 μ to 90 μ.

The first antenna (Plate 30, fig. 66) is uniramous and consists of five segments. There are four short setae on the distal margin of the terminal segment; the penultimate segment bears two setae distally on the median border of the antenna. The second segment bears two setae, one distally and one half-way along its length, both of these setae are also on the median border of the antenna. The second antenna (Plate 30, fig. 70) is uniramous and glabrous. It consists of four segments with the terminal segment chelate. Both the mandibles and maxillipeds are lacking.

It is perhaps interesting to note that the lines of fission indicating the position of emergence of the instar subsequent to the exuviae, occur on the the ventral surface of the head and are particularly in evidence around the area where one would expect to find the maxillipeds. As stated previously, the adult male possesses a pair of sub-chelate appendages which bear a close resemblance to the maxillipeds of the first and second copepodids and I can see no other explanation than that they have in fact evolved from the larval maxillipeds (compare Plate 6, figs 10 and 11, and Plate 13, figs 26 and 27 with Plate 21, fig. 47, and Plate 30, fig. 73). The maxillipeds of the adult male are very much larger than those of the first copepodid. This increase in size is perhaps connected with their use as an organ of penetration into the host.

The free thoracic segments are four in number and each bears a pair of biramous appendages. The protopodites, exopodites and endopodites of all of these appendages each consists of one segment. The exopodite of the first free thoracic appendage (Plate 31, fig. 83) bears four long setae,

two distally and two on the inner distal margin. The endopodite bears two long setae distally. The exopodites of the second and third free thoracic appendages (Plate 31, figs 84 and 85) each bears five long setae, two distally and three along the median margin. Each of the endopodites of these appendages bears two long setae distally. The fourth free thoracic appendage (Plate 31, fig. 86) has four long setae on the exopodite, two distally and two on the inner distal border, and two long setae distally on the endopodite.

The abdomen (Plate 31, fig. 88) is now clearly marked off from the thorax. It is three-segmented and there are two long setae on each of the two caudal furcae.

A summary of the appendages, and their segments and setae, of the larvae and the male exuviae appears in Table 2.

13. Early development in the adult (Plates 32-36, figs 89-97)

Only six immature adults of N. kilrymontis were obtained from the 627 parasitised specimens of Nucella lapillus examined. Of these six specimens none possessed an egg-string, five were without males attached at their posterior extremities, and the sixth possessed a male in an early stage of development. With so little material available it is difficult to come to any definite conclusions about this stage. The six immature adults were obtained at different times of the year and this, together with the fact that larvae are produced at all times of the year, indicates that reproduction in N. kilrymontis is not seasonal.

TABLE 2 The appendages of the larval stages and the male exuviae.

		CEPHALOTHORAX								THORAX												ABDOMEN
		1st ANTENNA	2nd ANTENNA			MANDIBLE			MAXILLIPED	1st APPENDAGE			2nd APPENDAGE			3rd APPENDAGE			4th APPENDAGE			
		Uniramous	Proto-podite	Endo-podite	Exo-podite	Proto-podite	Endo-podite	Exo-podite	Uniramous	Proto-podite	Endo-podite	Exo-podite	Proto-podite	Endo-podite	Exo-podite	Proto-podite	Endo-podite	Exo-podite	Proto-podite	Endo-podite	Exo-podite	
2nd METANAUPLIUS	Segments	1	2	2	4	2	2	4	-	1	1	1	1	1	1	1	1	1	1	-	-	1
	Setae	2	-	2	3	-	2	3	-	-	2	4	-	1	3	-	-	-	-	-	-	-
									Rudimentary beneath cuticle										Rudi-mentary			Rudimentary
3rd METANAUPLIUS	Segments	2	2	3	6	2	3	4	(2 beneath cuticle)	1	1	1	1	1	1	1	1	1	1	1	1	(2 beneath cuticle)
	Setae	2	-	2	3	-	2	3	-	-	2	4	-	2	4	-	2	3	-	2	2	-
																						Rudimentary
1st COPEPODID	Segments	3	3			-			2 (flexed)	1	1	1	1	1	1	1	1	1	1	1	1	1(3 beneath cuticle)
	Setae	8	-			-			-	-	2	4	-	2	4	-	2	3	-	2	2	2
			Uniramous 2 claws beneath cuticle on distal segment			Often rudimentary as a small projection, occasionally lacking																
2nd COPEPODID	Segments	4	?			-			2 (more flexion)	1	1	1	1	1	1	1	1	1	1	1	1	?
	Setae	?	?			-			-	?	?	?	?	?	?	?	?	?	?	?	?	?
			Uniramous Chelate			Lacking																
MALE EXUVIAE	Segments	5	4			-			-	1	1	1	1	1	1	1	1	1	1	1	1	3
	Setae	8	-			-			-	-	2	4	-	2	5	-	2	5	-	2	4	4
			Uniramous Chelate			Lacking			Lacking													2 caudal furcae

Two of the immature females without males measured 2.4 mm long by 0.5 mm broad, and 2.7 mm long by 0.7 mm broad. As mentioned earlier, these measurements overlap those of females already associated with males, but possessing no egg-strings, and with some of the smaller, fully mature adults. There is no sharp distinction between the dimensions of the three states, viz. (i) fully mature female with male; (ii) female with male but lacking an egg-string; (iii) immature female without a male. However it is true to say that the majority of the mature females (with egg-strings) are larger than the less mature specimens.

On considering firstly the immature female prior to the attachment of the male, it is straightway obvious that the two integuments surrounding the body are thicker than those in the mature adult (Plates 32 and 33, figs 89 and 91). Figure 92, Plate 33, is a transverse section of an immature female where the outer integument is missing. The inner integument can be seen to consist of two thick layers. The outer layer is of closely packed columnar epithelium while the inner layer has a spongy appearance, the cells not showing any definite organisation. The nuclei of both of these layers readily take up haematoxylin. If this figure is compared with fig. 37, Plate 16, a transverse section of a mature adult, then the striking differences in thickness of these two inner integuments is apparent.

The outer integument is again thicker than in the mature parasite. Although it does not show the presence of two layers it shows more nuclei than does the corresponding tissue in the mature adult. Figure 96, Plate 35, is of a female with attached male, the former not yet having produced eggs. The outer integument here (fig. 96) is seen to be much thicker

than in mature adults (fig. 37, Plate 16).

There appears to be some degree of organisation in the integument of the immature and young mature adults. At the anterior end of the immature female the outer integument is invariably drawn out in the form of two main processes or filaments, each branching several times into smaller filaments (Plate 34, figs 93 and 94) which ramify throughout the host tissues. In young mature adults these extensions of the integument are still present although there are less ramifications (Plate 36, fig. 97). In the fully mature adult these processes are lacking, although the outer integument at the anterior end of the female is more difficult to dislodge than elsewhere over the trunk. The outer integument at the anterior end of the fully mature female often appears to be elongated and wedge-shaped.

The exact function of these ramifications is not apparent. There appear to be three possibilities, that they act as an anchorage, or as a feeding mechanism, or perhaps even as both. In section III I considered the problem of nutrition for N. kilrymontis and came to the conclusion that it derived nourishment in some way via the two integuments.

Both in preserved and sectioned material the filaments of the immature adults do not show any internal organisation (although they do contain a high proportion of nucleated cells). In two living specimens I observed movement down the centre of some of the filaments. This movement was directed towards the anterior end of the female and closely resembled ciliary movement, although I have been unable to detect any cilia in these filaments. This might suggest the presence of a food current passing down each filament to the anterior end of the female, but the absence of

any digestive apparatus appears to contradict such a hypothesis. Conversely the possibility of external digestion by enzymes secreted by the female (prior to the absorption of digested food through the filaments) has not been investigated.

In the development of the female, together with the degeneration of the ramifications at the anterior end, there occurs a diminution in the thickness of the two body integuments. Thus in the mature adult female these integuments would be less of a barrier to any process of absorption of food than would the thicker integuments covering the young female.

The above observations are the only ones I can put forward to support the theory that N. kilrymontis feeds, at least in its young adult life, by means of anterior processes ramifying through the host tissues. Whether or not the ramifications in any way act as an anchorage for the young developing adult it is difficult to say. Certainly the mature adult shows no locomotory powers and is very firmly wedged in the host with no chance of it being dislodged. Perhaps the young adult is active to some extent and the anterior ramifications aid in maintaining the parasite in a favourable site once this has been obtained.

One abnormal condition I encountered is shown in fig. 98, Plate 36. This mature adult, which possessed a short egg-string, was taken from the left side of the mantle of a host specimen. On observing this animal alive, in Berkefeld-filtered sea-water, with the aid of a Beck Greenhough high power dissection microscope, I noticed that there was a vast network of tubules at the anterior end of the female. Again in these tubules the same movements as observed in the anterior ramifications of normal

immature females were apparent. In addition these "rootlets" swayed rhythmically in the surrounding water. I made a vaseline squash preparation of this specimen and stained it by the Mayer's haemalum technique. On studying the "rootlets" more closely they appeared to be very similar to the anterior ramifications of the immature females. I can only surmise that this was a case where the parasite had arrived in an unsuitable site and this was its reaction to the unfavourable environment. It would appear that the tissues of the mantle present a much more formidable obstacle to penetration than the tissues of the digestive gland and gonads, although I did find several normal specimens of N. kilrymontis in this region. In spite of this abnormality the female contained a male and had produced an egg-string containing active embryos.

At the posterior end of normal immature females the outer integument is continued as a long tube which also has some smaller branches, although these are fewer in number than those occurring anteriorly (Plate 34, figs 95 and 96). It is this tube which becomes the outer covering of the egg-string in the mature adult (see Plate 32, fig. 90). In the immature adult the wall of this tube is thicker than in the mature adult. This tube is further discussed in paragraph 14 of this section.

Internally the immature female contains the developing ovaries, oviducts, cement glands and accessory glands. The ovaries (Plate 32, fig. 89 and Plate 33, fig. 92) have the same structure as in the mature adult. The oviducts in virgin females are devoid of oocytes and are seen to be thick-walled tubes, the walls of which contain nuclei (Plates 32 and 33, figs 89 and 92). The specimen shown in fig. 96, Plate 35 is a young

female in which the male has recently arrived and is already fully developed. In this specimen the oocytes have commenced their passage down the oviducts and extend to about half way along the whole length of each oviduct. On the other hand, fig. 90, Plate 32 shows a female in which eggs are seen in the egg-string and yet the male is not completely developed, only the testes and the maxillipeds are apparent. In this specimen spermatozoa are present in the cement glands so that the production of spermatozoa and fertilization of the female do not necessarily occur subsequent to the complete development of the adult male.

The cement glands and accessory glands appear to have the same structure as in mature adults, although their ducts, particularly those of the accessory glands, are wider than in the adult (Plate 33, fig. 91). The vagina also is wider than in the mature adult.

Using the polyvinyl alcohol with chlorazol black E squash technique on one of the immature adults I again found that the integuments did not contain chitin. Furthermore I was unable to detect the presence of a muscular system or a nervous system in vaseline squash preparations, or sectioned specimens, of immature adults stained by the Mallory triple technique.

14. Discussion on the development of Nucellicola kilrymontis

Without any knowledge of the vitally important larval stage which attaches to the host, any further comments on the development of N. kilrymontis must be purely speculative. In both the metanauplii and copepodids there

is no evidence of separation into male and female types. I found no trace of a reproductive system in any of these forms. Since there are definitely two sexes in the adult phase then differentiation of the sexes must occur during or after the second copepodid stadium.

It did occur to me that there might be some mechanism in operation similar to that which occurs in some Epicaridia (Isopoda) where the bopyrid larvae are ambipotent for sex. The larva can develop into a male or a female adult, the sex being determined epigamically: if there is a female already present on the host then the larva becomes a male, and if no parasite is present then the larva becomes a female. Such a mechanism has not as yet, to my knowledge, been reported for the parasitic copepods. I came to the conclusion that this mechanism was not in operation in N. kilrymontis after observing that cases of more than one parasite occurring in one host were common and, particularly, in some of these cases each female had associated with it more than one male.

I believe that sexual differentiation occurs at some larval stage subsequent to those I have been able to rear. Whether or not there are altogether three or five copepodid stages in N. kilrymontis I am unable to say. Five copepodid stages have been reported for some parasitic copepods, but whether or not these five stages include the second and third metanauplii seems to be, as yet, an unsolved problem. If there are only three copepodid stages then the male exuviae, which are found trapped between the adult male and the adult female integument, are the remains of the exoskeleton of the third copepodid stage which has developed from the second copepodid. This would seem to be more probable than the occurrence

of five copepodid stages when the appendages of the first copepodid and the male exuviae are compared (see Plates 30 and 31, figs 65, 66, 69, 70 and 79-86). If this is the case then sexual differentiation must occur either at the third copepodid stage or during the second copepodid stage. Although sexual differentiation was not seen in the second copepodid larvae, it must be borne in mind that insufficient numbers of these larvae were obtained to enable me to make a thorough examination of this stage.

On the problem of the entry of N. kilrymontis into the host I think that the following postulation merits some consideration. The final free-living female copepodid stage attaches to the mantle of the host and moults. The soft inner parts of the larva are injected into the host through an incision made by the head appendages; it is possible that the frontal gland has some role at this stage. The form that enters the host either pushes or burrows (although there is no tissue reaction on the part of the host) its way through the host's tissues forming a tube as it passes to the visceral mass. The early adult female comes to rest at the end of this tube and ramifications are formed which act as an anchorage, a feeding mechanism, or both. The final free-living male copepodid enters the same aperture, perhaps being attracted by secretions from the female - this could be a possible function of the accessory glands. The male copepodid passes along the tube formed by the female, enters the female and moults giving rise to the adult male. After the introduction of spermatophores the egg-string is formed in the same tube whereby the female gained entrance to the host. Thus the most mature larvae in the egg-string (the second metanauplii) would pass to the exterior through a pore in the mantle.

In support of this hypothesis I offer the following four facts:-

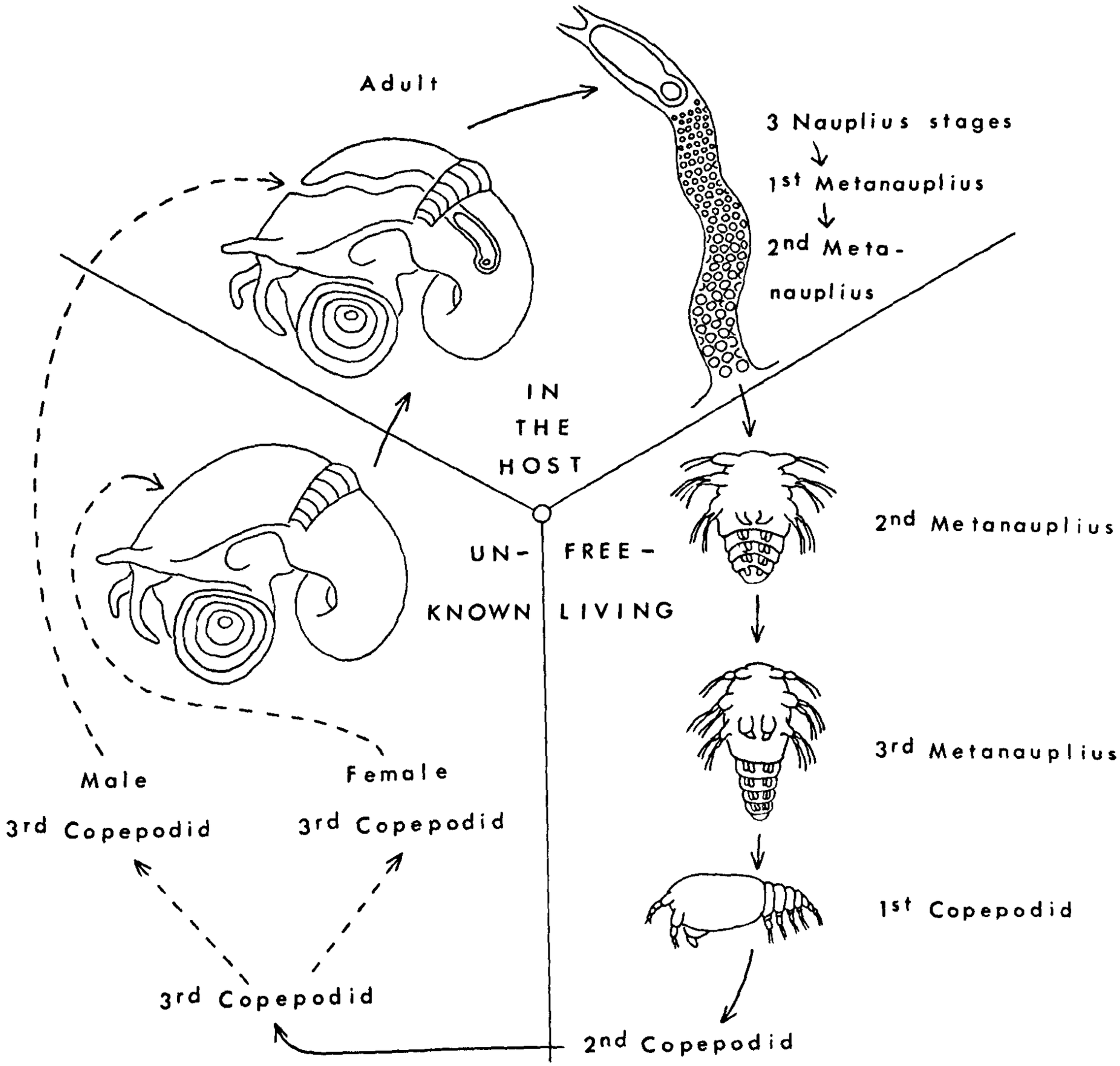
1. The presence of a tube is constant for all specimens of N. kilrymontis found. That this tube becomes filled with eggs is shown in Plate 32, fig. 90.

2. In mature adults the outer integument of the female is continuous with the covering of the egg-string.

3. I have never found exuviae of the female final copepodid stage. This indicates that the female copepodid must moult before penetrating the tissues of the host.

4. The only exuviae found are those of the male beneath the integument of the female. This indicates that the male copepodid does not moult until it comes into contact with an adult female.

A summary of the life cycle of N. kilrymontis is given in Text fig. 2.



TEXT FIG. 2. Diagram of the Life Cycle of *Nucellicola kilrymontis*.

V THE SYSTEMATIC POSITION OF NUCELLICOLA KILRYMONTIS

I have found it impossible to place Nucellicola kilrymontis in any known copepod genus. Furthermore it cannot with any certainty be placed in any family or sub-order since it possesses several characteristics which are not found elsewhere in this order. Of these the most striking are:

1. The high degree of degeneracy displayed by N. kilrymontis which parallels that of such forms as Xenocoeloma brumpti Caullery and Mesnil, 1915, Gonophysema gullmarensis Bresciani and Lützen, 1960, and members of the family Herpyllobiidae. In N. kilrymontis the body, in both sexes, is not articulated or segmented and neither is it divided into head, thorax and abdomen. Furthermore the integument is soft, there being apparently (after staining with Chlorazol black E) no trace of chitin in either sex, except for the maxillipeds of the male. Neither is there any trace of appendages in the adult female, and those of the male are reduced to a single pair.

2. The male is completely enclosed within the body of the female. Even in the most degenerate Lernaeopodoida the male, although in permanent intimate contact with the female, is always external to the female and a completely separate organism.

3. N. kilrymontis possesses only one egg-string; this is in direct contrast to the normal copepod complement of two. There are a few exceptions to this, the four very degenerate genera parasitic in echinoderms and ophiuroids have varying numbers of "egg-masses". A further exception is found in the Notodelphyoida where the egg-strings are replaced by an incubatory pouch on the dorsal surface of the thorax.

4. The female and male, together with the egg-string, are all completely enclosed within the host's tissues. Such a degree of endoparasitism is extremely rare in copepods; although there are several genera known where all, or part, of the body of the female is within the host, the egg-strings are always external to the host, except in three genera from ophiuroids.

5. Although the complete life cycles of only a few parasitic copepods are known, and although the infesting stage of N. kilrymontis is unknown, from the information that is available it is apparent that the life history of N. kilrymontis does not closely resemble any of the known families except the Lernaeopodids. A comparison of these life cycles is made in Table 3 (adapted from Baer, 1952).

TABLE 3

Correlation of hatching and habits with developmental stages in families of parasitic copepods and N. kilrymontis
(From Baer, 1952)

Group	Nauplius			Metanauplius			Copepodid			Adult		
	1	2	3	1	2	3	1	2	3	1	2	3
I Ergasilids	...	...	...	...	...	...	...	...	...	+++	+++	+++
II Caligids	...	...	...	...	...	...	...	.++	+++	+++	++.	...
III Pandarids	...	...	...	...	...	...	+++	+++	+++	+++	+++	+++
IV Monstrillids	...	+++	+++	+++	+++	+++	+++	+++	+++	...	...	...
V Lernaeids	ooo	ooo	ooo	ooo	ooo	...	+++	+++	...	+++	+++	+++
VI Lernaeopodids	ooo	ooo	ooo	ooo	ooo	ooo	...	+++	+++	+++	+++	+++
N. kilrymontis	ooo	ooo	ooo	ooo	o..	...	...	...	???	+++	+++	+++

Key:- ... free-swimming; +++ parasitic; ooo in the egg; ? not known

Despite the differences outlined above, N. kilrymontis does have some characteristics in common with members of the sub-order Lernaeopodoida, and more particularly with the family Lernaeopodidae (Wilson, 1932). For example:

1. In the Lernaeopodidae there is a very great disparity in size between male and female, in fact a greater disparity than elsewhere in the parasitic copepods. There is also great dissimilarity in body structure and appendages between the male and the female. Both of these characteristics are displayed by N. kilrymontis.

2. In the Lernaeopodidae there occurs, in the female, a fusion of the body regions due to degeneration. N. kilrymontis shows no division into body regions and indeed no trace of any segmentation. Furthermore, in the Lernaeopodidae the male is less degenerate than the female, and this is also true of N. kilrymontis.

3. The female Lernaeopodid loses her swimming legs, whereas the male does not, although they do become small and are of no further use in free locomotion, other than crawling over the female. In N. kilrymontis the male loses all vestiges of swimming legs when it penetrates the female. In this respect N. kilrymontis more closely resembles the Sphryiidae, the Lernaeopodoid family closely related to the Lernaeopodidae, since in this family the males, after attaching themselves to the females, lose all trace of their swimming legs.

4. Both male and female N. kilrymontis are fixed parasites; normally in the Lernaeopodoida the female becomes a fixed parasite, while the male clings to the female and can crawl about over her body. On the other hand, in most of the Caligoida, the only other sub-order containing such degenerate copepods, both males and females can move about at will, and most retain their ability to swim about freely.

5. In the ovaries of the Lernaeopodidae there is a uniform gradation in the size of the eggs from one end of the ovary to the other, and the eggs, in the early stages, are joined by filaments. Although the occurrence of such filaments in N. kilrymontis is not definitely established (see section IV. 1.), the eggs do increase in size from the beginning to the end of the ovary.

6. The larval stages of N. kilrymontis are similar in both morphology and mode of development to the larvae of the Lernaeopodidae. Particularly relevant is the suppression of the larval stages of Lernaeopodidae in the egg-string so that the emergent larva is at the 3rd metanauplius or 1st copepodid stage. The situation is much the same in N. kilrymontis where the emergent larva is the 2nd metanauplius which rapidly moults through the 3rd metanauplius to the 1st copepodid.

In the Lernaeopodidae the 1st copepodid only is free-swimming, all larval development prior to this being passed inside the egg, while subsequent to it the copepod is a fixed parasite, usually showing degeneration. According to several authors, notably Claus (1862) and Wilson (1911), some species hatch in an advanced metanauplius stage, rapidly moulting (within 10-60 minutes) to the 1st copepodid. N. kilrymontis very closely

parallels this, and although the rate of development beyond the 1st copepodid is not known, it certainly does not become parasitic at the 1st copepodid stage as do the Lernaeopodidae. However its development resembles that of the Lernaeopodidae more closely than any other family (see Table 3).

7. Wilson (1911) showed that the attachment filament of Lernaeopodidae commences development at the very beginning of larval development - in other parasitic copepods this structure appears at a much later stage. In Achtheres ambloplitis Kellicott (Lernaeopodidae) the filament develops slowly into a long coiled structure during the long period which the larva passes in the egg. This filament originates in the nauplius stage as an oval mass of glandular cells on the mid-line close to the frontal margin. There is a great similarity between Wilson's diagram of the filament at this stage and the frontal gland occurring in the larvae of N. kilrymontis, although in the latter the gland does not develop into a filament in the stages known. No comparable structure arises so early in any other parasitic copepod.

In addition to those listed at the beginning of this section, N. kilrymontis possesses other characteristics which differ markedly from those of the Lernaeopodidae:

One notable difference is that the female does not possess any elaborate oral apparatus; in the Lernaeopodidae the female has a proboscis and the 2nd maxillae, when present, are modified to form an attachment bulla. Even those genera where the 2nd maxillae are lacking possess well-developed mouth parts. The male and female Lernaeopodids each have two pairs of

antennae and four pairs of mouthparts; those of the female being extremely specialised in the functions of prehension and food uptake, those of the male are also specialised, but to a lesser degree. By comparison the female N. kilrymontis is completely wanting in mouthparts and appears to maintain its position by wedging itself in the tissues of the host (see section IV).

While the female Lernaeopodid feeds on the host blood via a mouth tube, N. kilrymontis most probably feeds by absorption through the integument. A further difference is that the Lernaeopodidae usually parasitise fish, whereas N. kilrymontis parasitises an invertebrate host.

In the Lernaeopodidae the females have complete alimentary canals consisting of mouth, oesophagus, stomach and intestine, while the males possess a reduced digestive system, there being no intestine or anus. In neither the male nor the female N. kilrymontis is there any trace of a digestive canal.

Although the sub-order Lernaeopodoida was created for copepods entirely parasitic on fish, several genera parasitic on invertebrates have been found which resemble the Lernaeopodoida more closely than any other sub-order and they have accordingly been included in this sub-order by Wilson. Such genera include: Xenocoeloma Caullery and Mesnil, 1915 and Herpyllobius Steenstrup and Lütken, 1861, both parasitic on annelids, Rhizorhina Hansen, 1892 on crustacea, and Pionodesmontes Bonnier, 1898 parasitic on ascidians.

In 1960 Bresciani and Lützen described a new highly modified endoparasitic copepod Gonophysema gullmarensis from the ascidian Ascidiella aspersa (O.M. Müller). From their discussion it would appear that N. kilrymontis

shares some features with Gonophysema and Xenocoeloma. Apparently these genera are the only two in which a nervous system has not been demonstrated, as is also the case for N. kilrymontis, neither do they, like N. kilrymontis, possess appendages, although Gonophysema does have a simple muscular system. Furthermore, of all known parasitic copepods, Gonophysema, Xenocoeloma, and a few species of the family Herpyllobiidae, are the only ones lacking a mouth, an intestine and an anus. To this list N. kilrymontis may now be added.

In certain Lernaeopodidae (and Herpyllobiidae) the males live as semi-parasites near the genital openings of the females, and in Gonophysema the males have become internal inhabitants of the internally parasitising female and the transfer of sperms has also become internal. An intermediate case is furnished by Ophioika asymmetrica Pyyefinch, 1940, an endoparasitic copepod of an ophiuroid in which the male has grown half-way into the body of the female, but where fertilization is still external. Of these three types N. kilrymontis certainly resembles Gonophysema more closely.

Bresciani and Lützen state "There is hardly any reason to follow the usual system, in which Xenocoeloma is placed among the Lernaeopodids (e.g. Wilson 1932). It is true that it shares the absence of an oviduct in the usual sense of the word with this group, but the division of the ovary in a germinal and a maturation portion is only found in Xenocoeloma and Gonophysema. In all other copepods the maturation of the eggs takes place in the ovary itself ..... The two hermaphroditic genera occupy such an isolated position that it should be justified to create a new sub-order for them. Nevertheless we refrain from so doing ....."

In N. kilrymontis there is no distinct division into ovary and oviduct, I have assumed, for convenience in nomenclature, that the two are continuous. If that part of the egg duct which I have named the oviduct is in fact the ovary, there being no oviduct, then the similarity between N. kilrymontis and these two genera is further increased. However there are many differences between Nucellicola and Gonophysema and Xenocoeloma, for example the nauplii of the latter two possess anal furcae, these are not present in Nucellicola. The developmental stages of Gonophysema also differ widely from those of Nucellicola, there being in the former a unique infesting stage called the onychopodid.

While disagreeing with Bresciani and Lützen that the erection of a new sub-order for degenerate parasites is at present justifiable, I do agree that such forms should not be placed in the Lernaeopodoida due to the remarkable dissimilarities between these forms and the Lernaeopodoida.

With regard to associations in general between copepods and molluscs, many copepod species either parasitic on, or commensal with molluscs have been described. Of these the majority of the relationships are of a commensal nature and most of the copepod members of such relationships belong to the sub-order Cyclopoida. Monod and Dollfus (1932), in their review of the copepods parasitic on molluscs, list 70 different copepods from 106 different hosts. Of these the majority are commensals and they list only three species (all belonging to the sub-order Caligoida) from prosobranch gastropods, namely, Cerastocheres trochicola Monod and Dollfus, 1932 from Trochus (Tectus) obeliscus Gmelin, Trochicola entericus Dollfus,

1914 from two species of Gibbula (L.) and from Calliostoma zizyphinum (L.), and Lichomolgus (Macrochiron) trochi Canu, 1899 from two Gibbula species. Only one of these, Cerastocheres, shows marked degrees of specialisation and degeneration and it belongs to the family Lernaeidae.

Since 1932 many new species living with molluscs have been described but in no case is there one so degenerate and specialised as N. kilrymontis. Although several degenerate forms from annelids, crustaceans and ascidians are known, N. kilrymontis is the first example of such a highly specialised parasitic copepod from the molluscs.

The diagnosis of N. kilrymontis is given below, the genus, for the present, remaining "incertae sedis".

Diagnosis

Nucellicola kilrymontis gen. et sp. nov.

(Nucellicola - inhabiting Nucella; kilrymontis - from the type locality Kilrymont, old Scottish name for St. Andrews).

Fixed, endoparasitic copepod with degenerate male, or males, enclosed within the body of the degenerate female. Parasitic in tissues of prosobranch gastropod.

Female

Body vermiform, cylindrical, unsegmented with thin, soft, transparent cuticle and with one or more anterior filamentous processes ramifying in the host's tissues. Appendages completely wanting.

Ovaries two, situated posteriorly, continuous with oviducts leading to vagina, and with wall of terminal region of oviduct specialised as cement gland. Vagina associated with four to six accessory glands and opening with sub-terminal aperture. Eggs arranged multiserially in a single egg-string.

Male

One or more, even five, dwarf, ovoid, unsegmented males situated posteriorly within the female in close proximity to the vagina. Male with two pairs of clavate antennae and with a pair of well-developed sub-chelate maxillipeds. Cuticle soft and transparent.

Testes two, large, pyriform situated postero-dorsally. Vas deferens continuous with testes and opening laterally to maxillipeds. Spermatophores formed in terminal region of vas deferens.

Development

Nauplius and 1st and early 2nd metanauplius passed within the egg-string. Eclosion from egg of 2nd metanauplius, of brief stadium, followed after ecdysis by 3rd metanauplius, also of short stadium. Succeeding 1st copepodid, of longer stadium, moults to 2nd copepodid instar. Third copepodid instar unknown.

Host

Occurs within tissues, usually digestive gland, of Nucella lapillus (L.) (Gastropoda, Prosobranchia).

Dimensions

Adult female, 2.5 mm to 7.1 mm long by 0.5 mm to 1.8 mm broad. Adult male, 0.53 mm to 0.69 mm long by 0.34 mm to 0.42 mm broad. Egg-string (estimated) up to 70 mm long.

Host locality

Rocky shore in the Bay of St. Andrews, and, to a lesser extent, along the immediate coastline southwards.

VI RELATIONSHIP WITH HOST AND HOST SPECIFICITY

1. Relationship with host

The relationship between Nucellicola kilrymontis gen. et sp. nov. and Nucella lapillus (L.) is of an intimate nature, the parasite being found firmly wedged in the tissue, usually the digestive gland, of the host. Neither organism appears to affect the other adversely and although microchemical investigations were not carried out, a study of stained sections, varying between 3 μ - 100 μ in thickness, revealed no noticeable difference between the tissues of parasitised and non-parasitised whelks (see plate 37, figs 99 and 100). There was neither any tissue reaction nor distortion of host tissue in any of the cases examined.

It is interesting to note that glycogen has been found in trematodes infesting fresh-water gastropods and that this glycogen has come from the host's tissues (Agersborg, 1924). Thus, such parasites need not be dependent on the blood of the host - they can live off the stored-up food of the body in general. Accordingly in N. kilrymontis it is possible, in view of the absence of mouthparts and the presence of a soft body wall, that the animal feeds by the absorption of materials through the body wall. Furthermore, since the presence of the parasite has no noticeable detrimental effect on the host, it is possible that N. kilrymontis lives on the stored foods in the host's digestive gland. I have observed that Nucella lapillus can live for long periods (at least eight months) without food, the only noticeable effect being a diminution in the size of the digestive gland (see section II). It is accordingly conceivable that N. kilrymontis could live on the same food reserves without detriment to the host.

In experiments involving parasitised and non-parasitised whelks I noticed that the former tended to die off before the latter, due possibly to the fact that reserves in the digestive glands of parasitised whelks were used up more quickly than in the non-parasitised whelks.

The degree of infestation varies considerably; I have found up to six adult females present in one host, and in one such instance I estimated that the parasites occupied some 50 per cent of the host's visceral mass without causing any apparent harm.

2. Host specificity

By far the most common prosobranch inhabiting the shore in the Bay of St. Andrews is the common periwinkle Littorina littorea (L.). Approximately 1,000 specimens of this prosobranch were examined and not one of these showed any trace of N. kilrymontis, although such specimens were taken from the regions of greatest infestation of Nucella lapillus. From deeper waters approximately 350 specimens of the whelk Buccinum undatum L. were examined and not one of these showed any trace of N. kilrymontis. Other local molluscs examined were various top shells and Mytilus edulis L. (particularly since these were used as a source of food for Nucella in early experiments). In no case did I find any trace of N. kilrymontis. I conclude that the relationship between Nucella lapillus and N. kilrymontis is a parasitism of a highly specific nature.

VII THE OCCURRENCE AND DISTRIBUTION OF NUCELLICOLA KILRYMONTIS

1. The Occurrence of Nucellicola kilrymontis in whelks from the rocks at the Castle Sands, the Bay of St. Andrews.

During the course of her investigations Miss Murdoch established that parasitised specimens of Nucella lapillus occurred on all of the rocky shores at St. Andrews. She obtained an average infestation of 15.6% having examined 507 host specimens from July 1954 to April 1955 and finding 79 of these parasitised. Of this total 41 out of 250 female hosts (16.4%), 26 out of 177 male hosts (14.7%), and 12 out of 80 hosts, sex undetermined (15%), were infested. The collections varied in number from 13 to 171 and were made at several different sites in the Bay of St. Andrews.

From October 1961 to April 1962 I took many random samples from six different rock-sites (numbered A to F) in the region of the castle sands. The objects of these random samples were, a) to locate a suitable site on which I might conduct a long-term survey, and b) to familiarise myself with the recognition of parasitised whelks so that my results over the succeeding two years would be statistically sound. In fact this precaution proved worthwhile since my first samples showed a noticeably lower percentage infestation than all later ones, indicating that in these early counts I had most probably not detected all of the parasites. A very close examination of host specimens was required since in several instances the only evidence of parasitism was a small section of the egg-string barely visible beneath the host integument. I will add, at this stage, that it is impossible to detect the presence of N. kilrymontis without first removing the shell of the host.

I finally selected site A which is a rock shelf fronting the castle ruins. Figure 101, plate 38, shows site A from above at mid-tide, and fig. 102, plate 38, is the same site photographed from point X on fig. 101. Specimens of Nucella lapillus are found all over the rocks and more particularly, especially in the winter months, in the deep clefts in the rock shelves. On these rocks there is an abundant supply of Balanus balanoides upon which the whelks feed.

I sampled site A at frequent intervals over a period of two years taking the same number of host specimens, 50, each time. In this way I hoped to be able to trace any seasonal variations in N. kilrymontis. Although 50 specimens is not a large number statistically I deemed it unwise to take more so that the population might not eventually become significantly lowered. I took my samples each time from the lower third (A/1) of the intertidal zone (spring tide levels), and the results are recorded in the graph Text fig. 3 and table 4.

In the graph there are no significant peaks or depressions which might indicate the presence of a breeding cycle in this parasite. In April 1963 and April 1964 there are two increases in percentage infestation and I attribute this to the fact that during the winter months host specimens congregate together in rock crevices where close proximity would result in more individuals becoming infested.

I kept separate records for male and female hosts and although the males generally show a higher degree of infestation by N. kilrymontis I can attach no significance to this.

TABLE 4

Analysis of samples of Nucella lapillus (L.) collected from site A/1 (the castle rocks, St. Andrews) during the period 31st May, 1962 to 27th April, 1964, showing the percentage infestation by Nucellicola kilrymontis.

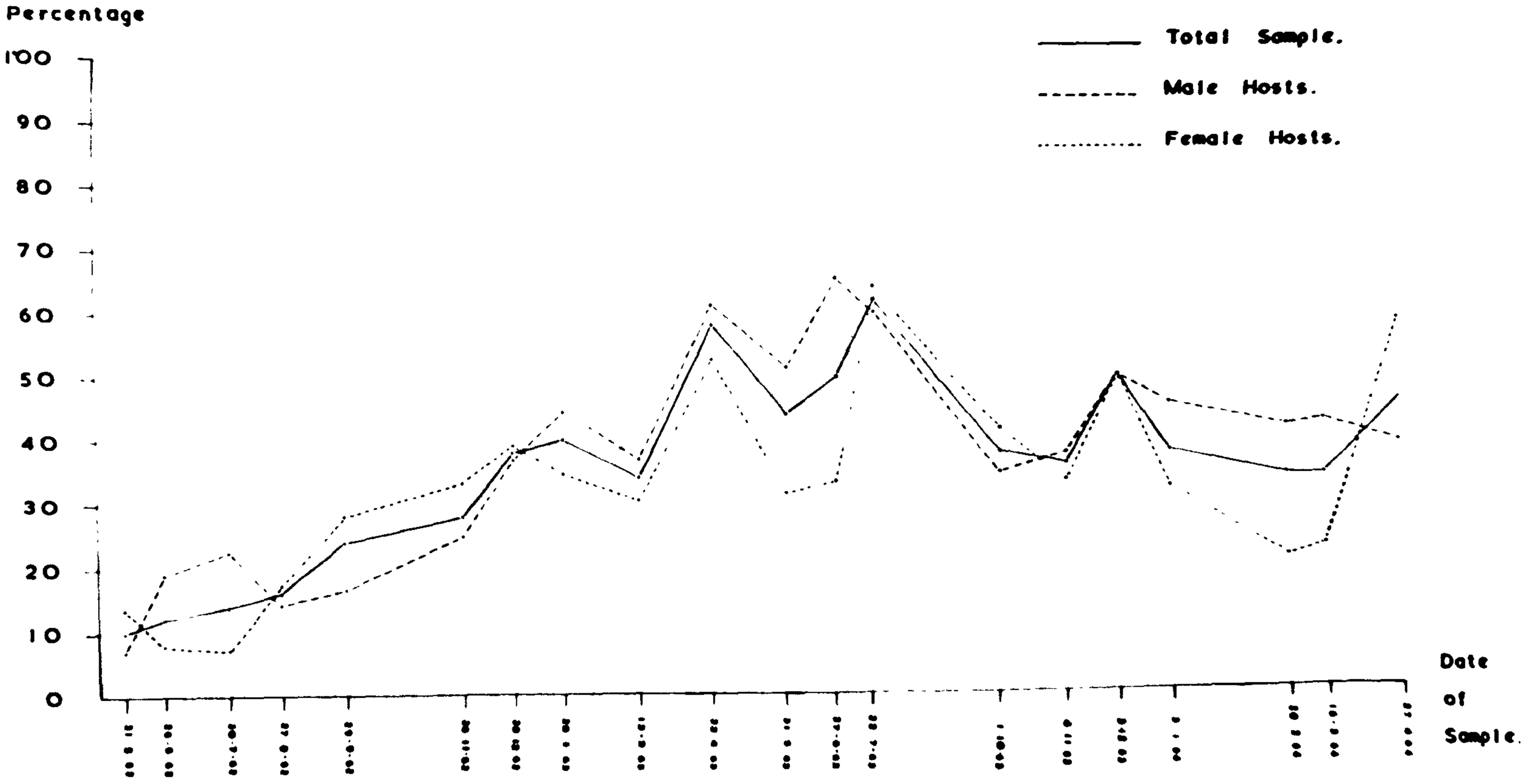
DATE	TOTAL HOSTS			MALE HOSTS			FEMALE HOSTS		
	Number examined	Number parasitised	Percentage infestation	Number examined	Number parasitised	Percentage infestation	Number examined	Number parasitised	Percentage infestation
31. 5.62	50	5	10.00	28	2	7.14	22	3	13.64
26. 6.62	50	6	12.00	21	4	19.05	29	2	6.90
30. 7.62	50	7	14.00	22	5	22.73	28	2	7.14
27. 8.62	50	8	16.00	21	3	14.29	29	5	17.24
29. 9.62	50	12	24.00	18	3	16.67	32	9	28.13
28.11.62	50	14	28.00	32	8	25.00	18	6	33.33
28.12.62	50	19	38.00	27	10	37.04	23	9	39.13
28. 1.63	50	20	40.00	27	12	44.44	23	8	34.78
13. 3.63	50	17	34.00	27	10	37.04	23	7	30.43
22. 4.63	50	29	58.00	31	19	61.29	19	10	52.63
31. 5.63	50	22	44.00	31	16	51.61	19	6	31.58
27. 6.63	50	25	50.00	26	17	65.38	24	8	33.33
22. 7.63	50	31	62.00	25	15	60.00	25	16	64.00
1.10.63	50	19	38.00	26	9	34.62	24	10	41.67
4.11.63	50	18	36.00	29	11	37.93	21	7	33.33
3.12.63	50	25	50.00	26	13	50.00	24	12	50.00
3. 1.64	50	19	38.00	22	10	45.45	28	9	32.14
20. 2.64	50	17	34.00	31	13	41.94	19	4	21.05
16. 3.64	50	17	34.00	28	12	42.86	22	5	22.73
27. 4.64	50	23	46.00	33	13	39.39	17	10	58.82
TOTAL	1,000	353	35.30	531	205	38.61	469	148	31.56

The Percentage Infestation of Nucella lapillus by Nucellicola kilrymontis.

Locality – Site A/1 (see text), the Castle Rocks, St. Andrews.

Period – May 1962 to April 1964.

Sample Size – 50



TEXT FIG. 3

TABLE 5

A comparison of the incidence of infestation of Nucella lapillus (L.) by Nucellicola kilrymontis in the three sites A/1, A/2 and A/3 (see text).

DATE	SITE A/1			SITE A/2			SITE A/3		
	Number examined	Number parasitised	Percentage infestation	Number examined	Number parasitised	Percentage infestation	Number examined	Number parasitised	Percentage infestation
28.11.62	50	14	28.00	28	7	25.00	19	1	5.26
13. 3.63	50	17	34.00	27	7	25.93	24	3	12.50
22. 7.63	50	31	62.00	50	9	18.00	50	3	6.00
1.10.63	50	19	38.00	50	6	12.00	43	2	4.65
20. 2.64	50	17	34.00	48	9	18.75	50	5	10.00
TOTAL	250	98	39.20	203	38	18.72	186	14	7.53

I divided the rock shelf A into three equal sectors, A/1, A/2 and A/3 corresponding to the region nearest L.W.S.T., the mid-tide region, and the region nearest H.W.S.T. On several occasions I took samples from A/2 and A/3 for comparison with A/1. The host specimens from A/2 and A/3 were smaller than those from A/1 although I was careful to examine only mature whelks. There were noticeably more immature whelks in A/3 than the other two sectors - this agrees with the observations mady by Moore (1938). I found that the percentage parasitism was highest in A/1, becoming progressively lower towards A/3 - see Table 5. I deduced that those whelks living in A/1 were submerged for longer periods than those in A/3 and were accordingly more prone to infestation.

2. The Distribution of Nucellicola kilrymontis in the Bay of St. Andrews

I took samples of Nucella lapillus from various rocks in the Bay of St. Andrews and these are shown in Table 6. At all sites (including those in 3 and 4 of this section) collections were made at low tide. The highest infestations are found in the rocks around the castle beach and the step rock bathing pool, the rocks of these two regions being continuous and only 100 to 150 yards apart. The rocks around the step rock pool are the furtheat north in the Bay, sandy beaches extending from this point to the Tay estuary.

The rocks around the pier, although continuous with, and only 100 yards south of those of site A (see Plate 39, fig. 103), harboured whelks with a lower incidence of infestation than those from site A/1 and the step rock pool rocks. Furthermore these rocks are of a more exposed type and the mature whelks collected there were smaller than those from the previous two

TABLE 6

Analysis of samples of *Nucella lapillus* (L.) collected from various regions in the Bay of St. Andrews during the period 31st May 1962 to 27th April 1964, showing the percentage infestation by *Nucellicola kilrymontis*.

SITE	Nature of habitat	Number examined	Number parasitised	Percentage infestation
Step Rock Pool Rocks	sheltered	280	93	33.21
Castle Rocks (site A/1)	sheltered	1,000	353	35.30
Pier Rocks	sheltered	256	24	9.38
Kinkell Ness Rocks	exposed	334	55	16.47

sites. This in spite of the fact that there is a more varied and abundant fauna in this region than elsewhere in the Bay - presumably due to the effluent sewage here.

Samples from the rocks around Kinkell Ness (approx. two miles south from the pier) have a percentage infestation lower than the step rock pool and castle rocks sites, yet higher than those from the pier-rocks. There are few rocks between the pier and the Kinkell Braes, which are continuous with, and north of, Kinkell Ness, the two sites being separated by the East Sands (approx. 400 yards long); there are also very few specimens of Nucella lapillus between these two sites. The rocks around Kinkell Ness are not deeply fissured as are those between the castle beach and the step rock pool - they are more flattened (plate 39, fig. 104 /background/) and thus the animals living on them are more exposed to the actions of the waves. In connection with this, the whelks from this region are smaller than those from the more sheltered rocks - presumably due to the lack of food since neither Balanus balanoides nor Mytilus edulis are found in such large numbers as they are in the regions of sheltered rocks (see below).

3. The distribution of Nucellicola kilrymontis along the coastline north and south of St. Andrews

Several sites both north and south of St. Andrews were sampled and the results are shown in Table 7 and the map, Plate 40. It is interesting to record here that nowhere were whelks found corresponding in size to those from site A, St. Andrews. The mature whelks were all noticeably smaller and found generally on exposed rocks. The shell heights of the specimens examined were recorded and these are listed in Table 7. From this table

TABLE 7

Analysis of samples of Nucella lapillus (L.) collected along the coastline north and south of St. Andrews during the period 3rd May 1962 to 12th September 1964, showing the percentage infestation by Nucellicola kilrymontis.

Map Ref. No.	Locality	Date	Nature of habitat	Shell height (in cms)	Number examined	Number parasitised	Percentage infestation
1	St. Andrews, Castle rocks (A/1)	31.5.62 to 27.4.64	sheltered	1.7-2.4	1,000	353	35.30
2	St. Andrews, Pier rocks	31.5.62 to 27.4.64	sheltered/ exposed	1.4-1.9	256	24	9.38
3	St. Andrews, Step Rock Pool rocks	31.5.62 to 27.4.64	sheltered	1.6-2.1	280	93	33.21
4	Carnoustie, West Haven	10.1.64	exposed	1.2-1.6	148	0	00.00
5	Arbroath, Whiting Ness	10.1.64	exposed	1.2-1.7	63	0	00.00
6	Montrose, Sillo Craig	10.1.64	exposed	1.1-1.6	89	0	00.00
7	Johnshaven	10.1.64	exposed	1.2-1.5	50	3	6.00
8	Stonehaven	10.1.64	exposed	1.1-1.8	256	0	00.00
9	Aberdeen, Cove Bay	11.1.64	sheltered/ exposed	1.2-1.6	195	0	00.00
10	St. Andrews, Kinkell Ness	31.5.62 to 27. 4.64	exposed	1.5-1.9	334	55	16.47
11	Boarhills, Buddo Ness	3.5.62 and 19.10.62	exposed	1.1-1.6	119	13	10.92
12	Fife Ness	8.9.64	exposed	1.2-1.5	100	4	4.00
13	Crail, West Ness	8.9.64	exposed	1.2-1.5	100	2	2.00
14	Pittenweem, Birnie Craig	8.9.64	sheltered/ exposed	1.4-1.9	100	4	4.00
15	St. Monance, Partan Craig	8.9.64	exposed	1.3-1.6	100	1	1.00
16	Elie, Chapel Ness	8.9.64	exposed	1.2-1.5	100	3	3.00
17	Isle of May, North Ness	11.5.63	sheltered	1.5-2.1	208	0	00.00
18	Isle of May, South Ness	11.5.63	sheltered	1.3-1.9	161	0	00.00
19	North Berwick	12.9.64	exposed	1.3-1.6	100	1	1.00

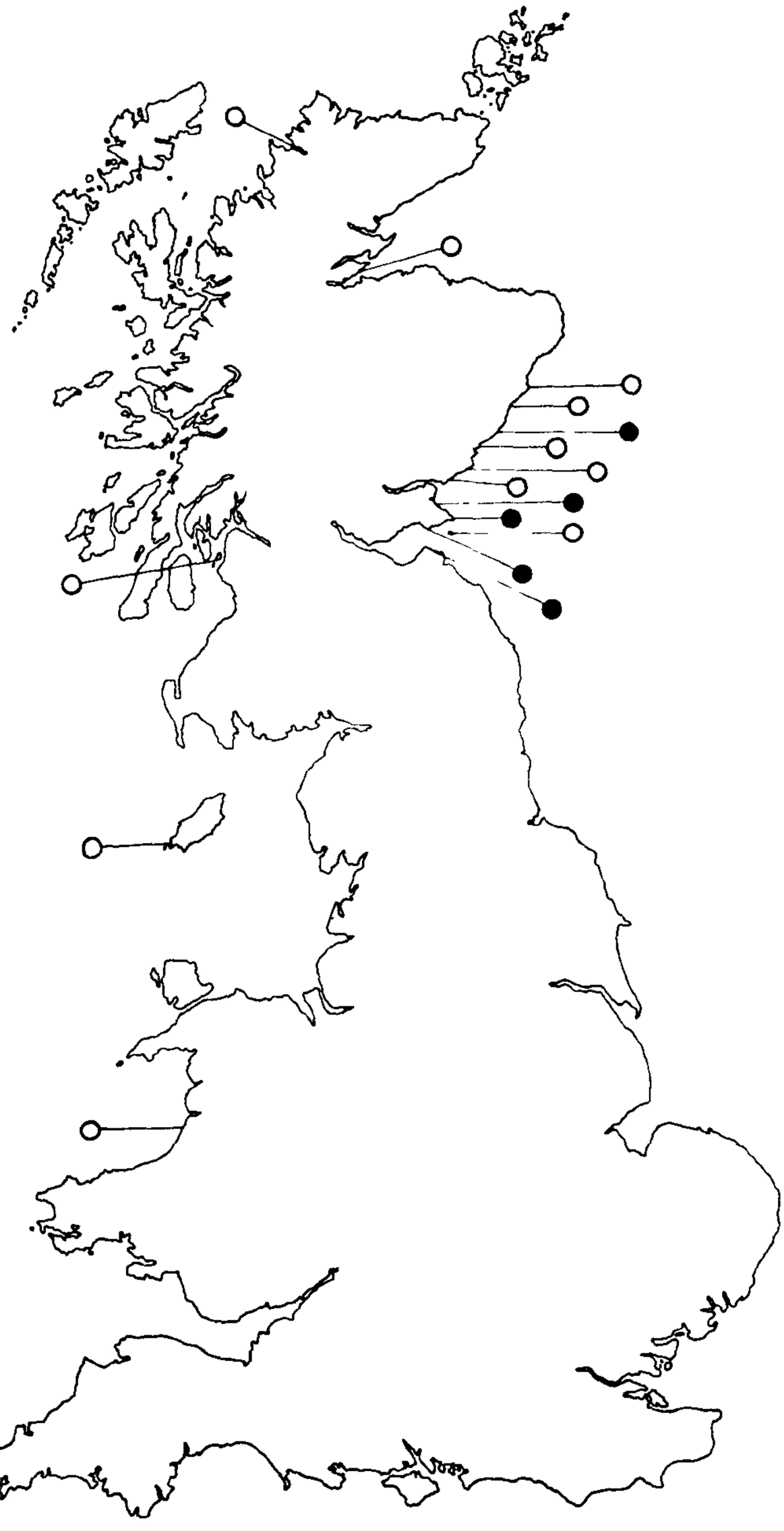
it is evident that whelks from exposed rocks are smaller than those from sheltered rocks.

In the map, Plate 40, the numbers refer to the different sites examined (see Table 7) and the asterisks indicate sites where parasitised whelks were recorded. The highest infestation was recorded in St. Andrews and those sites around the East Fife coast southwards from St. Andrews provided a lower percentage infestation. North of St. Andrews to the Tay there are very few rock-formations and the first convenient site for examination proved to be at West Haven, Carnoustie. From here northward to Cove Bay, Aberdeen, six sites were examined and from only one of these, Johnshaven, was N. kilrymontis recorded. This single record from Johnshaven appears to be inconsistent with other results north of the Tay. Having recorded several negative counts north of the Tay I had thought that this animal was restricted to the region south of the Tay but this is not the case.

The coastline south of St. Andrews to Elie is almost continuous rock and it is accordingly not difficult to imagine the spread of N. kilrymontis under such favourable conditions. The furthest point south along the coastline immediate to St. Andrews examined was North Berwick where there was an infestation of only one per cent. Although N. kilrymontis was found on the north and south shorelines of the Forth there is no record of its presence on the Isle of May. Neither was N. kilrymontis, or any similar parasite, recorded from Nucella lapillus in the Firth of Forth by Scott, T, 1892.

4. The distribution of Nucellicola kilrymontis around the British Isles

In addition to those sites listed in 3 above, five others around the British Isles were examined (see Table 8 and map, text fig. 4). Not one of these showed the presence of N. kilrymontis. Thus the investigations so far carried out indicate that, with the exceptions of Johnshaven and North Berwick, N. kilrymontis is restricted to the east coast of Scotland between the Firth of Tay and the Firth of Forth.



○ *N. kilrymontis* wanting.

● *N. kilrymontis* recorded.

TEXT FIG. 4. The geographical distribution of *Nucellicola kilrymontis*.

VIII SUMMARY

1. Professor H. G. Callan's discovery, in 1951, of a copepod living in Nucella lapillus (L.) was first investigated by Miss M. Murdoch as an honours exercise. She placed this animal in the genus Cerastocheres of the family Lernaeidae, but as the new species differs in so many respects from Cerastocheres and all other known genera a new genus, Nucellicola, has been erected and this parasite is described as Nucellicola kilrymontis gen. et sp. nov.

So far as can be ascertained N. kilrymontis is specifically parasitic in Nucella lapillus.

2. In this investigation the following techniques were used:-

i. Techniques were devised for identifying infested host specimens and for removing the parasite intact from the host.

ii. Fixation and staining methods for larvae and adults were the usual laboratory procedures using Zenker, Bouin and alcoholic sublimate for fixation and Mallory, Erlich, Mayer, Heidenhein and Chlorazol black E in P.V.A. etc. for staining.

3. The parasite is usually located in the digestive gland of Nucella lapillus, being completely enclosed within this gland.

An analysis of the incidence of infestation and the distribution of N. kilrymontis shows that:-

i. host specimens taken from the region of low water, spring tide, are more highly parasitised than those taken higher up the shoreline;

ii. mature host specimens show a higher rate of infestation than do immature ones;

iii. the highest rate of infestation, reaching 62% in a random sample of Nucella lapillus, is in the Bay of St. Andrews, and more particularly the rocks between the pier and the Step Rock pool at St. Andrews;

iv. a low rate of infestation obtains around the south-east coast into the Firth of Forth;

v. the only localities outside this region where N. kilrymontis has been recorded are at North Berwick and Johnshaven, near Aberdeen.

4. The adult females and males possess the following unusual features:-

i. there is complete absence of appendages in the female, and only one pair, the maxillipeds, is found in the male;

ii. chitin is absent from both sexes except for the maxillipeds of the male;

iii. there are two integuments around the adult female;

iv. there are four to six accessory glands, of unknown function, around the vagina of the female;

v. there is only one egg string;

vi. the male is very much smaller than the female and often there are several, up to five, males present, completely enclosed, in one female;

vii. there is no digestive system in either sex and it is assumed that N. kilrymontis feeds by absorption through the integuments;

viii. there is no trace of an excretory system, a muscular system, nor of a nervous system in the adults and immature adults of both sexes and thus neither do they possess any powers of locomotion;

ix. several immature adults are described, the immature females bearing anterior filamentous processes with possible nutritive or attachment functions.

5. The diploid chromosome number for the female N. kilrymontis is apparently 22.

6. Cyclical breeding activity does not occur in N. kilrymontis as all stages are obtained at all seasons of the year.

7. Development was successfully traced, from oocyte to 1st copepodid in those animals experimentally reared in the laboratory.

i. All stages prior to the 2nd metanauplius are passed within the egg string and the 2nd metanauplius emerges as the first free-swimming stage.

ii. An alimentary tract is lacking in all larval stages.

iii. A nervous system could not be demonstrated in any of the larval stages.

iv. The 1st, 2nd and 3rd metanauplii and the 1st copepodid have well-developed muscular systems.

v. Observations on the swimming behaviour of the larvae lead to the conclusion that the 1st copepodid is a distributive stage.

vi. the 2nd copepodid is described briefly.

vii. The male exuviae, presumed to be the remains of the exo-skeleton of the final male copepodid stage, are found between the integuments of the male and female.

8. During the course of experiments it was found that:-

i. Nucella lapillus can survive for long periods, up to eight months, without food and with no apparent detrimental effects;

ii. the use of antibiotics, following a technique described by Shelbourne, J.E. (1963), has an advantageous effect on the life span of Nucella lapillus under laboratory conditions;

iii. all of the larval stages up to, and including, the 1st copepodid are neither positively nor negatively phototrophic;

iv. larval stages beyond the 1st copepodid were not obtained.

9. With regard to the systematic position of N. kilrymontis it is concluded that although possessing several characteristics in common with those of members of the sub-order Lernaeopodoida and more particularly of the family Lernaeopodidae, the new genus Nucellicola cannot in the meantime be included in the current scheme of classification.

10. The following is a definition of the new species:-

Nucellicola kilrymontis gen. et sp. nov.

(Nucellicola - inhabiting Nucella; kilrymontis - from the type locality Kilrymont, old Scottish name for St. Andrews).

Fixed, endoparasitic copepod with degenerate male, or males, enclosed within the body of the degenerate female. Parasitic in tissues of prosobranch gastropod.

Female

Body vermiform, cylindrical, unsegmented with thin, soft, transparent cuticles and with one or more anterior filamentous processes ramifying in the host's tissues. Appendages completely wanting.

Ovaries two, situated posteriorly, continuous with oviducts leading to vagina, and with wall of terminal region of oviduct specialised as cement gland. Vagina associated with four to six accessory glands and with sub-terminal aperture. Eggs arranged multiserially in a single egg-string.

Male

One or more, even five, dwarf, ovoid, unsegmented males situated posteriorly within the female in close proximity to the vagina. Male with two pairs of clavate antennae and with a pair of well-developed sub-chelate maxillipeds. Cuticle soft and transparent.

Testes two, large, pyriform situated postero-dorsally. Vas deferens continuous with testes and opening laterally to maxillipeds. Spermatophores formed in terminal region of vas deferens.

Development

Nauplius and 1st and early 2nd metanauplius passed within the egg-string. Eclosion from egg of 2nd metanauplius, of brief stadium, followed after ecdysis by 3rd metanauplius, also of short stadium. Succeeding 1st copepodid, of longer stadium, moults to 2nd copepodid instar. Third copepodid instar unknown.

Host

Occurs within tissues, usually digestive gland, of Nucella lapillus (L.) (Gastropoda, Prosobranchia).

Dimensions

Adult female, 2.5 mm to 7.1 mm long by 0.5 mm to 1.8 mm broad. Adult male, 0.53 mm to 0.69 mm long by 0.34 mm to 0.42 mm broad. Egg-string (estimated) up to 70 mm long.

Host locality

Rocky shore in the Bay of St. Andrews, and, to a lesser extent, along the immediate coastline southwards.

IX ACKNOWLEDGEMENTS

I would like to record my thanks to the following:- Professor H.G. Callan, F.R.S., for placing the necessary facilities at my disposal in the department of Natural History; Mrs. Marion Clark (nee Miss M. Murdoch) for making available to me her original notes and drawings of Cerastocheres nucellae; Dr. Mary Parke, Plymouth Marine Laboratory, for kindly supplying me with cultures of diatoms and flagellates; Dr. C.H. Mortimer, F.R.S., director, Millport Marine Laboratory, Great Cumbrae, Dr. G. Russell, Marine Laboratory, Port Erin, I.O.M., and Dr. J.S. Scott and Mr. M.D.B. Burt of the department of Natural History, the University of St. Andrews, all for supplying me with collections of live Nucella lapillus; and Dr. Z. Kabata, Torry Marine Laboratory, Aberdeen, for helpful suggestions made during the course of my investigations.

I wish also to express my thanks to the Department of Scientific and Industrial Research for financial assistance in the form of a D.S.I.R. studentship awarded during the period of this study.

Finally, my deepest gratitude is to my supervisor, Mr. D.R.R. Burt, F.L.S., F.R.S.E., for his constant help and guidance throughout the course of this work.

X

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PLATE 11 — Nucellicola kilrymontis gen. et sp. nov.

Fig. 22 — Photograph of 15 μ sagittal section through the posterior region of a mature adult.

Fig. 23 — Photograph of 15 μ horizontal section through the posterior region of a mature adult.

PLATE 12 — Nucellicola kilrymontis gen. et sp. nov.

Fig. 24 — Drawing of a live adult male.

Fig. 25 — Composite drawing of an adult male.

PLATE 13 — Nucellicola kilrymontis gen. et sp. nov.

Figs 26 and 27 — Drawings of the maxillipeds of adult males.

Fig. 28 — Drawing of spermatophores.

Fig. 29 — Drawing of spermatozoa.

PLATE 14 Nucellicola kilrymontis gen. et sp. nov.

Fig. 30 Photograph of 10 μ horizontal section through the posterior region of a mature adult.

Fig. 31 Photograph of 10 μ horizontal section through the cement gland of a mature female.

PLATE 15 Nucellicola kilrymontis gen. et sp. nov.

Fig. 32 Photograph of oocyte from the early region of the oviduct showing chromosomes in prophase.

Fig. 33 Photograph of oocyte from the terminal region of the oviduct showing chromatids at metaphase.

PLATE 16 Nucellicola kilrymontis gen. et sp. nov.

Fig. 34 Photograph of 5 μ transverse section through the ovary of a mature adult female.

Figs 35 and 36 Photographs of 5 μ longitudinal sections through the oviducts of mature adult females.

Fig. 37 Photograph of 15 μ transverse section through a mature adult female in situ.

PLATE 17 Nucellicola kilrymontis gen. et sp. nov.

Fig. 38 Photograph of 12 μ transverse section of eggs at the "blastula" stage.

Fig. 39 Photograph of 12 μ transverse section of eggs at a later "blastula" stage.

Fig. 40 Photograph of 10 μ section of eggs at a later "blastula" stage.

Fig. 41 Photograph of 10 μ section of the egg-string containing early nauplius stages.

PLATE 18 Nucelliçola kilrymontis gen. et sp. nov.

Fig. 42 Photograph of 10 μ horizontal section of the 3rd nauplius stage.

Fig. 43 Photograph of 10 μ sagittal section of the 2nd metanauplius stage within the egg-string.

PLATE 19 Nucellicola kilrymontis gen. et sp. nov.

Figs 44 and 45 Photographs of sections of portions of egg-string in situ showing 1st and 2nd metanauplii sectioned in various planes.

PLATE 20 Nucellicola kilrymontis gen. et sp. nov.

Fig. 46 Drawing of 2nd metanauplius (free-living).

PLATE 21 Nucellicola kilrymontis gen. et sp. nov.

Fig. 47 Drawing of 3rd metanauplius.

PLATE 22 Nucellicola kilrymontis gen. et sp. nov.

Fig. 48 Drawing of 1st copepodid.

PLATE 23 Nucellicola kilrymontis gen. et sp. nov.

Figs 49 and 50 Photographs of live 2nd metanauplii.

PLATE 24 Nucellicola kilrymontis gen. et sp. nov.

Figs 51 and 52 Photographs of live 3rd metanauplii with emergent 1st copepodids.

PLATE 25 Nucellicola kilrymontis gen. et sp. nov.

Figs 53 and 54 Photographs of live 1st copepodids.

PLATE 26 Nucellicola kilrymontis gen. et sp. nov.

Figs 55 and 56 Drawings of 1st metanauplius showing the arrangement of the muscles.

PLATE 27 Nucellicola kilrymontis gen. et sp. nov.

Fig. 57 Drawing of free-living 2nd metanauplius showing the arrangement of the muscles.

Fig. 58 Drawing of 3rd metanauplius showing the arrangement of the muscles.

PLATE 28 Nucellicola kilrymontis gen. et sp. nov.

Fig. 59 Drawing of 3rd metanauplius showing the arrangement of muscles.

Fig. 60 Drawing of 1st copepodid showing the arrangement of muscles.

PLATE 29 — Nucellicola kilrymontis gen. et sp. nov.

Figs 61 and 62 — Photographs of male exuviae.

PLATE 30 — Nucellicola kilrymontis gen. et sp. nov.

Figs 63 to 73 — Drawings of the head appendages of the 2nd and 3rd metanauplii, the 1st copepodid and the male exuviae.

PLATE 31 — Nucellicola kilrymontis gen. et sp. nov.

Figs 74 to 88 — Drawings of thoracic appendages of the 2nd and 3rd metanauplii, the 1st copepodid and the male exuviae, and of the abdomens of the 1st copepodid and the male exuviae.

PLATE 32 — Nucellicola kilrymontis gen. et sp. nov.

Fig. 89 — Drawing of immature adult female possessing neither male nor egg-string.

Fig. 90 — Drawing of young mature female possessing an incompletely-developed male and an egg-string.

PLATE 33 — Nucellicola kilrymontis gen. et sp. nov.

Fig. 91 — Photograph of 10 μ horizontal section of the posterior end of an immature adult female without male.

Fig. 92 — Photograph of 15 μ transverse section through an immature adult female without male.

PLATE 34 Nucellicola kilrymontis gen. et sp. nov.

Figs 93 and 94 Photographs of 15 μ horizontal sections through the anterior region of an immature adult female, without male, in situ.

PLATE 35 Nucellicola kilrymontis gen. et sp. nov.

Fig. 95 Photograph of immature adult female including a recently-arrived male.

Fig. 96 Photograph of 12 μ longitudinal section through the posterior region of the specimen in fig. 95.

PLATE 36 Nucellicola kilrymontis gen. et sp. nov.

Fig. 97 Photograph of young adult female with included male.

Fig. 98 Photograph of an unusual mature adult taken from an "unsuitable" site in the mantle of the host.

PLATE 37 Nucella lapillus (L.)

Fig. 99 Photograph of 10 μ section through the testis and digestive gland of an unparasitised male.

Fig. 100 Photograph of 10 μ section through the testis and digestive gland of a male parasitised by Nucellicola kilrymontis gen. et sp. nov.

PLATE 38

Figs 101 and 102 Photographs of site A, the rock shelf fronting the castle ruins at St. Andrews.

PLATE 39

Fig. 103 Photograph of the rock shelves taken from the castle sands. "Sheltered" rocks.

Fig. 104 Photograph of the rocks at Fife Ness. "Exposed" rocks.

PLATE 40

Fig. 105 Map of part of the east coastline of Scotland showing the localities sampled.

PLATE 1 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 1 Photograph, from the left side, of a living specimen of *Nucella lapillus* (L.) with shell removed. A mature specimen of the parasite is visible in the digestive gland of the host, the egg-string passing into the renal organ of the latter.

a - egg-string

b - trunk of female

Fig. 2 Photograph, from the left side, of a preserved specimen of *Nucella lapillus* (L.) with shell removed. The presence of the parasite is denoted only by its egg-string which passes from the digestive gland towards the anterior of the host.

a - egg-string

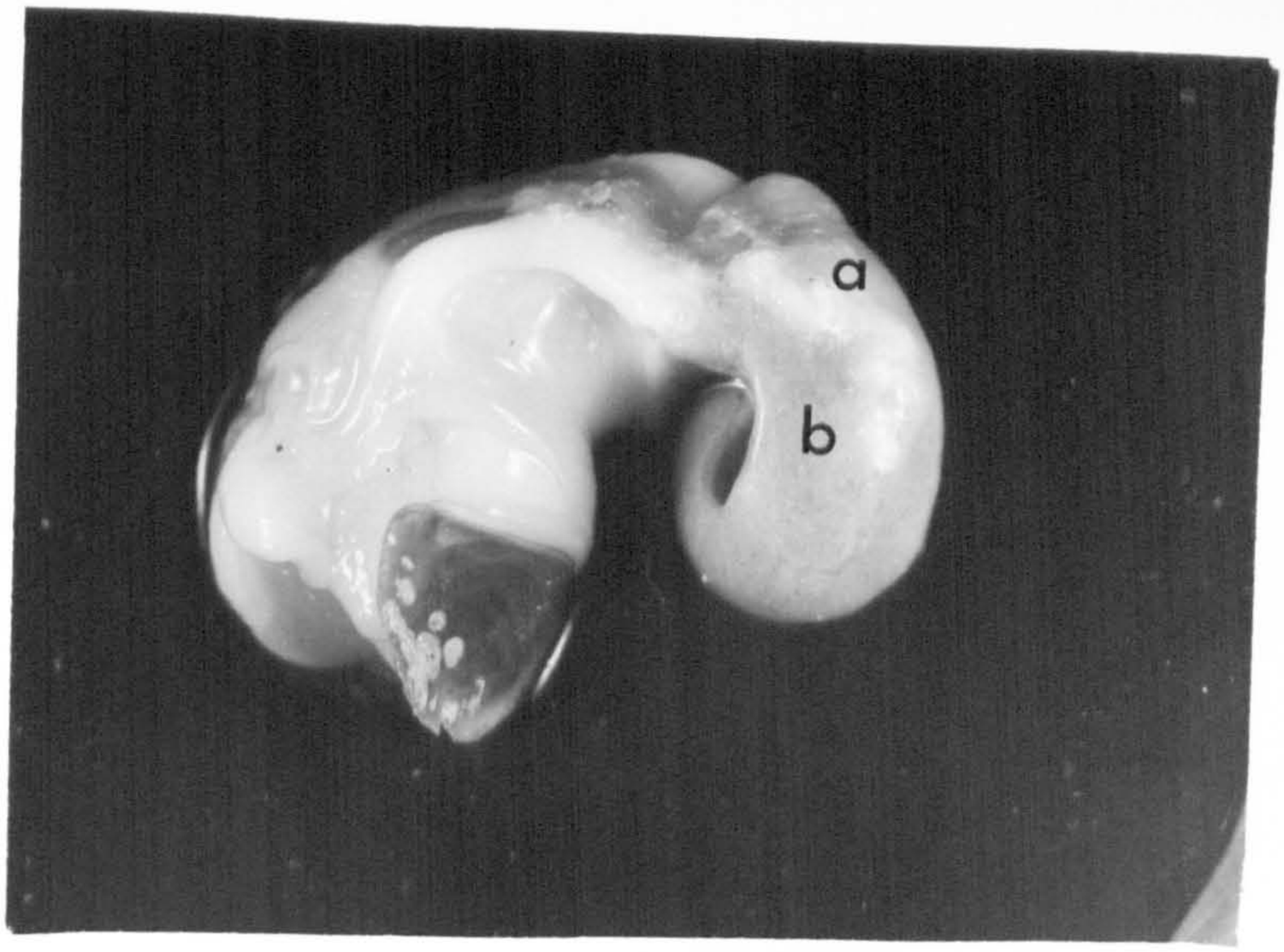


Fig. 1

1 cm.

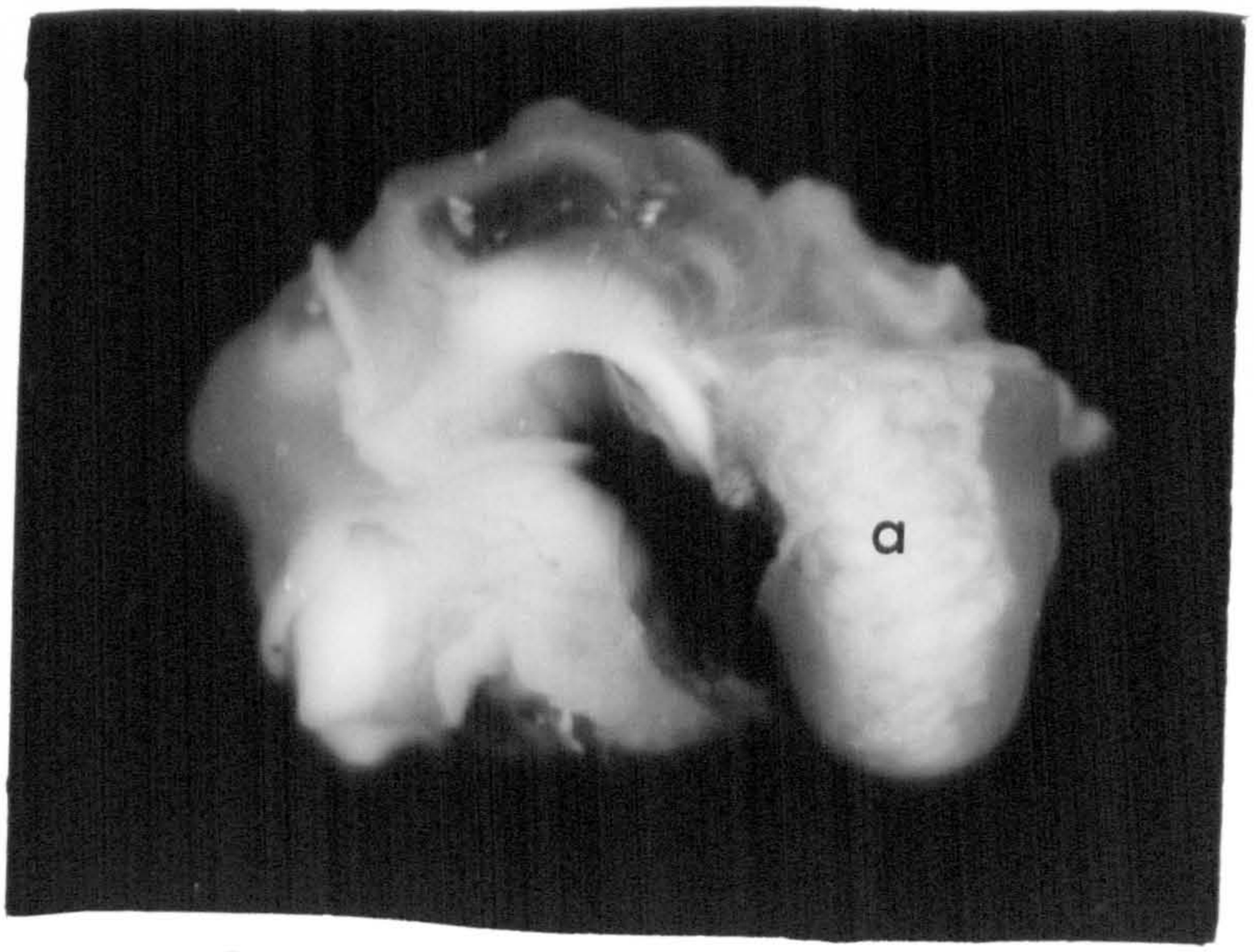


Fig. 2

PLATE 2 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 3 Photograph of live adult. Some of the internal structures are visible through the integument. The outer integument has ruptured and is 'curled up' at the anterior end of the trunk (on the right). The male is barely discernible and the egg-string is lacking.

Fig. 4 Photograph of live adult. Both integuments are intact and part of the egg-string remains (on the right). Internal structures not visible.

Fig. 3

⟵ 5 mm. ⟶

Fig. 4

PLATE 3 Nucellicola kilrymontis gen. et sp. nov.

Fig. 5 Drawing of mature adult.

a - male

b - cement gland

c - accessory gland

d - mature region of oviduct

e - ovary

f - early region of oviduct

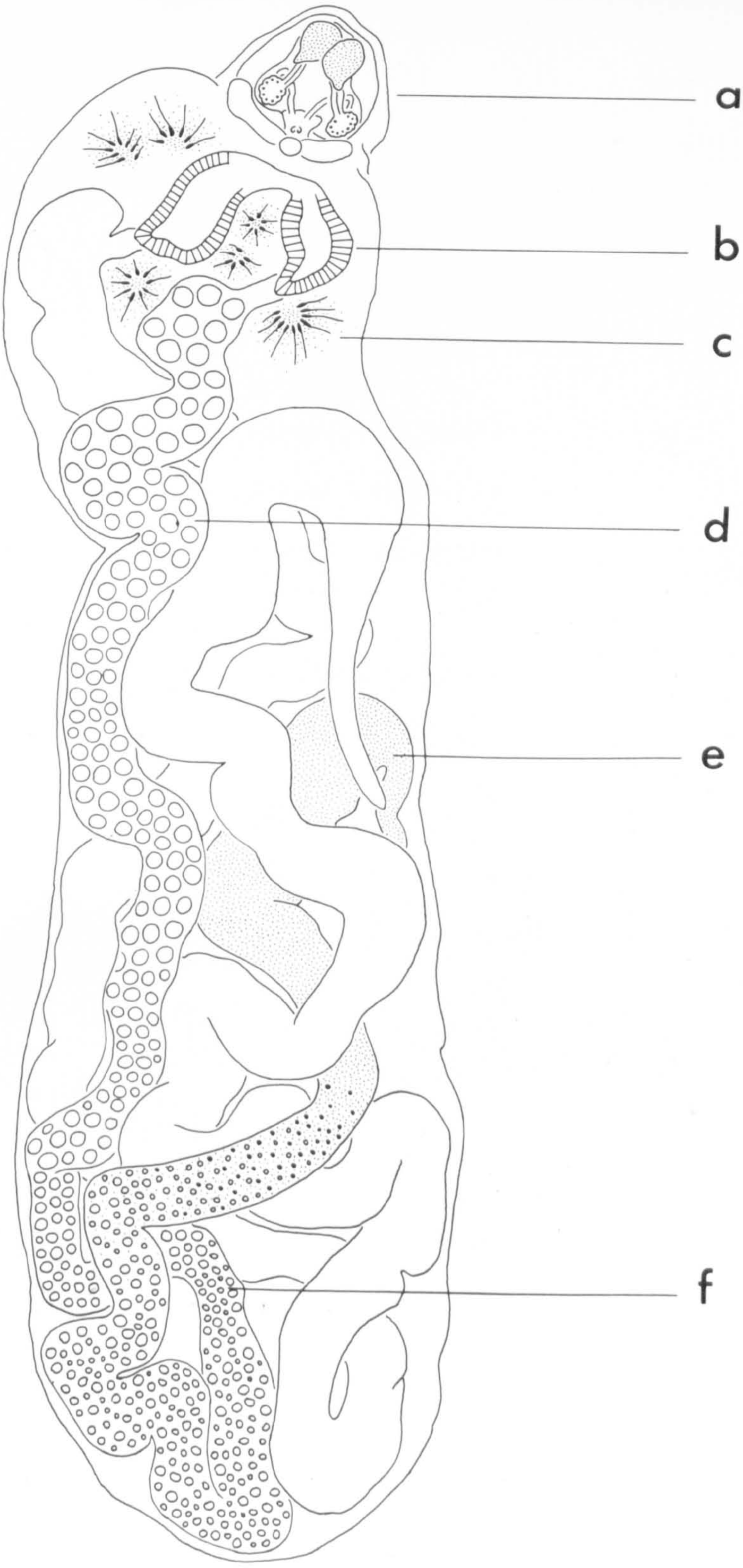


Fig. 5

PLATE 4 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 6 Photograph of a mature adult prepared by the squash technique. Two dwarf males are included at the posterior end of the female (to the right).

Fig. 7 Photograph of posterior end of a mature adult prepared by the squash technique. Three males are present and spermatophores are visible around the central male.

a - spermatophores

b - rostrum of male

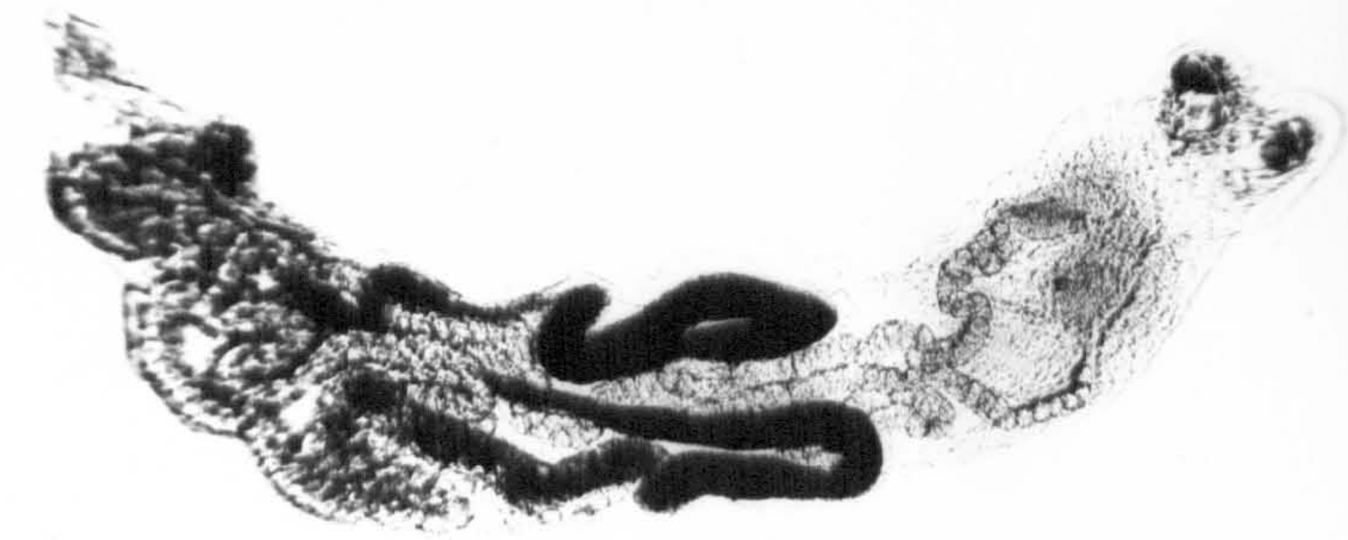


Fig. 6

1 mm.

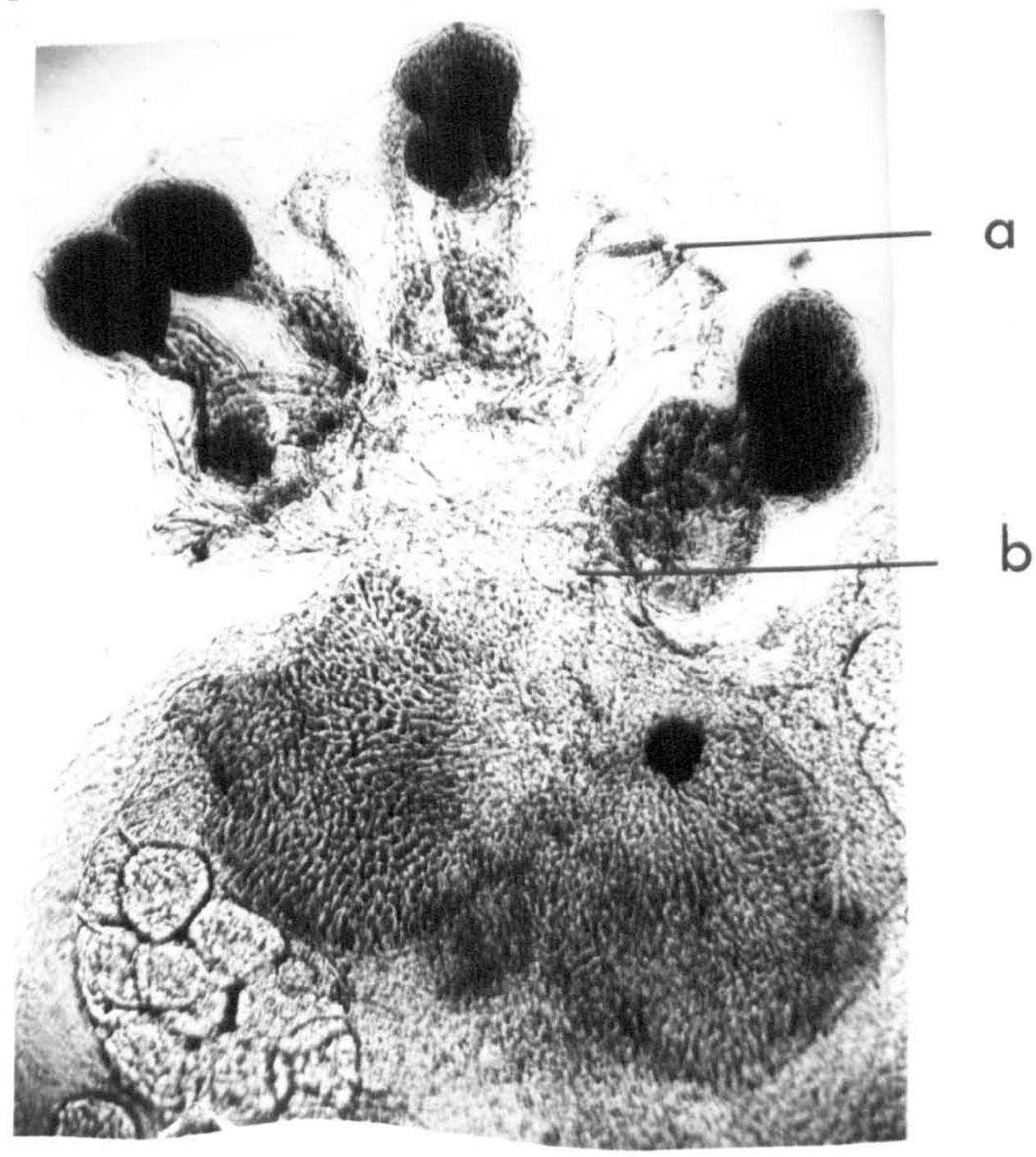


Fig. 7

PLATE 5 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 8 Photograph of mature adult prepared by the squash technique. The ovaries and oviducts of the female and four males are clearly distinguished.

Fig. 9 Photograph of mature adult prepared by the squash technique. Five males are present in this specimen.

Fig. 8

1 mm.

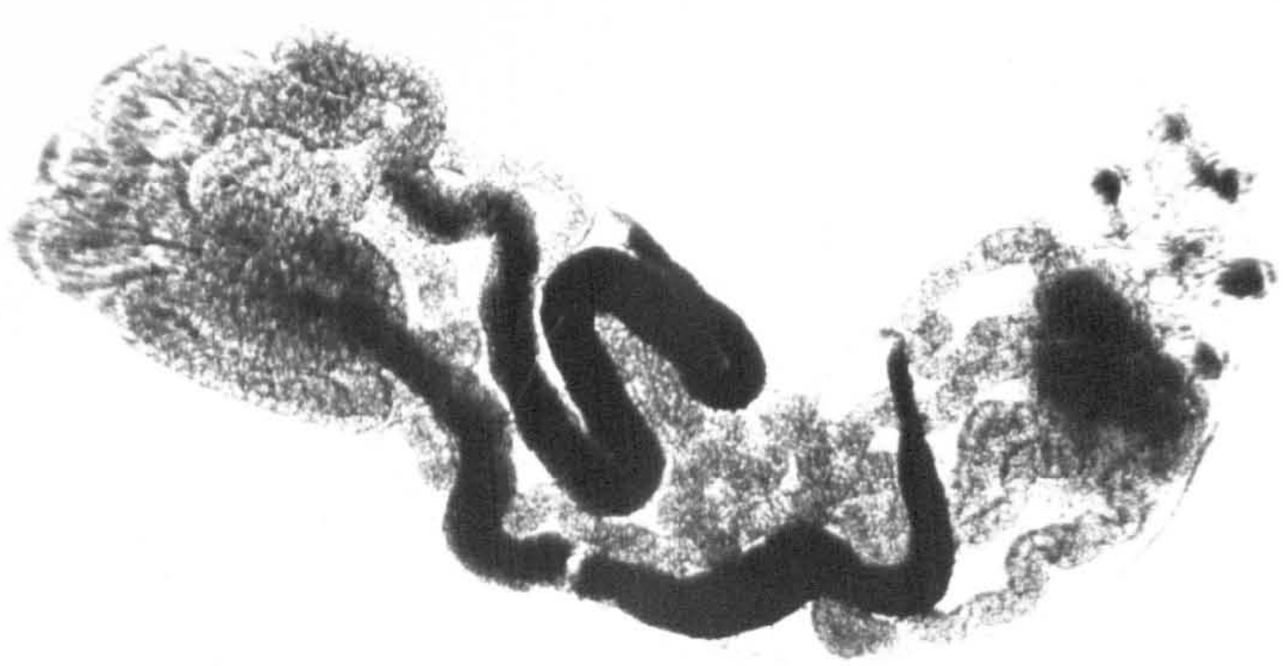


Fig. 9

PLATE 6 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 10 Photograph of posterior end of mature adult squashed and stained in polyvinyl alcohol and chlorazol black E.

Fig. 11 Photograph of posterior end of mature adult squashed and stained in polyvinyl alcohol and chlorazol black E.

Two males are present in this specimen.

a - exuviae of final male copepodid

b - maxillipeds of adult male

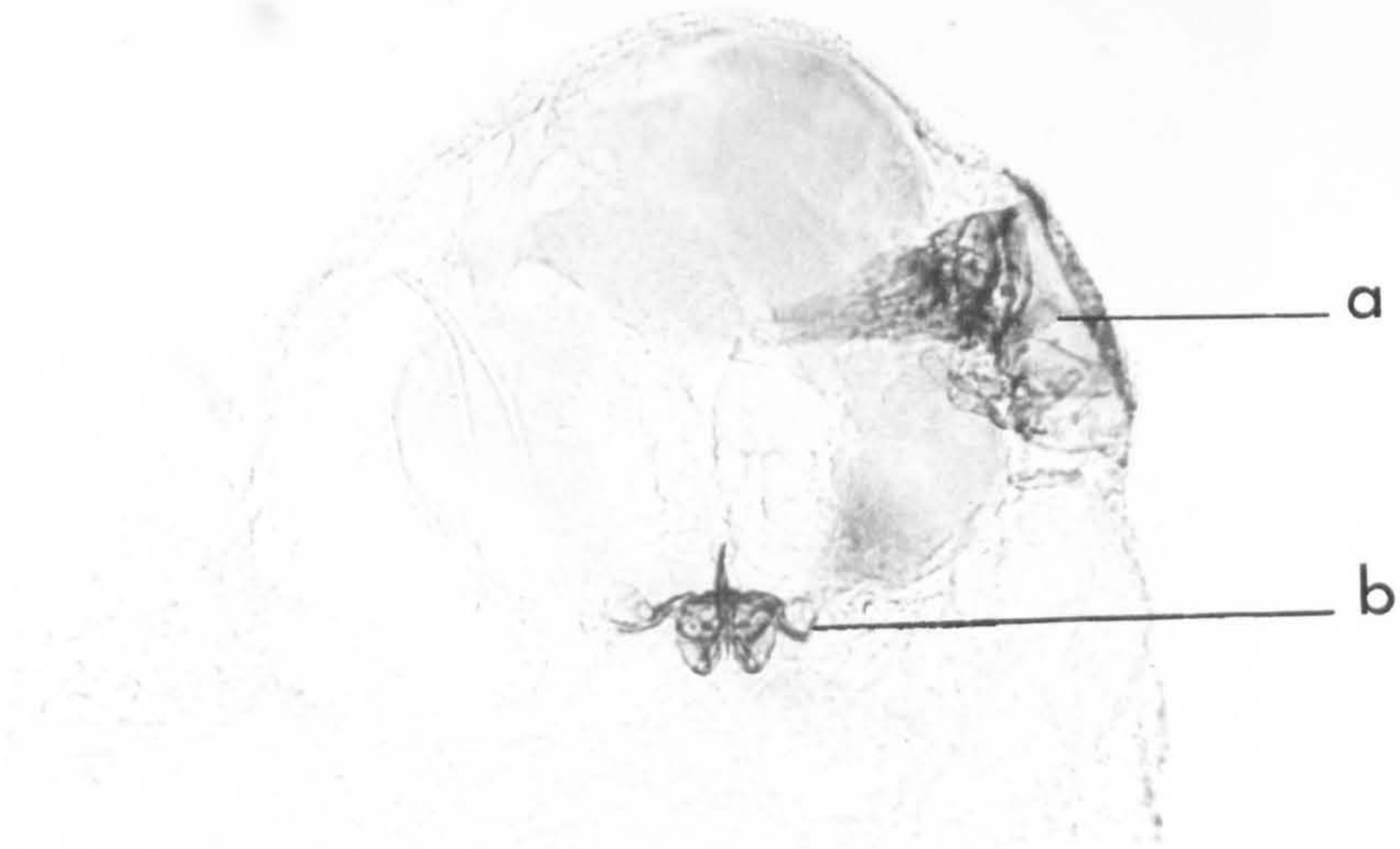


Fig. 10

0·1 mm.

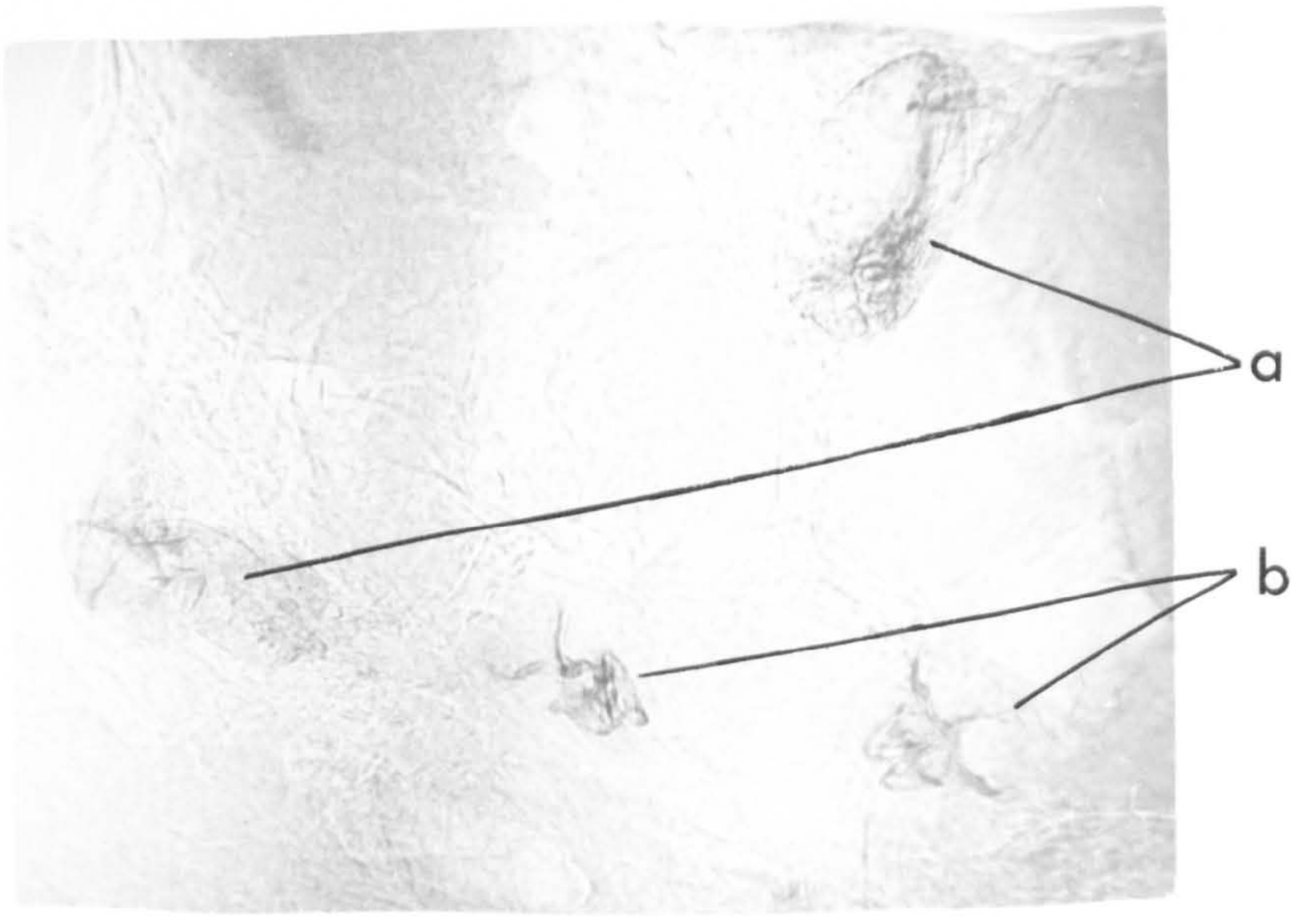


Fig. 11

PLATE 7 — *Eucellicola kilrymontis* gen. et sp. nov.

Fig. 12 — Photograph of 10 μ horizontal section through the posterior end of a mature adult in situ.

a - egg-string

b - male

c - host integument

d - vagina

e - cement gland

f - accessory gland

g - oviduct

Fig. 13 — Photograph of 10 μ horizontal section of the same specimen as in fig. 12 taken through a more dorsal plane. Both cement glands are apparent in this section.

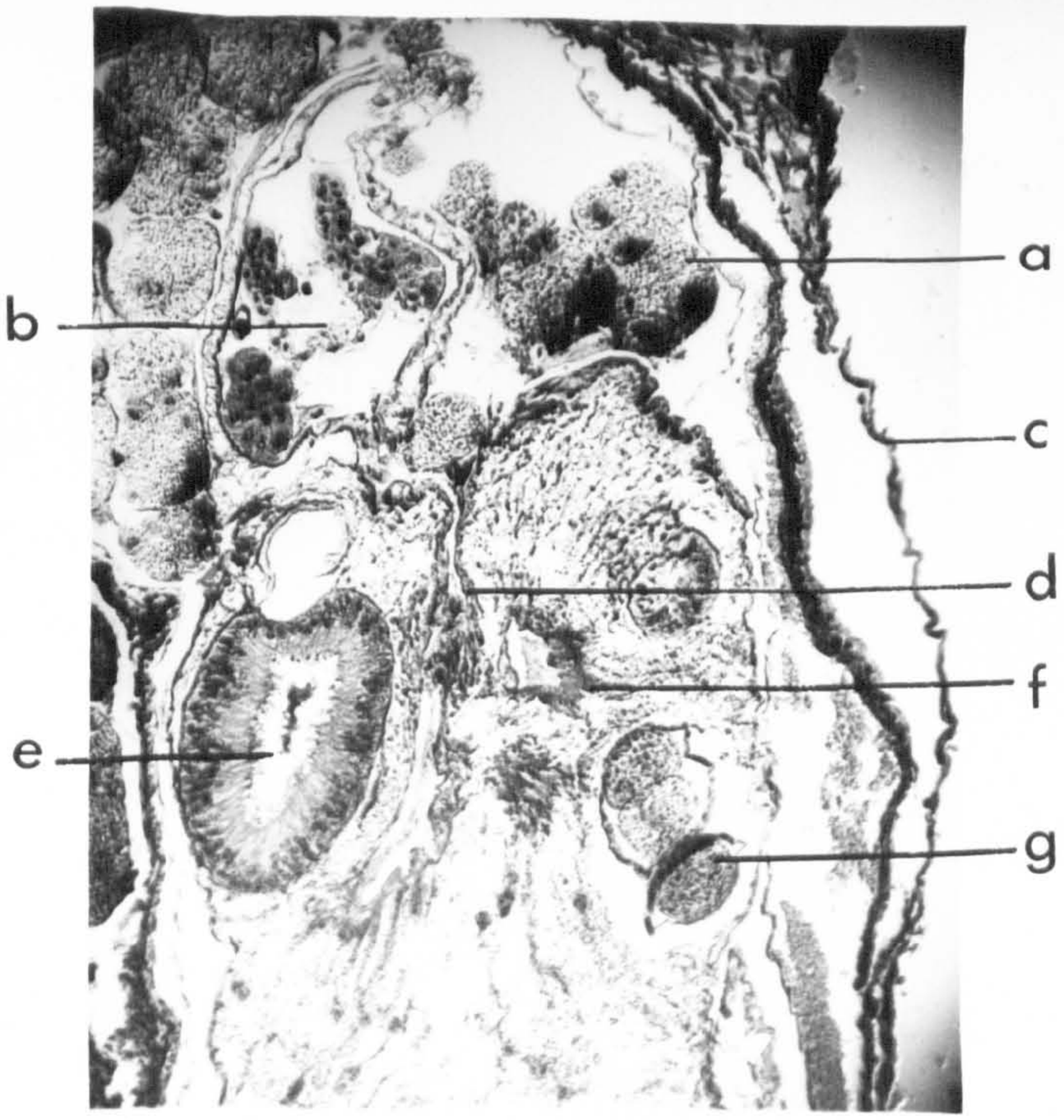


Fig. 12

0·1mm.

Fig. 13

PLATES 8 and 9 — Nucellicola kilrymontis gen. et sp. nov.

Figs 14 to 19 — Photographs of 25 μ transverse sections through the posterior region of a mature adult.

Figures 14 to 18 run consecutively from anterior to posterior and fig. 19 is taken through the region 50 μ posterior to fig. 18.

a - accessory gland

b - ovary

c - cement gland

d - vagina

e - mature region of oviduct

f - union of cement glands

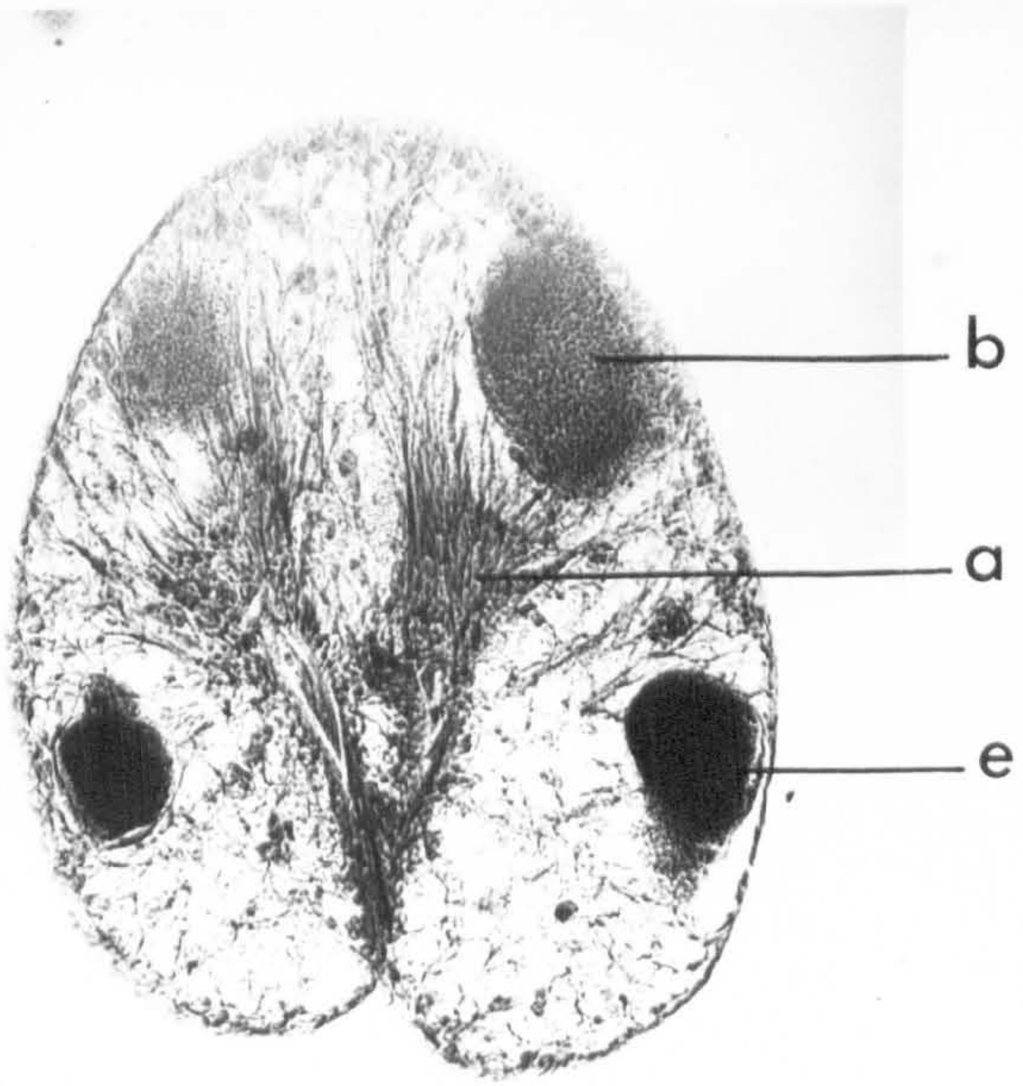


Fig. 14

0·1mm.

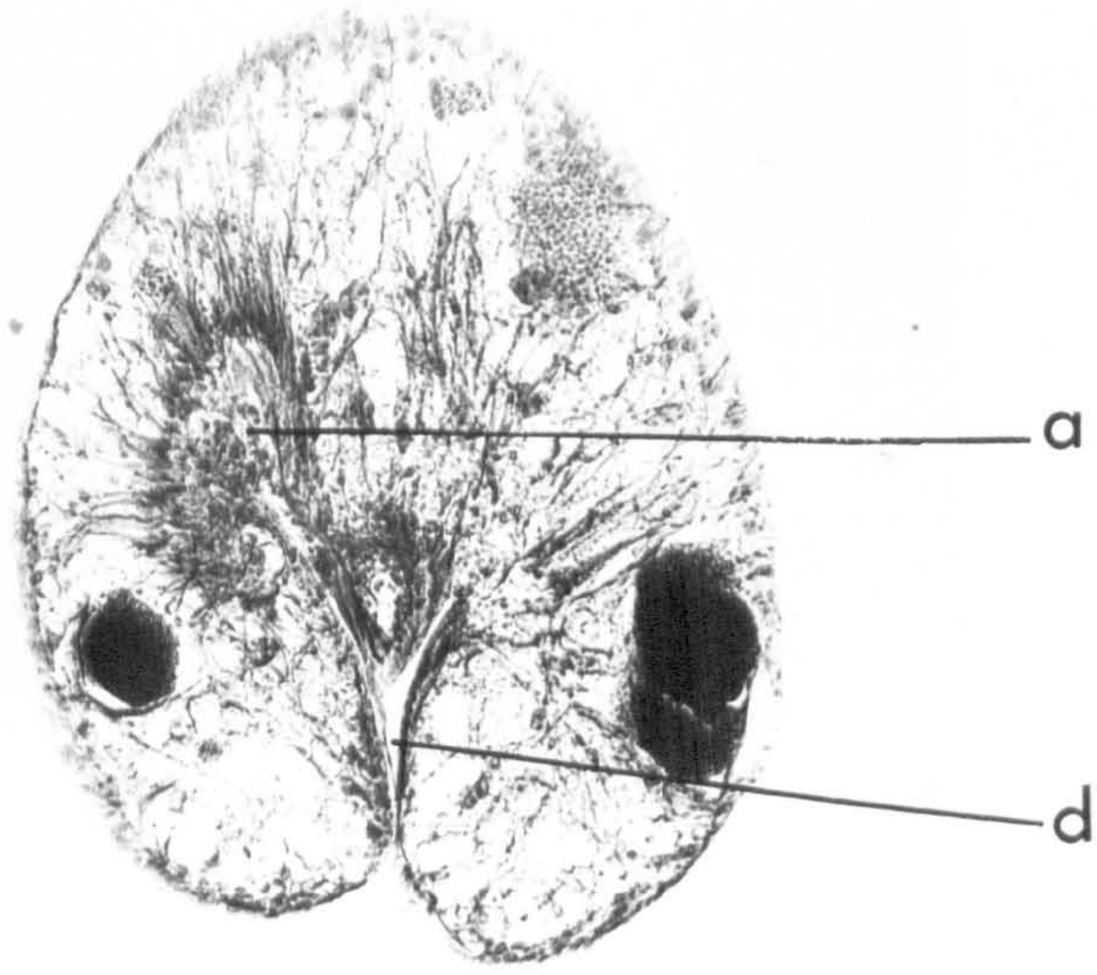


Fig. 15

Fig. 16

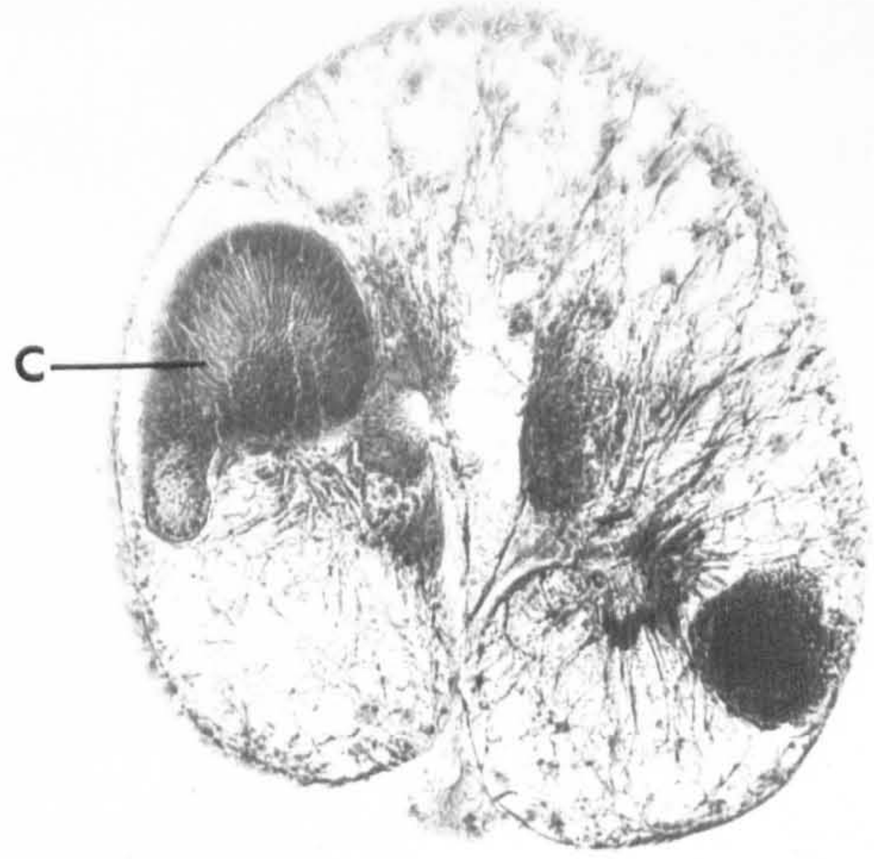


Fig. 17

O·1mm.

Fig.18

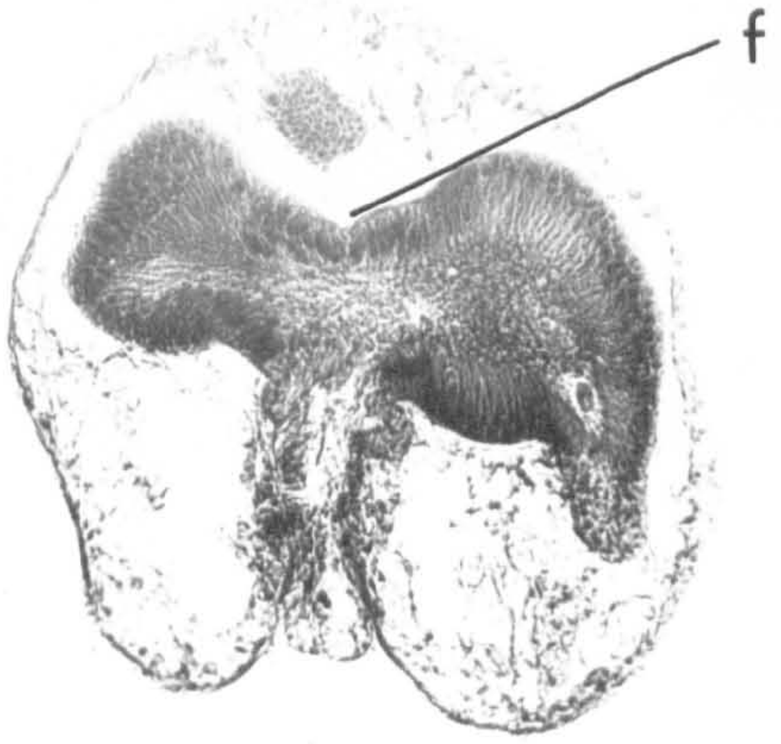


Fig. 19

PLATE 10 Nucellicola kilrymontis gen. et sp. nov.

Figs 20 and 21 Photographs of 15 μ transverse sections through the posterior region of a mature adult showing the presence of eggs in the vagina.

a - accessory gland

b - ovary

c - vagina

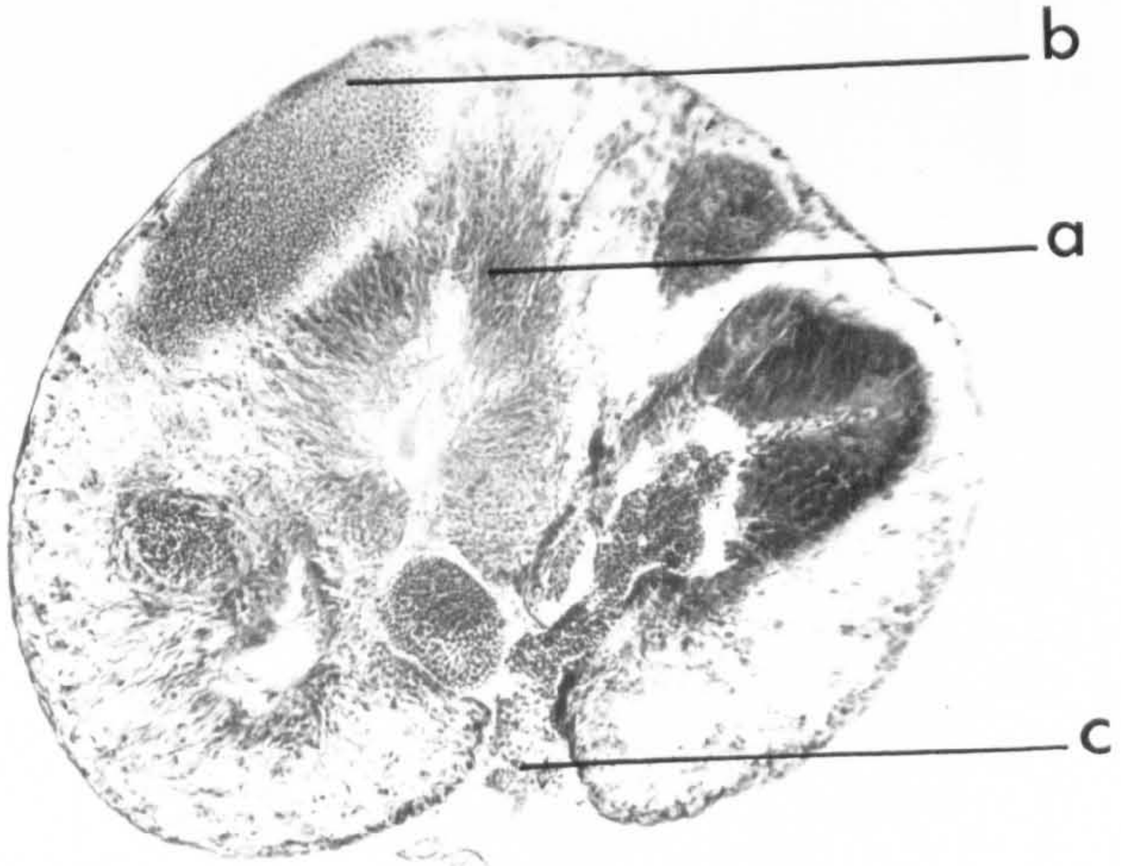


Fig. 20

0·1 mm.

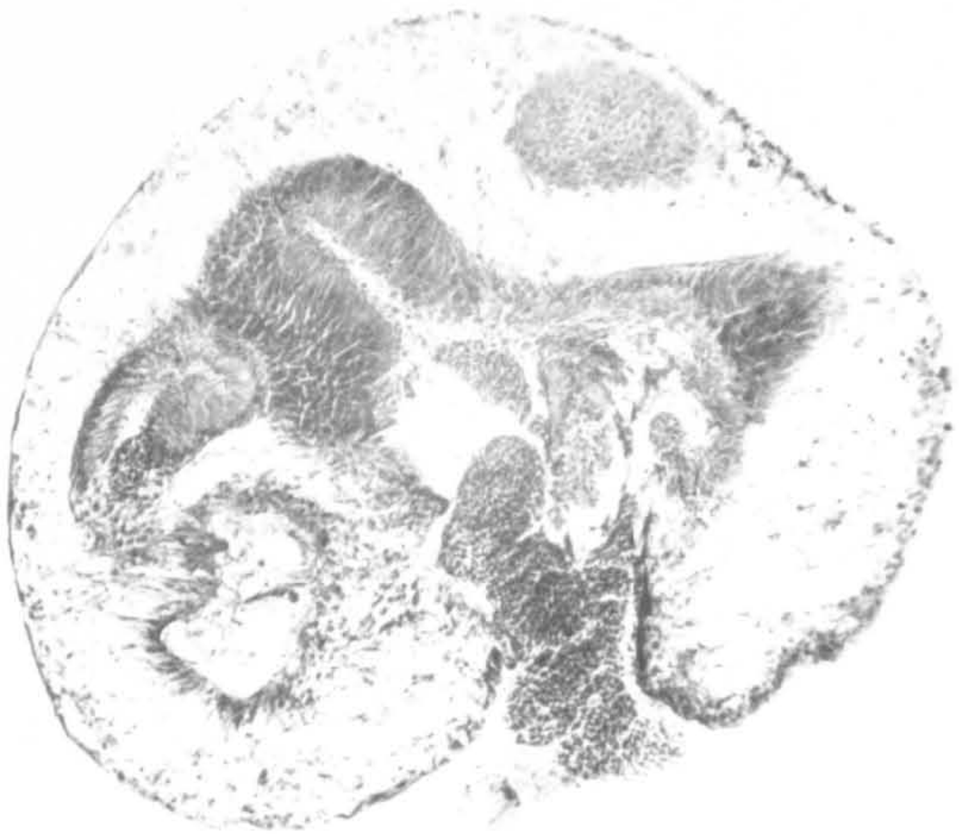


Fig. 21

PLATE 11 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 22 Photograph of 15 μ sagittal section through the posterior region of a mature adult.

a - accessory glands

b - vagina

c - eggs (distorted)

Fig. 23 Photograph of 15 μ horizontal section through the posterior region of a mature adult.

a - male

b - rostrum of male

c - egg-string

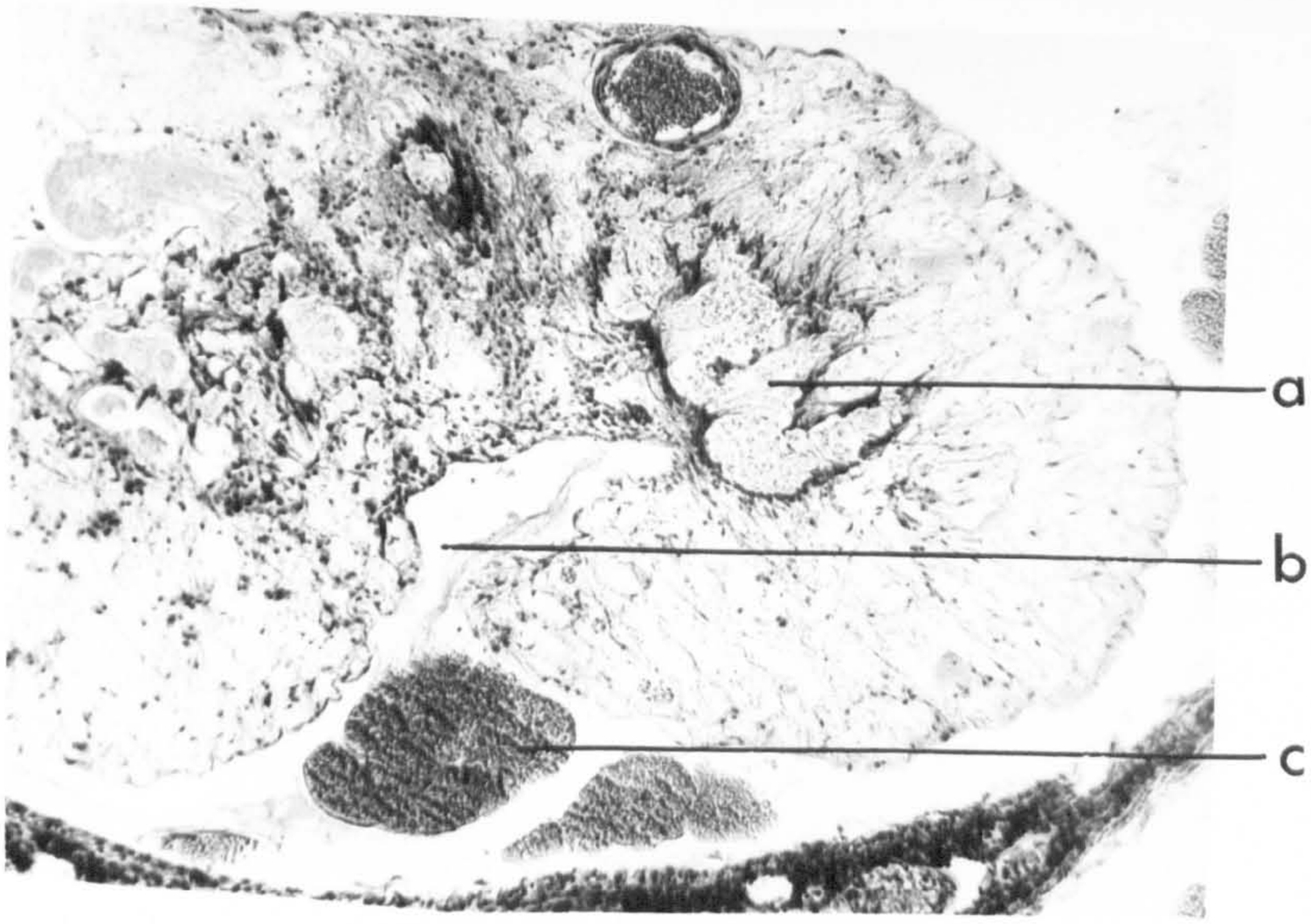


Fig. 22

O·1 mm.

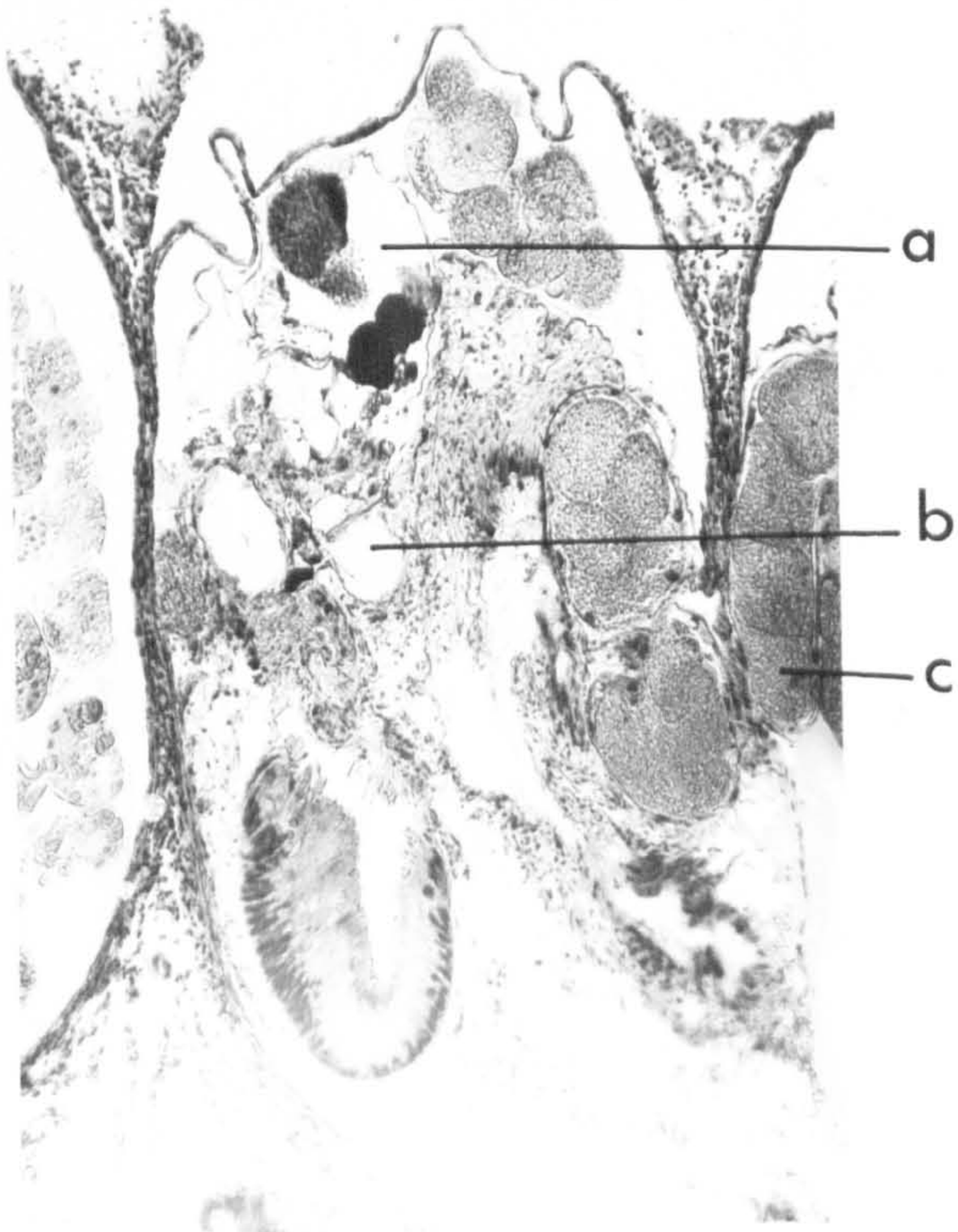


Fig. 23

PLATE 12 Nucellicola kilrymontis gen. et sp. nov.

Fig. 24 Drawing of a live adult male. Oil droplets are apparent in the centre of this specimen.

Fig. 25 Composite drawing, constructed from serial sections, of an adult male.

a - rostrum

b - 1st antenna

c - 2nd antenna

d - maxillipeds

e - region of spermatophore formation

f - spermatophore

g - vas deferens

h - testis

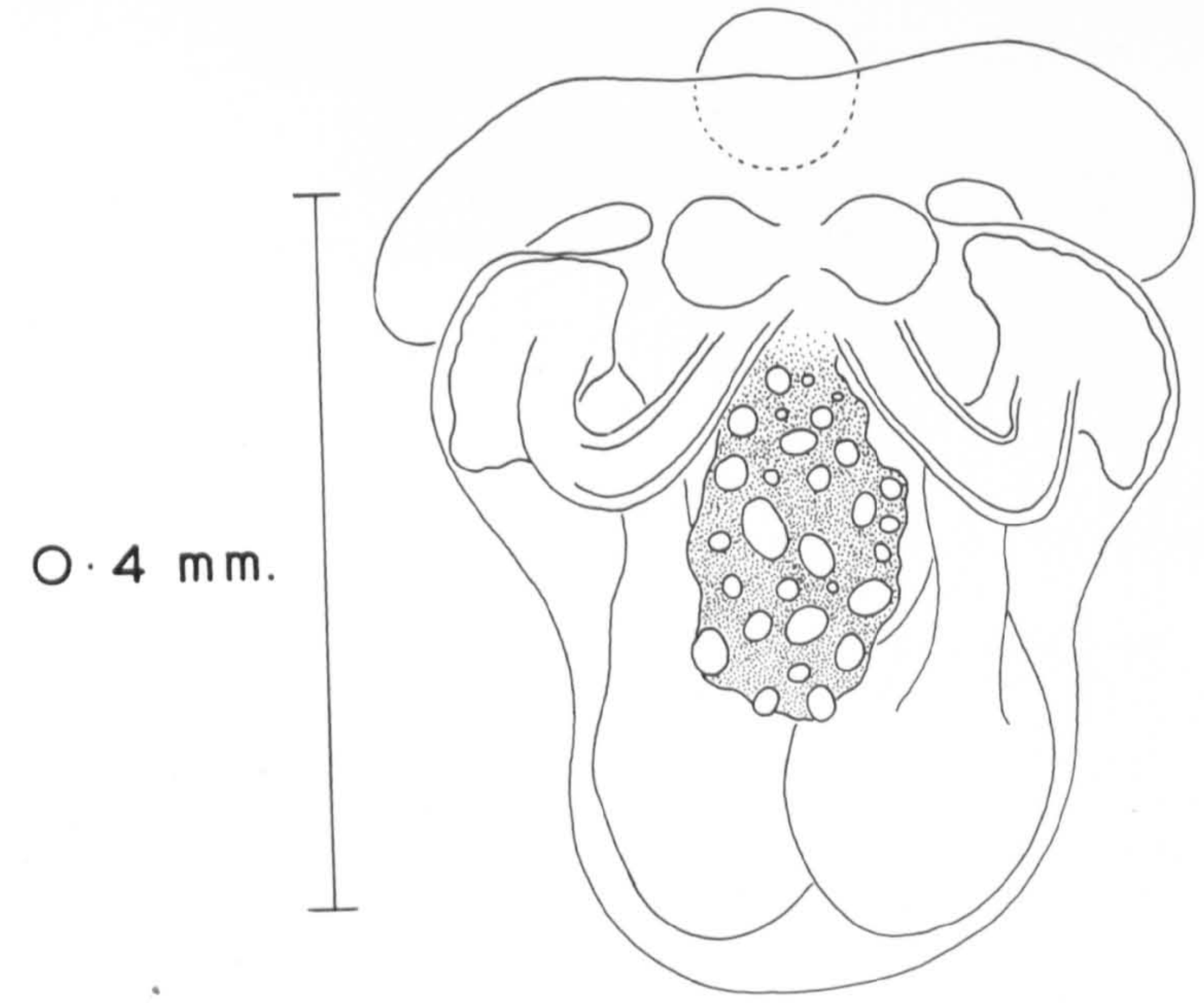


Fig. 24

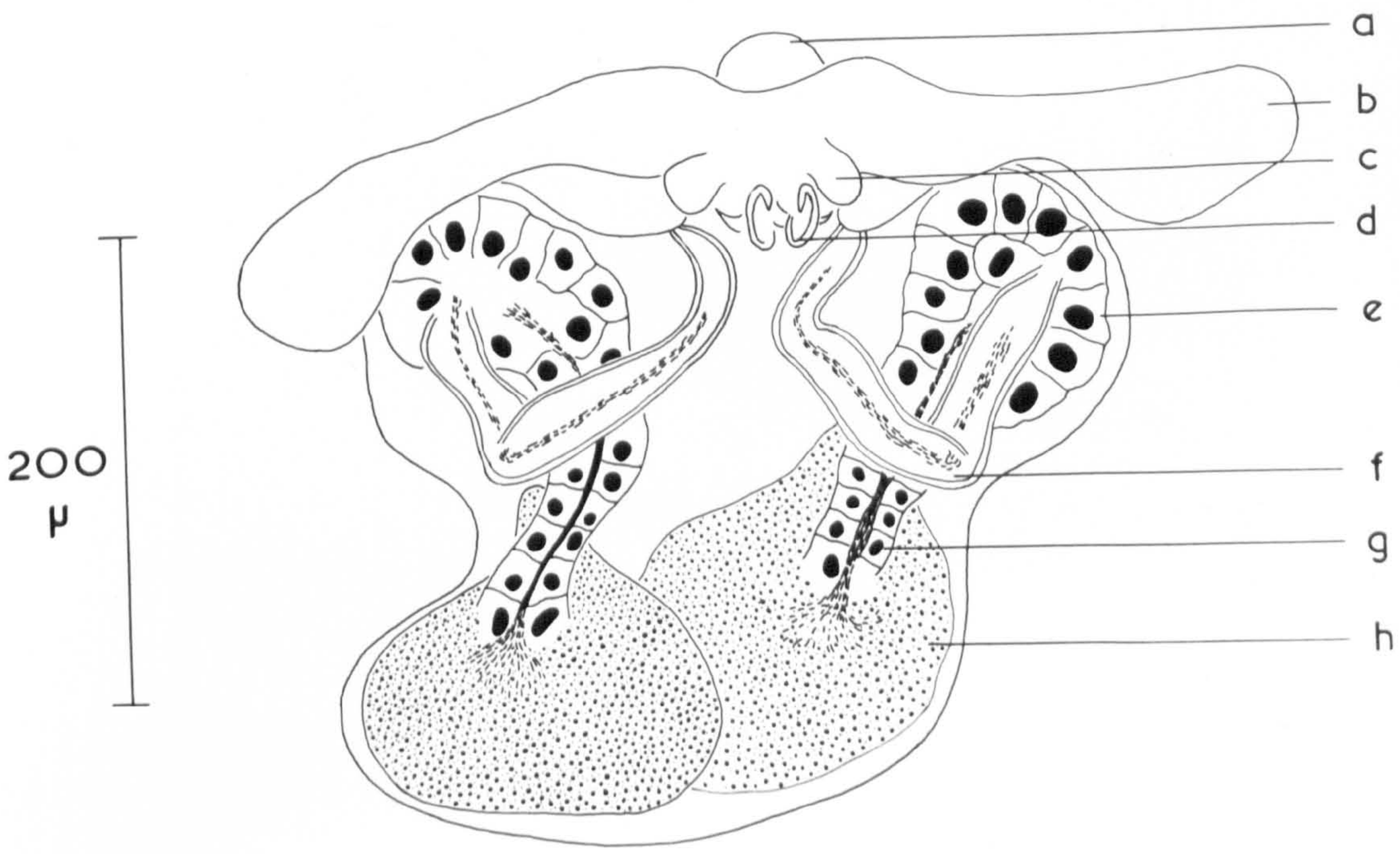


Fig. 25

PLATE 13 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 26 Drawing of the maxillipeds of an adult male from the dorsal aspect.

Fig. 27 Drawing of the maxillipeds of an adult male from the ventral aspect.

Fig. 28 Drawing of spermatophores.

Fig. 29 Drawing of spermatozoa.

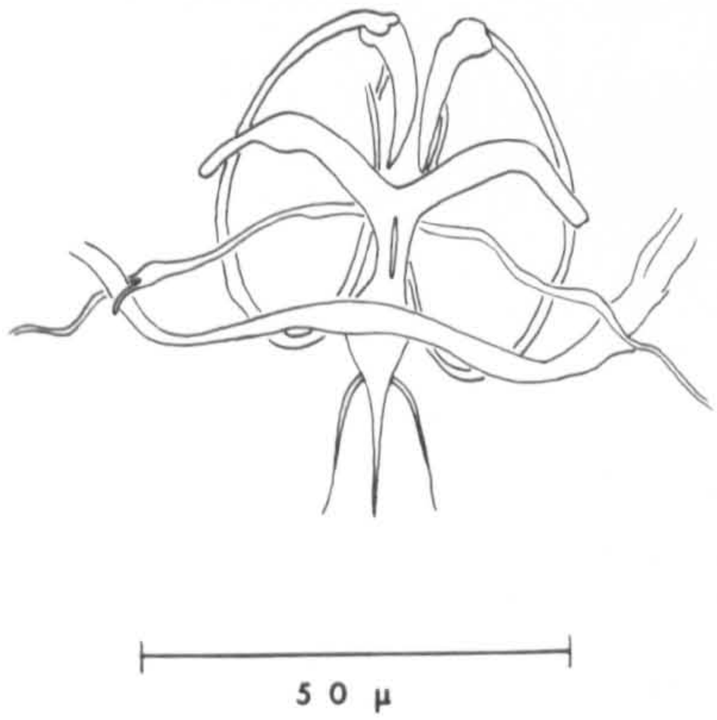


Fig. 26

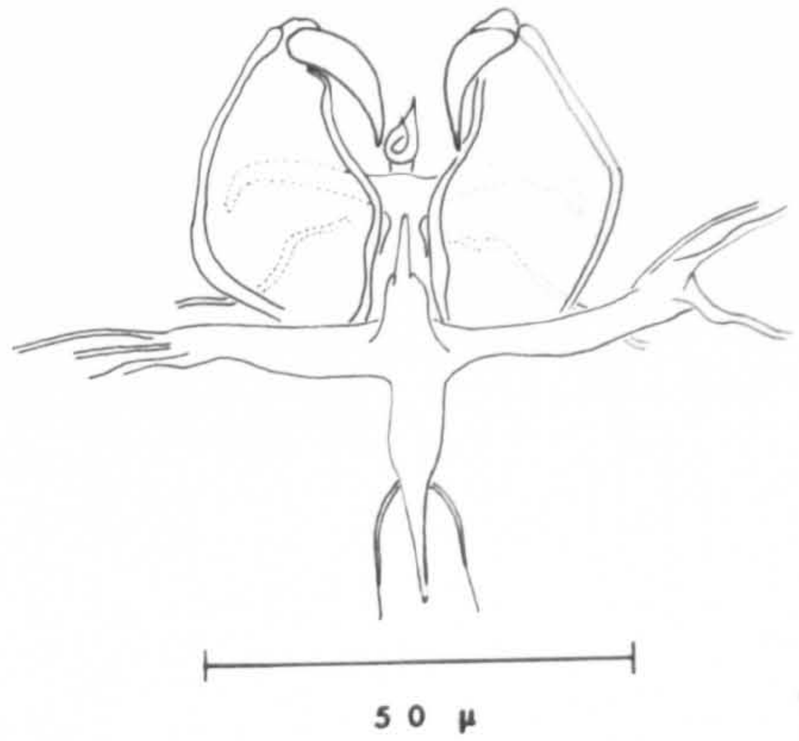


Fig. 27

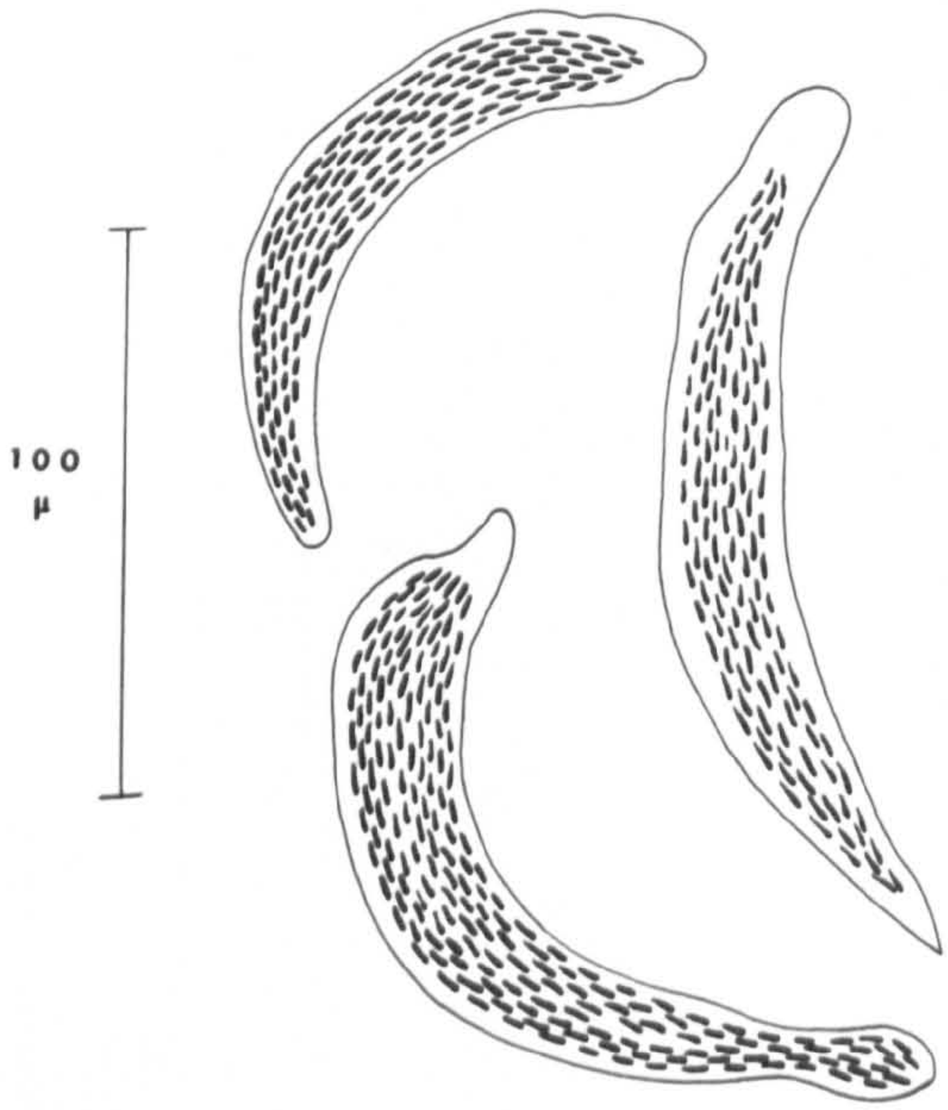


Fig. 28

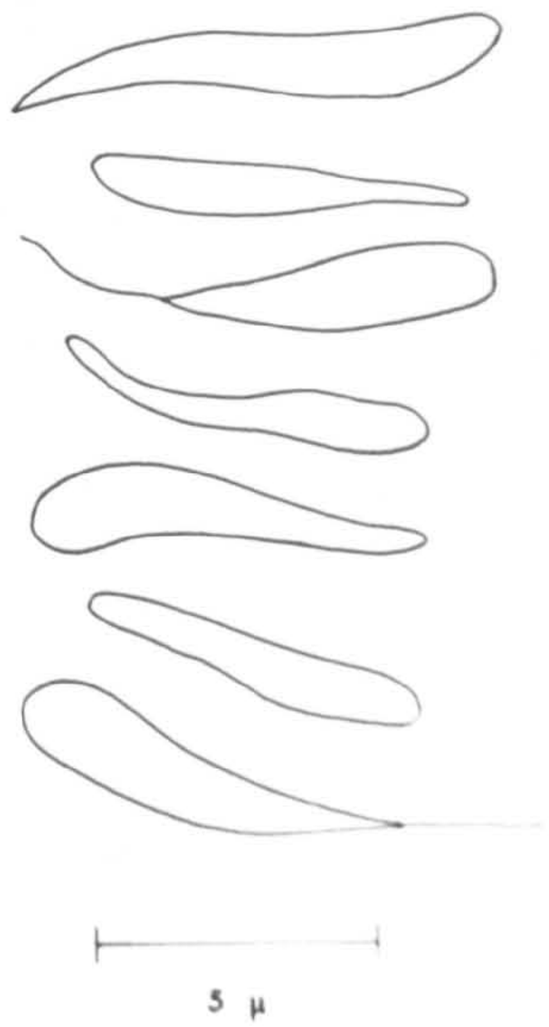


Fig. 29

PLATE 14 Nucellicola kilrymontis gen. et sp. nov.

Fig. 30 Photograph of 10 μ horizontal section through the posterior region of a mature adult. Spermatozoa are present in the vas deferens of the male and in the cement gland of the female.

a - spermatophores

b - vas deferens containing spermatozoa

c - cement gland containing spermatozoa

Fig. 31 Photograph of 10 μ horizontal section through the cement gland of a mature female showing the presence of spermatozoa in the lumen of the gland.

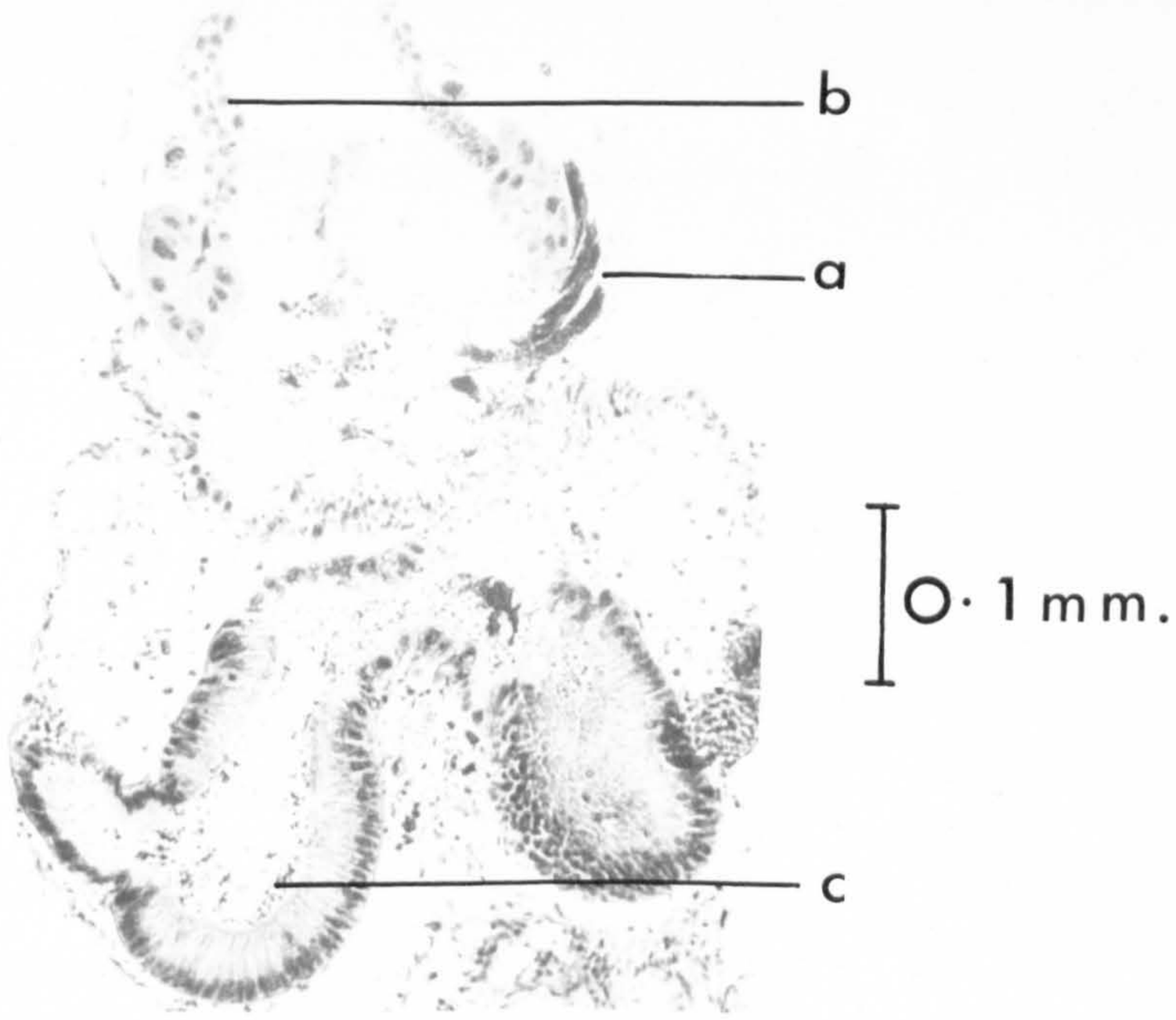


Fig. 30

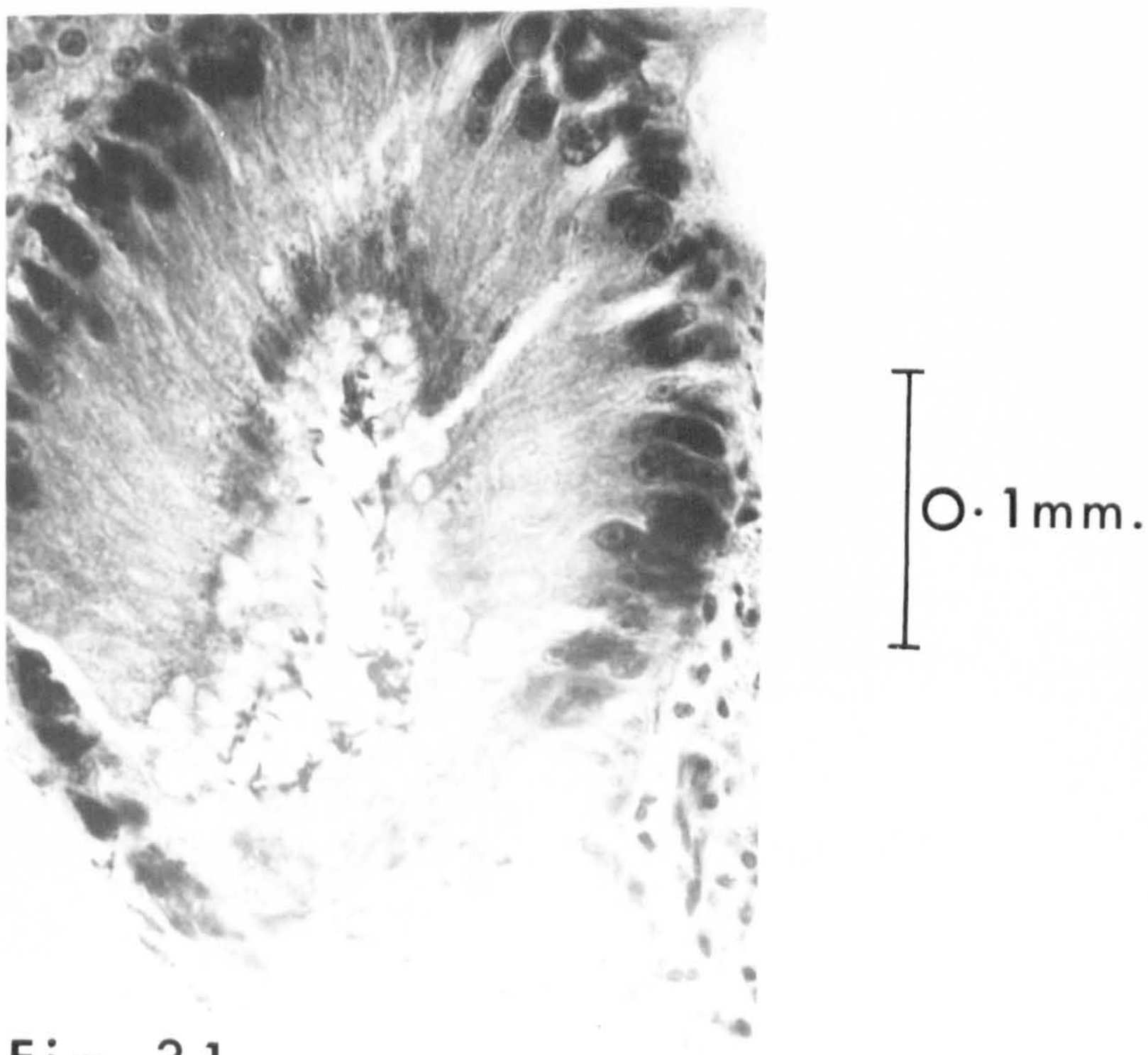


Fig. 31

PLATE 15 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 32 Photograph of oocyte from the early region of the oviduct prepared by the Aceto-orcein squash technique. About 21 chromosomes in prophase are apparent and the large nucleolus is quite distinct.

Fig. 33 Photograph of oocyte from the terminal region of the oviduct prepared by the Aceto-orcein squash technique. There are 11 pairs of chromatids arranged on the equatorial plate of metaphase (2nd Meiosis).

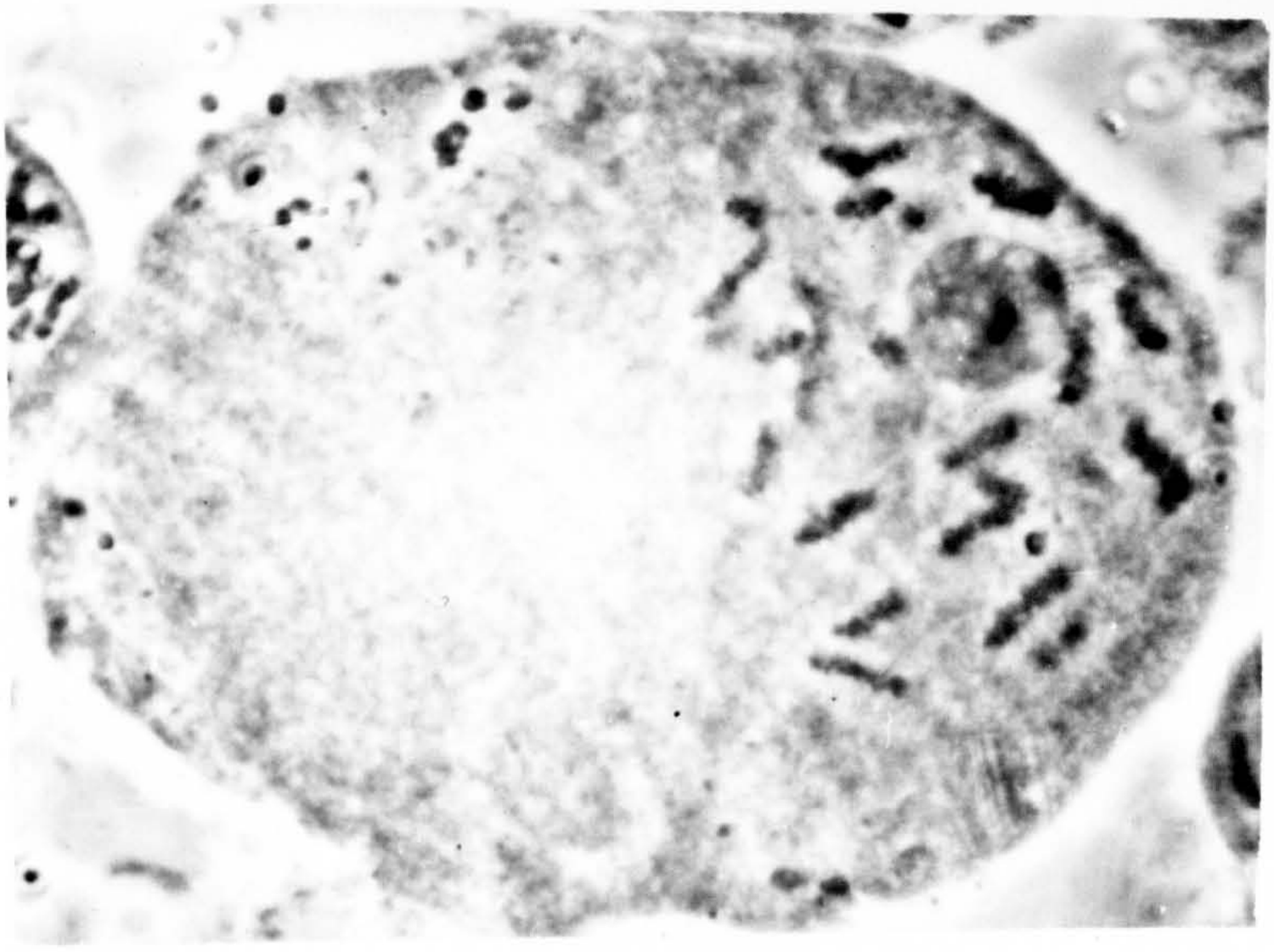


Fig. 32

20 μ

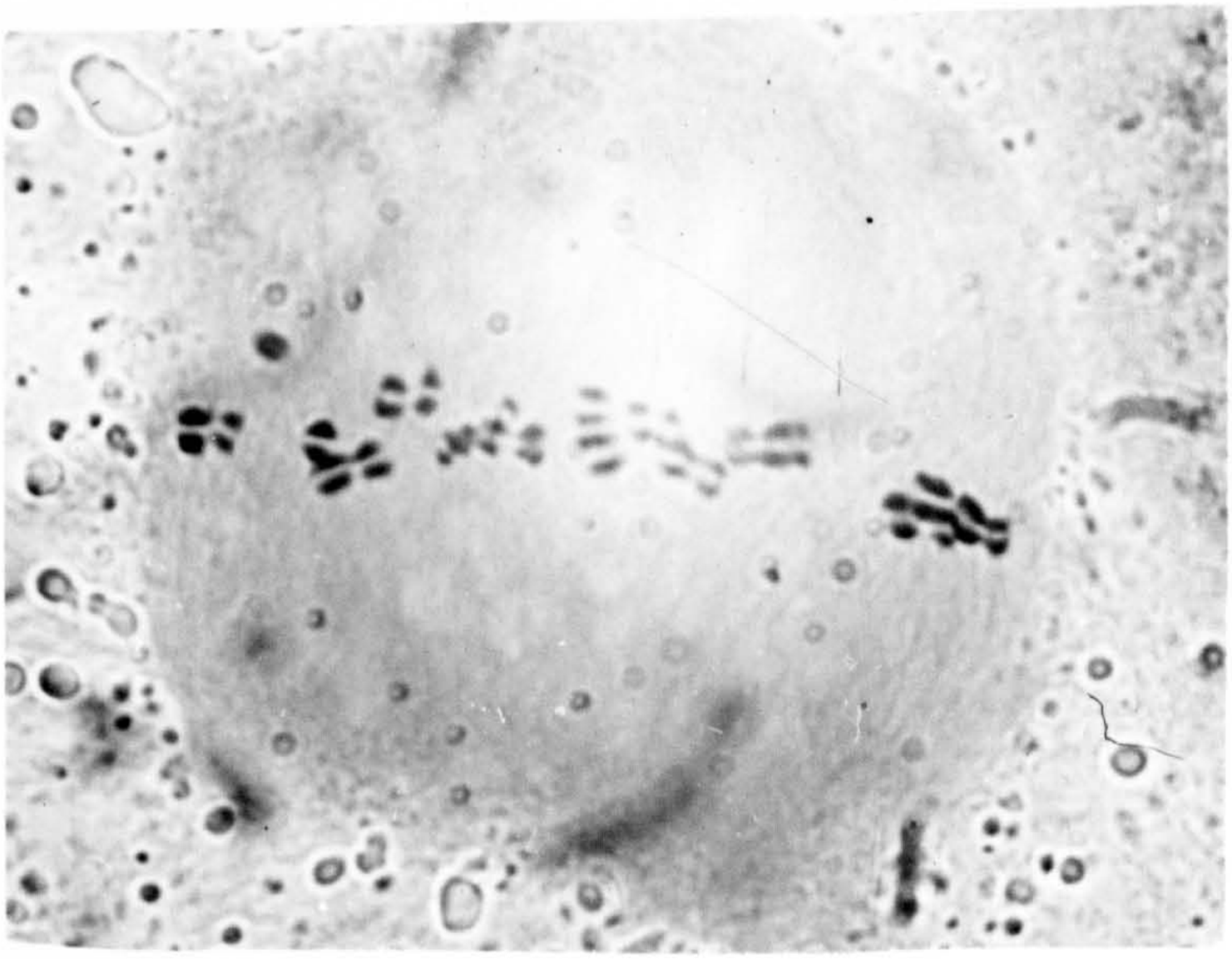


Fig. 33

PLATE 16 Nucellicola kilrymontis gen. et sp. nov.

Fig. 34 Photograph of 5 μ transverse section through the ovary of a mature adult female.

Fig. 35 Photograph of 5 μ longitudinal section through the early region of the oviduct of a mature adult female.

Fig. 36 Photograph of 5 μ longitudinal section through the early and mature regions of the oviduct of a mature adult female.

Fig. 37 Photograph of 15 μ transverse section through a mature adult female in situ showing the oviducts with oocytes at different stages in development. The two integuments are apparent, one around the female and the other lining the wall of the cavity.

Fig. 34

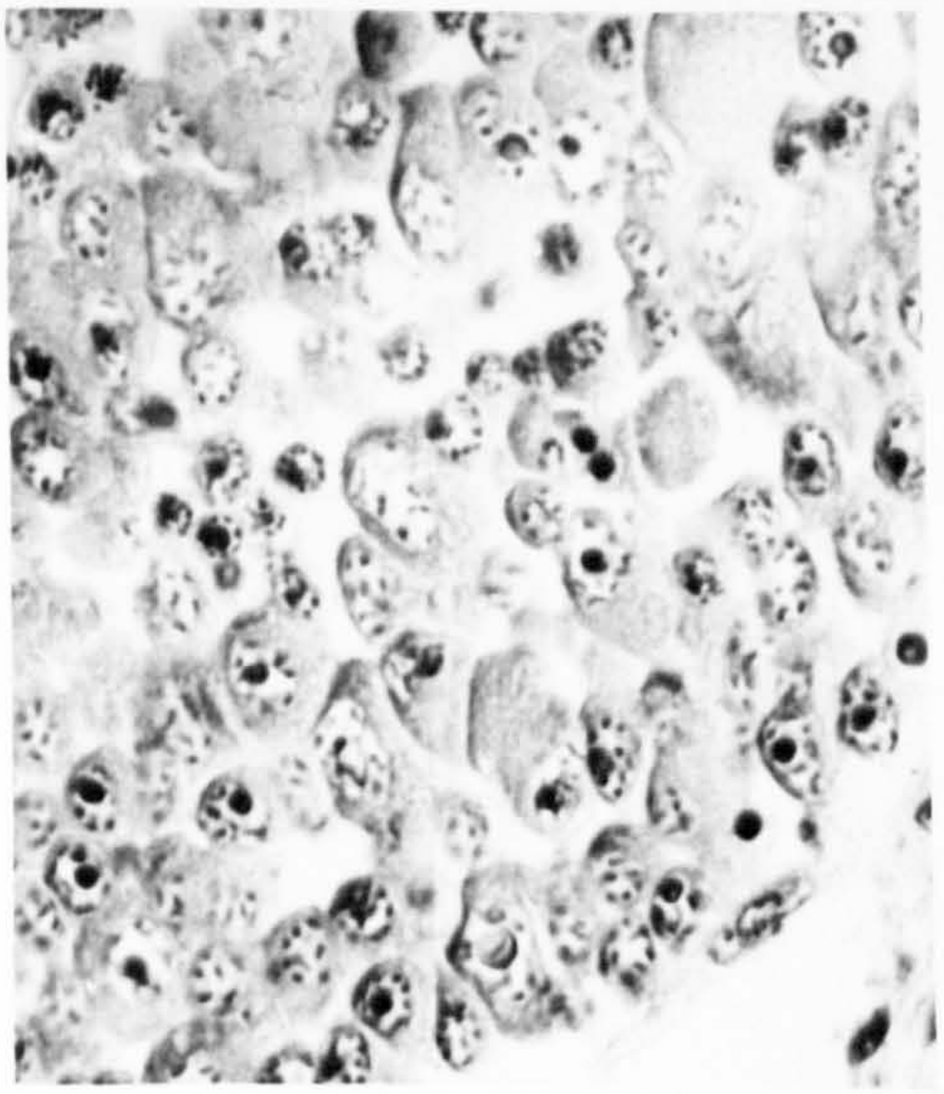


Fig. 35

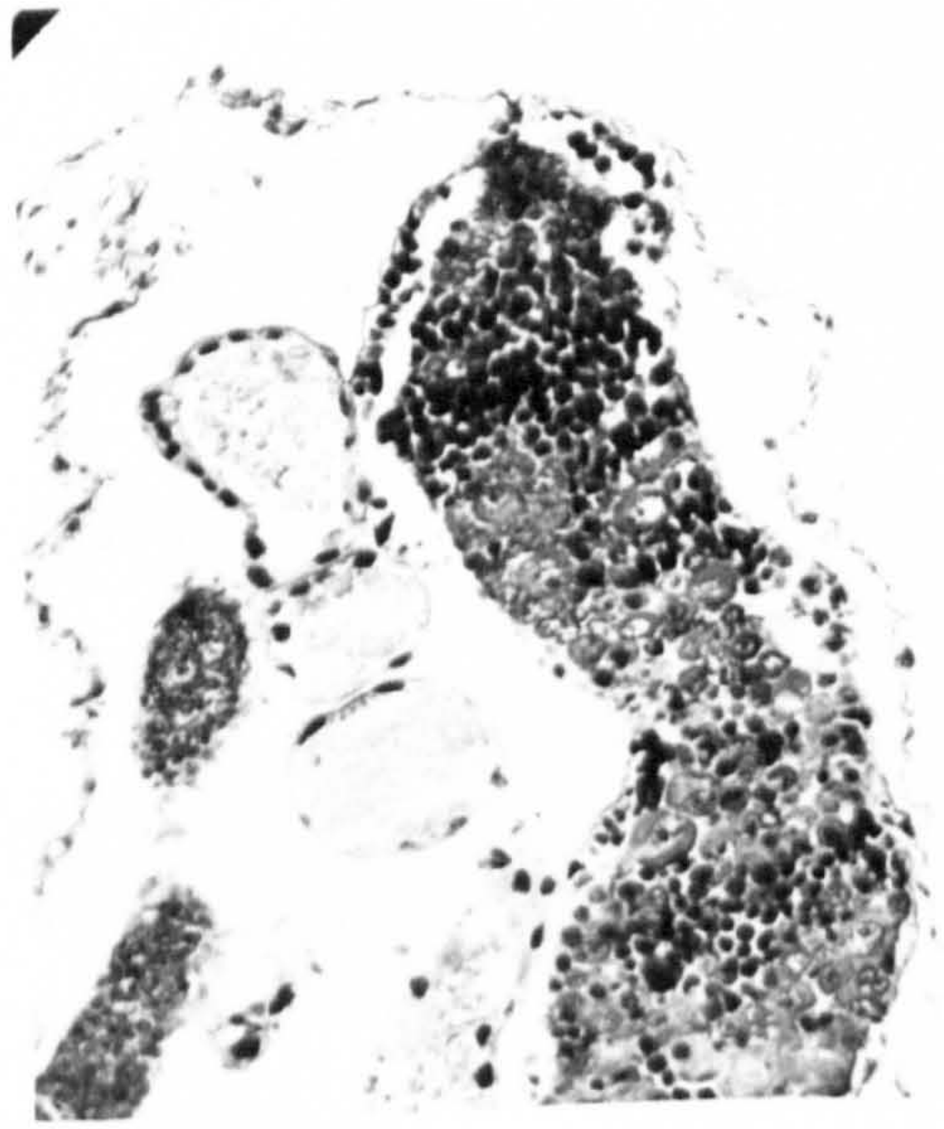


Fig. 36

O·1 mm.

Fig. 37

O·5 mm.

PLATE 17 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 38 Photograph of 12 μ transverse section of eggs at the "blastula" stage.

Fig. 39 Photograph of 12 μ transverse section of eggs at a later "blastula" stage showing differentiation at the future anterior end.

Fig. 40 Photograph of 10 μ section of eggs at a later "blastula" stage showing differentiation at both anterior and posterior ends.

Fig. 41 Photograph of 10 μ section of the egg-string containing early nauplius stages.

a - buds of 1st antennae visible on nauplii

b - a nauplius with three pairs of appendages

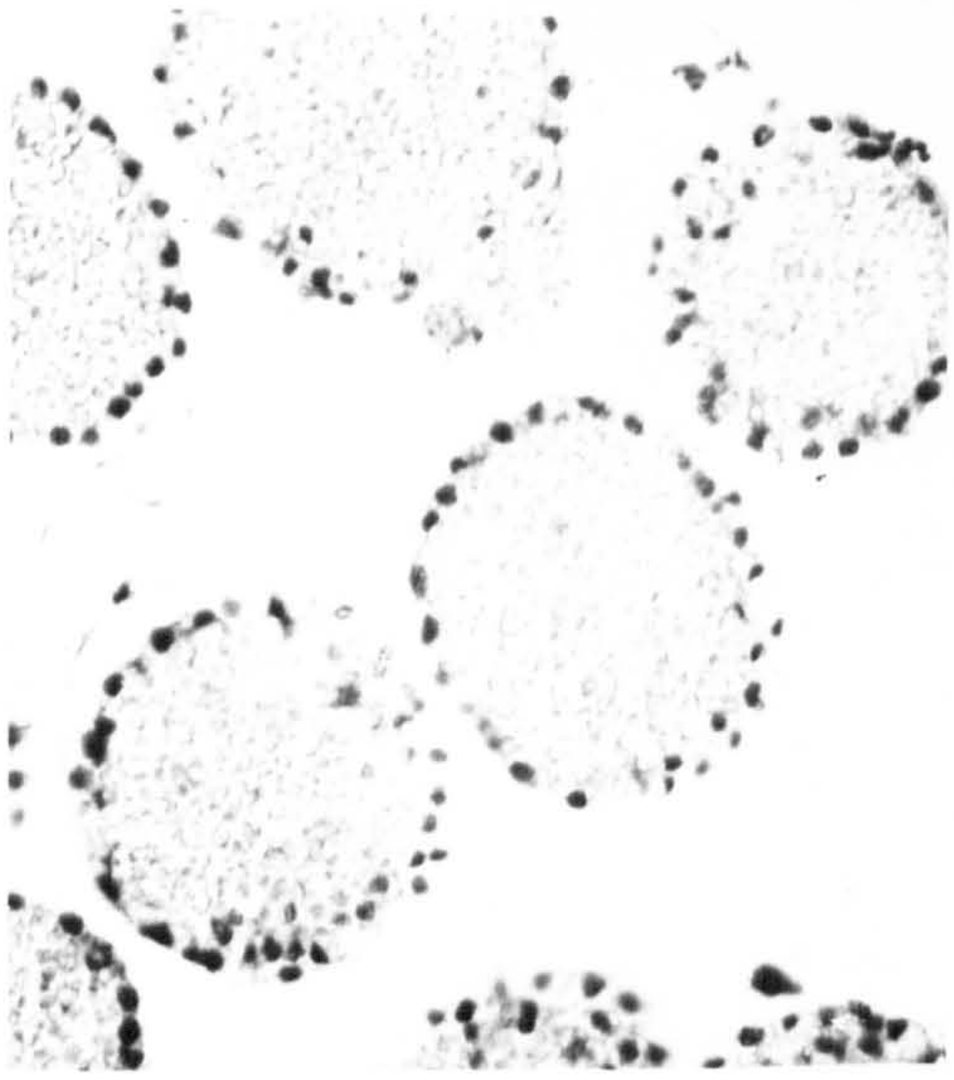


Fig. 38

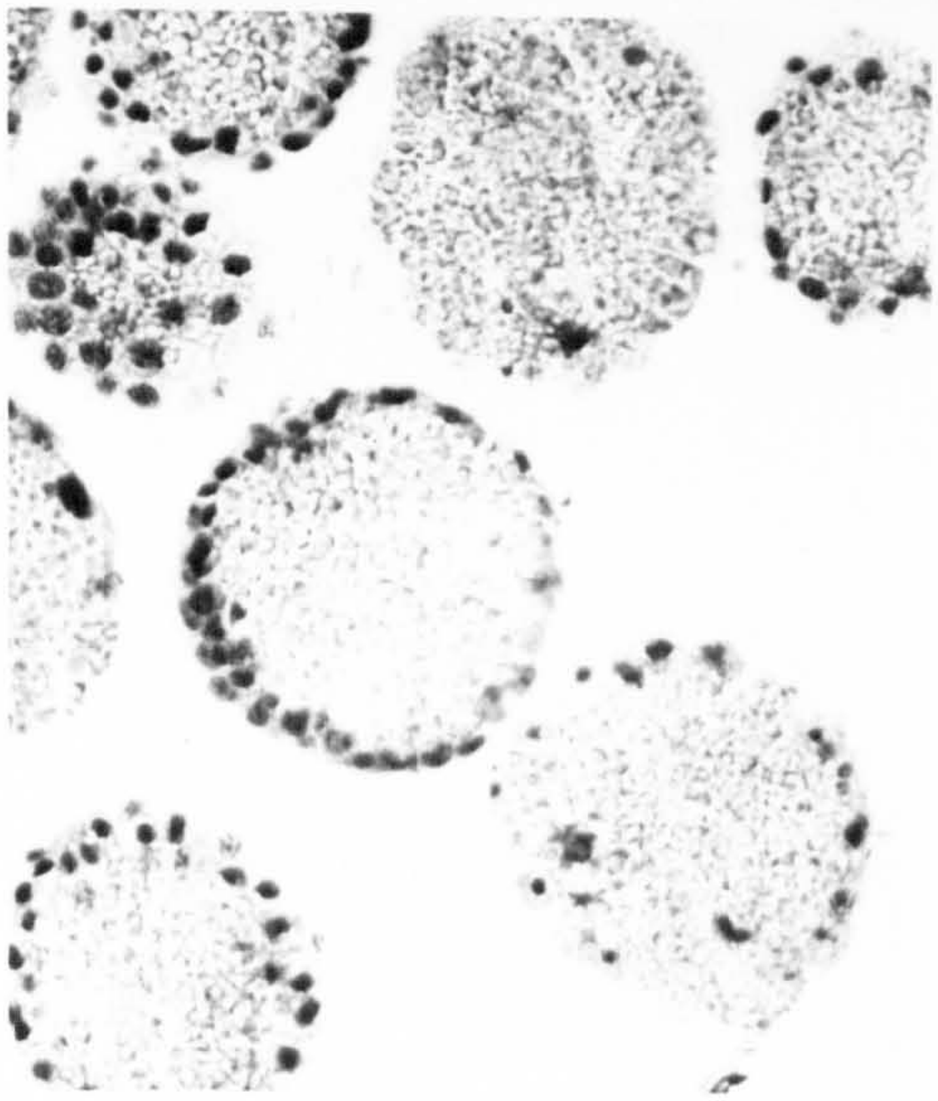


Fig. 39

O·1mm.

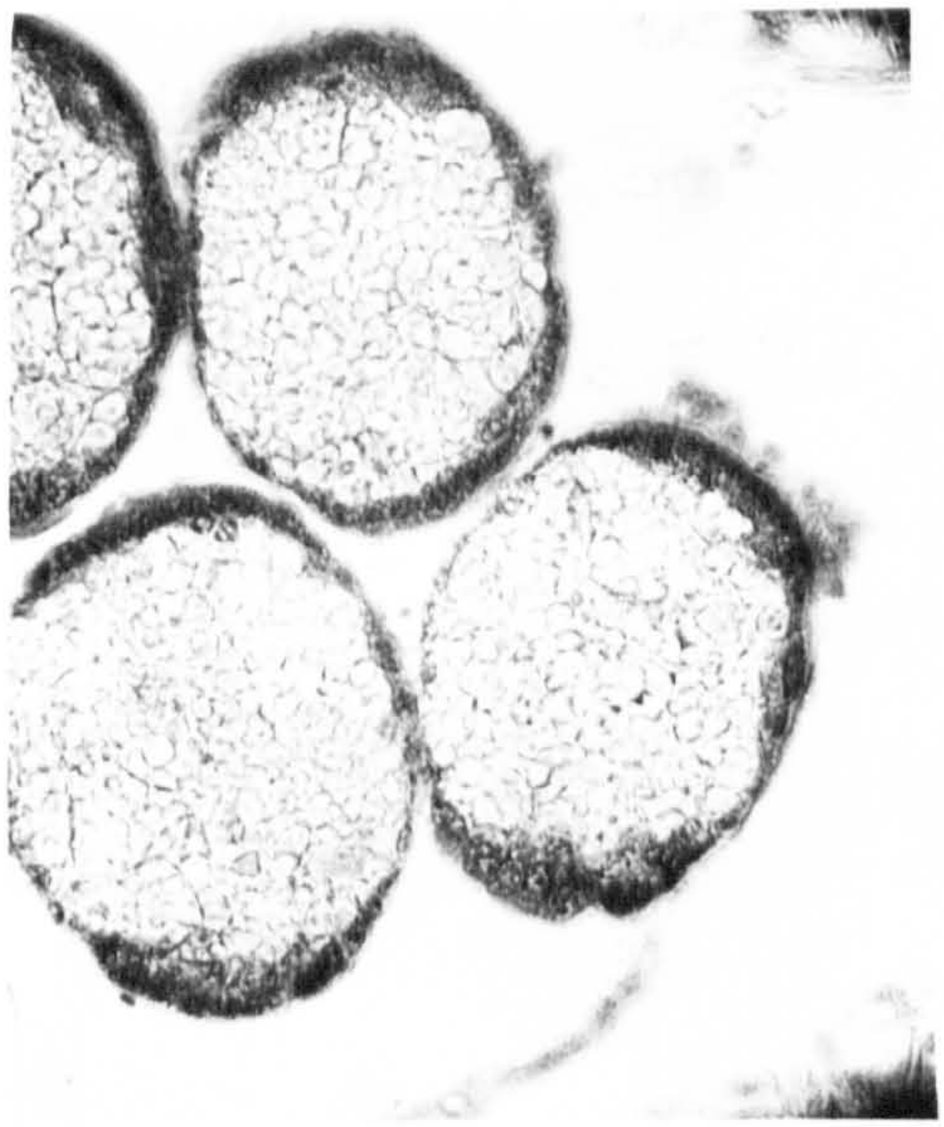


Fig. 40

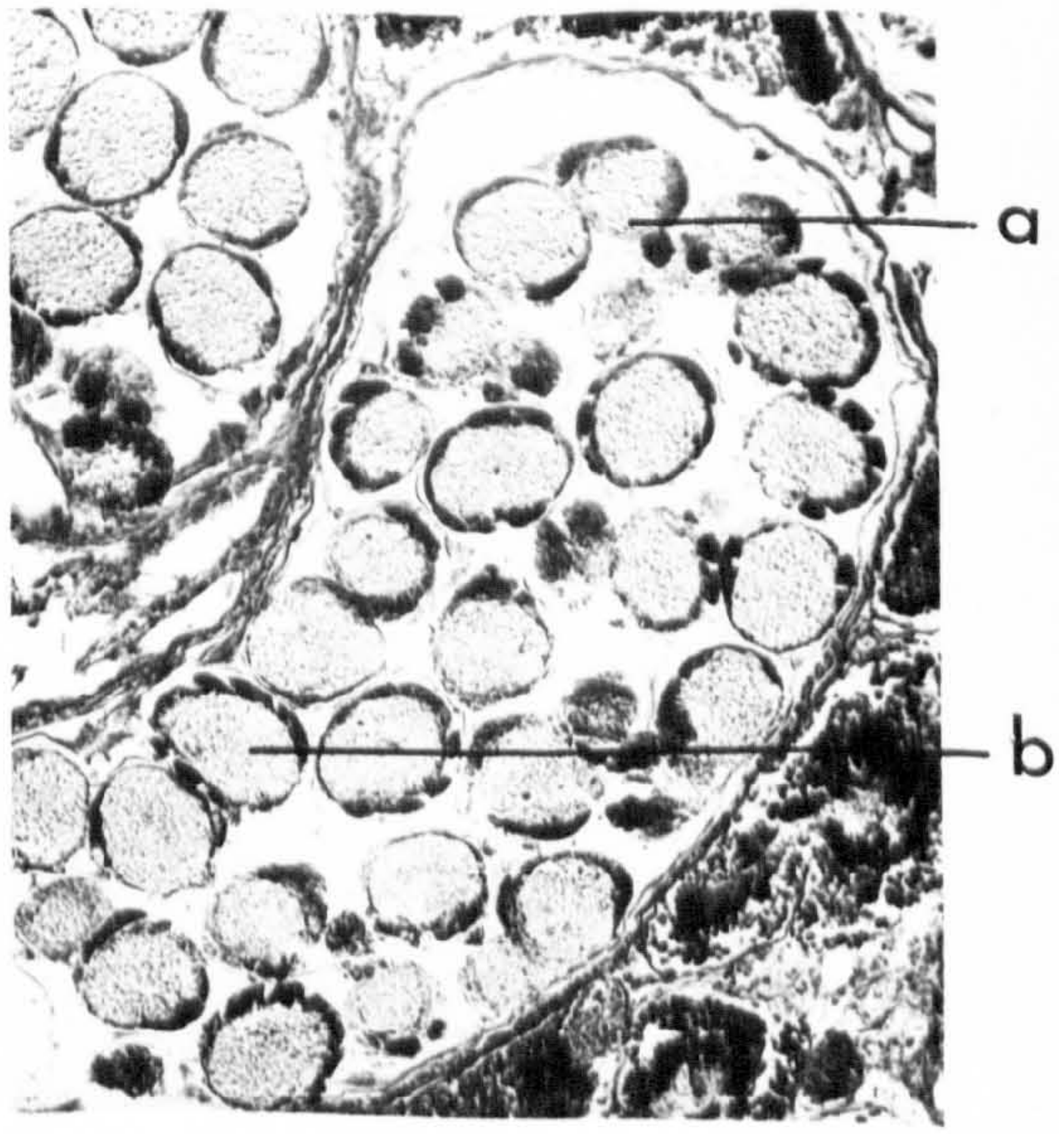


Fig. 41

PLATE 18 Nucellicola kilrymontis gen. et sp. nov.

Fig. 42 Photograph of 10 μ horizontal section of the 3rd nauplius stage. Three anterior appendages are apparent on the left side. Yolk is present in the centre of the specimen and the early stages of thoracic segmentation are visible.

Fig. 43 Photograph of 10 μ sagittal section of the 2nd metanauplius stage within the egg-string.

a - 1st antenna

b - 2nd antenna

c - mandible

d - origin of muscles supplying anterior appendages

e - yolk

f - dorsal longitudinal muscles

g - limb buds of the four thoracic appendages, the two anterior ones bearing setae

h - abdomen

Fig. 42

O·1mm.

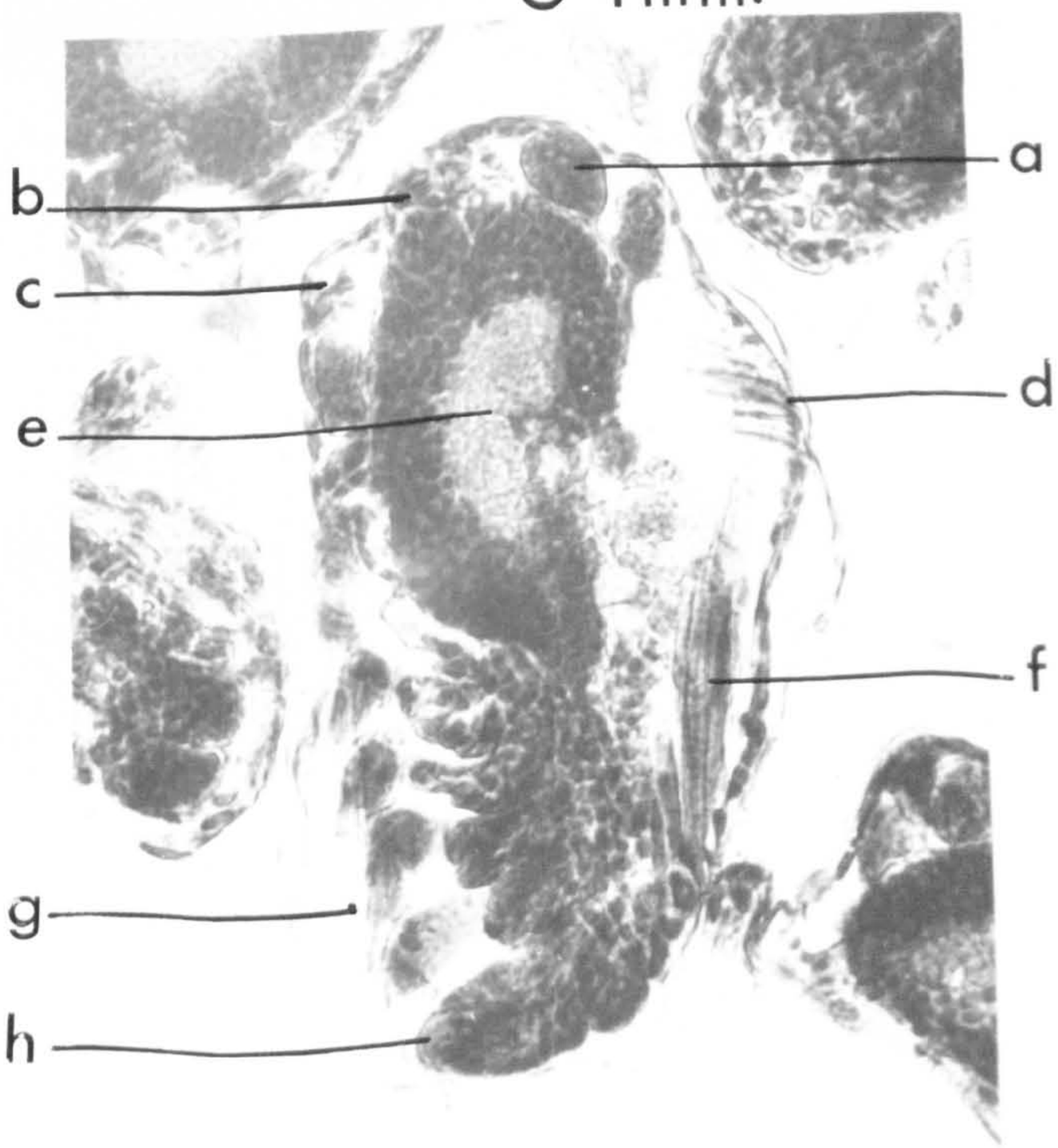


Fig. 43

PLATE 19 Nucellicola kilrymontis gen. et sp. nov.

Fig. 44 Photograph of 15 μ section of a distal portion of the egg-string in situ showing 1st and 2nd metanauplii sectioned in various planes.

Fig. 45 Photograph of 10 μ section of a distal portion of the egg-string in situ showing 1st and 2nd metanauplii sectioned in various planes.

a - 2nd metanauplius in sagittal section

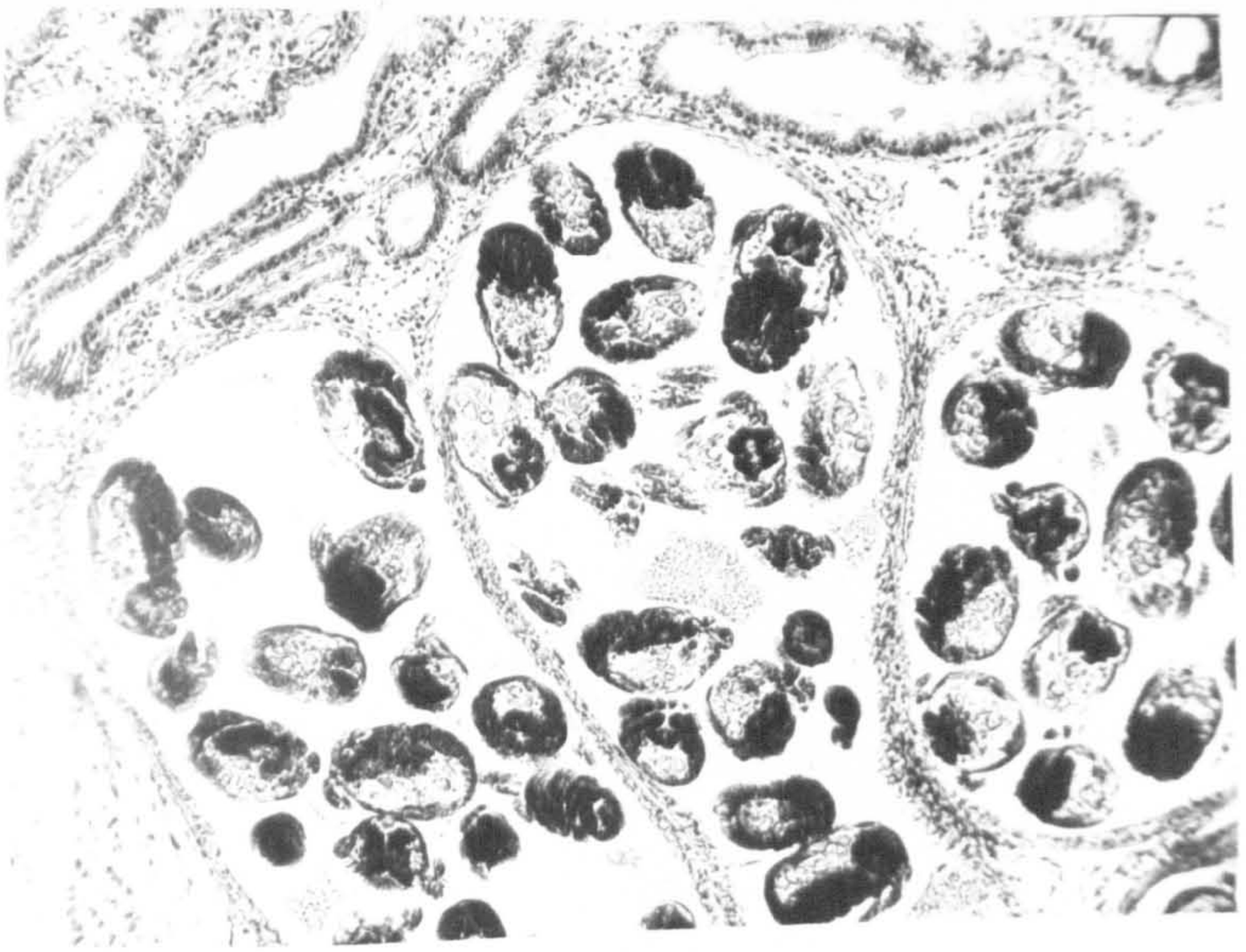


Fig. 44

O·1mm.

Fig. 45

PLATE 20 Nucellicola kilrymontis gen. et sp. nov.

Fig. 46 Drawing of 2nd metanauplius (free-living).

a - frontal gland

b - 1st antenna

c - 2nd antenna

d - mandible

e - rudimentary maxillipeds

f - thoracic appendages

g - rudimentary abdomen

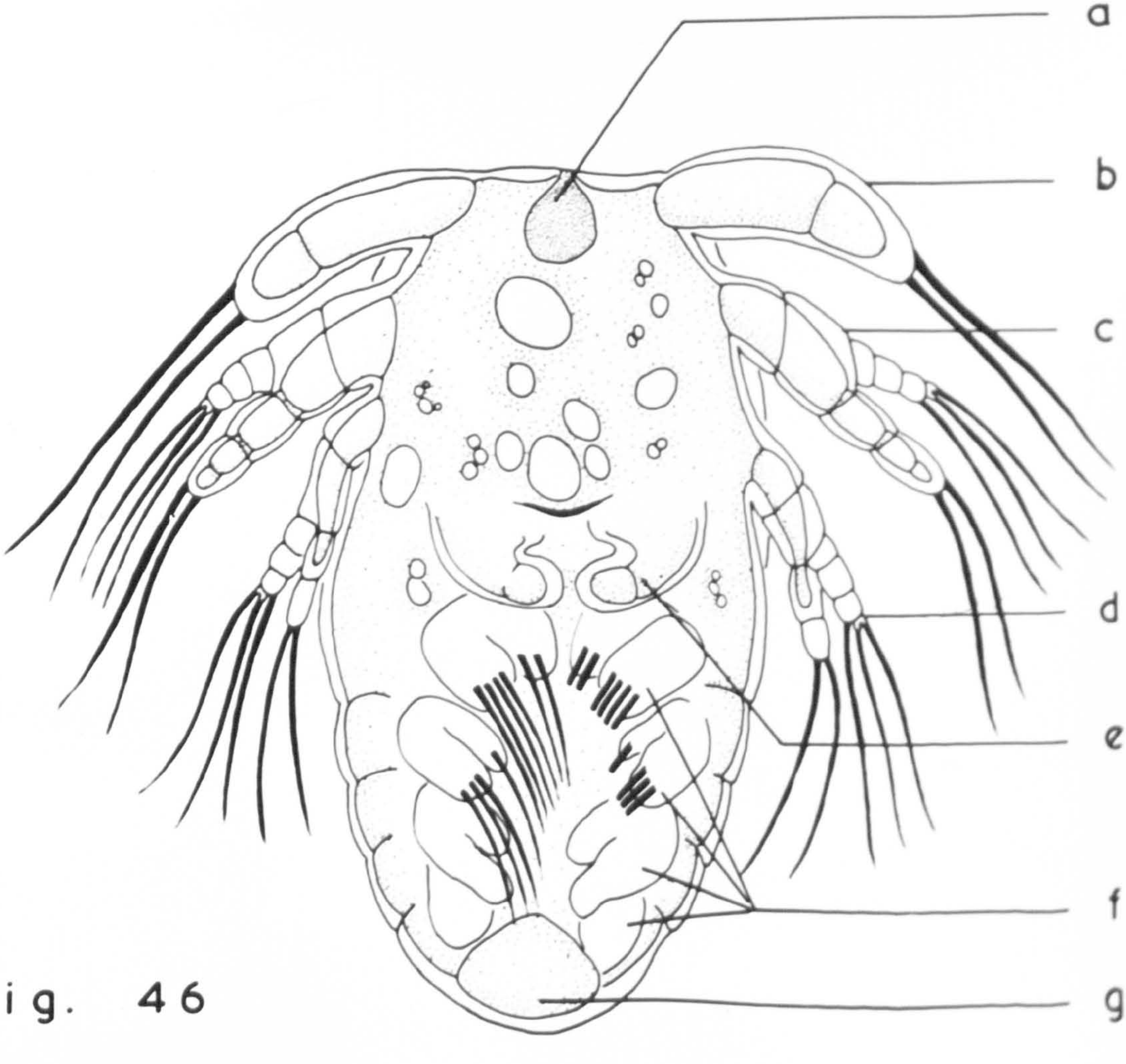


Fig. 46

100 μ

PLATE 21 Nucellicola kilrymontis gen. et sp. nov.

Fig. 47 Drawing of 3rd metanauplius

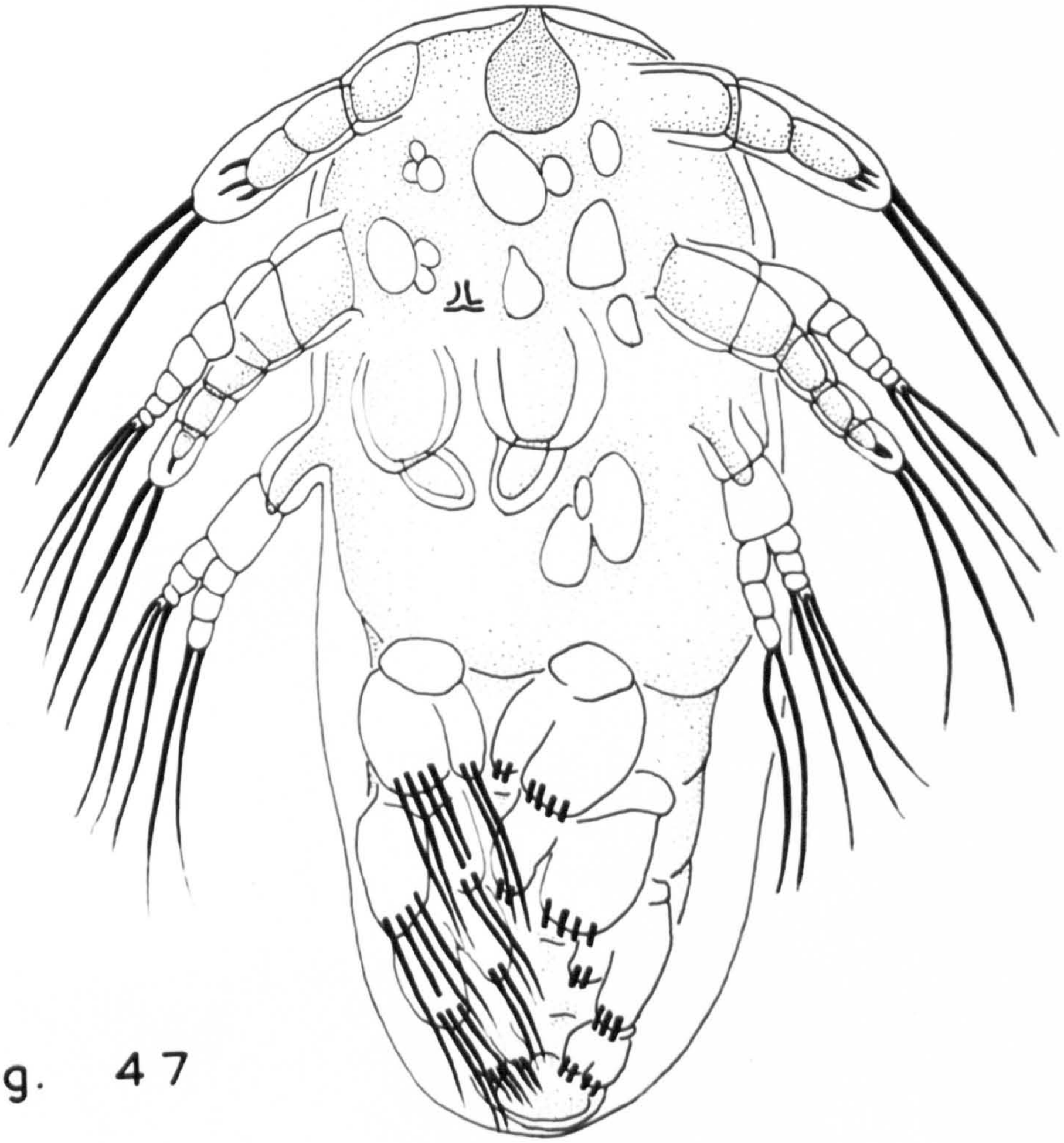


Fig. 47

100 μ

PLATE 22 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 48 Drawing of 1st copepodid

a - 1st antenna

b - 2nd antenna

c - vestigial mandible

d - maxilliped

PLATE 22

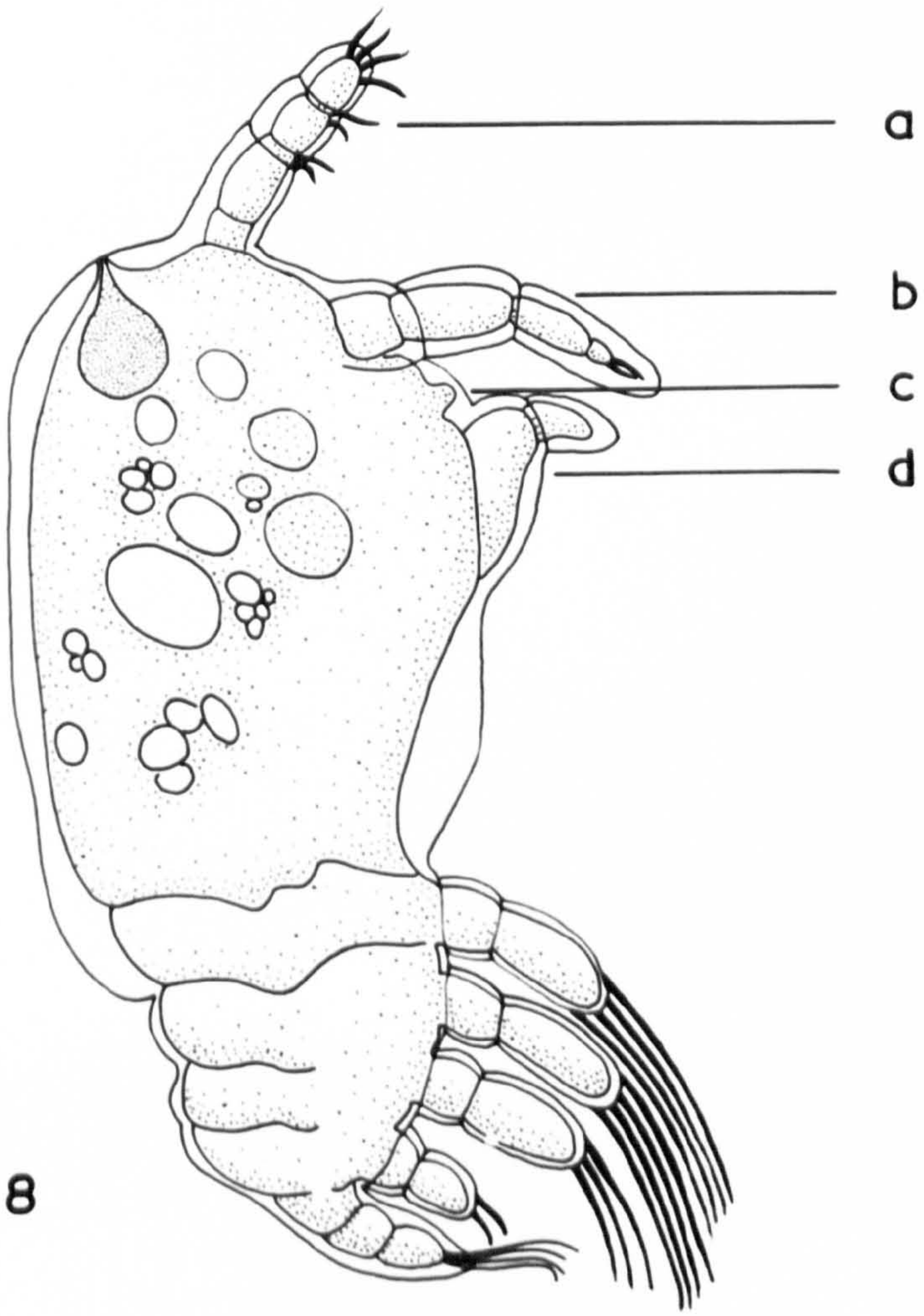


Fig. 48

PLATE 23 Nucellicola kilrymontis gen. et sp. nov.

Fig. 49 Photograph of live 2nd metanauplius, ventral aspect.

Fig. 50 Photograph of live 2nd metanauplius, lateral aspect.

Fig. 49

0·1mm.

Fig. 50

PLATE 24 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 51 Photograph of live 3rd metanauplius with the 1st copepodid beginning to emerge. Ventral aspect.

Fig. 52 Photograph of live 3rd metanauplius with the 1st copepodid emerging. Lateral aspect.

Fig. 51

O·1mm.

Fig. 52

PLATE 25 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 53 Photograph of live 1st copepodid. Lateral aspect.

Fig. 54 Photograph of live 1st copepodid, six days after emergence from 3rd metanauplius. Lateral aspect.

Fig. 53

O·1 mm.

Fig. 54

PLATE 26 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 55 Drawing of 1st metanauplius showing the arrangement of the muscles. Dorsal aspect.

Fig. 56 Drawing of 1st metanauplius showing the arrangement of the muscles. Lateral aspect.

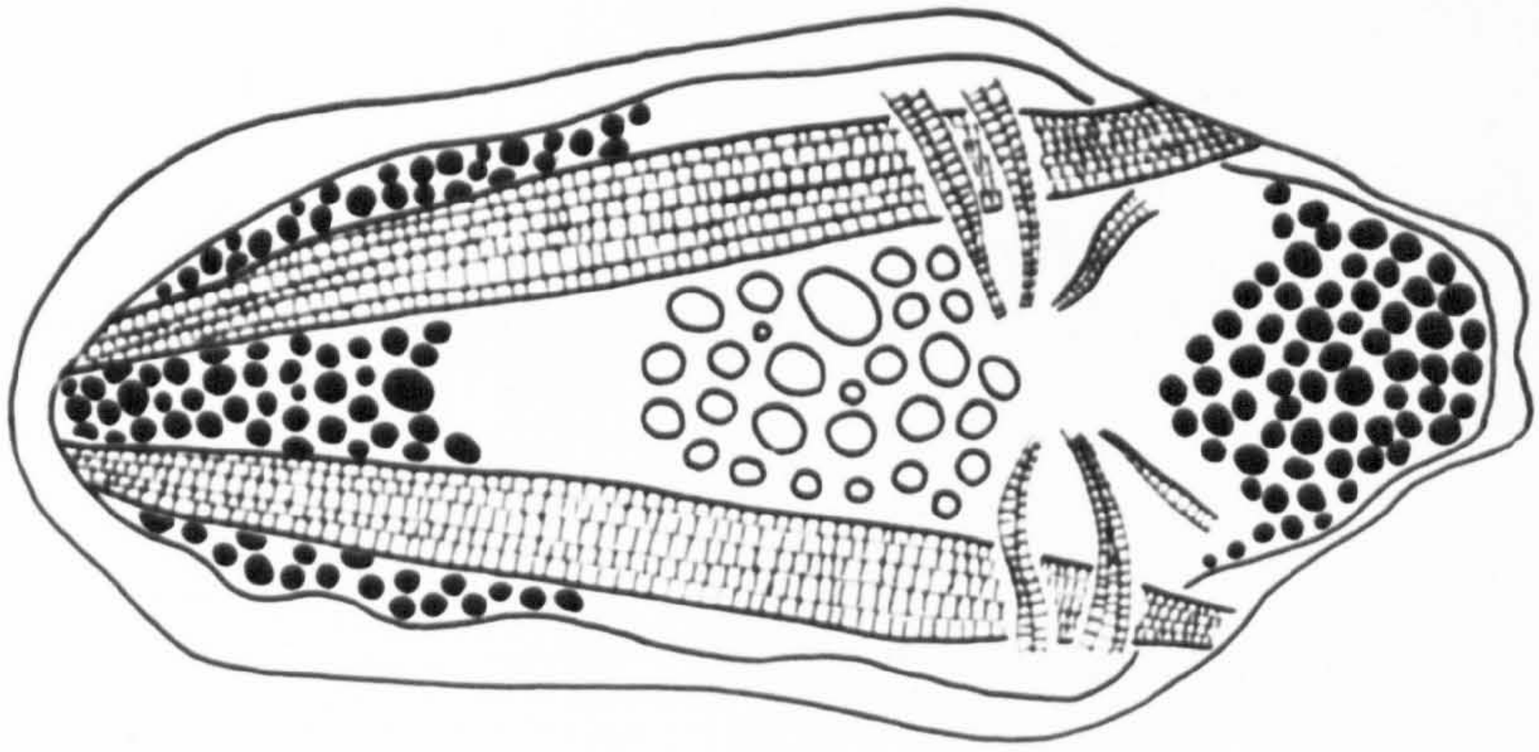


Fig. 55

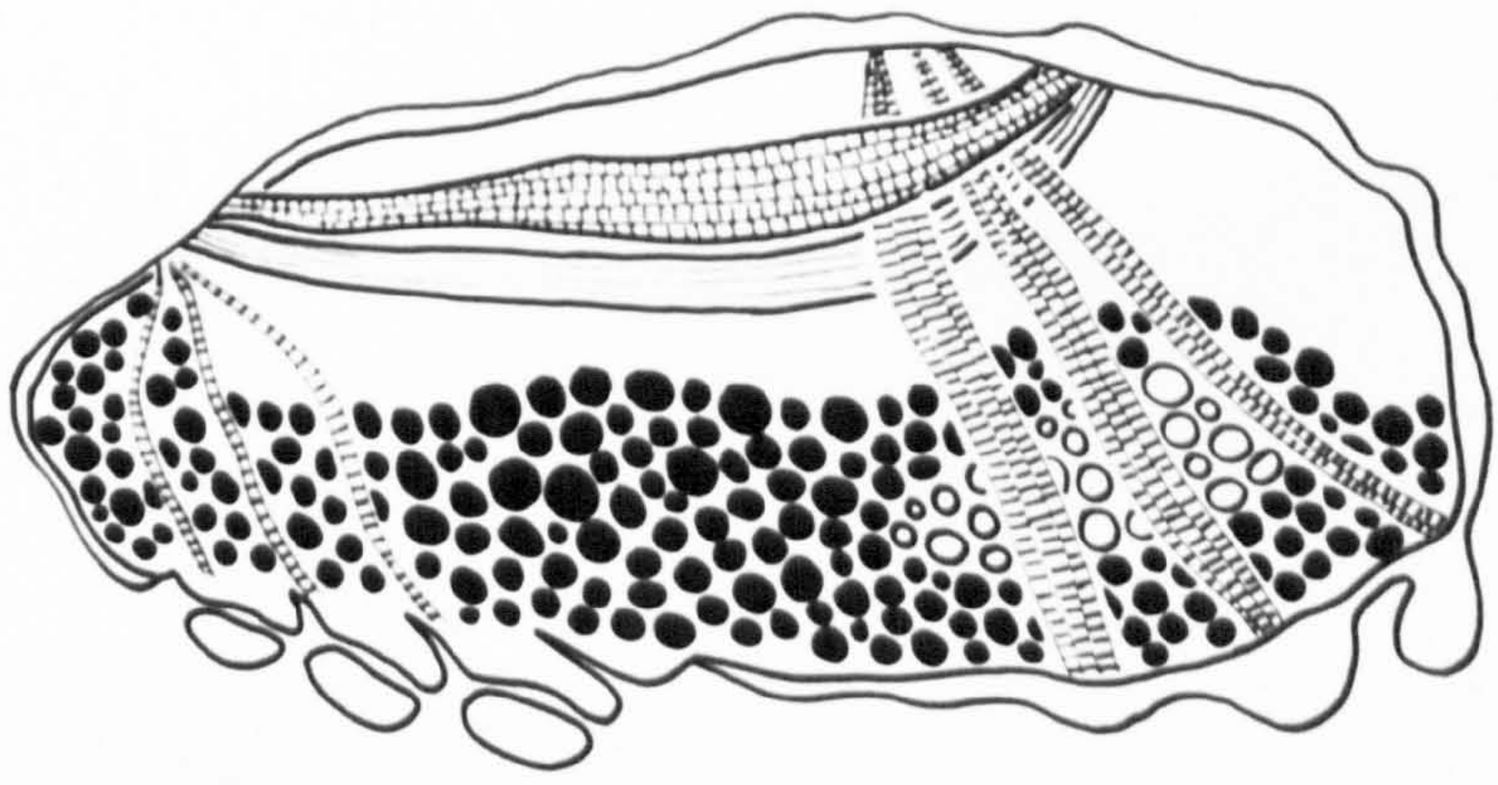


Fig. 56

PLATE 27 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 57 Drawing of free-living 2nd metanauplius showing the arrangement of the muscles. Lateral aspect.

Fig. 58 Drawing of 3rd metanauplius showing the arrangement of the muscles. Lateral aspect.

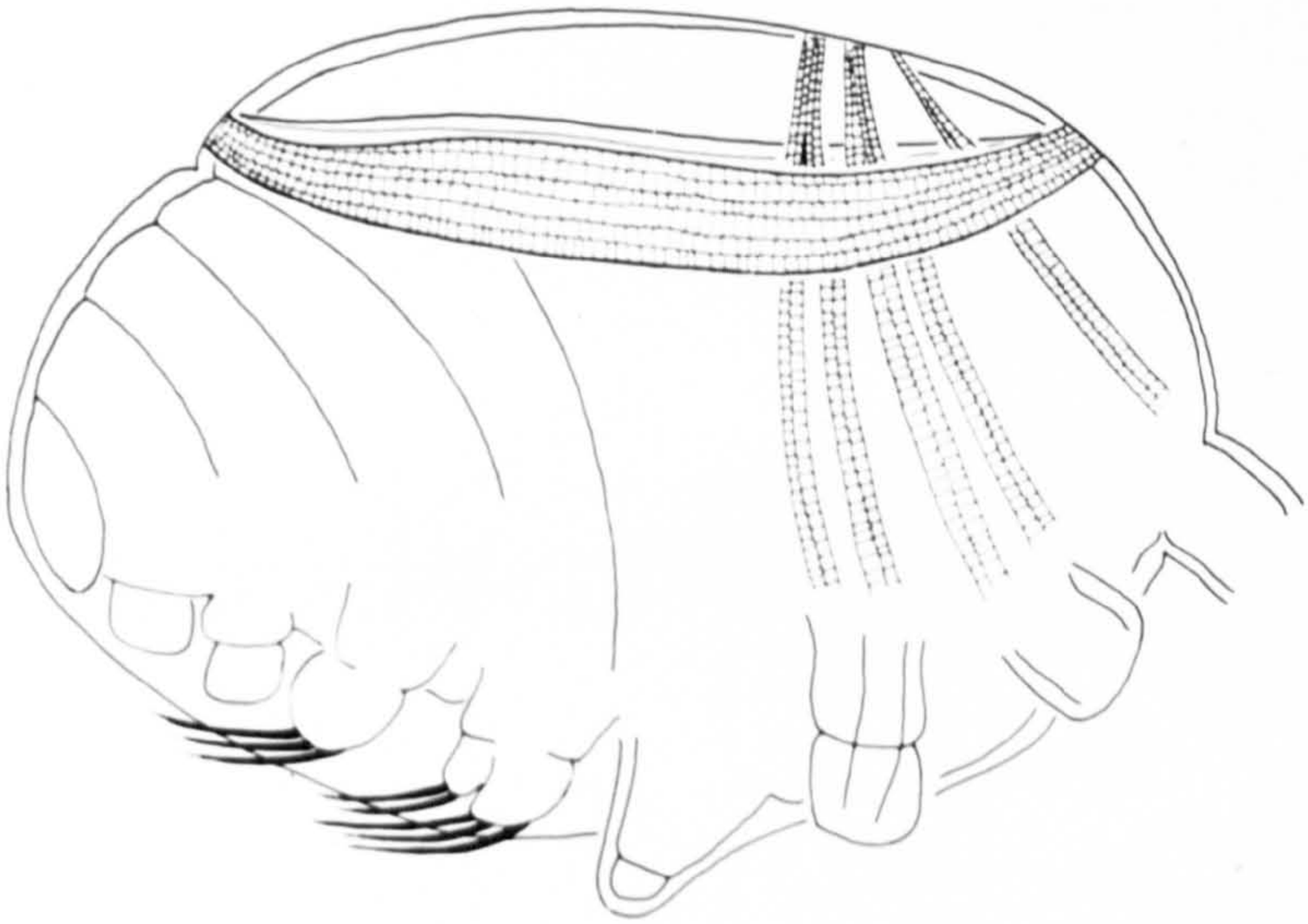


Fig. 57

100 μ

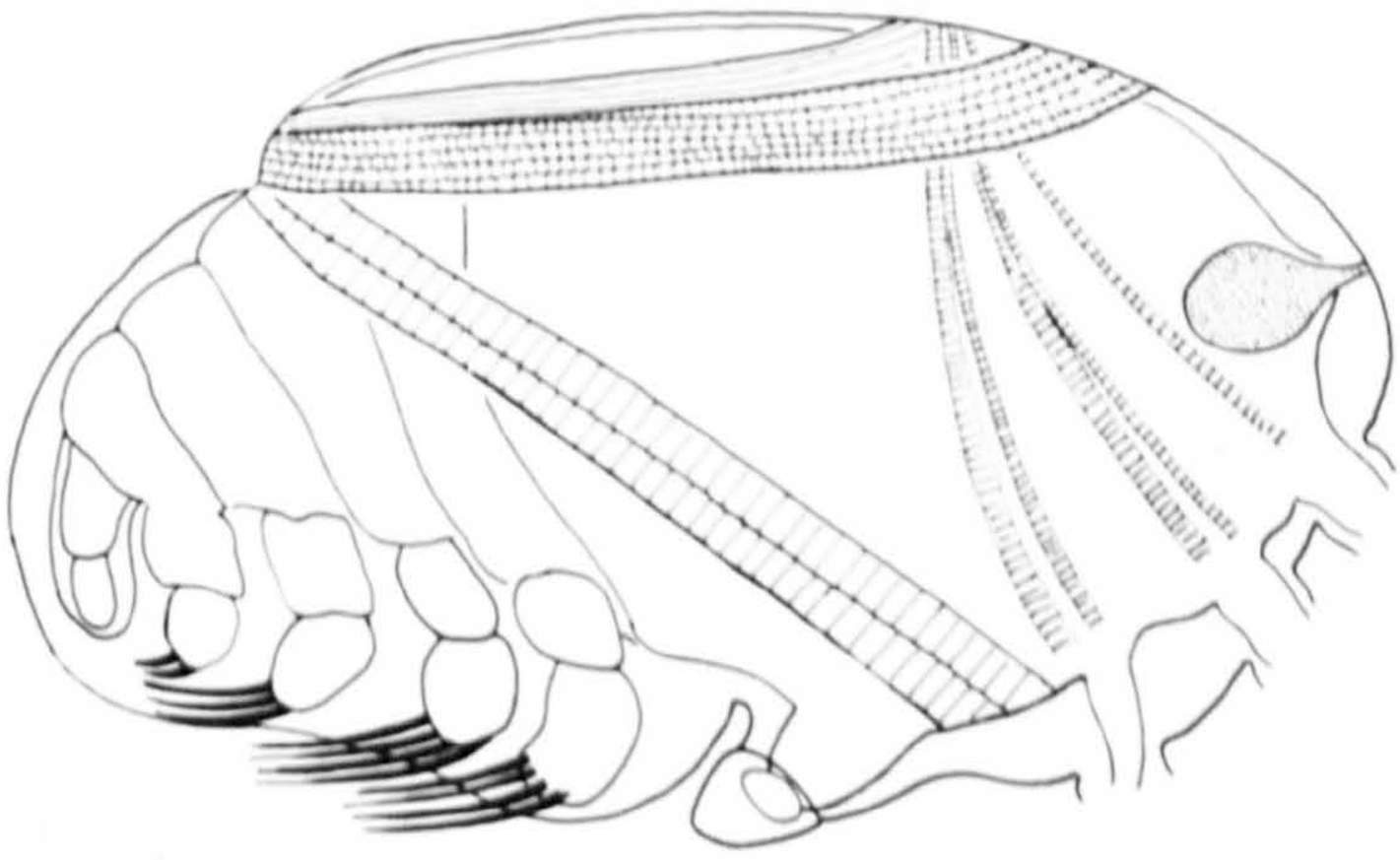


Fig. 58

PLATE 28 Nucellicola kilrymontis gen. et sp. nov.

Fig. 59 Drawing of 3rd metanauplius showing the arrangement of the muscles. Dorsal aspect.

Fig. 60 Drawing of 1st copepodid showing the arrangement of the muscles. Lateral aspect.

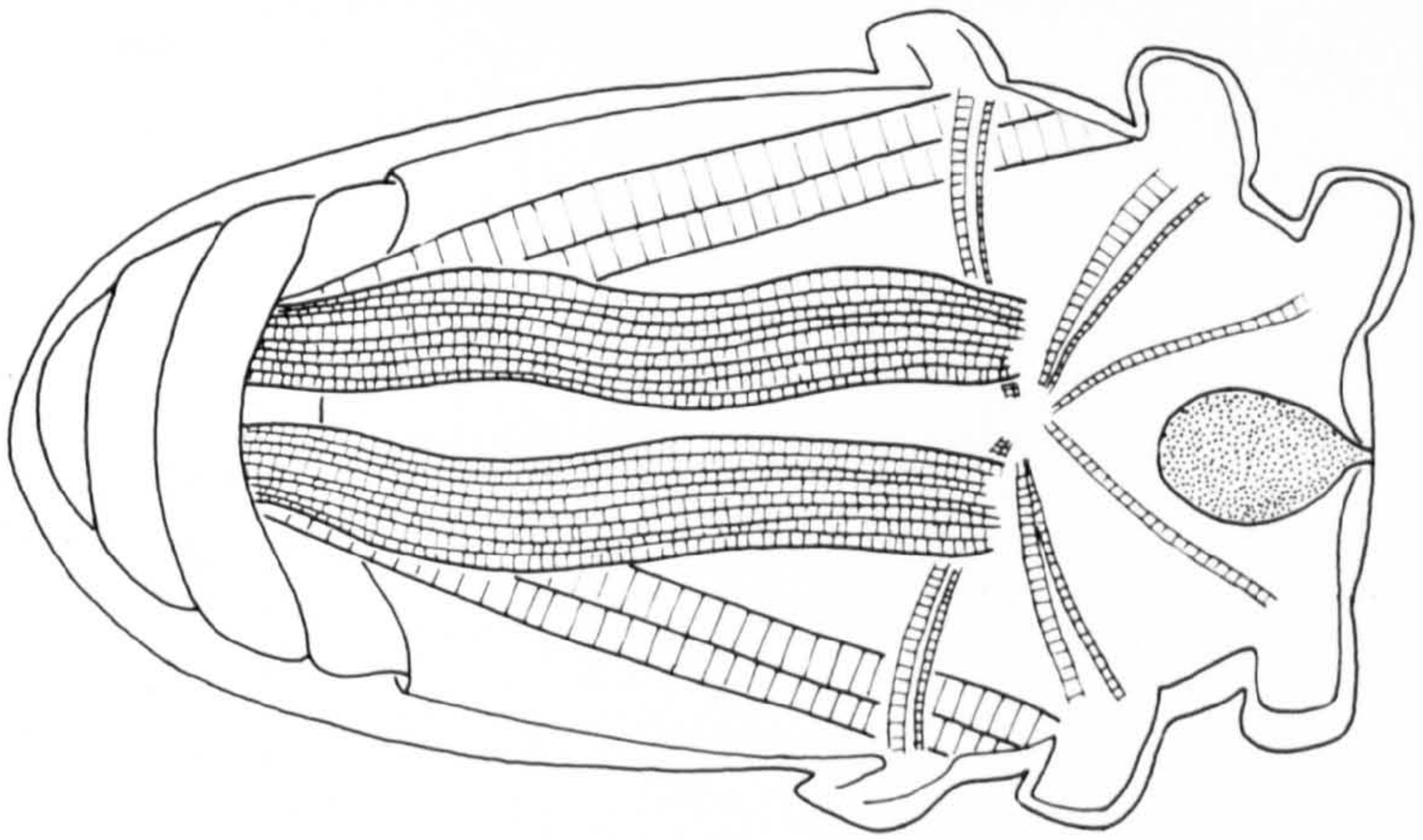


Fig. 59

100 μ

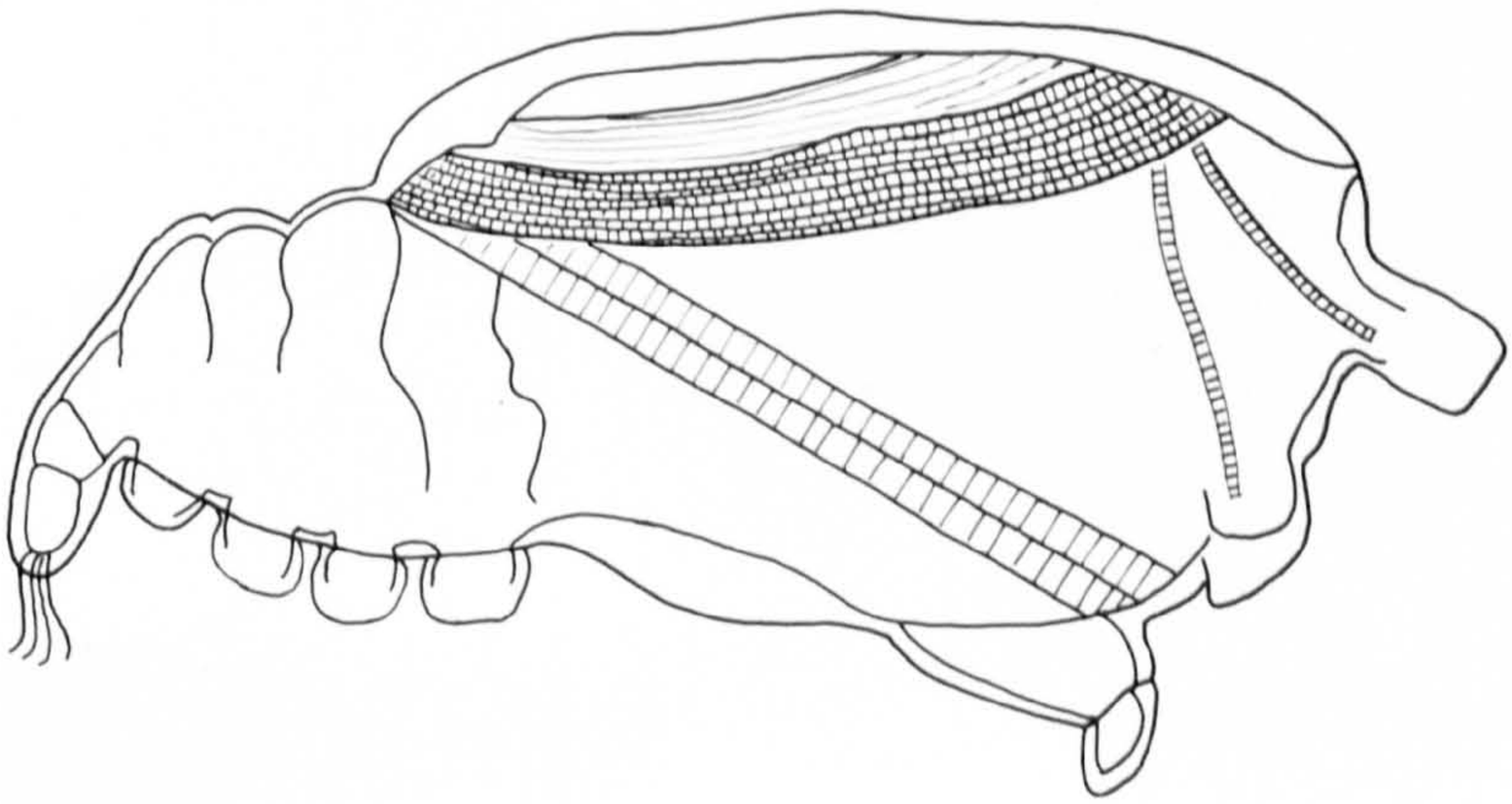


Fig. 60

PLATE 29 *Nucellicola kilrymontis* gen. et sp. nov.

Fig. 61 Photograph of male exuviae stained with polyvinyl alcohol and chlorazol black E. Ventral aspect.

Fig. 62 Photograph of male exuviae stained with polyvinyl alcohol and chlorazol black E. Lateral aspect.

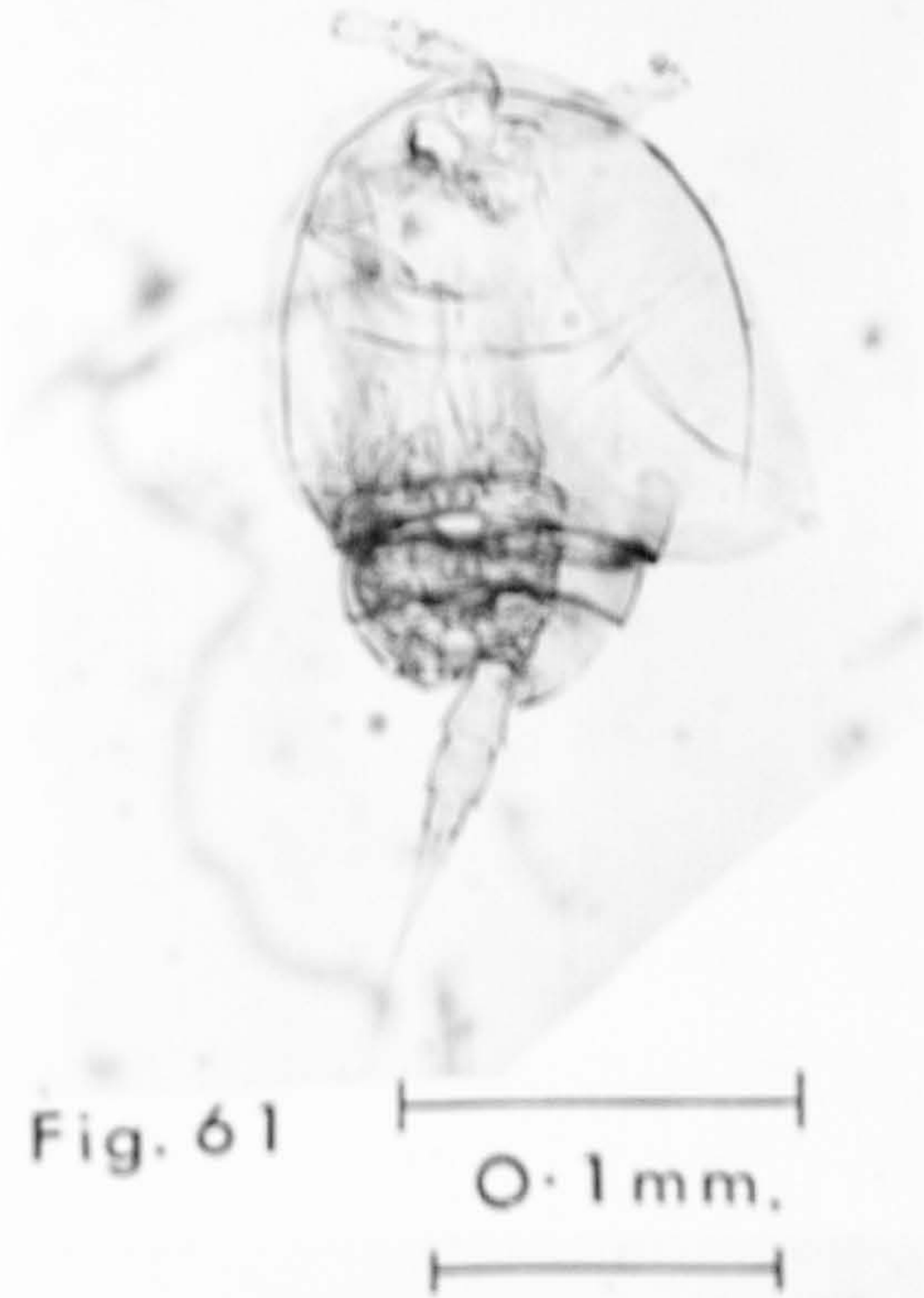


Fig. 61

0·1mm.

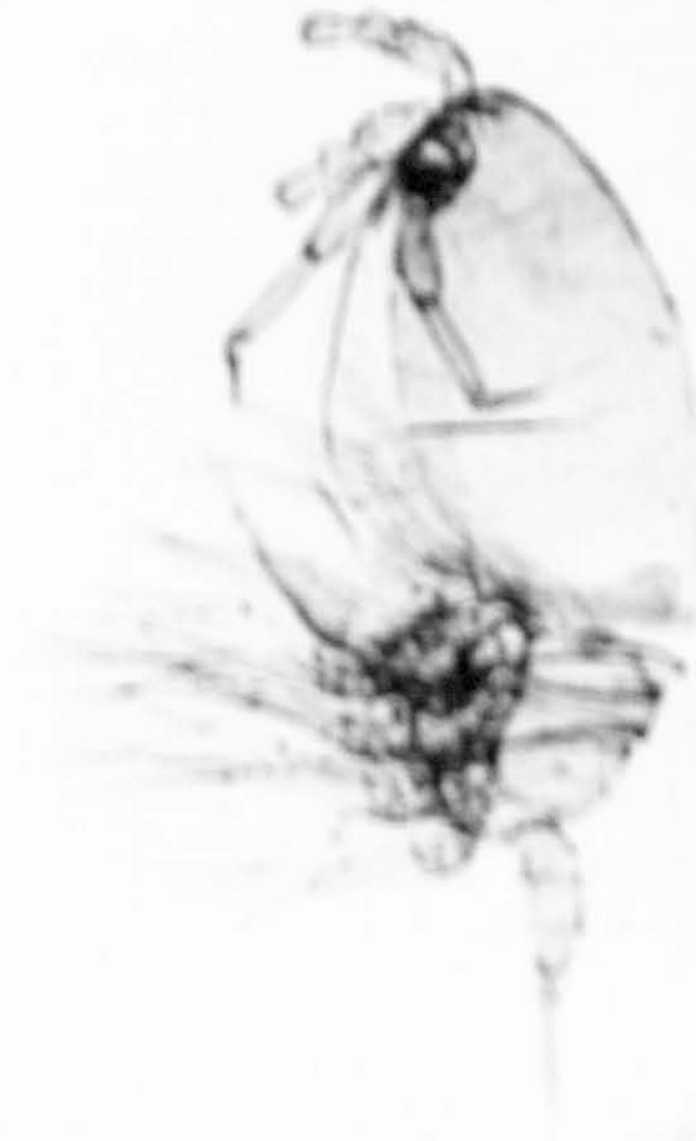


Fig. 62

PLATE 30 *Nucellicola kilrymontis* gen. et sp. nov.

Figs 63 to 73 Drawings of the head appendages of the 2nd and 3rd metanauplii, the 1st copepodid and the male exuviae.

Fig. 63 2nd metanauplius, 1st antenna.

Fig. 64 3rd metanauplius, 1st antenna.

Fig. 65 1st copepodid, 1st antenna.

Fig. 66 Male exuviae, 1st antenna.

Fig. 67 2nd metanauplius, 2nd antenna.

Fig. 68 3rd metanauplius, 2nd antenna.

Fig. 69 1st copepodid, 2nd antenna.

Fig. 70 Male exuviae, 2nd antenna.

Fig. 71 2nd metanauplius, mandible.

Fig. 72 3rd metanauplius, mandible.

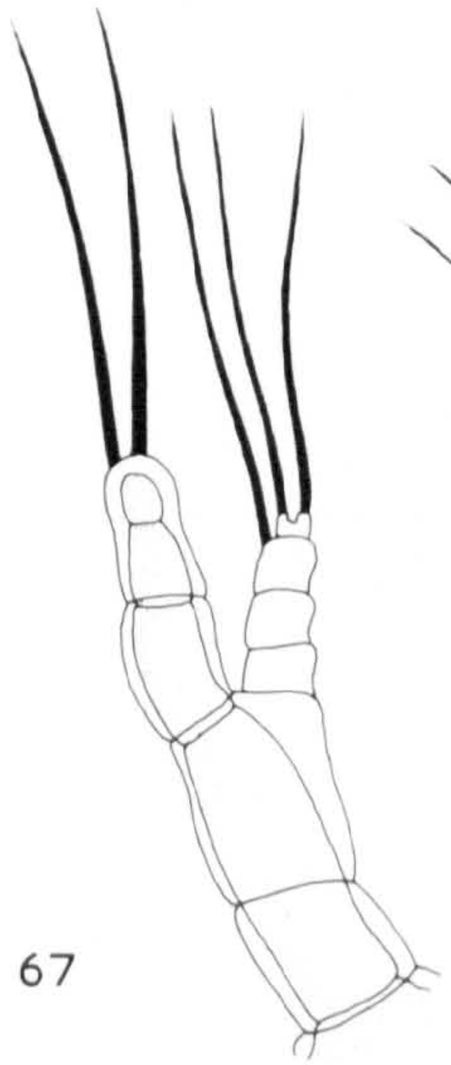
Fig. 73 1st copepodid, maxilliped.

63

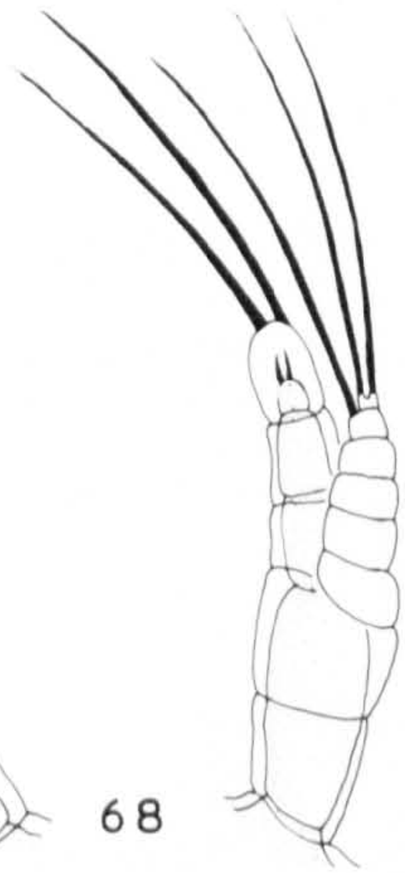
64

65

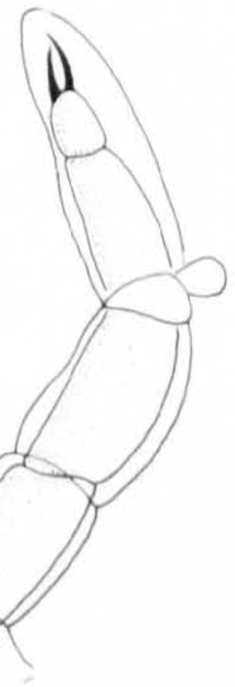
66



67

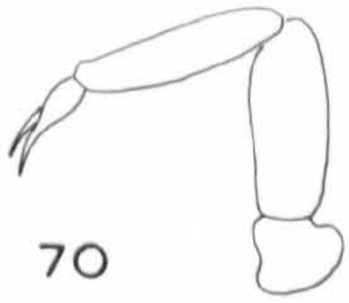


68



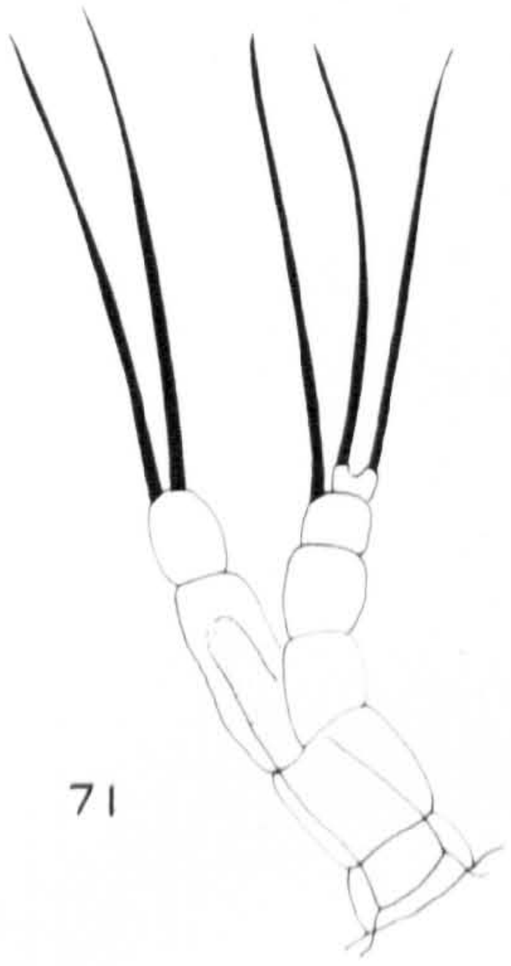
69

25 μ



70

Figs. 63—73



71

72

73

PLATE 31 — Nucellicola kilrymontis gen. et sp. nov.

Figs 74 to 88 — Drawings of thoracic appendages of the 2nd and 3rd metanauplii, the 1st copepodid and the male exuviae, and of the abdomens of the 1st copepodid and the male exuviae.

Fig. 74 — 2nd metanauplius, 1st thoracic appendage.

Fig. 75 — 2nd metanauplius, 2nd thoracic appendage.

Fig. 76 — 3rd metanauplius, 1st thoracic appendage.

Fig. 77 — 3rd metanauplius, 2nd thoracic appendage.

Fig. 78 — 3rd metanauplius, 3rd thoracic appendage.

Fig. 79 — 1st copepodid, 1st thoracic appendage.

Fig. 80 — 1st copepodid, 2nd thoracic appendage.

Fig. 81 — 1st copepodid, 3rd thoracic appendage.

Fig. 82 — 1st copepodid, 4th thoracic appendage.

Fig. 83 — Male exuviae, 1st thoracic appendage.

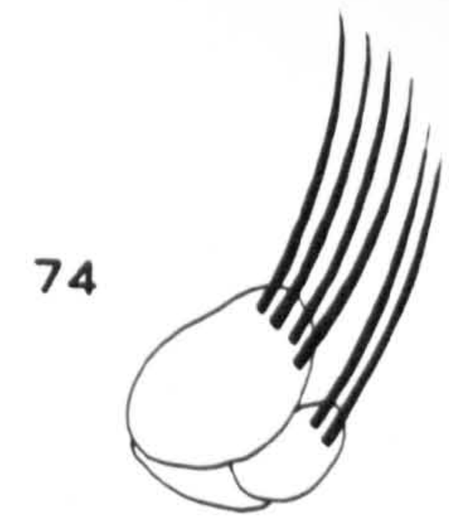
Fig. 84 — Male exuviae, 2nd thoracic appendage.

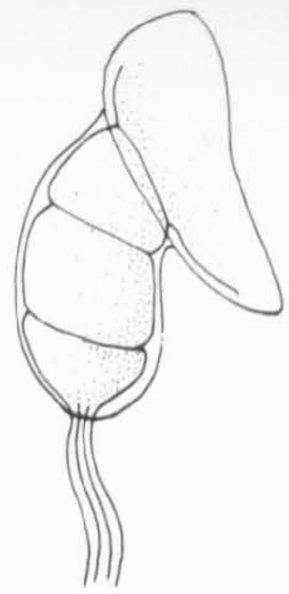
Fig. 85 — Male exuviae, 3rd thoracic appendage.

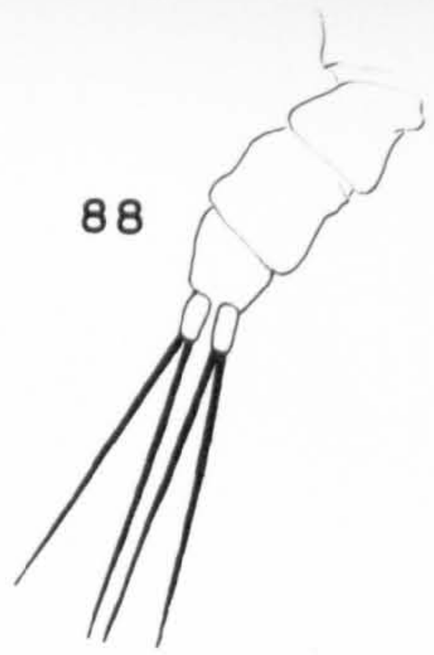
Fig. 86 — Male exuviae, 4th thoracic appendage.

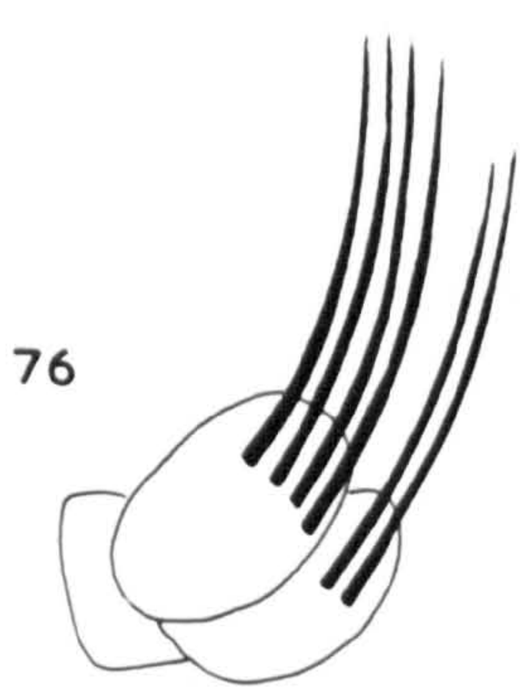
Fig. 87 — 1st copepodid, abdomen.

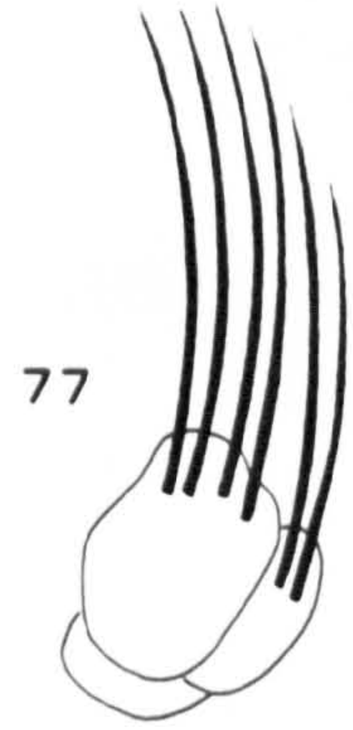
Fig. 88 — Male exuviae, abdomen.

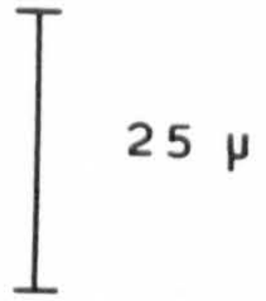




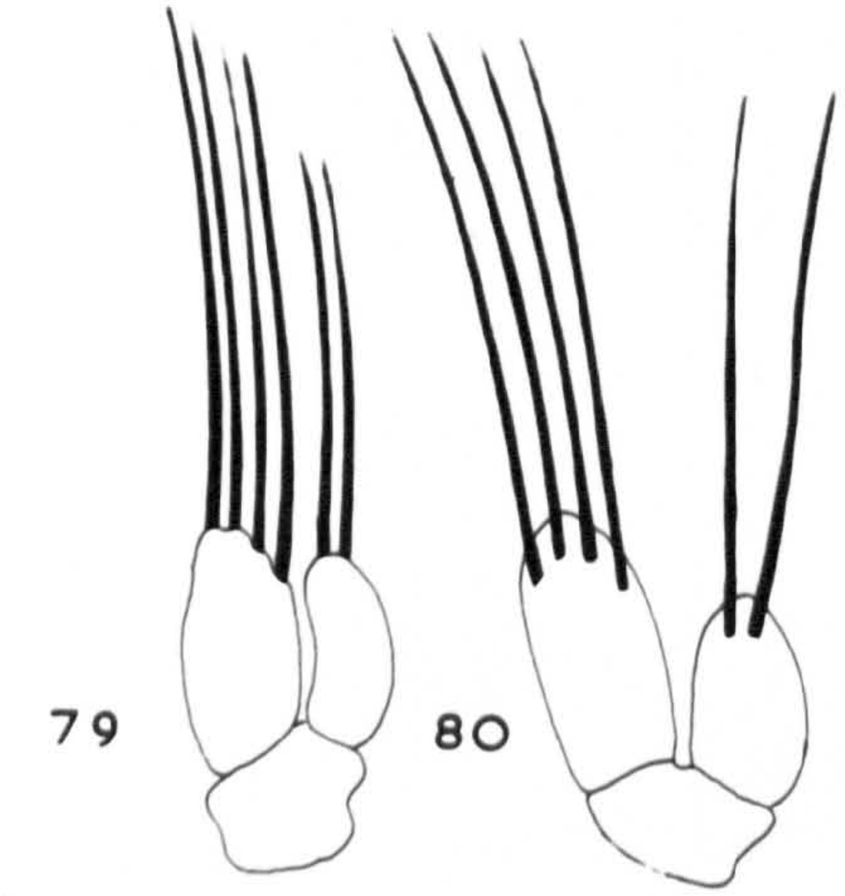


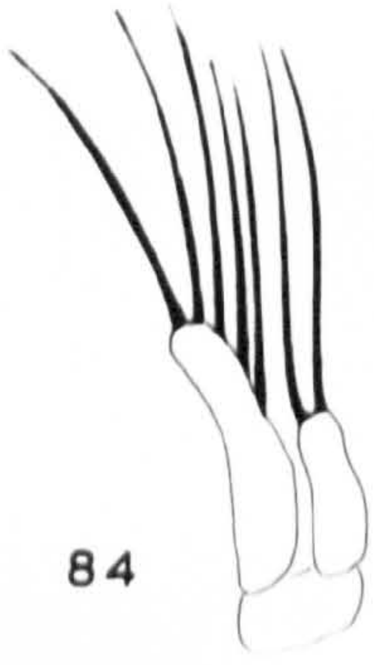






Figs. 74 – 88





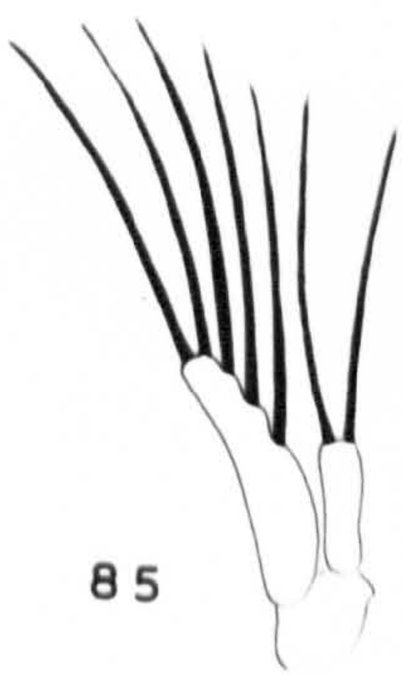


PLATE 32 Nucellicola kilrymontis gen. et sp. nov.

Fig. 89 Drawing of immature adult female possessing neither male nor egg-string. The terminal regions of the oviducts are void of oocytes.

Fig. 90 Drawing of young mature adult female possessing an incompletely-developed male and an egg-string.

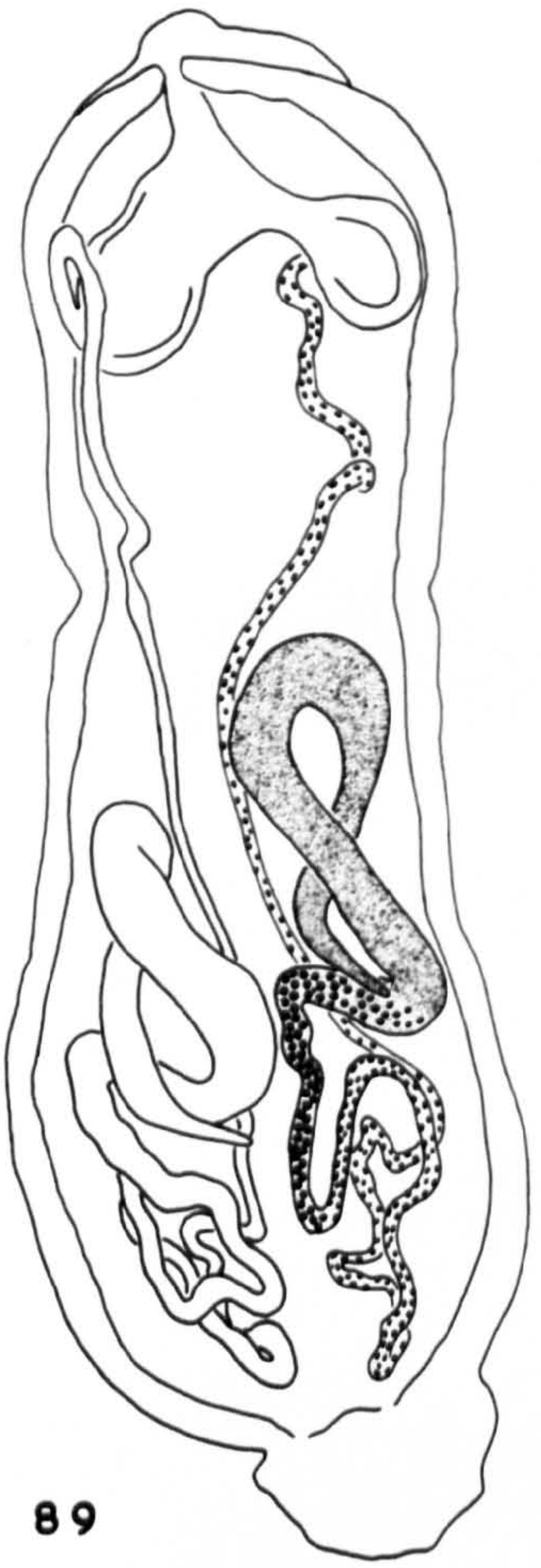


Fig. 89

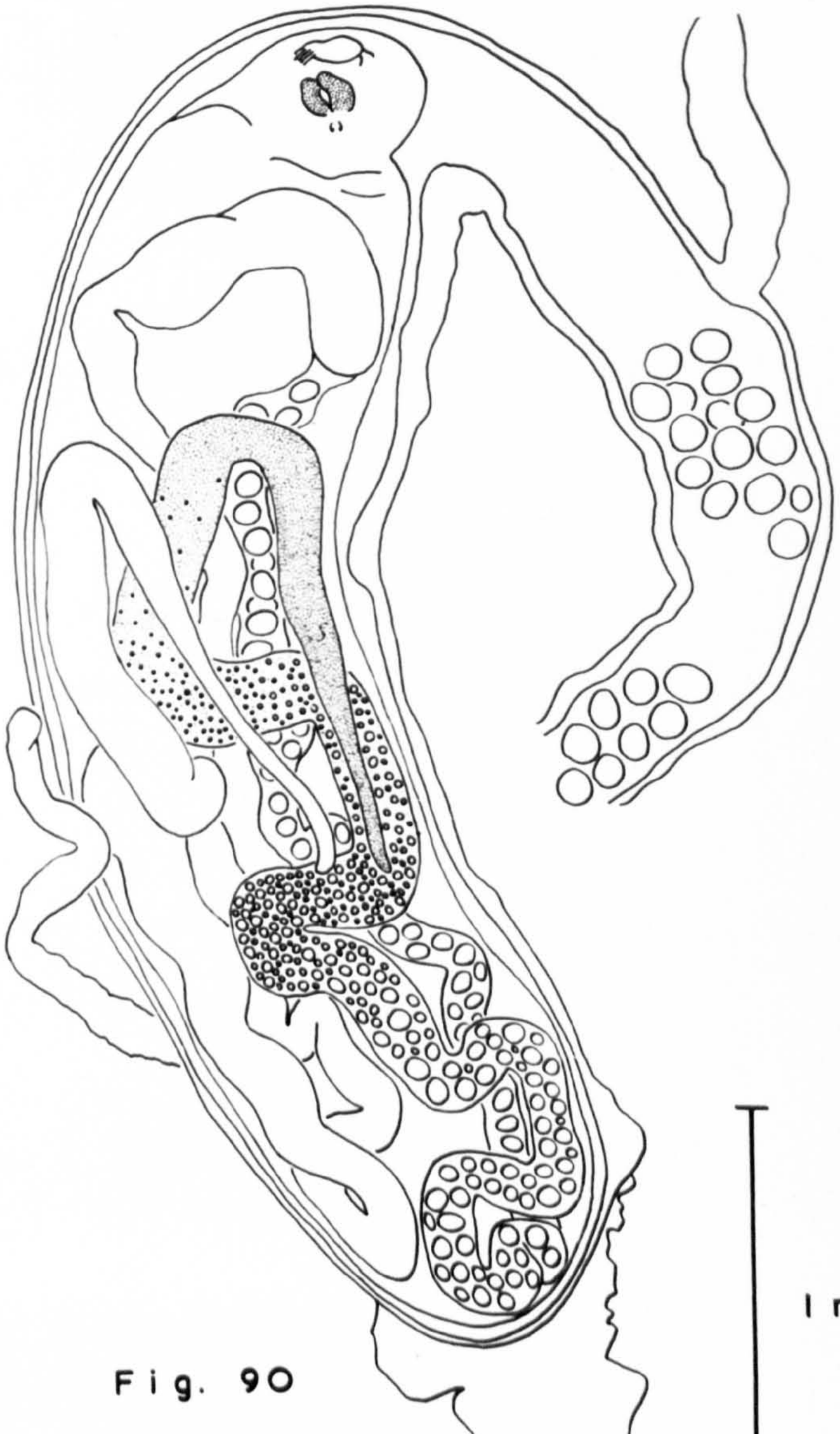


Fig. 90

1 mm.

PLATE 33 Nucellicola kilrymontis gen. et sp. nov.

Fig. 91 Photograph of 10 μ horizontal section of the posterior end of an immature adult female without male.

a - accessory gland

b - thick integument

c - cement gland

Fig. 92 Photograph of 15 μ transverse section through an immature adult female without male.

a - ovary

b - developing oviducts, empty and thick-walled

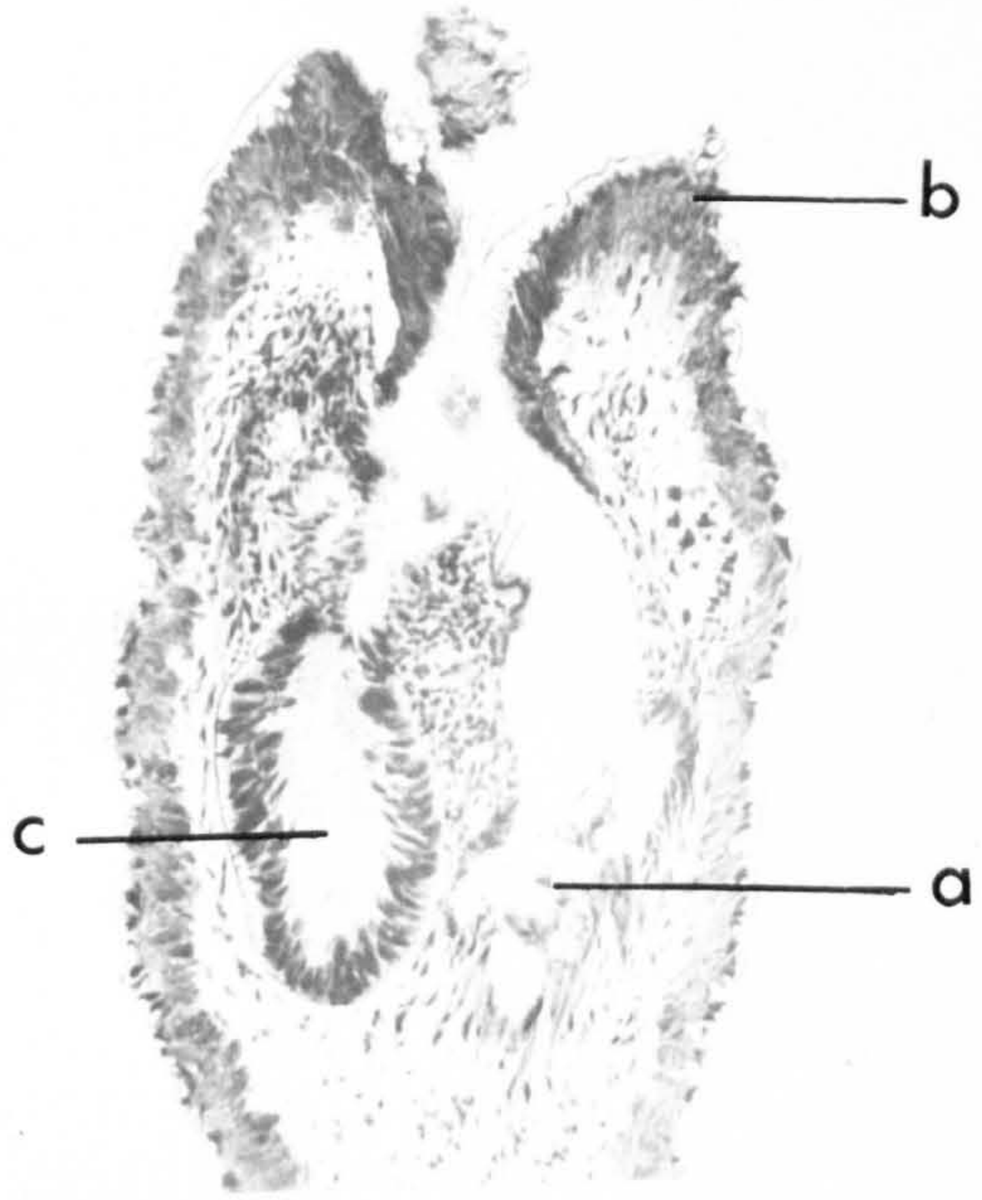


Fig. 91

O·1 mm.

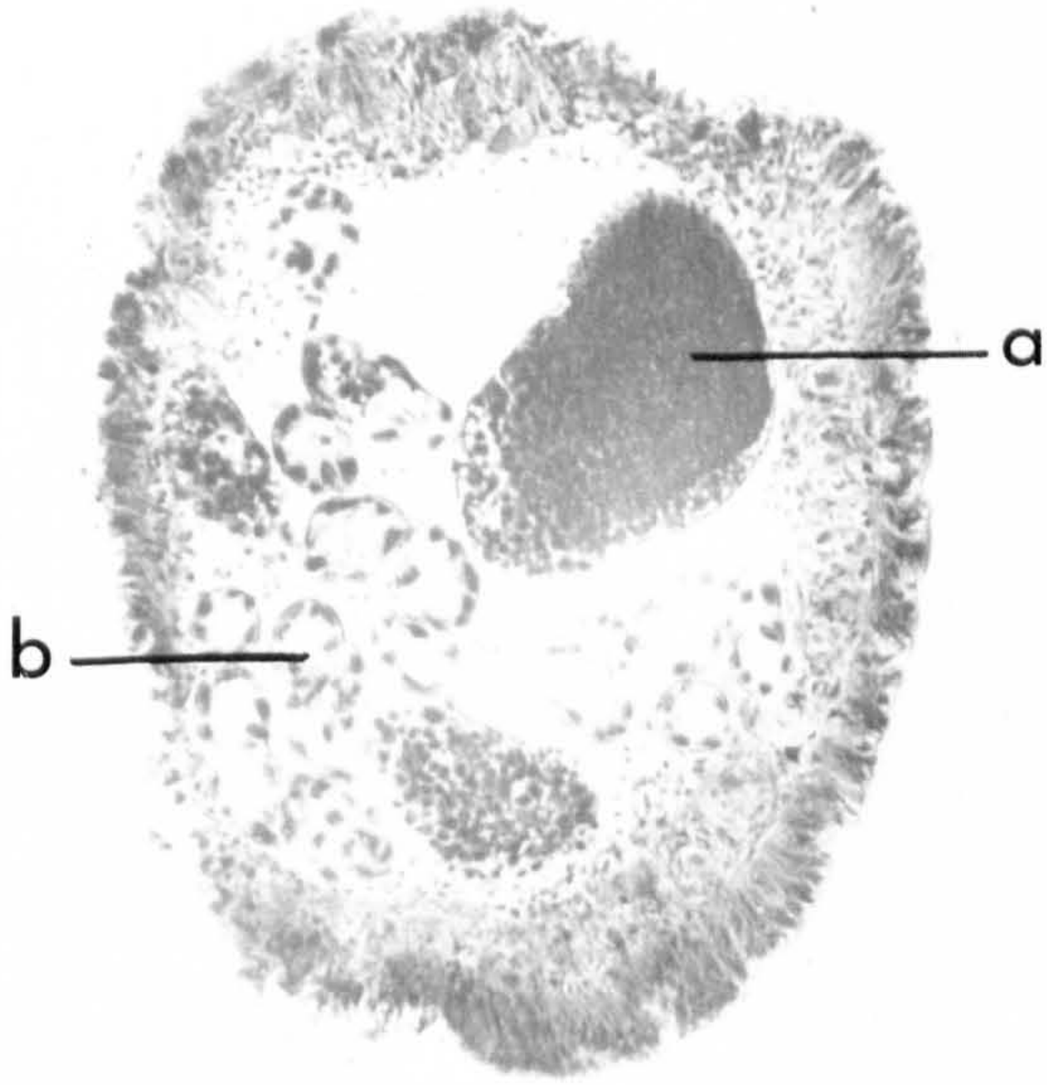


Fig. 92

PLATE 34 — *Nucellicola kilrymontis* gen. et sp. nov.

Figs 93 and 94 — Photographs of 15 μ horizontal sections through the anterior region of an immature adult female, without male, in situ, showing the anterior filamentous processes.

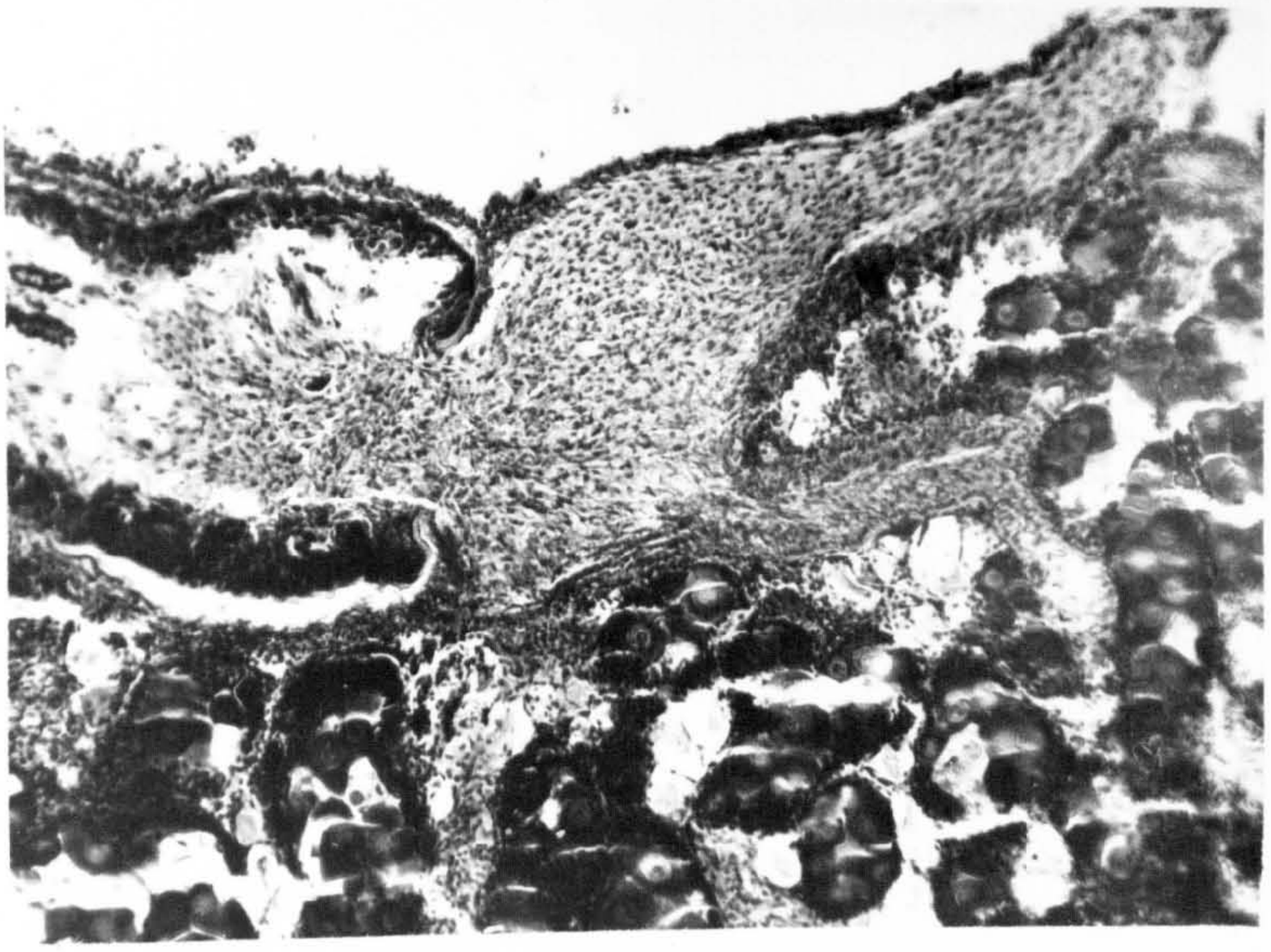


Fig. 93

0·1mm.

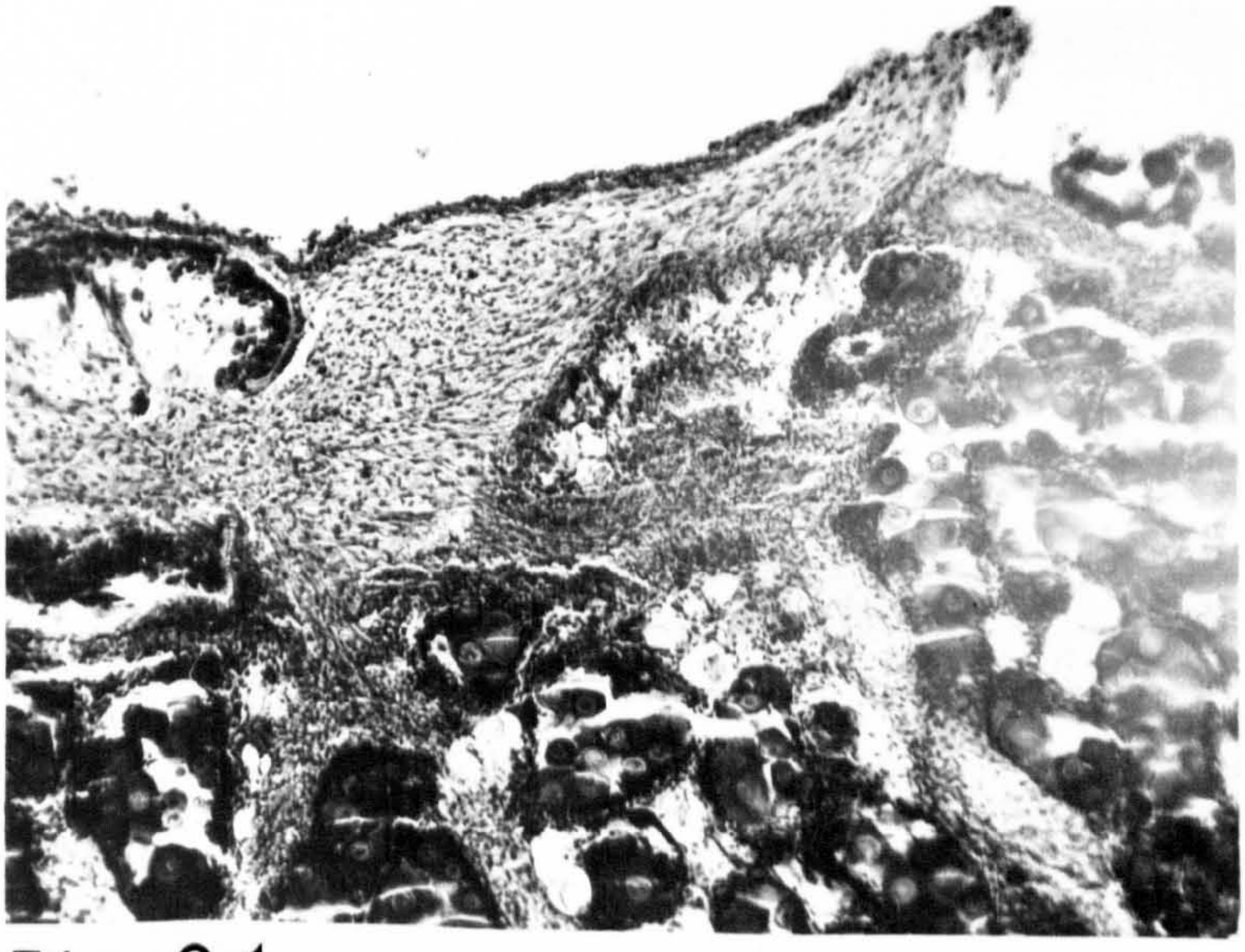


Fig. 94

PLATE 35 Nucellicola kilrymontis gen. et sp. nov.

Fig. 95 Photograph of immature adult female, including a recently-arrived male, showing the long posterior tube, with ramifications, which is the future covering of the egg-string.

Fig. 96 Photograph of 12 μ longitudinal section through X in fig. 95, showing that the posterior tube is continuous with the outer integument of the female.

Fig. 95

5 mm.

Fig. 96

0·1mm.

PLATE 36 Nucellicola kilrymontis gen. et sp. nov.

Fig. 97 Photograph of young adult female, with included male, prepared by the squash technique, showing the anterior filamentous processes of the female.

Fig. 98 Photograph of mature adult prepared by the squash technique but unstained. This specimen was taken from an "unsuitable" site in the mantle; the anterior processes are more numerous than usual and still persist although the female has been fertilized and is producing viable eggs.

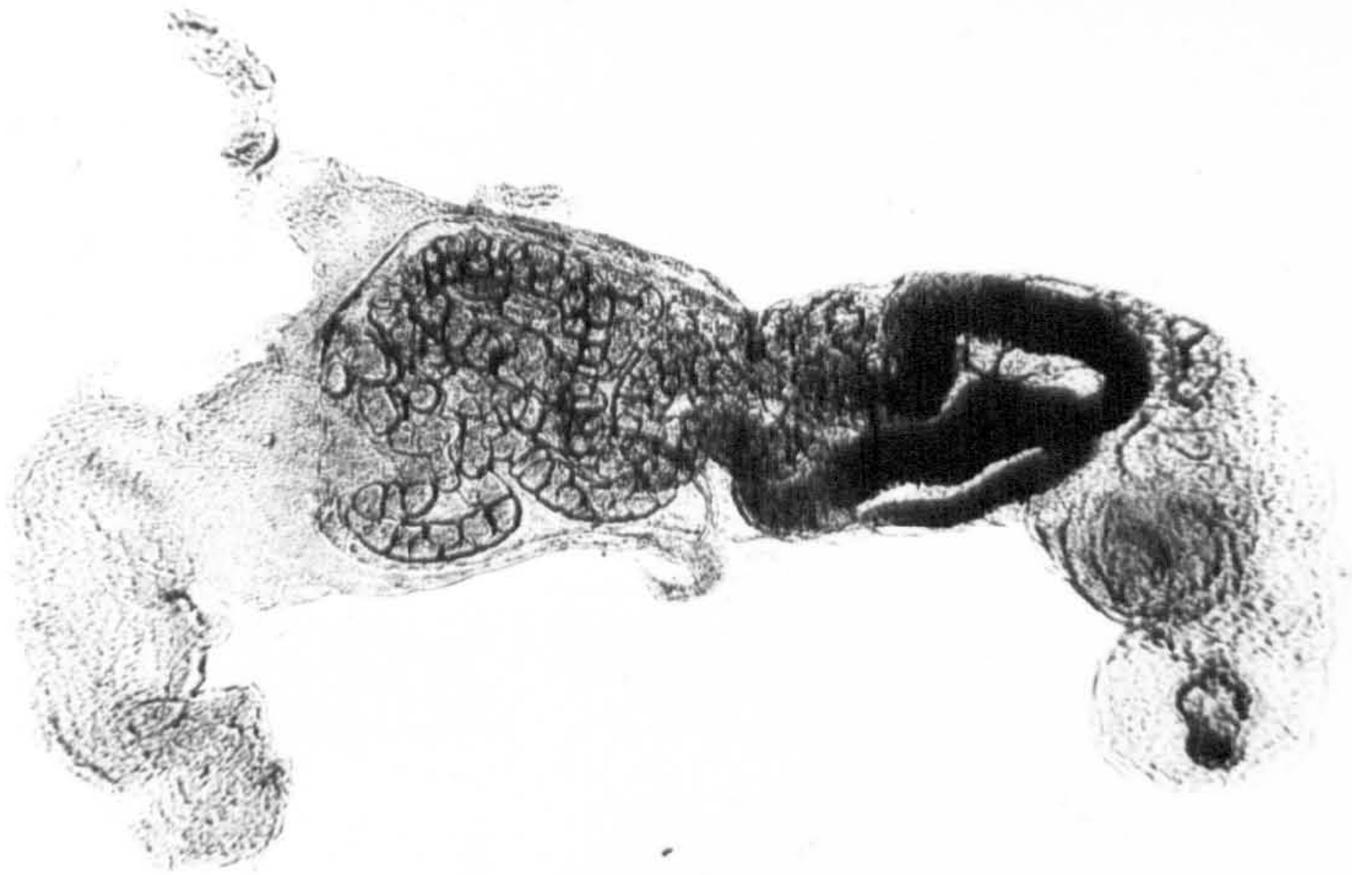


Fig. 97

1 mm.

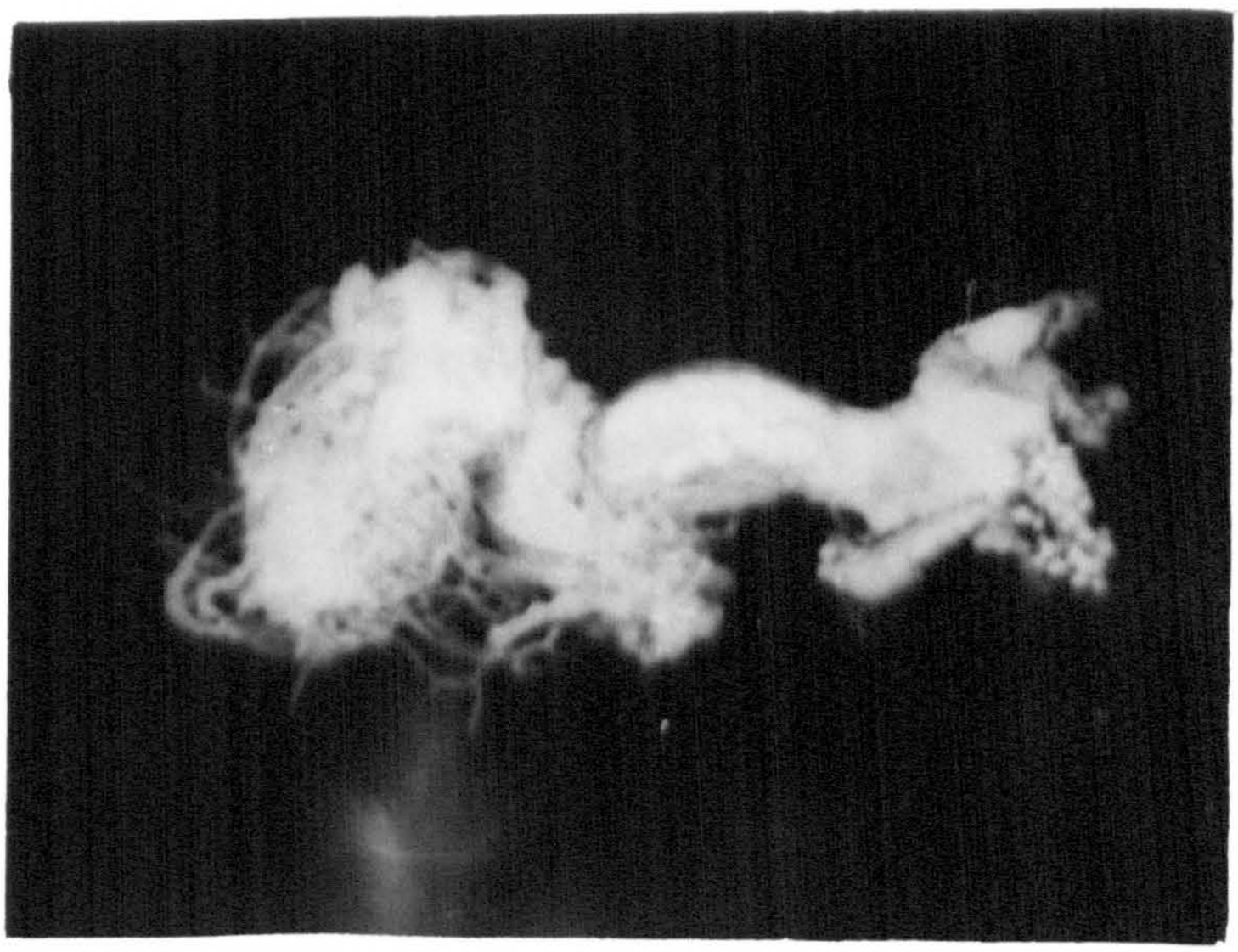


Fig. 98

PLATE 37 Nucella lapillus (L.)

Fig. 99 Photograph of 10 μ section through the testis and digestive gland of an unparasitised male.

Fig. 100 Photograph of 10 μ section through the testis and digestive gland of a male parasitised by Nucellicola kilrymontis gen. et sp. nov.

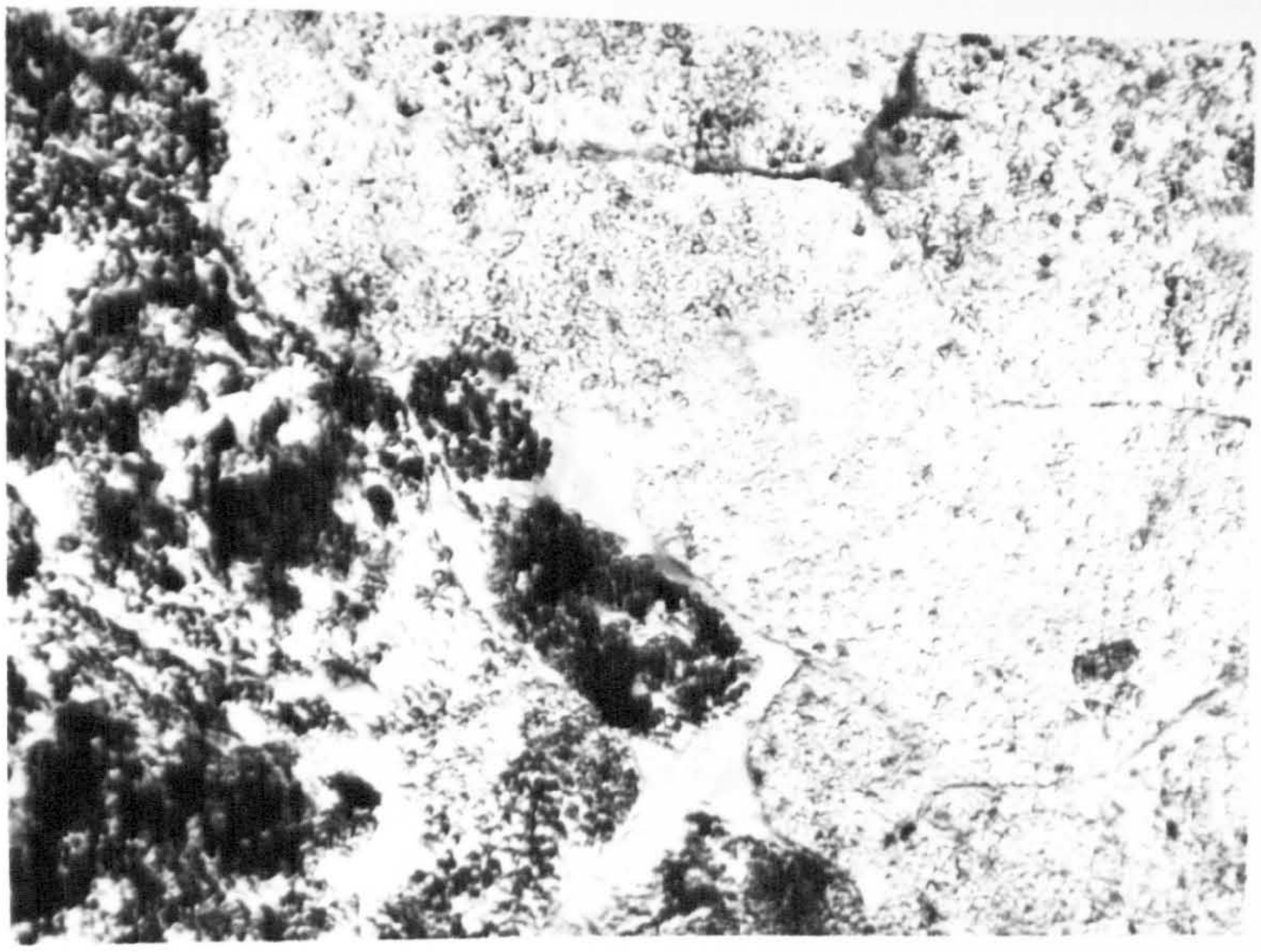


Fig. 99

0·1 mm.

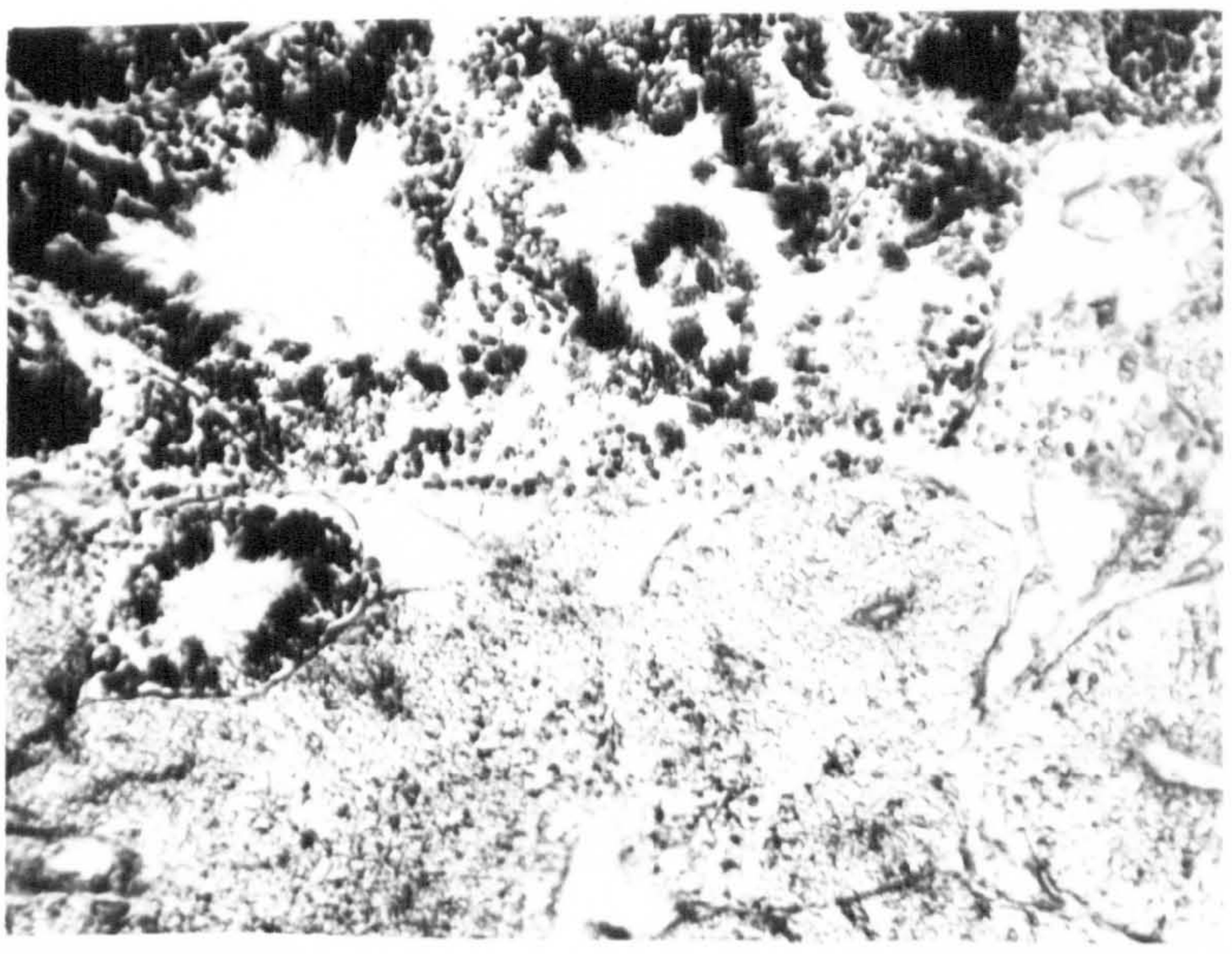


Fig. 100

PLATE 38

Fig. 101 Photograph of site A, the rock shelf fronting the castle ruins at St. Andrews.

Fig. 102 Photograph of site A taken from 'X' on fig. 101.

Fig. 101

Fig. 102

PLATE 39

Fig. 103 Photograph of the rock shelves, with the pier in the background, taken from the castle sands. These rocks are the type which afford a sheltered environment for Nucella lapillus (L.).

Fig. 104 Photograph of the rocks at Fife Ness. These rocks are the type which afford an exposed environment for Nucella lapillus (L.).

Fig. 103

Fig. 104

PLATE 40

Fig. 105 Map of part of the east coastline of Scotland showing the localities from which specimens of Nucella lapillus (L.) were taken for examination. Asterisks indicate localities where Nucellicola kilrymontis gen. et sp. nov. were recorded.

SCOTLAND

EAST AND SOUTH-EAST COASTLINE

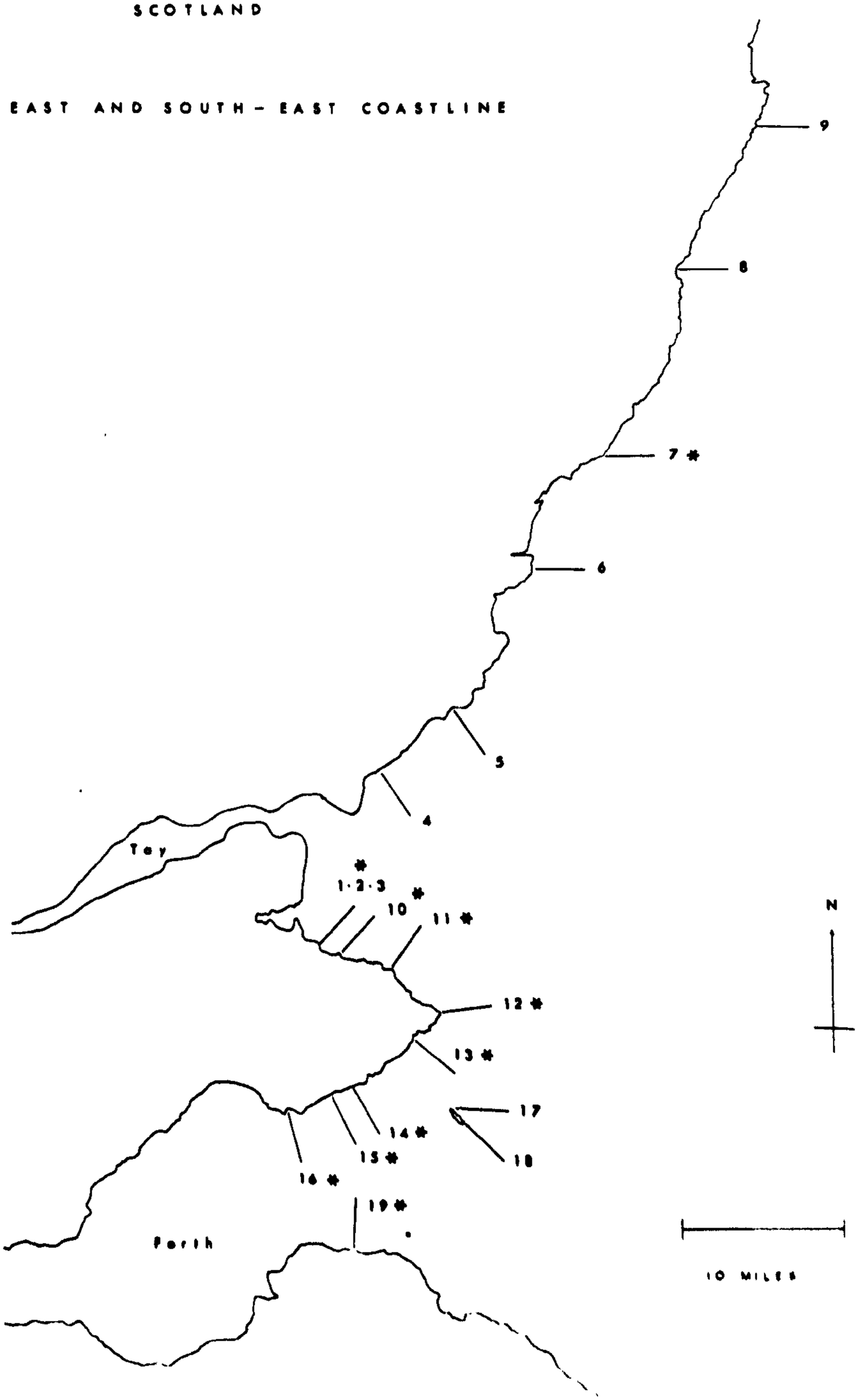


Fig. 105