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The Comparative Morphology of the Female Gonads of Two Members of the Nematode Order Tylenchida

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THE COMPARATIVE MORPHOLOGY OF THE FEMALE GONADS OF TWO MEMBERS OF THE NEMATODE ORDER TYLENCHIDA

(TITLE)

BY

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Presented by

OWEN GLENN COKER

a candidate for the degree of Master of Science in Zoology and hereby certify that in their opinion it is acceptable

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INTRODUCTION

The detailed anatomy of the female gonads has been described for only a few species of nematodes. Most of what is known relates to large animal parasitic forms such as *Ascaris lumbricoides* Linnaeus, 1758, *Necator americanus* Stiler, 1902, and *Parascaris equorum* Goeze, 1782. Very little information on gonad anatomy is available on the smaller forms. The detailed internal morphological study of these nematodes is difficult in both living and preserved specimens, because the internal organs are compacted in the body cavity, and one internal structure often obscures the other. The digestive tract, which runs the length of the nematode body, and which contains numerous globules and granules, makes *in situ* observation of the internal organs especially difficult.

The detailed cellular structure of the ovary of *Aphelenchus avenae* has been reported by Foreman and Taylor (8). Because a knowledge of the structure of the nematode gonad is of taxonomic value, this study was undertaken to determine whether the features described by Foreman and Taylor were common to any other members of the Order Tylenchida. *Ditylenchus destructor* Thorne, 1945, was chosen for comparison.

LITERATURE REVIEW

The general morphology and anatomy of the female gonad has been described by Chandler (5), Chitwood and Allen (6), Hirschmann (11), Hyman (13), Jenkins and Taylor (14), Pennak (20), Storer and Usinger (22), and Thorne (24). More specific detailed anatomical descriptions of the female gonad have been provided by Bastian (2), Chitwood and Chitwood (7), Looss (15), Mackin (16), Maupas (17), Pai (19), Seurat (21), Wu (25), Hirschmann (12), and Foreman and Taylor (8).

Hirschmann (11) stated that the typical female reproductive system consisted of six regions: vulva, vagina, ovary, oviduct, uterus, and post-vulvar uterine sac.

Pennak described the female reproductive system as consisting of a vulva, vagina, uterus, oviduct, ovary and seminal receptacle. Jenkins and Taylor stated that the female gonad consisted of a vulva, vagina, uterus, oviduct, ovary and spermatheca.

Chitwood and Chitwood describes the ovary as a tubular sac-like organ enclosed by epithelium in which the germinal cells develop. The ovary is divisible into two regions: a germinal region, or region of new cell initiation and division, and a growth region, or a region of development and maturation. At the germinal end of the ovary is a cap cell, the function

of which is still unclear. Several workers (7, 10, 17, 19) consider the cap cell to be a part of the ovarial epithelium. Musso (18) and Jenkins and Taylor consider the cap cell to be an undifferentiated germinal stem which gives rise to both germinal and epithelial cells. Hyman stated that the female reproductive system consisted of a vagina, vulva, uterus, ovary, seminal receptacle and oviduct which is formed from ovarial epithelium that continues from the ovary. Chitwood and Chitwood, Hirschmann, and Jenkins and Taylor describe the oviduct as a constricted thick-walled region of columnar epithelial cells that conducts eggs from the ovary to the uterus. Jenkins and Taylor suggested that the oviduct is a functional entity rather than a morphological one.

Following the oviduct is the uterus, which Caveness (4) defined as that portion of the oviduct modified to function as a place of development and maturation of the ova. Hirschmann, Hyman, Jenkins and Taylor stated that the function of the uterus is for storage of eggs, fertilization, shell formation, and some degree of embryonic development as the eggs pass along it. Wu, working with Ditylenchus destructor Thorne, 1945, divided the uterus into three separate regions: the seminal receptacle, that may or may not contain spermatozoa, the quadricolumella, and the uterus proper. Wu temporarily aseigned the term quadricolumella to the structure having four rows of cells with a highly glandular appearance which she suggested was secretory in function. Distally the uterus enters a common muscularized, cuticularized tube, the vagina, which is quite short and opens to the exterior by means of a midventral transverse slit, the vulva.

Goodey (9) described the reproductive system of Aphelenchus avenae Bastian, 1865, as consisting of a posterior vulva, a prodelphic outstretched ovary, post vulval sac, and a vagina with thickened walls, sloping forward from the vulva. Hechler (10) described the gonad of A. avenae as consisting of a single, anteriorly outstretched ovary that may be reflexed in well fed specimens, and containing oocytes in a single or double row. The vagina is composed of a thick wall, and an oval shaped vulva. The postvulvar uterine branch is wide near the vulva with a narrow appendage reaching to one-half the distance from the vulva to the anus.

Thorne described the gonad of D. destructor as being anterior and outstretched near the base of the esophagus with developing oocytes arranged in two lines, changing to tandem near the middle. The posterior uterine branch is rudimentary, and it has not been observed to function as a spermatheca.

Foreman and Taylor showed the ovary of A. avenae to consist of an epithelial layer composed of two rows of cells which form a tube enclosing a single row of oocytes.

MATERIALS AND METHODS

Aphelenchus avenae and Ditylenchus destructor were obtained from the Division of Nematology, Department of Plant Pathology, University of Illinois at Urbana, Illinois. These two species of nematodes were propagated on a fungus, Pyrenochaeta terrestris (Hansen) Gorenz, Walker, and Larsen, and maintained at room temperature on full strength potato dextrose agar in petri dishes.

The nematodes studied were extracted from seven-to ten-day old cultures. The extraction procedure and slide preparation were similar for both species. The cultures were rinsed with ten milliliters of distilled water and decanted to embryological watch glasses. Ten nematodes of each species were transferred with a picking needle under a binocular microscope to a drop of water on a slide. Separate slides containing nematodes of both species were heat-relaxed by passing the slides several times through the flame of an alcohol lamp. This served to immobolize the nematodes, but not to kill them. Overheating caused shrinkage of the nematodes. Nematodes of both species were transferred from the slides of distilled water to slides containing a drop of five percent formalin. So as to make observation easy, the specimens were aligned in the center of a drop of formalin so that their heads would be pointed in the same direction. Small glass rods of approximately the same diameter of the nematodes were placed in the drop of

formalin to prevent crushing of the nematodes when a cover glass was placed on the slide. After removing excess formalin, the cover glass was sealed with Zut, a microslide-coverglass adhesive, composed of two parts nitrocelluloee solution and one part ADM-100, a polymerized linseed oil product (23).

In order to prepare nematodes for sectioning, they were placed in small watch glasses containing a fixing solution composed of commercial formalin, fifty percent alcohol, glycerine, acetic acid and a trace of osmic acid (FAAGO). Dishes containing nematodes of both species were placed in an oven for twenty-four hours at 55 degrees Centigrade. The nematodes were then transferred to Baker Solutions (1) and processed to pure glycerine. Free hand sections of both species were made in the region of the ovary with a cataract knife. Sections were mounted for observation in glycerine jelly.

RESULTS

I. Aphelenchus avenae

Examination of mounted intact specimens showed the ovary to consist of three distinct regions (Fig. 1). Cross sections showed these three regions to be two rows of epithelial cells enclosing a single row of oocytes (Figs. 2-4). The exact relationship of the two layers of epithelial cells could not be determined. It is believed that the cells of both layers meet to form an envelope enclosing the oocytes. No attempt was made to locate and study the ovarial cap cell; nor was any attempt made to study the quadricolumella, although this structure was observed in some mounted intact nematodes.

II. Ditylenchus destructor

Observations of intact mounted specimens in the region of the ovary showed the oocytes arranged both in pairs and in tandem (Figs. 5-6). Cross sections showed the oocytes to be enclosed by a noncellular sheath (Fig. 7). Cross sections also revealed the arrangement of the oocytes within the sheath. Figures 7 and 8 both show a single oocyte enclosed by the sheath, and the paired arrangement of the oocytes can be observed in Figs. 9 and 10. The oocytes which were observed in pairs were seen in

cross sections of nematodes taken from the ovarial region located immediately below the esophagus. Since Ditylenchus destructor has a prodelphic outstretched ovary, this indicates that the immature oocytes are arranged in pairs, and that the more mature oocytes are in a single row in that part of the ovary nearest the vulva.

Figure 1. Lateral view of Aphelenchus avenae showing a section of the ovary. Ep. C., epithelial cell; Ooc., oocyte.

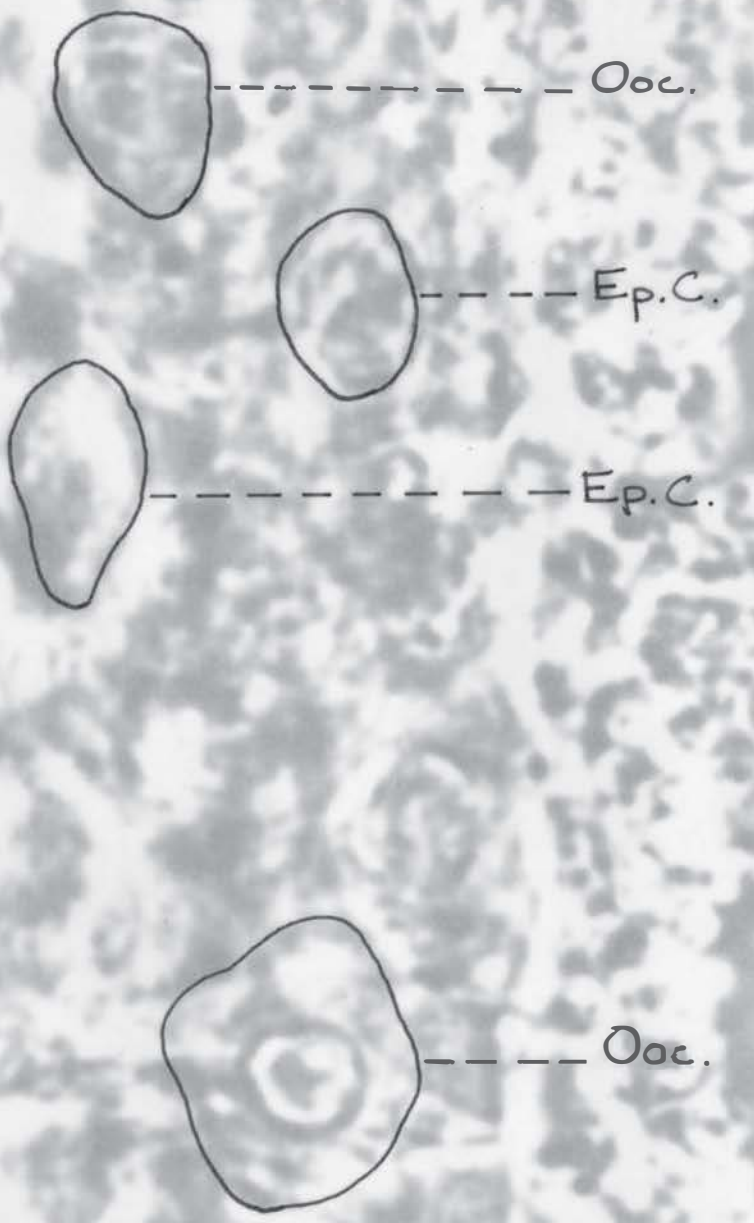
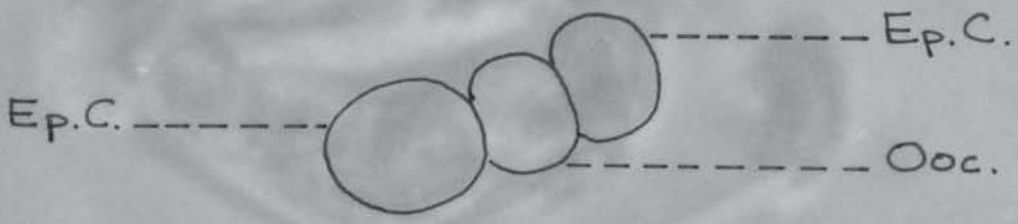

Ooc.
Ep.C.
Ep.C.
Ooc.

Figure 2. Cross section of Aphelenchus avenae through the ovary. Ep. C., epithelial cell; Ooc., oocyte.


Ep.C.
Ep.C.
Ooc.

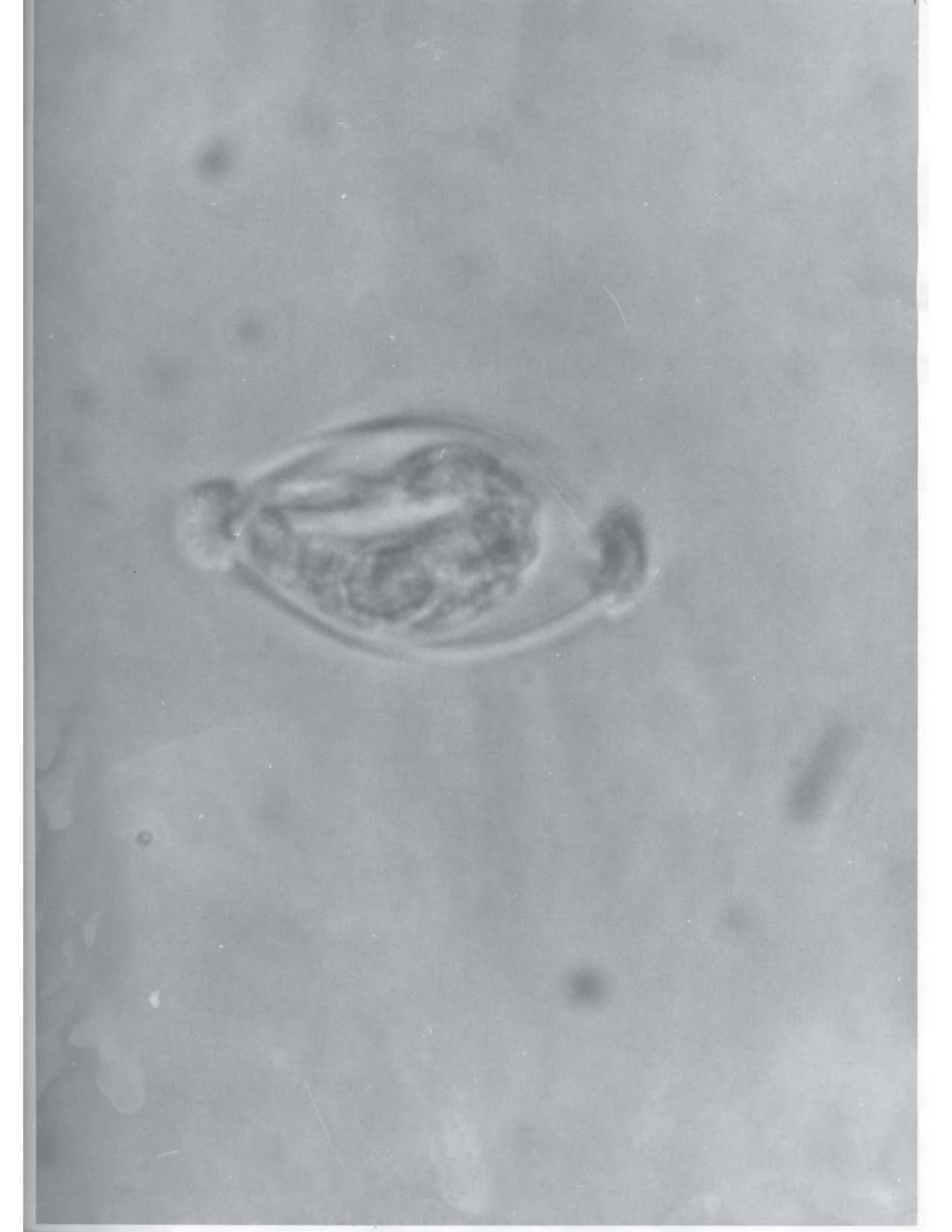
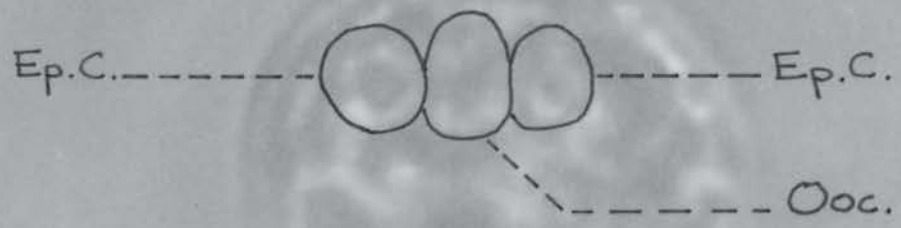


Figure 3. Cross section of Aphelenchus avenae through the ovary. Ep. C., epithelial cell; Ooc., oocyte.


Ep.C.
Ep.C.
Ooc.

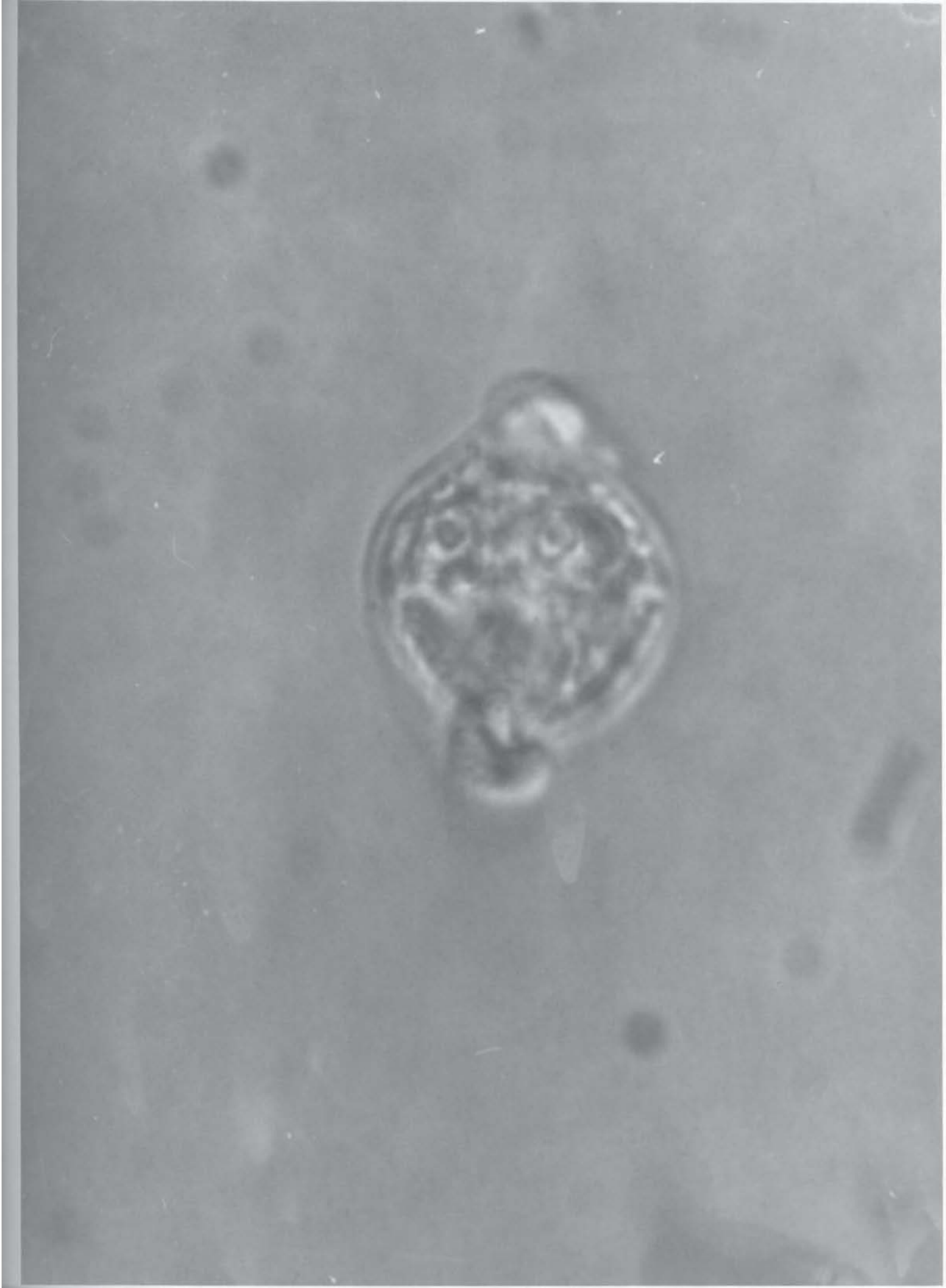


Figure 4. Cross section of Aphelenchus avenae through the ovary. Ep. C., epithelial cell; Ooc., oocyte.

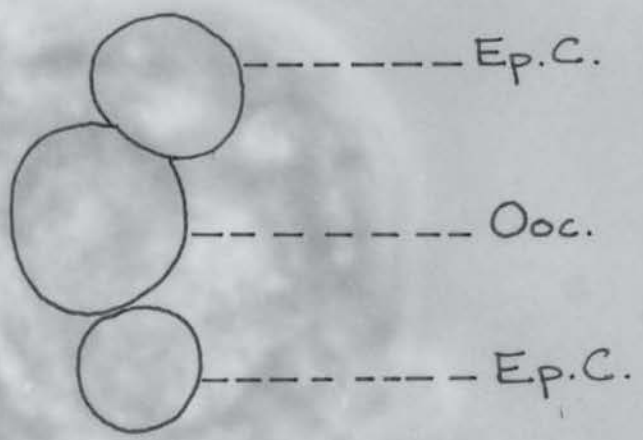
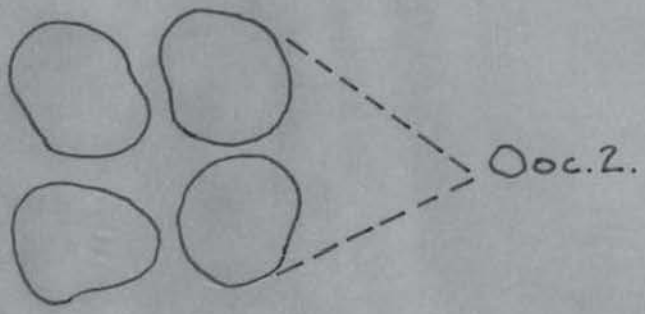
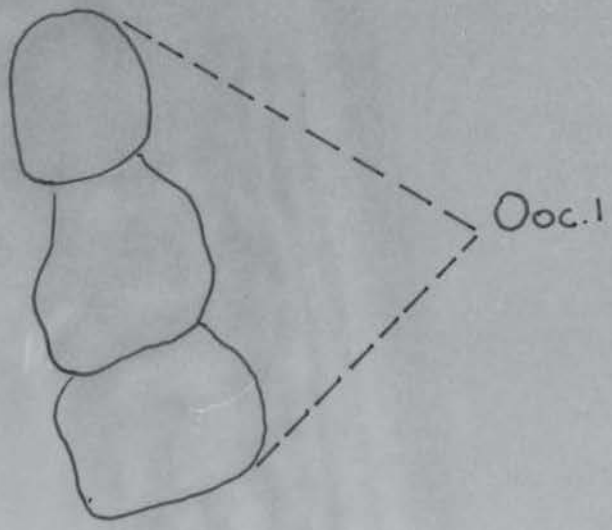

Ep.C.
Ooc.
Ep.C.

Figure 5. Lateral view of Ditylenchus destructor showing arrangement of oocytes in the ovary. Ooc. 1., oocytes in tandem; Ooc. 2., oocytes in pairs.


Ooc.2.


Ooc.1

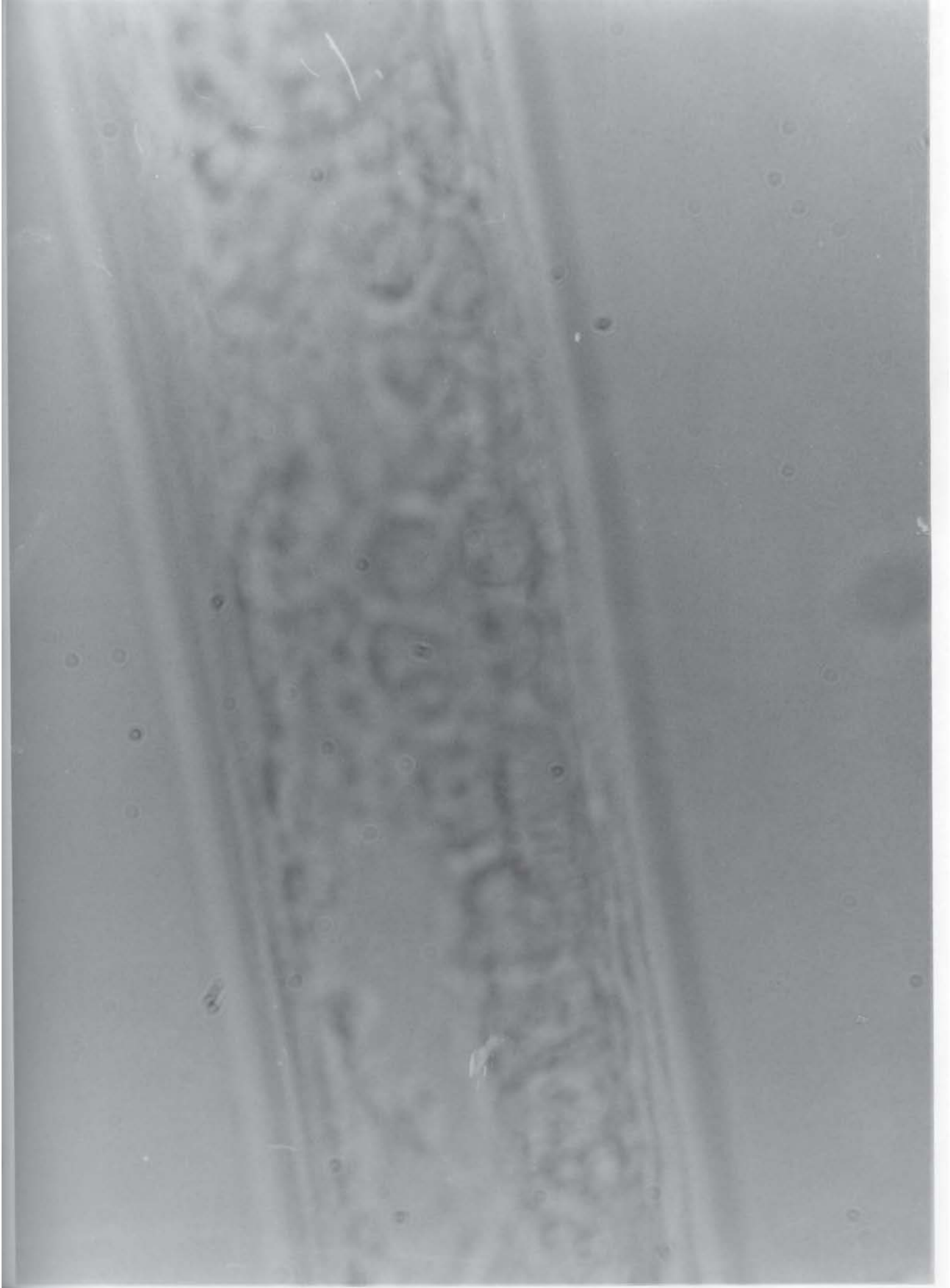
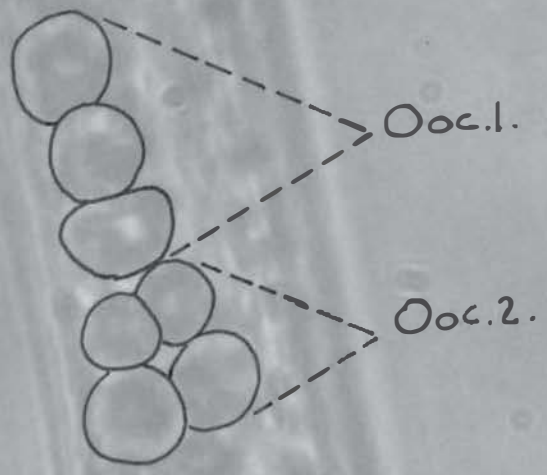


Figure 6. Lateral view of Ditylenchus destructor showing arrangement of oocytes in the ovary. Ooc. 1., oocytes in tandem; Ooc. 2., oocytes in pairs.


Ooc.1.
Ooc.2.

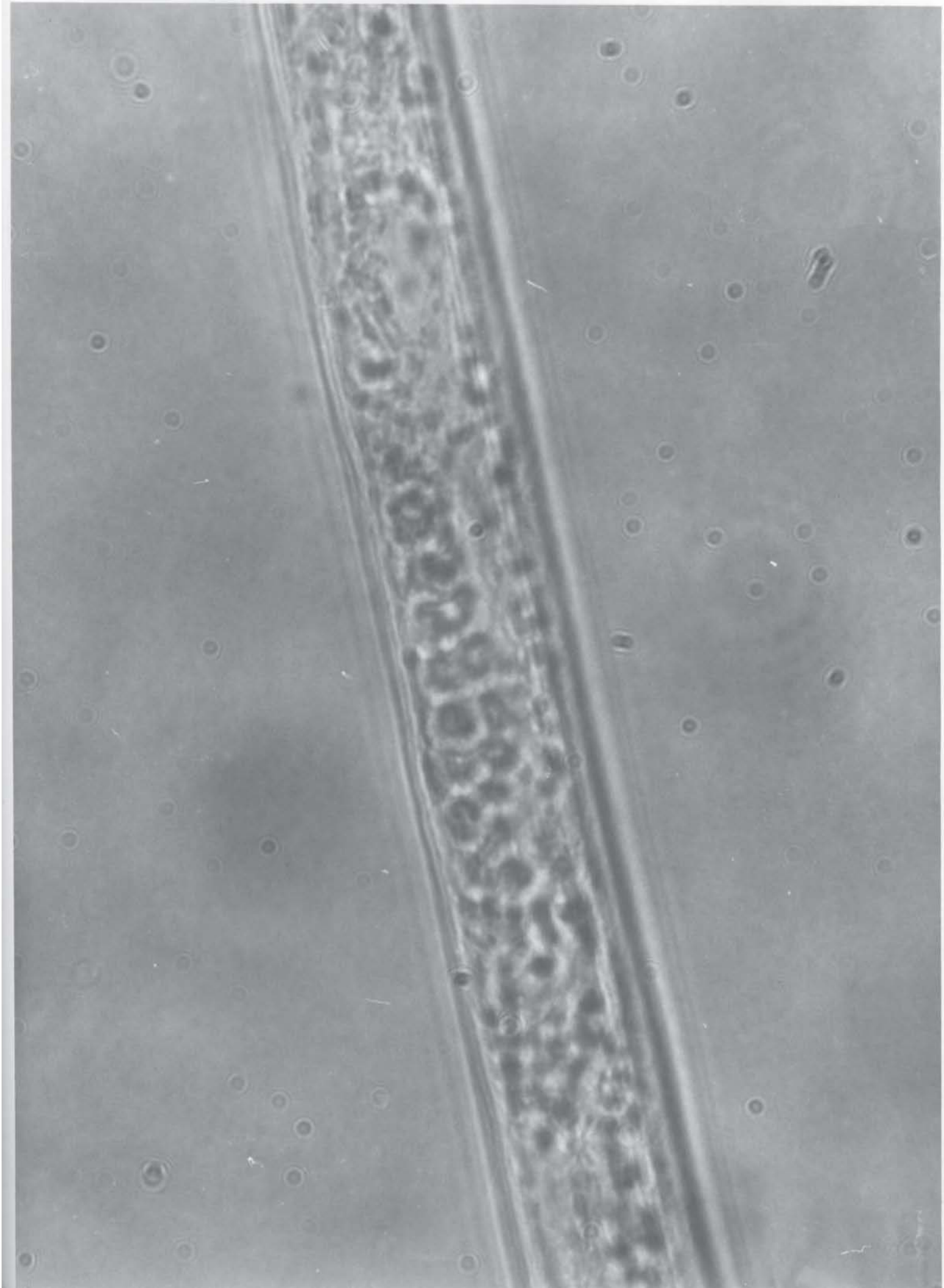
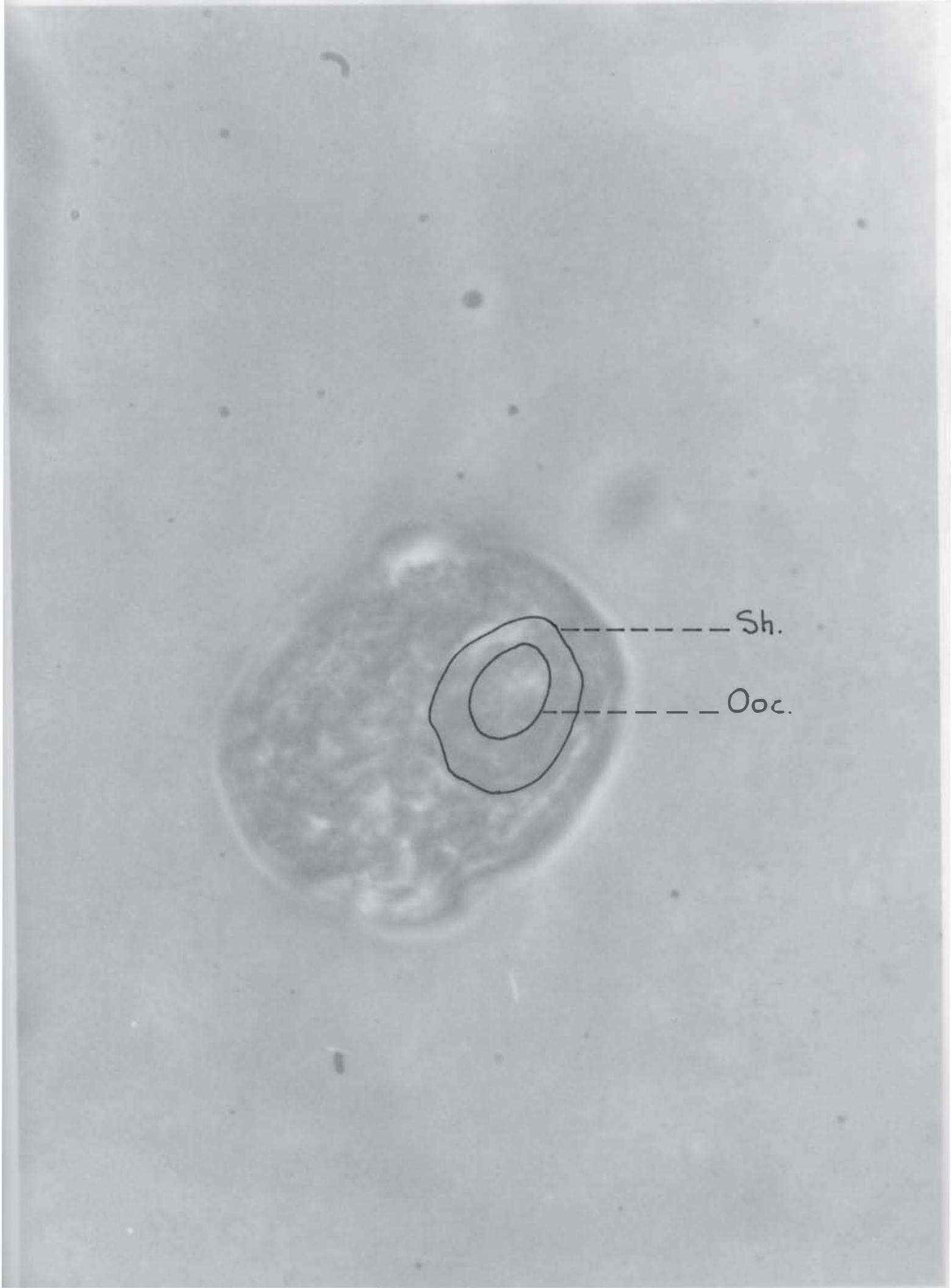


Figure 7. Cross section of Ditylenchus destructor through the ovary showing the noncellular sheath enclosing an oocyte. Sh., sheath; ooc., oocyte.


Sh.
Ooc.

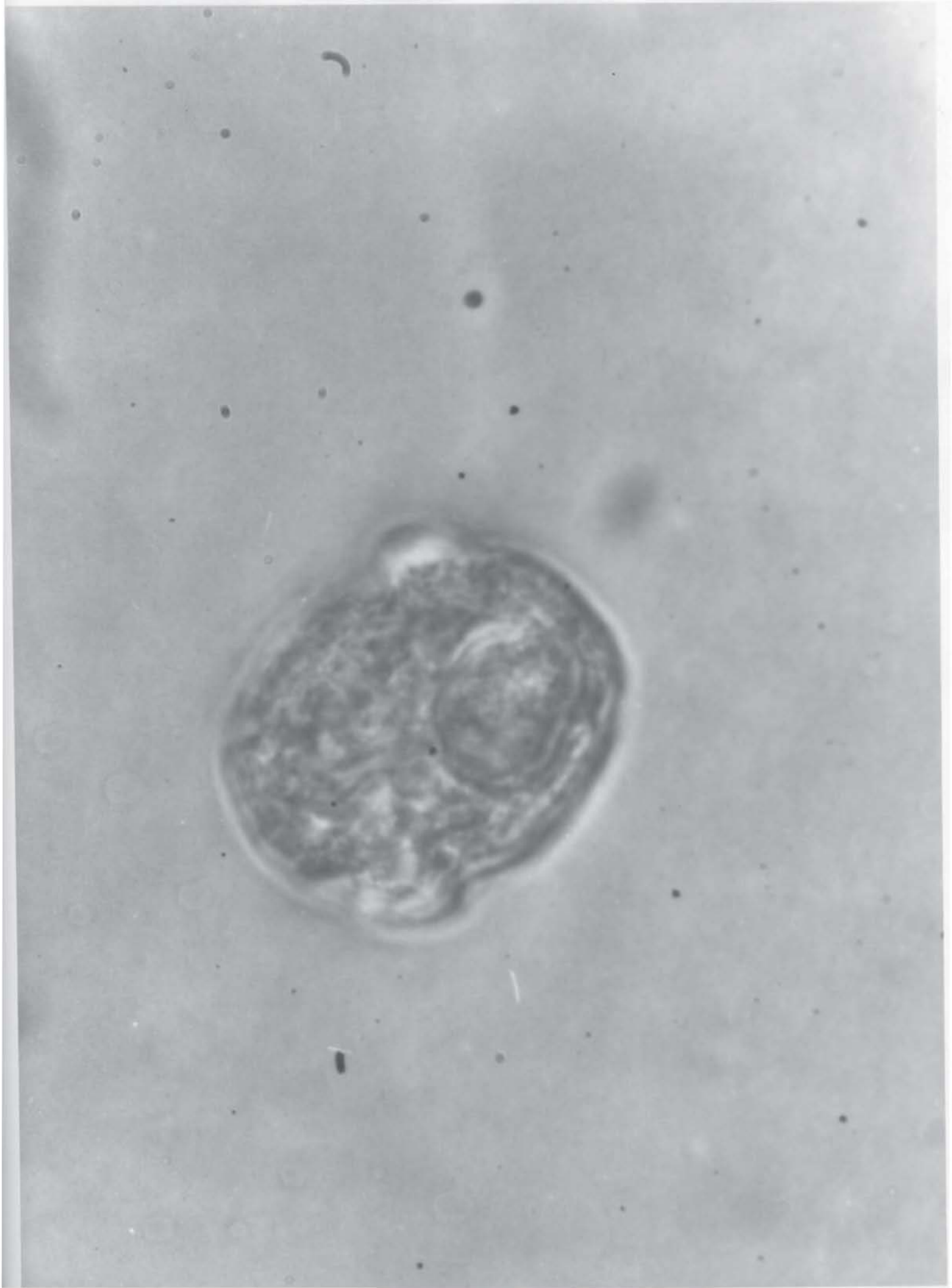


Figure 8. Cross section of Ditylenchus destructor through the ovary showing a single oocyte enclosed by the sheath. Sh., sheath; Ooc., oocyte.

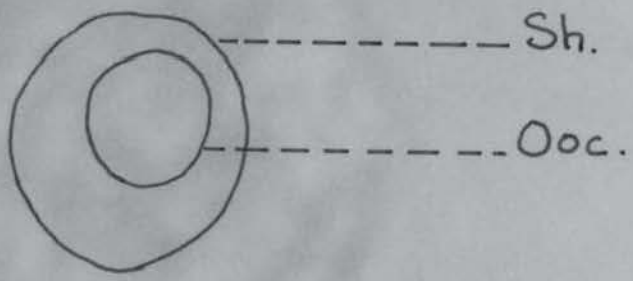
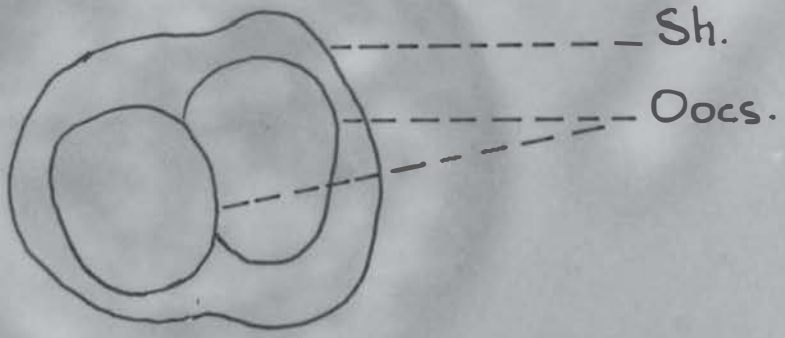

Sh.
Ooc.

Figure 9. Cross section of Ditylenchus destructor through the ovary showing two oocytes enclosed by the sheath. Sh., sheath; Oocs., oocytes.


Sh.
Oocs.

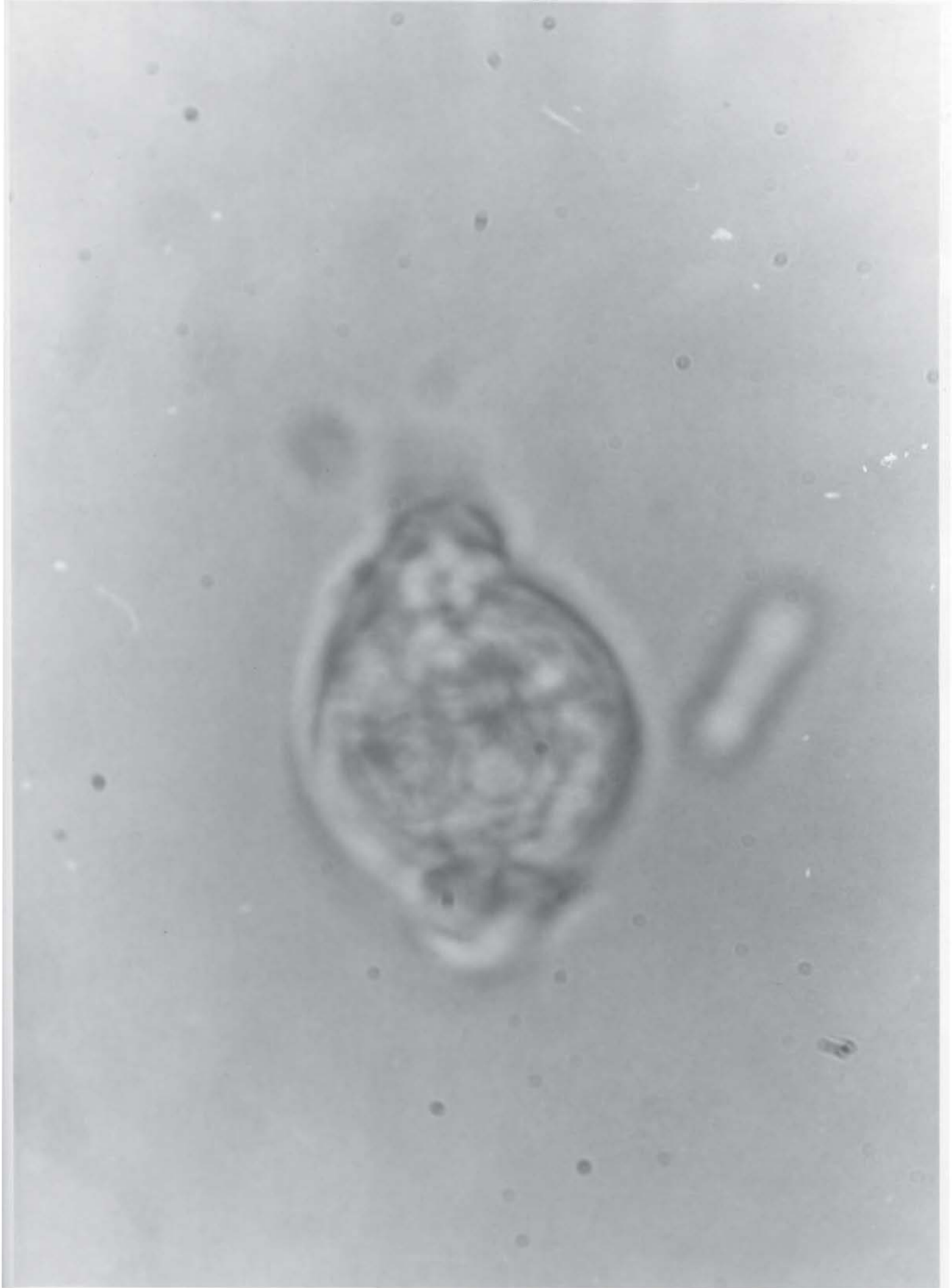
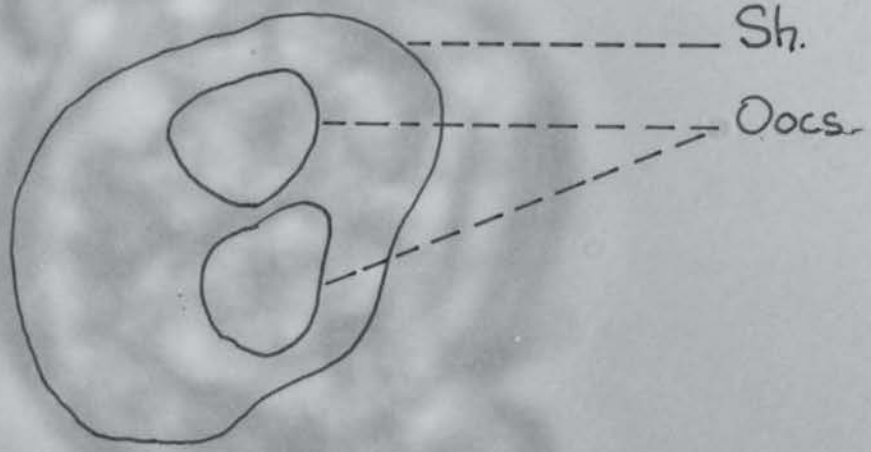
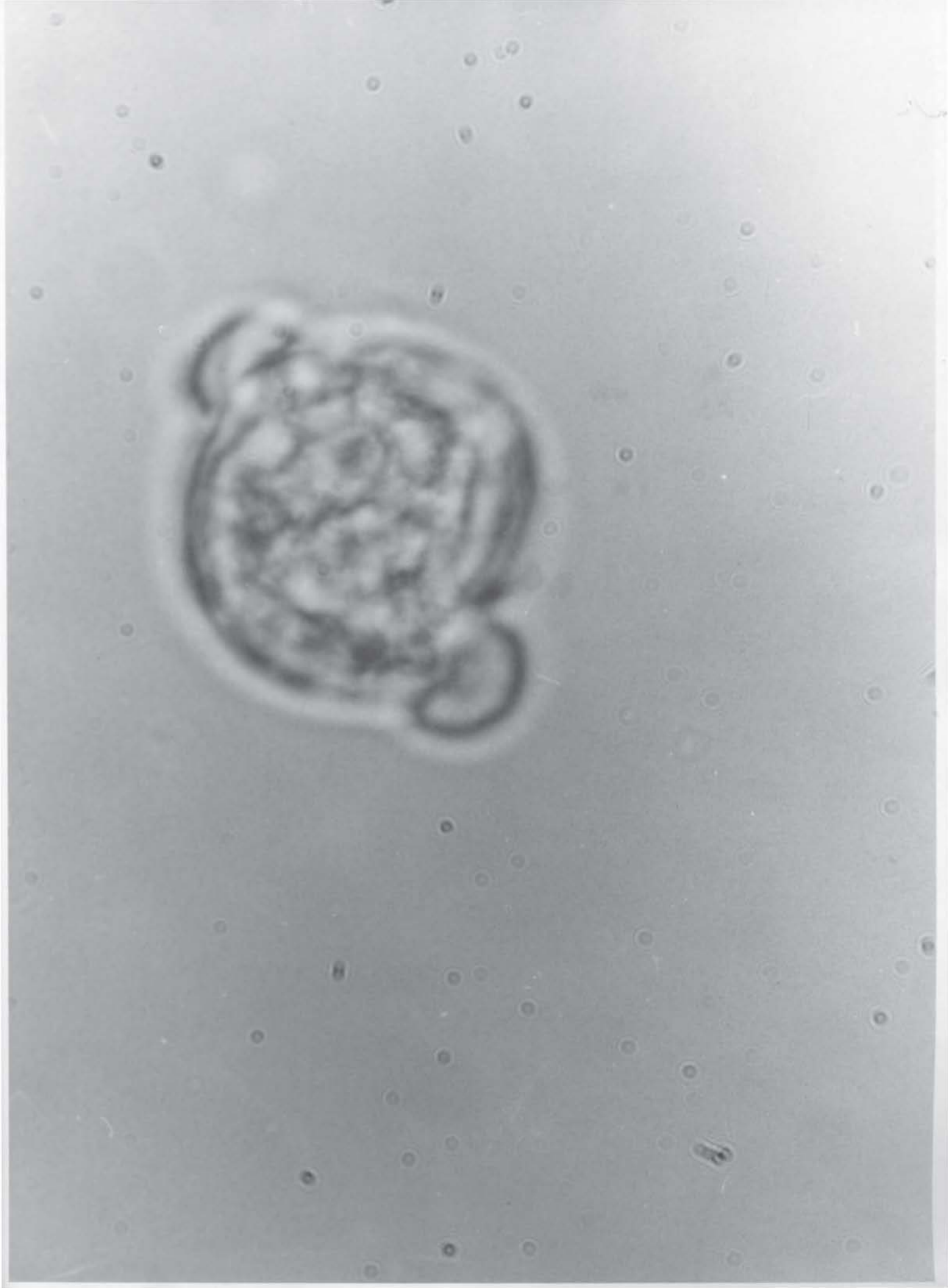


Figure 10. Cross section of Ditylenchus destructor showing two oocytes enclosed by the sheath. Sh., sheath; Oocs., oocytes.


Sh.
Oocs.



DISCUSSION

These investigations disclosed some features of nematode gonads which may be of significance in taxonomic studies of the Order Tylenchida. These studies provided evidence supporting the descriptions in the literature of the gonad of Aphelenchus avenae and Ditylenchus destructor. This work also disclosed, a previously undescribed feature, in which the oocytes are enclosed in Ditylenchus destructor.

The presence of a distinct cellular epithelial layer surrounding the oocytes as observed in Aphelenchus avenae is not a consistent feature of the Order Tylenchida, since this was not observed in Ditylenchus destructor. Neither is the arrangement of the oocytes similar in both species. Whereas the appearance of the oocytes in pairs indicated immaturity in Ditylenchus destructor, the age of the oocytes in Aphelenchus avenae could be approximated only by size and position along the ovarial tube.

The sheath surrounding the oocytes in Ditylenchus destructor was referred to as being noncellular, because cross sections showed no cells in this covering similar to that observed in Aphelenchus avenae. The precise structure of this sheath could possibly be determined by more refined sectioning techniques in conjunction with suitable biological stains.

The marked differences in the structures which enclosed the oocytes in both species is believed to be significant enough to warrant further study, and to justify their consideration as criteria in species delineation in the Order Tylenchida.

SUMMARY

Aphelenchus avenae Bastian, 1865, and Ditylenchus destructor Thorne, 1945, both members of the nematode Order Tylenchida, were chosen for a comparative morphological study of the female gonads. The detailed cellular structure of the ovary of both species was determined.

Both species of nematodes were cultured on a fungus, Pyrenochaeta terrestris (Hansen), Gorenz, Walker and Larsen, and maintained on full strength potato dextrose agar. Whole specimens were observed, as well as free-hand cross sections which were mounted in glycerin jelly for observation.

Observations of Aphelenchus avenae revealed a three layered appearance of the ovary. These three layers were determined to be two layers of epithelia enclosing a single row of oocytes.

The ovary of Ditylenchus destructor was observed to consist of a non-cellular sheath which enclosed oocytes arranged both singly and paired depending on their position in the ovarial tube. The non-cellular sheath enclosing the oocytes in this species had been previously undescribed.

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