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PRELIMINARY STUDIES ON THE ULTRASTRUCTURE  
OF THE FEMALE GONAD OF *XIPHINEMA INDEX*  
AND *X. MEDITERRANEUM* (NEMATODA: LONGIDORIDAE)

by

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Published information on the ultrastructure of the female gonads of nematodes is concerned either with animal parasites (Dick and Wright, 1973) or with members of the order Tylenchida (Yuen, 1971; Kisiel *et al.*, 1972) and Rhabditida (Epstein *et al.*, 1971).

We have investigated the ultrastructure of the female genital tract of two species of Longidoridae, *Xiphinema index* Thorne *et* Allen and *X. mediterraneum* Martelli *et* Lamberti. This group of plant parasitic nematodes has recently been shown of considerable economic importance and has excited the interest of many plant pathologists and agricultural nematologists.

*Materials and methods*

*Xiphinema index* was reared on grapevine (*Vitis vinifera* L.) and *X. mediterraneum* on Olive (*Olea europaea* L.) grown in sandy loam, in large pots, kept in a screenhouse.

Twenty females of each species were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. Each specimen was cut at the level of the oesophagus to facilitate the penetration of the fixative into the tissues. Fixation was achieved in two hours at 4°C. After overnight rinsing in cacodylate buffer at 4°C, the nematodes were post-fixed for two hours in 1% osmium tetroxide in veronal acetate buffered at pH 7.4 and containing 5% of sucrose. The specimens

were then carefully rinsed in veronal acetate and embedded in agar using the procedure of Wright and Jones (1965). The agar blocks containing the nematodes were again immersed in the buffer and kept under vacuum for five minutes. They were then dehydrated in the ethanol series and, after two passages through propylene oxide were embedded in « Araldite ».

Transverse sections were cut with LKB Ultratome III at a thickness of approximately 2  $\mu\text{m}$  (« semithin ») or 200 nm (« ultrathin »). Semithin sections were stained with toluidine blue and observed under a light microscope; ultrathin sections were treated with 5% uranyl acetate in water for one hour and lead citrate for 15 minutes (Reynolds, 1963) and observed in an Hitachi HU11B electron microscope at magnification up to  $\times 25,000$ .

### Results

The female gonad of *X. index* or *X. mediterraneum* occupies a large portion of the coelomic cavity (Bleve-Zacheo *et al.*, 1975). It is enveloped by a membrane embedded in connective tissue which appears as an amorphous layer constituted of mucopolysaccharides. This tissue is closely connected with cellular membranes of the surrounding organs. When the gonad has completed its development (this is when mature oocytes are present in the lumen of the oviduct) its volume is considerably increased and the surrounding tissues are compressed.

No fundamental differences have been observed between the structure of the gonads in the two species and therefore the description given below applies to both.

#### a) Ovary

The ovary comprises a short apical germinal zone followed by a more elongate growth zone. It is kept *in situ* by longitudinal and transverse muscular bundles (Fig. 2). The cells of the ovarian wall have little cytoplasm but a large vesiculous nucleus (Fig. 3). However, we have sometimes observed in these cells secretory granules and vacuoles with glycogen. Numerous oocytes are present in the germinal zone of the ovary (Fig. 1a). The cytoplasm of the oocytes is rich in organelles, especially ribosomes and mitochondria. Each

has a large nucleus with ribosomes associated with the external membrane.

In the growth area of the ovary, only two oocytes are present (Fig. 4 and 1b). The nucleus and nucleolus are well differentiated and the cytoplasm is again rich in ribosomes and mitochondria.

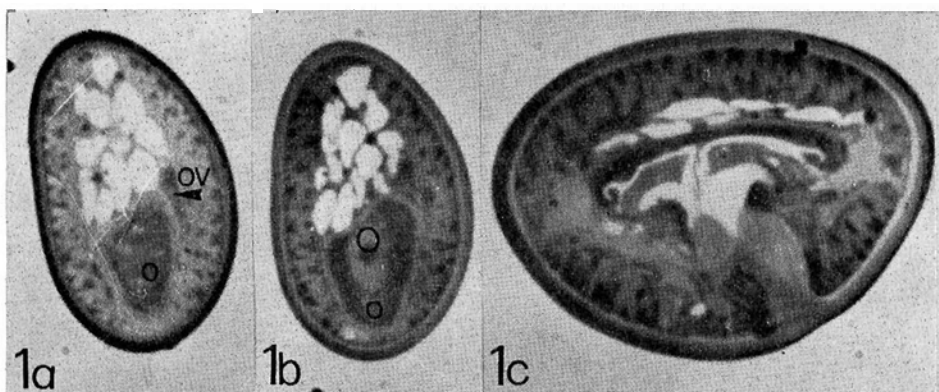


Fig. 1 - Cross sections of *Xiphinema index*: a) ovary with oocytes (o) and oviduct (ov); b) ovary (o) with a mature oocyte (O); c) vagina near the vulva. (x 750)

#### b) Oviduct

The tube-like oviduct is formed from a series of spindle-shaped uninucleate columnar cells (Fig. 4), and like the ovary, is enveloped by a membrane. The lumen is delimited by an L-shaped membrane thinner than the external one (Fig. 4). The cell nuclei are very large and invariably located at the periphery of the cell. Many secretory granules are present in the cytoplasm and the rough endoplasmic reticulum is very abundant. Structures resembling those observed by Yuen (1971) are present; they are considered to be glands secreting a fluid to lubricate the oviduct.

#### c) Sac of the oviduct

The sac of the oviduct also is enveloped in an amorphous structure (coeloma) which allows the expansion of the many folded tissues of which the sac is constituted, at the passage of the oocyte. This membrane, which is similar to that observed around ovary and oviduct, envelops all the invaginations of the sac (Fig. 5). The

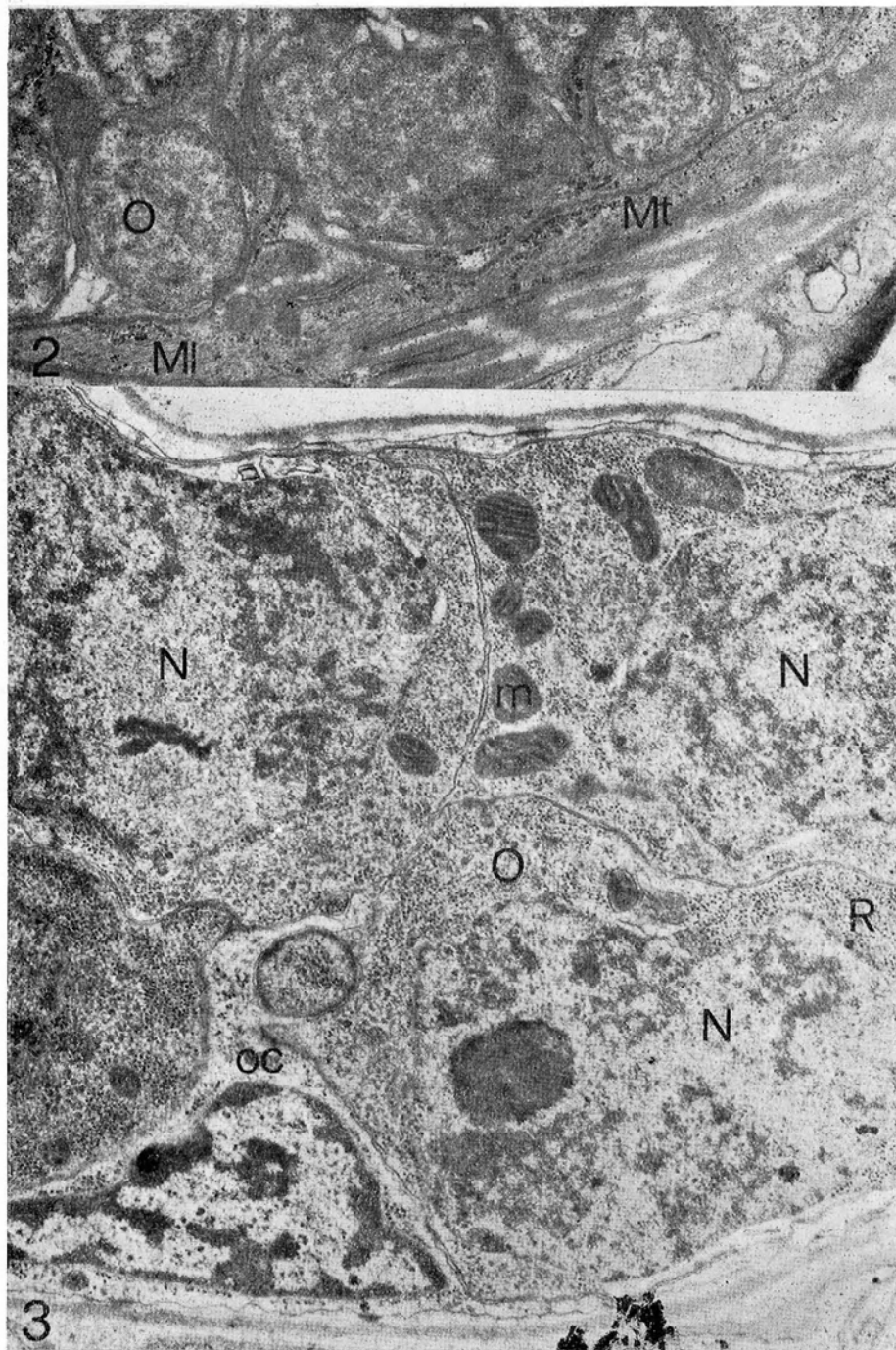


Fig. 2 - Cross section of the distal portion of an ovary of *X. mediterraneum*: longitudinal (MI) and transverse (Mt) muscles that keep the ovary *in situ*; O = oocytes. (x 20,000)

Fig. 3 - Cross section of the distal portion of an ovary of *X. index*: oocytes (O); m = mitochondria; N = nucleus; R = ribosomes; oc = ovary cell. (x 21,000)

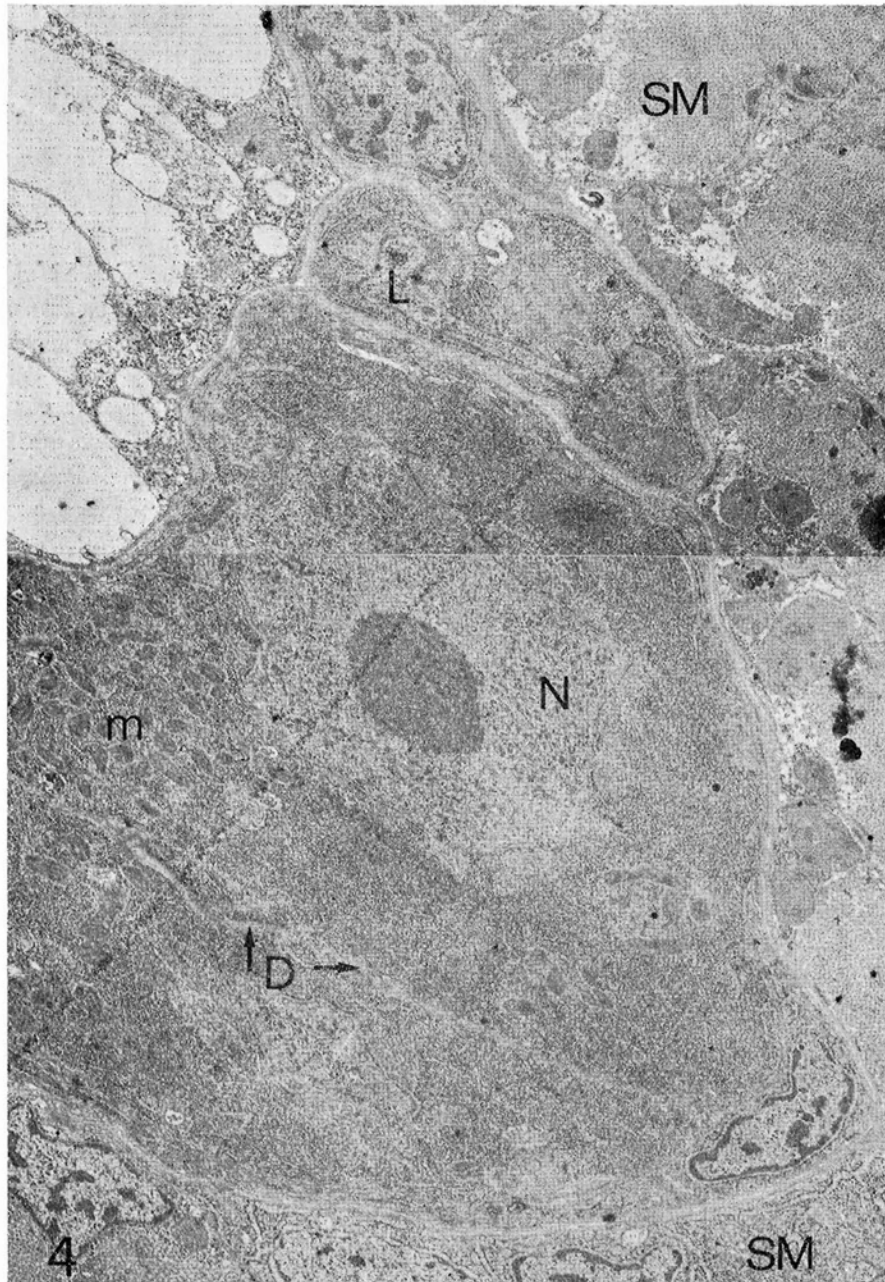


Fig. 4 - Cross section of ovary and oviduct of *X. index*: SM = somatic muscles; D = division between the two oocytes. In the mature oocytes note mitochondria (m), a large nucleus (N) with nucleolus and an ovary cell. L = lumen of the oviduct. (x 13,000)

cells in this region have a single spindle-shaped nucleus and cytoplasm with numerous ribosomes and some mitochondria. The presence of microtubules (neurotubules) enclosed within several lamellae (membranes) indicates nervous tissue (Fig. 5a). Another system of folded membranes associated with vacuoles containing either amorphous material or glycogen was also noted.

#### d) *Sphincter*

The sphincter connecting the oviduct with the uterus is shown clearly in Fig. 6. A sinuous folding of the epithelium allows the expansion of the sphincter when the egg passes through. The sphincter essentially consists of longitudinally and transversely oriented bands of muscles which intersect in a radiate way and appear like wedges toward the centre of the structure. Membranes connecting with this muscular system are probably nervous terminations. The cells still contain a single nucleus, the cytoplasm is sparse; the endoplasmic reticulum is in some places rough and the Golgi complex is present. Secretory granules are visible in the lumen.

#### e) *Uterus*

This region of the gonad is well provided with transverse and longitudinal bands of muscle throughout its length, but especially in the region near the sphincter (Fig. 7). The external envelope is folded. The uterus is constituted by eight cells with a large nucleus, many ribosomes, well developed endoplasmic reticulum but few mitochondria and little glycogen. Well evident is the Golgi apparatus (Fig. 8) consisting of a dense net of alveoli and vacuoles, forming a fenestrated plate approached by tubular structures connected with the endoplasmic reticulum. The cytoplasm contains smooth tubular membranes (Fig. 9) sometimes convoluted, otherwise linear in connection with alveoli containing secretory products. It is possible to observe alveoli with different quantities of material inside. Some of them are evidently being formed, but others are empty because the fluid has been discharged or has not yet been formed. Some alveoli appear reticulated. Globules of fat (Fig. 10) are present in the distal part of the uterus and ampulliform formations perhaps containing calcium can be seen.

The two uteri, innervated by the ventral cord, are connected to each other through the uterine vagina.

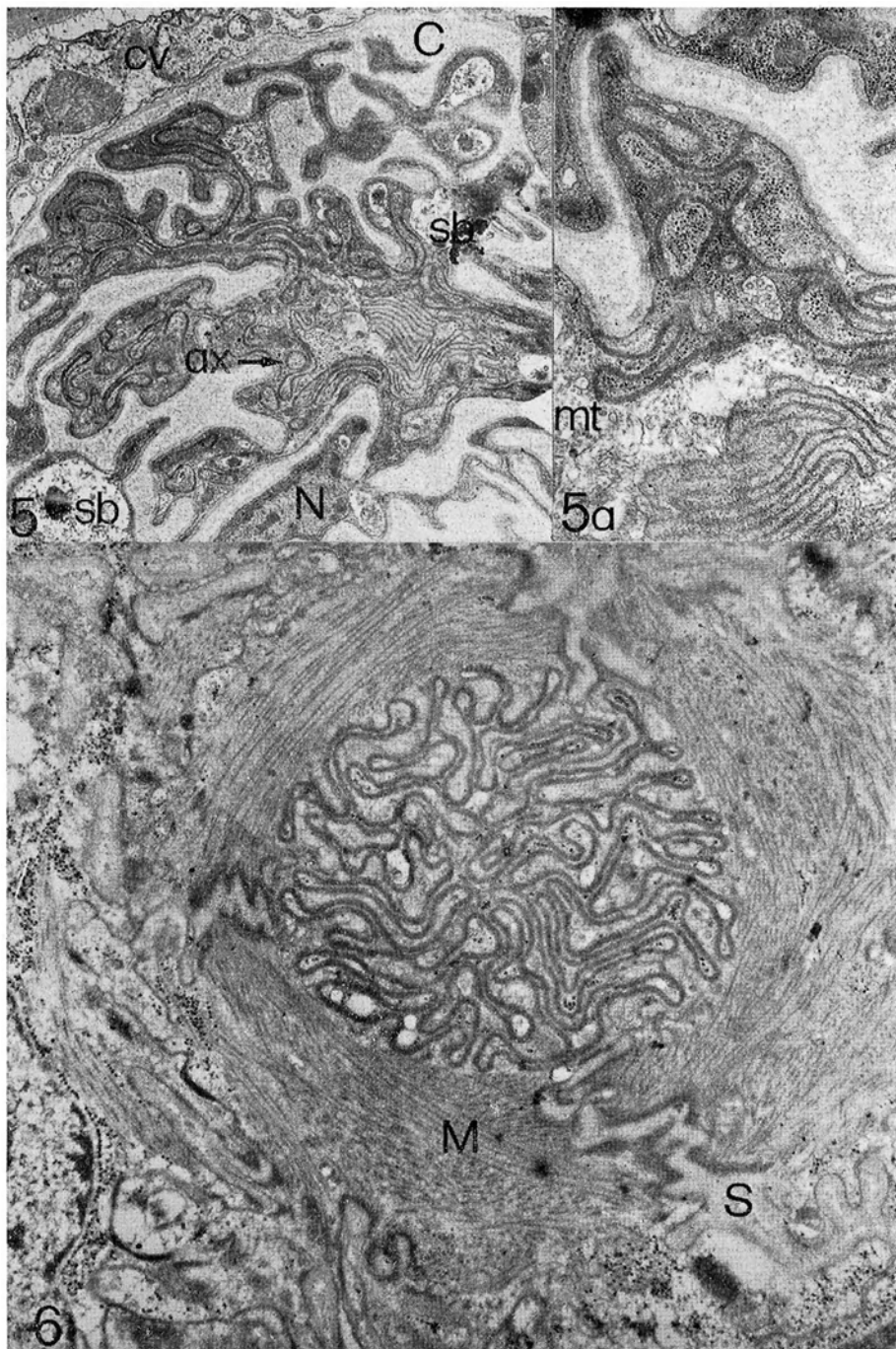


Fig. 5 - Cross section of the sac of the oviduct of *X. index*; C = coelomic cavity; N = nucleus; sb = secretory bodies; cv = ventral cord; ax = axones. (x 9,500)

Fig. 5a - mt = microtubules. (x 19,000)

Fig. 6 - Cross section of the sphincter of *X. mediterraneum*; S = sinuous contour; M = intersection of muscular bundles. (x 25,000)



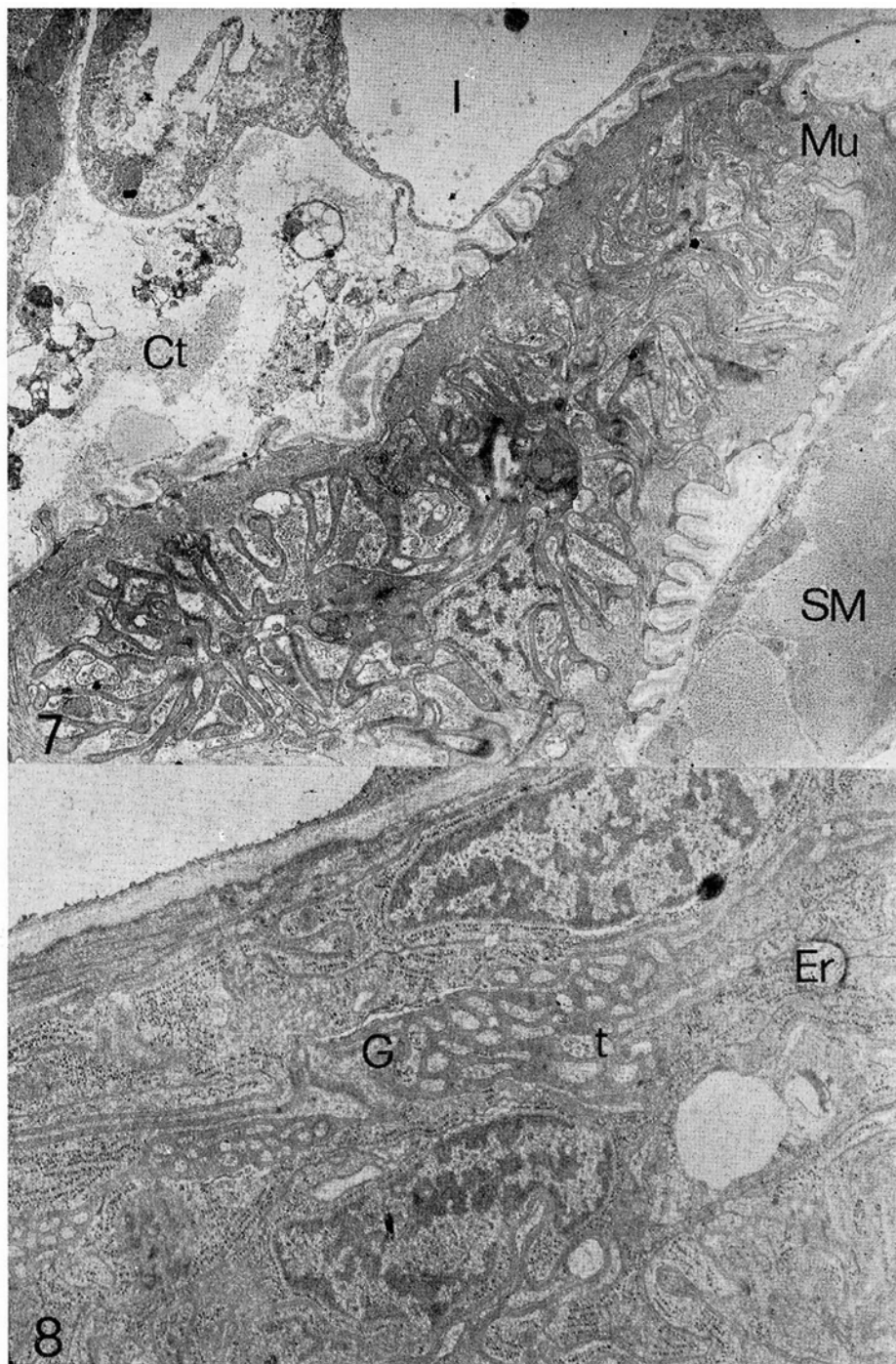


Fig. 7 - Cross section of the uterus of *X. index*: SM = somatic muscles; I = intestine; Ct = coelomocytes; Mu = uterine muscles. (x 12,500)

Fig. 8 - Golgi apparatus with perforated plates approached by tubular structures (t) connected with endoplasmic reticulum (Er). (x 22,000)



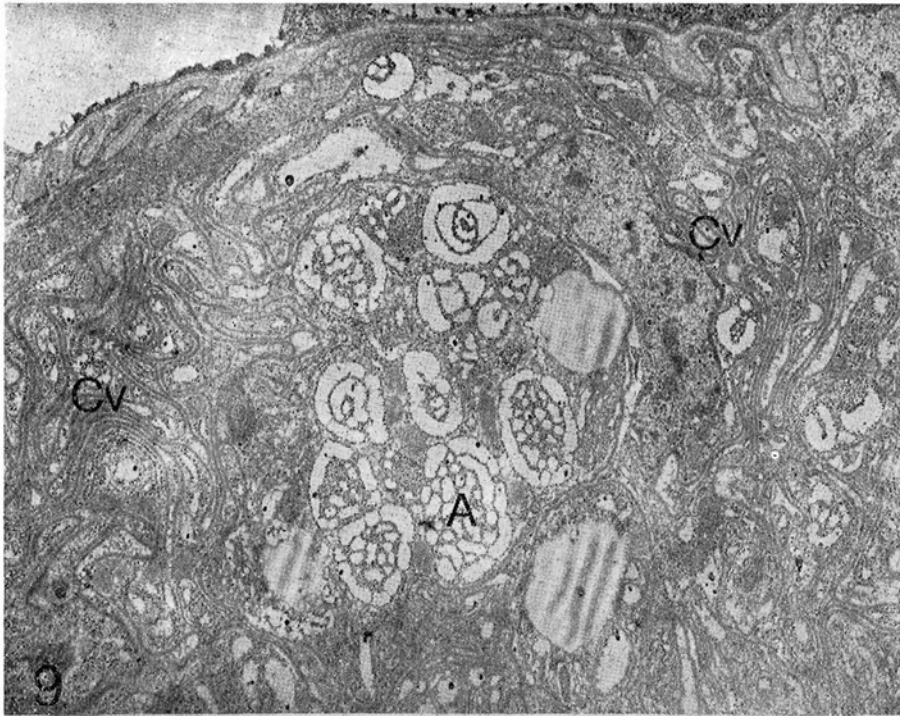


Fig. 9 - Cross section of the uterus of *X. index*: Cv = convoluted membranes; A = reticulated alveoli containing different quantities of secretion products. (x 22,000)

Fig. 10 - Cross section of the uterus of *X. index*: SM = somatic muscles; I = intestine; F = fat bodies; Ca = calcium concretions (?); A = alveoli with protein; G = Golgi apparatus. (x 24,000)

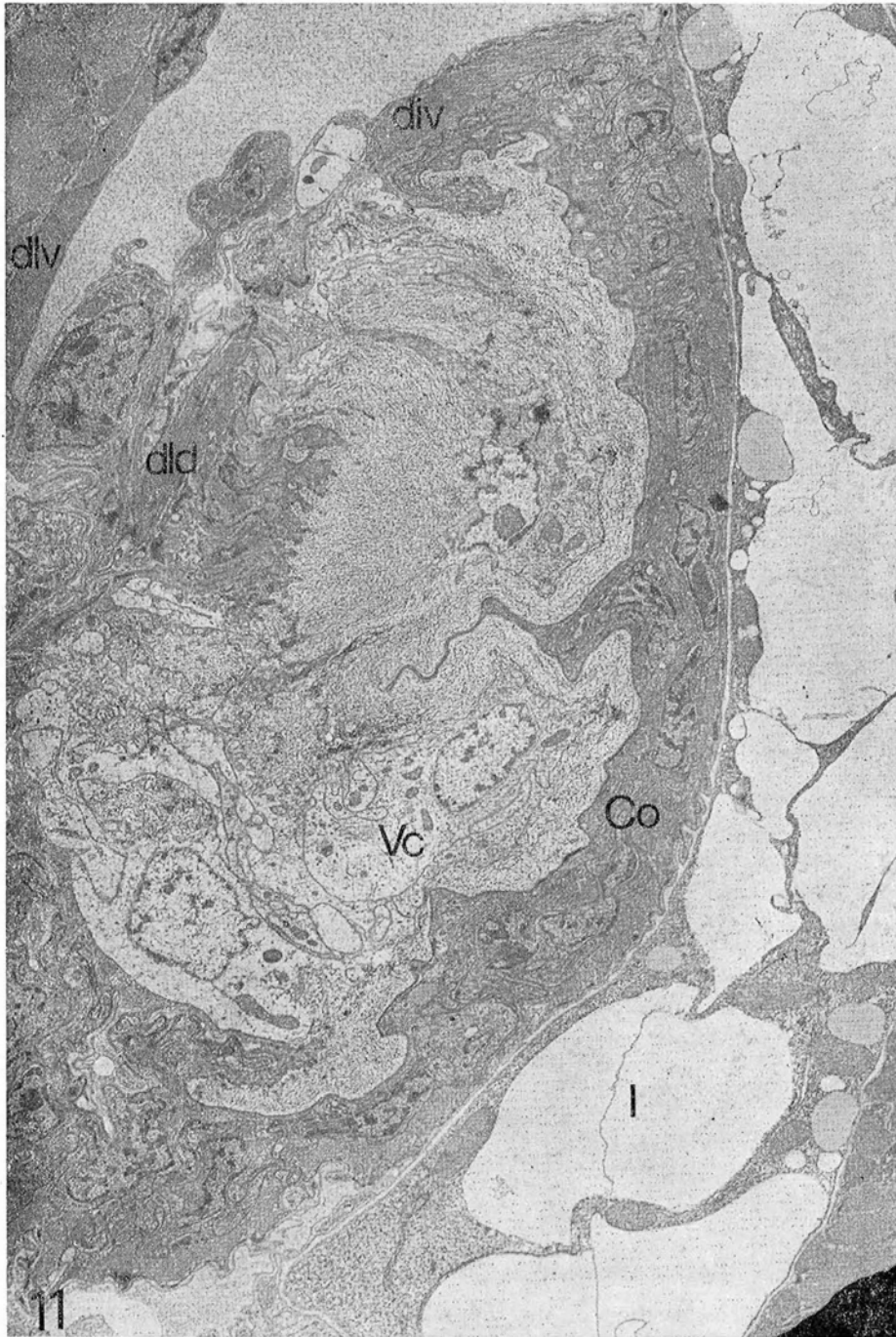


Fig. 11 - Cross section of the vagina of *X. index*: Co = compressores of the ovijector; div = *directores vaginae*; dlv = *dilatatores vaginae*; dld = *dilatatores dorsales*; Vc = vaginal cells; I = intestine. (x 20,000)

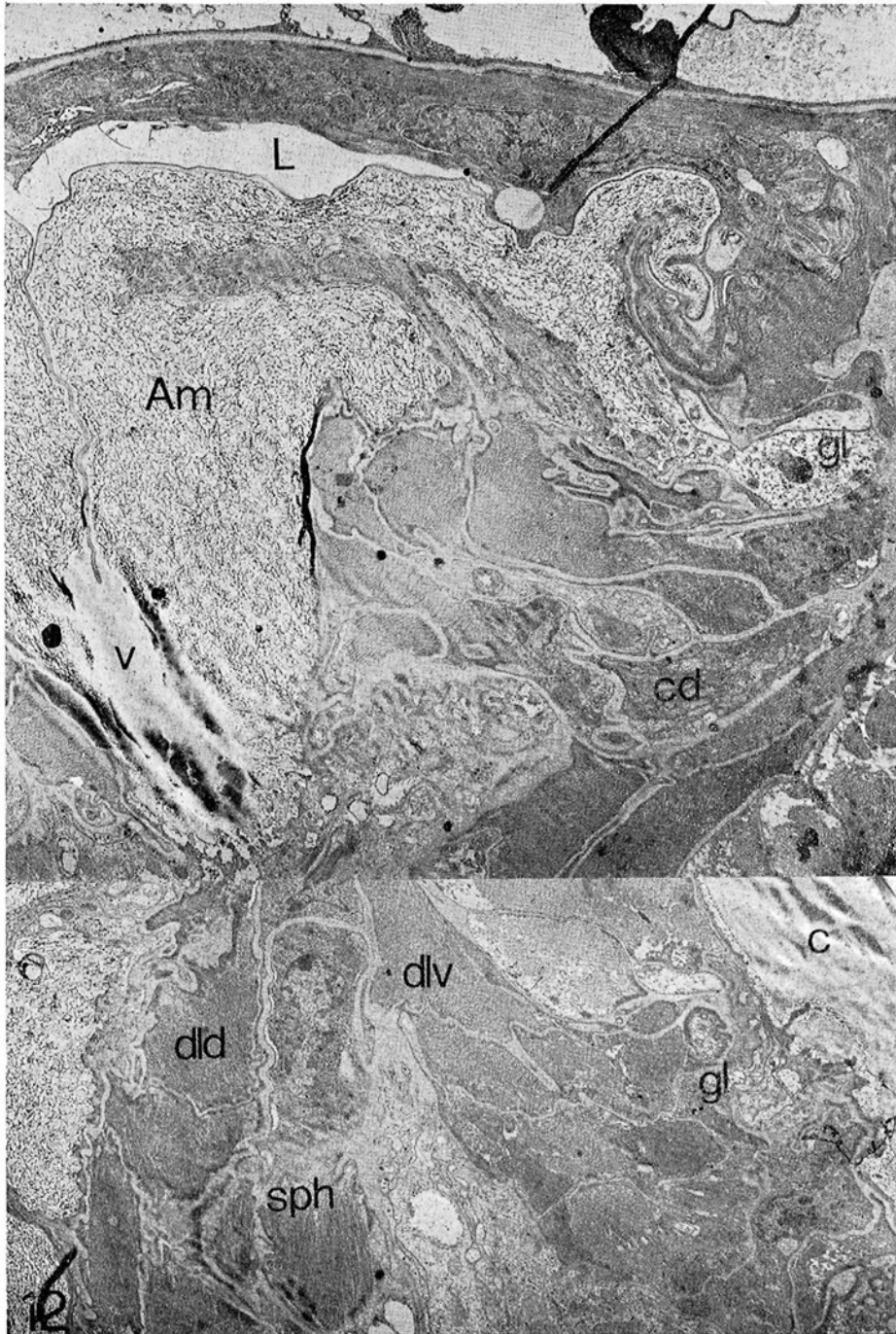


Fig. 12 - Cross section of the vagina of *X. index*: L = lumen; Am = amorphous matrix lining the lumen; gl = glandular structures; sph = sphincter; v = vulva; cd = cord; c = cuticle; dlv = *dilatatores vaginae*; dld = *dilatatores dorsales*. (x 20,000)

#### f) *Vagina and vulva*

The vagina essentially consists of a large band of muscles surrounding an inner structure. Dorsally (Fig. 11) there is a band of longitudinal muscles, compressor of the ovijector, which is continued by two bands longitudinally and transversally oriented on each side, the « *directores vaginae* ». Ventrally are the « *dilatatores vaginae* », which are also longitudinally directed and connected to the dorsal hypodermal cord. Dorsally to those are the « *dilatatores dorsales* » and in the middle the sphincter or « *constrictor vaginae* ». Inside this muscle mass is the tetraradiate lumen (Fig. 1c) surrounded by three ventral and three dorsal cells with granulated cytoplasm and large nuclei, ribosomes, a few mitochondria, glycogen and secretory bodies. The lumen is lined by a much sinuated cuticle and its cells are enveloped in a dense structureless matrix (Fig. 12). In these cells are vesicles full of glycogen. This region is innervated by nerves from the ventral hypodermal cord.

The vulva (Fig. 12) appears as a transverse slit-like opening surrounded by two lips constituted by « cortex » and « medulla » (Dick and Wright, 1973). The vaginal opening is closed by an electron unstained amorphous mass.

#### *Discussion*

The general structure of female gonad of nematodes is well known from the numerous light microscope studies made by various authors. Chitwood and Chitwood (1950) have reported on animal parasitic nematodes and Luc (1961), Coomans (1964), Yuen (1964) and Aboul-Eid (1969) on plant parasitic nematodes. Further information was obtained with the aid of the electron microscopy by Foor (1967), Epstein *et al.* (1971), Yuen (1971), Kisiel *et al.* (1972), Dick and Whight (1973) and Shepherd *et al.* (1973), but on other species as those dealt with by us. A few considerations on the function of the structures observed in *X. index* and *X. mediterraneum* might be, therefore, of interest.

The presence of secretory bodies in the germinal zone of the ovary suggests that material synthesized is rapidly translocated from the epithelium to the oocytes (Yuen, 1971). However, the function

of these bodies is not yet clear; they could contribute to the formation of a first involucre.

The ovary is without muscles as is the oviduct and its sac. We have noticed that the ovary reaches its largest size when mature oocytes are present which may stretch the epithelium. This expansion is readily accommodated in the oviduct which has a much folded epithelium. The epithelium of the oviduct also seems to have a secretory function to lubricate the passage of the oocyte.

The first innervation of the gonad is seen in the sac of the oviduct. They are similar to those reported for *Panagrellus silusiae* (Yuen, 1968) and derive from the ventral nerve (Roggen *et al.*, 1967). The glandular secretions, very evident in this portion of the gonad, probably form the second involucre of the oocyte.

The sphincter consists of a muscular mass intruding into the uterus. Its wall is much folded to allow for expansion.

The egg shell is formed in the glandular portion of the uterus where secretory granules are present in the cisternae and calcium in the vacuoles. The presence of the system of membranes and vacuoles in the uterus and the complexity of the Golgi apparatus support this observation. The system of membranes acts as a mechanism for deposition and discharge of material involved in the formation of the shell (Foor, 1967). The numerous vesicles present in these membranes are situated in areas poor of endoplasmic reticulum; therefore, it can be assumed that they are filled with protein substances produced by ribosomes (Narang, 1971). The complexity of the Golgi apparatus is related to the concentration of the secretion products, probably carbohydrates, obtained from a synthesis of simpler sugars linked to proteins produced by the ribosomes and distributed through the endoplasmic reticulum.

The vagina consists of a large many-folded surface which permits considerable expansion. Some vaginal cells show glandular activity with production of lubricating fluid. The lumen of the distal portion of the vagina is not tetra-radiate, but becomes so in its proximal part (Dick and Whight, 1973). The eggs are pushed into the « ovijector » by muscular contraction and during the process the tetra-radiate structure of the uterus becomes rounded.

The vaginal muscles have different functions: dorsally, a bundle of longitudinal muscles is present with the function of pressing on the « ovijector » (compressores of the « ovijector ») (Aboul-Eid, 1969).

Laterally, on both sides, longitudinal and transversal bundles keep the shape of vagina. Transverse and longitudinal groups are situated ventrally to the lumen, constituting the dorsal dilatores of vagina. The sphincter is at the convergence of these.

The ventral dilatores of vagina and vulva are contiguous with the somatic muscles attached to the lateral cords and enclosing the ventral hypodermal cord. The complex of muscles is innervated through the ventral nerve.

The vulva lips consist of an invagination and thickening of the cuticle whose different layers cross each other internally to allow a firm attachment of the muscles and a larger area of expansion. Other glandular structure noted here are probably involved in egg expulsion.

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#### S U M M A R Y

An electron microscope study of the female gonad of *Xiphinema index* Thorne *et* Allen and *X. mediterraneum* Martelli *et* Lamberti revealed no fundamental differences between the two species. The possible function of the structure observed is commented on.

#### R I A S S U N T O

*Studi preliminari sulla ultrastruttura della gonade femminile di Xiphinema index e X. mediterraneum (Nematoda: Longidoridae).*

In studi condotti con l'aiuto del microscopio elettronico non sono state notate differenze fondamentali tra la gonade femminile di *Xiphinema index* Thorne *et* Allen e quella di *X. mediterraneum* Martelli *et* Lamberti. Vengono fatti dei commenti sulla possibile funzione delle strutture osservate.

#### R É S U M É

*Etudes préliminaires sur l'ultrastructure de la gonade femelle de Xiphinema index et X. mediterraneum (Nematoda: Longidoridae).*

Des études de microscopie électronique on montré qu'il n'y a pas de différences fondamentales entre la gonade femelle de *Xiphinema index* Thorne *et* Allen et celle de *X. mediterraneum* Martelli *et* Lamberti. Des commentaires sur la fonction possible des structures observées sont apportés.



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