

## Structure of the female reproductive system of *Xiphinema americanum* (Nematoda: Longidoridae)

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**Summary** – The female reproductive system of *Xiphinema americanum* differs from that of other *Xiphinema* species in several respects. The ovary has branched oocytes surrounded by epithelial wall cells in which symbiotic bacteria are embedded. The oviduct has a long *pars dilatata* which is not clearly separated from the slender part; its wall is extensively folded and extendible. The sphincter between oviduct and uterus has a sinuous lumen and ‘internal musculature’. The uterus is extremely short and has a folded wall; the two uteri constitute together an extendible ovejector. Shortening of the uterus is considered to be an evolutionary trend in the *X. americanum*-group. © Orstom/Elsevier, Paris

**Résumé** – *Structure du système reproducteur femelle chez Xiphinema americanum (Nematoda: Longidoridae)* – Le système reproducteur femelle de *Xiphinema americanum* diffère en plusieurs points de celui des autres espèces de *Xiphinema*. L'ovaire présente des oocytes ramifiés entourés d'une paroi de cellules épithéliales dans lesquelles sont incluses des bactéries symbiotiques. L'oviducte présente une longue *pars dilatata* non clairement distincte de la partie étroite et dont la paroi, très repliée, est extensible. Le sphincter situé entre l'oviducte et l'utérus présente une lumière sinueuse et une "musculature interne". L'utérus est très court et sa paroi est également repliée; l'un et l'autre utérus forment ensemble un ovejecteur extensible. Le raccourcissement de l'utérus est considéré comme une tendance évolutive dans le groupe *X. americanum*. © Orstom/Elsevier, Paris

**Keywords:** bacteria, nematode, reproductive system, symbionts, ultrastructure, *Xiphinema americanum*.

The general morphology of the female reproductive system is illustrated in detail in descriptions of most *Xiphinema* species, but not in descriptions of the species in the *X. americanum*-group.

Several ultrastructural studies on the female reproductive system in *Xiphinema* have been published (Bleve-Zacheo *et al.*, 1976; Van de Velde *et al.*, 1990a, b; Coomans *et al.*, 1992), but, apart from a few details on the structure of the sphincter and the wall of the ovary in *X. pachtaicum* (Bleve-Zacheo *et al.*, 1976), no information is available on the ultrastructure of the female reproductive system in species belonging to the *X. americanum*-group. In this paper we report on the reproductive system of *X. americanum* Cobb, 1913.

### Materials and methods

Light microscopy observations are based on unstained specimens from various places in the USA, including topotype specimens, mounted on permanent Cobb slides.

For TEM, soil samples were collected in the USA by Drs R.T. Robbins and J. Halbrendt and shipped to Ghent. In the laboratory, the nematodes were extracted from the soil by the centrifugal-flotation method, using a non-toxic silica gel (Ludox AS, Du

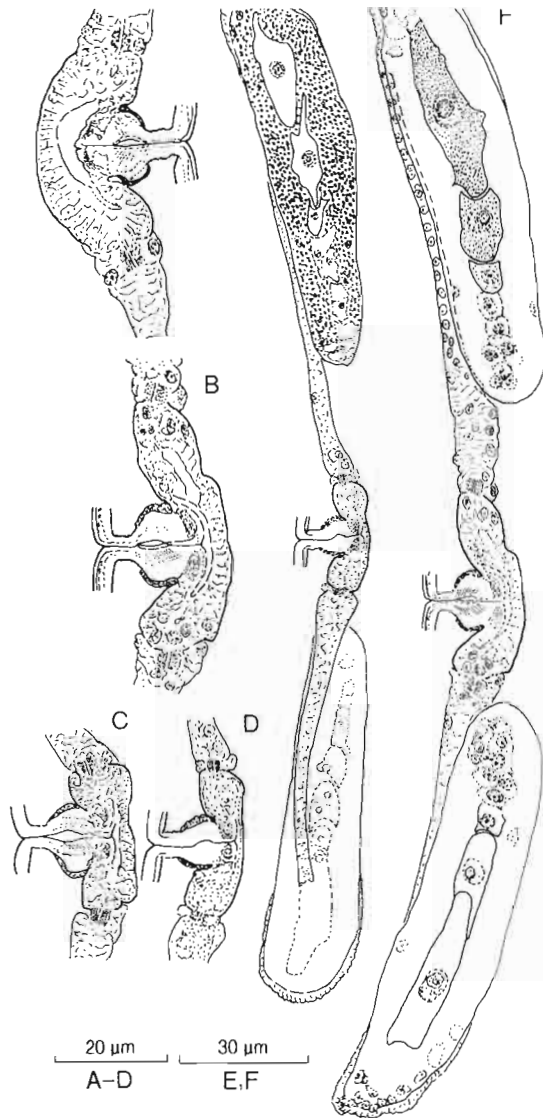
Pont de Nemours). Adult females were placed in an ice bath for a few minutes to relax. Then they were killed and fixed in ice-cooled fixative composed of 1% paraformaldehyde, 1.75% glutaraldehyde, and 1.5% acrolein in 0.1 M sodium cacodylate buffer pH 7.2. After 30 min, the nematodes were transferred to the fixative minus acrolein and were cut in pieces roughly 200–300 µm long, each piece containing one branch of the genital system. Cutting the specimens facilitates penetration of the fixative. Then, the pieces were incubated overnight in complete formula fixative at 4°C. After approximately 15 h of fixation, they were rinsed in 0.1 M sodium cacodylate buffer for 8 h. Postfixation was in 2% osmium tetroxide in 0.2 M sodium cacodylate buffer for 36 h and was followed by an *en bloc* staining of 1 h in 2% uranyl acetate. The specimens were dehydrated in a graded ethanol series and embedded in Spurr's resin.

Ultrathin sections were made on a Reichert ultracut S ultramicrotome and picked up on formvar-coated copper slot grids. The sections were post-stained in a LKB ultrastainer, for 30 min in uranyl acetate at 40°C and 5 min in lead stain at 20°C. The sections were examined with a Siemens Elmiscop 1A and a Jeol JEM 1010, both operating at 80 kV.

## Results

### LIGHT MICROSCOPY (Fig. 1)

The female reproductive system of *X. americanum* consists of two equally developed branches, lying either on the same side of the intestine (left or right) or on different sides. As in other species of the group,



**Fig. 1.** *Xiphinema americanum*. General morphology of female reproductive system. A-D: Variation in ovejector; E-F: Whole system (A, B, F: specimens from Wisconsin; C: specimen from Arlington National Cemetery, type locality; D, E: specimen from Rhode Island).

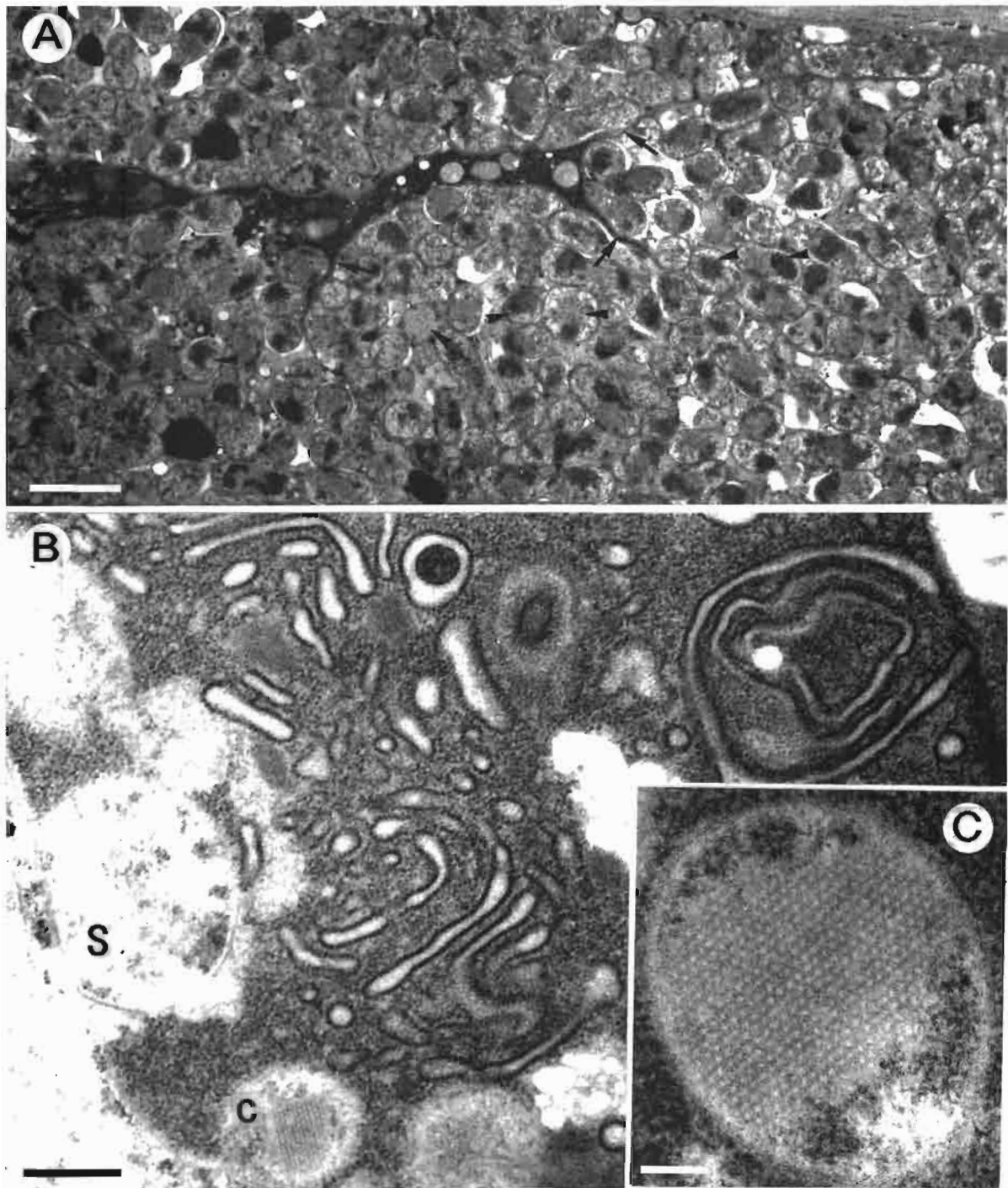
symbiotic bacteria are present around the germ cells. The youngest germ cells (oogonia?) are clustered in the apical region of the ovary; they are followed by a single row of oocytes. The oviduct consists of a slender part, with 18-22 ( $n=4$ ) nuclei, and a more expanded part (*pars dilatata oviductus*), with 16-20 ( $n=2$ ) nuclei. However, the boundary between the slender and expanded parts is difficult to establish under the light microscope. An obscure sphincter separates the oviduct from the very short uterus. The latter, combined with the uterus of the other branch forms a short ovejector, 30.1 (24-43)  $\mu\text{m}$  long ( $n=10$ ). The ovejector is very extensible since it can harbour an egg of roughly five times its resting length (egg size: 150-170  $\times$  24-31  $\mu\text{m}$ ,  $n=5$ ). The vagina has a wider proximal part with obliquely striated cuticle which is surrounded by the vaginal sphincter, and a narrower distal part to which the vulval dilator muscles attach. The vulva is a transverse slit.

### TRANSMISSION ELECTRON MICROSCOPY

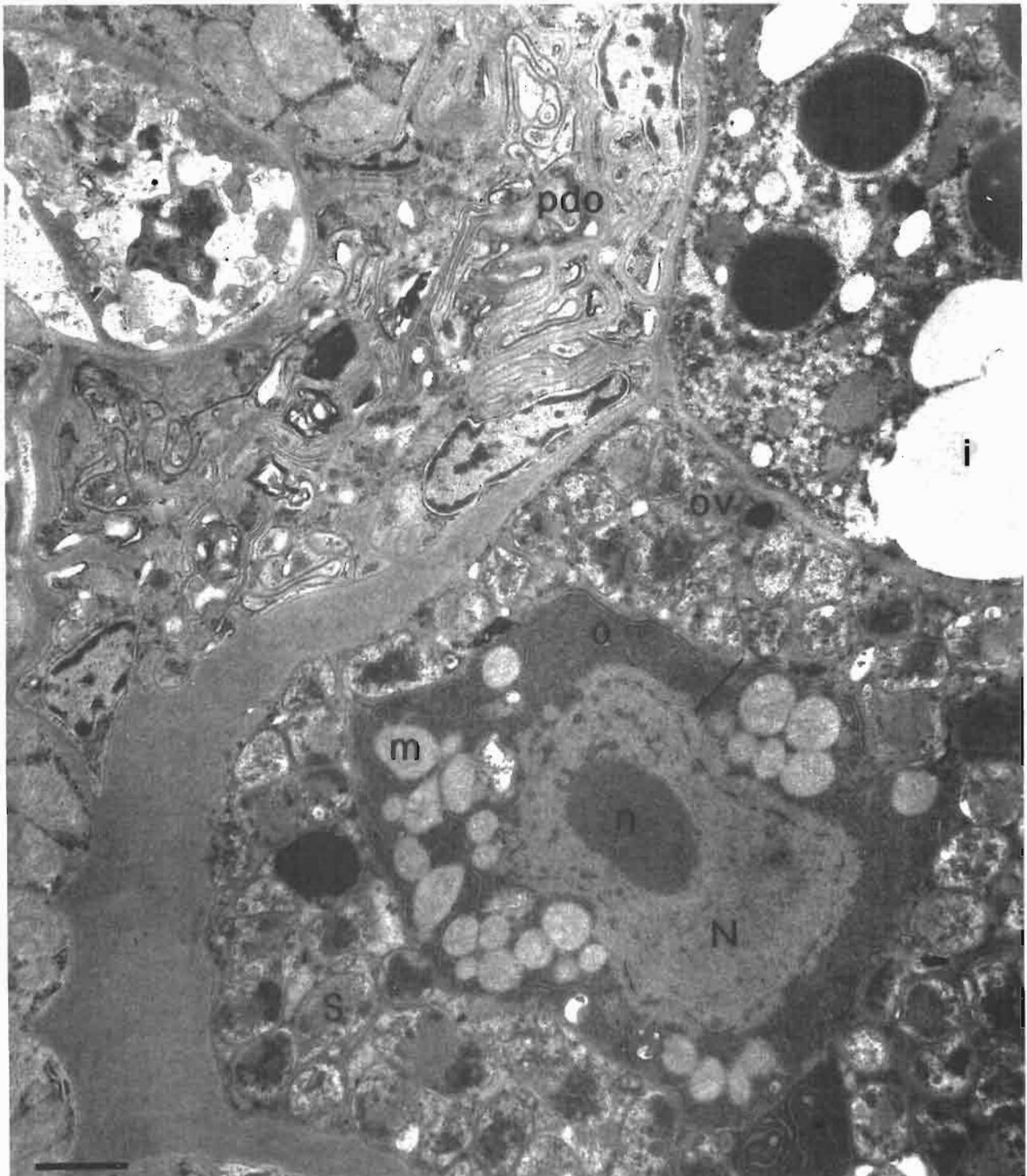
#### Ovary

The ovary of *X. americanum* consists of epithelial wall cells and germ cells as in other *Xiphinema* species, but, in addition, the ovary contains symbiotic bacteria. The outer membrane of the ovary is covered by a *basal lamina*, 60-80 nm thick. The central part of the ovary is occupied by a small number of irregularly-shaped oocytes. Narrow branched extensions of the oocytes lie between the bacteria (Fig. 2A). The oocytes have an electron-dense cytoplasm due to abundant ribosomes. The cytoplasm of the oocytes also includes clusters of electron-transparent mitochondria (Fig. 3), and, in some regions, elaborate configurations of rough endoplasmic reticulum (Fig. 2B). Some cisternae are always found close to the cell surface. The large central nucleus (about 10  $\mu\text{m}$  in diameter) has a moderately electron-dense nucleoplasm, denser scattered chromatin, and a large (3-7  $\mu\text{m}$ ) electron-dense round to oval nucleolus. Nucleoplasm-like material is present in the perinuclear region as a narrow (Fig. 3) or wider zone. The outer nuclear membrane is coated with ribosomes. Adjacent oocytes may overlap one another over a short distance (Fig. 3).

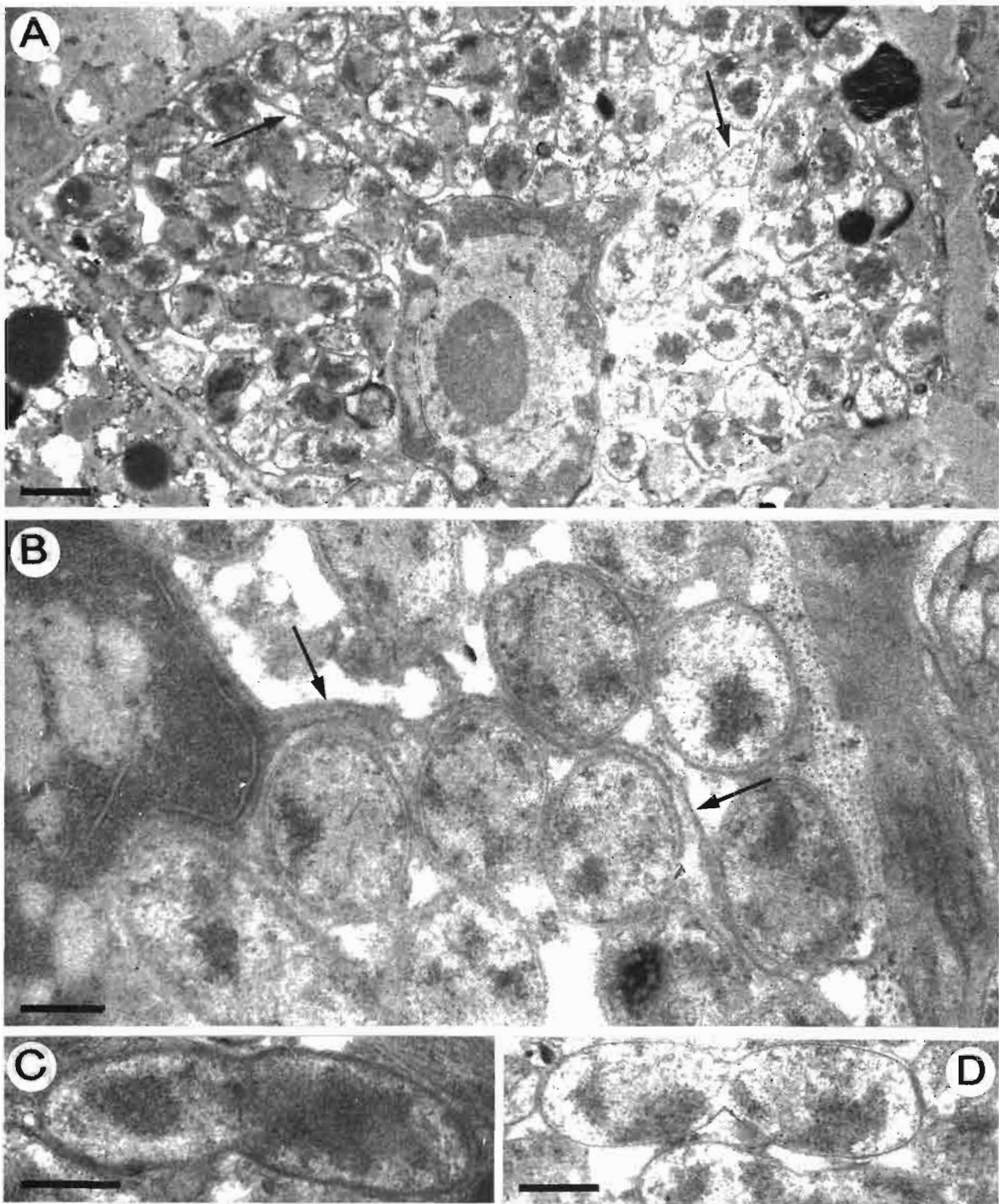
Near its apex, the ovary is mainly filled with bacteria embedded in the epithelial wall cells. The nuclei of the epithelial cells are irregular in shape and small (5-7  $\times$  3-5  $\mu\text{m}$ ), scattered, and few in number throughout the ovary itself. These nuclei contain a rather high amount of electron-dense chromatin, partly clumped against the inner nuclear membrane and partly in patches throughout the nucleoplasm. The cytoplasm of the epithelial cells is difficult to observe because of its fragmentation between the enclosed bacteria. It is less electron-dense than the



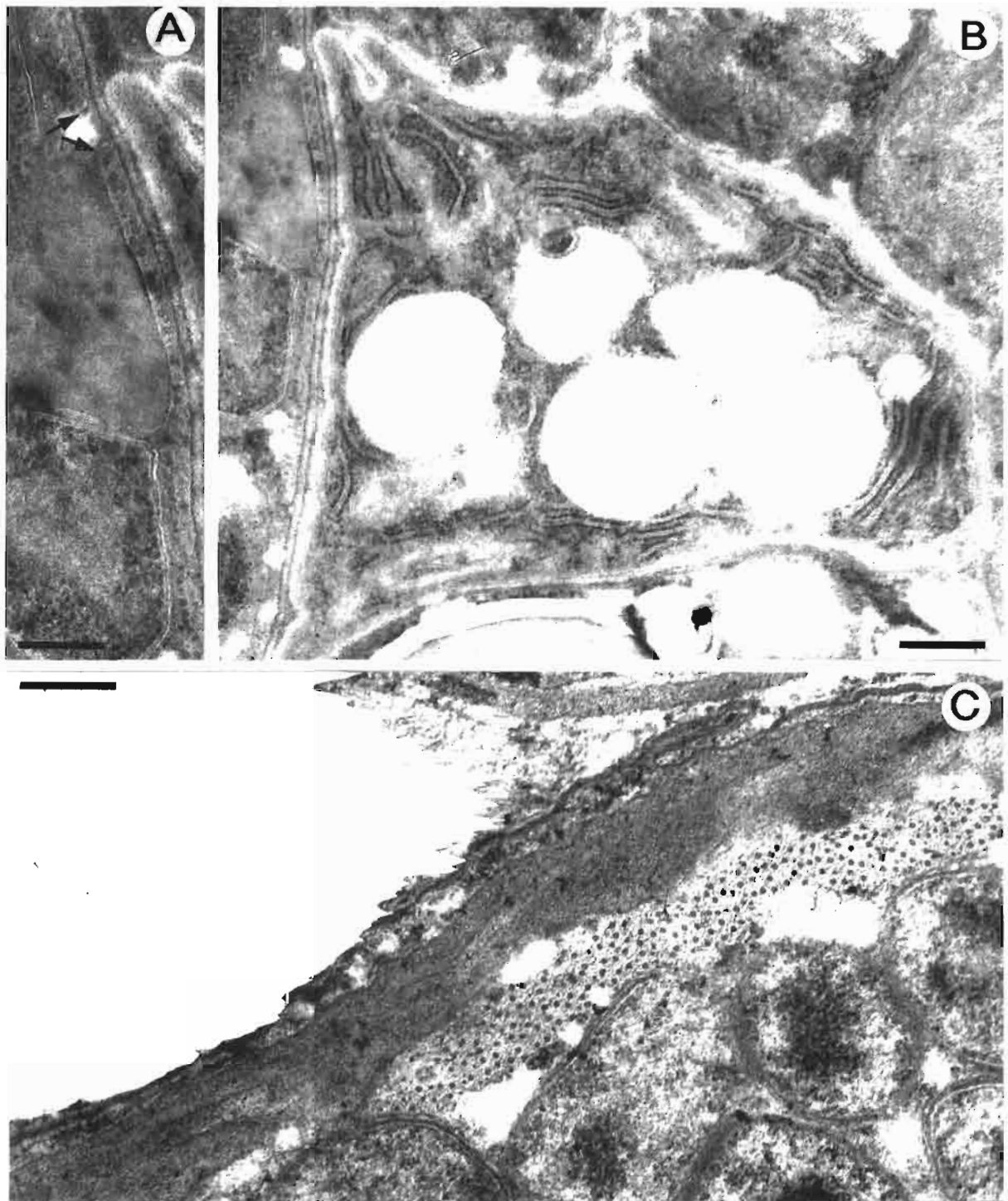
**Fig. 2.** *Xiphinema americanum*. *A*: L.S. through the ovary, showing branched oocyte (arrow) and symbionts (arrowheads); *B*: C.S. through an oocyte, showing RER, and enclosed symbionts; *C*: Larger magnification of the paracrystalline S-layer of a symbiont (Scale bar: *A* = 2  $\mu\text{m}$ ; *B* = 0.3  $\mu\text{m}$ ; *C* = 0.1  $\mu\text{m}$ . Abbreviations: c: paracrystalline S-layer, C.S.: cross section, i: intestine, L.S.: longitudinal section, m: mitochondrion, n: nucleolus, N: nucleus, o: oocyte, ov: ovary, pdo: pars dilatata oviductus, S: symbiont, sg: secretory granule).



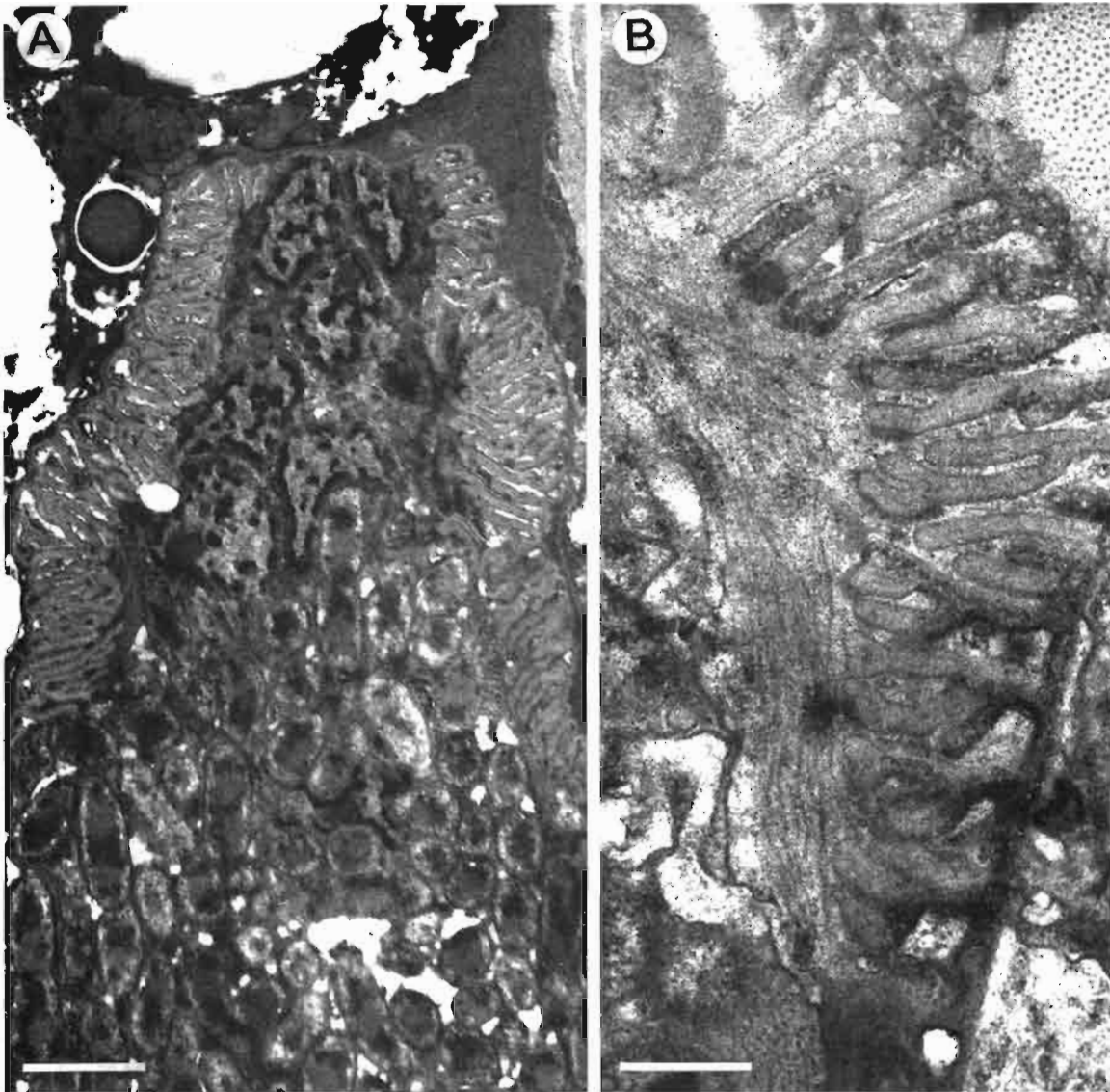
**Fig. 3.** *Xiphinema americanum*. C.S. through ovary and pars dilatata oviductus (Arrow: perinuclear nucleoplasm-like material; arrowheads: overlapping oocytes. Scale bar = 1  $\mu$ m. See Fig. 2 for abbreviations).



**Fig. 4.** *Xiphinema americanum*. *A*: C.S. through the ovary, with central oocyte surrounded by epithelial wall cells and symbionts; *B*: Membranes of adjacent myoepithelial cells, separating symbionts and connecting the outer wall of the ovary with the central oocyte; *C*, *D*: Dividing symbionts (Arrows in *A* and *B* point to plasmamembranes of adjacent epithelial and myoepithelial cells. Scale bars: *A* = 1  $\mu$ m; *B* = 0.3  $\mu$ m; *C*, *D* = 0.5  $\mu$ m. See Fig. 2 for abbreviations).



**Fig. 5.** *Xiphinema americanum*. A: C.S. through ovarian wall about halfway in the ovary (arrows point to myofilaments); B: C.S. through the slender part of the oviduct; C: C.S. through ovarian wall close to ovarian sac (Scale bar: A = 0.2  $\mu$ m; B, C = 0.3  $\mu$ m. See Fig. 2 for abbreviations).



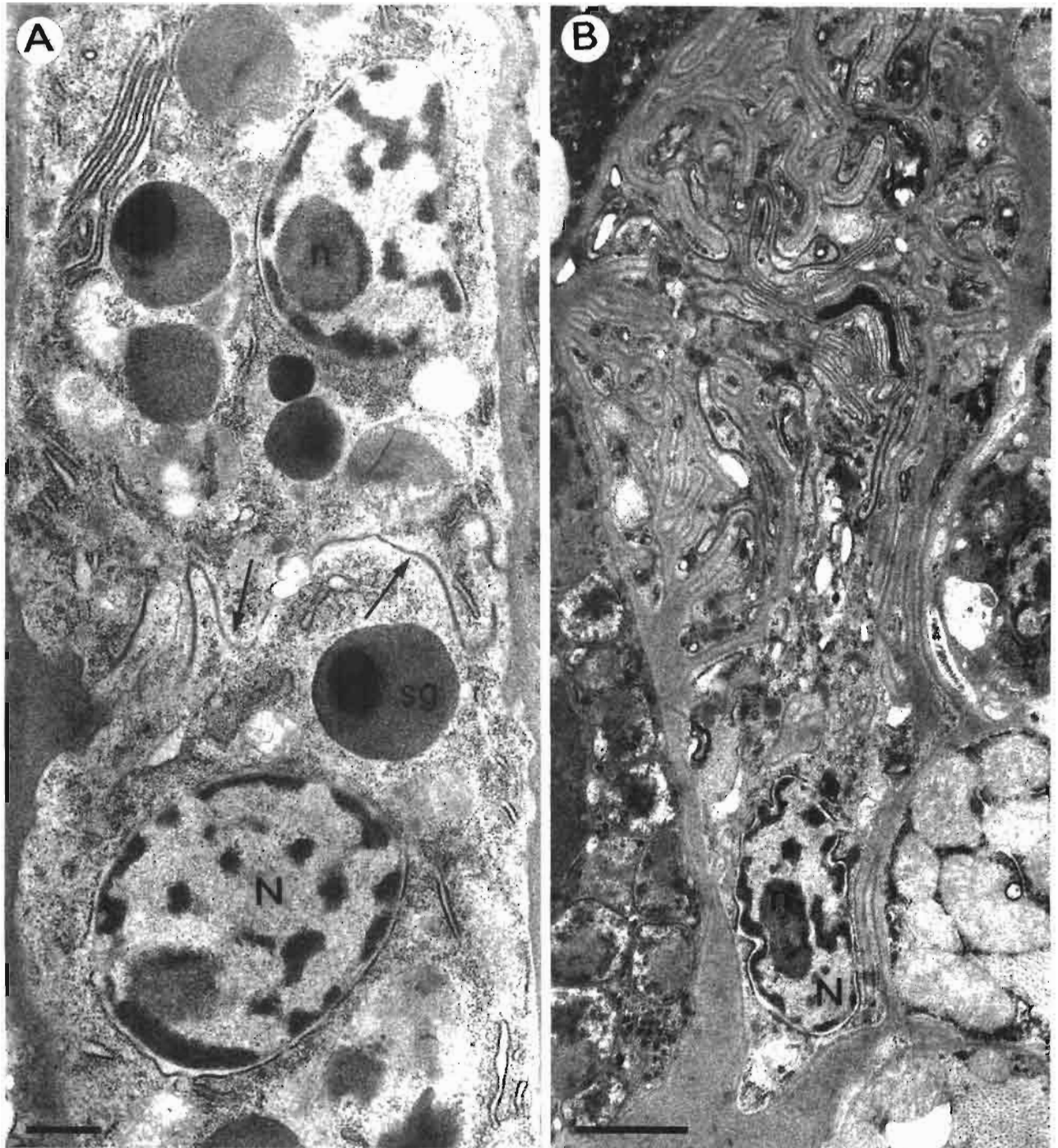
**Fig. 6.** *Xiphinema americanum*. A: L.S. through ovular sac; B: C.S. through wall of ovular sac (Scale bars: A = 2  $\mu$ m; B = 0.5  $\mu$ m. See Fig. 2 for abbreviations).

cytoplasm of the germ cell and contains scattered small granular inclusions.

The symbionts have the features of Gram negative bacteria; they fill all but the central part of the ovary. Fine tissue strands and membranes running between the germ cells and the ovular surface subdivide the bacterial mass in several sectors. These connections most likely represent the plasma membranes of adjacent epithelial cells which centrally join the plasma membrane of the germ cells (Fig. 4A, B). The bacteria are closely packed and embedded inside the epithelial cells, but occasionally a few bacteria can be

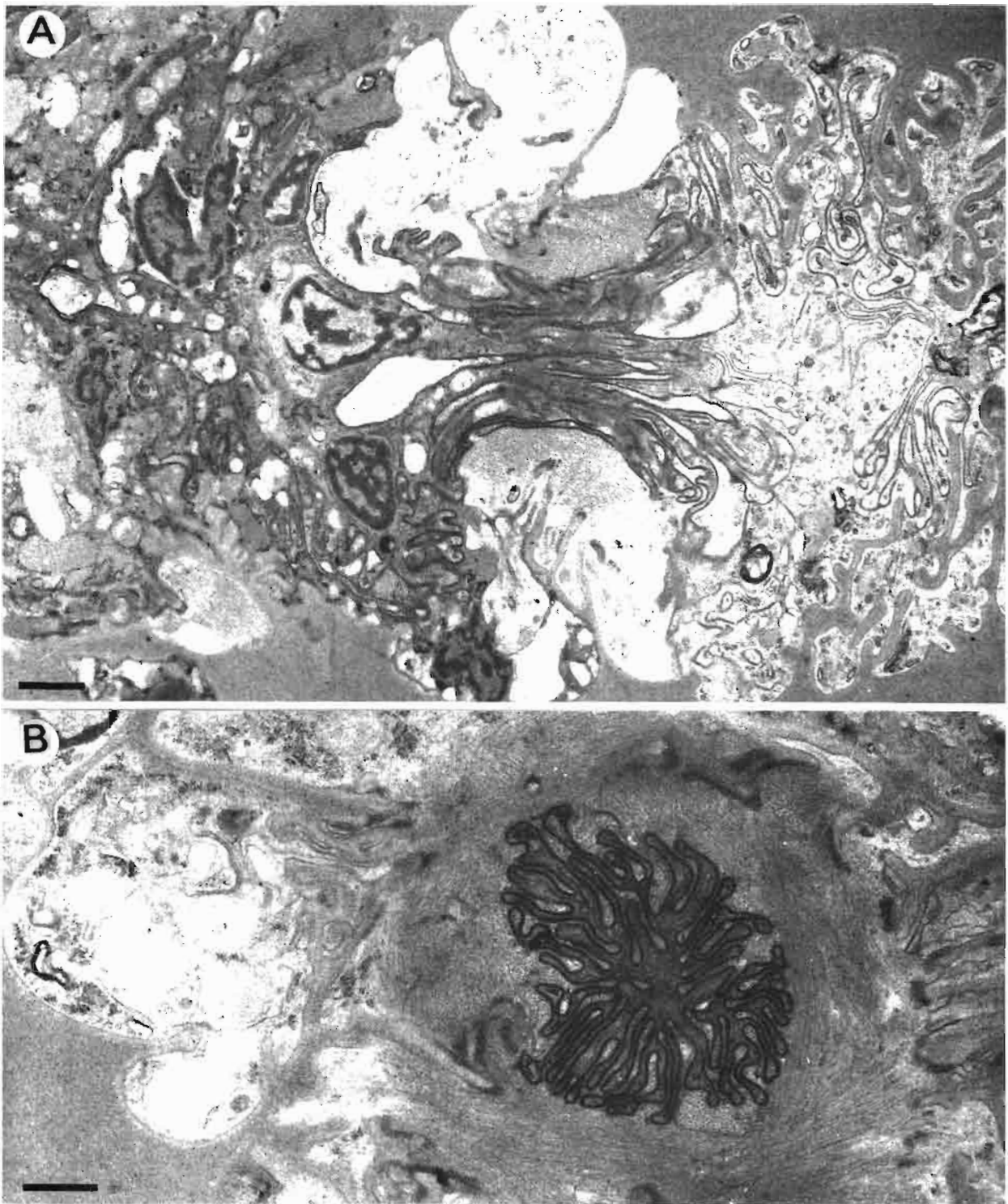
observed in close contact with or even encapsulated inside an oocyte (Fig. 2B). An exceptional tangential section (Fig. 2C) shows the S-layer of a bacterium, arranged as a 2D hexagonal close packing of spheres. Size and shape of the bacteria varies according to the section; some can be observed dividing (Fig. 4C, D).

Occasional myofilaments occur at the periphery of the ovarium in a single or double layer (Fig. 5A), from about halfway its length, becoming more numerous and more regularly distributed towards the ovular sac (Figs 4B; 5C), where the epithelial wall cells become myoepithelial cells. The myofilaments are mostly lon-

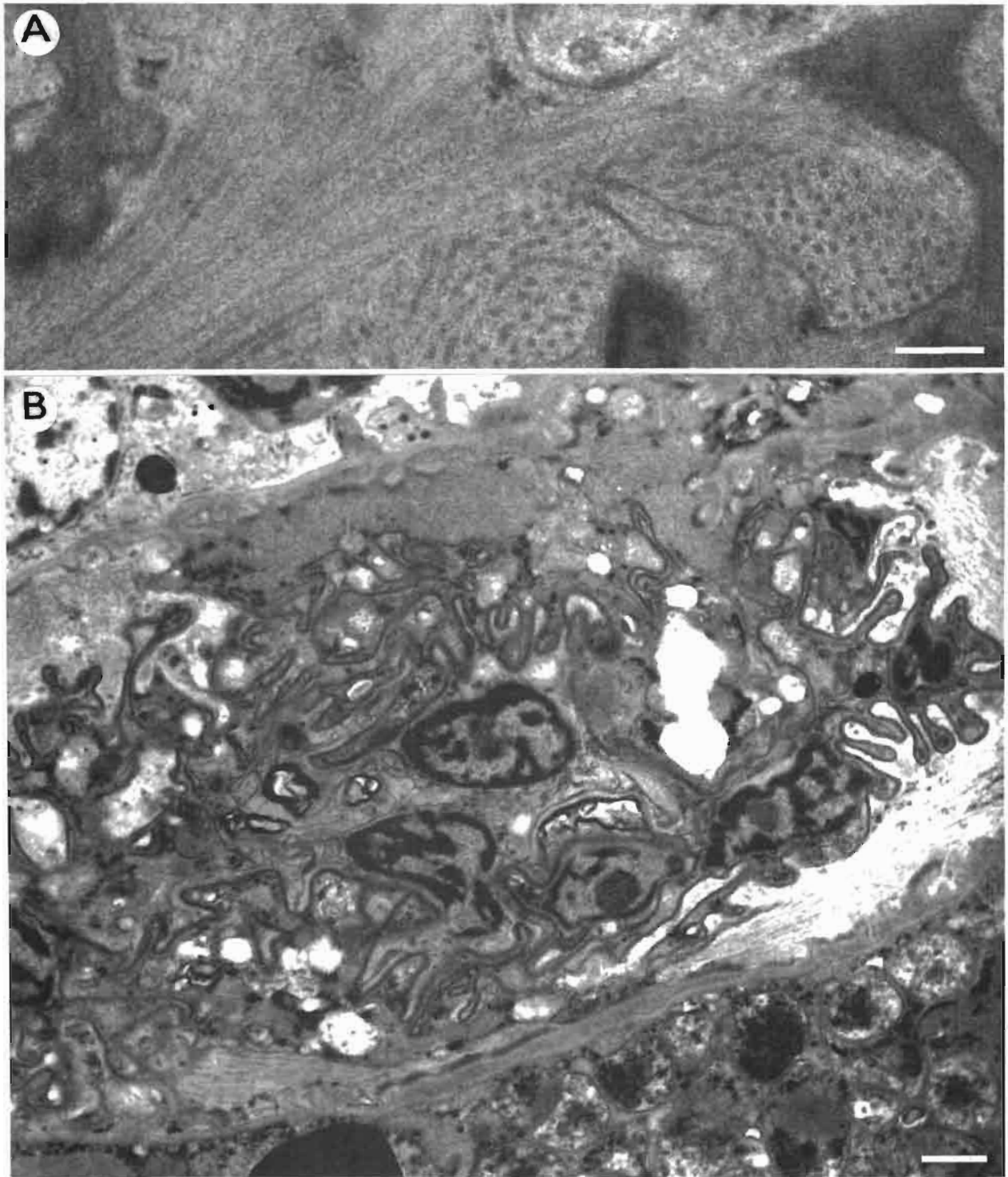


**Fig. 7.** *Xiphinema americanum*. A: L.S. through slender part of oviduct, showing two adjacent cells; B: C.S. of pars dilatata oviductus (Arrows indicate irregular plasmamembranes. Scale bars: A = 0.5  $\mu$ m; B = 1  $\mu$ m. See Fig. 2 for abbreviations).





**Fig. 8.** *Xiphinema americanum*. Sphincter between oviduct and uterus. A: L.S; B: C.S (Scale bars: A = 1  $\mu$ m; B = 0.5  $\mu$ m. See Fig. 2 for abbreviations).



**Fig. 9.** *Xiphinema americanum*. A: Myofilaments of sphincter; B: C.S. through uterus (Scale bars: A = 0.2  $\mu\text{m}$ ; B = 0.5  $\mu\text{m}$ . See Fig. 2 for abbreviations).

gitudinally orientated in the ovarian wall, but become variously arranged in the ovarian sac.

#### Ovarial sac

The ovarian sac is characterised by a highly folded cell membrane of the epithelial wall cells, covered by a 125-145 nm thick basal lamina. Contrary to the ovarian wall, the nuclei are closely packed (Fig. 6A). The myofilaments are more numerous, mostly located underneath the folds and variously directed (Fig. 6B). Clusters of symbionts are embedded inside the myoepithelial cells, though less numerous than in the ovary proper. This makes it easier to observe the epithelial plasma membranes between them (Fig. 6B). When present, the growing oocyte occupies a central position as in the ovary.

#### Oviduct

The narrow part of the oviduct is maximally 9.5  $\mu\text{m}$  wide and composed of a string of cells without any apparent lumen between them. A 70-90 nm thick basal lamina covers the outer surface. The adjacent cell membranes are in close contact with each other and have an irregular course (Fig. 7A). The cytoplasm contains well developed RER cisternae and globules of variable size and variable density (Figs 5B; 7A). The nuclei are roundish (5.5-6.5  $\times$  4-5.5  $\mu\text{m}$ ), with clear nucleoplasm and electron-dense chromatin. The

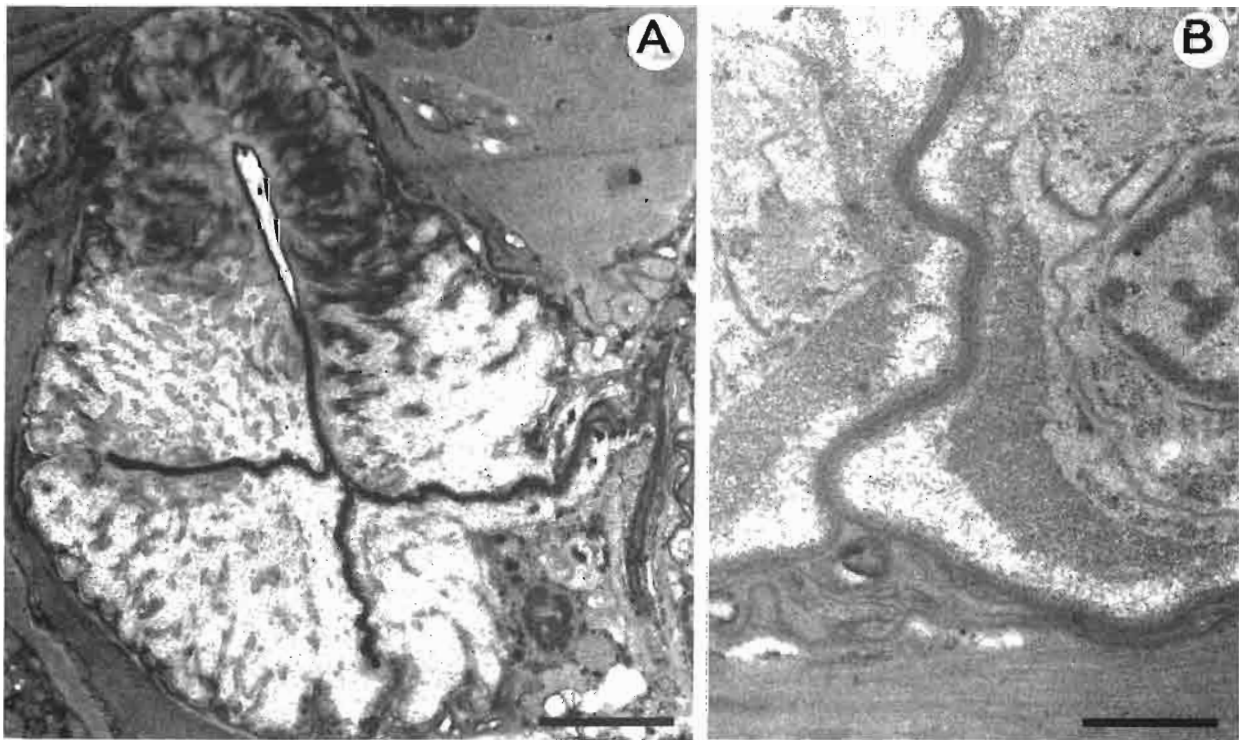
latter is mainly clumped against the inner nuclear membrane, but also occurs in irregular patches in the nucleoplasm. The nucleolus is about 2  $\mu\text{m}$  large and eccentric in position (Fig. 7A).

The *pars dilatata oviductus* is not clearly separated from the slender part, but its surface forms highly folded extensions into the pseudocoelom. The cell membranes of neighbouring cells are highly intertwined, forming intricate patterns. Cytoplasm includes rough as well as smooth endoplasmic reticulum (Figs 3; 7B), fine electron-dense material (glycogen?), and electron-dense irregular inclusions. The nuclei are oblong, more or less flattened, 6.5-9.5  $\times$  2.5-4  $\mu\text{m}$ , located at the periphery, and sometimes bulging out of the irregular contour. Although a central lumen is present, it is difficult to observe due to extreme folding of the whole *pars dilatata*. The latter is greatly extended when the lumen contains an egg cell.

Myofilaments are absent throughout the oviduct.

#### Sphincter

The sphincter between oviduct and uterus consists of *i*) a highly folded inner lining surrounding a narrow and multibranched lumen (Fig. 8B); *ii*) a muscular wall comprising inner myofilaments that are mostly longitudinally arranged and outer myofilaments that are mostly circular and partly obliquely arranged



**Fig. 10.** *Xiphinema americanum*. A: C.S. through vagina (junction with ovejector on the right); B: Detail of junction of vagina (above) with ovejector (below) (Scale bars: A = 5  $\mu\text{m}$ ; B = 0.5  $\mu\text{m}$ . See Fig. 2 for abbreviations).

(Fig. 9A); *iii*) several cell bodies that bulge into the surrounding pseudocoelom (Fig. 8). The plasma membrane is covered by a 100-125 nm thick basal lamina. The cytoplasm is rather electron-translucent except for the proximal part, adjacent to the uterus, where the nuclei are located (Fig. 8A). The central part (*i + ii*) is about 20  $\mu\text{m}$  long and 10-14  $\mu\text{m}$  in diameter, but the diameter value increases to 30  $\mu\text{m}$  if the bulging cell bodies (*iii*) are included.

#### Uterus

The short uterus (or ovejector, see below) has a strongly folded inner lining and less folded outer wall, but the folding is conspicuously less developed than in the oviduct and the lumen can be more readily observed (Fig. 9B). The wall musculature is well developed and myofilaments are variously directed, but with obliquely transverse orientation predominant. The nuclei are occurring centrally as well as close to the surface and may bulge out of the contour; nuclei are oval to irregular in shape, 3.5-6  $\times$  2-4  $\mu\text{m}$ , and contain patches of electron-dense chromatin. The chromatin may be particularly abundant on the inner nuclear membrane. The uterus is covered by a 100 nm thick basal lamina.

#### Vagina and vulva

The vagina has a cross-shaped lumen with electron-dense lining (Fig. 10A). Its cuticular wall contains many electron-dense fibres, running between inner and outer surface and forming irregular patterns. Proximally, the longitudinal arms of the cross open into the lumen of the ovejector. At this junction, each arm is surrounded by two pairs of cells with prominent nuclei. The pair closest to the lining contains electron-dense material arranged as a strand (Fig. 10B). Distally the longitudinal arms disappear while the transverse ones become the vulva. The cuticle becomes more dense and transverse oval in shape and merges with the body cuticle at the vulva opening which is a transverse slit.

#### Discussion

Bleve-Zacheo *et al.* (1976) described the ultrastructure of the female reproductive system in *Xiphinema index* and *X. pachtaicum* (= *X. mediterraneum*). In the light of the present observations on *X. americanum*, it is a pity that these authors gave a single description for both species, because "no fundamental differences have been observed between the structure of the gonads in the two species...". Fortunately, the species included in the plates were clearly identified.

The structure of the ovary and gonoduct described above for *X. americanum* markedly differs from the pattern that occurs in *Xiphinema* species outside of the *X. americanum*-group (Bleve-Zacheo *et al.*, 1976; Van de Velde & Coomans, 1988; Van de Velde *et al.*, 1990a, b; Coomans *et al.*, 1992).

The differences in the ovary are the most obvious and related to the presence of symbiotic Gram-negative bacteria inside the very thick wall. Although these symbionts can be readily observed under the light microscope, their exact location inside the ovary can be assessed only with TEM. They appear to be embedded inside the epithelial wall cells and, rarely, also in the oocytes. More information about these bacteria will be published elsewhere. The small granular inclusions in the epithelial cells are probably glycogen.

The slender part of the oviduct resembles that described for other *Xiphinema* species, except that it is narrower and comprises a lesser number of cells and larger nuclei. The well developed rough endoplasmic reticulum and the presence of globules demonstrates the secretory nature of these cells. On the other hand, the *pars dilatata oviductus* differs in several respects: it is comparatively longer, not clearly separated from the slender part, and it has an extensively folded wall.

The sphincter muscle between oviduct and uterus appears to be embedded into the surrounding cell bodies. This explains why it is difficult to observe the sphincter under the light microscope. The musculature and the sinuous lumen agree with those described for *X. pachtaicum* by Bleve-Zacheo *et al.* (1976).

The uterus is extremely short and in fact both uteri together constitute the muscular ovejector. Its outer wall is more folded than in species outside of the *X. americanum*-group, which is understandable in view of the considerable expansion needed to harbour a large size egg. Personal unpublished observations on other species indicate that the shortening of the uterus is an evolutionary trend in the *X. americanum*-group.

#### Acknowledgements

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